Biogeochemistry of Ancient and Modern Environments

Proceedings of the Fourth International Symposium on Environmental Biogeochemistry (ISEB) and, Conference on Biogeochemistry in Relation to the Mining Industry and Environmental Pollution (Leaching Conference), held in Canberra, Australia, 26 August – 4 September 1979.

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PREFACE

This volume contains a selection of papers presented to the Fourth International Symposium on Environmental Biogeochemistry (ISEB), and a conference on Biogeochemistry in Relation to Mining Industry and Environmental Pollution (Leaching Conference), held in Canberra, Australia on August 26-31 and September 3-4, 1979, respectively.

The ISEB were established to provide "a forum for uninhibited exchange of information and ideas among the biological, chemical, atmospheric and geological scientists working in the common area of biogeochemistry, encompassing soil and other earth sciences as well as the hydrosphere and atmosphere". By linking the fourth ISEB with the Leaching Conference the scope of discussions was extended to encompass the application of biogeochemical processes to the mining industry. This wide-ranging philosophy is reflected in the breadth and diversity of the subjects covered in this book.

The published papers are expanded versions of those presented at the meetings. They have all been scrutinized by at least one referee in addition to the editors. About 20% of the contributions to the meetings are not included, either because authors did not wish to publish or because the papers were not accepted by the editors.

The meetings were sponsored by the Australian Academy of Science and received financial support from the following sources:

Australian Government
International Geological Correlation Program
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Scientific Committee for the Protection of the Environment
The Ian Potter Foundation
United Nations Environment Program
Australian Consolidated Industries Ltd
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Western Mining Corporation
The Zinc Corporation Ltd

The interests of these organizations were represented in the program and, in particular, the following papers are contributions to the IGCP Project 157 on "Early Organic Evolution and Mineral and Energy Resources": J. Bauld *et al.* (p. 157), Y. Cohen *et al.* (p. 167), P. Cloud (p. 7), W. E. Ewers (p. 83), R. O. Hallberg (p. 145), D. M. McKirdy (p. 187), R. P. Philp (p. 173), M. Schidlowski (p. 47) and G. H. Taylor (p. 65).

The successful organization of international meetings requires help and cooperation from many quarters. We gratefully acknowledge the assistance given by the organizations listed above, by members of the International ISEB Committee, especially J. Skujins and H. R. Ehrlich, and by many colleagues in Australia. Particular thanks are due to our colleagues on the Organizing Committee, E. Bubela, J. R. Freney and N. Williams, to Mss P. Tart, B. Stewart (Australian Academy of Science) and R. Raison (Baas Becking Geobiological Laboratory) who bore the brunt of the secretarial work, to Ms M. May for typing the manuscript, and to all those who unselfishly spent time and effort on reviewing papers.

P. A. Trudinger
M. R. Walter
B. J. Ralph

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UNITS AND OTHER ABBREVIATIONS

Wherever possible SI (Système International d'Unités) are used. Those which are relatively unfamiliar are listed below. Unless otherwise stated TEMPERATURES are expressed in degrees celsius.

a	= atto = 10^{-18}
B.P.	= Before present
Bq	= Becquerel = 27 pCi
E	= exa = 10^{18}
G	= giga = 10^{9}
Gy	= Gray = 100 rad
J	= joule = 0.239 calories
lx	= lux
M	= mega = 10^{6}
p	= pico = 10^{-12}
P	= peta = 10^{15}
Pa	= pascal (101 k Pa = 1 atmosphere)
per mil	= parts per thousand
T	= tera = 10^{12}

AN OUTSIDE VIEW OF ENVIRONMENTAL BIOGEOCHEMISTRY*

L. T. EVANS

President, Australian Academy of Science

I have always regarded the name of these Symposia as a stroke of genius, perhaps even of commercial genius. It has everything. It promises a broad and integrated approach. It should appeal to all sources of funds. One senses also a field in active evolution, from chemistry to biochemistry to biogeochemistry to environmental biogeochemistry. It could still be prefixed by exo- to gather in the space industry, and then by neo- when it begins to tire.

The symposium program is like a good French menu, one wants to sample it all. But this is fare for polymaths, with broad scientific interests. Searching for a suitable father figure, I wondered about C.G.B. Daubeny (1795-1867) of Oxford, a geologist who became professor of Chemistry and then of Botany, and who worked about 150 years ago on the emissions of volcanoes, the effect of atmospheric CO_2 levels on Carboniferous vegetation and the origin of ozone (Anon, 1868; Oldroyd and Hutchings, 1979). He would have been in his element on this occasion , which I can hardly claim to be, so you will have to make do with some comments whose only virtue is that they are not constrained by any knowledge of the field.

Before making these however, may I, on behalf of the Australian Academy of Science which has sponsored this Symposium, add to Senator Webster's welcome to you all and express the hope that your discussions this next week will be stimulating and fruitful. The more formal presentations here in Becker Hall will set the stage for those unplanned conversations in the Fellows room over coffee or chance remarks by the moat which so often prove to be the most fertile moments of all symposia here. I trust there will be many of these for all of you because, as a young Academy, we are concerned to see young sciences such as yours, grow and interconnect the older disciplines.

I should like now to examine the four elements of your title (shades of the earth, air, fire and water of the old alchemists), and also their interaction.

THE ENVIRONMENTAL COMPONENT

Looking through the papers for this Symposium, any outsider would surely be struck by the high proportion devoted to seemingly harsh and hostile environments, Antarctic cold, desert dry, anaerobic, hypersaline, metalliferous etc. Lake and sea bottoms are prominent and I assume you would all join with E.S. Deevey (1970) "In defence of mud". As someone who began his research on the colonization of saline mud flats, I have every sympathy with this environmental masochism. You do not wholly ignore our more favoured urban and agricultural environments, but your concentration on oceans, estuaries, Antarctica, mine dumps and the atmosphere will surely help remedy past neglect and present ignorance of these important components of our biosphere, The extension of off-shore continental limits is making many countries more aware of the need for a better understanding of marine environments, just as the introduction of the SST and recognition of the possible effects of rising CO_2 concentration have made us more aware of the urgent need for greater understanding of atmospheric exchanges. This need was epitomised, for me, by Elsaesser's paper in which he shows how assessments of the effect of NO_x on stratospheric ozone have diminished and then reversed over the last decade.

Research on these less well known parts of our environment not only remedies past neglect but will also help us towards a more global and integrated approach to our environment. "Global" is a much misused word beloved of academics and politicians, so I should add that I mean global in the world-wide sense of the Global Atmospheric Research Program (GARP), and of this year's First GARP Global Experiment (FGGE), which seems to be globally squared, and not in a regional, national, sectoral or disciplinary sense. The better we understand atmospheric and oceanic circulations and exchanges the more we will approach effective global management of our whole environment. If our tectonic plates moved around a bit

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* *Address to the opening session of the 4th International Symposium on Environmental Biogeochemistry, August 26, 1979.*

faster we might even take a more global view of the populated land components of the biosphere.

Concentration on harsh environments leads also to understanding and speculation about past environments. These will, in fact, be the subject of Preston Cloud's Keynote address tomorrow morning. Biologists have argued that there is no grander subject for investigation than the full sweep of biological evolution, but when that is coupled with geochemical and atmospheric evolution, only the boldest spirits venture forth. Yet this combination has much to offer by way of insight as well as of intellectual excitement. Debate about the origins of the early rise in atmospheric oxygen, whether from photodissociation of atmospheric water vapour by ultra-violet radiation or from early photosynthesis, will undoubtedly continue, as will that on when during evolution the ozone screening of ultra-violet radiation built up to current levels. Several constituents of plants besides DNA require protection from the shorter wavelengths of ultra-violet radiation, and the biosphere as a whole - with the possible exception of some of the most primitive organisms - would be profoundly disturbed by greater penetration of such radiation.

THE BIOLOGICAL COMPONENT

Another consequence of your apparent emphasis on harsh environments is the preoccupation with relatively simple organisms. I see that my colleague John Langridge is listed to speak on Precambrian Genetics; had I not known him I might have assumed that his title referred to some rather primitive science, rather than to advanced science with primitive organisms.

Buick and Dunlop's abstract claims that the most ancient fossil remains yet described are from 3.5 G yr old chert-barites of the Pilbara Block at North Pole, Australia. This may confirm your views about Australians, and some of you might have placed these most ancient fossil organisms closer to this building.

The feature which struck me as most surprising about these primitive blue-green algal communities was the extremely high rate of primary production given by Cohen *et al.* of 8 g C m^{-2} day^{-1}, for the hypersaline Solar Lake near Eilat. This is comparable to the highest crop growth rates recorded for terrestrial crops like wheat or sugar beet. At your last symposium at Wolfenbüttel, Hirsch indicated that these Solar Lake communities are complex and highly stratified, with many species in each layer, but if such high rates of primary production can be sustained by these communities, the subsequent evolution of photosynthesis does not seem particularly impressive.

Indeed, it is often argued that Calvin cycle (C_3) photosynthesis in higher plants is limited by the behaviour of the key photosynthetic enzyme ribulose biphosphate carboxylase (RuBPc) which supposedly remains better adapted to earlier atmospheres than to present ones. This enzyme may constitute up to one quarter of leaf protein, and is probably the most abundant protein in the world, yet is often limiting to photosynthetic rate. It functions not only as a carboxylase, but also as an oxygenase, and present atmospheric levels of oxygen substantially reduce its rate of CO_2 fixation at present atmospheric CO_2 concentrations. At higher CO_2 and lower oxygen levels this competitive effect is greatly diminished. Given the important role of this enzyme in evolution, I find it difficult to accept that such behaviour of RuBPc is fossil-like. The alternative view is that, like sickle-cell anemia, it has advantages we may not be aware of. It may, for example, protect the photosynthetic apparatus from photo-oxidation, but this has yet to be convincingly demonstrated.

Two groups of plants, both of multiple origins, have evolved anatomical structures and biochemical mechanisms to circumvent the limitations of RuBPc. Higher plants from many families have evolved a mechanism by which CO_2 is first fixed by the enzyme phosphoenolpyruvate (PEP) carboxylase in an outer ring of mesophyll cells, then transported as dicarboxylic acids to an inner ring of bundle sheath cells where they are decarboxylated. The RuBPc in these inner cells is thus exposed to relatively high CO_2 as well as low oxygen concentrations, an environment in which it operates more efficiently. These C_4 plants, as they are known, thus have potentially higher rates of photosynthesis and growth, and include not only successful crops such as sugar cane, maize and sorghum, but also many successful weeds. By virtue of the C_4 pathway of photosynthesis, they use water more effectively than do C_3 plants, and are prominent in hot dry areas with high insolation.

In wetter, cooler and low insolation environments C_4 plants have no advantage over C_3 plants, and may even be at a disadvantage.

The other group of plants are the succulents with Crassulacean Acid Metabolism (CAM). They are too complex to discuss in detail here (cf Osmond, 1979). Typically, they fix CO_2 during the night by means of PEP carboxylase and then, with their stomata closed at least during the early part of the day, thereby conserving water, the CO_2 is released internally to be fixed by RuBPc. The CAM pathway is an effective way of conserving water, at the expense of high growth rates, especially in arid areas with cool nights.

In a way, these two pathways - C_4 and CAM - emphasize the extreme conservatism of the primary photosynthetic pathway, because they are both simply mechanisms for pre-concentrating atmospheric CO_2. My other reason for mentioning them is that they bear on the ratio of $^{13}C/^{12}C$ utilization in photosynthesis. Schidlowski, in his abstract, points out that the preferential fixation of ^{12}C in photosynthesis can be traced back through the fossil record for 3 Gyr, even as far back as 3.76 Gyr in West Greenland. That's real conservatism.

The bias in favour of ^{12}C in photosynthesis derives from the properties of the enzyme RuBPc. Whether derived from *Rhodospirillum, Hydrogemonas* or spinach, the extracted carboxylase gives $\delta^{13}C$ of about -34 per mil (Estep *et al.*, 1978), regardless of temperature, pH or bicarbonate concentration (Christeller *et al.*, 1976). Hence the high negative $\delta^{13}C$ of Calvin cycle (C_3) plant materials, of marine plankton and of most terrestrial plants. In C_4 photosynthetic plants, however, the initial CO_2 fixation is by the enzyme PEP carboxylase which displays no preference for ^{12}C, the purified enzyme from maize having a $\delta^{13}C$ of only -2 per mil (Reibach and Benedict, 1977). Since the CO_2 fixed by this enzyme in C_4 plants is released into a relatively closed pool for refixation by RuBPc, the $\delta^{13}C$ of C_4 plants approaches that of the air over terrestrial communities, being usually -13 to -19 per mil (Bender, 1971). Similarly, for CO_2 fixed by CAM plants, the $\delta^{13}C$ value is about -11 per mil, whereas that for CO_2 fixed in the latter part of the day was -26 per mil (Osmond, 1979). The overall mean value for CAM plants depends on day and night temperatures, light intensity and daylength. Both C_4 and CAM pathways arose relatively late in angiosperm evolution, so $\delta^{13}C$ values cannot be used to obtain clues to early environments.

Before leaving the subject of photosynthesis, I might add that man has done no better than nature. There is no evidence that photosynthetic capacity has been increased by selection in any crop. Indeed, the light-saturated photosynthetic rates of leaves of modern highly productive varieties of wheat are actually lower than those of the wild progenitor species from which they were derived ten thousand years ago (Evans, 1976). Selection by man has not increased the rates of photosynthetis or growth, but rather the proportion of assimilates which are allocated to the organs he harvests. Wheat crops still grow at rates similar to those mentioned by Cohen for the algal mats of Solar Lake.

THE GEOLOGICAL COMPONENT

This is not a symposium for the purely geophysical, but I have been impressed by the growing interpenetration of biological and geological studies evident in the abstracts. Of course, that geological resource which keeps rising in wells, in our collective consciousness and in price, namely oil, is a product of geological and biological partnership, whose origins are still debated. Twenty-five years ago my plant physiology professor from CalTech, Frits Went, visited his old friend in Australia, Laurens Baas Becking, one of the founding fathers of environmental biogeochemistry. He described the meeting succinctly in a letter to me: "Baas Becking told me his latest theory on the origin of oil, and then I told him mine" (that it derived from the terpenes of "vegetation smog", the blue haze over vegetation). It cannot be often that two men have exchanged new theories of oil formation.

Soon, of course, we will be more interested in biogeochemical processes producing substitutes for oil under the hand of man, using agricultural, forestry, industrial and urban wastes in place of those organic wastes of long ago. The materials and the processes may have much in common, but the time scale will be foreshortened. A more complete understanding of the original processes would help.

Other resources which may be partly biological in origin are the ferromanganese nodules formed at depth on the sea bed. There is much still to be learned about these possible

future sources of cobalt, copper and nickel. In view of the growth rates given for them by Ottow at the Wolfenbüttel symposium, of only 10^{-3} - 10 mm per thousand years, their mining could pose major problems of repopulation and initiation of regrowth on the ocean floor.

A complementary theme which will receive a lot of attention at this and its satellite symposia is the use of biological methods of processing mineral deposits. It seems that with the aid of those old microbiological favourites *Thiobacillus ferrooxidans* and *T. thiooxidans*, sometimes encouraged by a supply of inorganic nitrogen, phosphate and sulphur, it may be possible to win manganese, copper, nickel, uranium, vanadium and other elements from a variety of unpromising sources. This gleam in the biogeochemists eye may prove to be of increasing significance in the future as we are forced to shift from more concentrated, localized and purer ores to more diffuse ores of lower content. Lower energy input systems for treating such ores may become more and more attractive. Such treatments may also help the rehabilitation and revegetation of mine dumps.

In discussing the bacterial leaching of ores, Bosecker and Wirth speak of the use of "pregnant" solutions. To me, the pregnancy in this case, unplanned as happens so often in science and technology, lies in its being yet another clear example of how basic research may suddenly acquire relevance and usefulness. Looking to the future, our basic understanding of these biogeochemical processes is likely to be stimulated by the applied interest in them, and so the reciprocation process between pure and applied science will go on, emphasizing the need for a balance between these two faces of science. It also prompts the thought that in estimating the physical limits of the world's resources we should not overlook the greatest resource of all, human imagination and will.

THE CHEMICAL COMPONENT

One chemical aspect still receiving emphasis is that of "recalcitrance", which Alexander defines as persisting in natural ecosystems. In other words, recalcitrant molecules don't play the environmental biogeochemical game, and accumulate like DDT in eagles, Antarctic penguins and even mother's milk. On a Swansea wall I once read: "Don't eat people - they contain too much DDT."

The range of recalcitrant organic molecules is wide, and includes organo-metallics such as methyl mercury and tetra-ethyl lead. In fact, I was struck as an outsider by the apparent scope for more research on organo-metallic compounds, not merely those involved in our polluting activities, but also for example those produced in blue-green algal mats and other organic deposits. Clay-organic complexes may also deserve further study, not only in soils where they have long been recognized as important to soil structure, but also in sedimentary deposits where binding to clays may both protect and hasten the degradation of organic compounds.

Taken overall, my impression is that chemical pollution of the environment is currently attracting less attention than in earlier symposia. Is it under control, or have we lost interest, or are we quietly working away at it?

An exceedingly important component of environmental biogeochemistry is the continuing attempt to clarify and quantitate the interlocking cycles for carbon, nitrogen, oxygen, phosphorus, sulphur and other elements. These cycles constitute our ecological web, which we must understand if we hope to manage. Remember Charles Darwin's joke about how the British spinster kept the Royal Navy great, because she kept the cats, which kept down the village mice, which ate the honeycomb and larvae of bees: more honey meant more bees, which meant more red clover, which gave more beef, and therefore a better fed Navy. Biogeochemical cycles still share with Darwin's web some steps which are far less known than others, and a considerable range in time scales. And just as the widows had a major impact on his web, so are man's activities assuming an increasing significance in these cycles.

Consider the carbon cycle. According to Revelle and Munk (1977) man has added more than 200 Pg of carbon to the atmosphere since the Industrial Revolution, about two thirds of this coming from fossil fuels and one third from deforestation and the oxidation of soil organic matter. This total is equivalent to one third of the pre-industrial carbon content of the atmosphere, and we are currently adding about 5 Pg more of carbon as carbon dioxide from the burning of fossil fuels each year. How much additional carbon is added to the

atmosphere from deforestation and the oxidation of soil organic matter is a subject of current debate. Bolin (1977) and others believe that deforestation adds about 1 Pg of carbon each year, whereas Woodwell *et al.* (1978) estimate it to be more than 5 Pg, i.e. possibly greater than that from the burning of fossil fuel. Estimates of release from soil organic matter vary over an order of magnitude, up to 2 Pg per year. Even before petroleum combustion became important, man's clearing of land for agriculture in the latter part of the 19th century was sufficiently widespread to cause a fall in atmospheric $\delta^{13}C$ per mil (Wilson, 1978), but his impact has increased greatly since then.

This debate highlights our uncertainty not only about how much CO_2 is being added to the atmosphere as a result of man's activities - whether it is equivalent to one tenth or one quarter of net terrestrial primary production - but also as to what proportion of this is absorbed by the ocean. Atmospheric CO_2 is increasing annually by about 1 ppm, and the conventional wisdom has been that about 60% of the added CO_2 is taken up by the ocean, but if Woodwell's estimates are correct, absorption by the ocean must be much greater.

Man's impact on other biogeochemical cycles is also substantial. Nitrogen fixation in fertilizer manufacture and other industrial processes now exceeds half of that by terrestrial plant communities (Soderlund and Svensson, 1976). Man-made and natural sources now contribute about equally to the total amount of sulfur in submicron particles present at any time in the whole troposphere (Granat, 1976). The phosphorus applied in fertilizers each year is about one twentieth of the total uptake by terrestrial biomass (Pierrou, 1976).

Given the increasing impact of man on these cycles it becomes more and more important that our knowledge of them should reach the stage where we can predict with reasonable confidence the consequences of man's activities. That we still have a long way to go is evident from the changing perspective on the effects of nitrogen oxides on stratospheric ozone, and from our uncertainty about the net effects of rising CO_2 levels. Since the absorption bands for CO_2 and water vapour overlap, and because changes in temperature will influence evaporation, rising CO_2 levels will affect the hydrologic cycle as well as temperature regimes, and its effects will vary substantially from one region to another (Manabe and Wetherald, 1975). What the net effects of this complex of changes will be on climate and on agricultural production is difficult, but important, to foresee. As Churchill said, "It is essential to look ahead, but difficult to look further than you can see."

CONCLUSION

The four elements of your symposium title are all thriving, but the important question is, how much are they interacting? In places, the interactions still seem tenuous, but many of the examples I have mentioned show a welding of disciplines. As you continue to meet together, I am sure these interdisciplinary interactions will increase and prove fruitful. One local interaction of this kind, between micrometeorologists and soil chemists, has thrown new light on old problems concerned with the nitrogen cycle, and many other examples may spring to your mind. The particular significance of this symposium is, to my mind, that it is aimed at stimulating such interactions, rather than at deepening our respective ruts.

We become increasingly aware of the impact of man on the natural economy of our world, and of the need to predict its consequences. Such is the scale of man's activities, and the pace of change, that there is a growth industry within both government and community for extrapolations, predictions, scenarios, and technological assessments. These are difficult enough to supply at any time, but even more so when, as in environmental biogeochemistry, so many complex and interlocking cycles are involved. Only with modelling will comprehensive prediction be possible, but current knowledge will hardly support the modelling of even relatively simple systems, which may account for the rather notable paucity of papers presenting models at this symposium. Of course, the most difficult of all reactions to predict are the socio-economic consequences of change and the way in which people and institutions respond to them. In a recent book in which he looks at the consequences of 55 technological innovations, Lawless (1977) points out that in 40% of the cases early warnings of disaster were ignored. I believe it is not just our technological optimism that is to blame, but also the recognition by the community that most of these matters are exceedingly complex and that however much we must advance our scientific understanding, the outcome of change remains essentially unpredictable, often depending as much on human

reactions as on processes more amenable to scientific analysis.

We cannot ignore this human factor, indeed we should welcome it, but it should not daunt us from developing our understanding of environmental biogeochemical processes, or from using this to predict the impact of Man's activities. The need has never been greater, so I wish you a highly interactive symposium.

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EARLY BIOGEOCHEMICAL SYSTEMS

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INTRODUCTION

Biogeochemical systems are a product of the interacting evolutions of biosphere, atmosphere, hydrosphere, and lithosphere. They began with the appearance of life on Earth. But the appearance of objects we would unhesitatingly pronounce to be living is itself presumably only the end stage of a transitional process whose nature and antecedents are important parts of the story. If we include prebiotic chemistry, the process may have started in the space between the stars. That follows from the discovery by X-ray astronomers of interstellar hydrogen cyanide, formaldehyde, and the other important polyatomic organic molecules listed in Table 1.

TABLE 1

SOME ORGANOGEOCHEMICALLY INTERESTING MOLECULES FOUND IN INTERSTELLAR SPACE

hydrogen cyanide	HCN	isocyanic acid	HNCO
formaldehyde	HCHO	acetonitrile	CH_3CN
thioformaldehyde	H_2CS	methanol	CH_3OH
formic acid	CHOOH	methyl acetylene	CH_3C_2H
cyanimide	NH_2CN	methylamine	CH_3NH_2
formamide	NH_2HCO	acetaldehyde	CH_3CHO
cyanoacetylene	HC_3N		

The history of early biogeochemical systems is rooted in those processes that could have led to the formation of our planet, and the presence at its surface of substances and temperatures conducive to the origin and persistence of life. From there it extends for some billions of years toward the present.

The subject is huge, but I have only to introduce it. I shall interpret my role as a keynote speaker to be that of delineating broadly some major aspects of prebiotic and early biogeochemical systems about which there is disagreement, uncertainty, and need for further progress. I aim thereby to emphasize the connections between the various things we in this diverse audience love to do, to say a bit about method, and to suggest some opportunities for future investigation. Even the most obscure-seeming connections may harbor opportunity for new or broader insights, and any depth of understanding of a subject where the evidence is as fragmentary as it is in the case of early biogeochemical systems depends on perceptive attention to all possible linkages of the potentially relevant evidence.

Although we are confident that the results of our inquiries will turn out to be applicable in some sense to the improvement of the human condition, the driving force is simple curiosity. But we expect science to be predictive and therefore useful, if only in diminishing our ignorance. Its ability to predict is, in fact, a rough measure of how basic it is. Yet our most crucial questions, our most fundamental hypotheses, can be answered or tested only by more observations, by illuminating new discoveries. So observation, experiment, and theory go together like fingers, thumb, and eyes. It takes all three to grasp anything significant.

And, paradoxically, as Weisskopf (1979) has emphasized, the most significant advances in science are so often ones that were *not* foreseen: that failure to have predicted a discovery is in some sense a measure of its importance. Perhaps the discovery in recent decades of those polyatomic organic molecules in interstellar space is a case in point:

it could turn out to be as important in its own way as the discovery of the $3^{o}K$ universal black body radiation that won a Nobel prize for some of the same investigators. I know of no prediction of this remarkable chemistry and no accepted theory or hypothesis that would have predicted it. Yet it could transform our views about the beginnings of life (e.g. Hoyle and Wickramasinghe, 1979).

On an occasion like this it is customary to speak of past achievements and to anticipate future ones. And it is true both that significant advances have been made and that more are to be expected. Indeed many more advances are needed before we can fairly claim to have a good understanding of biogeological events on the primitive earth.

As a theme for this symposium, therefore, I stress opportunity rather than progress. I also stress the value of stochastic methods of reasoning, involving the simultaneous viewing of parallel or converging lines of evidence as a means of identifying likely processes and events that may be ambiguous or unrecognizable from any one of several individual lines (e.g., Gilbert, 1886). Progress has been made, to be sure, in identifying some key facts and proposing some alternative hypotheses about the main events of prebiotic and biospheric evolution, together with their biogeochemical consequences. But that is the easy part. The hard part is to define or redefine the problems so clearly as to bring out alternative hypotheses, to perceive the verifiable consequences of hypotheses that emerge, and to design and carry out the systematic inquiry and testing that sharpens our perceptions, eliminates untenable hypotheses, and modifies the viable hypotheses or invents new ones that more adequately or more elegantly reconcile the facts. Thus we generate the competition among hypotheses that highlights their weaknesses and strengths, generates changes for the better, and generally strengthens our chances of reaching a durable solution. That is where the opportunity lies. That is where method is important. Serendipity is an attribute of prepared and open minds. Insights occur to those habituated to think simultaneously along and blend different lines of parallel or convergent evidence.

To illustrate my theme I shall discuss briefly four central subject areas:

1. The initial earth.

2. The ancestral atmosphere and hydrosphere.

3. The conditions of biopoesis (or biogenesis if you prefer).

4. The biogeochemical consequences of early biospheric evolution.

In limiting myself to those subjects I thus also limit my discussion of early biogeochemical systems to the first 2.6 Gyr of Earth's history -- the interval that set the stage for the later and more familiar biogeochemical drama of eucaryotic evolution.

Originally I had thought also to include discussion on the beginnings of eucaryotic life, the origins of the Metazoa, and the onset of Phanerozoic history. Space, however, is a deterrent. And I have little to add, to my previously published views on those subjects (Cloud, 1968, 1976a,b).

THE INITIAL EARTH

We may turn, then, to the initial earth. All we know with assurance about the origin of the earth is that it exists, that it and its sister planets manifest characteristics that must be accounted for in any hypothesis of solar system origin, and that planets are somehow a product of stellar evolution. A stumbling block for most hypotheses of planetary origin has heretofore been the peculiar distribution of angular momentum in the solar system, with Jupiter getting the lion's share and the sun apparently a mere fraction. Geologists and planetary astrophysicists alike have long puzzled over this and over the question of Earth's initial temperature and its consequences. A half-century ago it was widely supposed that Earth had started in a molten state, wrinkling like a baked apple as it cooled and shrunk to form folded mountain ranges. That view was superseded by one that visualizes a cool, homogeneous, aggregation of the planet, concurrently puzzling over how to account in such a model for the scarcity of the nonradiogenic noble gases in Earth's atmosphere as compared to their solar abundances.

More recently, however, as the intensified exploration of space has brought new data of many kinds from planetary geology and galactic physics, the initial earth of our mind's eye has been heating up again. Concurrently, the variety of proposed solutions to the problem of angular momentum in the evolving solar system suggest that it may be another one of those questions for which we can think of so many different answers that it will never be possible to agree that any particular answer is the correct one. The elements of the new synthesis emerge from a sequence of papers by MacDonald (1959), Ringwood (1966, 1975), Turekian and Clark (1969), Wetherill (1971), and Cameron (1973, 1977). Although they differ in details, these authors concur broadly in visualizing a hot, inhomogeneous initial Earth and new constraints on the nature of the primordial atmosphere and hence on early biogeochemical systems.

Clearly the process of planetary genesis begins with a parent star, and evidence now is that star formation in our galaxy is a feature of its spiral arms, where dust and gas have been concentrated in gravitational troughs (Cameron, 1977). In the case of our sun, and perhaps of other stars, the trigger may have been a nearby supernovation. This is implied by isotopically anomalous inclusions in the Allende meteorite (Lee, *et al.*, 1976, 1978), some of which contain excesses of ^{26}Mg that are attributed to the decay of ^{26}Al in the early solar system. This radionuclide, a product of explosive carbon-burning in supernovae, has a half-life of only 720,000 years. Its presence in the solar nebula, implied by the ^{26}Mg anomalies in Allende, suggests the near cosmic synchronism of solar system origin and the supernovation responsible for the ^{26}Al. Such a coincidence supports the idea of supernovation as the initiating process in the condensation of new protostars such as our sun once was (Cameron, 1973, p. 431; Cameron and Truran, 1977; Schramm and Clayton, 1978). New supernovations, moreover, have now been observed with sufficient frequency to be considered as normal events in stellar evolution.

As for our sun, its surface temperature, luminosity and mass identify it as a main sequence star in the Herzsprung-Russell scheme of stellar evolution and show that it entered the main sequence from membership in a group of pre-main-sequence bodies known as T-Tauri stars upon ignition of the H_2 to the He burning cycle. Doppler shifts of T-Tauri stars indicate that ionized gas is streaming away from them, much in the manner of the solar wind but with far greater intensity. Angular momentum within the primitive solar nebula is considered to have had a random turbulent distribution, such that its later anomalous-seeming distributions could result from essentially random collisions together with outward transfer of both matter and angular momentum.

Following condensation of the ancestral sun from the solar nebula, Cameron's calculations imply that the T-Tauri wind would continue to blow away remaining gas at rates of up to a solar mass per million years until several tenths of a solar mass were lost to space. Meanwhile grains of nebular matter were probably colliding in the T-Tauri wind at high velocities (10 to 1500 m s^{-1}) and clumping or melting to form chondrules that fell toward the central plane of the flattening solar nebula over the course of a few centuries. The density of the primordial nebular disc would thereby increase, and larger bodies could grow by accretion to form proto-planets over the course of one to a few millenia.

In early stages of planetary growth and more distant parts of the solar nebula, accretion may have been mainly of gases and ices. In later stages and inner reaches of the ancestral nebula, rocky matter is swept up. In the absence of effectively cushioning gases, convected away in the T-Tauri wind, Cameron estimates that gravitational heating could raise temperatures to 1500°K or higher. At such temperatures, as potters know, even silicates start to vaporize and an atmosphere of mixed volatile components could form. Cameron (1973) hypothesizes that convection in the gaseous exterior of the proto-planet would mix in H_2 from the solar nebula to strip oxygen from iron oxides. That would yield steam and liquid iron, the iron settling out to form a core. Ringwood (1966) sees a similar reduction of iron, leading to core formation, but visualizes the reducing agent as carbon, derived mainly from methane, giving rise to a huge volume of carbon monoxide.

In either instance, the infall of reduced metallic iron would generate a further surge of gravitational heat, decrease Earth's moment of inertia, and increase its temperature and rate of rotation. The continuing T-Tauri wind, meanwhile, would remove all but traces of the primordial atmosphere. The atmosphere ancestral to ours was yet to be accreted by some later outgassing event or events. Ringwood also finds that, during the T-Tauri phase of the sun, Earth became somewhat depleted relative to chondritic abundances in its more volatile elements and relatively enriched in refractory ones. As a result of the

reductions it underwent, with subsequent loss of volatiles, it also inherited a number of potential oxygen sinks. Cameron's calculations imply that everything up to then happened very fast and at temperatures that favoured rapid outgassing, accompanied by continued stripping of gaseous components up to the atomic weight of xenon.

The still aggregating Earth, however, continued to sweep up dust and rocky particles that were stranded in space as the T-Tauri wind carried the surrounding nebular gases outward toward the Jovian planets and beyond. Such perhaps mainly chondritic matter was then itself presumably outgassed to produce additional secondary atmospheric components. And these, in turn, were lost from the hot planetary surface over the 0.1 to 1 Myr period during which Cameron's calculations indicate that the T-Tauri wind remained strong. After this a secondary atmosphere could have accreted, particularly around a planet like the primordial earth, with a partly molten core of iron whose internal motions give rise to a magnetic field that deflects the T-Tauri and later solar winds.

Such a hot, inhomogeneous, one-stage model for Earth's origin seems consistent with evidence presently available, including what is known about occluded meteoritic gases, the cosmically anomalous noble gas abundances of the terrestrial atmosphere, and the geochemistry of the oldest terrestrial sediments. It also avoids the need to invent both a special later core-forming event and a special explanation for the non-accumulation or loss of the hypothesized initial strongly reducing atmosphere of a cold-aggregated earth having a high content of metallic iron in its surface layers. Unlike a cold-aggregated earth, it also avoids the need to call on a later thermal event to explain the relative depletion in Earth's atmosphere of the noble gases (discussed below).

With all its remaining uncertainties, a hot, inhomogeneous, one-stage origin for our planet reconciles so much prior information that the hypothesis takes on a seductive quality. That is all the more reason why we should give hard thought to searching out and testing its verifiable consequences, whether they be found in recorded events of early earth history, in calculations or thought-experiments, or in the exploration of other planets. For, although a beginning has been made, we are still far from understanding the processes of collapse of the early solar nebula, the segregation of planets from the rotating solar disc, the transition from T-Tauri to present solar wind, the details of core formation, and early planetary evolution to the time of formation of our ancestral atmosphere - let alone the angular momentum problem. Indeed, in a recent paper, Ringwood (1977), contrary to his own earlier views, observes that the light elements required to explain the 8% density deficit of Earth's iron core at its estimated pressure and temperature may not be sulfur, as generally supposed, but oxygen. If so, ferrous iron could be a major constituent of the core, implying that a relatively low temperature homogenous accretion would be possible after all.

THE ANCESTRAL ATMOSPHERE AND HYDROSPHERE

A secondary origin for our *ancestral* atmosphere, in contrast to the preceding and now long vanished *primordial* atmosphere, seems to be required by the now well known and often remarked depletion of the nonradiogenic noble gases in the terrestrial atmosphere as compared to solar abundances (Russell and Menzel, 1933; Rubey, 1951). It is also implied by the similarity between meteoric and terrestrial patterns of noble gas abundances, allowing for radiogenic increases (Fanale, 1971; Holland, 1978, p. 258). And it is an inherent consequence of the hypothesis of planetary origin described above. In fact Walker (1977, p. 182) has estimated, using solar ratios relative to neon as an index, that remnants of the primordial atmosphere after thermal escape and T-Tauri stripping probably amounted to less than 1% of the present atmosphere.

Yet geologists know, quite unequivocally, that a substantial atmosphere and hydrosphere at least somewhat akin to ours, but without free O_2, already existed by the time the oldest water-laid sediments were deposited some 3.8 Gyr ago in the Limpopo Mobile Belt of Southern Africa, the Isua area of southwest Greenland (Bridgwater *et al.*, 1974), and elsewhere (Bridgwater *et al.*, 1973). Since 17% of Earth history had then elapsed, one might take 17% of the present hydrosphere and a corresponding atmosphere as minimal. It probably was substantially more than that.

Based on the evidence of these oldest known metasedimentary rocks, and considering the consequences of hot, inhomogeneous planetary accretion, we can deduce other interesting

things about the nature of the ancestral atmosphere. Such evidence excludes the possibility that that atmosphere could have been strongly reducing. Models that call for a strongly reducing ancestral atmosphere, rich in hydrogen and methane, must avert or discount the stripping of hydrogen by the T-Tauri wind and the loss of methane by the same event or events that took neon and heavier noble gases. Or they must hypothesize high concentrations of free iron in outer layers of the newly aggregated Earth. A strongly reducing atmosphere would demand cold accretion, with delayed descent of iron to the core.

But late formation of the core poses problems for accumulation of the secondary atmosphere indicated. New volatiles might be carried away from the reheated earth in the solar wind until that was deflected by Earth's magnetic field - - a product of core formation. Were core formation a very late event, we should also look for some evidence of it in the thermal and rotational history of the earth as a consequence of Earth's decreased moment of inertia. Given a hot origin for the planet, however, core formation becomes an early event, essentially simultaneous with planetary accretion. We also find independent geochemical support for early core-mantle differentiation. The oldest ultramafic and mafic extrusive rocks we know are so similar in their oxidation states, relatively high nickel content, and other geochemical characteristics to younger ones that we must judge core-mantle differentiation to have preceded the origin of the parent magmas of even the oldest of these rocks.

Walker (1976, 1977) summarizes other reasons for excluding a strongly reducing ancestral atmosphere. To be sure the ferrodolomites and the general excess of ferrous over ferric iron in the oldest known metasedimentary rocks imply that free oxygen was scarce or fleeting in the earliest ancestral atmosphere or at least the earliest for which we find a geologic record. Yet the oxygen-poor atmosphere signaled is better designated as anoxic than as reducing or anaerobic in the proper sense of those terms.

We can summarize evidence bearing on the ancestral atmosphere and hydrosphere as follows:

1. The noble gas anomalies and current planetary accretion theory imply that the ancestral atmosphere and the hydrosphere that condensed from it were both secondary.

2. The similarities between the terrestrial and meteoritic noble gas patterns imply that all ancestral volatiles, both atmospheric and hydrospheric, were out-gassed from materials of largely meteoritic (chondritic) origin.

3. The existence and nature of the most ancient sedimentary rocks yet known tell us that both an atmosphere and a hydrosphere of respectable dimensions already existed ca. 3.8 Gyr or more ago (Bridgwater *et al.*, 1973, 1974; Barton *et al.*, 1977).

And we are confronted with questions as follows:

I. After Earth cooled enough for a hydrosphere to condense, how was that hydrosphere prevented from freezing and staying frozen in the subdued radiation of the astronomer's faint early sun?

II. When and under what circumstances did life originate, especially in the presence of a sun presumed to have had only 70% or less of its present luminosity when it first became a main sequence star?

III. What interactions were initiated between biospheric, atmospheric, hydrospheric, and lithospheric evolution; and where and how might we seek evidence of these interactions?

In attempting to deal with such matters one might start with the cooling history of the Earth. Consider the model proposed by Jacobs (1961) as outlined by Cameron (1973). Following the stripping of combined oxygen by hydrogen in the T-Tauri wind and the settling out of the thus reduced liquid iron together with some light element to form Earth's core, heat is expected to convect outward in both liquid iron core and liquid silicate mantle. When cool enough, solidification starts to work outward from the centre of the core. But before the core is completely solid the base of the more viscous mantle freezes and solidification rises as heat is transferred upward through the liquid mantle

by convection. When solidification reaches depths of around 100 to 200 km, the surface will have cooled enough for it to start solidifying. Because common solids other than ice and iron sink in their liquid phases, turnover of the solidifying lithosphere proceeds until a more or less continuous solid crust is formed. The last region to solidify, effectively, the asthenosphere, is apparently the source for much of the mantle matter that has subsequently risen into the crust.

The cooling process was probably slow, considering that residual gravitational heat would have been supplemented by large thermal inputs from the decay of relatively short-half-lived radioactive nuclides such as ^{235}U and the even shorter lived heat-producing isotopes noted by MacDonald (1959). Such heat sources are greatly diminished after ca. 400 to 500 Myr from the time of origin, following which the rate of cooling would increase. Wetherill (1971) calculates that a solid crust could have formed not long after this, or ca. 4.1 to 4.2 Gyr ago, which approaches the age of the oldest Isua and Limpopo gneisses and supracrustals (Bridgwater *et al.*, 1974; Barton *et al.*, 1977).

Meanwhile, to judge from the impacted surfaces of other planets and their satellites, the primitive earth probably continued to sweep up substantial amounts of meteoritic debris. But whether such late-accreting matter was outgassed upon impact or incorporated into a still molten or plastic surface for later outgassing is unknown. After Fanale (1971) argued persuasively for essentially complete outgassing of atmospheric and hydrospheric volatiles concurrently with accretion of the planet, there was a strong swing to this viewpoint, coupled with growing evidence that a very high proportion of modern volcanic gases are recycled. But Fanale's instantaneous atmosphere would probably have been lost to the T-Tauri wind, while Holland (1978, p. 254) has recently summarized evidence demonstrating that primordial helium and probably other truly primordial gases are, in fact, still being outgassed from Earth's interior. In addition, inclusions of H_2O, CO_2, and N_2 in diamonds and upper mantle basalts imply that occluded juvenile volatiles are even now available for exhalation to Earth's surface. To be sure, a very substantial fraction of our secondary atmosphere (and hydrosphere) seems to have been outgassed rapidly as a volatile-rich veneer of meteoritic matter accumulated during late stages of Earth's aggregation. It is also likely, however, that even modern volcanic gases are not exclusively recycled, but include some fraction that has not been at Earth's surface before.

We do not know how much has been added over geologic time, so we grasp at any clues. Armstrong (1968) and Walker (1977) reason that the evidence of recorded sedimentary history for the last 2 Gyr implies little variation over that time in the elevation of continental cratons with respect to oceanic levels. This, in turn, is interpreted to indicate little growth of the hydrosphere and hence of new volatiles generally during that history. Such a view is also supported by the systematic variation in the ratios of the isotopes of oxygen with time (Chase and Perry, 1972). Currently then, it appears that the major part of Earth's surface volatiles may have been outgassed before about 2 Gyr ago and perhaps before 3.8 Gyr ago, with relatively minor but still continuing later additions.

What, finally, was the composition of our ancestral atmosphere? Although all views agree that there could have been little or no free oxygen in it, they differ in other respects, depending on the model of Earth origin assumed. Siever (1977) summarizes the consequences of a cold-aggregated planet, stripped of any primary atmosphere by the T-Tauri wind. He sees the ancestral atmosphere as a function of incomplete but catastrophic outgassing brought on by early core-mantle differentiation in the presence of free iron in the upper mantle, much as earlier proposed by Fanale (1971). Siever also finds that such an atmosphere would probably have been rich in CH_4, with substantial quantities of NH_3, H_2, and H_2S as well as oxidized molecules such as H_2O, CO_2, CO, and SO_2 "overlying an ocean of water with dissolved salts much like those of today."

A hot initial earth and the processes here visualized produce a different result. Degassing of a late aggregation of chondritic matter takes place on an already differentiated earth having a strong magnetic field that deflects a waning T-Tauri wind. An atmosphere so derived might consist mainly of H_2O, CO_2, N, probably CO, and traces of volcanic gases such as SO_2, H_2S, H_2, and CH_4. No source of primary free O_2 exists and any O_2 produced by the photolysis of water vapour by whatever mechanism, or by the very much lower rate of photolysis of CO_2 visualized by Shimizu (1979), would promptly combine

with its co-product or with any reduced atmospheric trace gases or CO then present. Our present atmosphere is seen as the evolutionary descendant of such an ancestral atmosphere, plus any additions from late mantle outgassing. It has undergone changes in volume and chemical proportions as a result of additions of O_2, oxidative reactions, volcanism, rock weathering, soil formation, and chemical sedimentation, most of these processes involving biogeochemical reactions at some stage.

Ferrodolomites and sideritic carbonates among the oldest known sedimentary rocks imply an aquatic chemistry of high ionic strength, a relatively high P_{CO_2}, and an anoxic milieu. I would hesitate to suggest a specific salinity or state the probable ratios of sulfate or other dissolved ions to chloride. But any sulfide or sulfite could serve as an oxygen sink, giving rise to sulfate by combination with evanescent O_2 from photolysis of water vapour. Thus some sulfate could have been present from the beginning of hydrospheric evolution and even locally concentrated. Nevertheless, I would expect sulfate to have been relatively low in the open sea in comparison to present abundances.

There is, then, reason to believe that our ancestral atmosphere was secondary and contained plenty of H_2O, CO_2, and probably N_2. Whether it was also strongly reducing, with H_2 and CH_4 as dominant gases, or merely anoxic, with CO as a principal secondary gas, depends on whether one thinks the planet to have accreted cold or hot. Perhaps advances in comparative planetology will resolve the differences to everyone's satisfaction. But there is much work and hard thinking still to be done on these problems.

THE CONDITIONS OF BIOPOESIS

Biopoesis, a word we owe to Pirie (1953), is the available unambiguous term for the origin of life, without prejudice as to mechanics of origin. Regrettably, the etymologically appropriate and more euphonious terms biogenesis and biosynthesis have been pre-empted for quite different ideas. The notion of biogenesis in the classical sense illustrates well how a probably mistaken universal can arise from the most meticulous research and rigorous logic, serve a useful function in particular circumstances, and yet ultimately become obsolete. You all know the story, of course. Louis Pasteur's famous experiments showed that, within the framework of ordinary experience on an oxygen-rich earth, life comes only from previous life and does not arise de novo. Biogenesis was the illustrious Thomas Huxley's term for this, in contradistinction to the origin of life from non-living antecedents, which he called abiogenesis. Although life arises only from other living things now, however, it may not always have done so.

Extensive published experimental work (summarized by Miller and Orgel, 1974) establishes the ease with which the chemical foundations of life can be generated under anoxic conditions by appropriate energy sources, given likely ancestral atmospheric gases. It transfers the problem of biopoesis from poetry to science. In fact, so much has been written about prebiotic geochemistry and the origin of life in recent years that any discussion of the subject is to some extent redundant.

Here I wish only to emphasize the maturity and sophistication of current and recent experimental work on the matter, beginning with the pioneering studies of Garrison *et al.* (1951), and of Miller (1953), and coming right on down to the present (e.g. Ferris *et al.*, 1978). In fact, so many of the essential large organic molecules have now been made under sterile conditions in the laboratory by energizing such a variety of plausible starting materials that the very nature of the question we started to ask has changed. We no longer ask whether life *could* have originated by chemical evolution from non-living materials. Instead we ask *by just what pathways* might it have originated, and under what primitive earth conditions.

The trick is no longer how to produce amino acids, proteins, nucleotide bases, and sugar-phosphate bonds but how to get them all working together inside of a lipid membrane with operating DNA-RNA sequences. The problem has two frustrating aspects that make it intractable in detail even though solvable in principle.

1. Even if one were to create a viable self-reproducing and mutating cell from nonliving materials, it would be impossible to say that this was the only way for life to have originated.

2. Even if one were to find the first living cells or their early descendants preserved

in sedimentary rocks as fossils, it would probably not be possible to distinguish them from similar coccoid objects of nonbiological or proto-biological origin such as those regularly produced in the laboratory by Fox and coworkers (e.g. Fox and Yuyama, 1963).

A question easier to cope with than just when and how the first cell arose involves the circumstances under which such an event may have occurred. Although we can't be sure of much about the origin of life we can be sure that anoxic conditions were essential. Free O_2 is lethal to all forms of life in the absence of suitable shielding mechanisms. Except for chlorophyll *a*, O_2 enters the most vital biosynthetic pathways, even of the most advanced organisms, at late stages in those syntheses (summarized by Schopf, 1978). All successful experimental production of important large organic molecules from simple precursors has involved the exclusion of free O_2. These organic molecules, moreover, do not survive and could not be assembled into a living cell in the presence of free oxygen (e.g. Miller and Orgel, 1974, p. 119). Nor could a truly primitive cell or noncellular glob of living protoplasm long survive in the presence of toxic free oxygen without O_2 defences. The fact that life is present on Earth is sufficient evidence that both our ancestral atmosphere and hydrosphere were anoxic -- or that life arose at and remained confined to some isolated anoxic site until biochemical oxygen defences evolved.

Liquid water and a source of energy were also needed for biopoesis, and both would have been present when Earth's surface first reached the temperature range within which liquid water could exist. For the absence or transient nature of any free O_2 would exclude the formation of an ozone screen, allowing high-energy UV radiation to bathe Earth's surface in the potentially lethal light of creation. Such an energy source has, in fact, repeatedly produced both HCN and CH_2O in a variety of anoxic mixes of plausible early atmospheric gases, thus providing the basic building blocks for amino acids, proteins, nucleotide bases, and ribose and deoxyribose -- leaving only phosphate as a critical chemical to be added in generating the large key molecules of life. As Calvin (1965) has emphasized, dehydration condensations are a critical aspect of the process. Some of the key dehydration steps are outlined in Fig. 1. In the absence of appropriate enzymes, the CN_2 radical of carbodiimides, or other products of cosmochemical evolution, such as cyanimide or cyanoacetylene (see Table 1), could have catalysed these condensations.

A challenging aspect of the conditions of biopoesis involves early Earth temperatures and the so-called "faint early sun". After a star enters the main sequence, assuming no variance in the gravitational constant, it gradually becomes hotter and brighter from the burning of H to He. Sagan and Mullen (1972) summarized evidence calling for an increase in luminosity of the sun toward the present that has been estimated by different astrophysicists at 30 to 60%, with a weighted mean of ca. 40%. Working backward and assuming only a 30% decrease, these authors calculate a global temperature, omitting local variations of -10°C some 4 to 4.5 Gyr ago. A 50% decrease gives -28°C, which is 10° below the C freezing point even of salt water. At the lesser 30% decrease, Earth's surface temperature would have risen above freezing for the first time less than 2.3 Gyr ago. Such temperatures are clearly not favourable for the origin of life, which was demonstrably present by 2.3 Gyr ago and probably present much earlier. Yet the sedimentary record offers clear proof of liquid water, both fresh and salty, as early as 3.8 Gyr ago and apparently continuously from then onward. So the astronomical expectation of a frozen early earth is not sustained. But why not? Perhaps the combination of residual gravitational heat and radioactivity kept Earth's surface hot until around 4 Gyr B.P. and warm somewhat longer. But Sagan and Mullen discount this. They call rather on an early major greenhouse effect: yet what could have accounted for such a greenhouse?

The greenhouse effect resulting from absorption of back-scattered radiation by the probably abundant CO_2 and H_2O in the ancestral atmosphere is discounted by Sagan and Mullen because of the lack of absorption between 8 and 13 μm. Instead these authors postulate very small quantities of NH_3, which absorbs strongly in this window, and which would achieve the needed greenhouse effect even if CO_2 and H_2O were not present in excess of their present atmospheric abundances. They find that an NH_3 mixing ratio somewhat greater than 100 times the present (10^{-6} compared to 10^{-8}) will provide the needed heat retention by means of a high-temperature equilibrium mesopause layer. This would also shield water vapour from photo-dissociation, while the NH_3 would presumably seek a low steady-state balance between production and destruction by UV photolysis and reaction with OH^-. But could active early volcanism have maintained the needed NH_3 budget until the evolution of NH_3-producing bacteria, and would the combination of volcanic and biological NH_3 thereafter have been able

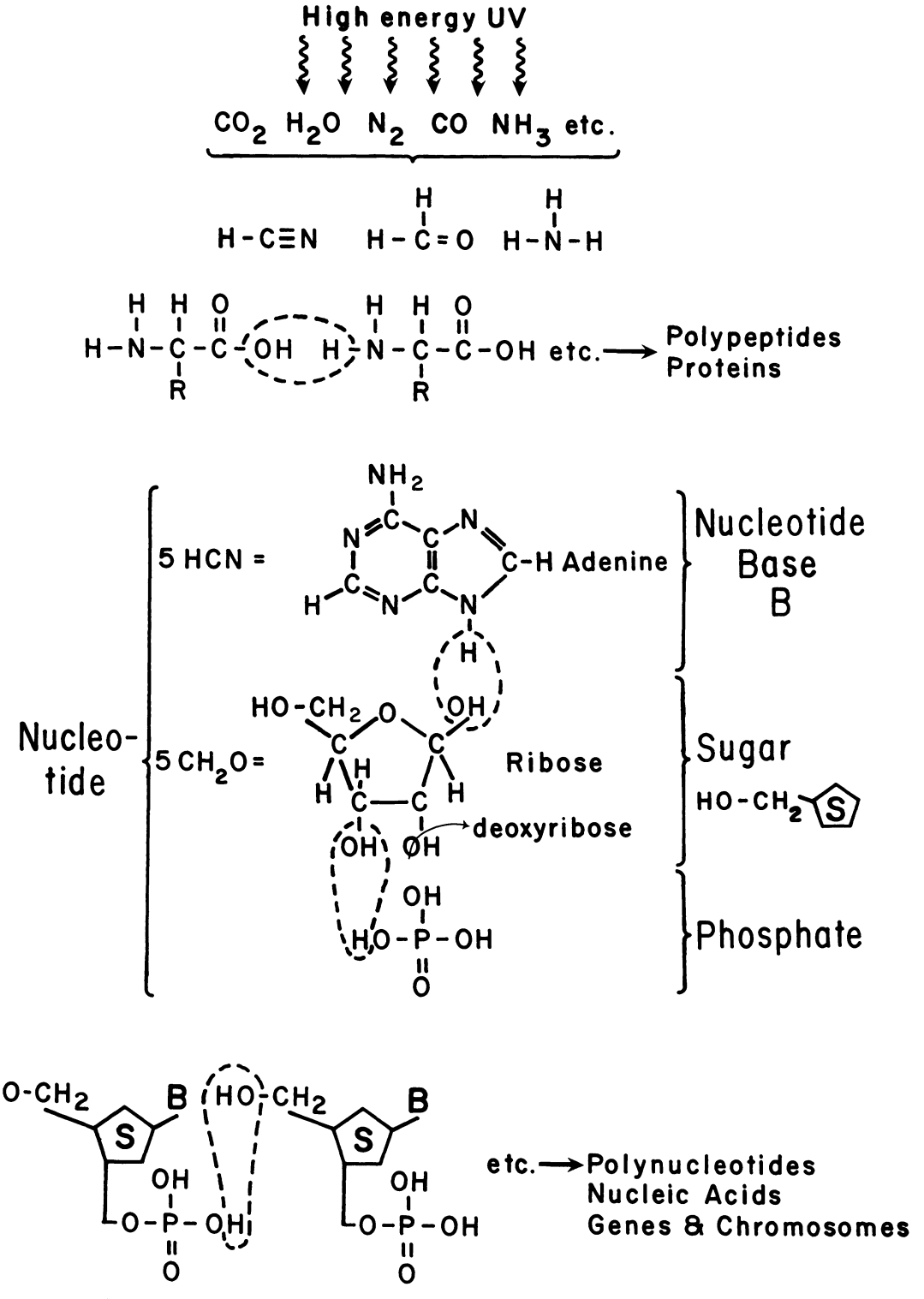

Figure 1: Importance of dehydration condensations in producing and linking key molecules of life.

to maintain the levels needed against continuing destructive effects? What evidence might be brought to bear on this question?

Although the geochemistry of early sedimentary rocks implies a negligible atmospheric NH_3 component and is also consistent with the high level of CO_2 called upon by Carver (1980) to generate the CO_2-H_2O greenhouse effect denied by Sagan and Mullen, it does not exclude the level of NH_3 required to sustain an ammonia greenhouse. Because of the thermodynamic instability of NH_3 in the presence of O_2, the maintenance of an NH_3 greenhouse until the sun became luminous enough to raise Earth temperatures above freezing less than 2.3 Gyr ago is also consistent with evidence summarized elsewhere (Cloud 1976a,b) favouring an essentially anoxic ancestral atmosphere until then. Meanwhile the combination of NH_3 with transient O_2 could have been compensated for by the introduction of new NH_3 of volcanic and bacterial origin.

Before leaving the subject of biopoesis, I would like briefly to extend my previous remarks on the interesting organic molecules that have been reported both in interstellar space (Table 1) and as early products of experimental prebiotic chemistry. Such observations have provoked new interest in the former panspermia hypothesis of Svante Arrhenius, by which universal life is continually colonizing suitable new sites. The destructive effects of unshielded cosmic radiation seem to me, however, to rule out simple undirected transport as traditionally postulated, and I find no evidence that is consistent with either directed transport or "advanced intelligence" within reach of our planet 4 Gyr ago. In fact one cannot but wonder why an intelligence sufficiently advanced for such an operation would have chosen to seed the planet with the simplest procaryotic microbes instead of more advanced forms of life (although it could be argued that this was a way of preparing the planet for oxidative metabolism).

Nevertheless this interstellar organic chemistry does tell us that we need no longer wonder whether the building blocks of life may have been available on the primitive earth. For, in addition to continuing new syntheses, large amounts of organic matter could have arrived here in chondritic debris that was swept up by Earth's warm surface in the late stages of accretion.

All of these ideas and others need to be further tested. Much remains to be done on the causes, possible pathways, and conditions of biopoesis before the problem can be considered even as well understood as the mechanisms of biologic evolution, about which such vigorous controversy still surges. Opportunities and challenges abound.

THE BIOGEOCHEMICAL CONSEQUENCES OF EARLY BIOSPHERIC EVOLUTION

The key event in biogeochemistry was, of course, the establishment of life on Earth, whether de novo or by the improbable route of directed panspermia. For after the successful establishment of terrestrial life, Earth's surface processes could never be the same again. Biospheric evolution would ever after interact with atmospheric, hydrospheric, and crustal evolution (including the formation of surficial ore deposits) through an array of biogeochemical systems and processes that had simply not existed previously (Cloud 1973, 1974, 1976a). I will limit my comments here to one central problem about which there are healthily variable views -- the source and history of atmospheric and hydrospheric oxygen. I refer, of course, primarily to free O_2 in the gaseous and dissolved states, and in lesser degree to atomic oxygen (O) and ozone (O_3). And I characterize O_2 as central because its presence, absence, level, and state so strongly influence the composition of the biosphere and the nature of biogeochemical processes.

Because we know of no primary sources for free O_2 and yet live in contact with and depend on an O_2-rich atmosphere, we are curious about where free O_2 may have come from, what its levels in hydrosphere and atmosphere have been at different times in the past, and to what degree it may have reflected and affected biospheric evolution. We expect to find evidence relating to such matters in the geologic record. And when we study that record, we do indeed find oxidized sedimentary iron formations among the oldest rocks we know, indicating that the hydrosphere, at least at some places and times, contained some free O_2 as far back in Earth history as we have evidence for water.

The banded iron formations (BIF) reflect unusual conditions of chemical sedimentation. As all have recognized, they denote the episodic introduction or precipitation of ferric and

ferro-ferric iron at sites where continuing sedimentation was of a different type -- commonly silica, now preserved as chert. In this way was generated the typical alternating iron-rich and iron-poor laminae of the cherty BIF (e.g. papers in James and Sims, 1973). After long reflection on this problem I evolved the hypothesis (Cloud, 1965, 1973) that the episodicity of the banding and lamination, its commonly remarkable continuity and areal extent, the scarcity of BIF among sedimentary rocks younger than 2 Gyr, and the rarity of typical ferric-oxide-rich continental to marginal marine red beds older than ca. 2 Gyr might all reflect some common relationship to an early local and probably biological source of oxygen. The source of O_2 had to be local and episodic. Free atmospheric O_2 above minimal transient levels would shut off the system by retaining iron in the weathering profile or precipitating it immediately at the edge of the sea as it does where iron reaches the sea today. W. W. Rubey later drew my attention to the fact that a similar but less comprehensive view had already been expressed in 1927 by A. M. MacGregor in a prescient early article in the South African Journal of Science.

In its important basic elements this hypothesis has both withstood the test of time and become the key element of a more far-reaching model of early Earth history (Cloud, 1976a,b). The ages I use here, however, are not the same as those originally proposed, for the model has evolved as new evidence has come to light. Its details will doubtless continue to change with future discovery, new observational detail, and theoretical refinement. That is the way of advancing science.

Observant critics have pointed to inconsistencies that call for attention -- for instance, red beds and sedimentary sulfates older than I had postulated, younger BIF, other banded iron formation that is older than convincing independent evidence for biological O_2 production, and even evidence of superficial oxidation on planets that have probably never developed a biosphere. These suggested flaws in my brainchild have required me to consider the new and reexamine the old evidence and to ask what modifications or alternative hypotheses would best reconcile the whole body of evidence. In particular, are there alternatives to photosynthesis as the major source of O_2 and to ca. 2 Gyr ago as the earliest time at which O_2, from whatever source, became a substantial permanent atmospheric component?

Well, yes, there are alternatives to both. Let us examine them. An obvious alternative to photosynthesis as a source of free O_2 is the well known photolytic dissociation of water vapour in the upper atmosphere (and perhaps to a trivial extent that of CO_2 as well, according to Shimizu, 1979). Although the photolysis of H_2O, followed by escape of hydrogen, is today a very limited process, Brinkmann (1969) has argued that it could have been much more important on the primitive earth, causing a filling of oxygen sinks and giving rise to 25% or more of Earth's free O_2 in relatively short order.

Omitting, for the moment, geochemical evidence that implies negligible O_2 levels during Archaean and earliest Proterozoic history (summarized by Cloud, 1973, 1976b), Brinkmann claims that an early major growth of photolytic O_2 cannot be eliminated on theoretical grounds alone. A nonbiological photolytic source for early O_2, however, does run into severe theoretical difficulties that would appear to call for a large reduction from Brinkmann's estimates. He assumed that every photolytic event is followed by escape of hydrogen, whereas today most (about 90%) of the hydrogen atoms so produced recombine with hydroxyl or oxygen to form water, not only on Earth, but, as Hunten (1973) has shown, on Mars and Venus as well. Processes that influence the rate of gravitational escape of hydrogen are described by Hunten (1973) and by Hunten and Strobel (1973). They find that loss of hydrogen from Earth's gravity field is controlled not so much by the rate of photolytic production as by the quantities of hydrogen and hydrogen-containing gases that are able to reach the stratosphere. This depends on the rate at which hydrogenous gases, mainly water vapour, are carried upward from the troposphere, a process that, as Mastenbrook (1971) has shown, is controlled by condensation at the tropopause, The temperature of the tropopause is, therefore, the key factor in the delivery of hydrogen-containing compounds to sites from which any hydrogen produced has a good statistical chance of escaping before recombining to form H_2O. Unless the primitive tropopause were warmer than now, which, from prior discussion, seems unlikely, O_2 of photolytic origin could probably not have been generated any more rapidly than it is today.

Three other considerations detract from the idea of a photolytic source for an early O_2-rich atmosphere.

1. When atmospheric O_2 rises to or above about 1% present atmospheric level an ozone layer is formed (Berkner and Marshall, 1965). This blocks the photolizing radiation and, together with the associated tropopausal cold trap, today limits further dissociation of H_2O vapour (Urey, 1959).

2. Given the previously discussed problem of preventing subfreezing surface conditions until after ca. 2.3 Gyr ago, a greenhouse effect of some kind is called for. Sagan and Mullen (1972) find that relatively modest NH_3 levels could have sufficed. Such a concentration of NH_3, however, blocks the photodissociation of water vapour at lower atmospheric levels and excludes the concurrent presence of significant levels of O_2.

3. All open O_2 sinks must be filled before free O_2 can accumulate. That includes any CO as well as NH_3 or other reducing atmospheric gases, new reduced volcanic gases and sulfides, ferrous oxides or hydroxides, and other O_2-scavenging materials at or above Earth's surface or in its circulating hydrosphere.

In view of these retardent effects on the accumulation of photolytic O_2, the geochemical evidence for easily oxidized but unoxidized terrestrial detrital sediments until ca. 2 to 2.3 Gyr ago, and the fact that we cannot write an objective geochemical balance for O_2 of photolytic origin, we must view the importance of that process with reserve. The evidence allows us to conclude with confidence only that non-biological photolysis accounts for some important fraction of Earth's historical production of O_2 perhaps even a large fraction, but probably not enough to saturate all then-existing oxygen sinks and lead to any significant or long-lasting accumulation of atmospheric oxygen.

Because of the near geochemical balance we find between carbon and oxygen from all atmospheric, hydrospheric, and sedimentary accumulation (Cloud and Gibor, 1970; Cloud, 1978, p. 123), it appears that photosynthesis alone can very nearly account for all of the now or once free O_2 we can identify (Fig. 2). That also contradicts the idea that simple photolytic dissociation of H_2O vapour was sufficient to overfill the O_2 sinks and produce an oxygen-rich Archean or very early Proterozoic atmosphere. No reason is found, therefore, for abandoning the view, implied by the range in time of BIF and red beds, of an early atmosphere and hydrosphere containing only trivial and transient free O_2. This view is also supported by the aforementioned wide occurrence of easily oxidized detrital pyrite and uraninite until at least 2.3 Gyr ago (notwithstanding reports of local occurrences of similar associations in modern peri-glacial deposits). And it is consistent with the very low positive (primordial) $\delta^{34}S$ values (avg. + 3.6 to + 3.8) described by Perry *et al.* (1971) for barite from the perhaps 3.3 Gyr old Swaziland System, and by Lambert *et al.* (1978) from the ca. 3.4 Gyr old North Pole sulfate deposits of the Pilbara Block in Western Australia.

Still, we have identified two potentially important sources of free O_2, and the degree to which each may have been involved in early atmospheric evolution comes down to a matter of rates of segregation of their coproducts, of timing, and of the possible existence of unrecognized O_2 sinks. Free O_2 of photolytic origin could have played an important role in converting CO to CO_2, NH_3 to N_2 and H_2O, and oxidizing other reduced gases and perhaps solids and dissolved substances, not all of which were tallied by Cloud and Gibor in writing their carbon-oxygen balance. The writing of more rigorous geochemical balances thus remains an important challenge for the future. A knotty aspect of this problem involves the meaning of the observed ratios of organic to carbonate carbon in the older rocks and the apparent near constancy of these and of $\delta^{13}C$ values over recorded Earth history. Discussion of this matter by Broecker (1970), Becker and Clayton (1972), and Schidlowski *et al*, (1979) leaves it clear only that the last word is yet to be said.

The time at which O_2 first became an important and persistent atmospheric component, regardless of origin, and the rate at which it accumulated to present levels is also not agreed upon. If my interpretation of the geochemical implications of the BIF is correct, the photosynthetic production of free O_2 is at least as old as the Isua supracrustals (3.8 Gyr). If not, the presence of stromatolites in 3.0 Gyr old sediments in eastern South Africa (von Brunn and Mason, 1977) is presumptive evidence that O_2-producing photoautotrophs were extant by then. Yet O_2 could not take up permanent residence in the pre-Phanerozoic oceans and begin to accumulate as a significant and sustained atmospheric gas until all major O_2 sinks were filled.

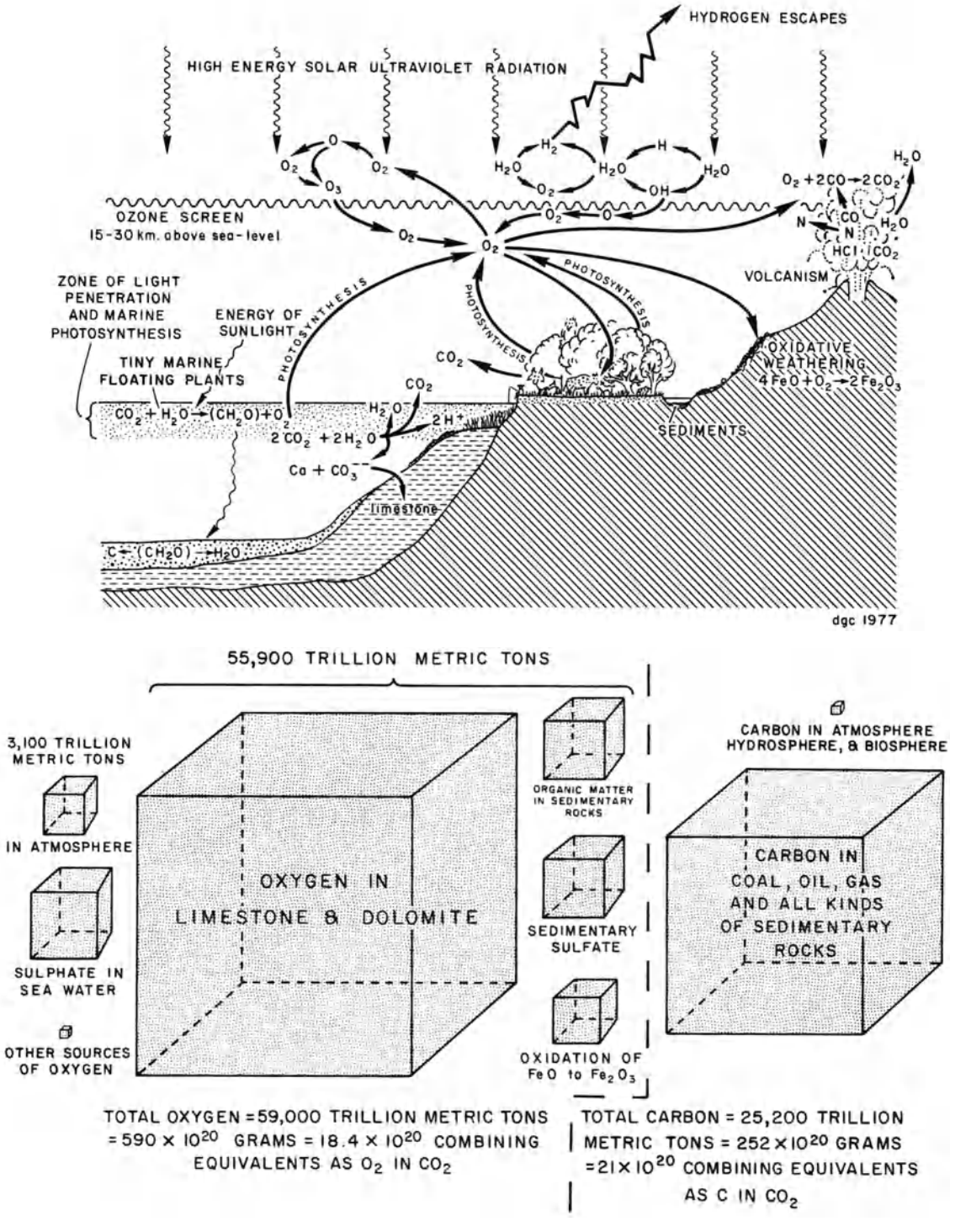

Figure 2: Sources of free oxygen and its apparent geochemical balances. Adapted from sketches by P. Cloud and A. Gibor (1970).

I have elsewhere presented (e.g. Cloud 1976a,b) evidence supporting a beginning of perceptible and persistent atmospheric O_2 about 2 Gyr ago, following a long interval of fluctuating balance between biologic sources in the sea and geochemical sinks. The accumulation of atmospheric O_2 of photosynthetic origin to present levels depends on reaction rates and the segregation of the thermodynamically unstable end products. That is, photosynthetic O_2 can accumulate no faster than carbon is buried, just as photolytic O_2 can accumulate no faster than H_2 is lost from the grip of Earth's gravity. Thus I suggest in Fig. 3 that biological events imply about 10% present atmospheric level of O_2 ca. 600 Myr ago and close to present atmospheric levels ca. 400 Myr ago.

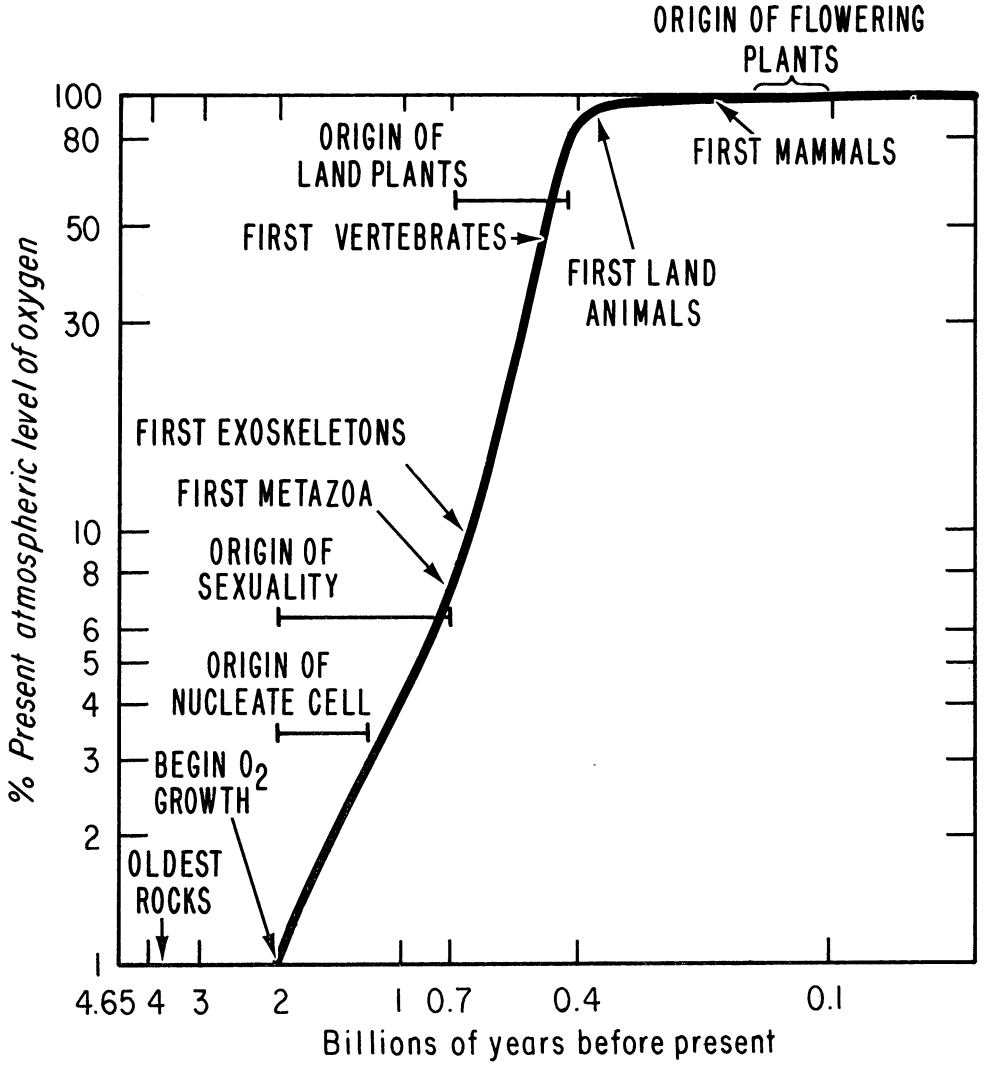

Figure 3: Growth of atmospheric oxygen inferred from biogeological events. Slightly modified after Cloud (1978).

Peer criticism, however, calls on me to modify and clarify my views. The currently ruling model of the primitive earth that has arisen from hypotheses that I originally presented in 1965 differs in important detail from those earlier proposals. Although its general thrust is not significantly changed, additional laboratory and field investigation will be needed to bring about and test the expected further evolution of this model.

Let us consider wherein my model has changed and in what respects it has either been misunderstood or is still challenged.

It has changed with regard to my views about the kinds and levels of oxygen toxicity and defenses. Critics sensibly insisted that an external oxygen acceptor such as ferrous iron cannot take the place of the internal oxygen defenses that are demanded at any level of biologically generated free O_2 and are, in fact, a part of the basic photosynthetic mechanism. I have now modified that part of the model which calls for a long-lasting balance between low and transient levels of biologically generated O_2 and banded iron formation to reduce emphasis on the O_2-mediating enzymes themselves while stressing the probably low tolerance to *external* O_2 of the early oxygen-producing microbiota. *Microaerophilic* photosynthesizers capable of tolerating only very low levels of ambient O_2 would have suited the niche envisaged had they also been able to switch to anaerobic metabolism during intervals of maximal O_2 depression.

What is called for is an O_2-producing microaerophile that is dependent on exogenous reductants to maintain a low P_{O_2}. It would not be surprising if such a microbe were also able to switch to anaerobic photosynthesis, utilizing primary election donors other than H_2O. A common primary electron donor for anaerobic microbial photosynthetic today is H_2S, but a reduced iron compound might have served such a function in the past.

To get to a state of microaerophilic O_2-producing photosynthesis from bacterial photosynthesis, limited to light reaction I, requires a second light reaction, light reaction IL The latter calls biochemically for the addition of chlorophyll-*a* and cytochrome-*f*. The blue-green algae are the simplest organisms that possess such attributes, and they include well-known living microaerophiles. Biochemically they are, in effect, O_2-releasing photobacteria that may also be microaerophilic and capable of facultative anaerobiosis. Although both light reactions are utilized in the presence of oxygen, a number of blue-green algae (cyanobacteria) can thrive and photosynthesize (without producing free O_2) under anaerobic conditions, utilizing photosystem I alone, with H_2S or other primary electron donors. Even where utilizing both photosystems, such cyanophytes are more oriented toward anaerobic than fully aerobic photosynthesis (Oren *et al.*, 1977; Towe, 1978). That is just the kind of creature it would have taken to maintain the postulated balance and perhaps seasonal episodicity between ferrous iron in solution and the ferric and ferroferric oxides of the BIF.

To this Towe (1978) has objected that the biosynthesis of chlorophyll-*a*, basic to photosystem II and O_2-producing photosynthesis, is initiated by an oxygen-requiring step in the oxidative decarboxylation of coprogen (Bogorad, 1966, p. 486), thus requiring the presence of photolytic free O_2 before cyanobacteria could evolve. With Demoulin (1979), I would, in turn, observe that Bogorad (1976, p. 68) also indicated alternatives to O_2 for the same decarboxylation, and Towe (1979) now agrees that: "The ability of some extant blue-green algae to sustain abundant growth in reducing conditions certainly implies that they can make the necessary decarboxylation step without free oxygen."

Of course we should not except absolutes. We must ask whether the earliest green-plant photosynthesizers could have been biochemically oess than complete blue-green algae. Could local and transient photolytic O_2 have started an O_2-dependent reaction that thereafter was sustained by locally produced and transient photosynthesic O_2? But Towe is also bothered by the apparent absence of the carbon needed to balance the oxygen responsible for the BIF. This carbon is, in fact, amply represented. Not only is there abundant real carbon in black shales at the centres of contemporary basins (up to 12% at places). In addition a substantial quantity of carbon was probably also returned to circulation as CO_2 released by the reduction of hematite to magnetite during diagenesis of the BIF -- $6Fe_2O_3 + C \longrightarrow 4Fe_3O_4 + CO_2$. Hydrodynamically we should not expect to find carbon equivalent to all or even a large part of the BIF at exactly the same depositional sites with it.

In evaluating a time scale for atmospheric O_2, one must separate oxidation of sediments

and transient oxidation from the establishment of a sustained aerobic atmosphere and the attainment of various levels of O_2 in it. The evidence is in the geologic record, but much has been lost and we know as yet only a fraction of what exists. We must use what we do know with discrimination, keeping in mind that geology, including evolutionary biogeochemistry, does not deal in absolutes.

Red beds, as well as sedimentary sulfates are important indicators of O_2 in the sedimentary environment -- one mainly atmospheric, the other mainly hydrospheric. Let us consider sulfate first. In earlier papers I did indeed state, as Walker *et al.* (1977) have corretly noted, that I knew of no *significant* deposit of well-established, primary, sedimentary gypsum older than ca. 800 Myr , although, of course, I was familiar with records of scattered clastic pseudomorphs. But science progresses, and I now stand corrected. Contrary to Walker *et al.*, however, no theoretical interpretations of mine were "*based* (my italic) on this contention." The presence even of thick and extensive sedimentary sulfates of great age in no way invalidates my earlier conclusions about the late appearance of an oxygenated atmosphere.

We can visualize several sources of early sulfate ion that involved no or only evanescent free O_2. SO_2 and perhaps SO_3^{2-} of volcanic origin were presumably components of the primitive atmosphere, where they scavenged trace quantities of photolytic O_2 to produce a steady or intermittent transfer of SO_4^{2-} ions to Earth's surface waters, Sulfides and sulfite in these waters could also have been a sink for biological O_2, either as depressants of ambient oxygen for microaerophiles or as acceptors for the nascent O_2 of bacterial photosynthesis (without the production of free O_2 at any stage of the process). There is, therefore, no theoretical reason why some sedimentary sulfate deposits should not have formed as far back as sulfide- or sulfite-oxidizing bacteria and evaporite settings existed. Thanks to the work of Lambert *et al.* (1978) we now know that sulfate deposits, whether indicative of transient free O_2 of photolytic origin or of bacterial sulfide oxidation, did exist early in Archean history. How extensive they may have been we do not as yet know.

As for true subaerial or marginal marine red beds, I judge them to be sensitive indicators of atmospheric oxygen and far more relevant to deciphering atmospheric evolution than are other familiar oxidized sediments, in spite of their obviously good opportunities for erosional removal. Such deposits virtually certify the presence of atmospheric O_2 *at the time of their oxidation*. Even very small and local quantities of O_2, of course, could, in the beginning, produce notable effects because of their conversion to highly reactive atomic oxygen and ozone under the high-energy UV irradiation to which Earth's surface was exposed before enough O_2 had accumulated to develop an ozone screen. Taken as a whole, the record suggests that red beds appear on a substantial scale rather abruptly following the last major and extensive development of BIF ca. 2 Gyr ago. Yet we also know red beds in older and BIF in younger rock. Both must be explained and the model either modified as may be needed to accomodate those explanations or abandoned in favour of a better one.

Let's start with the red beds. Red beds older than the big late early Proterozoic BIF (ca. 2 Gyr old) are reported from the Canadian shield and South Africa. Prominent among these are the red arkoses and conglomerates of the Chakonipau Formation in the Labrador Geosyncline, described by that splendid observer Eric Dimroth (1968, 1970, and elsewhere) as separated by ca. 900 m of sediments and an angular unconformity from the important overlying Sokoman Iron Formation, a true BIF. Rb-Sr isochron ages on metasedimentary rocks associated with the Sokoman and other iron formations of the Labrador Geocyncline, to be sure, give only ca. 1.7 to 1.8 Gyr , but this is probably a metamorphic age. It seems likely that their true age will prove to be close to that of the ca. 2 Gyr old Biwabik and Gunflint BIF (Goldich, 1973).

The red colouration of the pre-Sokoman Chakonipau Formation is clearly due to oxidation, as Dimroth recognizes. He, however, considers that alteration to have followed on the heels of sedimentation. In response to this I have asked elsewhere (Cloud, 1976a,b) whether this secondary oxidation might not reflect one of the known much younger episodes of deep subaerial oxidation that affected large parts of the Canadian Shield during the Proterozoic to Phanerozoic and Cretaveous time. Well-placed borings might resolve this issue, which I am less disposed to contest than formerly for reasons given below.

As for still older deposits, I do not accept as primary the purple and red stains, some

clearly favouring joint surfaces that are known from very ancient South African deposits (e.g. Cloud, 1976b). We do know, to be sure, of extensive primary South African red beds in the ca. 1.9 Gyr old Waterberg Group. But, in considering evidence for older red beds, it must be remembered that the Kaapvaal and other southern African cratons have a very long history of emergence and deep weathering. Local secondary oxidation, therefore, even penetrating to substantial depths along joints and faults, is to be expected, and one must be wary of it in evaluating records of oxidation in very ancient rocks.

Two records, nevertheless, do imply at least local episodes of subaerial oxidation older than I have heretofore hypothesized. One is described in an illuminating recent study by Button (1980) of a weathering crust that covers some 100,000 km^2 at the top of the 2.2 Gyr old Hekpoort Basalt, beneath the Dwaal Heuvel clastics of the Pretoria Group, Transvaal Basin, South Africa. This paleosaprolite displays high ferric-ferrous ratios throughout, along with much unoxidized iron, implying weathering by alkaline, O_2-bearing but O_2-deficient groundwaters. That means, pretty clearly, that some, if not much, O_2 was already showing up in the atmosphere about 200 Myr before it seems to have become generally osygenic. And that means that the transition from anoxic to oxygenous was either earlier or more gradual than I have previously supposed.

Well-established red sediments in the Huronian of southern Ontario also call for acknowledgment. Although contemporaneous very low oxygen pressures are implied by associated ferrodolomites and slightly older detrital pyrite and uraninite, thin red clastics of upper Huronian (Lorrain) age (>2.15 Gyr) are seemingly primary deposits. I once thought that they might be secondary derivatives from the reworking of abundant nearby BIF. But Button's Hekpoort weathering crust suggests that they may well represent early or local oxidation in place, and detailed sedimentological analysis by Grant Young and students (S.M. McLennan, oral communication, Sept. 1979) support a primary origin for red beds in the Gordon Lake and Bar River formations of latest Huronian age.

The presence of younger Proterozoic and even local Palaeozoic BIF (or similar rocks) is a further anomaly whose explanation is not yet clear. Although one can think of special explanations for such deposits, as I have done (Cloud, 1973), they need further and more detailed study -- especially the huge Jacadigo and Rapitan deposits of South America and northwestern Canada (discussed, with reference to original works by others, in Cloud, 1973).

Whether the answers we may find to questions raised by these anomalies destroy, or continue to support, or merely permit a modified version of the model of primitive earth history that I have been working on for the last two decades is of little consequence. The value of a model or an hypothesis rests not on whether it stands or fails but on the extent to which it stimulates and focuses inquiry in the process.

There is much still to be explained. As several have noted (e.g. Schidlowski and Eichmann, 1977), an O_2-rich atmosphere at any time in Earth history, and especially in the beginning, is thermodynamically improbable. The very presence of free O_2 is a manifestation of thermodynamic disequilibrium. The eucaryotic biogeochemical system would never have evolved but for a kinetic lag between sources and sinks of O_2. Yet all of these improbable things happened.

What I want to stress as I approach the end of this paper is the need in dealing with the many and subtle problems of palaeomicrobiology, biogeochemistry, and biogeology, broadly speaking, to combine a multifacetted approach to problems with caution about the evidence, boldness in interpretation, openmindedness about the unexpected, and a willingness to reconsider. It is no disgrace to be mistaken about an interpretation as long as none of the relevant evidence was distorted or concealed. Wrong interpretations are easily eliminated by vigilant colleagues in the same field (although often taken as gospel in distant or even allied fields). Wrong or misleading observations and experimental results have a disastrous staying power.

I am not saying that we should never reach out on slender grounds or denying the role of intuition. But I do wish to emphasize that the level of credibility of the evidence should always be clear, that a sharp distinction between evidence and hypotheses should be made and reflected in our terminology, and that our intuitions are scientifically valid only to the extent that we can subsequently test and relate them back to the common body of

established knowledge. Thus I urge equally the avoidance of genetic terms in a descriptive sense and the regular expression in some way of the degree of confidence that may be warranted for our observations, our experimental results, and the conclusions that are permitted, suggested, or demonstrated by them.

I have tried to use approximate percentages of credibility for this purpose but find that unrealistically precise. Instead it seems better to designate levels of credibility (in ascending order) by the use of qualifying terms such as permissive, consistent with, suggestive of, probably, presumptive, or demonstrable.

On viewing the major features of early biogeochemical systems through such a screen I find the following evidence and consequent conclusions worthy of your attention at the levels of confidence indicated:

1. Banded iron formation in the 3.8 Gyr old metasedimentary rocks of southwest Greenland and the Limpopo Fold Belt *permits* one to hypothesize *either* a generally oxygenous atmosphere at the time of the earliest hydrosphere we know *or* a common but local and episodic source of O_2 within that hydrosphere.

2. The apparent absence or great rarity of other oxidized sediments or minerals from rocks older than about 2 to 2.3 Gyr ago, and the presence in them at a number of places of abundant, easily oxidized minerals, *suggests* a local (most likely biological) source for hydrospheric O_2 where O_2 must be invoked. This *is consistent with* the interpretation that biogeochemical systems involving microaerophilic, oxygen-releasing photosynthesizers were already present and involved in BIF sedimentation as early as 3.8 Gyr ago.

3. Microbial remains showing cellular differentiation in the ca. 2.3 Gyr old Malmani Dolomite of the Transvaal System (Nagy, 1974, 1978) comprise the oldest evidence of a biogenic, indigenous, and primary microbiota. Earlier biogeochemical systems probably preceded that, including any that may have involved the very simple problematically life-like carbonaceous microspheroids of the Swaziland sediments and associated organochemical matter.

4. By Gunflint time, ca. 2 Gyr ago, there were abundant, *demonstrably* biogenic, filamentous microbial assemblages that display cellular differentiation and relations to the matrix that also *demonstrate* a primary incorporation of these fossils within the sedimentary rocks that enclose them.

5. The presence among these Gunflint filaments of enlarged, heterocyst-like and akinete-like cells and hormogonia-like chains of cells along with other features, is *presumptive evidence* of a nostocalean affinity, nitrogen fixation, and at least local free O_2 (for biological N_2 utilization occurs without special adaptation in the absence of O_2). It is *permissible* to extend a similar interpretation back to 2.3 Gyr B.P., based on the single enlarged cell found by Nagy in the Malmani Dolomite (item 2 above). These heterocyst-like bodies, if they are indeed heterocysts, *imply* that O_2 was then at least locally building up in the hydrosphere although not necessarily evading to the atmosphere. We must wait for demonstrably primary red beds as a clear signal that free O_2 was beginning to accumulate in the atmosphere.

6. Integration of all of the above with the data from carbon isotope ratios (Schidlowski and Eichmann, 1977), as well as evidence summarized above and elsewhere, strongly *suggests* that life began before the oldest sediments of record and that the major events in procaryotic biochemical evolution had probably been achieved by 2.3 to 2 G yr ago. It *suggests* also that, by the later time, sedimentary O_2 sinks were filling up and that the impending or ongoing evasion of residual O_2 to the atmosphere would soon wipe out any remaining NH_3 and shut off any Sagan-Mullen greenhouse effect. The timing would be about right. The calculations of Sagan and Mullen (1972) suggest that the sun would not have been warm enough to sustain life in the absence of such a greenhouse effect much before ca. 2.3 Gyr ago. The recent work of Button (1980) on a widespread weathering event in South Africa ca. 2.2 Gyr ago indicates that atmospheric O_2 was then already present but in low concentrations. It now seems, therefore, that the time from ca. 2.3 to 2 Gyr ago may have been a time of transition from an essentially anoxic atmosphere to one in which O_2 was generally present at concentrations greater than 1% present atmospheric level, or ca. 0.2 of an atmosphere.

Finally, I would ask, could the elimination of that early greenhouse effect at that time, while the sun was still faint, somehow be related to the cooling that produced the Huronian ice ages and perhaps contributed to the big 2 to 2.2 Gyr old BIF deposits? If we were to link the beginning of atmospheric O_2, the end of a greenhouse effect, glaciation, and the big terminal BIF, this could be taken to imply that biogenic O_2 produced during and before that last great episode of BIF was then already beginning to displace other atmospheric gases. That would also be consistent with the suggestion from Button's Hekpoort weathering crust that the transition to an oxygenous atmosphere stretched over some 200 Myr or so of imbalance before the final enduring conversion that opened a sustained gap between sources and sinks and paved the way to eucaryotic biogeochemistry, and to us.

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PRECAMBRIAN GENETICS

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INTRODUCTION

Although life is not readily definable, its essence is the multiplication of a certain sort of matter (animate) at the expense of different matter (inanimate) which does not so multiply. Energy is necessary for this and subsequent transformations, but is not considered in the present paper; it has been thoroughly discussed elsewhere (e.g., by Broda, 1971). The central feature of the animate type of matter is the transmission of a particular material order to succeeding entities; this is the genetic aspect.

This type of matter, here equated with "life", had its origin sometime before 3 Gyr ago as judged from the fossils preserved in the sediments. With time, the living state became progressively more elaborate and diversified, but for the present purposes it is convenient to divide its progression into successive stages: pre-organisms (molecules multiplying free in solution), proto-organisms (where the multiplying entity is enclosed in a boundary membrane), prokaryotes (bacteria, blue-green algae and their predecessors) and eukaryotes (all other living organisms except viruses and viroids). The first two stages have disappeared and are probably not even detectable as fossils. However, some tentative account of them may be given by extrapolating back the features of modern organisms and by considering the early environmental history of the earth. Since only the genetic aspect is considered here, the discussion will concern the chemical nature of the genetic information and the means whereby it may have been transmitted, accumulated, altered and exchanged in the successive stages of Precambrian life.

THE PRE-ORGANISMS

These hypothetical forerunners of contemporary life are believed to have been small circular self-replicating molecules of RNA for the following reasons.

It is known that in all organisms now living, including viruses, the transmission of genetic information rests on the complementary multiplication of nucleic acids. The information is contained in the linear order of bases (purines and pyrimidines) forming the nucleic acid molecule. A linear ordering of a few different subunits can provide a much greater amount of information than any two-or multi-dimensional structure without enormous complexity which would preclude its multiplication. However, it is not immediately clear that only nucleic acid bases could form suitable subunits for such molecules. At this stage of matter transformation of "evolution", the molecules forming the starting base for life would persist because of their ability to multiply themselves but they would have little or no other informational content. Judging from the organic molecules experimentally synthesized under the conditions of a reducing atmosphere, only three types of diversified compound capable of polymerization would be fairly common: nucleic acid bases, amino-acids and sugars. The possibility of a replicating protein, on the basis of pairing via opposite charges, has been discussed by Miller and Orgel (1974) but considered unlikely with the types of amino-acids probably then available. Similarly, polysaccharide chains are today formed by a non-template enzymic transfer process and do not appear to have the structure necessary for linear copying. Nucleic acid bases, on the other hand, are planar molecules because of their resonance characteristics with amino- and keto-groups appropriately situated for complementary hydrogen bonding. So the nucleic acid chain multiplies by acting as a template which selects the complementary base by forming hydrogen bonds with randomly colliding subunits. The complementary chain so assembled, then makes a copy of the original template molecule by the same process. All organisms, including the single-stranded viruses, have to make often useless complements during nucleic acid replication although it would seem possible to make identical (non-template) copies of nucleic acids by the attraction of like molecules as occurs in crystallization. A model for identical copying of nucleic acids via their phosphate groups has been proposed by Penrose (1960), but it may lack sufficient specificity.

Once self-replicating nucleic acid molecules arose, it must be assumed that step-wise changes occurred which made them capable of more rapid and precise growth and better interaction with accessory compounds affecting growth and stability. These advances all depend on self-reproduction to leave offspring containing the improvements. Populations of different molecules, slowly growing on a self-copying principle would undergo accidental changes which increased or decreased their rate of multiplication. This is comparable with known processes of evolution by mutation and selection, allowing those molecules to predominate that multiply most readily and most precisely. Whereas initially the mass ratio of animate to inanimate matter increased merely because the former had the capacity of self-multiplication, now it is supposed that a pool of replicating molecules comprises different structures and there is competition between them.

The molecules could grow to an optimal size, not by genetic duplication, which is an enzyme-catalyzed process of higher organisms, but by what is called "slippage". In the melting and reassociation of double-stranded nucleic acids, there may be displacement in pairing, especially in nucleic acids with only a few or repetitive bases (Kornberg, 1965). Successive cycles of such replication and slippage would cause a linear extension of the template nucleic acid.

The main source of the accidental changes in the self-replicating nucleic acid molecules which make them capable of evolving is likely to be the UV light of the sun, which is strongly absorbed by nucleic acid bases. According to Sagan (1973) the sun was some 30 to 40% fainter 4 Gyr ago than it is today, but the UV flux was not very different. For contemporary organisms, without protection, this would be enough to kill them in about 0.3 s (Zelle, 1955). At present, ozone absorbs the light between 220 and 320 nm but none of the main gases of a reducing atmosphere remove the light between 240 and 280 nm and nucleic acids adsorb most strongly at 250 nm. Even if the incident UV light were reduced in intensity by layers of water vapour, it must have been strongly mutagenic in the early Precambrian. In the absence of mechanisms for repairing radiation damage such as evolved later, the pre-organisms could only exist and multiply in regions shielded from UV light.

The pre-organisms are thus expected to be small self-replicating molecules of nucleic acid. They were probably circular for several reasons: in vitro polymerization shows that cyclization frequently occurs, an agent catalyzing polymerization would require to attach only once to a circular molecule; circular molecules tend to be more stable than linear ones and, except for some animal viruses, a basic feature of genetic organization is that the nucleic acid exists as circular molecules. These molecules are more likely to be of RNA than DNA because the simplest known natural self-replicating nucleic acids are the single-stranded ones of the viroids which have only about 350 bases in their RNA genomes. Moreover, the artificially-made nucleic acids that can be multiplied in vitro are RNA molecules, not DNA. These experiments (Biebricher and Orgel, 1973) on the enzyme-catalyzed replication of random copolymers of certain RNA bases show also that this type of multiplication of matter can occur extracellularly as postulated. The presence of uracil in RNA in place of thymine in DNA does, however, make the nucleic acid much more sensitive to radiation damage since the irradiation of RNA induces stable hydrates of uracil but not of thymine. According to Lesk (1969) DNA may have been selected to replace the original RNA because of the greater stability of genetic information in this form.

THE PROTO-ORGANISMS

The span of time occupied solely by the pre-organisms may have been quite short. An isolated pool of tangled incomplete molecules replicating very slowly, would be extremely vulnerable to accidents especially those leading to excessive dilution. Only when the molecules were separated by spherical boundary membranes from the dilute aqueous mass would they be able to evolve a coding function; that is, develop an informational aspect as well as a replicative one. With the appropriate materials, membranes can readily form by aggregation as has been demonstrated for coacervates, lipid-protein bilayers and protein microspheres. With both reactants and products then separated in a relatively concentrated form, an interaction between nucleic acids and proteins or amino-acids is possible. Such an interaction, frequently via messenger RNA and always requiring small RNA adaptors, gives the template nucleic acid its content of genetic information and has led to its division into distinct coding units or genes. The present-day genetic code, the correspondence between triplets of nucleic acid bases and specific amino-acids, is very highly evolved, but it must have been preceded by a type of direct templating or ordering influence on

spontaneous amino-acid polymerization. However, no convincing evidence for such an interaction has yet been produced.

By these means, protein catalysts, or enzymes, weak in activity and low in specificity could be at least partially specified by the nucleic acid and aid in its replication. Even a small ordering influence by the nucleic acid would be advantageous because some spontaneously formed proteins have catalytic activity, especially in hydrolytic reactions (Rolfing and Fox, 1969). The most essential gene activity of a developing organism would seem to be a transferase catalyzing group exchange in the polymerization of nucleotides and amino-acids. This is a function of most present-day hydrolytic enzymes of which more than 90% are relatively unspecific in their substrates. So the primordial polymerase with very little specificity could also have catalyzed many or most of the group transfer steps in different primitive biosyntheses.

The order of acquisition of genetic function, i.e. nucleic acid sequences specifying catalytic polypeptides, is expected to be, firstly, a group transferase active in replication; secondly, an oxidase capable of removing hydrogen from organic compounds, transferring it to a hydrogen acceptor and using the energy so derived to couple phosphate ions - the basis of anaerobic fermentation - and thirdly, an enzyme-catalyzed mechanism to repair UV-induced damage to the nucleic acids.

In the genetic build-up of a free-living organism, partial duplication of nucleic acid must have provided a range of genes from one or a few primordial genes. The present-day enzymes specified by genes have two important characteristics: a fairly distinct specificity for structurally-related substrates and a strict specificity for the type of bond rearrangement catalyzed. Substrate specificity has been shown to be alterable by mutation, but the type of bond change catalyzed appears very difficult to vary (Langridge, 1969). The maximum number of unique gene sequences that would need to be evolved in proto-organisms is thus likely to be no greater than the number of basic bond changes that are enzyme-catalyzed. This number is about six - for oxidoreductases, transferases, hydrolases, lyases, ligases and isomerases. Apart from the early requirement for an unspecific transferase, oxidoreductases would be needed for energy metabolism but lyases, ligases and isomerases represent more specialized catalytic activities which could have evolved later.

A further membrane function, which must have developed early in the evolution of the proto-organisms, is its role in separating nucleic acid molecules following their duplication. In order for the DNA molecule to be reproduced in bacteria, it appears to be necessary for it to be attached, at the point where replication begins, to the membrane of the cell (Jacob *et al.*, 1963). Then membrane growth, specifically the expansion of the mesosomes which are invaginations of the membranes to which the chromosomes are attached, moves the newly synthesized DNA helix away from the parental one. The bacterial membrane thus serves as a segregational apparatus, playing the part in prokaryotes of the cell spindle in eukaryotes. Present-day bacteria and presumably their predecessors do not possess a distinct nucleus bounded by a membrane as do eukaryotes, but their DNA is aggregated in the cytoplasm as "nucleoids".

It is not likely that the proto-organisms could exist in any other than very sheltered conditions nor could they make use of light energy until they evolved a mechanism to protect themselves from ultraviolet light damage. The main effect of UV light on DNA is to cause a dimerization of adjacent pyrimidine bases. Even with present levels of ultraviolet irradiation, about ten dimers per minute would be formed in an *Escherichia coli* chromosome (Singer and Ames, 1970). None of the known DNA polymerases is capable of synthesizing DNA past such a dimer which therefore constitutes a potentially lethal irradiation event. Contemporary bacteria have several mechanisms for repairing pyrimidine dimer damage, amongst which is a photoreactivation utilizing energy from the longer ultraviolet or shorter visible range (Kimball, 1978). The pyrimidine dimers are split into individual pyrimidines under the action of an enzyme using absorbed light energy. This repair system is the most generally distributed one and is thought to have preceded other mechanisms involving the excision of damaged bases and nucleic acid resynthesis and rejoining (Harm *et al.*, 1971). The latter repair systems make frequent errors during repair which largely account for the mutations induced in bacteria by UV light; photoreactivation, on the other hand, is an error-free process. It is by this stage, if not much earlier, that proto-organisms must have substituted DNA for RNA as the genetic material. Apart from the irrepairable hydrates of uracil produced in RNA by ultraviolet light, RNA is generally a more

labile molecule than DNA and its spontaneous mutation rate is probably much higher (Batschelet *et al.*, 1976).

THE PROKARYOTES

The trend towards an increase in the amount of genetic information carried by each proto-organism must have been greatly accelerated in establishing the conventional prokaryotes. Such trends may be observed even in existing organisms. Morowitz (1966) has calculated that about 500 genes would be the minimum necessary to allow an organism to be free-living except for the use of a preformed source of carbon. However, even *Mycoplasma meleagridis*, the simplest prokaryote with respect to amount of DNA, has enough DNA for twice that number of genes and certain other bacteria, such as *Serrata marcescens*, have nine times as much as *Mycoplasma*.

In the building-up in the Precambrian of the minimum genetic information for a free-living organism (500 to 1,000 genes), gene duplication, together perhaps with some contribution by the capture of genes of other organisms, must have been a primary evolutionary process. As suggested earlier, the genetic specification of the basic life processes of organisms may have evolved following the duplication of one or a few primordial genes. Then rare mutations could accumulate to provide the half-dozen or so enzyme types with different mechanisms of catalysis. Each of these enzymes is expected to have catalyzed bond re-arrangements in a large number of substrates, but with rather low efficiency.

A later role of gene duplication would be the multiplication of the small set of primitive genes to about 100 times their number to allow the evolution of the ranges of specific enzymes that are found in the simpler bacteria. Such evolution could occur by successive duplications followed by mutational change in substrate binding, as has been shown to have occurred in the evolution of certain dehydrogenases in *Bacillus subtilis* (Yoshida, 1965).

A further role of partial or complete gene duplication may have been one where larger enzymic proteins were evolved to provide greater specificity, or catalytic efficiency, or sites for regulatory molecules to attach, or regions for localizing the protein in the cell. A clear example of an increase in protein size by duplication occurs in the ferredoxin molecule, which was one of the earliest electron-transporting proteins, in anaerobic bacteria. In *Clostridium*, an original gene coding for a primitive ferredoxin of about 26 amino-acids appears to have been linearly duplicated to provide a protein of twice the size (Eck and Dayhoff, 1966).

In obtaining genetic material for the evolution of new functions, an alternative to the duplication of individual genes is the duplication of all or a major part of the genome. The distribution of genes on the chromosomes of some contemporary bacteria such as *Streptomyces coelicolor* (Hopwood, 1967) and *Escherichia coli* (Zipkas and Riley, 1975) supports the proposal that the genome has grown by complete duplication rather than by the gradual accretion of duplicated genes.

Because of the familiar morphology of most of the prokaryotic fossils of the Precambrian, it was thought that the prokaryotes had changed very little since they first originated and that the present-day species had extremely low rates of evolution. However, recent studies of prokaryotic relationships by means of DNA hybridization show that this view is incorrect. The rate of nucleotide substitution in *Xanthomonas*, for example, was found to be similar to that in rats and mice (Park and DeLey, 1967). Indeed, in terms of their nucleotide sequence relationships, some bacterial species are far more different from each other than are mammalian genera.

In the predecessors of the prokaryotes, including the pre- and proto-organisms, there would be high levels of genetic variation due to spontaneous mutation, as required for evolution to occur. In fact, all these primitive entities could survive only if they were sheltered from ultraviolet light or if they developed mechanisms to repair the damage it causes to nucleic acids. Moreover, the presence of oxygen increases the frequency of ultraviolet light-induced nucleic acid breaks (Dubinin, 1964). The rise of oxygen levels in the Precambrian would have forced the evolution of efficient protective mechanisms. The pre-organisms must have been shielded by rock, water depth or absorption to clays, the proto-organisms possibly by the evolution of the process of photo-reactivation and the immediate precursors of the prokaryotes by the evolution of systems of DNA excision and repair. The

last mechanism is one that could also be further developed for purposes of gene exchange. The advantage of a gene exchange process between individuals, each containing a different useful mutation, is that the chance of incorporating both mutations in the one individual is at least twice that of obtaining them by successive mutations. Extended to the whole genome, such recombination could provide new combinations of genes at rates hundred of times higher than sequential mutation.

Mechanisms for the recombination of genetic material are widespread in contemporary bacteria and blue-green algae and in viruses, both of DNA and RNA. In the bacteria, this recombination is brought about by gene exchange due to transduction (virus-carried prokaryotic genes), transformation (absorption of free DNA) or sexuality. In the early evolutionary forerunners of these organisms, transduction is unlikely because specialized viruses had probably not then evolved. Similarly, it is thought (e.g. by Hayes, 1968) that sexual recombination in prokaryotes may be a secondary consequence of the mechanism evolved by viruses and plasmids (self-replicating circles of DNA carrying genes for specific adaptation) for their transfer between cells. Again, these extra-nucleoid entities represent highly evolved molecules for specific parasitism or adaptation. There remains transformation as a likely first mechanism of gene exchange. While most contemporary bacteria will not take up DNA under normal circumstances, it has been shown that such bacteria as *Hemophilus, Bacillus* and *Neisseria* will do so under altered nutritional conditions. The next step, that of integrating the foreign DNA into the host genome, could be brought about by the DNA repair mechanisms earlier evolved against UV light damage. It has been shown by Rupp *et al.* (1971) that recombination can restore the integrity of DNA that has been damaged by UV light and by Radman *et al.* (1970) that UV-induced breaks in DNA stimulate recombination. The tentative conclusion, therefore, is that a gene exchange mechanism, that involving transformation, first appeared in living organisms as a by-product of their earlier-evolved repair mechanisms.

With the eventual evolution of a membrane-bound self-contained mass, capable of self-replication and the specification of amino-acid sequences, containing about 5,000 genes, a capacity for self-repair and the ability to exchange genetic material, the modern prokaryote in the genetic sense, is reached. However, this discussion takes no account of the kinetics and thermodynamics of organism origin and initial evolution which still provide formidable problems.

SUMMARY

From the genetic point of view, the ancestors of present-day organisms may be classified into three groups, each possessing certain primitive levels of information content, expression and exchange.

Pre-organisms. Small (less than 1,000,000 molecular weight) polymers probably of ribonucleotides. Circular in structure and with only the property of self-replication and perhaps of binding accessory organic or inorganic compounds which aid in replication. They had no self-generated source of energy, but relied for their primary reaction of polymerization on the presence of activated subunits such as phosphoramidates and catalysts such as cyanamide, both formed spontaneously by UV light.

Proto-organisms. The pre-organisms as above but with the nucleic acid enclosed in a semi-permeable boundary membrane composed of a layer of lipid, protein or lipoprotein. The consequent concentration of high molecular weight compounds enabled the evolution of a template influence or specification on amino-acid polymerization which in turn provided catalysts for replication, energy transformation and protection against radiation damage.

Predecessors of the prokaryotes. Organisms undergoing a rapid accumulation of genetic information by means of gene duplication and mutational divergence in catalytic specificity. In addition, gene exchange systems based on a combination of transformation and UV repair enzymes may have evolved as well as mechanisms for the control of adaptive gene expression. Although the prokaryotes have kept what is apparently the same shape and basic metabolism for several thousand million years, their DNA appears to have undergone change at rates similar to those of such rapidly evolving eukaryotes as the mammals.

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THE UNIVERSE OF PORPHYRINS

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PORPHYRINS

The simplest member of the porphyrin class of compounds is porphine - a planar molecule comprising four pyrrole rings linked by methene bridges. Strongly stabilized by aromatic conjugation, porphine absorbs in the visible range with electronic transitions giving rise to an intense absorption band in the optical blue: the Soret band just below 400 nm. Secondary absorption features appear in the red end of the spectrum, in most cases between 500 and 600 nm. The molecular weight of porphine is 310 in the unsubstituted and uncomplexed form.

Substitution in porphyrins is common at all the peripheral positions, and occasionally on the bridging carbon atoms. The most common substitution replaces the peripheral hydrogen atoms with alkyl groups. This gives rise to homologies involving variable numbers of methylene groups.

So, also, cation substitution of the two protons on opposite nitrogen atoms brings about formation of metal complexes. Substitution and complexation both result in changes in the Soret absorption. Shifts in the Soret absorption are frequently no more than 10 nm, and are usually toward longer wavelengths. Changes in the non-Soret bands are usually reductions in number - to two instead of four - with the two bands occurring in the same range as the four.

Mass spectrometry, along with absorption spectrometry, is of prime importance in characterizing porphyrins with small differences in molecular weight. Non-polar porphyrins are readily volatilized for mass spectral examination, while polar porphyrin molecules frequently require esterification with lower alcohols to be amenable to analysis by mass spectrometry.

Porphyrins are universal constituents of living organisms. In plants they provide for carbon fixation through photosynthesis. This process involves electron transfer and photolysis which may be regarded as the indirect transfer of oxygen. Both indirect and direct transfer of oxygen also take place through cytochromes and other tetrapyrroles in plants and animals. In all cases, porphyrins - the chlorophylls and haems - depend on supporting structures in the organisms, commonly directly on proteins in the case of cytochromes and haemoglobins, and on a variety of organic compounds, including proteins, in the chloroplasts of plants.

Porphyrins may be synthesized abiotically in a variety of ways. A simple condensation of pyrrole with formaldehyde was developed by Rothemund (1936) for the synthesis of porphine. An elegant 15-step method was developed for synthesizing chlorophyll *a* by Woodward *et al.* (1960). Biologically, porphyrins are synthesized via glycine, succinyl coenzyme A condensation and delta-aminolevulinic acid, yielding uro-porphyrin octacarboxylic acid (Sassa, 1972). From this highly-substituted porphyrin, secondary biosynthetic pathways result in the generation of specific porphyrins within the organism. Many questions of genetic selection arise in such a complex framework of biochemical architecture. The intent of this communication is to draw attention to the topic through the assembling of pertinent observations in the literature and the presentation of new data relevant to the genesis, function and diagenesis of porphyrins.

PREBIOTIC PORPHYRINS

Great advances have been made in understanding the principles involved in the origin of life on earth (Day, 1979). The fundamental processes involve substantial chemical evolution coupled with the emergence of functioning biochemical structures. The earth is only a speck in the cosmos, and it is perhaps best to begin the discussion in the larger

context.

In the Milky Way galaxy, hydrogen comprises the bulk of the mass, with carbon monoxide and water next in abundance. In this galaxy, cool dense clouds occupy up to 10% of the interstellar volume, and are believed to be the most likely site for chemical evolution (Gammon, 1978). The clouds are very large, accounting for the mass of 100,000 suns. The big bang background temperature in the clouds is 2.7°K, but the kinetic temperature is 10-30°K; temperatures in related diffuse clouds are about 100°K. In the protostellar cores of the dark clouds temperatures are thought to be about 300°K. Although hydrogen is by far the most abundant constituent of the dark clouds, at about a few hundred molecules (not atoms!) per cm^3, many other molecules exist there also. These are mostly organic, and about 50 compounds have been identified, largely by radio astronomy (Gammon, 1978). Nearly half of the organic compounds identified are N-containing; O-containing compounds are slightly more abundant. A surprising observation is that large molecules, e.g. nine-atom molecules, are nearly as abundant as much smaller molecules, e.g. five-atom molecules. This indicates that the reactions giving rise to the large molecules are more than gas-phase reactions. They almost certainly include either ion-molecule collisions or surface reactions on dust particles. Accordingly, the likelihood that molecules of even larger mass are being formed in interstellar space is very high.

Some of the larger molecules would be further compounds of C, H, and N. An obvious example is pyrrole - a stable nine-atom molecule that is within the size range of hitherto detected molecules. It is stabilized by significant resonance energy because of its aromatic character. The likelihood that it also exists in alkylated derivatives is very high. Perhaps one of the main reasons why pyrrole has not yet been detected is that its plane of symmetry makes radio-astronomy detection of its rotational absorption spectrum much more difficult than in the case of unsymmetrical molecules.

It is reasonable to suggest that porphyrins exist as such in interstellar space because (a) the Rothemund synthesis provides for simple condensation of formaldehyde and pyrrole, and (b) the porphyrin molecular skeleton is remarkably stable, with a resonance energy of about 1.7 MJ mol^{-1} (Longo *et al.*, 1970). The role of interstellar dust (Donn, 1968) in interstellar reactions is unclear but, in the laboratory, pyrrole and formaldehyde condense on the surface of graphite particles to form porphyrins in a matter of hours at room temperature (Hodgson, 1972).

If porphyrins are formed in interstellar dust clouds they should be detectable by absorption spectrometry. Optical measurements of light absorption in interstellar space have for many years revealed diffuse interstellar lines in the blue and red sectors of the optical spectrum. These lines were first discovered by Merrill (1938) and were discussed by York (1971). Johnson (1967) proposed that they be attributed to interstellar porphyrins, a far-out proposal that was made well before the current decade of discovery of the interstellar organics in this and other galaxies.

Evidence for extraterrestrial porphyrins in the interstellar regions is limited; the existence of diffuse interstellar absorption lines does not prove the existence of porphyrins. However, indications that extraterrestrial porphyrins exist may be found in other types of study. Meteorites are samples of extraterrestrial rock, and while they are not representative of interstellar regions they are clearly extraterrestrial in origin, and therefore may have something to contribute to the topic of the prebiotic universe of porphyrins. Though most meteorites contain virtually no C, a small number of stoney chondrites, the carbonaceous chondrites, contain up to 5% carbon. Four such meteorites - Orgueil, Murray, Cold Bokkeveld and Mokoia - contained porphyrins (Hodgson and Baker, 1969) as metal complexes, as might reasonably be expected in the presence of an inorganic matrix of silicate minerals. The absorption spectra of the free-base porphyrins after demetallation were similar, but not identical, to those of terrestrial pigments.

Pigments exhibiting porphyrin-like properties were detected in lunar surface samples from Apollo 11 and Apollo 12 (Hodgson *et al.*, 1972a). Those from Apollo 11 appeared to come from the rocket exhaust of the descent engine; those from Apollo 12, however, were indigenous lunar material (Hodgson *et al.*, 1972b). The Apollo 12 pigments were metal complexes and were present at about 0.05 ng g^{-1} lunar soil. Interpretation of the data led observers to believe that the Apollo 12 pigments may have been precursors of lunar porphyrins (requiring oxidation, as in the Rothemund synthesis). Such a finding is

consistent with the fact that condensation products on the surface of interstellar dust would be swept up by the moon in its orbiting in space without an atmosphere.

The evidence to this point indicates that porphyrins are likely to form in interstellar space, as deduced from interstellar synthesis of related compounds, and that they exist on extraterrestrial objects entering the earth's atmosphere and crust.

A major question remains. If it is possible for such pigments to be generated extraterrestrially, could they not have been generated on the surface of the earth itself before the appearance of life? The earth is presumed to have had a reducing atmosphere in its early stages of existence - largely methane, ammonia and water vapour. Miller and Urey (1952) eloquently demonstrated the generation of amino acids from such a milieu when it is exposed to ionizing radiation. Porphyrins were shown to form under the same circumstances (Hodgson and Ponnamperuma, 1968; Hodgson, 1972).

Prebiotic porphyrins on earth would almost certainly have had a variety of structures. While pyrrole and formaldehyde might be expected to condense directly to porphine with no side products, there is little reason to believe that pyrrole would be the sole condensing agent. The remarkable variety of C/H/N compounds in interstellar space could have led to a variety of substituted pyrroles, which in turn would lead to an even wider variety of substituted porphyrins. Further, additional syntheses would be possible since pyrrole is not a required intermediate. In this respect, other experiments have shown the condensation of unsymmetrical dimethyl hydrazine directly with formaldehyde to produce porphyrins (Hodgson, 1972). Direct condensation of pyrrole, however, gives many by-products besides porphine. A graphic demonstration of the indicated variability in prebiotic or abiotic porphyrins was provided in experiments involving pyrrole, formaldehyde and graphite - a simple vapour-phase transport to the graphite surface. The porphyrins appeared on the graphite in that experiment, and they were found to have been formed in homologous series with molecular masses ranging from 494 to 594 amu in increments of 14 amu (Hodgson, 1972).

BIOTIC PORPHYRINS

Scholars studying the origin of life on earth generally subscribe to theories that life arose from the generation of organic molecules, as indicated above, followed by the condensation of the small molecules to macromolecules and then to aggregates which are known as protobionts. Oparin (1964) developed a comprehensive theory involving coacervates to account for the evolution of the cell. As noted by Clark (1973), coacervates may represent a stage which actually occurred during the origin of living cells - a stage during which innumerable combinations of macromolecules were constantly being formed and reformed until several catalysts eventually came together in one droplet, allowing a complete biochemical pathway such as glycolysis to occur. It is clear that porphyrins would be available for incorporation into such protocells.

Part of the evolution of the cell was thus a simple coexistence of several biotic catalysts inside some kind of "container", with the catalysts operating concomitantly or sequentially to accomplish a transfer of energy. Another part of the same evolution saw the catalysts being enhanced in their functions by assuming certain preferred structural orientations. In some cases this was apparently achieved through charge-transfer complexes (e.g., involving pi-bonding of planar molecules). In other cases, the catalytic activity of the component was apparently enhanced by being supported through covalent bonding within structured macromolecules. In the latter cases, proteins probably provided support, with porphyrins being covalently bonded to the proteins. Such bonds are well known in vivo, and have been demonstrated both to form and to persist in vitro (Hodgson *et al.*, 1970) and to be essential in design (Calvin, 1979).

Evolving life forms functioned heterotrophically using organic carbon that was available as a result of prebiotic fixation. Thus the evolving organisms derived their energy from the organic carbon of Oparin's prebiotic "soup". Oparin s theory also states that the "organisms" were themselves direct and random agglomerations of matter in the same "soup". As long as the prebiotic supply lasted, replication of life catalysts was not necessary, but in due course replication processes developed to generate a wide variety of biochemicals including porphyrins.

Pigments required for electron transfer in evolving organisms, corresponding to the cytochromes of modern organisms, probably involved porphyrins complexed with readily available

iron and prebiotic proteins. The evolving organisms were then faced with the problem of inventing a synthesis for the pigments. Herein lay a problem of synthesizing, not just a single pigment molecule, but a number of molecules very similar to those which formed abiotically in the prebiotic times. Thus, the prototype pigment was not a single compound. The remarkable solution apparently was to generate, not a single pigment, but a mixture of homologous porphyrins similar to that of the prebiotic porphyrins. This solution was so successful that modern lower organisms still generate cytochrome porphyrins in homologous series, as indicated below.

Recent work in our laboratories demonstrates for the first time that tetrapyrrole (cytochrome) pigments from the non-photosynthetic bacterium *Desulfovibrio desulfuricans* are homologous. In this way they resemble the homologous chlorophylls of *Chlorobium* reported by Holt *et al.* (1966).

The mass spectrum of one porphyrin fraction from *D. desulfuricans* (eluate 6, see Appendix 1) is shown in Fig. 1. The dominant peaks were 572 and 574 m/e. These were iron complexes. Subtracting 54 for the iron (-56 for iron, +2 for two protons) indicates two homologous series. One was 518, 504, 490, 476, 462 and 448; the other was offset by two, 520, 506, 492, 478, 464, and 450. The spectrum of eluate 10 (see Appendix 1) was similar.

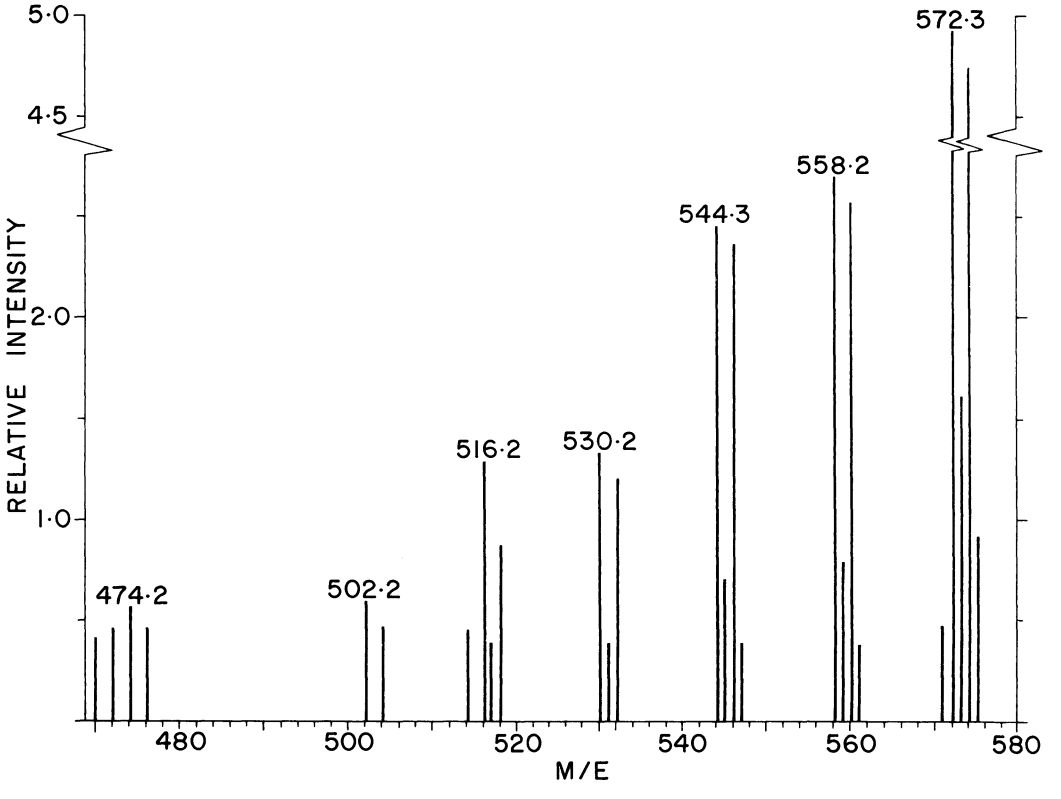

Figure 1: Computerized mass spectrum of porphyrins recovered from the cytochromes of *Desulfovibrio desulfuricans*. Note the two series corresponding to the aetio (478 ± n 14) and a vinyl-aetio series (476 ± n 14), when calculated to an iron-free basis, e.g. 530-56+2 = 476 m/e.

By way of reference, aetioporphyrins in sedimentary shales and petroleum occur in the 478 series, i.e. 478 ± n 14 m/e, in direct agreement with one series of porphyrins from *desulfuricans* cytochromes.

The other series from the same sulfate-reducing bacterium corresponds to the DPEP (deoxyphylloerythroaetioporphyrin) series of shales and petroleum. The reason for the difference of 2 mass units between the aetio and DPEP series in petroleum is usually attributed to the mass difference between a 4-ring porphyrin as in haems and a 5-ring porphyrin in chlorophylls. That explanation, however, is not valid in the case of chlorophyll-free *desulfuricans*. It seems reasonable, therefore, to attribute the difference to a residual vinyl group on the porphyrin rather than to a residual phyllo (fifth) ring.

As an aside, such an interpretation casts serious doubts on the validity of attributing all of the 476 ± n 14 pigments in shales and petroleum to DPEP rather than to vinyl-substituted aetioporphyrins.

To extend the foregoing observations regarding the existence of homologous porphyrins, tetrapyrrole pigments were prepared from the cytochromes of the alga, *Chlorella pyrenoidosa* (see Appendix 2). The cytochrome porphyrins from *Chlorella* occurred in a homologous series. The series was the vinyl-aetio array, 476 ± n 14. It was skewed toward the lower members, in the range 476-326 m/e.

The evolution of higher plants and animals appears not to have seen any major refinement in the biosynthesis of cytochrome porphyrins. Thus porphyrins prepared from ox-heart and horse-heart cytochromes (see Appendix 3 A-C) all showed homologies with the *Chlorella* cytochromes. The mass spectra of ox-heart porphyrins showed several homologies (the dominant series being 474 ± n 14), corresponding to doubly decarboxylated protoporphyrin IX, with two vinyl groups. The porphyrins from horse-heart cytochrome *c* were also homologous. The series corresponded to the 476 ± n 14 series, again indicating vinyl substitution on the porphyrin ring.

To test whether the cytochrome homologies of the horse-heart cytochrome porphyrins, and by inference those of ox-heart, might have been artifacts of the analytical procedures, haemin (Aldrich Chemical) was also treated with methanesulfonic acid as described in Appendix 3C. It showed a single molecular ion as shown in Fig. 2 with no indication of a homologous series, from which it was concluded that the cytochrome homologies were real.

If the above interpretation of the abiotic and cytochrome data is acceptable, prebiotic random synthesis of porphyrins is reflected in present-day cytochromes in organisms ranging from bacteria to vertebrates. It is significant, also, that haems with different functions, i.e. oxygen transport (haemoglobins) compared with electron transport (cytochromes) have sharply different molecular detail of alkylation.

With the rise of autotrophic processes, energy capture from light was of dominant importance. Thus, chlorophyll evolved from the prebiotic porphyrins. The evolution must have been very convoluted, however, to have settled on a porphyrin molecule with a fifth ring. Because there is no evidence that the fifth ring was available in the prebiotic "soup bowl" it is not at all clear how the ring evolved. The function of the ring is evidently to distort the porphyrin molecule for enhanced absorption of red light.

The surprising observation, again, is that chlorophylls in the evolving autotrophs adopted the homologous design of the prebiotic porphyrins. This was demonstrated for *Chlorobium* by Holt *et al.* (1966), who used an elegant analytical approach to show that six homologs existed with varying degrees of alkyl substitution on the porphyrin ring at position C_4 (isobutyl, n-propyl, ethyl), C_5 (ethyl, methyl) and C_8 (ethyl, methyl).

In the present study porphyrins derived from *Chlorella* chlorophylls (see Appendix 4) were examined. The mass spectrum indicated a similar homologous pattern to that described above for the tetrapyrroles isolated from the cytochromes. There was evidence for a ten-membered series 336 to 476. In addition there appeared to be a second, six-membered, homologous series centering about 478 m/e, possibly due to the aetio-type series.

It is important to recognize that the indicated homologies are not accountable in terms of the existence of the several forms of chlorophyll (i.e. *a*, *b*, *c*, and *d*).

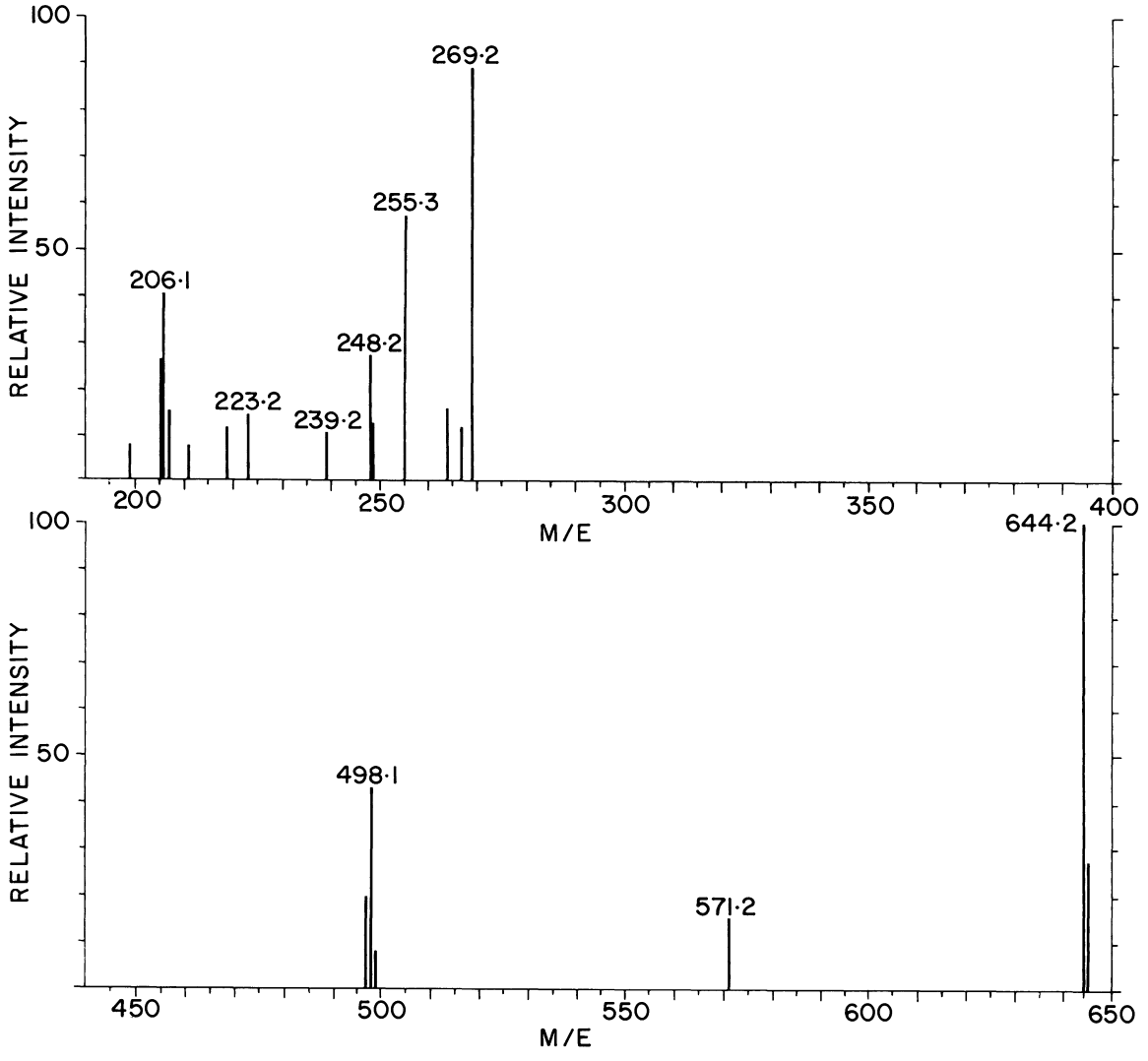

Figure 2: Computerized mass spectrum of porphyrin prepared from commercial haemin. Note the absence of homologous series. Mass 644.2 corresponds to 474 m/e when calculated to iron-free, decarboxylated basis.

Chlorophylls of higher plants are different, however. In the present study, porphyrins (more precisely, chlorins) were recovered from a mixture of Kentucky and Meriam blue grasses using the direct methanesulfonic acid method. The pigment exhibited a single molecular ion with a normal cracking pattern, thus revealing itself to be a single porphyrin molecule like the haemin pigment. Thus these higher plants showed no evidence of homologous series in their photosynthetic pigments.

The conclusion is that, in the case of chlorophylls, biochemical evolution from the lower plants to the higher plants has seen sharp refinements in the biosynthesis of the porphyrin molecule.

POST-BIOTIC PORPHYRINS

The basic structures of porphyrins persist strongly after the death of the organisms, in keeping with the resonance stability of the conjugated macrocycle of the porphyrins. Haems of cytochromes and haemoglobins readily enter soils and sediments. Bonded to substantial protein moeities, the pigments are much more soluble in water than are decarboxylated non-polar porphyrins. Thus they tend to behave in the environment as proteins or peptides rather than as pigments. Accordingly, they are readily leached from the soil litter. Being soluble in water, they are subject to substantial oxidation in surface waters. Under reducing conditions in the soils and sediments they tend to decarboxylate (Casagrande and Hodgson, 1976). The iron complexed in the haems is strongly bonded. It changes oxidation state in response to its environment. Haems of cytochromes tend to degrade to aetioporphyrins under diagenesis. Aetioporphyrins exist in near-surface sediments in the Black Sea, where they occur in a homologous series of four members with 492 m/e as the predominant porphyrin.

The dominant pigments in soils and sediments are direct decomposition products of chlorophylls. Pigments in recent marine sediments on the North Atlantic continental shelf indicate that the first diagenetic changes include loss of magnesium, resulting in pheophytin. Hydrolysis of the phytol alcohol then produces pheophorbide. The chlorins are remetalated either before or after dehydrogenation. In the North Atlantic the respective concentrations occur in the same order, with pheophytin most abundant (Baker and Hodgson, 1968). Traces of decarboxylated chlorins and porphyrins are also detectable in soils and sediments. The Black Sea studies showed two major series of homologous porphyrins: one, a chlorin series of five members ranging from 684 to 740 m/e, and the other, a porphyrin series of nine or more centred on 504 m/e. The dominance of chlorins relative to cytochrome pigments is undoubtedly related to the dominance of chlorophylls in the source material, but the relative insolubility of chlorophyll pigments in water may well be a contributing factor favouring the accumulation of the chlorophyll pigments in soils and sediments, as indicated by the concentration of 0.04 ng of chlorins l^{-1} of filtered river water (Peake *et al.*, 1972).

The evidence that cytochromes and chlorophylls from lower plants occur in homologies and that the chlorophylls of higher plants occur only as single compounds indicates that the pigments of recent marine sediments are derived only to a minor extent from the chlorophylls of higher plants.

Sedimentary processes transform soils and sediments into sedimentary rocks, and it is commonly recognized that the pigments of those rocks are derived directly from the pigments of the sediments. Porphyrins are common in sedimentary rocks, particularly in rocks that have never been extensively oxidized. In most instances the porphyrins are members of both the aetio series, and the series thought to be derived from chlorophylls (DPEP), with the latter predominating. The complexing metals are V (as vanadyl), Ni, Fe and probably other divalent metals including Cu and Zn (Hodgson *et al.*, 1969b). The porphyrins occur in homologous series (Dean and Whitehead, 1963).

One of the components of sedimentary rocks occurring under unusual geological/geochemical conditions is petroleum. All crude oils contain porphyrins, and almost without exception the porphyrins are homologous (Baker, 1966). The dominant series is DPEP; the most common complexed metal is vanadium. In oil from the Athabasca oil sands both series are present - the aetio series with 478 m/e and four homologs, and the DPEP series with 476 m/e and four homologs; both series are complexed with vanadium. Petroleum from Lloydminster has a ten-member aetio series (after demetalation) centering on 464 m/e (Casagrande and Hodgson, 1971).

While the foregoing data strongly indicate that homologous porphyrins in sedimentary rocks are simply preserved biotic homologies, a question remaining to be answered with respect to the origin of the homologies in sedimentary post-biotic porphyrins is: "Are the homologies a product of thermal diagenesis, as proposed by Baker *et al.* (1967), or are they due to something quite different from variable alkylation,, i.e. to residual protein fragments still bonded to the porphyrin molecule?"

That they may be products of thermal diagenesis has been indicated in laboratory experiments. A common porphyrin, mesoporphyrin IX dimethyl ester, was converted to a homologous series when exposed to elevated temperatures (250^{o}, 280 h) in a system containing

montmorillonite clay (Casagrande and Hodgson, 1971). The series comprised seven members. Not only was the series generated, but decarboxylation of the mesoporphyrin also took place. The experiments were extended to include other factors such as the effects of complexed metals on porphyrin production. Copper accelerated the reaction (Casagrande and Hodgson, 1974).

The diagenetic route, however, is unable to explain the appearance of homologous porphyrins in soils and recent sediments which have not been exposed to severe diagenesis.

It is possible that the diagenetic pathway is relevant in the prolonged exposures experienced by pigments buried at "geothermal" depth for very long periods of time. Under those conditions, one would expect the starting pigments to be homologous, and that each member of the homology would undergo transformation. The series would consequently be extended latterly, with the number of members increasing over those originally present in the biotic pigments. While there is some indication of such broadening in the older pigments, it does not appear to be very marked.

With respect to residual protein fragments as a possible explanation of homologous porphyrins, Hodgson *et al.* (1969a; 1970) showed that protein fragments are sometimes associated with porphyrin pigments. These, however, exist for porphyrin fractions from petroleum in which the porphyrins are distinctly larger (1000-2000 amu) than the normal petroleum porphyrins. Further, analytical oxidation of regular petroleum porphyrins to maleimides gave clear evidence of variable alkylation and no evidence of amino acid residues (Hodgson *et al.*, 1972).

While porphyrins are more stable than other heteroatom organic compounds, prolonged diagenetic exposure does destroy them, and their persistence in ancient sedimentary rocks of Precambrian age might be surprising. But porphyrins have been reported in Early Precambrian Swaziland System sediments more than 3 Gyr old (Kvenvolden and Hodgson, 1969). Of six formations sampled, two cherts and one shale showed measurable amounts of porphyrin pigments, though the abundances were low (about 0.1 pg g^{-1}). Special characteristics were similar to those of sedimentary porphyrins in general, but different in detail. Other investigators reported biotic amino acids (Kvenvolden *et al.*, 1968) and alkanes, including pristane and phytane (Oro and Nooner, 1967) in the same Onverwacht cherts.

The foregoing findings are consistent with the simple preservation of an established biochemistry at Onverwacht time. They are also consistent with suggestions that prebiotic systems were well developed more than 3 Gyr ago. It is interesting that the rise and fall of terrestrial porphyrins, along with other vital chemicals, should come full circle to the extent that prebiotic and postbiotic porphyrins, as they exist today, are probably analytically indistinguishable from each other.

In summary, porphyrins are almost certainly generated abiotically and extraterrestrially. With the origin of life on earth, the homologous randomness of the prebiotic synthesis persisted in the pigments adopted for oxygen transfer and photosynthesis. With the evolution of higher plants (and the vertebrates), some haem pigments and the chlorophylls were refined to unique single structures. Such was not the case for the cytochromes, and their homologous patterns persisted into the mammals. In the postbiotic periods diagenesis of the pigments takes place with little or no change in the fundamental structure of the pigments. Thus the homologies persist; diagenesis of postbiotic pigments tends to extend the homologies somewhat, but the effect appears to be minor.

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APPENDICES

1. Preparation of Porphyrins from *D. desulfuricans*

Sixty-five litres of cell culture of *D. desulfuricans* were centrifuged and freeze dried. To a 0.9 g portion of the dried cell mass was added about 5 ml of hydrobromic acid in glacial acetic acid (32%). This suspension was heated at about 90° for 2 hr. The hydrobromic and acetic acids were pumped off and the residue was partitioned between benzene and water. Testing of the benzene layer with dilute aqueous sodium hydroxide showed that the pigments in the benzene layer had been decarboxylated by the heat treatment. The benzene layer was washed with 6 M HCl which removed very little pigment, i.e. little or no free-base porphyrin, indicating that the tetrapyrrole pigments in the benzene layer were metal-complexed.

The benzene layer was then chromatographed on silica gel in a simple 1.5 x 20 cm column. Exhaustive elution with benzene removed substantial quantities of yellow pigments. Further elution with benzene to which 10% (v/v) methanol had been added produced two unresolved bands which were removed in a series of five eluates (eluants 6 to 10).

2. Preparation of Porphyrins from *C. pyrenoidosa*

The cell pellet from the harvesting of *C. pyrenoidosa* was washed thoroughly with methanol/acetone, recentrifuged and then suspended in Tris buffer (0.2 M, pH 7.5, 200 ml) and left at 0° for 12 h. This suspension was passed through a French press and then centrifuged in a SS34 Rotor of a Sorval refrigerated centrifuge (2500 g, 15 min) to remove the cell debris. The cell extract was brought to 35% with ammonium sulfate (0.35 g ml^{-1} extract), left at 2° (2 h), then centrifuged (2500 g, 30 min) to remove lipoprotein and subcellular particles. The yellow-brown supernatant solution was made to 25% with ammonium sulfate and left at 2° for 2 h. The suspension was centrifuged (2500 g, 30 min) and the brown suspension was dissolved in and dialysed against distilled water (48 h). This fraction was then subjected to chromatography on DEAE-cellulose.

The column (25 x 3.0 cm) was washed thoroughly with sodium hydroxide (1 M) then Tris buffer (0.02 M) until the pH equilibrated at 7.6. The crude protein fraction was applied to the column followed by a sodium chloride/Tris buffer gradient in 0.03 M 400-ml increments.

The red-brown fraction eluted down the column when the concentration of sodium chloride reached 0.12 M and was totally removed after the salt concentration had reached 0.3 M. The dissolved salts were then dialysed against running tap water (60 h).

The visible spectrum of this product was typical of cytochrome material showing a large Soret band at 410 nm and two smaller ones at 520 and 559 nm.

To degrade the crude cytochromes from *Chlorella* to simple porphyrins, the crude (slightly pink) solution of the cytochromes was made to 5% with formic acid; mercuric chloride (1.0 g) was added and the whole solution was stirred under nitrogen (37°, 20 h). The mixture was cooled, acetone (50 ml) was added, and the solution was filtered and extracted into ether. This ethereal solution was evaporated, the residue was dissolved in glacial acetic acid (5.0 ml), ferrous sulfate (10 mg) was added plus concentrated HCl (0.1 ml) and nitrogen was bubbled through the solution (20°, 2 h). The whole was poured onto ether and water and saturated with sodium acetate, and the ethereal extract was washed with sodium chloride and evaporated.

The residue was esterified with diazomethane and subjected to a dehydration procedure with *p*-toluene sulfonic acid in refluxing benzene. The final product was purified on alumina using chloroform/benzene (1:10) as an eluant. The eluate was evaporated and redissolved in ether and the visible spectrum was recorded. The mass spectrum was obtained.

3. Preparation of Porphyrins from Cytochrome *c*

A. Cytochrome *c* (ex. beef heart) (5.0 μmol, 62 mg) was dissolved in aqueous formic acid (5% v/v, 5.0 ml) and mercuric chloride (50 mg) was added. The mixture was stirred (37° 20 h) under a stream of nitrogen. Acetone (5.0 ml) was added and the turbid brown mixture was thoroughly extracted into ether. This ether was evaporated and a small portion of the

residue was dissolved in pyridine (0.5 ml); isooctane (2.0 ml) was added and the whole was left at -20° overnight. The precipitated pyridine haemochrome was isolated and the visible spectrum was recorded.

To prepare haematoporphyrin from the haematohaem, the ether fraction from above was dissolved in acetic acid (30 ml); ferrous sulfate (45 mg) and concentrated HCl (0.5 ml) were added and the whole was stirred (2 h, 20°) under a stream of N_2. The cherry red ether solution was then poured onto water saturated with sodium acetate. The organic layer was extracted 3 times with ether, washed successively with water and 5% NaCl, and the visible spectrum recorded.

To esterify the haematoporphyrin, the ether layer from above was treated with an excess of diazomethane, as an ethereal solution; the solution was then stirred (30 min, 20°) and concentrated to dryness.

To prepare protoporphyrin IX dimethyl ester, the residue was dissolved in benzene (20 ml), *p*-toluene sulfonic acid (6.0 mg) was added and the whole was refluxed (16 h). The mixture was cooled, concentrated and then treated with water saturated with sodium acetate. The aqueous layer was thoroughly extracted with ether and the extract, after solvent evaporation, was chromatographed on an alumina column eluting with chloroform/benzene (1:10). The eluate was evaporated and redissolved in ether and the visible spectrum was recorded.

B. Horse-heart cytochrome *c* (Aldrich Chemicals, 0.1 mg) was dissolved in a few ml of water and absorbed into about 10 g of silica gel. The moist silica gel was extracted in a small soxhlet extractor with an inventory of 150 ml of methanol plus 5 ml of 12 M HCl to hydrolyze the protein. After 16 h of extraction the extract was evaporated to dryness. The residue was partitioned between benzene and water. The pigment entered the organic layer and was washed twice with 1% NaOH and twice with water.

C. Porphyrins from horse-heart cytochromes were recovered using a methanesulfonic-acid method developed for the recovery of geochemical porphyrins in sedimentary rocks including shales and petroleum (Hodgson *et al.*, 1969b). Thus, cytochrome *c*, 0.1 mg, was added to 2 ml of methanesulfonic acid for several hours at room temperature. The reaction mixture was taken up in methanol and chromatographed directly on DEAE-cellulose. After elution with methanol, the column was eluted with methanol/water (90/10 v/v) which removed a major red-brown band which showed the absorption spectrum of a free-base porphyrin. The pigment was esterified by reaction with methyl iodide for mass spectral analysis.

4. Prepartion of Porphyrins from *Chlorella* Chlorophylls

A crude chlorophyll fraction from *Chlorella* was prepared by evaporating the supernatant and methanol/acetone washings obtained during harvesting of the microorganism, dissolving in acetone, and precipitating the chlorophylls by dilution with petroleum ether. The pigment was collected by filtration, redissolved in acetone, reprecipitated, and then transferred to ether (200 ml).

To degrade the chlorophylls to their methyl pheophorbide derivatives, the ethereal solution from above was stirred with concentrated hydrochloric acid (12 M, 100 ml, 30 min, 20°); the mixture was diluted with several volumes of water and the aqueous phase was thoroughly extracted by ether in order to remove phytol groups and unchanged pheophytin. The HCl was then diluted with 3 vol of water (overall 15% HCl), the crude methyl pheophorbide derivative was extracted into ether and the ether layer was washed free of acid with water.

The phylloerthyrin derivative was prepared as follows: the pheophorbide (100 mg) was heated in hydrobromic acid/acetic acid (32%, 20 ml, 140°, 8 h). The mixture was then cooled and poured onto water made 10% with hydrochloric acid. The aqueous layer was thoroughly extracted into ether and washed free of acid with water. This product was subjected to esterification with ethereal diazomethane and the visible and mass spectra were recorded.

ANTIQUITY OF PHOTOSYNTHESIS: POSSIBLE CONSTRAINTS FROM ARCHAEAN CARBON ISOTOPE RECORD

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INTRODUCTION

Carbon is stored in the earth's crust principally as two carbon species: reduced or "organic" carbon (C_{org}) and oxidized or "carbonate" carbon (C_{carb}). With most of the element concentrated in the sedimentary shell, the reduced and oxidized carbon constituents of terrestrial sediments make up the bulk of total carbon residing in the outer skin of the planet.

During the last decade, an impressive collection of sedimentary carbon isotope data has been assembled by several geochemical working groups (Oehler *et al.*, 1972; Perry and Tan, 1972; Eichmann and Schidlowski, 1975; Schidlowski *et al.*, 1975, 1976a, 1976b; and others). The principal result of these surveys is that the isotopic composition of sedimentary carbon (notably of carbonates) did not vary significantly over the earth's history, showing minor oscillations around a mean rather than any distinct unidirectional trend with time (Fig. 1). Such a unidirectional trend towards heavier carbonates might for instance, be expected to have accompanied the buildup of a sedimentary reservoir of organic carbon during the early history of the earth since preferential concentration of light carbon in the organic depository should have progressively increased the $\delta^{13}C$ values of contemporaneous carbonates (Broecker, 1970).

CARBON ISOTOPE GEOCHEMISTRY OF THE ISUA META-SEDIMENTARY SERIES

Recently, it has been possible to extend the carbon isotope record to almost 3.8 Gyr ago by investigating the carbon constituents of the Isua supracrustal belt of Western Greenland (Schidlowski *et al.*, 1979). As a result of this isotope investigation, a $\delta^{13}C$ mean of -2.5 ± 1.7 per mil vs PDB (Peedee Belemite) was obtained for 124 carbonate samples from over the whole eastern division of the Isua belt. With the values of average marine carbonates lying close to zero permil, and the probable greenstone-belt association of the Isua meta-sediments suggesting a marine origin of these latter, the above mean for the Isua carbonates differs by some 2 per mil from the value normally to be expected. The observed isotope shift towards more negative values is considered to be caused by the low to medium-grade metamorphism (mostly of the amphibolite facies) to which the Isua supracrustals have been subjected. As is testified by abundant calc-silicate minerals (notably tremolite) in these rocks, the Isua carbonates have undergone extensive metamorphic decarbonation reactions which, in other areas, have been shown to lower $\delta^{13}C$ values of the residual carbonate in about the same amounts as those observed in the Isua meta-sediments (cf. Shieh and Taylor, 1969; Sheppard and Schwarcz, 1970; Hoefs and Frey, 1976). This is in agreement with calculated fractionation factors for carbon isotope exchange in the system CO_2-$CaCO_3$ at metamorphic temperatures between 500-600°C (Bottinga, 1969).

Likewise, it is considered that the $\delta^{13}C$ mean of -15.3 ± 6.2 per mil (PDB) yielded by 13 samples of reduced (in part graphitic) carbon recovered from the Isua suite is also due to secondary processes. It is well known that metamorphism tends to increase the $\delta^{13}C$ values of sedimentary organic matter from original values of -25 ± 5 per mil to less negative ones, sometimes approaching the -10 per mil range (McKirdy and Powell, 1974; Eichmann and Schidlowski, 1975; Hoefs and Frey, 1976). Four samples with $\delta^{13}C_{org}$ values between -21.6 and -24.9 per mil contained in the above suite either represent basically unaltered relics of the original organics or isotopically light differentiation products generated during metamorphism. If on further investigation the "light" carbon values prove to be coupled with low concentrations of organic carbon in the rock samples (C_{org} <100ppm), the possibility of recent contamination would have to be considered (cf. Oehler and Smith, 1977).

If the proposed interpretation of the carbon isotope geochemistry of the Isua sediments in terms of a later metamorphic overprint is correct, then both the carbonate and organic

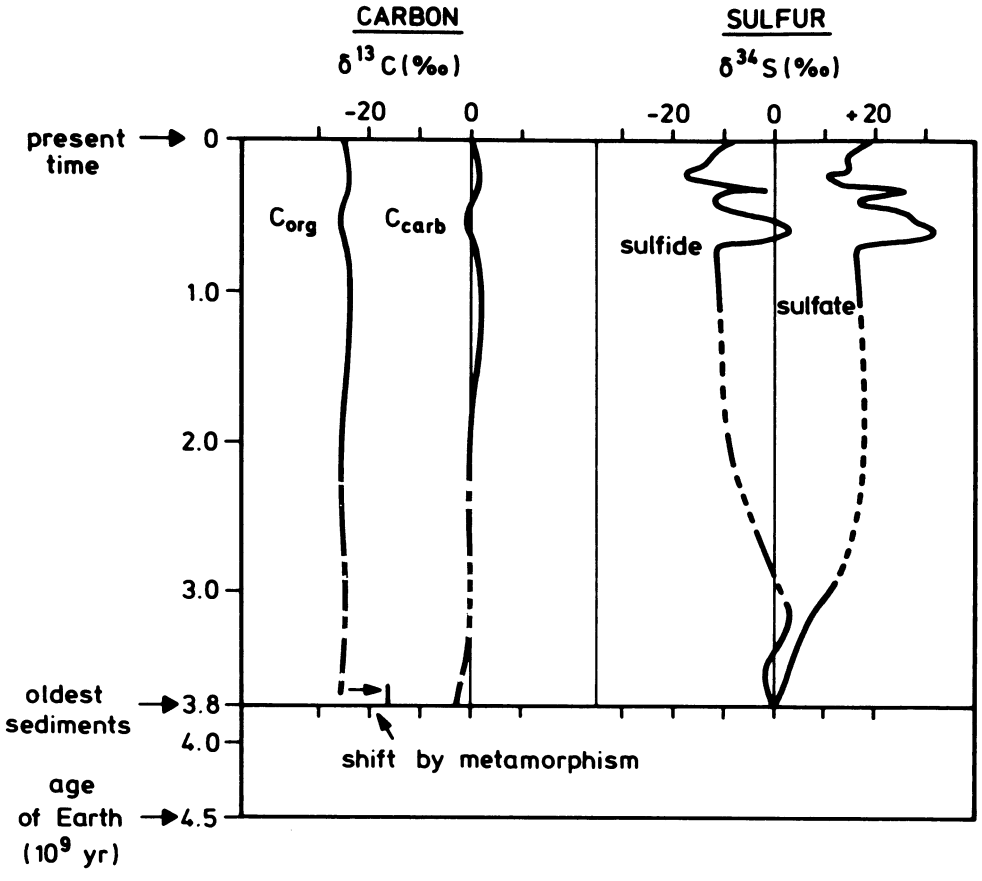

Figure 1: Isotope fractionation between organic carbon and carbonate carbon (left) and sulfide sulfur and sulfate sulfur (right) as a function of time. $^{13}C/^{12}C$ and $^{34}S/^{32}S$ ratios are expressed as δ-values, indicating differences in ^{13}C and ^{34}S contents (per mil) relative to conventional standards (*Peedee Belemite* for carbon and *Canyon Diablo Troilite* for sulfur). Sedimentary carbon and sulfur are stored in the above twin reservoirs, with one partial depository being always of biological origin. Since biological processes (photosynthetic carbon fixation and bacterial sulfate reduction) tend to concentrate the light isotopes, the non-biological reservoirs are enriched by 20-30 per mil in heavy ^{13}C and ^{34}S, respectively (see positive δ-values for carbonate and sulfate in above graphs).

Carbon of photosynthetic origin (C_{org}) commonly displays $\delta^{13}C$ values of -25 ± 5 per mil, i.e., it is depleted by some 25 per mil in ^{13}C as compared to carbonate carbon (C_{carb}). As is apparent from the graph, this characteristic difference can be traced back to the very start of the sedimentary record (shifts observed in the 3.8 Gyr old Isua rocks can be ascribed to metamorphism).

In contrast to the carbon isotope curves, dichotomy of the two sulfur functions develops as from about 3 Gyr B.P. only, the underlying process (dissimilatory sulfate reduction) thus being younger than photosynthesis. The conspicuous oscillations of the sulfur curves during the Phanerozoic reflect periodic shifts in the relative proportions of sulfide and sulfate in the sedimentary sulfur reservoir which cannot be traced back into the Precambrian because of the scarcity of the record (dashed parts of graph).

carbon originally possessed $\delta^{13}C$ values close to those of their geologically younger counterparts, and the long-term continuity of the sedimentary carbon isotope record dates back to 3.8 Gyr ago.

IMPLICATIONS OF ARCHAEAN CARBON ISOTOPE RECORD FOR THE ANTIQUITY OF PHOTOAUTOTROPHY

The basic constancy of the average sedimentary $\delta^{13}C_{carb}$ and $\delta^{13}C_{org}$ values over the last 3.8 Gyr of the earth's history as implied by the above interpretation of the Isua data will have far-reaching geochemical consequences. Since some principal implications for the evolution of the terrestrial carbon cycle have already been addressed elsewhere (Schidlowski *et al.*, 1979), it is proposed that the present discussion be confined to questions bearing specifically on early organic and environmental evolution. The simplified graph of the sedimentary carbon isotope record given in Fig. 1 is to illustrate the following discussion.

It should be noted at the outset that the isotopic composition of carbon, and notably the ^{12}C-enrichment in reduced carbon compounds, does not, per se, constitute evidence of biogenicity. It is known, for instance, that the trace amounts (~200 ppm) of reduced carbon contained in basalts and other common igneous rocks lie well within the range of biological carbon. This coincidence is, however, a fortuitous one since the isotopic composition of the carbon of igneous rocks is the result of progressive outgassing of ^{13}C-enriched carbon dioxide from the parent magma, thus shifting the original magmatic $\delta^{13}C$ mean of -5 ± 2 per mil (PDB) to about -26 ± 3 per mil in the residual carbon fraction (Pineau *et al.*, 1976; Javoy *et al.*, 1978). Our following considerations refer, on the other hand, to carbon in *sedimentary environments*. Such environments are constrained by a well-defined set of physico-chemical and geological parameters characterized, inter alia, by

1. a low-temperature range consistent with the presence of liquid water,
2. a facies-dependency in the distribution patterns of both principal carbon species, and
3. a common coexistence of reduced (organic) and carbonate carbon within the same sequence.

The left part of Fig. 1 is a graphic representation of the almost constant isotope fractionation of some 25 per mil between organic carbon (C_{org}) and carbonate carbon (C_{carb}) over the presently known sedimentary record. As pointed out before, the conspicuous shift, notably of the $\delta^{13}C_{org}$ values, at the very base of the graph may be accounted for by the amphibolite-grade metamorphism to which the Isua supracrustals have been subjected. Naturally, coverage of the curve by actually measured data is quite different, the record for the time span 3.0-3.8 G yr B.P. being based as yet on sediments from three occurrences only (Swaziland System, Rhodesian schist belts, Isua).

For today, there is direct evidence that the fractionation observed between the reduced and oxidized carbon species is inherent in the process of photosynthetic carbon fixation, being the result of a kinetic fractionation effect which favours preferential metabolization of the light carbon isotope (e.g., Park and Epstein, 1960; Smith and Epstein, 1971; Wong *et al.*, 1975; Wong and Sackett, 1978). Because of this evidence, it is commonly assumed that similar fractionations encountered in the geological past between sedimentary organics ($\delta^{13}C_{org} = -25 \pm 5$ per mil vs. PDB) and carbonate carbon ($\delta^{13}C_{carb} = +0.3 \pm 1.5$ per mil vs. PDB) are due to the same process. This interpretation is supported by the virtual identity of the isotopic composition of reduced sedimentary carbon of unquestionable biological origin (such as coal, lignite, etc.) with that of recent and subrecent plant matter. Since the finely dispersed kerogen constituents (which account for > 90% of the reduced carbon in the average sediment) fall into the same range, it is reasonable to assume that the bulk of the organics stored in the sedimentary shell must ultimately stem from photosynthesis.

However, as we approach the benchmark of about 3 Gyr B.P., a certain reservation as to the biological nature of the isotopic fractionation between the two sedimentary carbon species might appear legitimate. Such reservations were certainly reinforced by the isotope data recently accrued for the Isua rocks which would suggest a minimum age of 3.8 Gyr for the advent of photosynthesis if we were prepared to accept the isotope shifts observed for both C_{org} and C_{carb} as being caused by metamorphism.

With the conspicuous constancy of the sedimentary carbon isotope record providing as yet the principal indication for the presence of photoautotrophic life during the early Archaean, attempts to interpret the isotopic evidence should be made with due caution. Nevertheless, the statement might seem warranted that photoautotrophy probably dates back to 3.8 Gyr ago, *provided* the isotopic fractionation observed between reduced and oxidized carbon in Archaean sediments was not caused by an inorganic geochemical process which, by some peculiar incidence, brought about the same direction and magnitude of fractionation as later characterized photosynthesis. Admittedly, such inorganic process cannot be ruled out for certain, but is perhaps unlikely. It is known that fractionations between reduced and oxidized carbon in Fischer-Tropsch processes are considerably larger than those of photosynthesis, lying mostly in the range between 50 and 100 per mil (Lancet and Anders, 1970). Also, reactions of this type are high-temperature processes (~400°K and more), which are unlikely to occur in normal sedimentary environments. Furthermore, the facies-dependency in the distribution patterns of reduced carbon in some of the oldest sediments (inclusive of the Isua suite where members of the banded iron formation are often conspicuously enriched in carbon; Schidlowski, unpublished results) may be difficult to explain in the case of a non-biological origin of Archaean sedimentary organics.

Data hitherto obtained for carbon isotope fractionation in Miller-Urey type electric discharge syntheses have also shown that fractionation factors in these abiogenic processes are mostly different from those governing biological carbon fixation (S. Chang, personal communication, 1980). The bulk of the reaction products, notably the bydrocarbons, are enriched in ^{12}C by about 4-7 per mil only as compared with the parent methane. The amino acid fraction as a whole shows a 8-10 per mil ^{12}C-enrichment, with just single members like glycine climaxing at about 17 per mil. Apart from such limited overlaps with the biological field by some quantitatively unimportant reactions products, fractionations in these processes stay well below those known for photosynthetic and chemosynthetic pathways of biological CO_2-fixation.

Hence, with the presently available information, it seems most likely that the Isua organic material is biological rather than non-biological. As was discussed above, it is generally considered that the kerogen in sediments is ultimately of photosynthetic origin. However, it should be noted that chemoautotrophs, particularly methanobacteria, also bring about carbon isotope fractionations of about 25 per mil (Fuchs *et al.*, 1979). So the carbon isotope fractionation postulated for the pre-metamorphic Isua sediments would also be consistent with the presence of chemoautotrophs only. There are doubts, however, whether the hydrogen concentration in the contemporaneous environment (Walker, 1978) would have been sufficient to permit the prolific growth of chemoautotrophs that would have been needed to fix 20% of the total exogenic carbon as organic carbon (this figure necessarily results from a carbon isotope mass balance - see Schidlowski *et al.*, 1979). If we conclude that the Isua organic material is photosynthetic, then we must ask whether the data allow the products of the various forms of bacterial photosynthesis to be distinguished. Anoxygenic photosynthetic bacteria yield higher carbon isotopic fractionations (30-35 per mil; Wong *et al.*, 1975) than do cyanobacteria (11-24 per mil; Pardue *et al.*, 1976), but these differences are likely to be blurred during the early stages of diagenesis, let alone metamorphism.

The possible emergence of photoautotrophy by Isua times is consistent with the presently available morphological record. The recent discovery of stromatolites in the approximately 3.5 Gyr old Warrawoona Group of the Pilbara Block, Western Australia (Dunlop *et al.*, 1978; Walter *et al.*, 1980) has pushed back by some 500 Myr the earliest occurrence of biosedimentary structures attributable to benthic bacterial mats. Cellular evidence of primitive life has also been described from rocks dating back to about 3.4 - 3.5 Gyr B.P. (Muir and Grant, 1976; Knoll and Barghoorn, 1977). As these forms already reflect fairly advanced levels of biological complexity, life must have originated a substantial time before. On the other hand, microstructures of suspected biological affinities recently reported from the Isua meta-sediments themselves (Pflug, 1978) are unlikely to stand up to critical scrutiny (J. W. Schopf, E.S. Barghoorn, personal communication, 1979).

CONSEQUENCES FOR THE TERRESTRIAL REDOX BALANCE

In this context, a brief discussion of possible implications of the isotope data for the redox state of the atmosphere-ocean-crust system is appropriate. If the reduced carbon constituents present in Archaean sediments really represent photosynthetic carbon stemming

from the reduction of carbon dioxide by the oldest photoautotrophs, there should have accrued oxidation equivalents of this reduced carbon, which should be recogniseable in the geochemical record. Irrespective of the particular pathway utilized in biological CO_2-reduction, for each C-atom fixed as organic matter four electrons have to be supplied by a reductant which itself emerges in oxidized form from the reaction (Table 1).

TABLE 1

REDUCTION OF CO_2 BY THE MOST COMMON INORGANIC ELECTRON DONORS IN PHOTOSYNTHESIS (CH_2O STANDS FOR CARBOHYDRATES SYNTHESIZED)

$0.5\ H_2S + H_2O + CO_2 \xrightarrow{h\nu} CH_2O + H^+ + 0.5\ SO_4^{2-}$	$\Delta G_o' = +116.7$ kJ	(1)
$2H_2S + CO_2 \xrightarrow{h\nu} CH_2O + H_2O + 2S$	$\Delta G_o' = +50$ kJ	(2)
$2\ H_2O^* + CO_2 \xrightarrow{h\nu} CH_2O + H_2O + O_2^*$	$\Delta G_o' = +68.9$ kJ	(3)

Equations 1 and 2 represent two principal bacterial photosynthesis reactions utilizing H_2S as a reductant, the resulting oxidation products being either sulfate or elemental sulfur. Equation 3 represents O_2-releasing green plant photosynthesis, the top achievement in the evolution of photoautotrophic life where water serves as electron donor. Reaction 3 is also utilized by procaryotic blue-green bacteria (cyanobacteria) which were the only oxygen producers during the Precambrian prior to the appearance of eucaryotic algae. Note pronounced difference in energy requirements between oxygenic and non-oxygenic photosynthesis (standard free energy changes according to Broda, 1975, p.75).

With the organic carbon in the oldest sediments testifying as to the release of contemporaneous oxidation equivalents, the only point negotiable is whether these early oxidation products can be explained in terms of free oxygen - as in the case of cyanobacterial photosynthesis - or whether these ancient processes had yielded oxidized species other than free oxygen as does other bacterial photosynthesis. By speaking of *oxidation equivalents* rather than of *oxygen equivalents* of these ancient organics we would, however, arrive at a minimum statement acceptable to most workers. As pointed out by Cloud (1976), the presence in the Isua supracrustals of oxide-facies banded iron formation is likely to provide evidence of cyanobacterial (i.e., oxygen-releasing) photosynthesis since oxidation processes of the magnitude involved in the generation of Precambrian iron formation could hardly be sustained by inorganic photochemical oxygen sources (Walker, 1978). If such inferences are correct, O_2-producing photosynthesis as the top achievement in the evolution of photoautotrophy (Table 1, Equation 3) must have appeared at a very early stage. Subsequent work will have to show whether or not such postulate stands up to critical review.

Results of assays hitherto carried out on total reduced carbon from the Isua sediments (Moore and Welch, 1977; Oehler and Smith, 1977; Schidlowski, unpublished results) have shown that the C_{org}-content of these rocks lies approximately within the range of geologically younger sediments. Irrespective of current uncertainties regarding the evolution of the present sedimentary mass of some 2.4×10^{24} g through time (with constant mass and linear accumulation models as possible extremes, cf. Garrels and Mackenzie, 1971, p. 261), we are bound to end up with a reservoir of organic carbon on the order of 10^{21} g for the Archaean sedimentary shell if the C_{org}-content of the average sediment (ca. 0.5%) also holds for the oldest sedimentary formations (see also Reimer *et al.*, 1979). Since any substantial increase in biomass (both living and fossil) was contingent on a sufficient supply of electrons for the reduction of carbon dioxide (Table 1), there may be serious doubts whether electron donors other than water could have provided the reducing power for the accumulation of a crustal C_{org}-reservoir of about modern size. With water constituting the most ubiquitous reductant available, it has already been proposed elsewhere that only its universal utilization as a source of electrons would have met the quantitative requirements for a large-scale production and accumulation of terrestrial biomass (Schidlowski, 1978).

If this were actually the case, the conclusion would be inevitable that a major part (if not the bulk) of the oxidation equivalents released by the oldest photoautotrophs during Isua times already consisted of molecular oxygen. This would not necessarily imply a contemporaneous oxygenic atmosphere since it is fairly obvious that, prior to the saturation of the principal oxygen sinks (notably the Fe^{2+}-burden of the Archaean seas), free oxygen was unlikely to accumulate in the atmosphere in appreciable quantities. Although recently challenged by Dimroth and Kimberley (1976), the relatively late establishment of oxygenic conditions on Earth seems to be consistent with most geological evidence presently available. According to this evidence, the conspicuous redox imbalance imposed on the terrestrial environment by the presence of free oxygen dates back, in all probability, to some 2 Gyr ago.

SULFUR ISOTOPE RECORD AND TIME OF EMERGENCE OF SULFATE RESPIRATION

Parenthetically, the principal implications of the sedimentary sulfur isotope record included in Fig. 1 will be briefly summarized here.

Similar to carbon, isotopic fractionation in the terrestrial sulfur cycle is also biologically mediated, being the result of bacterial sulfate reduction ("sulfate respiration"). Due to preferential metabolization of light sulfur (^{32}S), bacteriogenic sulfide appears to be depleted, on average, by some 30 per mil in the heavy sulfur isotope (^{34}S) as compared to the parent sulfate (Holser and Kaplan, 1966). Consequently, the $\delta^{34}S$ age curves of the two principal sedimentary sulfur species (sulfide and sulfate) differ by about this amount, the conspicuous oscillations of the graphs during the Phanerozoic indicating periodic changes in the relative proportions of the sedimentary sulfide and sulfate reservoirs (fluctuations during the Precambrian are probably obscured by the scarcity of the record).

With respiratory processes generally reflecting a higher level in bioenergetic evolution than does photosynthesis, sulfate respiration may be expected to have postdated the rise of photoautotrophy. It is, therefore, by no means surprising that the probable beginnings of bacterial sulfate reduction can be tracked down to the time span between 2.8 and 3.1 Gyr B.P. when isotopic disproportionation between the sulfide and sulfate branches of the terrestrial sulfur cycle started to develop (Lambert, 1978; Monster *et al.*, 1979; Schidlowski, 1979). It is obvious from the hitherto available (though very scanty) Precambrian sulfur isotope record that there is very little fractionation between sulfide and sulfate sulfur as from the start of the record at Isua times until about 3 Gyr ago. It is in keeping with these findings and the general trend of bioenergetic evolution that photosynthetic (green and purple) sulfur bacteria are presently being regarded as the most probable ancestors of sulfate respirers (cf. Broda, 1975, p. 112).

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OXYGEN AND OZONE EVOLUTION IN PALAEOATMOSPHERES

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INTRODUCTION

The two main processes that have been responsible for releasing O_2 to the earth's atmosphere are photosynthesis and photodissociation. Both involve the decomposition of water molecules and the removal of hydrogen from the atmosphere. The energy for photosynthesis in plants and bacteria is provided by visible light while UV photons of wavelength shorter than 240 nm are required for the direct, abiotic, photodissociation of atmospheric water vapour.

Under present conditions, the predominant O_2-producing process is plant photosynthesis and the present atmospheric level (1 PAL) of O_2 is maintained by an almost exact equilibrium between photosynthetic production of O_2 and its utilization in processes of respiration, combustion and decay. Atmospheric O_2 is at present cycled through the biosphere with a time constant of about 2000 yr (Meadows 1972). The small net loss of O_2 from the atmosphere-biosphere system through surface oxidation must be replenished by the burial of sufficient organic carbohydrate to remove a stoichiometrically equivalent amount of hydrogen from the atmosphere-biosphere system. In the absence of biological processes surface oxidation would consume all the O_2 at present in the atmosphere in a period of a few million years (Holland, 1973; Walker, 1974).

Prior to the development of living organisms, the only O_2-producing process was the direct photodissociation of atmospheric water vapour by solar UV radiation. For this abiotic process the build-up of O_2 requires that hydrogen gas be permanently lost to the palaeoatmosphere through the exosphere in an amount exceeding any input of hydrogen from volcanoes or from the solar wind. The rate of O_2 production depends on the rate at which photodissociated hydrogen is transported to, and escapes from, the upper atmosphere, and the atmospheric oxygen level achieved in this way is governed by competition with the loss rate through surface and gaseous oxidation. Estimates of the rates of these processes will be made later.

The earth's atmosphere is generally assumed to be secondary and to have had its origin in the efflux of gases retained within the primeval solid earth (Rubey, 1955). If the earth accreted homogeneously there may have been a short period prior to the formation of the core when there was a highly reducing palaeoatmosphere (Holland, 1962) rich in methane and ammonia. There are, however, strong geochemical arguments against such a model (Ringwood, 1959) and it is more probable that the atmosphere was formed by the efflux of gases from an inhomogeneous earth which had no free iron in the upper mantle. Under these circumstances the palaeoatmosphere would have consisted mainly of water vapour, carbon dioxide and nitrogen (Walker, 1976; Hart, 1978). The purpose of this paper is to discuss the possible levels of O_2 and O_3 that may have been produced by the action of solar UV radiation on such a palaeoatmosphere (Carver, 1974; Blake and Carver, 1977).

PALAEOTEMPERATURES

The quantity of water vapour reaching photodissociating regions of the atmosphere depends on the surface temperature and the temperature profile throughout the troposphere and stratosphere. Geological evidence of ripple marks on rocks shows that there were substantial bodies of water 3.5 Gyr ago while an even earlier presence of abundant liquid water is implied by the existence of ancient sedimentary deposits (Cloud, 1972). Thus the geological evidence indicates that throughout most of its history the earth's mean surface temperature has been between 0° and 100°. Theories of stellar evolution, however, suggest that the sun has brightened by ca. 30% (Newman and Rood, 1977) since it first joined the main sequence some 4.5 Gyr ago, but there appears to be no evidence for a faint young sun in the geological record. Sagan and Mullen (1972) suggested that an ammonia greenhouse might have provided the additional infra-red blanketing needed to keep the earth's mean surface temperature above the freezing point of seawater but, as Owen *et al.* (1979) have remarked, it is unlikely

that an ammonia-rich atmosphere could have persisted long enough to maintain the greenhouse effect needed throughout the early Precambrian.

A more likely possibility is that the greenhouse effect was provided by a palaeoatmosphere containing much more CO_2 than does the present atmosphere. In his computer simulation of atmospheric evolution, Hart (1978) models the efflux of CO_2 from the solid earth to the atmosphere, its solution in the oceans, and deposition as carbonates. The model suggests that the CO_2 content of the atmosphere was ca. 1000 PAL at 4.25 Gyr B.P. (Before Present) and ca. 100 PAL at Gyr B.P. Hart's values for the variation with time of the atmospheric CO_2 content have been used to calculate the variation in the earth's mean surface temperature assuming that the sun brightened linearly with time from an initial value equal to 75% of the present solar constant. The resulting temperature history is shown as curve (a) of Fig. 1, which is similar to that calculated by Owen *et al.* (1979). The calculations assume a constant surface albedo equal to its present value, and ignore climate-induced changes in the cloud cover content. Also shown in Fig. 1 are two alternative temperature histories (curves b and c). Curve (b) assumes that the sun has been constant and that the CO_2 content has varied according to Hart's (1978) model; curve (c) assumes that the solar constant was only 75% of its present value when the sun first joined the main sequence, that the brightening has been linear with time and that the CO_2 content of the atmosphere has always had its present value. It has been assumed in all cases that CO_2 and water vapour (in an amount determined by the surface temperature) were the only gases contributing to the greenhouse effect. As may be seen from Fig. 1, curve (b) (sun constant, CO_2 varies) indicates a runaway greenhouse effect at times earlier than 2 Gyr B.P. while curve (c) (sun varies, CO_2 constant) implies that the oceans would have been frozen for most of the Precambrian. Only curve (a) in which both the solar constant and the atmospheric CO_2 content are assumed to have varied leads to a sensible surface temperature history consistent with the presence of large areas of liquid water throughout most of geological time.

Knauth and Epstein (1976) have provided some geological evidence which places closer limits on Precambrian temperatures than those implied just by the presence of the oceans. Their work indicates that Precambrian surface temperatures may have been significantly warmer than at present. Knauth and Epstein (1976) measured hydrogen and oxygen ratios in cherts and obtained the results plotted in Fig. 1 which suggest the possiblity that earth surface temperatures may have exceeded the mild warming in the early Precambrian indicated by the model atmosphere calculations (curve a of Fig. 1). If Precambrian surface temperatures were as warm as the results of Epstein and Knauth indicate, the increased atmospheric water vapour content would have important implications for the rate of O_2 production by photodissociation.

PREBIOTIC ATMOSPHERIC OXYGEN LEVELS

The earliest known microfossils are at least 3 Gyr old and include what are usually considered to have been O_2-producing cyanobacteria. Cloud (1972) has argued that O_2 produced in the hydrosphere by ancient organisms was not released to the atmosphere but was instead used to oxidize ferrous iron in solution which was then deposited to produce the banded iron formations characteristic of the period from about 3 to 2 Gyr B.P. On this model, arguments for which have been reviewed by Margulis *et al.* (1976), the O_2 content of the atmosphere was low before 2 Gyr B.P. Oxygen-mediating enzymes are assumed to have developed at the end of this period leading to a sudden increase in O_2 production, and the oxidation of the remaining iron in solution with the deposition of the red beds. The transition to the modern O_2-rich atmosphere then began as, in the absence of further oceanic sinks, photosynthetically produced free O_2 escaped to the atmosphere.

While there can be no doubt that the O_2 of the present atmosphere is photosynthetic in origin, there are difficulties in the photosynthetic model for Precambrian O_2 production. Van Valen (1971) has pointed to the problem of identifying the large deposits of reduced carbon needed to account for the red beds while Towe (1978) has questioned the predominant role of photosynthesis as an O_2 source in the early Precambrian on both biochemical and geochemical grounds. Towe suggests that photodissociation should be considered as an alternative source with some cyclic atmospheric-hydrospheric instability modulating the O_2 supply and thereby banding the iron formations.

What O_2 level could have been produced in the early Precambrian atmosphere by purely abiotic means? Berkner and Marshall (1965) assumed that abiotic O_2 would be limited to $<10^{-3}$ PAL because the O_2 so produced would shield atmospheric water vapour from further photodissociation owing to the "Urey effect". Although the water vapour and molecular oxygen photo-

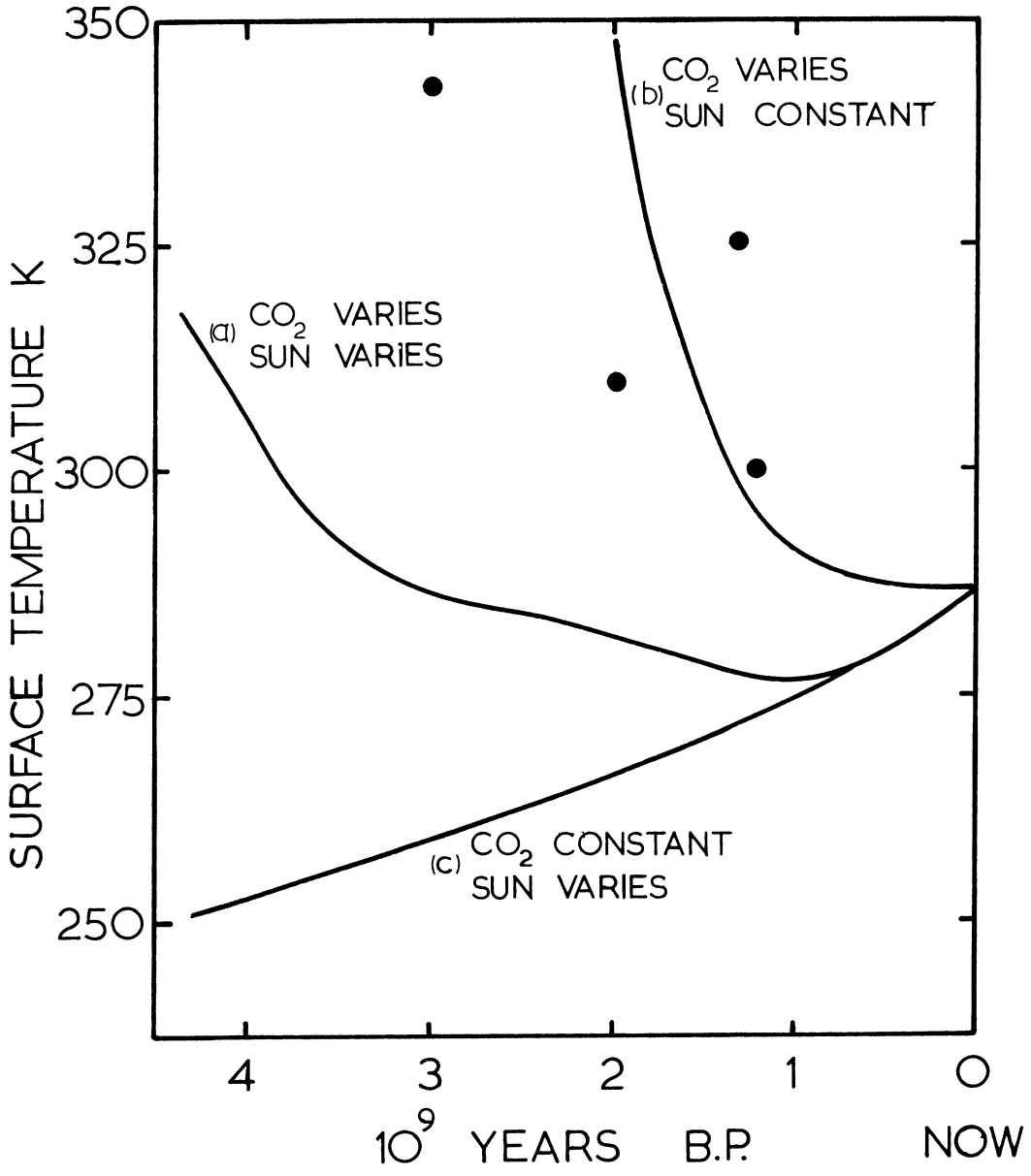

Figure 1: Calculated surface temperature histories of the earth assuming: (a) Atmospheric CO_2 varies according to Hart's (1978) model and sun brightens by 25% during geologic time; (b) CO_2 varies as in (a) but sun remains constant; (c) CO_2 remains constant but sun brightens as in (a). Precambrian temperatures estimated by Knauth and Epstein (1976) are shown by the solid circles.

absorption cross sections do overlap in wavelength range, Brinkman (1969), using the laboratory results of Blake *et al.* (1966), was able to show that O_2 was not as effective a shield for water vapour, as had been assumed by Berkner and Marshall, and that substantial amounts of O_2 would be released into the atmosphere by the photodissociation process. A large photodissociation rate does not, however, of itself ensure a significant accumulation of free oxygen in the atmosphere since most of the O_2 so released recombines with hydrogen

radicals in a series of chemical reactions in the lower atmosphere. For oxygen-deficient palaeoatmospheres the photochemical region begins in the lower atmosphere and there is a maximum in the water vapour photodissociation rate at an altitude of ca. 5 km.

The factor limiting the accumulation of atmospheric O_2 is not the water vapour photodissociation rate but the rate at which hydrogen is transported from the lower atmosphere to escape through the exosphere. Hunten (1973a and b; see also Hunten and Strobel, 1974; Liu and Donahue, 1974) has calculated the escape flux for atmospheres containing a mixture of hydrogen and a heavier gas. There is a diffusive upwards flux due to buoyancy, and the hydrogen escape rate is limited by the transport processes provided the exospheric temperature exceeds about $500^{o}K$. For a wide range of atmospheric conditions the hydrogen escape rate is given approximately by Equation 1,

$$\phi = \frac{b}{H} \frac{f}{(1+f)} \qquad 1$$

where b is the binary diffusion parameter, H is the atmospheric scale height and f is the fraction of hydrogen atoms (including hydrogen combined in water vapour and other compounds) to all other molecules in the lower atmosphere. The result is largely independent of the details of the photochemistry and, perhaps even more surprisingly, the escape flux is not very sensitive to variations in the solar UV flux. Liu and Donahue (1974) found, for example, that a factor of three reduction in the UV intensity caused a 28% change in escape flux while a doubling of the UV flux increased the escape flux by only 6%. Thus for the purpose of the present exploratory estimates, we may use the simple Equation 1 for the escape rate even at times when the solar flux may have been significantly different from its present value. For hydrogen transport through a terrestrial atmosphere $(b/H) \simeq 2 \times 10^{13}$ atoms $cm^{-2}\ s^{-1}$ and, if the hydrogen is a minor constituent ($f \ll 1$), the hydrogen escape flux can be written in the convenient approximate form (Equation 2; Walker, 1976):

$$\phi \simeq 2 \times 10^{13}\ f\ (\text{H atoms})\ cm^{-2}s^{-1} \qquad 2$$

If we can neglect any alternative sources of hydrogen, Equation 2 corresponds to an O_2 production rate given in Equation 3:

$$P \simeq 4 \times 10^{-5} f\ (\text{PAL of } O_2)\ yr^{-1} \qquad 3$$

In the present atmosphere, with its cold trap at the tropopause and its dry stratosphere, f is ca. 5×10^{6} and Equation 2 indicates an escape flux of ca. 10^{8} (H atoms) $cm^{-2}s^{-1}$, in fair agreement with observations (Liu and Donahue, 1974). (We can alternatively regard this agreement as an experimental calibration of the ratio b/H for the terrestrial atmosphere). The present escape rate is of the same order as the input of hydrogen and other reduced gases from volcanoes (Holland, 1973) which shows that abiotic processes are now a negligible source of atmospheric O_2 compared with photosynthesis. In the past, a net production of O_2 by photodissociation could only have occurred for palaeoatmospheres containing significantly more water vapour or other hydrogen compounds than does the present atmosphere.

The model atmosphere calculations discussed earlier suggest that, during the early Precambrian, surface temperatures may have been warmer than at present owing to the atmospheric greenhouse effect provided by a heavy CO_2 blanket. Epstein and Knauth's (1976) chert measurements suggest surface temperatures as high as 52^{o} at 1.3 Gyr B.P. and 70^{o} at 3 Gyr B.P. In the presence of liquid water the total amount, Q, of water vapour in the atmosphere varies approximately exponentially with the change in surface temperature, ΔT. A convenient numerical approximation (Hart, 1977) for the atmospheric water vapour content is given by Equation 4,

$$Q = \exp 0.0698\ \Delta T\ \text{PAL} \qquad 4$$

where ΔT (in degrees Kelvin) is the change in surface temperature from its present mean value ($288^{o}K$) and Q is measured in units of the water vapour content of the present atmosphere (1 PAL).

Morss and Kuhn (1978) have calculated temperature and water vapour profiles as a function of altitude for palaeoatmospheres with CO_2 contents of 5, 8 and 10 PAL and O_2 contents of 10^{-2}, 10^{-3} and 10^{-4} PAL, respectively. No tropopause, and thus no cold trap, was apparent in their calculated temperature profiles so that water vapour would most likely have been transported to higher altitudes. Their model 6 for example (CO_2 ca. 10 PAL, O_2 ca. 10^{-4} PAL) shows a $15\text{-}20^{o}$ increase over present atmospheric temperatures throughout the 0-15 km altitude

range and an order of magnitude increase in the water vapour mixing ratio near 15 km. The models of Morss and Kuhn all assume a solar constant equal to its present value. In the absence of detailed calculations of temperature profiles for the models under consideration here it has been assumed that the hydrogen (water vapour) fraction f appropriate to calculate H escape has the value 5×10^{-6} for the present atmosphere and that for other atmospheric models, f scales proportionately to Q, the total atmospheric water vapour content. For the Morss and Kuhn models this procedure underestimates f by about a factor of 2.

Assuming that f is proportional to Q, the palaeoatmosphere model calculations imply a value for f of ca. 2×10^{-5} in the very early Precambrian and the temperature estimates of Knauth and Epstein (1976) imply that f was ca. 7×10^{-5} at 1.3 Gyr B.P. and ca. 2×10^{-4} at 3 Gyr B.P. There is no intention to suggest that these values of f are any more than estimates based on a number of simplifying assumptions. The aim is solely to indicate the range of possible values based on our present limited knowledge of Precambrian temperature profiles.

While these larger values of f imply increased rates of hydrogen escape (and hence O_2 production), the O_2 level actually achieved in a palaeoatmosphere by these processes will depend on the loss rate. The present arguments are based on the premise that the palaeoatmosphere consisted principally of CO_2, water vapour and nitrogen, i.e. that it did not contain large quantities of reduced gases such as hydrogen, ammonia or methane. Under these circumstances, the main loss process is surface oxidation. For present atmospheric conditions the oxidation rate depends on such factors as the rate of exposure of surface material as well as on the intrinsic properties of the surface, and the oxidation rate is, therefore, likely to vary as only a low fractional power of the atmospheric O_2 content. At low O_2 levels, such as those pertaining in palaeoatmospheres, the situation was probably somewhat simpler since it was the supply of O_2 rather than the exposure of fresh material that was the limiting factor. Under these conditions of low O_2 level the oxidation rate L is proportional to the O_2 content $n(O_2)$ (Brinkman 1969) as shown in Equation 5,

$$L = \frac{n(O_2)}{\tau} \qquad 5$$

where τ is a characteristic time for surface oxidation.

The abiotic O_2 production rate P, determined by the hydrogen escape flux ϕ, is given by Equation 3 and, at equilibrium P=L, so that the equilibrium abiotic O_2 level is as expressed in Equation 6,

$$n(O_2) = 4 \times 10^{-5} \; f \; \tau \quad \text{PAL} \qquad 6$$

where τ is in years.

Equation 6 provides an estimate of the equilibrium abiotic O_2 level for various assumed values of the atmospheric hydrogen (water vapour) fraction, f, and the surface oxidation time τ years. Brinkman (1969) investigated Precambrian oxygen production and surface oxidation for a range of τ between 1 and 100 Myr. Hart (1978) assumed that τ was about 20 Myr. In the present atmosphere Holland (1973) has estimated that the oxidation time is about 3 Myr. Equation 6 applies only when f exceeds its present value so that exospheric hydrogen escape overwhelms the input of hydrogen from volcanic or other emissions. We can simply take account of this effect by replacing f in equation 6 by [f - f (present)] on the assumption that volcanic emission has been approximately constant and that it at present balances exospheric escape. With these assumptions, Fig. 2 shows the way in which the abiotic O_2 level depends on the surface oxidation time and atmospheric hydrogen fraction for a range of possible values of these parameters. The solid lines enclose an area limited by τ values of 1 and 10 Myr (bracketing the present value), and by the present f value and that implied by the Precambrian surface temperatures measured by Epstein and Knauth (1976).

Fig. 2 does no more than indicate a range of possibilities for the equilibrium abiotic O_2 content of a palaeoatmosphere, and any conclusions based on our limited knowledge of Precambrian conditions must be very tentative. Bearing this caveat in mind, the evidence presently available about Precambrian surface temperatures suggests that, unless oxidation times were an order of magnitude faster than at present, equilibrium O_2 levels in the range 10^{-3} to 10^{-2} could have been achieved by purely abiotic means. There is a possibility that O_2 levels may have gone as high as 0.1 PAL during any very warm and moist Precambrian periods but O_2 levels exceeding this could not have been achieved without a biological source, and abiotic O_2

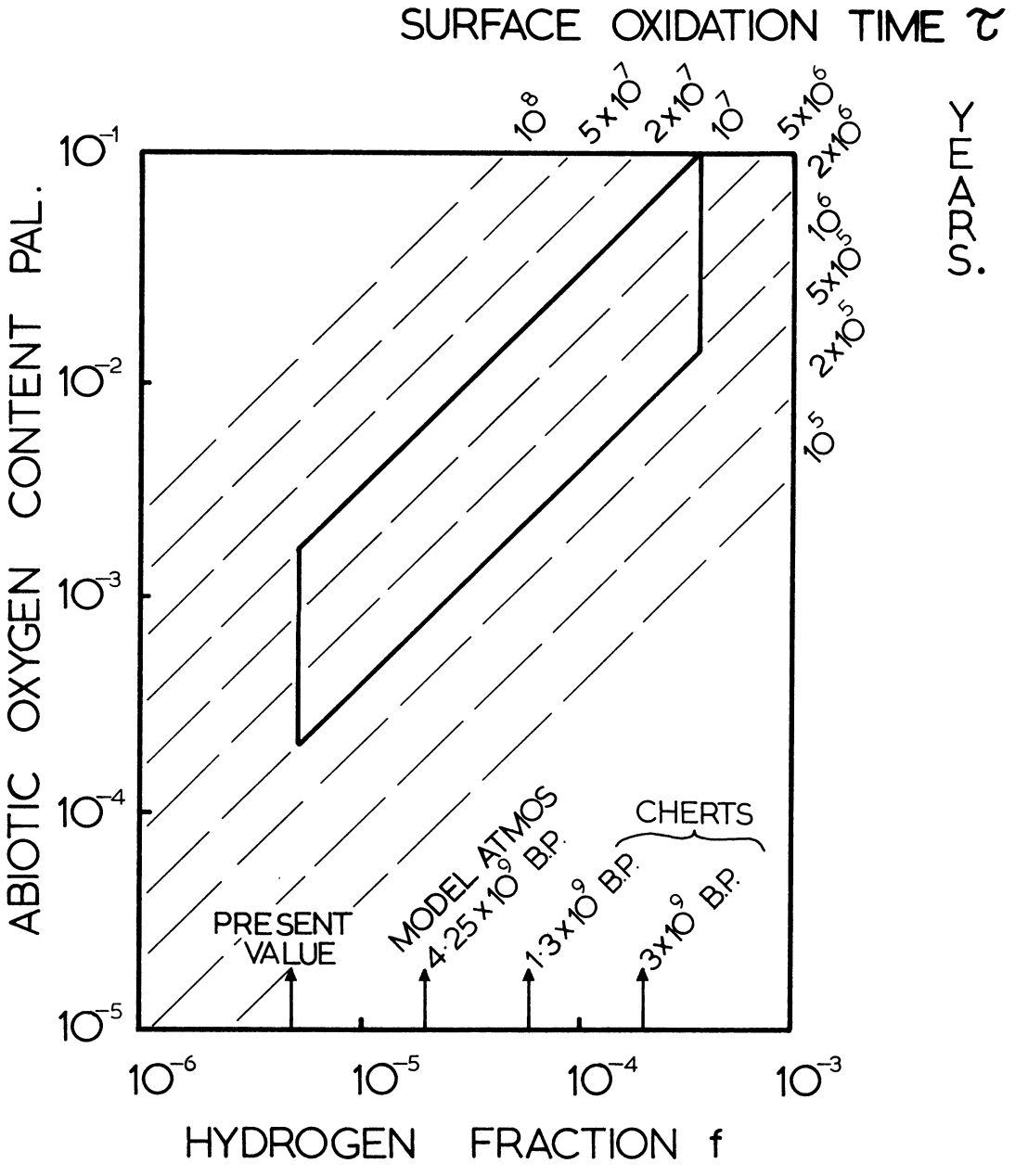

Figure 2: Equilibrium abiotic O_2 content (PAL) as a function of surface oxidation time (τ years) and hydrogen fraction f (mainly water vapour) in the lower atmosphere. The solid lines enclose an area limited by oxidation times τ of 10^6 and 10^7 and by the present f value and that implied by the Precambrian surface temperature (chert) measurements of Knauth and Epstein (1976). Note that the O_2 content is negligible unless f exceeds its present value so that exospheric hydrogen excape overwhelms volcanic hydrogen input.

levels would have been entirely negligible at times when the atmospheric hydrogen (water vapour) fraction f did not exceed its present value.

OZONE LEVELS IN PALAEOATMOSPHERES

Ozone is an important minor constituent of the present atmosphere because it is the principal absorber of solar UV radiation in the 220-330 nm band. This band of radiation interacts strongly, and mainly deleteriously, with living matter (the ozone and DNA absorption curves have similar shapes) and the O_3 in the present atmosphere provides an effective protective screen which appears to be essential for the continuation of life as we know it at the earth's surface.

Berkner and Marshall (1965) argued that solar UV radiation penetrating through an increasing O_2 and O_3 screen exerted a dominating effect on biological evolution but the major change in the oxygen content of the atmosphere probably occurred much earlier than they assumed (Cloud, 1972, Margulis *et al.*, 1976). What, if any, was the role of atmospheric O_3 in the evolution of biological systems? In atmospheres lacking significant quantities of O_2 and O_3, solar UV radiation penetrating to the earth's surface might be a source of the energy needed for the prebiotic syntheses of organic molecules (Berkner and Marshall, 1965 and references therein). For how long could such conditions have persisted on the earth? Did living systems evolve without the protection of an atmospheric ozone screen? How did the O_3 content of the atmosphere vary as the O_2 content increased to its present value from the low prebiotic level over 3 Gyr ago? These are questions of considerable evolutionary significance and it is possible to gain some further understanding of them by means of model atmosphere calculations.

The O_3 content of a palaeoatmosphere is clearly related, but not necessarily proportional, to the O_2 content. Blake and Carver (1977) have calculated the O_3 content of various model palaeoatmospheres with O_2 contents in the range 10^{-6} to 1 PAL. These calculations indicate how the atmospheric O_3 content may have changed during the period when the O_2 content was increasing to 1 PAL from its low prebiotic level in the early Precambrian. The model atmosphere calculations take account of reactions involving water vapour, hydroxyl and hydroperoxyl radicals, nitrogen oxides, methane and carbon dioxide as well as the "classical" ozone chemistry, considered by Ratner and Walker (1972), of a pure oxygen atmosphere interacting with solar radiation and chemically inert "third bodies" (nitrogen molecules). As the O_2 content of the model palaeoatmosphere is reduced from 1 PAL, the peak in the O_3 density profile is found to occur at lower altitudes but the total O_3 column density does not decrease rapidly with decreasing O_2 content. Oxygen-deficient palaeoatmospheres contain a much higher fraction of oxygen in the form of O_3 than does the present atmopshere.

Calculated O_3 column densities are shown in Fig. 3 as a function of the assumed molecular oxygen densities. The O_3 results are sensitive to the density of molecular nitrogen (or the inert "third bodies") which depends on the assumed rate of outgassing. If the earth outgassed instantaneously then the appropriate Precambrian nitrogen density would be 1 PAL; if the outgassing was continuous and uniform a nitrogen density of ca. 0.3 PAL would have been reached at about the time of the earliest microfossils (3 Gyr B.P.). The spread in O_3 values shown in Fig.3 corresponds to a range in molecular nitrogen density between these two limits of 0.3 and 1 PAL. These calculations indicate that the total O_3 content of the atmosphere does not decrease significantly until the O_3 amount falls below 10^{-2} PAL and, at O_2 concentrations of 10^{-3} and 10^{-4} PAL, the O_3 still amounts to about 0.3 and 0.03 PAL, resp.

In order to estimate the adequacy of different ozone screens, the relative biological damage has been calculated for solar ultraviolet radiation penetrating to the earth's surface through various model palaeoatmospheres. These results are also shown in Fig. 3. In making these estimates (Blake and Carver, 1977), the solar flux transmitted to the earth's surface has been weighted by the shape of the UV absorption coefficient of DNA (Lindstrom *et al.*, 1966) which is assumed to be representative of the spectral sensitivity of biological material (Booker, 1975). The biological damage curve shown in Fig. 3 has been normalized to unit damage for the screen provided by a typical mid-latitude O_3 column of 8.5×10^{18} cm^{-2} in the present atmosphere (Nicolet 1975) and a solar zenith angle of 30^{o}. Also shown in Fig. 3 is the relative biological damage caused by UV radiation penetrating a typical equatorial ozone column of 6.5×10^{18} cm^{-2} in the present atmosphere (Nicolet 1975) with an overhead sun.

The present equatorial level clearly gives a lower limit to the maximum tolerable exposure to biologically damaging UV radiation. Fig. 3 shows that, for an O_2 content of only 10^{-3}PAL,

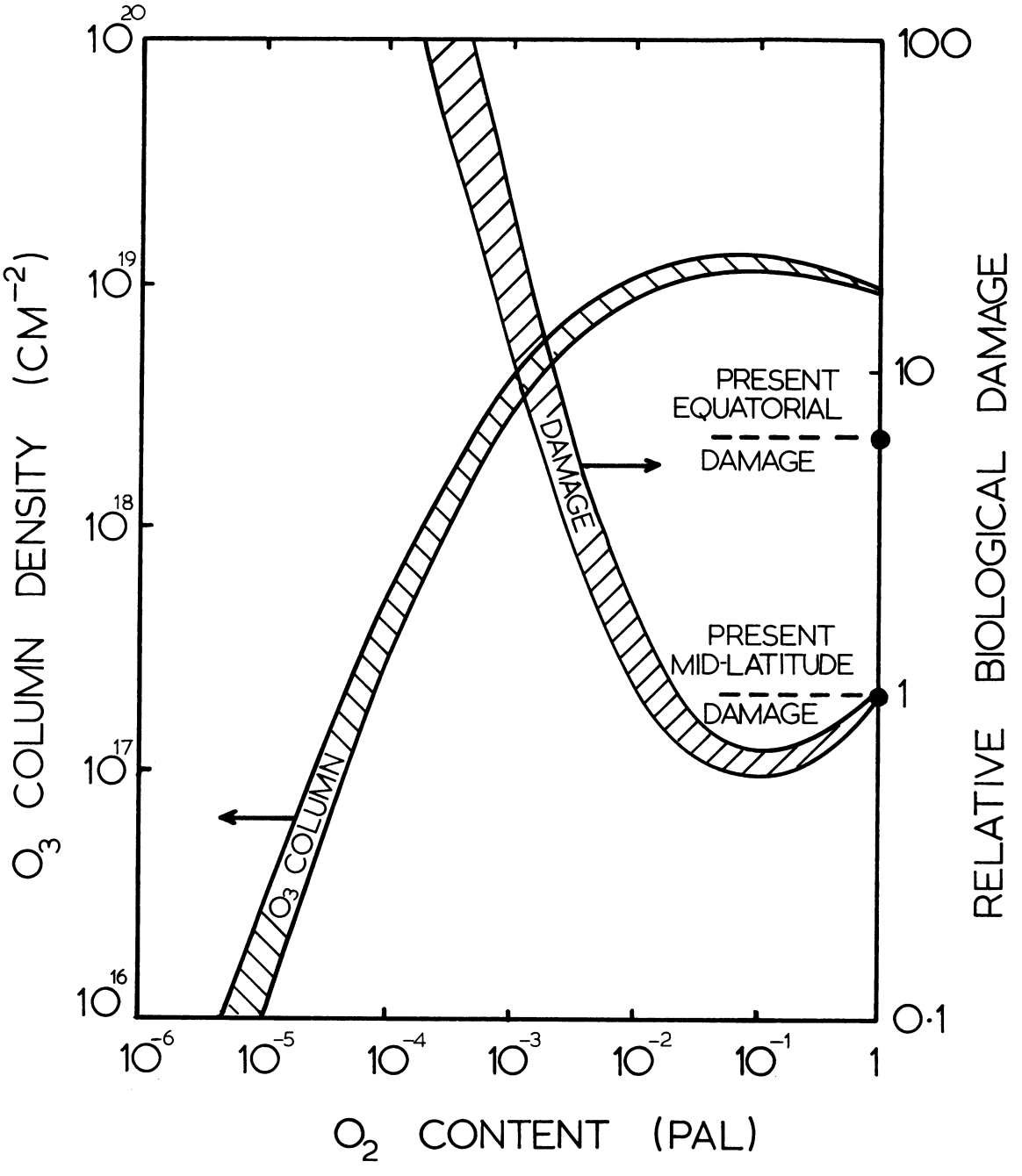

Figure 3: Calculated O_3 column densities (left hand scale) as a function of atmospheric O_2 content (Blake and Carver, 1977) with a spread in values corresponding to assumed nitrogen contents between 0.3 and 1 PAL. The relative biological damage produced by solar UV radiation penetrating these O_3 screens is also shown (right hand scale), normalized to unit damage for 30^o zenith angle under a typical present-day mid-latitude O_3 screen. The relative damage occurring for an overhead sun penetrating a typical present-day equatorial O_3 screen is indicated.

the biologically damaging effect of the solar UV radiation is expected to be not much greater than for a typical present-day equatorial O_3 shield. This comparison has been made on the assumption that the Precambrian solar flux was the same as at present. The conventional theory of stellar evolution implies, as discussed previously, that the solar flux in early Precambrian times was fainter than at present. An entirely opposite view of the history of the solar flux has been discussed by Hoyle (1972) who has considered some of the evolutionary consequences of a varying gravitational constant which, he suggests, would imply a solar flux at the earth 3 Gyr ago some 5 times its present value. Even under these improbable conditions an oxygen level of 5×10^{-3} PAL would provide a biologically adequate O_3 screen.

CONCLUSIONS

There are of course many uncertainties in this or any other attempt to estimate the properties of the earth's palaeoatmosphere and to chart the possible evolution of atmospheric oxygen and ozone. The model atmosphere calculations of the O_3 content (Blake and Carver, 1977) depend on details of the assumed photochemistry, and the estimated abiotic oxygen equilibrium level depends sensitively on the water vapour content of the atmosphere and on the surface oxidation loss rate. Bearing these qualifications in mind, we are led to the conclusion that the earth may have possessed a biologically adequate ozone screen throughout most of the Precambrian. If Precambrian surface temperatures were as warm as suggested by the measurements of Knauth and Epstein (1976) abiotic production through the photodissociation of water vapour could have produced oxygen levels in the range 10^{-3} to 10^{-2} PAL. Oxygen levels in this range provide enough O_3 to prevent solar UV radiation reaching the earth's surface in sufficient intensity either to cause lethal damage to living organisms or to provide the energy needed for the prebiotic synthesis of organic molecules. The transition from the abiotic O_2 level of the early Precambrian atmosphere to that of the present atmosphere probably began about 2 Gyr ago when photosynthetically produced oxygen started to enter the atmosphere following the deposition of the red beds (Cloud, 1972). The main conclusion which can be drawn from the model atmosphere calculations is that once the oxygen content of the palaeoatmosphere reached ca. 10^{-3} PAL, by either abiotic or biotic means, the evolution of living organisms would have been protected by a Precambrian O_3 shield.

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PAST OXYGEN LEVELS: SOME EVIDENCE FROM URANIUM DEPOSITS

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INTRODUCTION

Cloud (1965; p.33) wrote: "..... it is now generally agreed that the components of the present atmosphere came ultimately from within the earth, mainly by volcanism. Free oxygen, however, is not directly available from such sources; nor, in the absence of green plant synthesis, is it formed secondarily except in trivial and readily scavenged quantities from photolytic dissociation of CO_2 and H_2O. All who have considered the problem critically, therefore, agree that at an early stage in its history the terrestrial atmosphere was essentially anoxygenic, or reducing. Only at a later date, after the appearance of a photosynthetic source of oxygen could the atmosphere evolve towards its present oxygenic (and oxidizing) state." It is fair to add that there is not universal agreement as to the nature of the earth's atmosphere during past geological time. For example, Dimroth and Kimberley (1976) did not agree that an O_2-free atmosphere had existed at any time during the span of geological history recorded in well-preserved sedimentary rocks. However, such a stance is difficult to maintain in the face of abundant evidence which has been reviewed by workers such as McKirdy (1974), Biochenko *et al.* (1975) and Schidlowski *et al.* (1975) all of whom, and others, have contributed to the hypothesis of a primordial atmosphere, possibly largely of methane, subsequently modified to being CO_2-rich by the addition of juvenile gases from the outgassing of the earth. Oxygen, generated both photolytically and photosynthetically, was probably a quite minor component of the atmosphere for a considerable period before oxidizable minerals became fully accessible to O_2 in the atmosphere and in the hydrosphere.

Much of the evidence on the changing atmosphere of the earth has come from observations of the nature and mineralogy of rocks; this approach, restricted to uranium mineralization, is the subject of the present paper. Schidlowski *et al.* (1975, pp.49-50) stated that "The assumption of an extremely low redox potential of the ancient atmosphere - ocean system (particularly the atmosphere) is justified on the following grounds:

(a) Unoxidized condition of pyrite and uraninite in ancient detritals

(b) Occurrence of banded iron formations

(c) Absence of red beds from the oldest sedimentary formations

(d) Retarded evolution of higher life forms."

The authors elaborate these four grounds.

Elements such as iron, manganese, copper and uranium, capable, in near surficial environments, of existing in two or more valence states, are particularly useful indicators of redox conditions affecting the distribution of economically significant minerals. Iron must be considered very important because of its abundance in surface and near-surface rocks, and because of its occurrence in sediments as oxides, sulfides, carbonates, phosphates and other forms. Uranium is also of special interest because of strongly contrasting chemical properties and behaviour in its two relevant valence states. The International Uranium Symposium on the Pine Creek Geosyncline (the proceedings of which will appear in 1980) provided a recent opportunity to consider some of these aspects of Precambrian geology.

THE CHEMISTRY OF URANIUM

The low-temperature aqueous geochemistry of uranium has recently been the subject of reviews by Langmuir (1978) and Taylor (1979), to which the reader is referred for more information and literature references than can be included in the following summary.

Metallic uranium does not occur naturally. Uranium in chemical combination exhibits several valence states of which only two, U(IV) and U(VI), are important in the present

context. The oxide of U(IV),as a mineral, is known as uraninite or by the varietal name of pitchblende. This is by far the most commonly occurring mineral of uranium and, in many of the major uranium ore bodies of the world, is the only mineral of economic importance. The ionic radii of Th^{4+} (10.2 nm) and some other ions are similar to that of U^{4+} (9.7 nm); uraninite and thorianite form a solid-solution series, and substitution of rare earths occurs. Unlike uranium, there are no Th(VI) compounds; thorium and rare earth substitution is thus restricted to uranous (U^{4+}) compounds. Uranous compounds - and especially uraninite - are, in general, exceedingly insoluble in water. Various suggestions have been made as to how tetravalent uranium is transported in natural aqueous systems; the most probable forms appear to be halide complexes and colloids.

There are many minerals in which uranium occurs in the hexavalent state. In naturally occurring compounds hexavalent uranium invariably occurs as the uranyl ion $(UO_2)^{2+}$, which is stable over a wide range of Eh-pH conditions. A high degree of mobility is conferred on the uranyl ion by the presence of CO_2 which allows the formation of stable uranyl carbonate complex ions (Tugarinov, 1975). The carbonate complexes are obviously relevant to biological systems, in which CO_2 is to be expected. In such systems a decrease in CO_2 concentration may result in uranium precipitation, although the increase in alkalinity which accompanies CO_2 loss may inhibit uranium precipitation. Many other complex ions, including organic complexes, are known but I do not consider them to have been of great importance for the transport of uranium in processes leading to mineralization.

The transformation of uranous to uranyl ion obviously involves oxidation and in natural systems it is nearly always, directly or indirectly, O_2 from the earth's atmosphere which is implicated. In recent times, at least, oxidizing conditions have not been restricted to the earth's surface but, through diffusion and by the movement of water, may occur at depths hundreds of metres below the earth's surface.

The transformation of the uranyl to the uranous ion depends, of course, on reduction. By far the most important reductants in low pressure and temperature aqueous systems of the kind under discussion are the high-carbon compounds. The most important components of this family are materials collectively called carbonaceous matter (also known as 'kerogen', 'organic matter', and by other names). Most carbonaceous matter in sedimentary rocks is not graphite (which tends to be restricted to high-grade metamorphic rocks and some igneous rocks) but a non-crystalline solid composed essentially of carbon, oxygen and hydrogen. The reduction of uranyl ion is, thus, in general, accompanied by the oxidation of organic matter to CO_2. (Probably some carbonate minerals associated with uranium mineralization are formed in this way.)

I have recently reviewed the biogeochemistry of uranium (Taylor, 1979). The most important connection between organisms and uranium mineralization is in the role of carbonaceous matter of biological origin as a chemical reductant. It does not seem necessary to deal here with the leaching of uranium in an oxygenated environment through bacterial oxidation of pyrite to form ferric sulfate and sulfuric acid. Such leaching must have been involved in weathering processes in later Precambrian, as in Phanerozoic times.

URANIUM MINERALIZATION AND GEOLOGICAL TIME

Many geological classifications of uranium ores have been attempted. None is yet universally acceptable since the origin of some of the largest orebodies is disputed. Table 1 shows some of the most characteristic types, which will be considered below. Together, these ore types account for the great majority of known uranium deposits. While uranium enrichments in black shales, phosphorites and some other deposit types are not considered here, these do not appear to add greatly to our understanding of the earth's past atmosphere. Similarly, calcrete deposits, of which Yeelirrie in Western Australia is easily the best known (Mann and Deutscher, 1978), are products of a comparatively O_2-rich atmosphere. Since only modern examples of calcrete mineralization have been reported they do not at present add to our understanding of the earth's past atmosphere.

CONGLOMERATES

There is an extremely large literature (e.g. Pretorius, 1974) on the conglomeratic ores which have been very extensively worked in South Africa and Canada for both uranium and gold. In some papers the impression is given that this mineralization results only from the presence of detrital grains which have been concentrated by mechanical sorting at the

TABLE 1

SOME IMPORTANT URANIUM ORE TYPES

Ore Type	Typical Occurrence
Conglomerate	Witwatersrand, South Africa; Blind River, Canada
Alligator Rivers ("Vein-type")	Jabiluka, N.T., Australia
Hydrothermal	Schwarzwalder, U.S.A.
Sandstone	Colorado, U.S.A.

time the conglomerate was being deposited or reworked. While it is certainly true that rounded, and probably stream-abraded, grains of pyrite and uraninite occur in these conglomerates (Minter, 1976), there is disagreement as to how much of the metallic mineral content can be attributed to a placer origin. In fact, Reimer (1975) concluded that most of the Witwatersrand uranium was transported to the general depositional site in dissolved form, later forming nodular grains which were concentrated at particular sites by normal sedimentary processes.

My conclusion from microscopic observation is that a significant proportion (most, in the samples available to me) of the uranium in the Witwatersrand ores cannot owe its origin to a placer mechanism. Much fine uraninite is enclosed in carbonaceous matter, which, on the basis of morphology, texture and chemical composition, is assessed as being of biological origin. Hallbauer and van Warmelo (1974) reported the isolation of lichen-like fossil organisms from the Witwatersrand rocks, but doubt has been expressed as to the validity of these discoveries (P.E. Cloud, personal communication, 1979). Whatever the affinity of this carbonaceous matter, it appears to be of organic origin. It seems almost certain that the fine uraninite enclosed in carbonaceous matter (thucholite) must have arrived in solution. I have considered the possibility that this part of the uranium may have been immobilized by reduction subsequent to the deposition of the rock, either by the introduction of uranium via formation waters or by redistribution of uranium originally present as detrital grains. However, the detrital grains of uraninite and pyrite show no sign of the solution attack which would be expected if post-depositional formation waters had been oxidizing. Moreover, the presence of fine uraninite within the thucholite means that the latter must have been permeable to aqueous solutions. Such permeability is typical of freshly deposited organic matter having a high moisture content, but not of organic matter which has undergone diagenesis. I therefore conclude that post-depositional emplacement of the fine uraninite, if it occurred at all, must have been at a very early stage after the deposition of the conglomerate.

It has been suggested that some carbonaceous matter associated with uranium is the product of radiogenic degradation of gaseous or liquid hydrocarbons. However, the textures and properties of thucholite (and all other carbonaceous matter associated with uranium ores which I have observed) are inconsistent with such an origin. In fact, on the basis of chemical composition, texture and isotopic evidence, virtually all carbonaceous matter in sedimentary rocks (except perhaps some from the very early Precambrian) appears likely to have been derived from organisms, although extensive biological degradation may occur prior to deposition (Deuser, 1971). There is, in general, an association between the metallic minerals and thucholite where the latter is present. However, there is good evidence for genuinely detrital uraninite in that the thorium content of apparently detrital grains is up to 10% and there is variation from grain to grain (Kimberley, 1978).

In summary, we have evidence, in the same rocks, of transport of uranium in solution and of transport of pyrite and uraninite as detrital grains. The migration of uranium in solution at the surface must have been of the uranyl ion and implies a degree of oxygenation; the precipitation of such dissolved uranium in a carpet-like mat on the surface implies very ready reduction and thus only a low activity of O_2 in the water and, therefore in the atmos-

phere. The detrital grains of oxidizable minerals are evidence that, at the stream floor, oxidation was occurring more slowly than mechanical concentration. It is reasonable to infer that, because of the low O_2 content of the atmosphere and the presence of abundant 'sinks' for O_2, substantially reducing conditions were maintained below even the shallow depths of water indicated by the sediment type. Such inferences are not, perhaps, so readily drawn from studies of the Canadian Blind River deposits (Robertson, 1976). While there is an association between uranium mineralization and the occurrence of thucholite at Blind River, this thucholite (at least in the sample available to me) has form and properties suggesting that there may have been heat alteration, which would certainly have eliminated some of the remarkably well-preserved morphological features of the Witwatersrand thucholite. Such heat alteration would be consistent with evidence from the Canadian rocks for a degree of regional metamorphism.

Conglomeratic uranium deposits are limited in time from about 2.8-2.2 Gyr B.P. and this prompts two questions: (i) why are there no earlier and (2) why are there no later examples? Rocks more than 2.8 Gyr old are not so abundant that we can speak with assurance. One reason as to why older conglomerates may not exist could have been the lack of uraniferous igneous rocks. I suggest that an additional factor was the absence of a certain minimum (but low) level of atmospheric O_2 to permit oxidation and transportation of uranium as a soluble uranyl species which could subsequently be concentrated by reduction on (presumably) the very organisms, or their remains, which yielded the necessary oxygen. A small content of O_2 in the atmosphere would also help in the incipient weathering of rocks and encourage the liberation of single mineral grains. I therefore suggest a possible change in atmosphere from virtually no O_2 in the atmosphere, to a low level, at about 2.8 Gyr.

The other end of the range, 2.2 Gyr, must represent a time when conditions in near-surface waters became distinctly more oxidizing. It seems reasonable to infer that at least those shallow bodies of water where conglomerates deposited had become sufficiently oxygenated that no stable concentrations of oxidizable minerals could form and persist. This increase in O_2 may have been, in part, the result of using up some readily available O_2 'sinks' but, because there are other, contemporaneous changes in the sedimentary record, there was probably an increase in the photosynthetic yield of oxygen. This time of about 2.2 Gyr seems to have been important for deposition of banded iron formation and for the first appearance of massive carbonate sequences and red beds.

ALLIGATOR RIVERS

The recent International Uranium Symposium on the Pine Creek Geosyncline (1979) showed that there is no agreement yet as to the origin of the large ore bodies which occur in the East Alligator region of the Northern Territory Australia and in Saskatchewan (although there appear to be differences between the two groups of deposits). The large concentrations of uranium in the East Alligator region occur mainly in metasedimentary rocks of Lower Proterozoic age. The ore itself postdates the metamorphism, i.e. the ore is younger than about 1.75 Gyr. Many authors postulate (and I agree) that the uranium in East Alligator deposits was probably concentrated from supergene sources. In my view, therefore, the most likely time of mineralization is at, or soon after, 1.7 Gyr. The host rocks typically contain carbonaceous matter (including graphite); there is a spatial relationship, although no precise correlation, between carbonaceous matter and ore. The ore, essentially uraninite, occurs up to hundreds of metres below the unconformity, so that even taking account of possible karst formation (Ferguson *et al.*, 1979), there must have been a considerable thickness of rock in which oxidizing conditions prevailed. Regoliths occur beneath the unconformity. There is thus good evidence of appreciably higher levels of O_2 at, or soon after 1.7 Gyr (assuming the correctness of that time of deposition) as compared with the 2.2 Gyr environment.

HYDROTHERMAL VEINS

There is some confusion in the literature since uranium mineralization occurs both in true veins and in so-called vein-type deposits (actually the Alligator Rivers type of mineralization referred to above). True veins appear usually to be related to metamorphic and, perhaps less commonly, to igneous intrusive episodes. Such uranium appears to have been transported in the reduced state as chloro-complexes and, with other metallic elements, deposited as a result of changes in the chemistry of the host solutions. No redox reactions appear to have been involved. Veins of this nature were formed during the

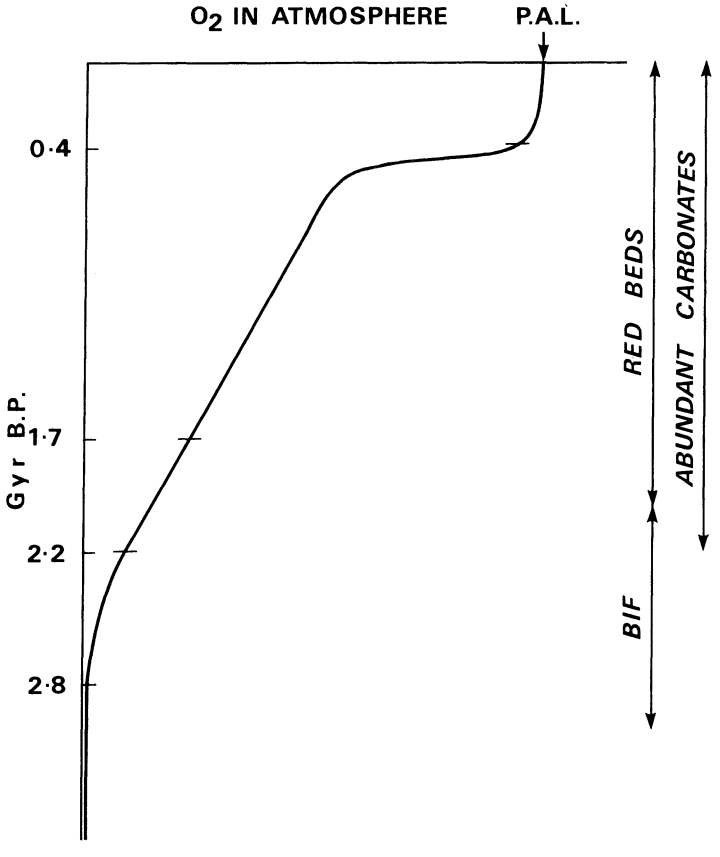

Figure 1: Schematic diagram showing variations in O_2 content of atmosphere from evidence of U mineralization.

Proterozoic and Palaeozoic eras; their emplacement appears to have been unrelated to the earth's atmosphere, except perhaps in so far as there may have been some preconcentrating redox mechanism before the vein-forming solutions existed.

SANDSTONE

Uranium deposits of the sandstone type are agreed to be the result of redox reactions fixing uranium as UO_2 following the migration of uranyl species into a zone where carbonaceous or sulfide reductants were present. All economic deposits of this type occur in rocks of late Palaeozoic, Mesozoic and Tertiary age (Robertson *et al.*, 1978). Robertson *et al.* attribute the absence of older economic deposits (minor occurrences are known) to the flushing of uranium by ground and surface waters over geological time. I find this explanation unconvincing in view of the potential for the occurrence and preservation of such mineralization in Proterozoic and early Palaeozoic rocks, for example in Australia. The widespread development of land plants during the Palaeozoic era provided a large additional supply of O_2 to the atmosphere. The late Palaeozoic and younger sandstone-type deposits imply the presence of oxygenated waters at considerable depths.

DISCUSSION AND CONCLUSIONS

Others (e.g. Dahlkamp, 1978; Robertson *et al.*, 1978) have noted the apparently time-limited nature of uranium deposits. In this paper I have attempted to show that much of the variation in style of uranium mineralization may be a consequence of changes in the O_2 content of the atmosphere. Such inferences, of course, constitute only one input to any general consideration of the subject.

Fig. 1 shows the inferred trend, in schematic form, of changing O_2 content of the atmosphere. The "landmarks" are

- ca. 2.8 Gyr B.P. Increase from no significant activity of O_2 to a low but stable amount in surface waters.
- ca. 2.2 Gyr B.P. Increase in depth of water in which oxygen available, presumably correlating with higher content of O_2 in the atmosphere.
- ca. 1.7 Gyr B.P. Oxygen content of atmosphere had increased considerably because there must have been a higher activity of O_2 in near-surface water and rocks than at 2.2 Gyr.
- ca. 0.35 Gyr B.P. Attainment of present atmospheric level of O_2 following development of land plants.

It does not appear possible, on the sole basis of evidence from uranium mineralization, to quantify the proportion of O_2 in the earth's atmosphere between 2.8 Gyr and 0.35 Gyr B.P.

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TIMING AND RELATIONSHIPS AMONG PRECAMBRIAN CRUSTAL AND ATMOSPHERIC EVOLUTION AND BANDED IRON-FORMATIONS

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INTRODUCTION

Current thoughts on the early history of the earth have been greatly influenced by the models of Cloud (1972, 1976). These models have attempted to explain, in an internally consistent fashion, the evolution of the atmosphere, hydrosphere, biosphere and crust of the earth. Our concepts of the origin of one of the most enigmatic rock types in the geologic record - the early Precambrian banded iron formations - have also been greatly influenced by Cloud's models. During the past several years, many advances have been made in the fields of isotopic age dating, geochemistry and sedimentology which are significant to our understanding of the early history of the earth and to the origin of early Precambrian banded iron formations. In this context, a review of some of these advances and their implications seems warranted.

PRECAMBRIAN CRUSTAL EVOLUTION

Geochemical Considerations

Many workers have studied sedimentary rock composition in order to evaluate possible secular changes in upper crustal composition (see review by Schwab, 1978). Through time there have been decreases in Al, Na and Mg and increases in Si, K and Ca in sedimentary rocks. It is uncertain, however, if these trends are primarily controlled by chemical evolution of the upper crust, recycling of sedimentary debris, diagenesis and metamorphism, or changes in sedimentary styles.

Trace element studies in sedimentary rocks can add greatly to our understanding of crustal evolution. To this end, the study of rare earth elements (REE) has proven particularly useful since they are not severely affected by most sedimentary and metamorphic processes and their distribution in fine-grained sediments is thought to be fairly representative of upper continental crustal abundances.

There is a distinct difference in REE patterns for Archaean and post-Archaean sedimentary rocks (see review by Taylor, 1979). These trends were precisely defined in detailed studies of Australian sedimentary rocks (Nance and Taylor, 1976, 1977; Taylor, 1979) where it was shown that two suites of Archaean and post-1.5 Gyr B.P. sedimentary rocks had consistent and significant differences in REE distributions. The most recent estimates (Taylor, 1979) of average Archaean (AAS) and post-Archaean (PAAS) sedimentary rock REE patterns are plotted, normalized to chondrites, in Fig. 1. Both of these patterns reflect an efficient sampling of the contemporaneously exposed upper crust (Taylor and McLennan, 1980). Such patterns support a compositional change in the exposed crust from andesite during the Archaean to granodiorite during the post-Archaean.

These studies lacked sampling during the important period of 2.7-1.5 Gyr B.P. McLennan *et al.* (1979a) attempted to fill this 'gap' in a study of the Lower Proterozoic Huronian succession of Canada. These rocks were deposited some time between about 2.6-2.16 Gyr B.P. (Roscoe, 1973). Lower Huronian REE patterns were similar to typical Archaean sedimentary rocks while upper Huronian samples were virtually indistinguishable from post-1.5 Gyr B.P. sedimentary rocks. The interpretation suggested that an essentially episodic change in the composition of the upper crust occurred at the end of the Archaean, related to widespread intrusion of K-rich granitic rocks into the upper crust (Fig. 2). Modelling of the REE data indicates that a mixture of 1/3 late Archaean K-rich granites to 2/3 early Archaean upper crust is necessary to produce the post-Archaean sedimentary REE pattern (Taylor and McLennan, 1980). Recent research in the Lower Proterozoic Pine Creek Geosyncline of Australia is in full agreement with the Huronian data (McLennan and Taylor, 1980).

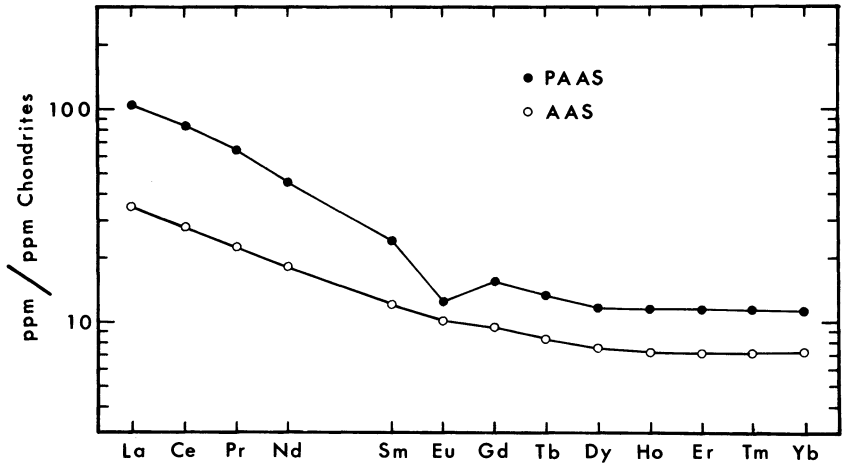

Figure 1: Rare earth element (REE) diagram, normalized to chondrites, for Post-Archaean average Australian sedimentary rock (PAAS) and average Archaean sedimentary rock (AAS). Data from Taylor (1979), Taylor and McLennan, 1980). AAS resembles modern calc-alkaline andesites while PAAS resembles typical granodiorite.

The uniformity in REE patterns in post-Archaean sedimentary rocks is difficult to explain in terms of a slowly evolving upper continental crustal composition. Instead, the REE patterns support a two stage model with a sharp break in composition at the Archaean-Proterozoic boundary (current work in Greenland may suggest a more complex history before about 3.0 Gyr B.P. on some continents).

Sr Isotopes in Seawater

Veizer (1976a) and Veizer and Compston (1976) have made estimates, from carbonate rocks, of Sr isotopic composition of seawater through time. Archaean $^{87}Sr/^{86}Sr$ ratios are not dissimilar to upper mantle values. There is, however, an extremely sharp increase of the radiogenic component (approximate increase in $^{87}Sr/^{87}Sr = 0.0025$) between about 2.5 - 2.1 Gyr B.P. followed by a gradual increase during the Proterozoic and Phanerozoic. The sharp increase corresponds to the REE change and was interpreted as being a result of significant fractionation of the crust. Veizer and Compston (1976) suggested the extremely low $^{87}Sr/^{86}Sr$ of Archaean seawater was due to a high mantle influence and concluded that there was very little 'conventional' continental crust being eroded. This is in agreement with other geochemical information on the nature of Archaean oceans (Fryer *et al.*, 1979).

Sm-Nd Systematics

Sm-Nd dating in the Canadian Shield (McCulloch and Wasserburg, 1978) suggests that much of the rock exposed in the Churchill Province formed at about the same time as the Superior Province (2.7-2.5 Gyr B.P.) - a fact long recognized by geologists but never before clearly demonstrated by geochronology. These authors suggested that this event represented a major crust-forming period because the material must have come from a source with

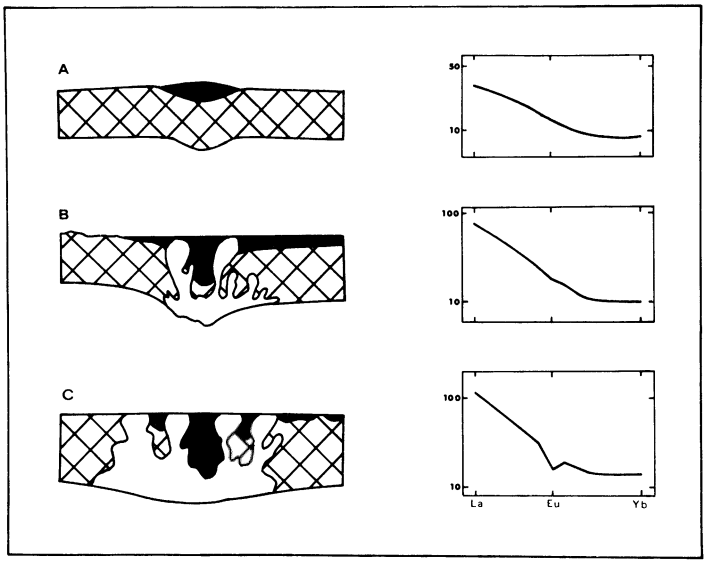

Figure 2: Generalized model of crustal evolution as indicated by REE evidence (McLennan *et al.*, 1979a). A. Archaean REE patterns resulted from an early upper crust dominated by greenstones (black) and Na-rich granitic material (hatched). B. At the end of the Archaean (ca. 2.7 Gyr B.P.; earlier in South Africa), K-rich granitic rocks (white) were intruded into the crust. Erosion and gradual unroofing of these rocks resulted in rapidly changing sedimentary REE patterns. C. When erosion cut sufficiently deep, typical post-Archaean sedimentary REE patterns appeared (indicating a granodioritic upper crust) and these have remained essentially unchanged.

chrondritic-like Sm/Nd (0.31; i.e. - the mantle). They noted that, "it appears that the activity around the period 2.5 to 2.7 AE is unique in geologic history and that some truly major periods of continental growth are sharply episodic." (McCulloch and Wasserburg, 1978, p.1011).

TIMING OF CONTINENTAL EMERGENCE AND STABILIZATION

An intriguing model of crustal evolution has been proposed recently by Hargraves (1976) who argued that the sialic and simatic portions of the earth separated during the early Archaean but remained submerged beneath a globe-encircling sea until much later. Land areas were small and ephemeral during the Archaean and were related to the rise of greenstone piles above sea-level. On the basis of geochemical and heat flow evidence, Hargraves suggested that the continents did not stabilize and rise substantially above sea-level until about 1.4 Gyr B.P.

Although there is some merit in the idea of submerged sialic crust during the Archaean (see Windley, 1977), Hargraves' (1976) precise timing of continental emergence and stabilisation is clearly at odds with most evidence. Windley (1977) has shown that the continental crust was thick and extensive during the Early Proterozoic. Local areas show crustal thicknesses of >30 km, comparable to modern values (Tarney and Windley, 1977). Such a thickness could easily sustain land masses about 2 km above sea level (Hargraves, 1976; Windley, 1977). Windley (1977) also pointed out that extensive,

relatively stable epicontinental basins first appeared on the North American craton during the Early Protozeroic (Fig. 3A), clearly indicating exposed land. Such stable epicontinental basins also first appeared during the Early Proterozoic in Aus ia (Fig. 3B) and most other continents. Another important point, not previously empl ed is the evidence for extensive Early Proterozoic glacial deposits throughout North America (Young, 1970, 1973). Stratigraphic correlations suggest these rocks were deposted as part of a single continental glaciation (Fig. 3A). Such a phenomenon would require extensive exposures of land.

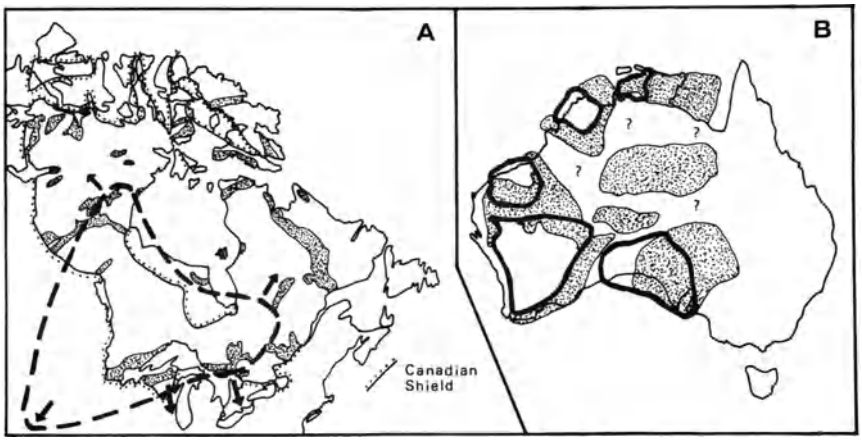

Figure 3: A. Map of the Canadian Shield showing areas of Lower Proterozoic deposits (stippled). Evidence from Sm-Nd dating (McCulloch and Wasserburg, 1978) indicates that much of the Canadian Shield may have been part of the Archaean basement. Heavy broken lines are possible extent of Lower Proterozoic glaciation. Arrows show major palaeocurrent trends. These features indicate that much of the Canadian Shield was exposed and stable by the end of the Archaean (after Windley, 1978; Young, 1973).

B. Map of Australia showing major basins of Lower Proterozoic deposits (stippled). Archaean cratons are shown by heavy lines and possible extensions are indicated by question marks. Such widespread Lower Proterozoic deposits strongly suggest that an extensive continent was exposed at the end of the Archaean (after Plumb, 1978).

REE and isotopic evidence support a major change in the composition of the exposed crust, probably related to the intrusion of late Archaean granitic rocks. It is also now clear that the continents were emergent and began to stabilize during the late Archaean - early Proterozoic. The widespread development of relatively stable epicontinental basins at about this time (see Cloud, 1976; Frarey, 1976) was probably in response to the dramatic change in the chemical and structural nature of the crust.

South Africa

Cloud (1976) drew attention to the 'diachronous nature of cratonisation' which brought to an end the Archaean style of crustal evolution. In particular, he noted the initiation of Proterozoic-style basin development (Pongola, Dominion Reef, Witwatersrand "Systems") during the late Archaean of South Africa (3.1-2.5 Gyr B.P.). The term "Zuluan Wedge" was introduced to incorporate these rocks into a uniform time scale (Cloud, 1976). If the above interpretation of the geochemical and isotopic data is correct, we may predict that these upper Archaean sedimentary rocks of South Africa should show similar evolutionary

trends to Lower Proterozoic rocks in Canada and Australia. Preliminary trace element studies of sedimentary rocks from the Pongola and Witwatersrand Systems indicate that typical post-Archaean REE patterns may be common, thus reinforcing the above interpretation of crustal evolution and the concept of the Zuluan Wedge proposed by Cloud (1976).

OXYGEN IN THE PRECAMBRIAN ATMOSPHERE AND HYDROSPHERE

It has been argued by many workers that the early Precambrian (>2 Gyr B.P.) atmosphere and hydrosphere were deficient in free O_2, although several workers have recently disputed this claim (e.g. Dimroth and Kimberley, 1976). It is beyond the scope of this paper to discuss, in detail, evidence for an O_2-deficient early Precambrian atmosphere and hydrosphere, but the most important points can be summarised as follows:

1) *paucity of well-documented, syngenetic, subaerially oxidised hematitic sediment before about 2.2-2.3 Gyr B.P. (see Cloud, 1976, for discussion).* This line of evidence remains ambiguous since tectonic conditions favourable to red bed development were not common during the Archaean (Bridgwater and Fyfe, 1974). Also, it remains to be proven what levels of free O_2 are necessary to allow the formation of such deposits. It is interesting to note, however, that lower Huronian sedimentary rocks (>2.16 Gyr old) are characteristically drab coloured even though they were deposited in environments where oxidised, hematitic sediments would be expected to form (Roscoe, 1969). On the other hand, upper Huronian sedimentary rocks (Cobalt Group) are brightly coloured due to hematitic alteration (Roscoe, 1969).

2) *apparent iron mobility and high Fe^{2+}/Fe^{3+} ratios in early Precambrian weathering profiles (Rankama, 1955; Pienaar, 1963; Fryer, 1977).* This line of evidence must also be considered somewhat ambiguous since some evidence for iron accumulation is present in pre-Huronian palaeosols (Fryer, 1977; Holland, 1979).

3) *enrichment of iron (particularly Fe^{2+}) and manganese in Archaean and Lower Proterozoic carbonates (Veizer, 1973, 1976b, 1978).* It is well known that carbonates can be enriched in Fe^{2+} during diagenesis and metamorphism and that this could cause the trends of increasing iron (and Fe^{2+}/Fe^{3+}) with increasing age. However, Veizer (1978) has carefully and systematically considered these secondary effects and concluded that they cannot be the dominant control. A primary origin for these features is strongly indicated and suggests a much lower amount of free oxygen in the hydrosphere and atmosphere (Veizer, 1978).

4) *evolution of the dominant heavy mineral suites in Lower Proterozoic sedimentary rocks (Roscoe, 1969; 1973) from those thought to be unstable during oxidising weathering (pyrite, uraninite, etc.; the "yellow sands" of Roscoe, 1973) to normal suites, similar to those found today.* It is well known that pyrite is a physically resistant mineral and is a common heavy mineral even in river deposits forming at the present time, but its abundance, as a detrital mineral, is invariably trivial (Pettijohn *et al.*, 1973). Pyrite cannot be expected to survive during extended periods of oxidising weathering (Pettijohn *et al.*, 1973). In lower Huronian sandstones, however, pyrite is a major heavy mineral. Its origin as, primarily, a detrital mineral is firmly established (Roscoe, 1969, 1973; Frarey and Roscoe, 1970; see below). These rocks were derived from a weathered source (Roscoe, 1969; 1973; Frarey and Roscoe, 1970; McLennan, 1977) and some of these rocks even bear evidence of weathering occurring penecontemporaneously with sedimentation (Long, 1976).

5) *presence of extensive uraninite-pyrite bearing quartz-pebble conglomerates in late Archaean - Early Proterozoic sedimentary rocks.* Important occurrences include the Blind River, Witwatersrand, Montgomery Lake Group and Serra de Jacobina deposits. A detrital origin for much of the uraninite and pyrite has been firmly established by field relations (Pienaar, 1963; Gross, 1968; Roscoe, 1969; Robertson, 1976), sedimentological and petrographic studies (Pienaar, 1963; Roscoe, 1969; Minter, 1976), geochemistry (Pienaar, 1963; Roscoe, 1969; Grandstaff, 1974; Robertson, 1976) and isotopic studies (Roscoe, 1969; Rundle and Snelling, 1977). In the case of the Blind River deposits, it is known that the sericitic matrix of the conglomerates is virtually indistinguishable from the underlying sericitic palaeosol and was probably derived from it (Robertson, 1976). This supports the contention that these rocks were derived from a weathered source. Kinetics of uraninite oxidation at the earth's surface (Grandstaff, 1974) indicate upper

oxygen partial pressures for the atmosphere, at that time, of about 10^{-7} to 10^{-3} atm.

The idea of an early O_2-deficient atmosphere and hydrosphere is also attractive because many other features of the geologic record can be explained by such conditions. Holland (1973) and Drever (1974) pointed out that the oceans are the most likely source of the iron in Precambrian iron formations. They noted that a massive iron reservoir in the oceans would be possible if the amount of O_2 in the atmosphere were much lower than at present. Establishment of a stable oxygenic environment during the early Proterozoic is compatible with palaeobiological observations (Schopf, 1979). Initiation of *widespread* sulfate-reduction at ca. 2.3 Gyr B.P. (Lambert, 1978; Donnelly and Lambert, 1979) is consistent with the absence of widespread oxidising environments before this time.

Indus River Uraninite

The presence of minor amounts of detrital uraninite in the Indus River (Zeschke, 1961) led several authors (e.g. Simpson and Bowles, 1977) to discount the large Precambrian uraninite deposits as evidence for an O_2-deficient environment. However, the Indus River occurrence is two to three orders of magnitude smaller than the Blind River occurrence, and is found in a mountainous area in one of the most tectonically active regions of the world. This area contains *no* weathering mantle and has abundant glacial deposits (Snead, 1972). Under such conditions, one can easily visualize unweathered uraninite being incorporated into a stream where oxidation reactions would be extremely sluggish. On the other hand, the source of Huronian rocks appears to have had gentle relief (Frarey and Roscoe, 1970). Tectonic activity was comparatively mild and mainly related to fault movements (Roscoe, 1969). Furthermore, the uraninite deposits of the Huronian succession appear to have been derived from an intensely weathered source (Pienaar, 1963; Roscoe, 1969; Robertson, 1976). It seems most unlikely that uraninite (or pyrite for that matter) could withstand such conditions in an oxidising environment.

Timing of Oxygen Introduction

The timing of O_2 introduction is most important. Careful examination of the Lower Proterozoic Huronian succession of Canada is instructive. The base of the Huronian is marked by high Fe^{2+}/Fe^{3+} palaeosols (see above) and extensive U-pyrite conglomerates. Calcites of the Espanola Formation contain abundant Fe which cannot easily be explained by diagenetic or metamorphic controls (McLennan *et al.*, 1979b). Lower Huronian sandstones and conglomerates typically contain pyrite-rich heavy mineral assemblages (Roscoe, 1973). These observations indicate an O_2-deficient environment. As pointed out above, Lower Huronian rocks are invariably drab coloured even though depositional environments are such that red beds could be preserved (Roscoe, 1969). On the other hand, Upper Huronian sedimentary rocks (Cobalt Group) display heavy mineral suites typical of young sedimentary rocks. Red coloured rocks are found in all units of the Cobalt Group (Roscoe, 1969). This colouration does not appear to be inherited and probably formed in situ (Roscoe, 1969). If these characteristics are recording a significant influx of O_2, then it seems likely that it occurred near the lower boundary of the Cobalt Group.

The Huronian lies unconformably on Archaean basement and is thus younger than about 2.6 Gyr. It is clearly older than about 2.16 Gyr (all Rb-Sr dates have been recalculated using $\lambda Rb^{87}=1.39\times10^{-11}yr^{-1}$), since it is cut by diabase of this age (Fairbairn *et al.*, 1969). Sedimentary rocks of the Gowganda Formation (Cobalt Group) have been dated at about 2.3 Gyr B.P. (but with a large error; Fairbairn *et al.*, 1969). Oxygen was clearly introduced before 2.16 Gyr B.P. and a tentative age of greater than 2.3 Gyr B.P. (but less than 2.6 Gyr) seems consistent with all the data. This age is significantly older than the usually quoted age of 2.0 Gyr B.P. Roscoe (1973) quoted a date of 2.3 Gyr B.P. using arguments similar to those given above.

PRECAMBRIAN IRON FORMATIONS

Precambrian iron formations have been traditionally divided into two broad categories based on age, lithological and tectonic association and size (Gross, 1965). Algoman-type iron formations were generally deposited in unstable volcano-sedimentary basins (greenstone belts) of Archaean age, in association with turbidites. These deposits are typically smaller and less continuous than Superior-type deposits. Superior-type iron formations are typically represented by the immense Early Proterozoic deposits familiar

to most Precambrian geologists. These rocks were deposited in stable epicontinental basins and are commonly associated with continental sedimentary rocks such as carbonates. The iron formations, themselves, are usually almost entirely devoid of a clastic component, attesting to the stable nature of the basins. Individual deposits are at least an order of magnitude larger than most of their Archaean counterparts. Iron formations of Late Precambrian age, such as the Rapitan iron-formation of north-west Canada, have not been emphasized previously by geologists but are now known to be quantitatively important (Young, 1976; Yeo, 1977; Delaney *et al.*, 1980). These iron formations are much different than those deposited during the earlier Precambrian and deposition from upwelling hydrothermal solutions has been suggested (Delaney *et al.*, 1980).

Distinguishing Algoman and Superior iron formations on the basis of age and other criteria no longer seems valid. Previously, several authors had argued that the Superior-type iron formations were deposited over a very brief interval of between 2.1 and 2.0 Gyr B.P. (Cloud, 1973; Goldich, 1973). Recent U-Pb zircon dating of volcanic and tuffaceous units of the Hamersley Basin indicate an age of deposition, for the iron formations, of about 2.49 Gyr B.P. (W. Compston *et al.*, unpublished results). This is substantially greater than previously accepted dates of 2.2-2.0 Gyr B.P. If the correlations between the Hamersley and Transvaal Basins (Button, 1976a) are correct, then a much older age is indicated for the South African iron formations. Examination of iron formations in the Archaean Gorge Creek Group of the Pilbara region led Fitton *et al.* (1975, p.14) to note, "The lateral persistence and order of thickness of this iron and chert group compare with those of the similar lower Proterozoic, Hamersley Group ....".

The minimum age of deposition for the large Early Proterozoic iron-formations is not well established. Dating of the iron formations of the Lake Superior region is plagued with uncertainties (Goldich, 1973). Conservative interpretations suggest deposition at between 2.2-1.9 Gyr B.P. (Goldich, 1973). Iron formations in the Labrador Trough, of eastern Canada, may be the youngest of the Superior-type. Fryer (1972) has given an age of deposition of about 1.85 Gyr B.P. Goldich (1973) presented evidence suggesting this is a metamorphic age but could only confine deposition to between about 2.2 and 1.9 Gyr B.P.

Understanding of the ages of iron formations is clearly inadequate and several interpretations are still viable. It seems fairly clear that deposition of very large iron formations commenced during the Archaean.Deposition, in various basins, probably continued until at least 2.0 Gyr and perhaps until 1.85 Gyr B.P.

DISCUSSION

It seems fairly well established that the early Precambrian atmosphere and hydrosphere were deficient in free oxygen. Exact levels of O_2 are not known but *upper* limits of pO_2 of 10^{-7}-10^{-3} atm may be set for the atmosphere on the basis of rates of uraninite oxidation (Grandstaff, 1974). Significant quantities of O_2 were introduced into the atmosphere-hydrosphere system between 2.6-2.3 Gyr B.P. This is significantly before the often quoted age of 2.0 Gyr B.P. The precise amount of O_2 added is not known: however, it was enough to allow frequent production of oxidised species. Radiometric ages indicate that very large (Superior-type?) iron formations occurred well before, and well after the time of O_2 introduction.

With the present understanding of Precambrian crustal, atmospheric and hydrospheric evolution, a tentative hypothesis for the development of early Precambrian iron formations can be proposed. Prior to about 2.3 Gyr B.P., under conditions of very low pO_2 in the deeper oceans, dissolved Fe^{2+} could have been present in appreciable quantities (Holland, 1973). At this time, precipitation of iron formations (mainly, but not exclusively, magnetite-chert) commonly occurred. Owing to the unstable nature of depositional sites at this time (greenstone belts), these iron formations were typically relatively thin, of small lateral extent, and restricted to breaks in sedimentation and volcanism (Dunbar and McCall, 1971; Beukes, 1973). Rarely, when large, relatively stable basins formed during the Archaean, such as in the Pilbara of Western Australia, extremely large iron formations, comparable to Lower Proterozoic examples, could accumulate (Fitton *et al.*, 1975).

Near the end of the Archaean (earlier in South Africa), a dramatic crustal event occurred

which resulted in a fundamental change in crustal composition. This was also a period of major crustal growth (Veizer and Jansen, 1979). Probably coeval with this, the continents stabilized on a large scale allowing the formation of large, relatively stable epicontinental basins. As these basins developed, major accumulations of iron formation could occur during periods of minimal clastic influx. Several models, such as those of Holland (1972), Drever (1974) and Button (1976b), seem to adequately explain the depositional processes. In general, conditions were analogous to carbonate and evaporite deposition.

Sometime before about 2.3 Gyr B.P., significant amounts of O_2 were introduced into the atmosphere and hydrosphere, resulting in an unstable ocean chemistry due to the presence of large quantities of reduced iron. The amount of Fe^{2+} added to the oceans decreased significantly after this time (Garrels *et al.*, 1973). Deposition of large iron formations continued after the introduction of O_2 until the large ocean reservoirs of Fe were extracted. The time span of this activity could have been as much as 300-450 Myr. After about 2.0-1.85 Gyr B.P. there was insufficient dissolved iron in the oceans (or it was at too great a depth) to allow further development of such deposits.

Such a scenario seems compatible with the lithological associations observed for the Precambrian. It also is in agreement with available geochemical, isotopic age and geological constraints on the timing of crustal evolution, O_2 evolution and iron formation development.

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CHEMICAL CONDITIONS FOR THE PRECIPITATION OF BANDED IRON-FORMATIONS

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INTRODUCTION

Of all the geological sources of evidence that have been quoted as to the composition of the atmosphere and surface waters in the Archaean and early Proterozoic, banded iron-formations (BIF) deserve special attention. Not only to they occur in vast quantities and widely distributed on the continents, not only are they virtually restricted to rocks older than about 2 Gyr, but they are chemical sediments that exhibit extraordinary lateral continuity in individual bands, and also sudden changes in composition from one band to another that must ultimately depend on simultaneous changes in the composition of the water from which they formed.

The data presented here relate to the Dales Gorge Member of the Brockman Iron Formation. Much of it comes from the work of A.F. Trendall, and some from the author's laboratory.

Geological Setting and the Composition of the Rocks

Trendall and Blockley (1970) give a full account of the setting of the Dales Gorge Member in the lower Proterozoic Hamersley Group; its outcrop is indicated in the locality map (Fig. 1). It contains 17 BIF macrobands, ranging in thickness from a few to about 15 m, characterized by the presence of magnetite and comprising mainly chert and iron oxides with minor carbonates and silicates. These alternate with 16 generally thinner "S" macrobands, which contain some shales but also chert and siderite. This stratigraphy is shown in Fig. 2. The same nomenclature will be used in this paper but it should be emphasized that much of the material comprising the S macrobands is also a banded chemical sediment containing more than 15% Fe. Within the BIF macrobands there are two finer scales of banding, defined by Trendall (1965) as mesobands and microbands. Microbands, which consist of an alternation of a silica-rich and an iron-rich layer, range in thickness from less than 1mm up to about 2mm, and there may be many of them, usually of uniform thickness, in a single chert mesoband.

There is considerable variety of mesoband types with a wide range in the proportions of the main components giving on a millimetre scale extreme contrasts in composition. When average compositions of whole macrobands are determined, however, values are obtained that are typical of BIF and S macrobands respectively. Such averages derived by Trendall and Pepper (1977) from Wittenoom and by Ewers and R.C. Morris (unpublished results) from Paraburdoo show remarkable similarity.

The averages do, however, obscure variational trends in the composition of BIF macrobands adjacent to the neighbouring S bands. This is shown in Fig. 3 in which selected components are compared with total iron, as Fe_2O_3, through the stratigraphic interval from the top of BIF 16 to the top of S11. These are based on analysis of short lengths of a core through fresh BIF from Paraburdoo (Ewers and Morris, unpublished results). It will be noted that CO_2, MgO, Al_2O_3 and TiO_2 are consistently more abundant in S bands and that the last two of these vary in sympathy with one another. The "shoulders" of increased CO_2 in BIF above and below S bands is mainly due to an increase in siderite at the expense of iron oxides.

THE SUPPLY OF IRON

Trendall analysed 22 chert mesobands and found that the average amount of iron in 1 cm^2 of each microband ranged from 8 to 43 mg Fe with an overall average of 22.5 mg. He suggested that the most probable depositional period corresponding to each microband, that is to each alternation of a silica-rich with an iron-rich lamina, was 1 yr and on this rate of sediment accumulation calculated that the whole Dales Gorge Member (142 m thick) would

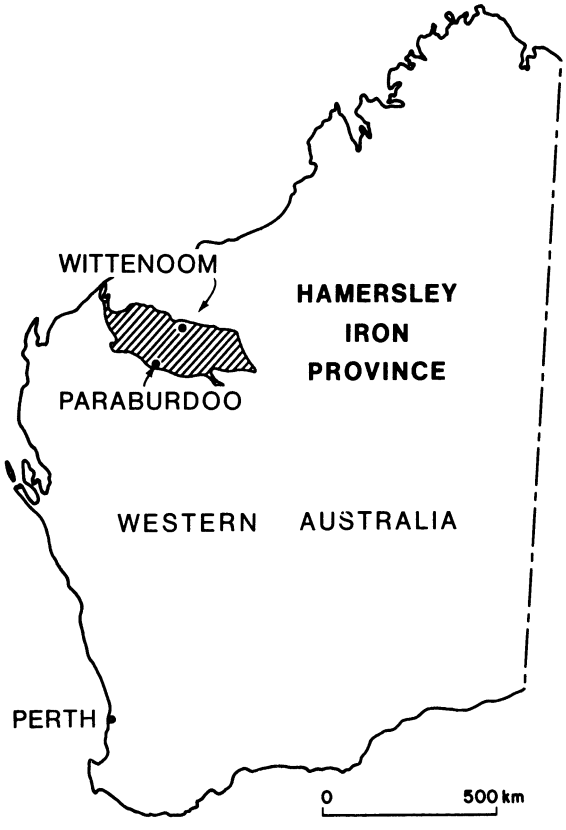

Figure 1: Locality map showing the minimum area of deposition of the Brockman Iron Formation.

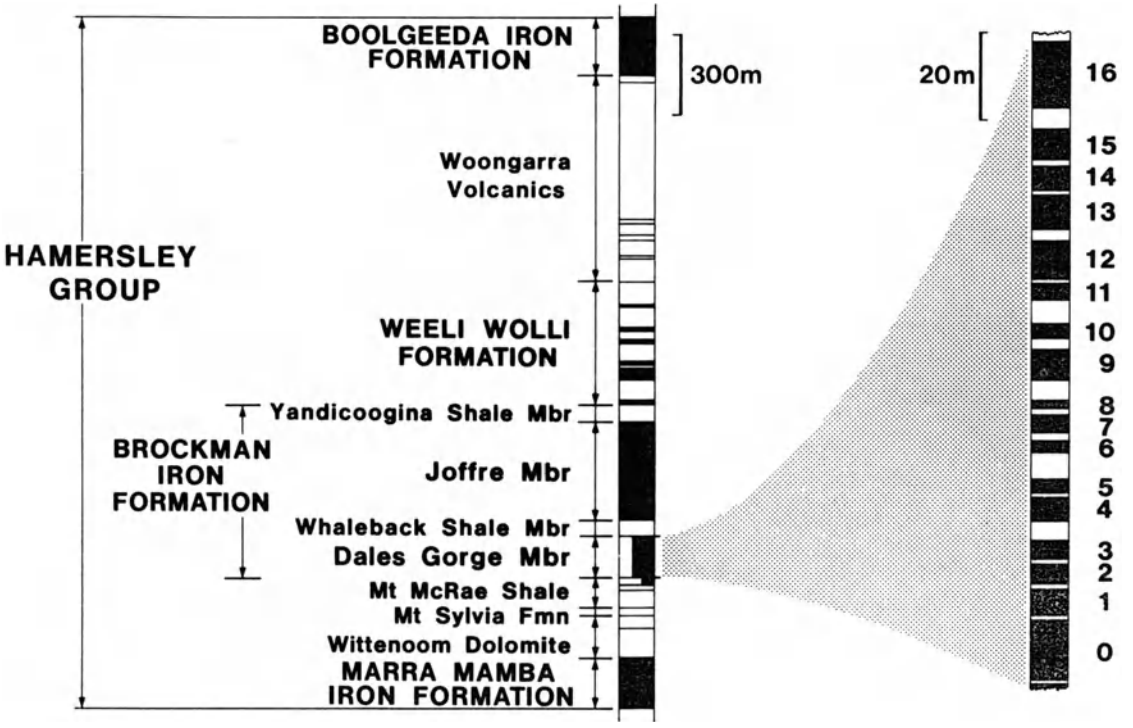

Figure 2: Stratigraphic column of the Hamersley Group showing the Brockman Iron Formation and the detail of the Dales Gorge Member. Modified from Trendall and Blockley (1970).

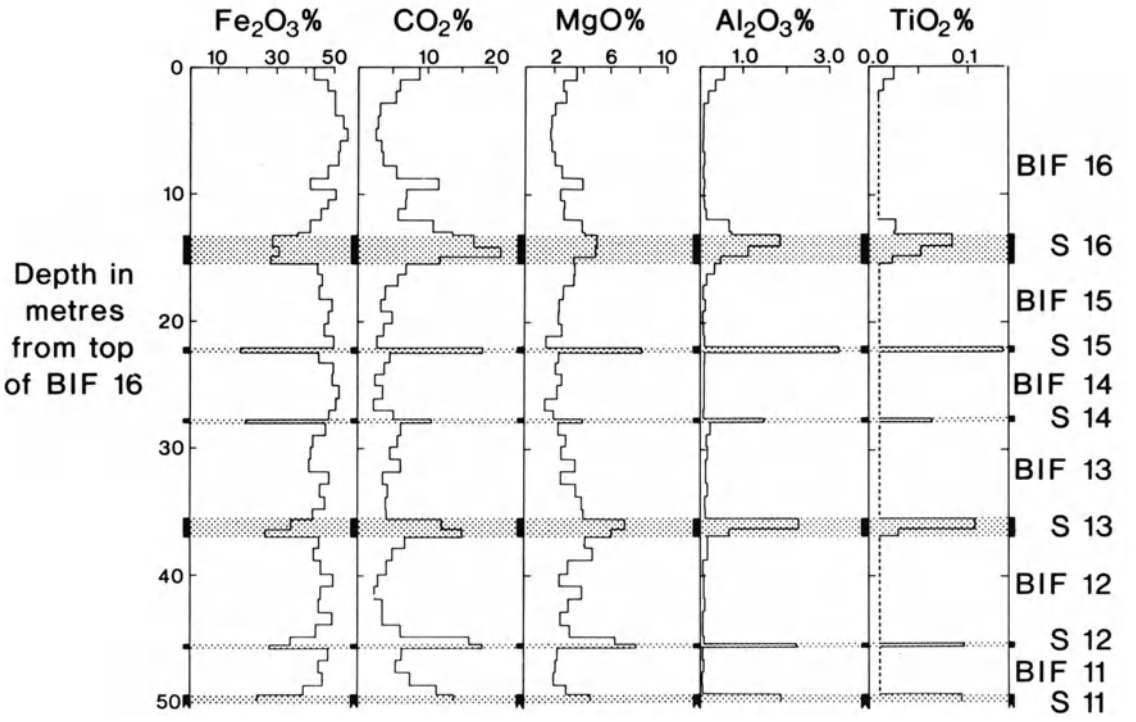

Figure 3: Compositional variation through the upper 11 macrobands of the Dales Gorge Member. Based on analyses of core 75/1 from Paraburdoo.

have deposited in about 0.6 Myr. Another calculation with different assumptions gave an estimate of 1.6 Myr (Trendall 1972). If we accept that microbands are true varves then we must accept that each year the body of water which gave rise to the sediments yielded, on average, 22.5 mg Fe for every cm^2 of the total area of deposition - probably at least 100,000 km^2.

Without entering into the question as to whether the sediments deposited in a lake, a closed basin, an open basin or a shelf open to the sea, some idea can be gained by simple arithmetic of the reciprocal relationship of amount of water and concentration of iron required to meet this required supply. If we make the quite improbable assumptions that the deposition occurred in a static basin of uniform depth and that all of the iron was precipitated each year and then replenished for the next year, we arrive at the numbers in Table 1. The depths given in Table 1 are simply the lengths of columns of 1 cm^2 cross section to give the required volume to be delivered by whatever flow process of each cm^2 each year.

TABLE 1

VOLUME-CONCENTRATION RELATIONSHIP FOR 22.5mg Fe
TO BE PRECIPITATED FOR EACH cm^2

Concentration Fe		For each cm^2 of deposit	
		Volume	Effective Depth
μM	mg l^{-1}	l	m
1	.056	402	4020
10	.56	40.2	402
100	5.6	4.02	40.2
1000	56	.402	4.02

If, as is most likely, only a fraction of the total iron was precipitated from the body of water in any one year, for any given concentration the volumes and effective depths would need to be increased correspondingly. Presuming, whatever the mechanism, that precipitation originated near the surface, the notion of effective depth should also include an element of access by circulation to the surface zone. Even so, effective depth should probably be assumed to be tens rather than hundreds of metres so that the average concentrations of iron would need to have been at least 5 mg l^{-1} and probably higher.

How was the iron carried in solution? Reactions with silicate and carbonate rocks deny the possibility that such a large body of water could have been sufficiently acid ($pH \leq 2.2$) for iron(III) to reach the required concentration in solution either as the free ion or as a complex ion. Quite concentrated colloidal solutions of iron(III) hydroxide can be prepared in the laboratory but their stability is dependent on the absence of the electrolytes that would be expected in natural waters. Mel'nik (1973) has summarized the literature on iron(III) colloids and has properly decided that they are not relevant to this transport problem. Another possibility is that iron(III) hydroxide flocs, precipitated when acid solutions of volcanic origin (see Zelenov, 1958) oxidized and hydrolysed on admixture with more alkaline water, were widely distributed by currents. Modern examples of this behaviour are discussed by Mel'nik (1973) and they are seen as essentially local phenomena. It is most unlikely that mechanical distribution of flocs could have produced the remarkable lateral continuity and relative uniformity of thickness of the banded iron-formations over the whole of the Hamersley Iron Province.

One is led then, as others have been led before, to the conclusion that iron was distributed in true solution and as divalent iron. The limits on Fe^{2+} solubility are set by the redox conditions (here expressed as fugacity of oxygen, f_{O_2}), by pH and by activities (a_x) of such precipitants as sulfide, silicate and carbonate, none of which can be considered independently of the others. Complexing of iron(III) with ligands is relatively unimportant, but the $Fe(OH)^+$ complex contributes to total solubility at higher pH values.

The solubility relationships relevant to the transport of Fe^{2+} are summarized in Fig. 4, based on an attempt to set realistic limits on the area on an f_{O_2}-pH diagram in which Fe^{2+}_{aq} can exist at activity ≥ 0.1 mM. Allowing for an activity coefficient of about 0.26,

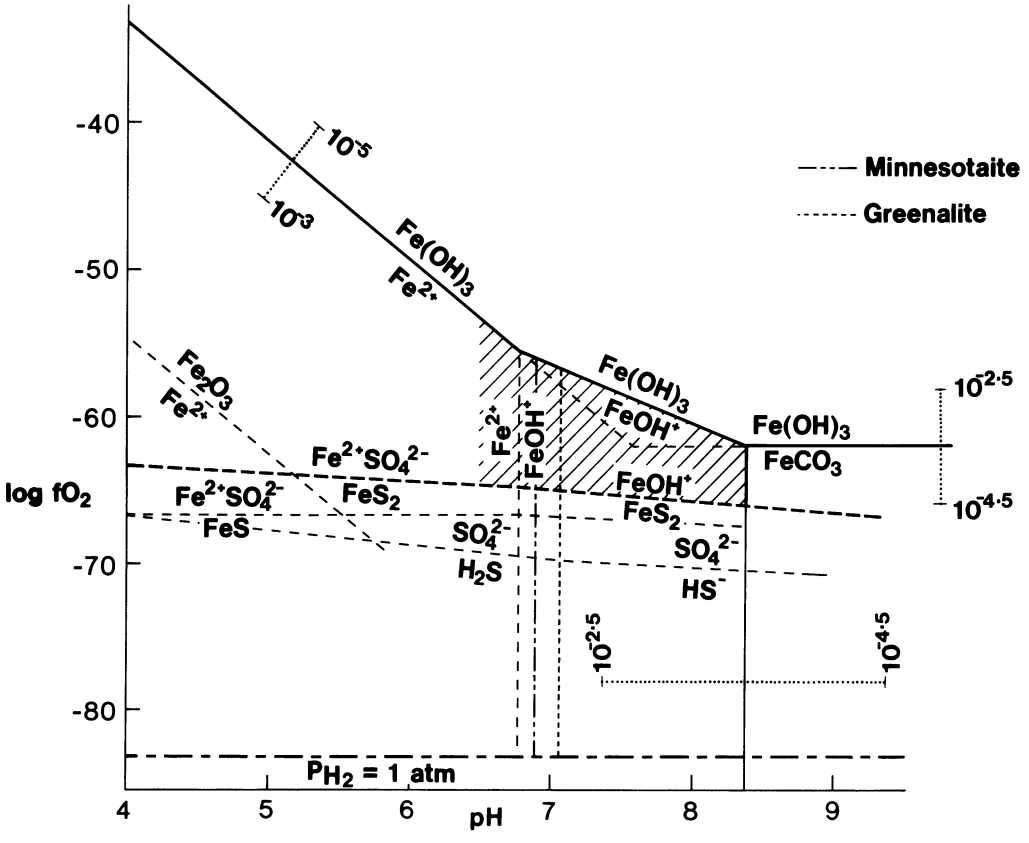

Figure 4: pH/f_{O_2} diagram defining the limits for Fe^{2+}_{aq} to exist in aqueous solution at an activity ≥0.1 mM at 25°. Based on data of Wagman *et al.* (1969), and assuming $a_{SO_4}{}^{2-} = 10^{-2.76}$M, $f_{CO_2} = 10^{-3.5}$ atm. The bar across the boundary Fe^{2+}, $Fe(OH)_3$ indicates shifts when $a_{Fe}{}^{2+}$ ranges from 1-0.01 mM. Bars on boundaries of $FeCO_3$ field show shifts when f_{CO_2} ranges from $10^{-2.5}$ to $10^{-4.5}$ atm.

this activity limit corresponds to a concentration of ≥21.5 mg l^{-1}, which, on the basis of the above discussion, I would suggest is a plausible estimate of what would be needed to supply the required amount of iron. In calculating Fig. 4 the activity of SO_4^{2-} has been taken as $10^{-2.76}$M and the fugacity of CO_2 (f_{CO_2}) as $10^{-3.5}$ atm.

This diagram represents a deliberate compromise between equilibrium thermodynamics, what can be deduced from the chemical and mineralogical compositions of the rocks, and what is known about the ability of the systems to achieve equilibrium. The solid lines represent observable reactions of Fe^{2+} (and $FeOH^+$) to form the metastable "precipitated $Fe(OH)_3$" and siderite, $FeCO_3$; the solid products of these reactions could have been primary components of the sediments. Dashed lines, on the other hand, represent equilibria which would be relevant only when conditions applied that allowed theoretical equilibrium to be achieved. Thus there is no evidence to suggest that either hematite or magnetite formed directly as a primary phase. Hematite, the more stable of the two at 25° at all oxygen fugacities above $10^{-69.5}$ atm is shown in Fig. 4. Magnetite is omitted in contrast to the treatment by Klein and Bricker (1977) who, as pointed out by Frost (1978), gave quite undue prominence to this phase. Klein and Bricker (1977, p. 1462) invoked "a poorly crystallized hydrated magnetite type phase" but, as they have pointed out, its relationship to $Fe(OH)_3$ would probably be similar to that of magnetite to hematite and would not have altered Fig. 4. One cannot, however, accept Frost's claim that the solubility relationships of hematite are relevant to the precipitation of BIF: it is clear from Fig. 4 that, if hematite controlled the solubility of Fe^{2+}_{aq}, siderite could not have formed. Other evidence can be drawn from the mineral assemblage in the BIF-macrobands. Thus, although traces of barite in BIF support the assumption that sulfate was present in solution, pyrite would have been more plentiful if conditions had existed for Fe^{2+}_{aq} solubility to be limited by the presence of sulfide. In fact, pyrite is sparse in the BIF macrobands. The silicate equilibria, either with minnesotaite, $Fe_3Si_4O_{10}(OH)_2$ (after Mel'nik, 1973), or with greenalite, $Fe_3Si_2O_5(OH)_4$ (after Eugster and Chou, 1973), are more problematical. These silicates occur in BIF but one expects much greater supersaturation in silicate systems than in carbonates and, in any case, silicate formation does not appear to have precluded siderite formation.

These considerations lead to a probability that the pH and f_{O_2} conditions of the solution from which BIF formed lay in the hatched area of Fig. 4, probably close to the boundary with the siderite field, and possibly, though not necessarily, above that for pyrite.

There is a considerable uncertainty as to pH. Analogy with modern oceans and their silicate buffer system would suggest a pH near 8. However there are conflicting data on the change in free energy for the reaction:

$$Fe^{2+}_{aq} + H_2O \rightarrow FeOH^+_{aq} + H^+_{aq}$$

(cf. data of Wagman *et al.*, 1969 and Langmuir, 1969). If the data selected by Langmuir are shown to be correct, the pH for the boundary of the siderite field in Fig. 4 would move to pH 7.55.

Neither can one be certain of a realistic value of the concentration of ferrous iron. Mel'nik (1973) proposed 100 to 400 mg l^{-1}, Holland (1973) 3 mg l^{-1}, and I have suggested here about 20 mg l^{-1}. It is instructive to calculate the potential reservoir of iron represented by these concentrations throughout a world ocean assumed to be of the same bulk as the modern world ocean. The amounts are 512 Eg Fe using Mel'nik's upper figure and 3.84 Eg using Holland's. These compare with an estimated total iron of about 50 Eg in the Brockman Iron Formation alone. An oxygenated atmosphere would not have allowed these concentrations to accumulate, and, if they had accumulated, circulation of water even from ocean deeps (ca. 10^3 yr residence time in modern oceans (Broecker, 1971)) would have brought much of the iron to the surface and caused it to precipitate in a relatively short time. It seems inescapable that the atmosphere must have been anoxic, and also that the supply of soluble iron was being replenished, possibly from local sources.

THE PROCESS OF DEPOSITION

Starting with iron present in solution as Fe^{2+} or $FeOH^+$, with pH and f_{O_2} described by the shaded area in Fig. 4, a change in these conditions toward more oxidizing conditions would cause $Fe(OH)_3$ to precipitate, whereas a change towards higher pH would precipitate carbonates.

The BIF-macrobands are characterized by iron oxides that are the final product of oxidizing Fe^{2+} to Fe^{3+} followed by hydrolysis to precipitate a hydrous hydroxide gel. Some silica will inevitably be coprecipitated in this gel (Harder, 1964; Harder and Fleming, 1970), and additional silica could be precipitated by a variety of processes that do not involve oxidation and will not be considered here.

The oxidation of Fe^{2+} could have occurred by two different mechanisms:

(1) as a by-product of photosynthesis (Cloud, 1973)
and (2) by inorganic photochemical oxidation (Cairns-Smith, 1978).

These two mechanisms are not mutually exclusive. Each has attractive features and some difficulties which suggest that they may indeed be complementary.

Carbon dioxide is essential to photosynthesis which would be favoured by an $f_{CO_2} \geq 10^{-3.5}$ atm, high enough in fact to restrict the solubility of Fe^{2+}. A low f_{CO_2} would enhance solubility of Fe^{2+} and is not precluded by the photochemical process. Carbon which is probably organic (Becker and Clayton, 1972) is observed in the S macrobands and is sometimes associated with high phosphorus (Ewers and Morris, unpublished results). The absence of carbon in BIF macrobands could be explained by reaction with $Fe(OH)_3$ to form magnetite and siderite.

The second mechanism suggested by Cairns-Smith (1978) was based on photo-oxidation of Fe^{2+} and the concomitant evolution of hydrogen in solutions irradiated with UV light in the range of wavelength 200-300nm. The experimental demonstration of this process has been restricted to acid solutions (pH $\lesssim$ 3) by experimenters who evidently wished to avoid precipitation of $Fe(OH)_3$ (Jortner and Stein, 1962). On the other hand, adsorption of UV by ferrous iron has been demonstrated by the oxidation of ferro-cyanide, $Fe(CN)_6^{4-}$, at pH 11.8 in the presence of a suitable electron scavenger (Airey and Dainton, 1966). The experiments with Fe^{2+}_{aq} at pH 4 to 8 need to be done.

The light wavelengths required for photo-oxidation are in the part of the ultra-violet range that is almost entirely filtered out by O_2 and O_3 in the present atmosphere. An anoxic atmosphere is essential therefore, but, given that, Cairns-Smith (1978) has calculated that more than sufficient light of the required wavelength, and at measured quantum yields, would be available to oxidize Trendall's 8-43 mg Fe cm^{-2} yr^{-1}. The absorption of UV light by Fe^{2+} in solution could have provided an effective UV screen for micro-organisms so that, in this respect, photo-oxidation and photosynthesis may indeed have been complementary.

It is noteworthy that, whereas the oxidation of Fe^{2+} to Fe^{3+} results in consumption of H^+, its subsequent hydrolysis and precipitation releases H^+ and there is a nett gain of $2H^+$ for each Fe^{2+} precipitation as $Fe(OH)_3$. Similarly precipitation of Fe^{2+} or Mg^{2+} as carbonate or silicate releases acid, so that the formation of chemical sediments will tend generally to lower pH. This effect was presumably countered by inflow of silicate-buffered water.

It has been suggested by several workers that the S-bands represent interruptions to BIF deposition caused by volcanic events (La Berge, 1966; Trendall and Blockley, 1970). La Berge recognized strata containing volcanic shards within the S-bands and transitional zones below and above S-bands which he ascribed to the onset and decline of volcanic activity. We have found that Al_2O_3 and TiO_2, in almost constant proportion to one another, show a distribution that can be interpreted in the same way (Ewers and Morris, unpublished results). These components are unlikely to be transported in solution and will have been quickly incorporated in the sediments after their addition to the body of water. Trendall and Blockley summarized the arguments against the S-macrobands being deposited normally as clastic silts, and it seems most likely that the shaly parts of the S bands represent an addition of volcanic dust. Perhaps the best evidence, however, is the effect that this material had on the style of precipitation of other components. Whereas water-borne terrigenous sediments would be relatively inert chemically, the addition of finely divided silicate glass to an electrolyte solution would inevitably increase pH as cations such as Na, K and Mg are dissolved and the silica formerly bonded to them forms $Si(OH)_4$ or SiO_2. All of the chemical precipitation in the S bands is consistent with an increase in pH and/or f_{CO_2}, the solution composition in Fig. 4 moving to the right into the siderite field. Increased pH would also increase

supersaturation with respect to silicates of iron and presumably of magnesium. The carbon found in S bands probably represents pelagic organisms brought down with the dust, and sulfate incorporated in the sediments is reduced, possibly by sulfate-reducing bacteria in the presence of this organic material, to give an average 0.3% of sulfur as pyrite in the S bands.

Less obvious is the cause for the apparent cessation of oxidative precipitation of iron, but the general absence of oxides containing Fe^{3+} in the S bands could be the result of reaction of precipitated $Fe(OH)_3$ with organic matter. On the other hand chemical precipitation in the more alkaline medium may have been more rapid than that of the BIF and may have caused periodical depletion of iron at least from the surface layers in which either photosynthesis or photo-oxidation would operate.

SUMMARY

This chemical study of the Dale's Gorge Member with its interesting alternation of BIF bands and S bands suggests a continuum of precipitation in which the alternation of the major macroband types was caused by pH variation resulting from protracted additions of reactive volcanic dust. In interludes without major volcanism, BIF precipitation proceeded with iron(II) in solution being oxidized either photosynthetically or photochemically. Such a model requires a vast and periodically replenished reservoir of Fe^{2+} and a connection to the area of precipitation such that the iron was maintained at a reasonable concentration (perhaps 20 mg l^{-1}) over a long period. One necessary element of an environment for this to occur would have been an essentially anoxic atmosphere, with the water at low oxidation potential and at a pH such that siderite was close to saturation. Acidity released by the precipitation of iron hydroxide and of iron and other carbonates and perhaps silicate, must have been buffered by circulation of silicate buffered water.

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ENVIRONMENTAL BIOGEOCHEMISTRY

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INTRODUCTION

With the holding of the Fourth International Symposium on Environmental Biogeochemistry in Canberra, Australia, the field of biogeochemistry can perhaps be said to have come of age. It is the purpose of this article to provide a brief overview of this burgeoning field, with the aim of indicating some of the general principles and practical applications.

If biochemistry is the study of the chemistry of living organisms, and geochemistry is the study of the chemistry of the earth, what then is *biogeochemistry*? Is the emphasis chemical, as in biogeoCHEMISTRY, geological, as in bioGEOchemistry, or biological, as in BIOgeochemistry? Actually, biogeochemistry is at once all and none of these. Clearly it is an interdisciplinary field, and as such immediately arouses suspicion from firm disciplinarians. The biogeochemist might be looked upon suspiciously as one who is capable of doing nothing well, and therefore does a lot of stuff poorly, compensating for this by spreading activities over a wide area. Although this idea might seem reasonable, we can perhaps derive some comfort from the fact that at one time Biochemistry was also an interdisciplinary field, but has now become a discipline in its own right, with journals, societies, and university departments named after it. Fortunately or unfortunately, biogeochemistry does not yet have this baggage to contend with, and is free to develop in whatever direction those carrying its banner choose.

What then is *biogeochemistry*? The term was first coined by the Russian geochemist, W.I. Vernadsky (1939), and was introduced into the English language by G. E. Hutchinson (1943). Much of the early work on biogeochemistry concerned the accumulation of trace elements by organisms, and on a comparison of element ratios of organisms with their environments. The work was primarily analytical, and was rather static in its approach. Biogeochemistry became a dynamic subject, and one capable of developing a real following, when it became *environmental biogeochemistry*. Because in environmental science we are concerned with the rates at which processes take place, environmental biogeochemistry is greatly concerned with chemical transformations which are taking place, and especially with those transformations that are catalyzed by living organisms. And it is because of the critical role of living organisms as catalysts of chemical reactions that biogeochemistry is of such great practical importance. Chemical reactions that might occur at exceedingly slow rates spontaneously may occur at rates one million or more times faster if an appropriate living catalyst is present and functional.

GLOBAL ASPECTS

In recent years it has become fashionable to think and talk about global chemical cycles, and numerous conferences and books have appeared on this subject. Although many aspects of global chemical cycles can be considered in the complete absence of biological influence, whenever a *volatile* component is involved, biological influences become important, often even paramount. Actually, in most discussions of geochemical transformations, two kinds of cycles are often intermixed, which I call the *redox* and the *transport* cycles. A redox cycle is a cycle based on changes in the oxidation state of an element. Many elements have more than one stable oxidation state, and some, such as carbon, nitrogen, and sulfur, can have many oxidation states. The most oxidized form of the element can undergo stepwise reduction, and the most reduced form can undergo stepwise oxidation. A cycle can thus be described, with the most oxidized and most reduced forms at the nadir and zenith, and intermediate oxidation states at the equators. Such a cycle is actually only a conceptual cycle, since it would never be the case that all of the transformations would take place at a single place at a single time. In addition to the redox cycle, a *transport* cycle can also be defined, which describes the movement of an element from one place to another on earth, as for instance from land to air or air to water. Such a transport cycle

may or may not also involve a redox cycle. For instance, when oxidation or reduction of an element leads to the conversion of a nonvolatile substance to one that is volatile, the latter can then be transported to the air, so that the transport cycle is coupled to the redox cycle. In other cases, oxidation or reduction does not lead to a change in state, and has no influence on transport. It is clear that for those elements which have volatile oxidation states an understanding of the redox cycle will be of great importance for predicting the extent and nature of the global transport cycle. It is probably for this reason that so much current interest in biogeochemistry has developed.

Biological and Nonbiological Reactions

Living organisms are only catalysts of chemical reactions. A chemical reaction will not occur in the presence of an organism that would not also occur in its absence, although the rates under the two conditions may be vastly different. In fact, it is well known that many important biogeochemical reactions will occur at quite significant rates under strictly chemical conditions. One of the tasks of the biogeochemist is to sort out chemical from biochemical reactions in natural systems. How can this be done?

It is sometimes possible to make inferences from analyses of the products being formed (using knowledge gained from studies on other systems), or from the measurement of the fractionation of stable isotopes (for instance, $^{12}C/^{13}C$ or $^{32}S/^{34}S$), making the assumption that since fractionation is primarily a low-temperature process, it is likely that it will only have occurred at a significant rate in nature if it is catalyzed by a living organism. For many geochemical reactions, the role of living organisms is virtually obvious, such as the importance of terrestrial green plants in carbon dioxide fixation (photosynthesis). However, many geochemical reactions occur in hidden habitats, such as soils, sediments, ocean waters, the depths of lakes, and it is not obvious whether the reaction in question involves the activity of a living organism or not. In most of these hidden habitats, the organisms involved are microorganisms and, for this reason, much of biogeochemistry is really microbial biogeochemistry. It is for this reason that a number of ecologically-oriented microbiologists have undergone a transformation into biogeochemists.

In summary, then, biogeochemistry can be considered to be the field that deals with chemical transformations in nature, especially in those habitats and under those conditions that are of interest to the geochemist. It deals to a great extent with the kinetics of chemical reactions, although thermodynamic considerations are also involved. The biogeochemist is often microbially oriented, because so many of the reactions of interest are catalyzed by microorganisms. The biogeochemist must be (among other things) a skilled analyst, an experimentalist, a field-oriented ecologist, and a visionary. In addition, although the interest is primarily in the present, the past cannot be ignored, because in many ways, and to turn around a well-worn phrase, the past is a key to the present. Thus, evolution and the origin of life are two other topics that come under the purview of the biogeochemist.

BIOLOGICAL INTRODUCTION

Life seems infinitely diverse, and biologists spend a lot of time trying to simplify this diversity so that it can be handled in research studies in a reasonable way. One broad generalization is the distinction between procaryotic and eucaryotic organisms. Procaryotic organisms are more commonly known as bacteria (including the blue-green algae, now called the Cyanobacteria), and constitute the organisms with the simplest cell structures. It is commonly believed that the procaryotes arose first on earth, and were the forerunners, directly or indirectly, of the eucaryotes. Eucaryotes are the group which contains all of the higher organisms, including green plants and man, although many simple organisms (e.g. protozoa, algae) are also eucaryotes.

Unique Features of Life

It is because of the uniqueness of living organisms that a discipline such as biogeochemistry arose in the first place. What are the unique features of living organisms, considered from the viewpoint of the geochemist? Three major categories can be listed: stereospecific catalysis, membrane transport, self-reproduction. We have already indicated that a critical property of living organisms, from a geochemical viewpoint, is their ability to greatly speed up the rates of many chemical processes. The catalysts of living

organisms are enzymes, and one of the features of enzyme catalysis is that it is usually stereospecific. Significantly, biological catalysis is not only rapid and effective, but occurs at low temperatures. Low-temperature reactions are of great geochemical significance, because most of the earth is at a low temperature, and many important reactions would probably not occur at all (or at least, at very minimal rates) if it were not for living organisms.

Unique Features of Procaryotes as Geochemical Agents

Although all microorganisms present properties that make them of critical importance biogeochemically, the procaryotic microorganisms are especially significant. This uniqueness arises partly because of the great genetic pliability of procaryotes, and partly because they are capable of carrying out many biochemical reactions that are carried out poorly or not at all by eucaryotes. In addition, a number of procaryotes are uniquely tolerant to extreme environments such as high temperature and low pH, and are consequently able to catalyze geochemical reactions under conditions where reactions might otherwise not occur. A number of important biogeochemical reactions are only catalyzed by procaryotes. These include a number of reductive processes, such as carbon dioxide reduction to methane, dissimilatory sulfate reduction to hydrogen sulfide, and nitrogen gas reduction to ammonia. In addition, certain oxidative reactions are carried out primarily by procaryotes, including the oxidation of methane to carbon dioxide, oxidation of sulfide minerals and elemental and sulfur to sulfuric acid. A number of important photochemical reactions are also catalyzed primarily by procaryotes, including the anaerobic oxidation of sulfide and elemental sulfur to sulfate, reactions that in the dark will only occur if a highly oxidised electron acceptor is present. Because of the activity of procaryotic phototrophs, a completely anaerobic sulfur cycle is possible, which is quite common in permanently anaerobic marine and fresh-water environments.

Procaryotic microorganisms are also the main agents in a number of inorganic transformations in nature, including the transformations of sulfur, nitrogen, and iron compounds. Because so many of these transformations are critical for global cycles, and hence for our understanding of biogeochemical processes, an understanding of procaryote ecology is of great significance. It is perhaps for this reason that biogeochemistry had its roots in the studies of the great bacteriologically-oriented scientists, Sergei Winogradsky and Laurens Baas Becking.

Elements of Living Organisms: Concentration and Rejection

The ability of living organisms to concentrate various chemical elements to levels considerably higher than those found in their environment is a well-known phenomenon, the original recognition going back to 1913 and the fascinating book written by Lawrence J. Henderson entitled "The fitness of the environment". It was from this viewpoint that the Vernadsky school of biogeochemistry began, and the use of biogeochemistry in geochemical exploration is based on this idea. Although the original idea derived from a rather static viewpoint of living organisms, in recent years there has been a resurgence of interest in these studies, because of the realization that the chemical compositions of living organisms reflect their chemical environments, making it possible to predict under many cases the consequences of artificial increase or decrease in the level of a particular element.

In the present discussion, we are concerned not with the exotic elements that organisms do not require, but with the elements which make up the main constituents of protoplasm. Chemicals can be divided into groups, depending upon whether they are or are not used by living organisms. Many chemicals, such as aluminium, strontium, radium, and barium, are not essential ingredients of protoplasm of any organism, and although they may be taken into cells, they are of significance biologically only if they are present in such high amounts as to be toxic. Other chemicals, such as silicon, chlorine, iodine, selenium, and molybdenum, are required elements for only certain organisms, or only under certain kinds of conditions, and the vast majority of organisms can develop quite well without them. Then there are the elements which are essential for all living organisms: sodium, calcium, magnesium, carbon, sulfur, iron, potassium, nitrogen, manganese, zinc, phosphorus, hydrogen, and oxygen (and perhaps copper and cobalt).

Relation of Chemical Composition to Environment

The biologically essential elements must be considered in relation to their natural abundances, and to the concentrations present in protoplasm. Data for the average chemical composition of a typical microbial cell, *Escherichia coli*, are given in Table 1, along with the concentrations of the same elements present in fresh-water and sea water. Table 2 gives similar comparisons for algal cells. It should be emphasized that the values presented are global averages, and localized differences can often occur.

However, it is not enough to compare the concentrations of elements in water with their concentrations in organisms. We must consider the amount of that organism present in the water, and how much of the element in question is tied up in the organism, as compared to the concentration present in the water. To carry out such an analysis, I have calculated what I call an Element Excess Concentration, which is the concentration in the water which is in excess (or deficient, under certain conditions) of the concentration required if all of the element in the organism came from the water. These calculated values are also given in Tables 1 and 2.

From this analysis it can be seen that, on the average, all of the elements are present in the environment in excess of those required by these organisms, except for *nitrogen* and *phosphorus*. In seawater, *iron* is possibly marginally deficient. This analysis clearly shows what has been already well established limnologically (Schindler, 1978), that phosphorus is frequently a limiting element for biological growth in aquatic systems. Although nitrogen also appears to be limiting, when it is realized that many organisms are able to fix atmospheric nitrogen, it can be estimated that there would be sufficient nitrogen present in gaseous form dissolved in the water to provide needed nitrogen for growth. However, since nitrogen fixation is an energy-demanding process, nitrogen limitation may still develop under many conditions.

THERMODYNAMICS

One of the great generalizations of biology relates the laws of thermodynamics to biological metabolism. It is clear that the formation of biological material from chemical substance is an energy-demanding process, and the energy required must be derived either from light or from the oxidation of chemical materials. Both light and chemical energy are obtained only from the environment. From a biogeochemical viewpoint, however, we are not so much concerned with cellular metabolism as with the chemical modifications of the environment which organisms create as a result of their life processes. Overall, chemical reactions will only occur if the free energy of the reaction is negative. Living organisms are not able to get around the second law of thermodynamics. Taken at its most basic state, what we have in an environment is a cluster of potential electron donors and electron acceptors, and the possibility that various donors and acceptors will react. Whether or not a donor and an acceptor will react will depend first upon thermodynamics, and second upon kinetic considerations. It is in relation to these kinetic considerations that we must consider the role of living organisms.

Although a given environment may have a variety of electron donors and electron acceptors, it is often necessary, for scientific convenience and to simplify study and analysis, to concentrate on only certain chemical species. Usually, the chemical species present in the largest concentrations may be looked at first, with the minor species being ignored unless they are of unusual interest. When we do approach biogeochemical reactions in this way, we find that a matrix of reacting species can be constructed, and within this matrix the free energy of reaction can be calculated for each of the matrix pairs. Such a matrix, shown in Table 3, reveals a number of interesting things. First, certain donor-acceptor pairs react with positive free energy, and these reactions will hence be unlikely to be important biogeochemically. Second, some electron acceptors are much more likely to be important than others, because the free energy yield is higher. It is of considerable fundamental importance that precisely the chemical reactions which the matrix predicts would be important are actually those which are most commonly observed to occur at high rates in nature (although there are a few important exceptions). Since thermodynamics does not have anything to say about reaction rates, but only about whether reactions will or will not occur, how does it happen that those reactions predicted from the matrix to be most important are those that appear to take place at the most rapid rates in nature? To answer this question, we must digress a bit and discuss an important aspect of microbial physiology, viz. cellular growth yield.

TABLE 1

ELEMENT CONCENTRATION IN AQUATIC SYSTEMS

Element	Bacteria (g/100 g dry cells)	Concentration (mg l^{-1}) in bacteria assuming 10^7 cells ml^{-1}	River Water chemistry (mg l^{-1})	Sea Water chemistry (mg l^{-1})	Element excess (or deficiency) assuming 10^7 cells ml^{-1}		
					Freshwater	Seawater	Remarks
Carbon	50	5	11.4 (bicarbonate)	28	2.3	5.6	
Oxygen	20	2	857,000 (water)	857,000	428,500	428,500	
Nitrogen	15	1.5	0.23 (nitrate) 12.5 mg l^{-1} (N_2, 25°)	0.5 10.5 mg l^{-1} (N_2, 25°)	(6.5) 8.3 (N_2 fixation)	(3.0) 7.0 (N_2 fixation)	Deficiency No deficiency if N_2 fixation
Hydrogen	10	1.0	108,000 (water)	108,000	108,000	108,000	
Phosphorus	3.2	0.32	0.1 (phosphate)	0.07	(3.2)	(4.6)	Deficiency
Sulfur	1.1	0.11	3.5 (sulfate)	885	31.8	8,045	
Potassium	0.97	0.097	2.3	380	23.7	3,917	
Calcium	0.49	0.049	15	400	306	8,163	
Magnesium	0.36	0.026	4.1	1,350	158	51,923	
Sodium	0.19	0.019	6.3	10,500	331	552,631	
Iron	0.089	0.009	0.67	0.01	74	1.1	Possible seawater deficiency

All weights are given as weight of the element.

Bacterial composition data from Luria (1960).

Seawater data from Horne (1969).

Freshwater data (average, world rivers) from Livingstone (1963); phosphate data for freshwater from Stumm and Stumm-Zollinger (1972).

Element excess calculated by dividing water concentration (column 4 or 5) by bacterial concentration (column 3). If calculated value is less than 1, then deficiency calculated by dividing bacterial concentration by water concentration.

TABLE 2

RELATIONSHIP BETWEEN CONCENTRATION OF ELEMENTS
IN ALGAE AND CONCENTRATION IN A FRESHWATER SOURCE

Element	Algae (g/100 g dry cells)	Lake concentration ($mg\ l^{-1}$) assuming 8 mg l^{-1} algae	Average water concentration ($mg\ l^{-1}$)	Relative excess or deficiency in water	
				Excess	Deficiency
Carbon	49	3.9	11.4	2.9	
Oxygen	31	2.5	857,000	342,800	
Nitrogen	6.2	0.50	0.23 (12.5 N_2)	25 (N_2)	2.17 (nitrate)
Hydrogen	7	0.56	108,000	192,857	
Phosphorus	1.2	0.096	0.1	1.04	
Sulfur	0.67	0.054	3.5	65	
Potassium	1.9	0.15	2.3	15	
Calcium	0.98	.078	15	192	
Magnesium	0.6	.048	4.1	85	
Sodium	0.6	.048	6.3	131	
Iron	0.67	.054	0.67	12	

The data on algal composition are obtained from Healey (1973) and are averages of many measurements for both eucaryotes and procaryotes. The lake concentration of algae is representative of a eutrophic lake (Lake Mendota, WI). The average water concentration is a world average value, from Livingstone (1963).

TABLE 3

FREE ENERGY OF REACTIONS FOR A MATRIX OF ELECTRON ACCEPTORS AND DONORS. VALUES ARE GIVEN AS KJ PER ELECTRON EQUIVALENT TRANSFERRED

Electron donors (Donor/Oxidized) product	Electron acceptors (Acceptor/Reduced product) $\frac{1}{2}O_2/H_2O$	Fe^{3+}/Fe^{2+} (pH2)	$NO_3^-/\frac{1}{2}N_2$	SO_4^{2-}/HS^-	HCO_3^-/CH_4
H_2/H_2O	-118.62	-79.9	-112.1	-19.04	-16.99
HS^-/S^o	-104.73	-16.1	-98.7	-2.64	-3.93
S^o/SO_4^{2-}	-97.9	-60.25	-91.2	+0.79	+2.18
NH_4^+/NO_2^-	-43.1	-5.4	-39.29	+55.65	+56.9
NO_2^-/NO_3^-	-38.1	-3.43	-30.96	+60.7	+62.3
Fe^{2+}/Fe^{3+} (pH2)	-71.1	0.00	-31.97	+96.65	+67.8
CH_4/HCO_3^-	-100.0	-67.8	-94.98	-1.30	0.00

Free energy values and method of calculation described in Brock (1979). All reactions considered at pH 7 except for those involving Fe^{2+} or Fe^{3+}. Those values underlined are unlikely to occur in biogeochemical systems because of unfavorable thermodynamics.

GROWTH YIELDS ON VARIOUS SUBSTANCES

It has been known for a long time, and is currently of great industrial importance, that the cell yield available during the growth of a microorganism on a substrate is a function of the amount of energy available when this substrate is metabolized. The more energy available from a given donor-acceptor pair, the larger the cell yield realized. The reason for this is that putting a cell together requires a considerable amount of energy in the form of high-energy phosphate bonds, both to synthesize the various building blocks, and to polymerize the building blocks into the macromolecules of which cells are composed. The molar yield of high-energy phosphate bonds which can be formed when a mole of substrate is metabolized is determined in part by the amount of energy potentially available when a substrate is oxidized. Thus, if a donor-acceptor pair releases a large amount of energy, then more high-energy phosphate bonds are formed and a greater amount of cell yield can be realized during growth on this pair.

Relation of Cell Yield to Reaction Kinetics

How does cell growth yield relate to the rate of biogeochemical reactions? In most natural environments, reaction rate is limited by the amount of catalyst present, at least until the concentrations of the reactants get to such low levels that other factors enter the picture (see below). Thus, if more catalyst is present, the reaction rate should increase. Since the catalyst is the living organism, and since the amount of living organism present will depend upon the potential growth yield for the donor-acceptor pair, it is clear that thermodynamics and reaction kinetics are, in biogeochemistry, coupled phenomena. If the donor-acceptor pair have a high free energy yield, then a lot of cell material can be formed, and a lot of reaction can occur. If the donor-acceptor pair have a low free energy yield, then not much cell material will be formed, the amount of catalyst in the system will be low, and the reaction will proceed at a lower rate.

Another consequence of the above idea, of more concern for the microbial ecologist interested in cell numbers than for the biogeochemist interested in reaction rates, is that energetically unfavorable reactions will lead to low cell yields, so that it may be difficult to quantify the numbers (or biomass) of organisms catalyzing such reactions, even though considerable amount of chemical change may be taking place. This is most readily seen in the case of energetically unfavorable reactions such as nitrification and methanogenesis, where it is much easier to measure change in the relevant chemical species than to count the numbers of organisms involved.

I do not want to imply that the concept just advanced is the sole explanation of varying reaction rates in nature, because there are obviously many other factors. Insolubility of substrate, for instance, is an important factor limiting reaction kinetics. For instance, even though the cellulose-oxygen donor-acceptor pair shows favorable thermodynamics, the rate of reaction is slow even in the presence of a good cellulose-digesting organism, due to the insolubility (and probably the crystallinity) of the cellulose substrate. Another major consideration is the differential effect that environmental conditions may have on one or another type of biogeochemical reaction, so that differential rates of reactions may be due to the existence of particular environmental attributes (for instance, high temperature, low pH). However, this concept does explain very well many of the observations made on chemical species present at different levels in sediment cores, especially the relative rates of disappearance of the various electron acceptors: oxygen, ferric iron, manganese (IV), sulfate, and carbonate.

MICHAELIS-MENTEN KINETICS AND BIOGEOCHEMISTRY

An important concept, first applied to microbial growth by Jacques Monod, is that at low substrate concentrations, the growth rate of an organism is proportional to the concentration of the growth-limiting substrate. Enzyme reactions show similar substrate-limited reaction rates, as first described in detail by Michaelis and Menten, and similar Michaelis-Menten kinetics can be generally applied to substrate-limited growth of microorganisms. The analysis in these terms, so widely used in microbial physiology, is equally applicable to biogeochemistry.

The physiological reason for Michaelis-Menten kinetics in microbial growth probably relates to the fact that transport of the growth-limiting substrate into the cell is the rate-limiting step, and transport is mediated by enzyme-like carriers. From an ecological viewpoint, the significance of Monod-type kinetics is that organisms deplete their substrates as they grow, so that eventually they limit their own growth rates. Growth rate does not, directly, affect population density, and it would be theoretically possible to have a very slow-growing population that eventually reached a high cell density, but in actuality this does not seem to be the case, probably because population density is influenced not only by growth rate but also by rate of loss of cells from the population due to lysis, predation, and grazing. If all loss factors were eliminated, then it might be possible to get a large population density even with a slow-growing organism, but in many cases the loss factors play a major role in population growth. (It might be possible that loss factors are of less importance in anaerobically-growing populations, because animal grazers are absent, although this has not really been studied.)

Relating cell growth kinetics to biogeochemical reactions, we must first think of the range of substrates available for living organisms. Either directly or indirectly, the biogeochemical processes in sediments and soils are driven by organic matter decomposition, since organic matter provides the reducing power necessary to drive various reactions. As the major organic molecules break down, other organic compounds of intermediate oxidation state are often formed, and these compounds are then acted upon by other organisms in the system. If there are electron acceptors present, then the electrons removed from organic molecules will be transferred to these acceptors, and reductive processes will occur. If all available electron acceptors have been used up, then strictly fermentative processes will occur, leading ultimately to the conversion of organic matter into methane and carbon dioxide. Any compounds that are not readily broken down by organisms will accumulate. Under steady-state conditions, the concentrations of substrates or intermediates will be determined by the rate at which they are broken down. The compounds that are broken down most rapidly will be present in very small concentrations, if at all, whereas those broken down slowly will be present in higher concentrations. Thus, an important principle of biogeochemistry suggests itself: if a compound is found in significant amounts in a

biogeochemical system, then it is not very significant (as a reactant) in that system! This is a strange reversal: that which is present is not important and that which is absent may be important! This is an extremely important idea, because it has considerable influence on how biogeochemical research is carried out.

As an example of the implications of this concept: Methanogenesis in lake sediments is an important end process of the anaerobic decomposition of organic matter. The intermediate materials on the way from source organic matter to methane are hydrogen gas and acetate. Significantly, neither one of these materials can be detected readily by direct assay of sediment. Superficially, it might have been thought that because hydrogen and acetate were absent, that neither one of these substances was involved in methanogenesis, but pure cultures studies of the methane bacteria have shown that only a very few substances were involved in methane formation, among which were hydrogen and acetate. Because of the pure culture data, it was natural to determine if these substances might indeed be important in sediments. It could be easily shown (Winfrey *et al.*, 1977) that both hydrogen and acetate stimulated methanogenesis in lake sediments. Further, if radioactive carbon dioxide or radioactive acetate was added to lake sediments, these compounds were rapidly converted to radioactive methane. Winfrey and Zeikus (1979) then showed that there was a detectable, albeit small, pool of acetate in lake sediments which was turning over very rapidly. From the pool size and the turnover rate, the importance of acetate as an intermediate in methanogenesis could be shown. A similar situation could also be shown for hydrogen, although the pool size of this gas was even lower than that for acetate.

These results emphasize the importance of carrying out pure culture studies of important components of biogeochemical processes, because only in this way will it be possible to recognize intermediates that might be important in various processes. However, it is obviously not sufficient to do only pure culture studies, because the pure culture tells us what might occur in nature, but not necessarily what is actually occurring.

Returning to the idea of Monod kinetics, the knowledge of pool sizes is important for understanding the factors limiting biogeochemical reaction rates, because the affinity which an organism has for a substrate is a more or less defined property, and if the concentration of the substrate in the environment is much lower than the affinity constant, then it is unlikely that this substrate-organism combination is of much significance. One reason for obtaining pure cultures is so that affinity constants can be determined, although it is important to note that the values obtained may be influenced by the environmental conditions under which the organisms are growing.

MEASURING REACTION RATES IN NATURE

Because geologists deal with events that took place over long periods of time, they often do not consider the direct measurement of geochemical reactions important. The biogeochemist, on the other hand, thinks in much shorter time spans, and is naturally inclined to measure rates. The importance of direct measure of reaction rates should be emphasized: only in this way can we tell what reactions really are important in geochemical systems. There are three distinctly different approaches to the measurement of biogeochemical reaction rates: 1, by incubation procedures, usually by means of radioisotopes; 2, through time series measurements of chemical changes; and 3, by chemical analyses at single times in time-stratified systems.

Incubation methods often provide the most convenient and sensitive way of measuring reaction rates, and if a convenient radioisotope is available, then this approach should always be considered. Incubation methods without the use of radioisotopes suffer because long incubation times are often necessary, and secondary changes may take place in the incubation mixtures. The advantage of radioisotope methods is that they are so sensitive that incubation times can be kept very short, thus avoiding any secondary effects that might develop during the incubation procedure itself. Radioisotope methods are especially valuable for determining turnover rates of substances, as mentioned earlier in this paper.

Time series measurements can often be used to obtain reaction rates for whole systems, provided it is known that the changes in concentrations observed are due primarily to chemical reactions within the system, rather than to additions from or subtractions to the outside. Such measurements are most effectively measured in lakes, where chemical changes within the water column, or in some portion of the water column (e.g. the hypolimnion) can

be related to reaction rates.

Chemical analyses in time-stratified systems, such as sediment cores, have the advantage that, under favorable conditions, the reaction rate measured is known to have taken place under strictly in situ conditions. (There is always the worry that disturbance of samples by incubation methods may change reaction rates considerably, either increasing or decreasing them.) However, for analyses in stratified systems to make possible the calculation of rates, the system under study must be well controlled and well understood. Sediment systems are often amenable to analysis in this way. Cores are taken and cut into segments which are analyzed separately for particular chemical species. Changes which take place with depth can be interpreted in terms of reaction rates, provided that the rate of sedimentation is known. It is possible to measure sedimentation rates directly, with sediment traps, or indirectly, by use of internal markers such as pollen grains or natural radioisotopes.

The ideal thing is to be able to measure reaction rates by more than one method, and if the various methods agree, then more confidence is given. It cannot be emphasized enough that for any of these approaches to work, the system under study must be well understood, which means that it must be followed over a long period of time. Annual cycles must especially be understood, so that it can be known whether or not particular chemical changes seen are related to nonbiological events such as mixing processes, rather than to biogeochemical activities.

EDUCATION FOR BIOGEOCHEMISTRY

I think it safe to say that biogeochemistry has a much greater future than it has had a past. Although the initial impetus for the present series of conferences was concern for the environment, it seems likely that in the future a more significant motivation will be the energy crisis, with all that this implies. We are concerned, in biogeochemistry, with interpreting past geochemistry in terms of present processes, and in understanding how things got the way they are today in terms of the evolution of past processes. Because so much of energy research relates in one form or another to fossil fuels, and because fossil fuels represent organic material which did not undergo decomposition, an understanding of production and decomposition processes may help us to explain how fossil fuels got the way they are, and where they are.

However, biogeochemistry is not an applied field, but a very fundamental science. Although the practical considerations may provide some impetus for the rapid development of the field, it is not because of these practical situations that biogeochemistry should develop, but because of the vast number of important and fascinating basic problems that are now amenable to attack by fresh and modern methods. Biogeochemistry provides an insight, a way of looking at things, that is new and different. Analytical tools, isotopic procedures, microbiological culture techniques, are now available that have never been available before, and the biogeochemist can make the most of them.

Perhaps the most difficulty, over the next generation, will be the establishment of the teaching tools needed to indoctrinate a new line of students who will be needed to carry this research forward. At the moment, biogeochemistry falls through the cracks in a modern university, just as biochemistry did at the turn of the 20th century. Because universities in the Western World are no longer expansionist minded, it will not be possible to develop biogeochemistry departments de novo. Thus, courses, graduate programs, textbooks, and the like, will perforce be developed within the existing university departmental structure. This means that biogeochemists will be found in a variety of existing departments: bacteriology, geology, soils, chemistry, limnology, botany, environmental engineering, and perhaps others. What we will have to do is make a conscious effort to bring people with biogeochemical interests from these various departments together for courses and research projects. Out of such interdisciplinary involvement will eventually emerge a new discipline. In the meantime, we should take every opportunity we find for promoting the development of this science. Journals should be established, review books should be edited, textbooks should be written, societies should form. Ultimately, if the discipline really has viability, such activities will attract notice and be successful, and the field will emerge.

What if all this activity does not lead down the charted path? What if a generation of

concerted effort by a variety of interested scientists does not lead to the formation of a vigorous intellectual discipline? Well, then, it would probably be safely said that we have been wrong in thinking that we really do have a group of new insights that the whole world is waiting for. Then, we might be able to say that biogeochemistry does not really deserve to exist as a major scientific discipline, but only as one of that never-ending series of minor or peripheral sciences that always seem to come and go along the long and fascinating path of scientific development.

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LAKE SEDIMENTS: CHEMICAL COMPOSITION AND SOME ASPECTS OF THEIR FORMATION AND DIAGENESIS

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INTRODUCTION

Sediments are a major sink for chemical components of water bodies. As part of on-going limnological studies of two man-made, urban lakes in Canberra (Cullen *et al.*, 1978a, b and Rosich *et al.*, 1978), the chemistry of the sediments has been studied for comparison with other lakes and to attempt to elucidate some of the processes that control the formation of sediments and transformations within sediments. The biological availability of sediment components is an important issue, especially in water management, and the results of some studies of nutrient availability are presented in Rosich and Cullen (1980).

Lake Burley Griffin (Figs. 1 and 2) was formed in 1964 and its catchment land-use is about 3% urban, 76% light agricultural and 31% native forests. Secondary treated sewage effluent from Queanbeyan (population about 20,000) enters the river just upstream of the lake. There is an abandoned metal sulfide mine in the catchment from which, until 1976, metal-contaminated leachate entered the lake system. Completion (in 1978) of the Googong Dam upstream of the lake is expected to double the mean water residence time in the lake (to about 4 months).

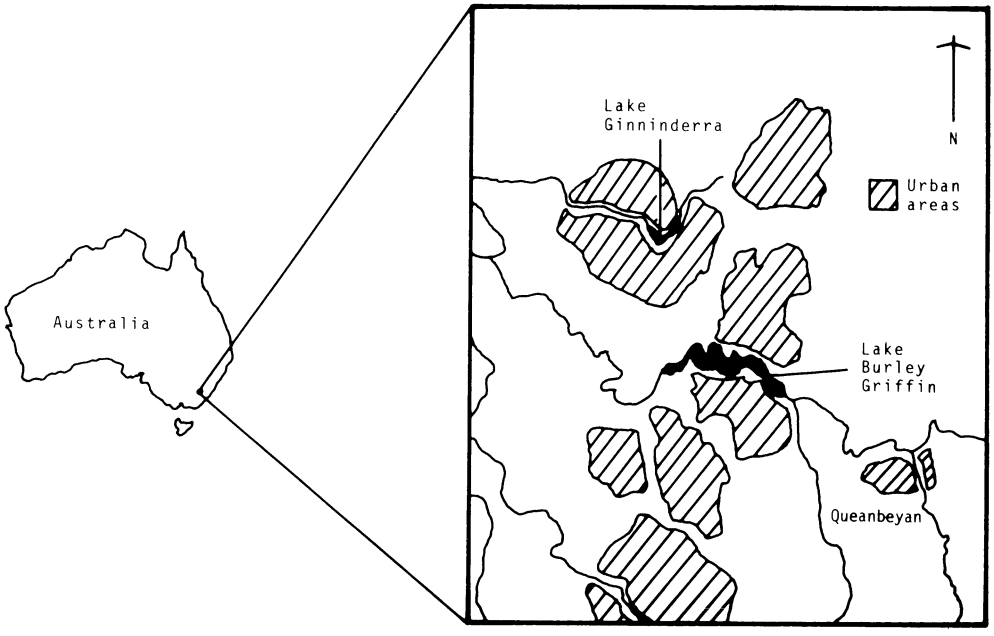

Figure 1: Location of Lakes Burley Griffin and Ginninderra.

Lake Ginninderra (Figs. 1 and 3) first filled in 1974 and its catchment land-use is about 16% urban, 64% light agricultural, 2% native forest and 17% urban parks. The mean water residence time is about 10 months.

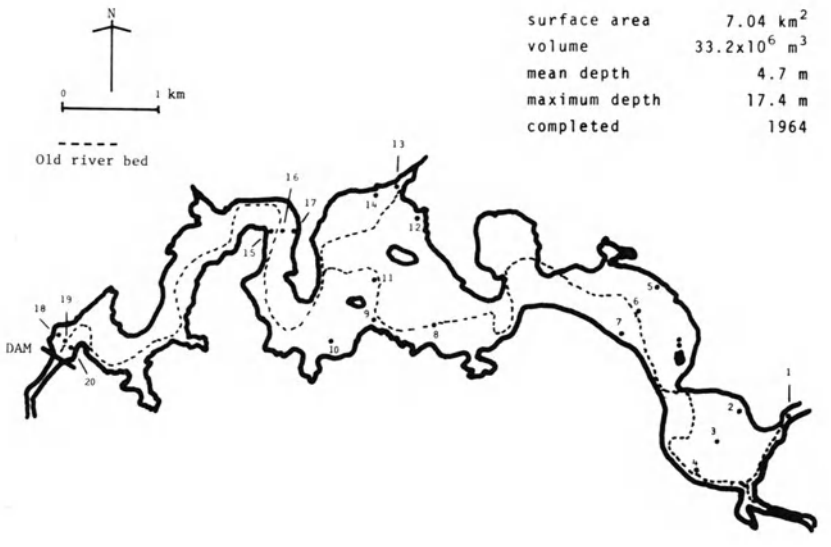

Figure 2: Lake Burley Griffin sediment sampling sites.

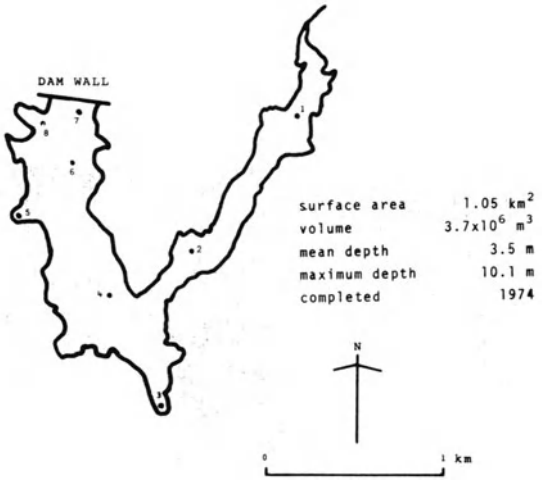

Figure 3: Lake Ginninderra sediment sampling sites.

MATERIALS AND METHODS

Surface sediments, which were dark-grey muds, were sampled to a depth of no more than about 100 mm with an Eckman grab and the samples stored frozen until analysed. Mass losses on sequentially heating to 105, 550 and 950° were used to estimate moisture, organic matter and carbonate + clay-lattice water contents, respectively. Kjeldahl nitrogen was determined after Lively (1974) using the mixed indicator described in A.P.H.A. (1971).

After digestion of dried samples with nitric and perchloric acids, total metals and total phosphorus were determined by standard AAS techniques and colorimetrically (phosphomolybdate with ascorbic acid as reductant), respectively. Forms of phosphorus (Rosich and Cullen, 1980) and metals were determined after sequentially extracting undried sediment overnight with 0.1 M NaOH and 1 M HCl.

SEDIMENT CHEMISTRY OF LAKES BURLEY GRIFFIN AND GINNINDERRA*

Overall Comparisons

A comparison of a range of parameters for the two Canberra lakes and several other lakes of different types is given in Table 1. The two Canberra lakes are non-calcareous. Mass loss on ignition (550-950°) is very likely accounted for largely by loss of clay-lattice water (Dean, 1974).

The sediments of the Canberra lakes contain levels of P, N and C at the lower end of the range and are typical of those lakes in which the inflowing waters carry a high load of suspended solids (compare with Mulwala, Australia; Kamloops, Canada; and Kinneret, Israel).

As commonly observed, the N:P ratios in the sediments are lower than those of phytoplankton (which have N:P ratios near 5-8) while the C:N ratios are higher (3-10 for phytoplankton). These results indicate an overall loss of nitrogen from sediments as a result of mineralisation and denitrification. It is noteworthy that Bantam Lake, which is in a cool temperate climate, has a higher N:P ratio (and lower C:N ratio) than the other lakes for which data are available and which are in warm-temperate climates. Variations in the other parameters probably depend largely on the properties of the soils of the catchment.

The samples from Lake Burley Griffin appeared to be true lake sediment while in the case of Lake Ginninderra the bottom half of the samples appeared to be original lakesite soil. Nevertheless, the P, N and C values were relatively uniform in this sediment reflecting high mobility of these elements in bottom muds (see also p. 112).

Five years after filling, the sediment of Lake Ginninderra has similar levels of total P (455 $\mu g\ g^{-1}$) and organic carbon (16 $\mu g\ g^{-1}$) to those of Lake Burley Griffin (450 and 16 $\mu g\ g^{-1}$, respectively). However, it seems likely that these levels reflect the recent climatic conditions. In October 1976 a major (1-in-24 year) flood occurred in the Lake Burley Griffin catchment which resulted in an *increase* in total sediment P (compare September 1976 with May 1977). This was unexpected since the soils of both catchments are poor in phosphorus (see e.g., the topsoil data in Table 5). This increase may have resulted from a decrease in organic carbon leading to a decrease in dissolved Fe(II) and hence increased P levels (see also p.114 and Table 5). The flood resulted in small decreases in the other components listed in Table 1 and also in some variations in zinc (445 to 465 $\mu g\ g^{-1}$), lead (130 to 94 $\mu g\ g^{-1}$) and copper (29 to 28 $\mu g\ g^{-1}$).

In Lake Ginninderra, a significant decline in the Kjeldahl nitrogen level occurred in spring (November 1977) compared to autumn (March 1977, not shown in Table 1) and winter (June 1977) while, by June 1979, the level had increased again. Similar data available for Lake Burley Griffin are consistent with this trend. It is suggested that the spring decline resulted from increased growth of the lake biota and by increased denitrification. The data for Lake Ginninderra represent a loss, from the top 100 mm of sediment, of about 100 g N m^{-2}. This nitrogen does not appear in the water column and indeed there is also a loss of nitrogen from the water column of about 3.5 g N m^{-2}.

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* A complete listing of the data for both lakes is available from the authors (requests should be directed towards R.S. Rosich).

TABLE 1

SEDIMENT CHEMISTRY IN LAKES BURLEY GRIFFIN AND GINNINDERRA COMPARED TO OTHER LAKES

Lake	Total P µg g^{-1}	Kjeldahl-N µg g^{-1}	Organic[a] Carbon mg g^{-1}	Mass loss on ignition 550°-950° mg g^{-1}	N:P	C:N	Fe mg g^{-1}	Mn	Na	K µg g^{-1}	Ca	Mg
BURLEY GRIFFIN [b]												
Sept. 1976	340	(d)	34 [a]	14.5	1.0							
May 1977	415	1300	27 [a]	16.2	3.0	24.5	25.6	1570	505	3540	1650	3760
June 1979	450	680	15 [a]	15.9	1.6	23.1	25.7		340	4080		4020
GINNINDERRA [c]												
June 1977	340	1490	16 [a]	32.8	4.7	10.7	32	1430		880	1500	2380
Nov. 1977	355	340			1.0		29	1210	130	350	1770	2740
May 1979	455	1040	16 [a]	23.7	2.2	15.3	30.4		710			3090
MULWALA [e]	770	2140	32.9		2.9	18.8						
KAMLOOPS [f]	750						40	700		1000	1500	10000
KINNERET [g]	600	300	8		1.7	10.3	18.5	480	500	2500		
16 NON-CALCAREOUS WISCONSIN [h]	2550						38.5	990				
BANTAM [i]	1900	11300	108		5.9	9.6						
WINDERMERE AND ESTHWAITE [k]							46.5	1500	6600	18100		

a Organic carbon estimated by $\frac{\text{mass loss (105-550°)}}{2.13}$ (Dean, 1974).
b Means for sites 2-4, 9-11, 13, 18-20. Note that the means for these 10 sites differ by less than 10% from the means for all 19 sites (2-20).
c Means for sites 1-4, 6.
d Values for sites 2 and 13 are 500 and 330, respectively, giving N:P ratios of 1.0 and 1.0, respectively.
e Hart *et al* (1976). On the Murray River, downstream of Albury, S.E. Australia.
f Approximate values - from graphs in St. John *et al* (1976). On the Thompson River, near Vancouver, S.W. British Columbia.
g Serruya (1971). On the Jordon River, Israel.
h Williams *et al*. (1971).
i Frink (1969). Connecticut, U.S.A.
k Gorham and Swaine (1965). In the Lake District, England.

Forms of Sediment Phosphorus and Other Components

The various chemical forms in which sediment components can exist are of interest both from the point of view of diagenetic processes and the availability of components to the lake biota (Syers *et al.*, 1973).

The Canberra lakes have generally lower proportions of inorganic (and hence higher proportions of organic) forms of P than the other lakes listed in Table 2. Reasons for the lower levels of inorganic P in Lake Burley Griffin in the 1979 samples are not readily apparent.

TABLE 2

FORMS OF PHOSPHORUS, IRON AND MANGANESE IN SEDIMENTS

Lake	P				Fe		Mn	
	Total	NaOH ext	HCl ext	Organic [a]	Total	HCl ext	Total	HCl ext
	μg g^{-1}				mg g^{-1}		μg g^{-1}	
Burley Griffin [b]								
May 1977	415	140	105	170				
June 1979	450	75	80	295	25.7	12.4	365	42
Ginninderra [c]								
May 1979	455	35	25	395	30.4	11.3	350	34
16 Non-Calcareous Wisconsin [d]	2550			650	38.5		990	
Erie [e]	880	330	445	110	38.4		870	
Bantam [f]	1900	780	220	900				

[a] Calculated from Total-P minus (NaOH-extractable P + HCl-extractable P).

[b] Means for sites 2-4, 9-11, 13, 18-20.

[c] Means for sites 1-4, 6.

[d] Williams *et al.* (1971).

[e] Williams *et al.* (1976); their non-apatite, inorganic P, and apatite, inorganic P are considered to be equal to NaOH-extractable P and HCl-extractable P, respectively.

[f] Frink (1969).

Significant amounts of iron (37-48%) and manganese (10-12%) are extracted by HCl while few of the other metals listed in Table 1 are extracted to any great extent (magnesium about 4% and potassium 1-2%). About 6% of the zinc was extracted. Only insignificant amounts of any of the metals were extracted by NaOH.

PROCESSES INFLUENCING SEDIMENT CHEMISTRY

The elemental composition of sediments is governed by the chemistry of soils in the catchment area (especially for major elements such as Fe, Al, Mn, Na and K), and both

biological processes (for the macronutirents P, N and C) and physico-chemical processes (for P, N and C, and also for trace elements such as Zn, Pb and Cu) are important.

Forms of Phosphorus

Table 3 lists selected linear correlation coefficients between various components of Lake Burley Griffin sediment samples. Although several authors have noted a correlation between total P and the depth of water from which sediment samples are taken (e.g., Frink, 1969 and Hart *et al.* 1976), there is only a weak correlation for Lake Burley Griffin. Zn, Fe, Mn and K correlate reasonably well with the depth of the water column.

The most significant correlations for the forms of P (especially the NaOH-P) are with Fe. Good correlations were found for at least some forms of P and Al, Na, Mn, K and Mg but, since even stronger correlations exist between Fe and these five metals, it is probable that the level of Fe (probably in the form of mixed Fe(II) and Fe(III) hydrated oxides together with the mineral vivianite, $Fe_3(PO_4)_2.8H_2O$) is the major control on the level of P in sediments. These results and conclusions are similar to those reported by Syers *et al.* (1973), Williams *et al.* (1971), Stumm and Morgan (1970), Bray *et al.* (1973), Nriagu and Dell (1974) and Emerson (1976).

It is suggested that, in Lake Burley Griffin, sedimentation of Fe (and Al) components will be influenced by depth inasmuch as some of the components (particularly the hydrated oxides) will be finely dispersed and hence will settle only slowly, and in less turbulent water. If, however, there is insufficient P to saturate the adsorption capacity of the Fe and Al components, then a strong correlation between forms of P and depth need not exist.

Other Interrelations

The correlation between Fe and Al is to be expected because of the chemical nature of soils and simultaneous coagulation of Fe and Al oxides. Interrelation between Fe and Mn is well documented (Syers *et al.*, 1973; Williams *et al.*, 1971; Stumm and Morgan, 1970). On the other hand, interrelation of Mg with Fe is less common but is consistent with observations on the adsorption of Mg onto alumina (W. Stumm *et al.*, unpublished results). The interrelation of Fe with Na and K probably reflects simultaneous coagulation of the Fe oxides and clays.

The strong correlations of Zn with Fe (and Al and Mn) and with water depth suggest that, to a significant extent, Zn is removed from solution by adsorption onto Fe (and possibly also Al) oxides. On the other hand, Pb and Cu, both of which are less stable in solution than Zn (with respect to solid hydroxy-carbonates and hydroxy-sulfates), are distributed more uniformly over the bottom of the lake.

Similarly, the high correlations between Pb and Cu and organic matter probably reflect the greater stability of complexes between these metals and organic complexing agents (including fulvic and humic acids). All three metals (Zn, Pb and Cu) show high correlations with Kjeldahl N and this might indicate significant uptake of these metals by the bacterial biomass in the sediments.

LEVELS OF ZINC, LEAD AND COPPER

Until control measures were applied in 1976, the Lake Burley Griffin system received a substantial input of trace metals leached from an abandoned metal sulfide (Cu, Pb, Zn, Ag and Au) mine approximately 60 km upstream on the Molonglo River (Fitzgerald and Haldane, 1977).

Table 4 compares the levels of Zn, Pb and Cu in several Australian lakes. The levels of copper are similar (probably within experimental precision) in all sediments and indicate either no pollution, or similar pollution, in each case. Lead levels in sediments of both urban lakes (Ginninderra and Burley Griffin) are higher than in Lake Hume and are presumably due to contamination from automobile fuel combustion. The high level of zinc in Lake Burley Griffin sediments is the result of contamination from the Captain's Flat mine effluent. This effluent may also contribute some lead to Lake Burley Griffin sediment.

TABLE 3

SELECTED LINEAR CORRELATION COEFFICIENTS FOR COMPONENTS IN LAKE BURLEY GRIFFIN SEDIMENTS COLLECTED ON 10 MAY 1977 [a]

	Depth [b]	Total P	NaOH P	HCl P	Inorg P	Org P	Kjeld N	Org Matter	"Carb-onate"	Fe	Al	Mn	Na	K	Pb
Depth [b]		0.17	0.28	0.29	0.31	-0.09	0.21	0.05	0.26	0.53	0.32	0.49	0.24	0.55	
Organic Matter							0.40								
Fe	0.53	0.86	0.89	0.65	0.89	0.47	0.53	0.45	0.56		0.94	0.84	0.73	0.97	
Al		0.91	0.88		0.81					0.94					
Mn										0.84	0.82				
Na											0.82				
K		0.82	0.85		0.84					0.97	0.93	0.86	0.80		
Mg										0.83	0.79				
Zn	0.71						0.61	0.28		0.76		0.78		0.83	
Pb	0.14						0.58	0.64		0.32					
Cu	0.27						0.60	0.71		0.49					0.81

[a] Data for all 19 sites (2-20). Correlations significant at the 0.05 level. All correlations greater than 0.80 have been included together with selected correlations lower than 0.80 for comparison purposes.

[b] Depth of water from which the sediment samples were taken.

TABLE 4

LEVELS OF Zn, Pb AND Cu in SURFACE SEDIMENT OF SEVERAL AUSTRALIAN LAKES ($\mu g\ g^{-1}$)

Metal	Lake Ginninderra [a]	Lake Burley Griffin [b]	Lake Hume [c]	Lake Mulwala [c]
Zn	50-100	140-1020	53-95	64-89
Pb	11-96	38-131	22	
Cu	20-44	23-45	21-32	14-22

[a] Data for sites 1-7.

[b] Data for all sites 2-20.

[c] From Hart (1977).

SOME ASPECTS OF DEPTH PROFILE VARIATIONS AND DIAGENESIS OF SEDIMENT COMPONENTS

Well defined layers within three Lake Ginninderra sediment samples (from sites 2, 4 and 6; about 100 mm total depth) were subsampled as indicated in Table 5.

The phosphorus concentration in the sediments remains relatively uniform with depth and is higher than in the original topsoil. Clearly, phosphorus is quite mobile in the anaerobic interstitial waters and migrates downwards (even against a presumed hydraulic gradient). This suggests that the actual concentration of phosphorus in sediments is controlled by physico-chemical factors *within* the sediments themselves, rather than by simple gravitational settling from the water column. The concentration of sediment nitrogen is also uniform with depth but is much lower (in the November samples) than in the topsoil.

Organic matter content is more uniform with depth than the above two constituents but organic matter also seems to be migrating downwards (compare the concentration in the three layers with the concentration in the topsoil).

As expected from the behaviour of hydrated iron (III) oxides and manganese in oxygenated surface waters, the levels of iron and manganese are mugh higher in the top layer. On the other hand, zinc, which is apparently removed from the water column predominantly through adsorption onto hydrated iron (III) oxides, is much less adsorbed in the top layer than manganese. The probable drop in pH in this thin layer (since interstitial waters are typically lower in pH than the overlying lake water) would cause this lack of adsorption. Thus zinc is forced to migrate down into the lower layers where its solubility must be controlled by other adsorption phenomena or precipitation.

There are no significant differences in the levels of sodium, calcium and magnesium in the three layers and even variations in the potassium level are probably within normal geochemical limits. Thus the levels of these constituents are presumably controlled mainly by the geochemistry and erosion of the soils of the catchment.

Lead and copper appear to be removed from surface waters mainly as insoluble compounds and are in slightly higher concentration in the top layer. Overall, the main sources of lead, zinc and copper are likely to be the soils of the catchment, but with some input resulting from urbanisation.

CONCLUSIONS

The chemistry of the sediments of Lakes Burley Griffin and Ginninderra is comparable with that of a wide range of lake types but most closely compares with other lakes whose inflowing waters carry a high suspended solids load. The sediments are deficient in nitrogen but the levels of the macronutrients (P, N and C) appear to depend on both the

TABLE 5

DEPTH PROFILES OF CHEMICAL COMPONENTS OF LAKE GINNINDERRA SEDIMENTS

	Moisture Content %	Total P	Kjeld N	N:P	C:N	.ORG MATTER [a]	"CARB-ONATE" [b]	Fe	Mn	Na	K	Ca	Mg	Zn	Pb	Cu
		µg g^{-1}				mg g^{-1}		mg g^{-1}				µg g^{-1}				
LAKE GININDERRA - November 1977 - means for sites 2, 4 and 6																
Top Layer [c]	62	435	405	0.9	100	86.9	7.9	48	9140	170	320	2410	2670	11	85	32
Middle Layer [c]	53	375	330	1.2	94	82.9	7.4	29	460	150	245	1680	2780	77	58	22
Bottom Layer [c]	50	330	315	1.0	119	71.2	12.0	22	175	145	150	1640	3190	69	55	20
Topsoil at Lake Ginninderra site before the lake was filled [d]																
Mean		8	1240	176	10.4	22.6					142					

[a] Organic matter = mass loss on ignition, 105-550°.

[b] "Carbonate" = mass loss on ignition, 550-950°.

[c] Top layer: reddish-coloured - a few mm thick.
Middle layer: light coloured - appeared to be true lake sediment
- about 30 to 50 mm thick.
Bottom layer: dark coloured - appeared to be original lakesite soil
- about 70 to 50 mm thick.

[d] Laurie and Montgomerie (1972).

seasonal and recent climatic conditions.

Evidence is presented which supports the now widely-accepted role of iron in determining the distribution of phosphorus in lake-sediment systems. The data further suggest a role of iron oxides in affecting the distribution of other elements such as Mn, K, Na, Mg and Al. Whereas iron appears to influence the distribution of Zn, it has a lesser influence on Pb and Cu.

The levels of zinc, lead and copper are generally similar in the sediments of several Australian lakes. One exception is a higher level of zinc in Lake Burley Griffin due to contamination from an abandoned mine in its catchment. Urban lakes appear to show higher levels of lead.

Depth profile data for Lake Ginninderra sediment showed that P, N and organic C are surprisingly mobile in the (anaerobic) sediment and appear to penetrate into the original lakesite soil. It is suggested that the actual level of phosphorus in sediments is controlled by physicochemical factors *within* the sediments themselves.

Higher levels of Fe, Mn, K, Ca, Pb and Cu occurred in the microlayer at the sediment-water interface. On the other hand, zinc was much lower in this layer and it is suggested that this is due to a lower pH, resulting in desorption.

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LAKE SEDIMENTS: ALGAL AVAILABILITY OF LAKE BURLEY GRIFFIN SEDIMENT PHOSPHORUS

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INTRODUCTION

This paper reports an experiment to estimate the amounts of phosphorus in lake sediment that may be available to phytoplankton. It is known that sediment can continue to maintain a polluted lake in an eutrophic condition (Bjork *et al.*, 1972; Bengtsson *et al.*, 1975). This is obviously an important issue when decisions are to be made about the diversion of sewage or other external sources of phosphorus to lakes.

The phosphorus cycle in lakes can be considered as a dynamic, steady-state system in which changes in the levels of P in the various compartments are rather small under constant environmental conditions (Stumm and Morgan, 1970; Stumm, 1975). Interchange between compartments can, however, be both very rapid and extensive (Mortimer, 1941, 1942; Hayes and Phillips, 1958; Pomeroy *et al.*, 1965; Lean *et al.*, 1975) even where the P levels in the compartments remain unchanged.

In this study we sought to assess the extent to which Lake Burley Griffin sediments could support algal growth under laboratory conditions of ample light, oxygen, stirring and all nutrients other than phosphorus. The ability of the sediments to supply "all other nutrients" was also assessed. Bioassay tests such as these are time consuming and so we also tested the correlation between the amount of P taken up by the algae and the amount of P taken up by less time-consuming chemical extraction with sodium hydroxide followed by hydrochloric acid.

MATERIALS AND METHODS

Availability of phosphorus to algae can be assessed by measuring the increase in algal biomass (by cell numbers, turbidity, chlorophyll *a*, etc.) of the test system compared with a standard test in media containing known amounts of orthophosphate. However, deviations from the standard growth curve may occur for a variety of reasons (E.P.A., 1971) and we elected to use an indirect approach (based on Golterman *et al.*, 1969 and Sagher, 1974), in which changes in inorganic, extractable P in the sediment were followed during growth of the algae. Tests were made to ensure that cessation of growth was ultimately due to limitation by P and to assess the extent of any bacterial mineralisation of sediment organic P.

Sediments from three different regions of the lake were collected with an Eckman grab (Rosich and Cullen, 1980):

inflow end - site 2, 3.0 m depth
mid lake - site 9, 7.0 m depth
outflow end - site 19, 17 m depth (deepest area of the lake)

The natural populations of algae in the lake water (from the outflow end, unfiltered) and sediments were used. Counts of algae <10 μm in size were made using a haemocytometer. Supplemental nutrients were provided to the final concentrations recommended for the algal growth media in E.P.A. (1971).

For the bioassays, duplicate sets of 250 ml conical flasks, stoppered with cotton wool, were prepared by mixing 18 ml of sediment suspension (with a concentration of total P of about 6.2 g P m^{-3}), 3.6 ml of a P-minus medium, a complete medium, or no added nutrient medium as required, and lake water to bring the total volume to 180 ml. The flasks were incubated in a growth cabinet at 24° with continuous fluorescent light giving an illumination of about 36 lx. The flasks were swirled twice daily and the pH adjusted as needed (about every 2-3 days) to between 7.5 and 8.0 by the dropwise addition of 0.05 M HCl.

Forms of P were determined at 5 or 7 day intervals in a 10 ml aliquot taken from each test flask as it was stirred with a magnetic stirrer. The aliquot was centrifuged at 15,000 rpm for 15 min and the supernatant analysed for molybdate reactive phosphorus (H_2O-extractable P). The residue was shaken overnight with 15.0 ml of 0.1 M NaOH (NaOH-extractable P), centrifuged and the resulting residue then shaken overnight with 15.0 ml of 1 M HCl (HCl-extractable P) and centrifuged. Carry-over of extractants (which was up to about 2%) was determined by weighing the centrifuge tubes at appropriate stages. Molybdate-reactive phosphorus was determined by standard methods using ascorbic acid as reductant while total P at the end of incubation was determined after digestion with acid-persulfate (Cullen *et al.*, 1978).

Accurate estimation of the amount of sediment P utilized by algae requires corrections for the amounts of P extracted from algal cells during the NaOH and HCl extractions. The corrections were calculated from data obtained by incubating lakewater containing P-plus medium but without sediments (Table 1). Green algae dominated these test flasks and it is assumed that similar proportions of P are extracted from the algae present in all tests.

TABLE 1

PROPORTION OF PHOSPHORUS EXTRACTABLE FROM ALGAE
(Lakewater containing P-plus medium, but no sediment, incubated for 31 days)

	Total P	P extractable by:			Total extractable P
		H_2O	NaOH	HCl	
mg P m^{-3}	970	2	117	37	156
% of total P			12%	4%	16%

Thus:

(i) Actual P-uptake by algae = apparent uptake x $\frac{100}{(100-16)}$

(ii) P extracted from algal cells by NaOH = actual P-uptake by algae x 0.12.

(iii) P extracted from algal cells by HCl = actual P-uptake by algae x 0.04.

RESULTS AND DISCUSSIONS

The test situation most applicable to actual lake conditions is the bioassay without supplementation of the nutrients already present in the sediment-lakewater mixture (the test headed no added nutrient medium). The data for this and the other tests on sediment from site 9 are shown in Table 2 while Fig. 1 shows some results for sediment from site 2. Note that in the no added nutrient medium tests blue-green algae were dominant, as might be expected since sediments are deficient in nitrogen.

In the no added nutrient medium test in light, 480 mg P m^{-3} was initially extracted from the sediment, but after 31 days of algal growth this decreased to 225 mg P m^{-3}. This apparent uptake by the algae of 255 mg P m^{-3} was corrected for the phosphorus extracted from algal cells themselves (Table 1) to give an actual P uptake of 305 mg P m^{-3}. However, the (unfiltered) lakewater itself contains 40 mg P m^{-3} phosphorus which is partitioned as H_2O-extractable (15 mg), NaOH-extractable (15 mg), and HCl extractable (5 mg), and when this, together with P in the nutrient media, is subtracted from the initial levels, the initial P extracted from sediment is obtained. These levels are shown for each test situation in Table 2.

From Table 1 we see that 12% of the total phosphorus in algae is extracted by NaOH, and 4% by HCl. If these proportions of the 305 mg P m^{-3} actual P uptake are then subtracted from the final figures, the actual P left in the sediment in each form is obtained (0, 30

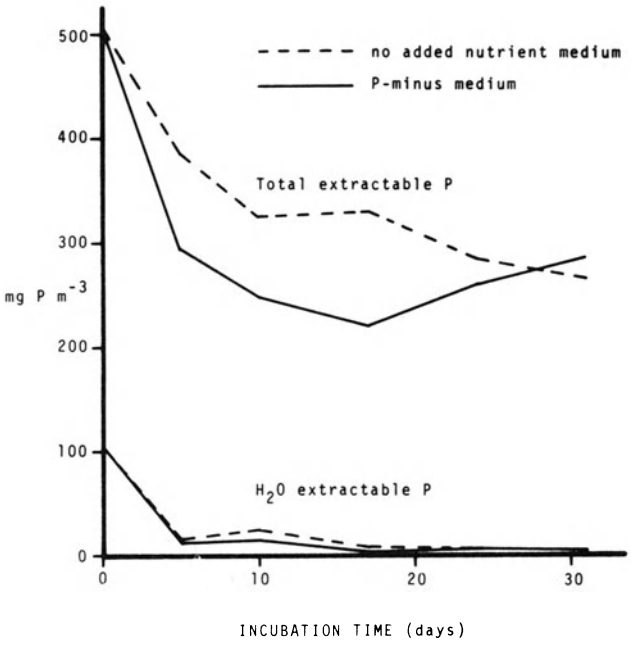

Figure 1: Sediment bioassay results - site 2.

and 150 mg P m^{-3} for the no added nutrient medium test; Table 2).

The corrected phosphorus decreases in the sediment for each test situation in the light for the 3 lake sites, and the percentages of P extracted, are shown in Table 3. Where no added nutrient-medium was used the algal populations extracted 85-88% of the phosphorus that would be extracted from sediment by NaOH. Assuming that there are no chemical changes in the nature of P during incubation, in two of the sediments (from sites 9 and 19) 21 to 22% of the HCl-extractable P was also available to the algae. For the other sediment there was an apparent 15% *increase* in the level of HCl-extractable P. Similar results were reported by Golterman *et al.* (1969), whereas Sagher (1974) reported that 55 to 100% of the NaOH-extractable P was available to the algae over a 3 to 4 week incubation period but that little, if any, of the HCl-extractable P was available to the algae.

The results show that with no added nutrient medium the algae extracted 57-64% of the phosphorus that could be removed from the sediment by combined NaOH and HCl treatments. When P-minus medium was supplied, the proportion of P extracted by algae was 45-72%. When phosphorus was included in the medium the proportion extracted from sediment was 28-35%.

This last test confirmed that phosphorus concentration limited uptake of P by the algae in the P-minus test system since about twice as much P was taken up by the algae in the P-plus test system (Table 2). Green algae dominated the culture flasks and even though the H_2O-extractable P was initially at a much higher level, it was still depleted within 10 days (compared to 5 days from the P-minus tests).

To express the algal uptake of sediment P (data headed "Total-ext P" in Table 3) in terms of the total sediment P (that is, inorganic-P plus organic-P) it is necessary to subtract the contribution of the lake water (40 mg P m^{-3}) from the total P of the test systems. The results for the test system with no added nutrient medium are given in Table 4.

TABLE 2

RESULTS OF SEDIMENT BIOASSAY TESTS ON SEDIMENT FROM SITE 9

Time	Algae: Relative counts [a] Greens	Algae: Relative counts [a] Blue-Greens	Total P	P extractable by: H_2O	P extractable by: NaOH	P extractable by: HCl	P extractable by: Total	P-uptake by algae: Apparent [b]	P-uptake by algae: Actual [c]
			mg P m^{-3}						
P-minus - in lake water - in light									
Initial	1	-	615	70	215	195	480		
From Sediment [d]				55	200	190	445		
Final [e]	55	-		2	65	165	230	250	300
From Sediment [f]				0	30	155	185		
No added nutrient medium - in lake water - in light									
Initial	1	-	615	70	215	195	480		
From Sediment [d]				55	200	190	445		
Final [e]	9	80		<2	65	160	225	255	305
From Sediment [f]				0	30	150	180		
P-plus - in lake water - in light									
Initial	1	-	1235	690	215	195	1100		
From Sediment [d]				55	200	190	445		
Final [e]	33	-		2	205	230	435	665	725
From Sediment [f]				0	120	200	320		
P-minus - in lake water - in dark									
Initial			615	70	215	195	480		
From Sediment [d]				55	200	190	445		
Final [e]				40	160	160	360	120	

[a] Algal counts are relative to the initial count for the lake water used in all the bioassay test flasks. Note that severe clumping, attachment to the walls of the flask and the size selectivity (<10 μm) of the haemocytometer used for cell counting invalidate the cell counts as an absolute measure of biomass.

[b] Calculated as the difference between the initial total-ext-P and the final total-ext-P.

[c] Calculated using the equation: actual algal P uptake = apparent algal P uptake x 1.19.

[d] Extractable P available from sediment alone. Calculated from the initial data by subtracting the contribution from the lake water and, where used, the P-plus medium.

[e] Data after 31 days incubation.

[f] Calculated from the final data by subtracting the amount of P extractable from algae by NaOH and HCl.

TABLE 3

ALGAL UPTAKE OF FORMS OF SEDIMENT PHOSPHORUS

All results are for sediments suspended in lake water and incubated in the light	Corrected decrease in sediment P fractions[a]					
	NaOH-ext P[b] mg P m^{-3}	(%)	HCl-ext P mg P m^{-3}	(%)	Total-ext P mg P m^{-3}	(%)
SITE 2						
with P-minus medium	280	(82)	-65	(50)	210	(45)
no added nutrient medium	290	(85)	-20	(15)	265	(57)
with P-plus medium	185	(54)	-50	(38)	130	(28)
SITE 9						
with P-minus medium	225	(88)	35	(18)	260	(58)
no added nutrient medium	225	(88)	40	(21)	265	(60)
with P-plus medium	135	(53)	-10	(5)	125	(28)
SITE 19						
with P-minus medium	280	(93)	50	(31)	330	(72)
no added nutrient medium	260	(87)	35	(22)	295	(64)
with P-plus medium	175	(58)	-15	(9)	160	(35)

[a] Corrected for the amount of P extracted from algal cells by NaOH and HCl and for the P composition of the lake water used in the tests.

[b] The sum of H_2O-ext P and NaOH-ext P at the start of the tests was used as the initial value of NaOH-ext P in calculating the decrease at day 31.

TABLE 4

PROPORTION OF SEDIMENT TOTAL PHOSPHORUS TAKEN-UP BY ALGAE IN "NO ADDED NUTRIENT-MEDIUM" TEST SYSTEM

Site	% Uptake of Total-P
2	47%
9	46%
19	43%

The final test system (Table 2) was a culture that contained P-minus medium but was incubated in the dark. The data for sediment from all 3 sites showed an uptake (presumably by bacteria) of 13 to 25% of the total extractable P although dissolved P (H_2O-extractable P) was not eliminated from the systems. There was no apparent increase in biomass (total organic carbon in the dark at the end of 31 days was about 1/4 to 1/7 of the values under light). However, if it is assumed that all of the total P (40 mg P m^{-3}) present in the lake water is taken up by bacteria, then the amount of sediment P taken up by bacteria becomes only 4 to 18%. If it is further assumed that bacterial mineralisation of sediment P under light is much lower, due to an ample supply of fresh algal detritus, then bacterial

release of sediment P under light is probably insignificant.

CONCLUSIONS

Nearly half (43-47%) of the total phosphorus contained in the top 100 mm of sediment from Lake Burley Griffin is available, during 3-4 weeks, to algae growing under aerobic laboratory conditions. Should this relationship apply to the lake itself,and assuming the same rate of loss of P in the outflow, we calculate that the lake could maintain its present level of primary productivity for about 23 yr even if *all* external sources of phosphorus were eliminated (Cullen *et al.*, 1978). This proportion of the sediment phosphorus is available with or without supplemental nutrients but, in the latter case, nuisance blue-green algae grow at the expense of the less troublesome green algae since sediments are deficient in nitrogen.

Nearly all (82-93%) of the phosphorus extractable from Lake Burley Griffin sediment by NaOH was available to algae, while up to about 31% of the phosphorus extractable by HCl was available, during a 31 day period. Thus these expedient chemical tests would provide an adequate indication of the potential for algal uptake of sediment phosphorus in many management situations.

It must be acknowledged that the conditions of the bioassay used in this work probably favour the release of the maximum amount of rapidly available sediment phosphorus. At the same time, the amount of phosphorus that might be released under the anaerobic conditions that often occur in the bottom waters of lakes is likely to at least be comparable with the amount released under aerobic conditions.

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BACTERIAL POPULATIONS OF TWO SALINE ANTARCTIC LAKES

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INTRODUCTION

The Vestfold Hills are an ice-free area of approximately 520 km^2 between the coast of Antarctica and the edge of the polar ice cap, located between 68° 22'S and 68°40'S, and 77°49'E and 78°33'E. Davis Station, established in 1957 by the Australian National Antarctic Research Expedition (ANARE), lies on the coast at 68°34'S, 77°58'E. The biology of the area has been summarized by Johnstone *et al.* (1973).

The hills contain numerous lakes and ponds of varying salinity (Fig. 1). Many of these lakes formed when sea water became trapped during isostatic uplift after the edge of the Antarctic ice cap retreated several thousand years ago. The varying salinities observable now are products of a number of factors, including proximity to the ice cap and the sea, the catchment area and topography, and the volume to surface area ratios of the water bodies. This paper describes the bacterial populations of the saline Ace Lake, and of the hypersaline Deep Lake, which has been the subject of several years limnological study by biologists from ANARE (cf. Kerry *et al.*, 1977).

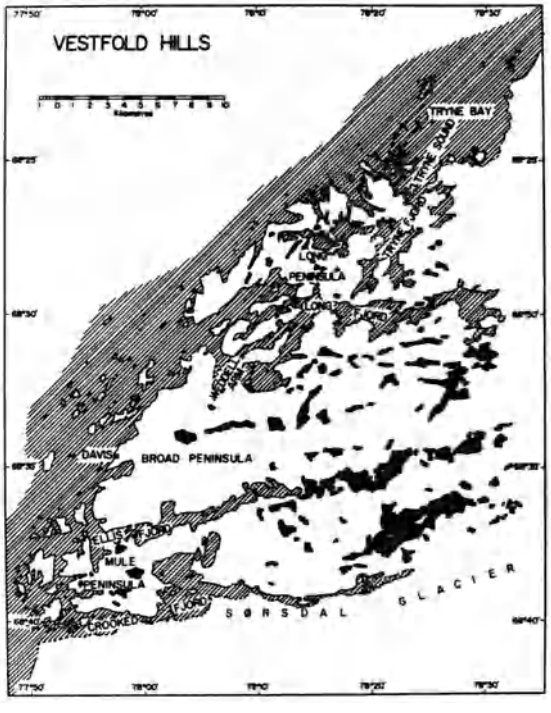

Figure 1: Map of the Vestfold Hills locating the major lakes.

METHODS

Water samples were collected using a Niskin (sterile bag) sampler or a Van Dorn sampler.

Temperatures were measured with a thermistor.

Oxygen concentration was measured by the method of Major *et al.* (1972); hydrogen sulfide concentration was determined by the titrimetric method of Golterman and Clymo (1969).

Light penetration was measured with a Kahl Scientific Instrument Corporation submarine photometer, by comparing transmitted light with incident light as determined by a deck cell.

Bacterial pooulations were studied using total and viable counts, and enrichment cultures. Total numbers were determined at a magnification of 500X by phase contrast microscopy using a haemocytometer. Viable counts were obtained by spreading 0.2 ml or 1.0 ml of the water sample on pre-dried plates of nutrient agar or tryptone soya agar (Oxoid Ltd.) which had approximately the same salinity as the lake. The inoculated media were incubated at 8° for eight weeks and illuminated for 12 h day^{-1}. Identification at genus level of some of the bacteria was based on cultural and morphological characteristics, the Gram-staining reaction, motility, and the physical and chemical properties of the environment from which they were isolated. The presence of methane was taken to indicate the presence of methane-producing bacteria, since there is no evidence for recent volcanic issues, which could be the alternate origin of methane.

RESULTS AND DISCUSSIONS

Ace Lake

Ace Lake (68°24'S, 78°11'E) is a meromictic (permanently layered) lake with a surface area of 0.13 km^2 and a maximum depth of 23 m. It is covered by up to 2 m of ice for more than half the year. The bacteria of Ace Lake were investigated during 1977 and 1978.

The surface water to a depth of approximately 7 m is oxic (Fig. 2) and has been termed the oxylimnion (Burton, 1980).

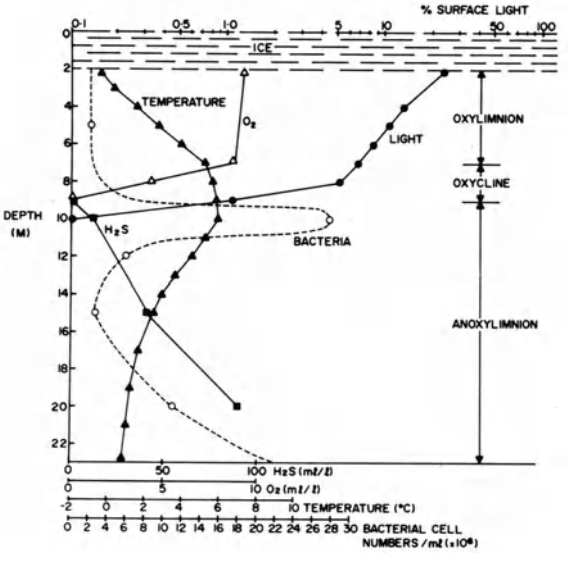

Figure 2: November profiles of oxygen and hydrogen concentrations, bacterial cell numbers, temperature and light for Ace Lake.

The lower, anoxic water from 9 m to the bottom is the anoxylimnion. The zone between 7 m and 9 m, where the oxygen content of the water decreases rapidly to zero, is the oxycline. The anoxylimnion contains hydrogen sulfide and methane, and is discussed by Burton (1980).

The salinity at the surface of the lake is approximately 28 g l^{-1}, about 80% that of sea water. Salinity increases evenly to nearly 40 g l^{-1} at the bottom. Sunlight penetrates to approximately 10 m, which is about 1 m into the anoxylimnion.

Total counts of bacteria were made at various depths down to 20 m. At 5 m in the oxylimnion the numbers were generally constant at near 2 x 10^6 cells ml^{-1}, with a slight increase to 2.5 x 10^6 cells ml^{-1} towards the end of summer. Preliminary evidence indicated the presence of algae in high concentrations (M. Burch, personal communication).

Greater numbers of bacteria were present near the interface of the oxycline and anoxylimnion at about 8-9 m and the types of bacteria suggested that a dynamic cycling of sulfur compounds was occurring (Fig. 3). At 9-10 m, in the irradiated top meter of the anoxylimnion, at least two genera of photosynthetic bacteria, *Rhodospirillum* and *Chromatium*, were represented. Oxidation of sulfide by photosynthetic sulfur bacteria produces elemental sulfur and sulfate which are reduced back to sulfide by sulfate-reducing bacteria (genus *Desulfovibrio*). Dissolved and particulate organic carbon resulting from this process supports populations of heterotrophs; aerobes in the oxycline and anaerobes in the anoxylimnion. The aerobic heterotrophs between 7 m and 9 m could be the major cause of the oxycline by reducing dissolved oxygen in the oxidation of the organic materials.

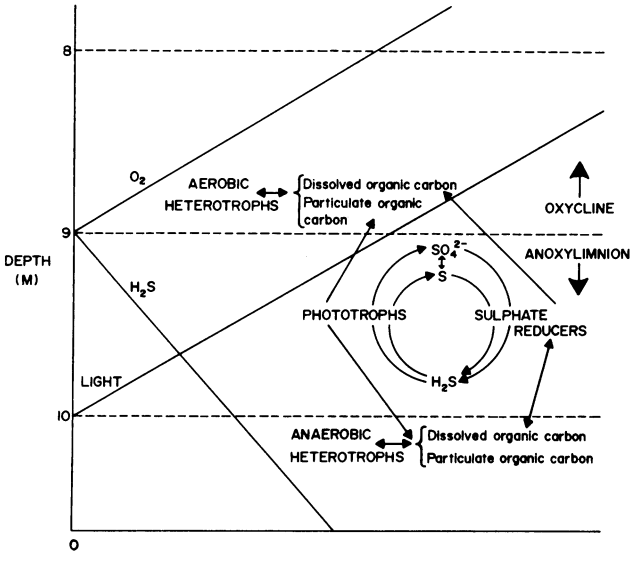

Figure 3: Suggested sulfur and carbon-cycling in the region of the oxygen-hydrogen sulfide interface in Ace Lake.

Other work on meromictic lakes has provided evidence for the cycling of sulfur compounds near the oxic-anoxic interface. In Ain-ez-Zauia in Libya, Butlin and Postgate (1954) found sulfate-reducers and sulfide-oxidizing phototrophs which, they considered, combined to cycle sulfur. The work by Kuznetsov (1968) and Sorokin (1970) on Lake Belovod in the USSR shows a situation where the physical, chemical and bacteriological properties at the oxycline in Lake Belovod, resembled those of Ace Lake. Cohen *et al.* (1977) showed the presence of bacterial phototrophs and cyanobacteria (as well as several other types of bacteria), but not sulfate reducers, in the water column of Solar Lake. However, both photosynthetic sulfide-

oxidizing, and sulfate-reducing organisms were found at the surface of the lake sediment where they were shown to be involved in sulfur cycling (Jørgensen and Cohen, 1977).

In Ace Lake, environmental and cultural data indicated the presence below 10 m of sulfate reducers, methane producers, photosynthetic sulfur bacteria, and anaerobic heterotrophs. Since light does not pass below approximately 10 m, phototrophic organisms cannot operate below this depth, and their presence in the anoxylimnion must have been due to sedimentation.

The total population increased quite considerably near the bottom. Burton and Barker (1979) suggested that this was due to bacteria settling out from the water above, but more recent evidence (Burton, 1980) indicates that there is an active mixed population confined to the lowest part of the water column where it is involved in the conversion of organic material to methane.

Total counts obtained during 1977 and 1978 for the zone of high population at 9-10 m show that the population reached a maximum density (and presumably peak activity) during the middle of November in each year (Fig. 4), even though the lake was still ice-covered.

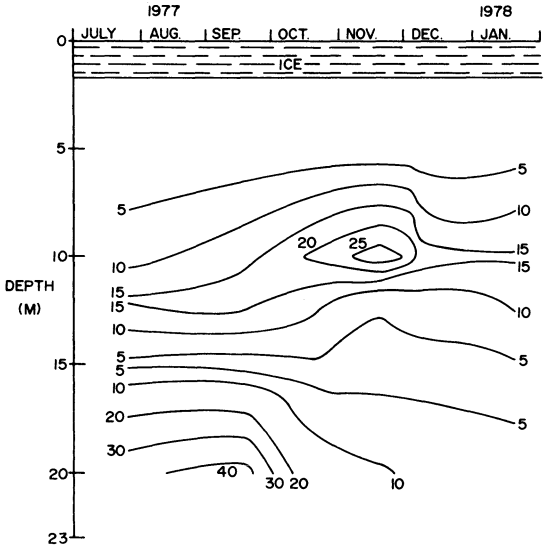

Figure 4: Isopleth diagram of bacterial population densities (in 10^6 cells ml^{-1}) in Ace Lake from the surface to 20 m, between July 1977 and January 1978.

The population rise coincided with the increasing daylength of the Antarctic spring. Conversely, the population declined in late summer as winter approached. Since the basis of the metabolic activity of photosynthetic organisms is the input of energy from sunlight, it would seem that the sulfide oxidation part of the sulfur cycle (carried out by photosynthetic bacteria) would be the rate-limiting step in the cycle during periods of less than optimal incident radiation. However, the substantial production of algae in the oxylimnion of Ace Lake demonstrated by Burton (1980) is likely also to be directly related to the seasonal changes of daylength. When these algal cells die organic matter rains down to the oxycline and during the approach of summer an increase in this organic debris could stimulate an increase in non-phototrophic bacteria. Thus, this descending organic matter could stimulate the resumption of the sulfur cycle at the top of the anoxylimnion. The total population of bacteria at 10 m was as high as 2.8×10^7 cells ml^{-1} during summer but

dispersed in winter. The water temperature at this depth changed little throughout the year, being between 5.8° and 7.0° (Fig. 5). The high population near 20 m in the middle of the year must be the result of sedimentation during the previous six months.

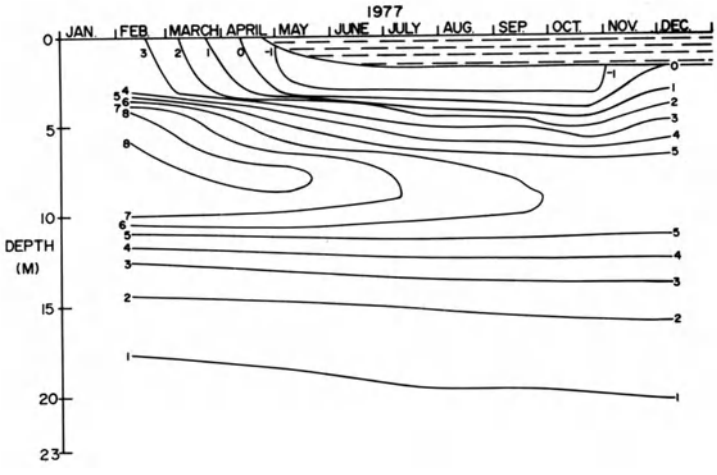

Figure 5: Isothermal diagram for Ace Lake for 1977. Isotherms are given in °C.

Somewhat similar situations to that in Ace Lake appear to occur in Clear Lake and Pendant Lake, two other meromictic lakes in the Vestfold Hills, although these have been less well studied than Ace Lake. In both Clear Lake and Pendant Lake, maximum densities of bacteria occurred just below the oxycline during summer (Table 1). However, in neither of these lakes does the bacterial population increase near the bottom, nor is methane produced in the anoxylimnion.

Deep Lake

Deep Lake (68°34'S, 78°11'E) is a hypersaline lake with a salinity of approximately 265 g l^{-1}, a surface area of 0.58 km^2 and a maximum depth of 36 m. It is monomictic, becoming completely mixed once each year during winter. The freezing point of the highly saline water is so low (ca. -28°C) that an ice-cover never forms over the lake.

Bacterial population densities throughout 1977, estimated by the total count method, were around 10^5 cells ml^{-1} at all depths except during winter when they approached 10^6 cells ml^{-1}. The population increase coincided with the onset of holomixis in the lake, suggesting that this was a purely physical phenomenon due to the resuspension of sedimented cells. Further evidence that the peak was not the result of biological processes was that it occurred during the coldest and darkest time of year, when any biological activity would be minimal. In fact, there is no evidence for bacterial activity in Deep Lake at any time of the year. Bacteria could not be cultured in vitro from Deep Lake when the salinity of the medium was more than half that of the lake itself. As well, the levels of total

organic carbon (approximately 50 mg l^{-1}) and total nitrogen (approximately 7 mg l^{-1}) were extremely high, indicating that no break-down of organic material was occurring. It seems that all the bacteria in Deep Lake have been washed from the surrounding catchment during the summer thaw, most coming in with a stream that flows through freshwater ponds. The bacterial population in the stream was up to 100 times that in the lake.

TABLE 1

BACTERIAL CELL NUMBERS IN RELATION TO OXYGEN AND HYDROGEN SULFIDE IN TWO LAKES IN THE VESTFOLD HILLS, ANTARCTICA

Lake	Depth	O_2 (ml l^{-1})	H_2S (ml l^{-1})	Cells (ml^{-1} x 10^{-6})
Clear Lake (18 Oct. 1978)	5	10.4	0	0.03
	20	10.3	0	0.05
	28	6.3	0	0.15
	30	0	17	32.5
	35	0	20	4.8
	40	0	27	6.0
	45	0	31	7.0
	50	0	34	4.5
	54	0	36	7.3
Pendant Lake (25 Dec. 1978)	5	9.5	0	0.10
	7	7.3	0	0.15
	8	9.5	0	7.3
	9	6.8	0	10.9
	10	0	0	22.9
	11	0	38	61.5
	12	0	105	2.1
	13	0	180	5.0

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MEASUREMENT OF BACTERIAL BIOMASS IN SANDY SEDIMENTS

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INTRODUCTION

Bacteria have important roles in sediment processes such as nutrient cycling, decomposition of organic matter and as a food source for many animals. In shallow marine environments where macrophytes are the main primary producers, bacteria are a dominant component of the food chain because much of the organic matter cannot be digested (Fenchel, 1977). Accurate estimates of bacterial biomass are needed in studies on their trophic role, but these are difficult to obtain in sandy sediments. Direct measurement of bacteria stained with acridine orange has been used after homogenising the sand to release attached organisms, but these are minimum estimates because an undetermined number of bacteria can remain attached (Dale, 1974; Meyer-Reil *et al.*, 1978).

Measuring the volumes of bacteria embedded in sediment is difficult, particularly as there is great variation in size. As it is tedious to count and measure more than a few hundred cells out of a population of perhaps 10^{10} cells g^{-1} of sediment, errors may result from small sample size and patchy distribution. I have attempted to use muramic acid (MA) to estimate biomass, because it should not be subject to errors due to operator fatigue, small sample size or the inability to see or recover all bacteria embedded in or attached to particles (Moriarty, 1975, 1977, 1978). This method, however, has other problems, which relate to the technique and the principle of the method. Measurement of trace amounts of MA in hydrolysed sediment is difficult and detailed procedures are given here for techniques that should overcome problems experienced previously.

Muramic acid was chosen as an estimate of biomass because it is a cell wall component unique to most prokaryotes (Rogers and Perkins, 1968). Being a constituent of the cell wall polymer peptidoglycan, its amount is directly correlated with surface area rather than biomass and thus some estimate of cell size is necessary to convert what is actually a surface area measurement to volume. There are two different types of cell wall structure in bacteria. Most of those that are termed a Gram-negative type (based on a standard staining reaction) have a single thickness of peptidoglycan, whereas the Gram-positive types may have from 2 to 10 layers (Rogers and Perkins, 1968). In earlier work, I had to make an assumption about the proportions of these types of bacteria in sediments because the Gram stain could not be applied directly. A combination of direct microscopy and MA determinations is shown in this paper to provide an estimate of the proportions of Gram-negative and Gram-positive bacteria that are released from the sand grains by homogenisation, and thus to provide more accurate estimates of sediment biomass. One more problem in using MA is that, if the cell wall degrades more slowly than cytoplasm in dead bacteria, an overestimate of biomass will result. In surface marine sediments, the effects of a wet, aerobic environment should lead to rapid autolysis and, combined with predation by animals, few empty wall sacs should accumulate (Moriarty, 1977). This problem is now being investigated by electron microscopy and preliminary results show that few wall sacs accumulate. In deep sediment layers, and in terrestrial soils, cell walls might be expected to accumulate and thus invalidate the use of MA to estimate biomass.

The participation of bacteria in the decomposition of seagrass detritus and in the cycling of nutrients has been discussed by Fenchel (1977). Moreton Bay, Queensland, contains many seagrass shoals and, as part of a study of their productivity, I wished to determine whether bacterial biomass in the sediment was influenced by seagrass cover. Sediments were analysed from two sites with seagrass and one without, although the latter site was surrounded by seagrass.

METHODS AND MATERIALS

Sediments

Sediments were from shoals in Moreton Bay, Queensland that were dominated by *Zostera capricorni* Aschers. The sediments were mainly siliceous sand, median grain size about 0.2 mm, with some heavy minerals. A corer, 50 mm diameter and 250 mm length, was used. For analysis, three layers of each core were placed in weighed containers on ice immediately after collection. The top aerobic layer (0-1 cm), the zone at which the redox potential changed as shown by black colour (usually between 1 and 3 cm), and a deep anaerobic layer (20-21 cm) were sampled. Two cores were taken intact on ice to the laboratory where redox potential was measured 30 min after collection.

At the laboratory, the samples were weighed, a 10 g subsample was removed from each and the remainder was freeze-dried and dry weights were determined.

Correlation of Direct Counts with Muramic Acid

The 10 g subsamples were diluted with 10 ml seawater (previously filtered through 0.2 µm membrane filter), and homogenised at 20,000 r.p.m. for 30 s using an 'Ultra-Turrax' (John Morris Scientific, Sydney). Sand grains were allowed to settle for 1 min and the suspension was decanted into a measuring cylinder. The sand grains were washed twice with filtered seawater and the total suspension of silt and bacteria was made up to 50 ml. A 1 ml sample was removed, diluted to 18 ml with filtered seawater, and 2 ml of concentrated (35%) formalin added. This suspension was stored at 4° until examined microscopically. The remaining 49 ml were centrifuged and the pellet was hydrolysed for MA determination. The technique for direct counts was modified from that of Daley and Hobbie (1975). A portion of the preserved suspension, usually 200 µl, but adjusted if necessary to give a relatively even distribution of bacteria and maximum count rate, was added to a filter funnel containing 5 ml of filtered (0.2 µm) seawater and 20 µl of 0.1% (w/v) acridine orange. After 2 min the mixture was filtered through a black o.2 µm cellulose acetate (Sartorious) filter, which was then mounted with oil under a coverslip. It was examined with a Leitz Dialux microscope using 12.5X eye pieces and a 50X water immersion objective, the Leitz Ploemopak epifluorescence apparatus and the K filter system. With bacteria embedded in detritus the cellulose acetate filters gave better results than polycarbonate types. Filamentous bacteria and large rods were counted in units of 1 µm length. If a filament was, say, 10 µm long, it was counted as 10 bacteria, not one.

Organic Carbon and Nitrogen

Organic carbon and nitrogen were determined using a Perkin-Elmer model 240 elemental analyser. Carbonates were a negligible proportion of these sediments.

Muramic Acid

Muramic acid values for the total sediment were determined on weighed portions of the freeze-dried sediment. The method has been published elsewhere (Moriarty, 1977; 1978), but as modifications have been made, a complete list of the steps in the procedure is given in Appendix 1.

RESULTS AND DISCUSSION

Relationship of Muramic Acid and Cell Numbers

Muramic acid values in the surface layers of all sediment cores were higher than values at 20 cm depth. Not only were there greater numbers of bacteria in the surface layers than in the deep layers, but there were more bacteria per µg MA at the surface (Table 1). Muramic acid content and numbers of bacteria in the layers at which the sediment changed from aerobic to anaerobic were more variable, although in most cases, the ratio of numbers to MA was less than at the surface. The very low ratio of numbers of cells to MA in deeper layers is probably due to two main factors; viz, an increase in proportion of Gram-positive cells and an increase in dead cells, which do not fluoresce, but which have walls containing MA. Preliminary observations with an electron microscope (unpublished) show that, at a depth of 20 cm, many intact walls are present without contents,

whereas most cells in the surface layers are intact. It was estimated that about 40% of MA in the deep layer might be present in the empty wall sacs. In calculating the ratio of cells to muramic acid and the bacterial biomass, therefore, the MA values of the 20 cm layers were reduced by 40%. Even with this refinement, there were still much lower ratios of cells to MA in the anaerobic layers, which suggests that the proportion of Gram-positive bacteria was higher. It is not possible to use the classical Gram stain on bacteria embedded in clay and organic matter. The approximate proportions of Gram-positive to Gram-negative cells can be estimated from the following formula (Equations 1-2), where n and p are the proportions of Gram-negative and Gram-positive bacteria, respectively (Moriarty, 1977):

$$mgC = \mu gMA/(8n + 44p) \quad (1)$$

where

$$n + p = 1 \quad (2)$$

Thus Equation 1 can be rewritten as follows

$$p = ((mgC/\mu g\ MA) - 8)/36 \quad (3)$$

It has been estimated from microscopic observation that the average biomass of cells in these sediments if 8×10^{-11} mg C. By combining this value with the ratio of the number of cells per µg MA, Equation 2 becomes:

$$p = ((1/(\text{No. cells} \times 10^{8}\ \mu g^{-1}MA \times 8\times10^{-11})) - 8)/36 \quad (4)$$

TABLE 1

MURAMIC ACID (MA) IN RELATION TO BACTERIAL CELL NUMBERS

Depth cm	E_h mV	Total MA µg.g^{-1}	No. Cells Per µg MA cells x 10^8 µg^{-1}	Gram-positive type cells approx. %	Recovery %
Site 1 : Seagrass Sediment					
0 - 1	-	4.4 ± 0.4	8 ± 1	20	33 ± 10
1 - 2	-	3.6 ± 0.6	7 ± 1	30	50 ± 5
20 - 21	-	2.4 ± 0.4	6 ± 1	40	60 ± 5
Site 2 : Seagrass Sediment					
0 - 1	+150 ± 50	3.8 ± 1.4	15 ± 10	0	50 ± 20
1 - 2	+ 40 ± 60	3.0 ± 1.0	8 ± 3	20	60 ± 30
20 - 21	- 80 ± 30	2.3 ± 0.1	6 ± 0	40	70 ± 10
Site 3 : Bare Sand					
0 - 1	-	0.9 ± 0.4	10 ± 2	10	70 ± 30
2 - 7*	-	1.0 ± 0.2	10 ± 3	10	85 ± 15
20 - 21	-	0.3 ± 0.1	6 ± 1	40	20 ± 10

Cells were mounted in the supernatant of the homogenised sediment and expressed as a proportion of MA in the same homogenate. The recovery of MA in the homogenised suspension is expressed as a percentage of that in the total sediment. Approximate percentages of cells with walls of a Gram-positive type are estimated as described in the text. Means and ranges of values for 3 cores are shown for sites 1 and 2, and for 4 cores at site 3. The sediment changed from mainly oxidising to reducing at about 1-2 cm depth, as shown by colour change from light to black, at sites 1 and 2. * denotes that 1 cm was sampled in the zone where colour changed from light to black, which varied in these cores from 2 to 7 cm in depth.

The values for the proportion of Gram-positive bacteria in Table 1 have been estimated from this equation. It provides only a very rough guide to proportions, because these values are influenced strongly by variations in the size of cells. Nevertheless, the similarity in results from all cores in three different areas, supports the conclusion that bacteria with cell walls of a Gram-negative type are more common in the surface aerobic layers than in deeper anaerobic sediments. Although it has not been possible to release all bacteria by homogenisation, it is assumed that these results apply to the total bacterial population. Methanogenic bacteria, which do not contain MA and thus could lead to confusing results, are unlikely to be present in the deeper layers studied here, because the sediments are subject to some aeration with tidal movement and thus the redox potential does not fall very low (Table 1). A study is now underway using transmission electron microscopy to investigate the types of cell wall structure in these sediments. Preliminary results indicate that Gram-positive bacteria constitute 12% of bacteria in the top layer and 30% in the 20 cm layer. There is reasonably good agreement between the numbers of bacteria and amount of MA in the homogenised samples, especially in the surface layers. These results verify the methods used to assay MA, and further support is given by the predicted proportions of Gram-positive bacteria and the values thus far obtained by electron microscopy.

King and White (1977) have criticised the method used here because D-lactate dehydrogenase is not entirely specific. As the results presented here show, the MA procedure does work, but ether extraction to remove D-lactate and glycollate formed after acid hydrolysis and the use of internal standards is necessary. The main advantage of the method is that small quantities of sample can be analysed, e.g. 1 mg organic matter. Purification of MA by thin layer chromatography is necessary when the starting material contains large amounts of polysaccharide because cellulose was found to degrade during hydrolysis to yield about 25 µg D-lactate (or the equivalent as glycollate) for each 100 mg cellulose. This error would not be detectable in the sediments analysed here. Fazio *et al.*, (1979) have developed a method using GLC, but it is not simple. A procedure using high pressure liquid chromatography that measures MA without degradation to lactate is being investigated at present, and thus far appears to be very simple and sensitive.

It is difficult to obtain an accurate estimate of bacterial biomass with any technique in sandy sediments. Direct microscopic counting of bacteria on sand grains is almost impossible, so direct microscopy after homogenisation was used by some workers (e.g. Dale, 1974; Meyer-Reil *et al.*, 1978). Counting bacteria embedded in detritus is very difficult, and I have found it even more difficult to measure their sizes. Not all bacteria were removed by the vigorous homogenisation used here; the amounts recovered were variable and unpredictable as shown by MA measurements (Table 1). Some estimates of total biomass based on direct counting only would have been seriously in error. As the results presented here show, MA values only would also not give an accurate estimate without knowledge of average cell sizes and proportions of Gram-positive and -negative cells. In order to estimate the biomass of bacteria from a factor based on surface (such as MA or lipopolysaccharide), the calculation should take into account the size of the cells, as well as the proportion of Gram-negative and Gram-positive bacteria. King and White (1977) proposed a factor, derived from the average MA content of a few organisms of both types, which is arbitrary and could lead to considerable under- or overestimation of biomass, depending on the composition of the population. Some marine Gram-negative bacteria have been shown to contain between 5 and 10 µg MA $mg^{-1}C$. This variation is due to differences in the sizes of the cells, as they all have the same thickness of peptidoglycan. Cells with a diameter of about 1.0 µm have about 5 µg MA $mg^{-1}C$; cells with a diameter of 0.6 µm contain about 10 µg MA $mg^{-1}C$ (Moriarty, 1977). Gram-negative cells 0.2 µm in diameter would contain about 28 µg MA $mg^{-1}C$, but there were few in these sediments.

Thus, as most Gram-negative bacteria in these sediments have diameters within the range 0.5 - 1.0 µm, a value of 8 µg MA $mg^{-1}C$ was selected to convert muramic acid values to biomass. If there were no Gram-positive bacteria present the error in biomass estimation should be less than 25%. Gram-positive bacteria however are present and, from values measured on a few types, an average of 44 µg MA $mg^{-1}C$ has been used in the calculations above. These bacteria also vary greatly in size and thus, for a given thickness of peptidoglycan, in their MA content. They vary much more in MA content, because they may have perhaps 2 to 10 or more layers of peptidoglycan. If they are a low proportion (e.g. 10% as in the surface layers here) the extra variability due to cell size and

composition should not have much effect on the error in estimation biomass. If they are a high proportion of a population, then many more determinations than I made of MA content and variation with size, should be made on cultures from the environment under investigation. Likewise, if other types of prokaryotes, such as sulfur bacteria or blue-green algae, are present in high proportions, the simplified formula (equation 1) may need to be modified. For the seagrass flats studied here, the formula is applicable. The blue-green algal population was low, and would have contributed less than 1% of the MA. Spores were present in very low numbers, compared to bacteria (Moriarty, 1975); this result was confirmed by electron microscopy, where no clearly identifiable spores were seen. The proportions of Gram-positive bacteria probably range from 10% at the surface to 40% at 20 cm, but even if subsequent more detailed work shows that the true values are say 40% at the surface to 80% in deeper layers (and this is unlikely), the biomass calculations would be in error by only a factor of 2.

Bacterial Biomass

To convert total MA values to bacterial biomass, the proportion of Gram-positive bacteria has been taken as 10% at the surface, 20% at 1-2 cm depth and 40% at 20 cm, giving factors of 85, 65 and 45, respectively, by which MA was multiplied to give carbon (equation 1 above). Muramic acid values at 20 cm were reduced by 40% to allow for dead cell walls. Preliminary results with electron microscopy indicate that there is little MA in dead walls in the surface layers. The work discussed here was carried out mainly to test the usefulness of MA as a measure of bacterial biomass. The results below are presented as an indication of the amount and variation in bacterial biomass in these sediments.

Bacterial biomass was greatest at the surface in most cores, although in some cores taken around seagrass roots a higher biomass was found at 1-2 cm, apparently in association with the roots (Table 2). Biomass was lowest in the deeper layers. At the surface, biomass

TABLE 2

BACTERIAL BIOMASS IN RELATION TO ORGANIC CARBON AND NITROGEN

Depth	Organic C		Bacterial C			C:N
cm	mg g^{-1}	g m^{-2}	mg g^{-1}	g m^{-2}	% Org C	
Site 1						
0 - 1	2.1 ± 0.2	30 ± 3	0.37 ± 0.04	5.2 ± 0.5	18 ± 2	10 ± 1
1 - 2	2.5 ± 0.3	35 ± 5	0.23 ± 0.04	3.2 ± 0.5	9 ± 3	10 ± 1
20 - 21	2.4 ± 0.3	34 ± 4	0.11 ± 0.02	0.15 ± 0.2	4 ± 0	11 ± 1
Site 2						
0 - 1	2.0 ± 0.4	27 ± 5	0.32 ± 0.12	4.2 ± 1.0	16 ± 4	9 ± 1
1 - 2	1.7 ± 0	22 ± 0	0.19 ± 0.06	2.6 ± 0.8	12 ± 4	9 ± 1
20 - 21	1.1 ± 0.3	15 ± 4	0.10 ± 0.01	1.2 ± 0.1	9 ± 1	10 ± 1
Site 3						
0 - 1	0.43 ± 0.08	6.9 ± 1.3	0.08 ± 0.02	1.3 ± 0.4	18 ± 5	7 ± 1
2 - 7[a]	0.29 ± 0.01	4.8 ± 0.1	0.06 ± 0.01	0.9 ± 0.1	20 ± 4	6 ± 0
22 22	0.32 ± 0.13	5.1 ± 2.2	0.01 ± 0.005	0.02 ± 0.1	3 ± 2	8 ± 1

Biomass was calculated as described in Results and Discussion. Organic nitrogen values in the sediment are expressed as a ratio of organic carbon. Values per m^2 are calculated over 1 cm depth. Means and ranges of values for 3 cores are shown for sites 1 and 2, and for 4 cores at site 3. [a]denotes that 1 cm was sampled in the zone where the colour changed from light to black.

was up to 10 times and, on average, about 5 times greater in sediments containing seagrass than in sediments from bare sand. The bare sandy areas were a few hundred m^2 in extent and were surrounded by the seagrass flats. The bare sand flat had lower biomass values and in general the oxidising zone penetrated deeper (down to 7 cm). Total organic carbon and nitrogen values were distributed similarly. Bacterial biomass varied from about 5% up to 25% of organic matter in the upper layers, with an average of about 18% at the surface (Table 2). These high values, together with the rather low redox potential and the presence of sulfide at about 1 cm in depth, especially around the seagrass roots, suggest that the bacteria were actively growing. Thus, there is likely to be a high rate of bacterial productivity in these flats, which is probably supported by exudation from seagrass roots in addition to sources such as leaching from leaves, epiphytes and benthic algae and dead material. As very little organic matter has accumulated in these sediments and the C:N ratios are not high, it seems that most of the organic matter that is not carried away by currents is used by the microbial community and not deposited. No dead leaves or fragments of leaves were observed below the surface of the sediment, so root exudation and decomposition may well be important. Marshall (1970) discussed a similar situation that occurs on shoals with stands of *Zostra marina* in New England. He argued that the low organic carbon content of the sediments was a direct result of high microbial productivity. The low proportion of dead cell walls in the surface layers, which is indicated by the good agreement between direct counts and MA values and is confirmed by preliminary results with an electron microscope, is further evidence that most of the bacteria are active and that there is high predation on them.

ACKNOWLEDGEMENTS

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APPENDIX 1

DETAILED PROCEDURE FOR DETERMINATION OF MURAMIC ACID

1. Weigh out 500 mg - 2 g dry sediment (freeze-dry for storage).

2. If sample contains $CaCO_3$, shake (overnight if necessary) in 10% acetic acid (2 ml glacial acetic $g^{-1}CaCO_3$). Centrifuge, discard supernatant: wash in distilled water, centrifuge.

3. Transfer sample to tube (e.g. prescored ampules, 5 ml capacity), then add 2-4 ml HCl, 4M.

4. Seal the tube and hydrolyse at 100° for 8 h. Hydrolyse for 18 h if TLC is to be used for further purification.

5. Transfer to 30 ml centrifuge tube and remove HCl in vacuo, (e.g. over NaOH pellets in acid-resistant freeze-dryer).

6. Dissolve the sample in distilled water, add 0.2 ml Na_2HPO_4 (0.5M), adjust to pH 8.0 with NaOH; centrifuge any precipitate, wash pellet and keep supernatant and washings. Adjust pH to 1-2 with HCl and freeze-dry.

7. Extract with diethyl ether. Vortex for 1 min with 10 ml ether; centrifuge. Repeat once. Discard ether. Dry off remaining ether, dissolve in distilled water, adjust to pH 9.0 with NaOH and make up the known volume, e.g. 2 ml.

8. Freeze half of the sample (-15°) for the residual D-lactate and glycollate assay (e.g. 1 ml).

9. Adjust the other half (e.g. 1 ml) to pH 12.5 and incubate at 35°-40° for 2 h.

10. Reduce pH to about 9.0 with HCl, make up to twice volume in previous step (e.g. 2 ml).

11. Volume after alkali hydrolysis should be twice that after ether extraction. Set up 3 tubes for each assay as follows:

    (a) 50 µl acid hydrolysate (after ether extraction);
    (b) 100 µl alkali hydrolysate;
    (c) 100 µl alkali hydrolysate + 50 µl (1 ng μl^{-1}) D-lactate as internal standard.

    (These volumes may be increased if lactate levels are low, or decreased if high or if inhibition is high).

    Make (a) and (b) up to 150 µl total volume with distilled water. Make a set of standards (in range to be measured), final volume of 150 µl (usually 0, 25, 50, 100, 150, 200 ng).

    It may be best to assay 10 samples (30 tubes + 5 standards) at one time. If more are to be done, they can all be set up and kept in the freezer and removed in batches of 10 for enzymatic assays.

    Each sample tube (acid and alkali treated) contains the same amount of original mixture (with the above procedure) and thus inhibition should be the same in all. Correct acid hydrolysates for inhibition by same amount as alkali hydrolysates. Sediments commonly give about 10-40% inhibition. If inhibition is much more than this, dilute samples to decrease inhibition, and repeat.

12. Now prepare the following lactate reaction mixture sufficient for the following number of samples desired.

Glutamate buffer 0.1M pH 9.4	0.5 ml/assay
NAD 33 mg Ml^{-1} in H_2O	10 µl/assay
D-LDH 5 mg ml^{-1}	4 µl/assay
GPT 10 mg ml^{-1}(80 U mg^{-1})	2 µl/assay

    Check pH after adding enzymes to buffer: $(NH_4)_2$ SO_4 can cause it to drop below

pH 9.0. If so, raise to pH 9.0 with NaOH (1M).

13. Add 0.5 ml of the D-lactate reaction mixture to the small test tubes containing sample and mix rapidly.

14. Place the test tubes in a water bath at 30° for 20-30 min.

15. Transfer the tubes to crushed ice.

16. Prepare a volume of the following reagents (phosphate mixture) sufficient for triplicate analyses and equilibrate the mixture at 30°.

Phosphate buffer 0.1M pH 7.5	2 ml/assay
FMN 0.5 mg ml^{-1} H_2O	10 µl/assay
Tetradecylaldehyde saturated in ethanol	10 µl/assay

17. Prepare 2 mg of bacterial luciferase in 1 ml of phosphate buffer, then mix with bovine serum albumen (2 mg), centrifuge at 15 min at 2000 x g at 0° and keep in ice.

18. Place 2 ml of phosphate mixture into a small polypropylene vial then add 20 µl of luciferase and mix.

19. After 15 s (or similar known interval), add 200 µl of the lactate reaction mixture into the propylene vial containing phosphate mixture plus luciferase.

20. Cap the tube, mix and place in a scintillation vial.

21. Start the counting sequence precisely 30 s later. Prepare next tube while waiting.

22. Measure standards first, then samples, then standards again. If the time period is short (about 15 min) between standards, little variation should be noticed. If it is long (about 1 h), the standard curve can alter (Moriarty, 1978); in this case a computer program is used to add increments to slope and/or intercept. NADH levels are not stable; they rise gradually to a maximum in the ice bath, then fall, so it is better to assay a few (approx. 10-12) samples (30 tubes) at a time: i.e. incubate a new batch of standards and samples with D-LDH as necessary. Check new batches of D-LDH and GPT to ensure that the levels of "NADH oxidase" is low.

23. Variations may be required in the volumes of sample, concentration and volume of glutamate buffer mixture and concentration of luciferase, in order to accommodate differing requirements for amount of lactate in samples, or variations in enzyme activity. High blanks, i.e. in the acid hydrolysate tubes (step 11a) indicate that divalent or heavy metals were present during ether extraction: add more phosphate at step 6, and carry out ether extraction again.

* D-LDH = D-lactate dehydrogenase; GPT = glutamate pyruvate transaminase.

CARBON FLOW WITHIN THE SOUTH WEST ARM OF PORT HACKING (N.S.W., AUSTRALIA)

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INTRODUCTION

In August 1973 some 15 scientists from CSIRO's Division of Fisheries and Oceanography, and from several other organizations, began a study aimed at understanding the flow of carbon through various biological and chemical compartments in a small marine embayment: the South West Arm of Port Hacking (located about 30 km south of the center of Sydney, New South Wales, Australia : Fig. 1). Following the termination of the project in 1978

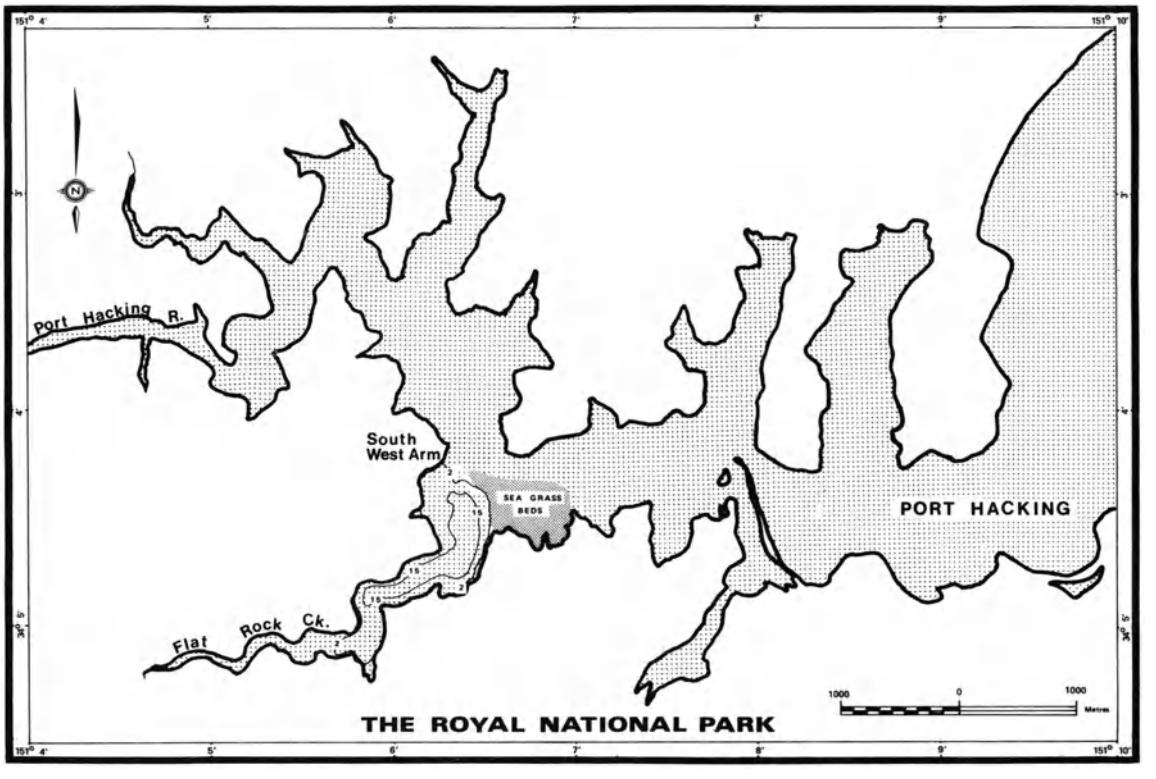

Figure 1: Map of Port Hacking, showing the location and general features of South West Arm. The location of two depth contours in SWA and the seagrass beds are shown.

an attempt was made to synthesize the available data and, to this end, both the carbon budget and compartment modelling approaches were found useful. The former can be interpreted as a static, or time-invariant, model and the latter as a dynamic, or time-varying, model. The essential features of a static and a dynamic model of carbon flow in South West Arm are presented below.

STUDY AREA

South West Arm (SWA) has a surface area of about 0.8 km^2 (low water) and is about 2 km long and 700 m across at its entrance (Fig. 1). It has a maximum depth of about 19.2 m (ISLW) and a tidal range of 2 m (spring tide; 1.3 m neap tide). For about 1 km upstream from the entrance there is a deep basin which rises from its maximum depth steeply (within 100 m) to a wide shallow (<2 m deep) sill of sand, as illustrated in Fig. 1.

For about 75% of the time the salinity of SWA is that of an arm of the sea. During the remaining 25% of the time there are a series of short transient states following the input of freshwater from rainstorms. As little quantitative data are available relating to these states, our models represent only that 75% of the time when the water has marine salinities.

A large seagrass bed (>80% *Posidonia australis*) exists just outside of SWA (Fig. 1). Given the proximity of such a highly productive region, we felt it necessary to evaluate its influence on the behaviour of South West Arm.

STATIC MODEL

South West Arm was conceptualized in terms of separate water column and sediment subsystems. In each subsystem the following compartments were used: dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), detritus, autotrophs (with subdivisions), and heterotrophs (also subdivided). The information that exists about annual-averaged compartment sizes and about the flow of carbon through various compartments in SWA is summarized in Fig. 2. (For the preparation of this illustration it was assumed that SWA has a uniform depth of 10 m.) In this figure the seagrass and benthic microalgal compartment sizes and flow rates apply only to the seagrass beds; the flow rates from water column autotrophs to sediment epifauna and macrobenthos apply only to the intertidal and shallow subtidal areas - a small fraction of the area of SWA; the flow rates from DOC to heterotrophs are for photosynthetically-derived DOC (i.e. PDOC) only.

A general perspective of Fig. 2 shows that most of the water column carbon is in the form of DIC (53% of total carbon); in the sediment subsystem the largest compartment is detritus (29%), although seagrasses also retain a substantial amount of carbon (12.5%). Only 15% of total carbon is contained within living organisms. Further, there is a total flow rate of 450 g C $m^{-2}yr^{-1}$ in the water column (including inflows and outflows). Seagrasses and microalgae account for a flow of 130 g C $m^{-2}yr^{-1}$ in the sediment subsystem and other compartments cycle 120 g C $m^{-2}yr^{-1}$. About 220 g C $m^{-2}yr^{-1}$ flow between subsystems due to the seagrasses and another 280 due to other compartments.

There is no evidence that significant amounts of carbon are imported into SWA from the seagrass beds. Particulate carbon, in terms of floating seagrass leaves, is exported from the seagrass beds at a rate of 11 g C $m^{-2}yr^{-1}$ and only about half of this carbon is likely to enter SWA and sink to the sediments. The 77 g C $m^{-2}yr^{-1}$ of water column DOC that is produced by the seagrasses has an unknown fate but it is known that the water velocity in the seagrass beds is low.

A more detailed perspective of Fig. 2 suggests that the dominant path of carbon flow in the water column is from DIC to autotrophs to heterotrophs. We know that a substantial amount of detrital carbon in the water column (274 g C $m^{-2}yr^{-1}$) settles to the sediment subsystem and that this carbon will be generated by flows from the water column autotroph and heterotroph compartments. The fate of the majority of the newly introduced sediment detritus is largely unknown; the obvious path is to sediment heterotrophs, probably micro-/meiobenthos, as decomposition organisms. The only known flow from sediment heterotrophs is to DIC.

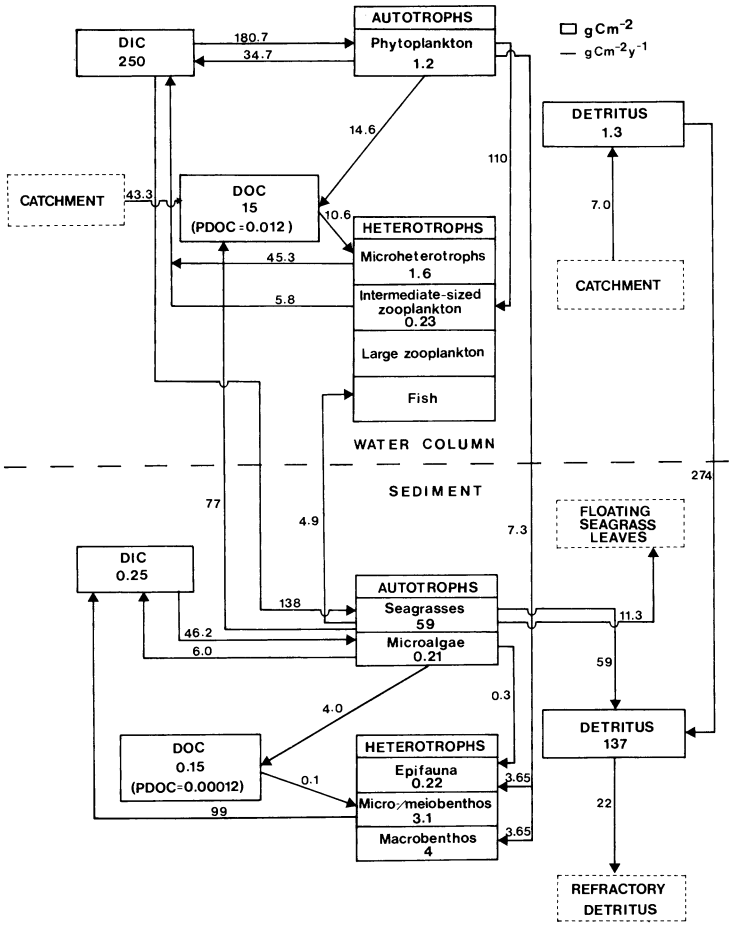

Figure 2: Annual-averaged compartment sizes (g C m^{-2}) and flow rates (g C $m^{-2}yr^{-1}$) for the flow of carbon through South West Arm. Inflows and outflows are indicated by the dashed boxes.

DYNAMIC MODEL

Given the main flow path as identified above and invoking an assumption of annually-balanced compartments, it is possible to estimate the unknown flow rates (see Fig. 3). Since the available data allowed us to deal only with intermediate-sized zooplankton, the flow rate from the water column to sediment detritus was reduced.

A mass balance differental equation was written for each compartment. Where better data from SWA were not available it was assumed that the flow rates could be best characterized

as being constant (flow to refractory detritus) or being linear donor- or recipient-dependent, as suggested by knowledge of the mechanisms determining the flow rates. This allowed the rate constants to be easily derived from the data given in Fig. 3. Wiebe and Smith (1977) showed experimentally that flows between water column PDOC, micro-heterotrophs and DIC could be adequately described by such relationships. Griffiths and Caperon (1979) showed experimentally that the flow from water column autotrophs to intermediate-sized zooplankton is a function of the product of the two compartment sizes, at least for phytoplankton concentrations of from 0.32 to 3.2 g C m^{-2}. Data on SWA collected by Scott (1978,1979) were adequate to allow us to apply a theoretical equation derived by Bannister (1974) for predicting gross primary production, vis.,

$$\frac{12\phi_{max} I_{max} k_c C \lambda}{k_c C + k_w} \int_{-\frac{1}{2}}^{+\frac{1}{2}} \left[e^{-TI_o/I_{max}} - e^{-I_o/I_{max}} \right] dt'$$

where ϕ_{max} is the maximum quantum yield [=0.06 (mol C) (einsteins absorbed)$^{-1}$]; I_{max} is the illumination at which the light saturated photosynthetic rate occurs (=40 einsteins m^{-2} day^{-1}; 117 J m^{-2} s^{-1} inside the integral; Scott, 1979); k_c is the extinction coefficient per unit of phytoplankton (=0.048 $m^2 mg^{-1}$ Chl *a*; Scott, 1978); C is chlorophyll *a* concentration (mg Chl *a* m^{-3}) calculated from the model output assuming C/Chl *a* =60; k_w is the non-phytoplankton component of the extinction coefficient

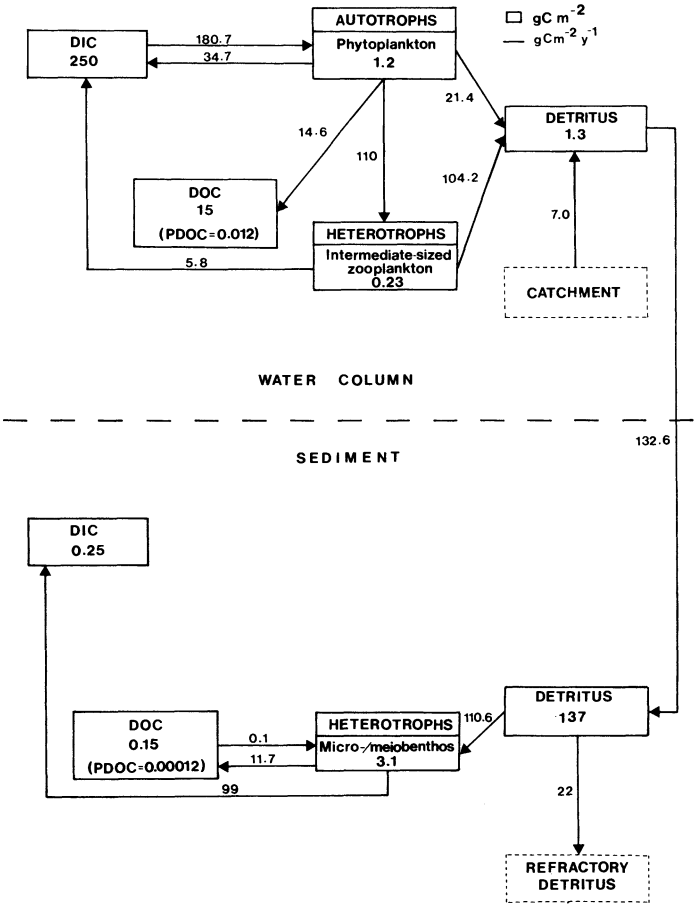

Figure 3: The dominant path of carbon flow through South West Arm

(=0.220 m^{-1}; Scott, 1978); λ is the day length (=0.507+0.093 cos(2 Π(t+9)/365)) where t is day number from January 1; T is the fraction of incident subsurface illumination transmitted to 10 m, calculated using Beer's Law; and I_o is the incident subsurface illumination. We assumed that 50% of total irradiation is photosynthetically active and 15% is reflected by the water surface. The total surface irradiation was assumed to vary as 0.5 $I_{o\,max}(1+\cos 2\Pi t')$ where t' is dimensionless time (see Vollenweider (1965)). $I_{o\,max}$(in J $m^{-2}s^{-1}$) was calculated from a fit to local data as 856+283 cos(2Π(t+12)/365).

The model was implemented and run without parameter adjustment. For average phytoplankton densities, gross primary production was too large by a factor of about 3. The effects of limiting nutrient concentrations are not included in the model. Some preliminary calculations with the Michaelis-Menten expression suggest that average nutrient concentrations in SWA, and in particular nitrate concentration, could account for this discrepancy. A second result from our simulations is that, using the experimentally-determined function for the flow rate from water column phytoplankton to zooplankton, all light-induced production above the average value is taken up by zooplankton: the phytoplankton mass stays constant over the year while the zooplankton mass shows an annual, light-induced cycle. Griffiths and Caperon (1979) obtained measurements of the rate constants ranging from 0.12 to 0.42. Each estimate was simulated - after recalculating the unmeasured flows from each of autotrophs and heterotrophs to detritus. The value of the constant did not affect the predicted phytoplankton mass but for 0.12 and 0.30 (all phytoplankton eaten by zooplankton) the peak zooplankton masses were 0.95 and 0.52 g C m^{-2}, respectively. The degree to which zooplankton mass varies over the year in SWA is unknown.

DISCUSSION

The flow of carbon through various biological and chemical compartments in South West Arm is very complex. In spite of the considerable experimental effort expended in understanding these flows there remain many gaps in our knowledge. In this situation we found it useful to construct and use a variety of models for synthesizing the available information.

The static model was most consistent with the data but also most limited in its predictions. The dynamic model was more powerful in describing the temporal pattern of variation but was based less on solid information than the static model. It is obvious that a third level can be added - an ecosystem model containing dynamic models describing both carbon flow and water circulation. Such a model has, in fact, been developed for SWA by Cuff *et al.* (1978). In this model we included all flows thought to be of potential importance and those special, freshwater-induced transients which occur in SWA for about 25% of the year. This model is highly desirable in terms of the number of processes it simulates but very far indeed from a solid information basis for South West Arm.

ACKNOWLEDGEMENTS

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IN SITU EXPERIMENTATION WITH ANAEROBIC SEDIMENTS: SOME BIOGEOCHEMICAL APPLICATIONS

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INTRODUCTION

The biogeochemistry of sediments is mostly confined to the upper part of the sediment. It is very rare to find biological processes of major importance in the geochemistry of sediments below a depth of half a meter. More often the major chemical changes take place at the uppermost centimetres of the sediments. In anaerobic sediments (sediments with the redoxcline at or above the sediment/water interface) biological effects on chemical transformations are usually restricted to even more shallow depths than with aerobic sediments. In the Baltic Sea anaerobic sediments are unconsolidated with a water content of 70% or more (Fig. 1). These sediments occur at various water depths but are mainly situated below the halocline, that is below about 55 m. The biogeochemistry of these sediments is being studied with the use of an in situ simulation technique. The experimental site is at a water depth of 10 m. This depth was chosen to facilitate the necessary SCUBA diving.

The in situ technique is applied under conditions which resemble as closely as possible the natural environment. The intricate structure of the sediments is maintained and is thus not affected by compression or stirring forces as is the case with more generally used sampling techniques. The compression effect was demonstrated in an early experiment where an unusually high content of ammonium ion was observed in the enclosed bottom water immediately after the start of the experiments (Fig. 2). This illustrates a common error introduced in analyses of bottom water and pore water from sediment cores where the sediment is compressed by the choke-wave in front of the corer. In later experiments this error has been eliminated by a careful procedure during the installation of the experimental systems. The system which employs a plexiglas box is gently pushed into the sediment with its top part open. It is left a few days to equilibrate with the surrounding environment and is then closed with a lid.

THE CLOSED BOX SYSTEM

The closed experimental system was developed 1970 and was described by Hallberg *et al.* (1972, 1976). It was aimed to simulate the chemical change between sediment and water in the stagnant basins of the Baltic Sea. During the stagnant periods these basins can be considered as closed systems. Their "life cycle" starts with a rapid decrease in oxygen concentration followed by anaerobiosis and increasing concentrations in the water mass of, for example, hydrogen sulfide and phosphate and ammonium ions. Periodically the water mass is washed out by oxic and more saline water intruding into the basins from the North Sea, and the cycle is terminated. An average duration of this "life cycle" is between six and twelve months. An example of the results obtained with the simulated system is shown in Fig. 3. The kinetics and total amounts of compounds released from the sediment and accumulated in the water mass depend on the time of the year at which the experiments are conducted. This is because of seasonal variation in nutrient levels of the sediments. Organic matter is the driving force for the biogeochemical reactions in the anaerobic sediments. During the spring the sediments are rich in diatoms while during autumn they are comparatively depleted in easily degradable organic matter. An example of this seasonal effect on phosphate release from the sediments is shown in Fig. 4.

The results of the closed box experiment agreed well with those of an investigation of a stagnant fjord on the Swedish west coast (Dyrssen and Hallberg, 1979). This was in spite of the fact that the fjord has a continuous supply of sediment material and organic matter, and an almost constant concentration of sulfate. In the box experiments sedimentation is eliminated and the sulfate concentration decreases markedly. Temperature and salinity of the fjord and box experiments were 5 - 6°, 31 per mil and 1 - 16°, 7 per mil respectively. The kinetics of ammonium ion release were practically the same (0.55 mmol m^{-2} day^{-1}: Fig. 5). Phosphate release was more rapid at the beginning of the box experiment because of

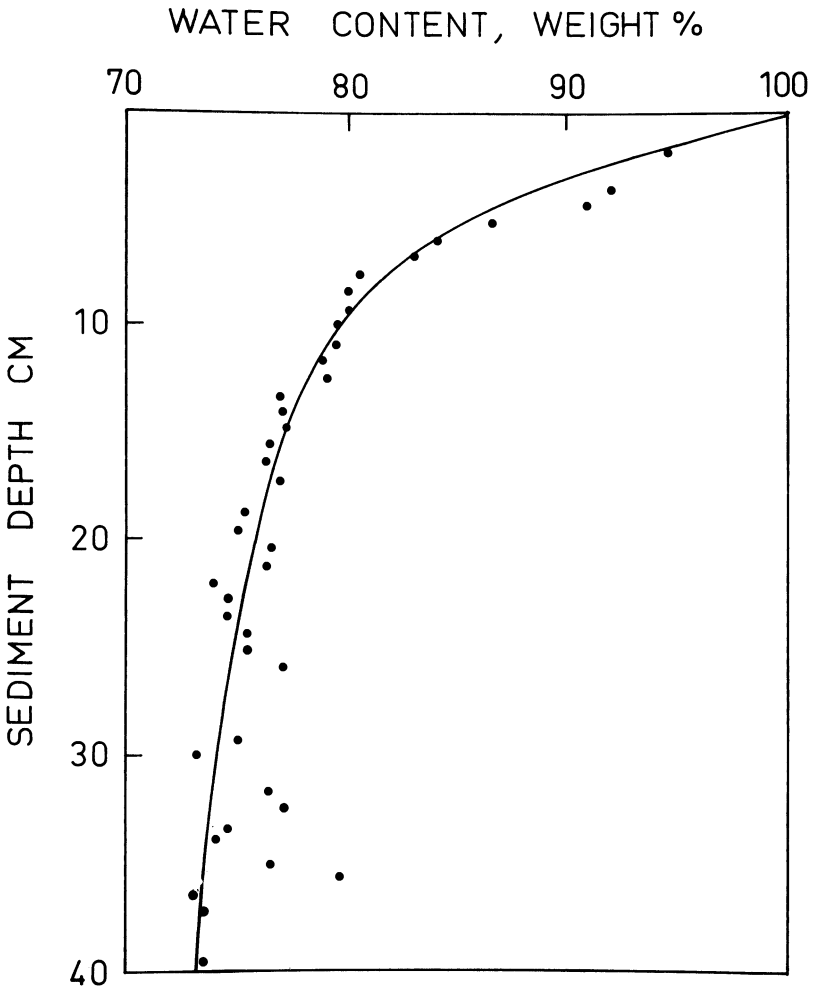

Figure 1: Water content of the sediments of the Gotland Deep, Baltic Sea. The graph is a schematic representation of the data of Niemistö and Voipio (1974).

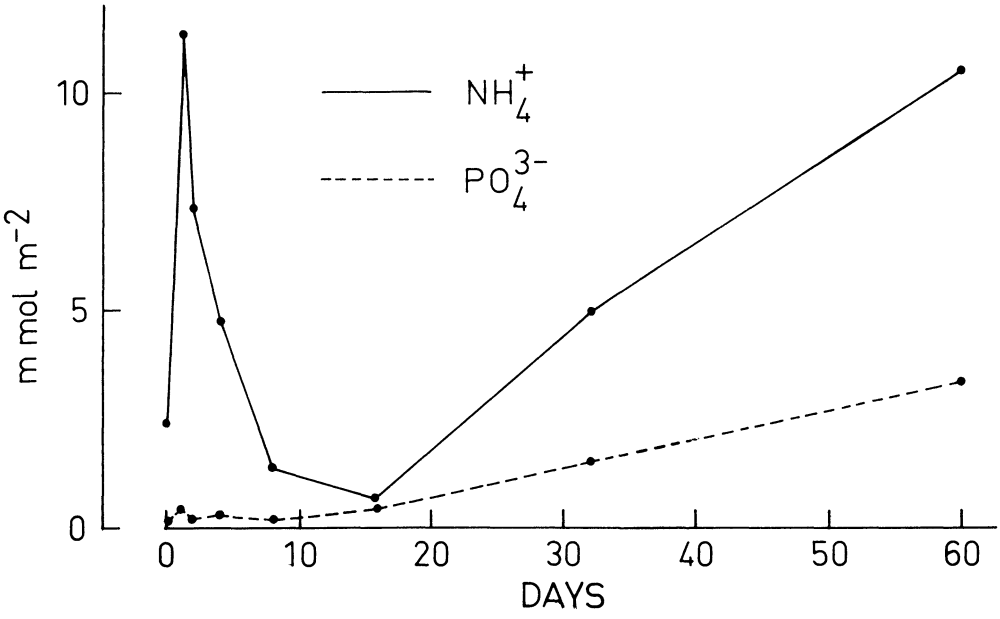

Figure 2: Variation of ammonium and phosphate during a redox turnover in the closed box system (Hallberg *et al.*, 1972). The ammonium ion peak at the beginning is an artefact (see text).

the redox turnover effect. After the first rapid release the reactions slowed down but a seasonal variation was observed (Holm, 1978), with the lower rates occurring during autumn. The rate of phosphate release from the fjord (0.083 mmol m^{-2} day^{-1}; Almgren *et al*, 1975) was within the range (0.03-0.3 mmol m^{-2} day^{-1}) found with closed box system (Holm, 1978).

The box experiments are less expensive than a corresponding investigation with the same sampling frequency from a vessel to cover the natural fluctuation in the Baltic Sea basins. During the stagnant periods the deep water of the basins can be considered homogeneous although they sometimes show vertical gradients. The confined water of the boxes has to be homogeneous in order to facilitate the interpretation of the sampling results. The boxes contain 32 l of water and a sediment area of 0.16 m^2. The water removed during sampling is compensated for by an equal volume of surrounding sea water. If a smaller box volume had been used, frequent sampling would have given rise to noticeable environmental changes inside the boxes. On the other hand, a significant volume should probably generate gradients unless a stirring system was used. We have occasionally used stirring systems in our boxes but found that the homogeneity of the confined water was maintained without stirring.

The environmental changes in the boxes are most significant at the beginning of the experiments, that is during and following the redox turnover of the chemical system. The sampling has, therefore, to be most frequent during that period. It is also important that

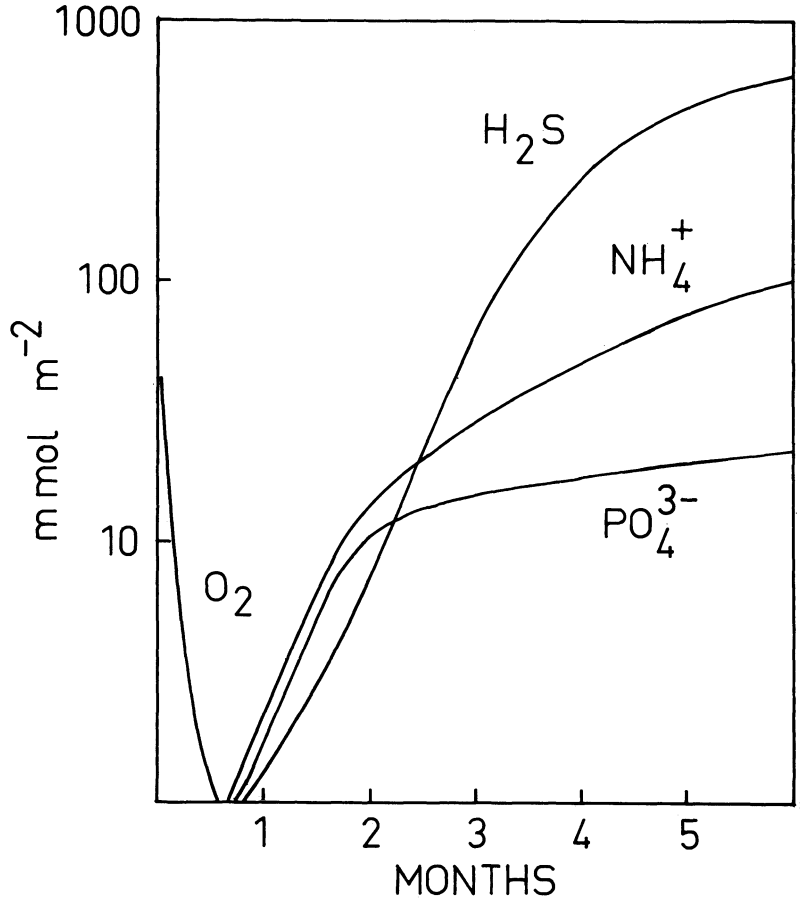

Figure 3: Schematic representation of the physico-chemical variation in the closed box system. This is aimed to represent the first part of the "life-cycle" in a stagnant basin of the Baltic Sea (see text).

the samples are analysed immediately upon collection, as the biological system is very active and the redox buffering capacity is very low.

The closed box system has advantages over the in situ bell jar system used by the Oceanographic Institute in Kiel (Balzer, 1978). The latter encloses 2094 litres of sea water and 3.14 m^2 of sediment and has a very advanced sampling system whereby the samples are taken automatically and stored at the experimental site. One package of ten samples is usually collected once a week. However, chemical and biological reactions in the samples will continue during storage and the obtained data will, therefore, exhibit a high scattering. Only the last sample of each package can be considered representative. In practice an additional sample for analysis was usually taken manually at the time of automatic collection of the sample package, indicating that the automatic sampling technique had limitations (Balzer, 1978). A technically complex system also requires a large

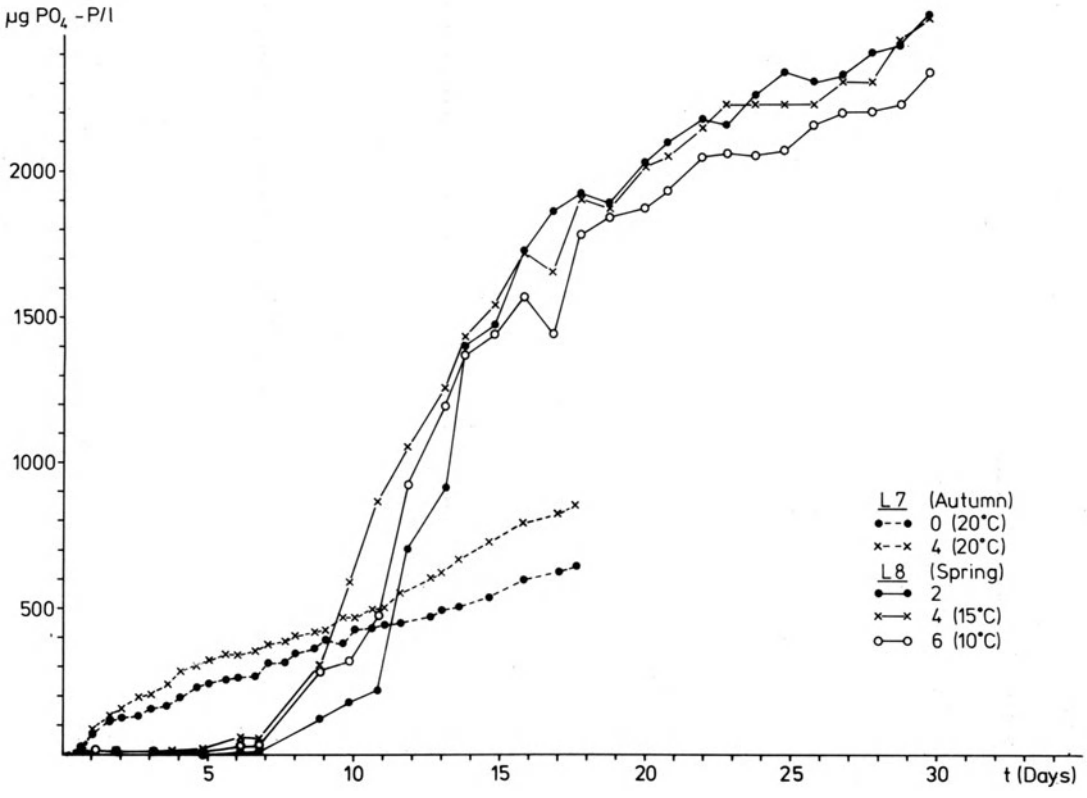

Figure 4: The release of phosphate from sediments in the closed box system. The early part of the release is at a much higher rate during spring than during autumn (Holm, 1978).

budget, which usually limits the number of systems and experiments. The relatively simple closed box systems allow experiments to be carried out in duplicate. Moreover several experiments, in which parameters such as temperature and organic matter may be varied, can be run in parallel. Excellent agreement between the results of duplicate experiments has been obtained (Fig. 6).

THE OPEN CYLINDER SYSTEM

The disadvantage of the closed system is that, while it represents the dynamics of a basin with a stagnant water mass, the continuous input of material from the overlying water body is eliminated. We have, therefore, initiated a study with an open cylinder system, though on a smaller scale than that of the "corals" used in Lake Eire (Lean and Charlton, 1976). The open system consists of a plexiglas cylinder (height above sediment 1.2 m and diameter 0.3 m). The water close to the sediment/water interface inside the cykinder is replaced by water of approximately double the salinity of surrounding waters by introduction through an opening at the lower part of the cylinder, thus forcing a corresponding amount of the original water to overflow. Thereby a halocline is established in the cylinder and a reducing environment is created below the halocline with a continuous contribution of material from above. Two biogeochemical gradients are achieved, one at the halocline and one at the sediment/water interface. In the first experiment the halocline was stable for a little over two months when it became disturbed by strong currents above the cylinder. To determine the input of materials from the overlying water without disturbing the

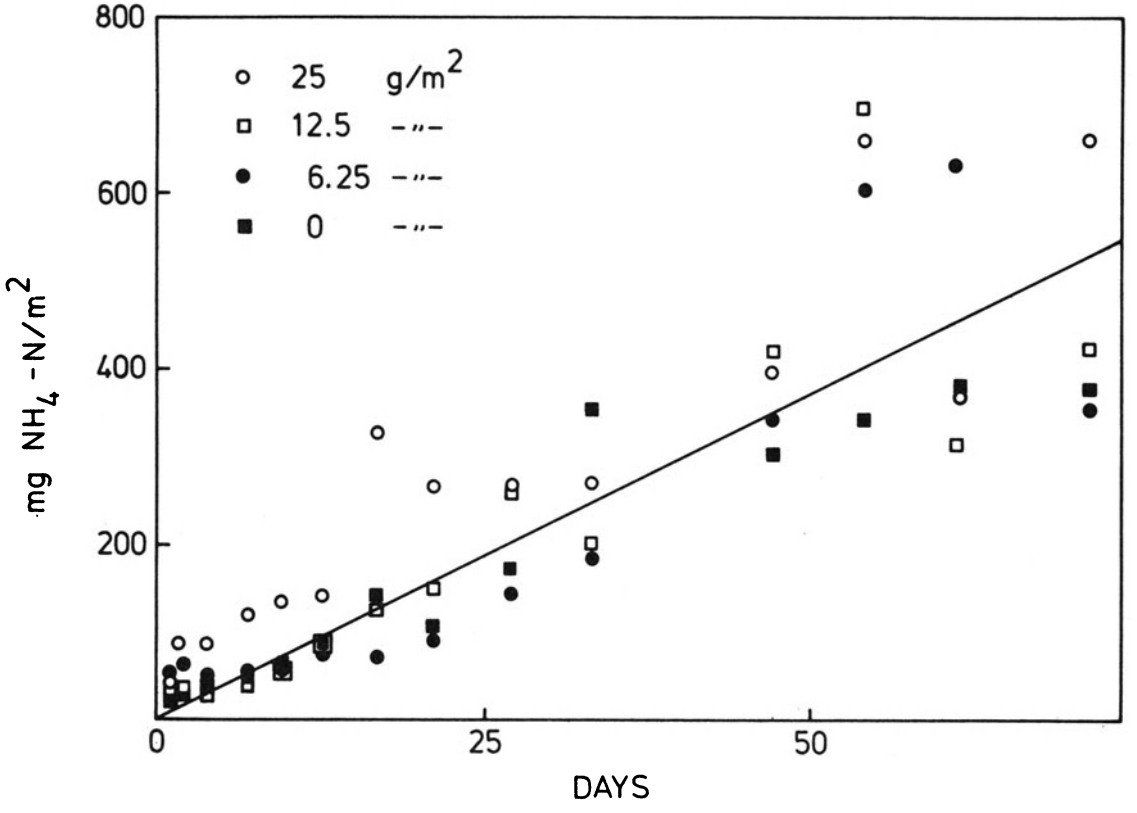

Figure 5: Ammonium ion release with different amounts of added detritus (symbols) in the closed box system (Hallberg *et al.*, 1976). The straight line represents the zero-order release rate constant of 7.7 mg N m^{-2} day^{-1} obtained by Almgren *et al.* (1975) during the By Fjord investigation (Dyrssen and Hallberg, 1979).

experimental system a separate cylinder is employed. This is identical to the experimental cylinder except that it has a sampling device positioned at the level of the halocline (Fig. 7). The sediment trapping effect of the cylinder was investigated by S. Blomqvist and C. Kofoed (unpublished results). They concluded from their investigation in the actual experimental area that the height/diameter ratio of cylindrical sediment traps should exceed 3 to obtain valid samples (Fig. 8). Diameters of less than 45 mm show a preference for trapping of organic matter (Fig. 9).

In the open system, the redox-potential and conductivity will be registered electronically every two hours at 15 levels from below the sediment surface to a height of 0.75 m above the sediment surface (Fig. 10). Water samples can be taken through self sealing rubber membranes at 10 levels. The first complete experiment will start in the spring of 1980.

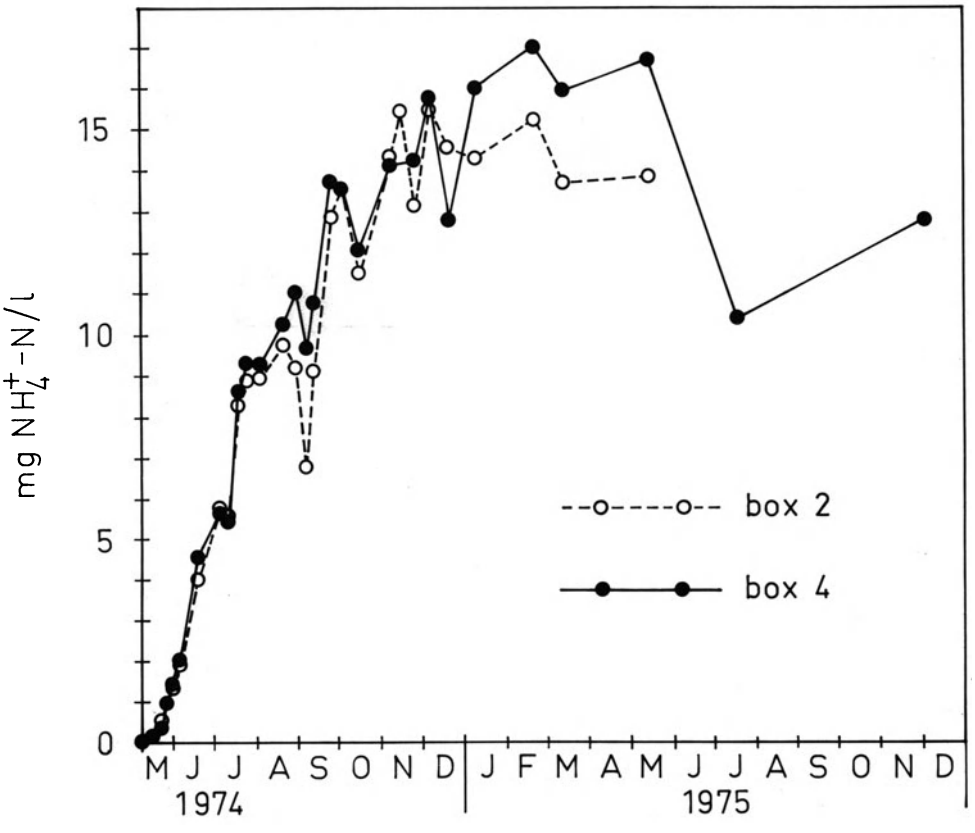

Figure 6: Ammonium ion release in duplicate box-experiments (Engvall, 1978).

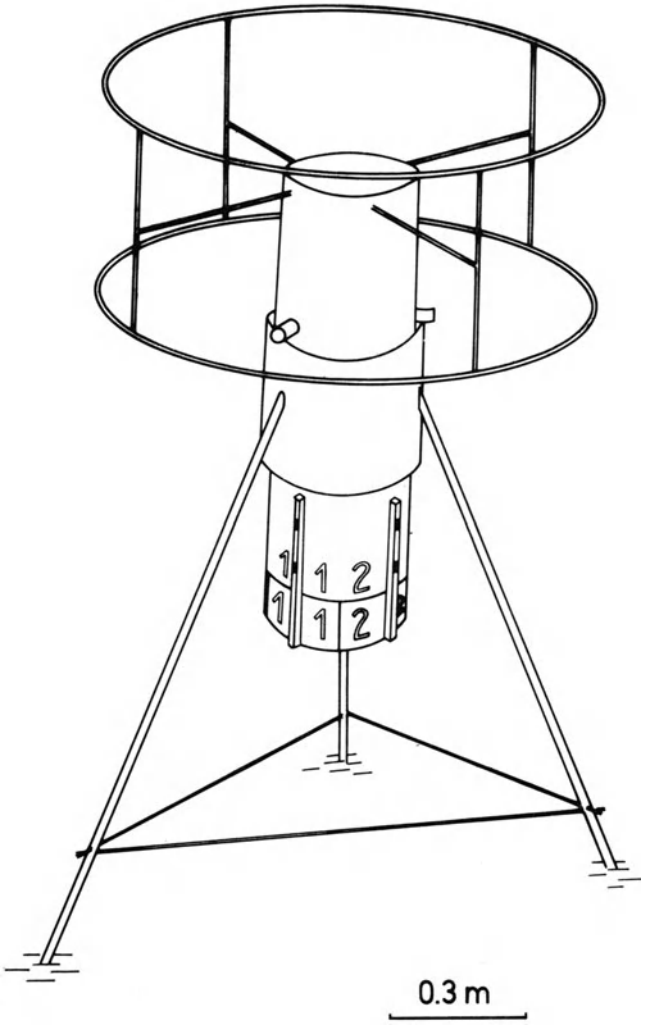

Figure 7: Sediment sampling system run parallel with the open cylinder system. Subsamples can be taken by SCUBA diving at the bottom of the sampling system (by courtesy of S Blomqvist and C. Kofoed).

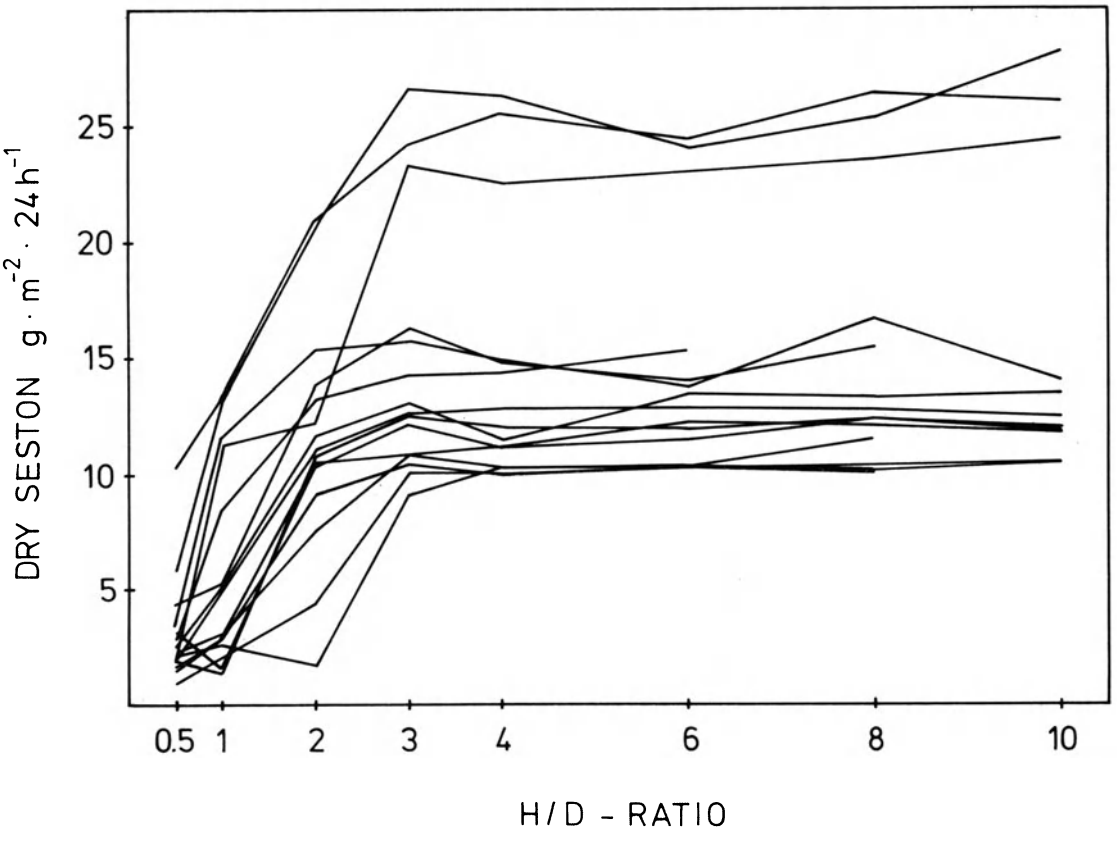

Figure 8: Amount of seston in the open cylinders vs. their height/diameter ratio (H/D) during five sampling periods. The data show that the H/D value should exceed 3 (by courtesy of S. Blomqvist and C. Kofoed).

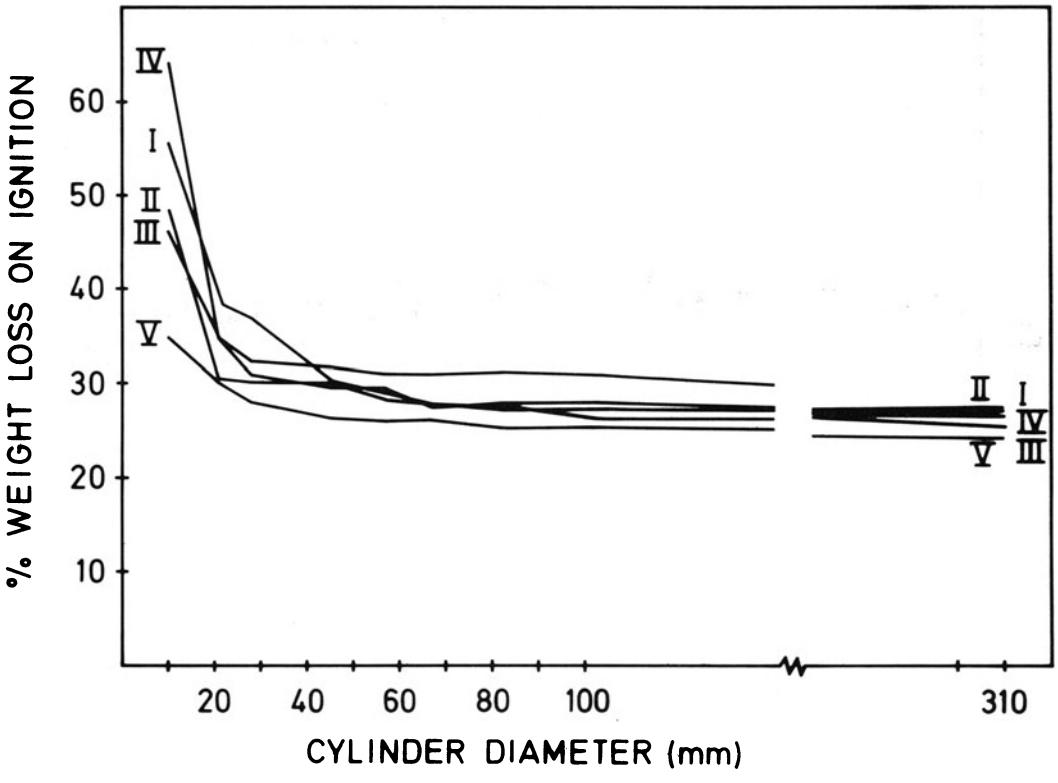

Figure 9: Percent weight loss on ignition of seston vs. interior cylinder diameter (H/D-ratio 8) (by courtesy of S. Blomqvist and C. Kofoed).

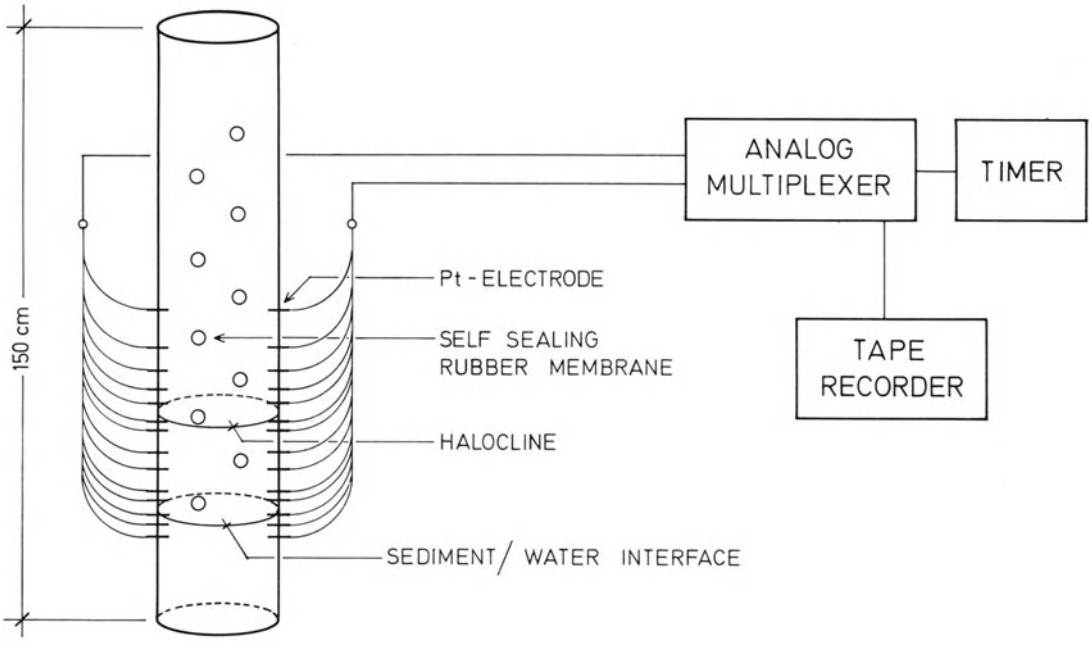

Figure 10: The open cylinder system consisting of a Plexiglas cylinder (diameter 0.3 m, total length 1.5 m). A halocline is produced in the cylinder. 15 pairs of Pt-electrodes are connected to an electronic system for registration of redox potential and conductivity. Water samples can be taken through self sealing rubber membranes.

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SEDIMENTOLOGICAL AND GEOBIOLOGICAL STUDIES OF INTERTIDAL CYANOBACTERIAL MATS IN NORTH-EASTERN SPENCER GULF, SOUTH AUSTRALIA

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INTRODUCTION

Stratiform stromatolites, which occur in some modern intertidal and lacustrine environments, are organo-sedimentary structures commonly produced by the sediment-trapping activities of the cyanobacteria (blue-green algae). Stromatolites were prominent in analogous Proterozoic environments and are often associated with sulfide mineral deposits (Mendlsohn, 1976). It has been suggested that this association implies a role for the biological activities of stromatolitic ecosystems in the formation of some base metal sulfide deposits (Renfro, 1974; Trudinger and Mendelsohn, 1976). This suggestion appears feasible since the accumulated organic matter in the stromatolites would have been a potential source of energy for sulfate-reducing bacteria and, furthermore, fractionation during bacterial sulfate reduction could account for particular sulfur isotope distribution patterns in some deposits.

In this paper we describe the ways in which sedimentary processes govern cyanobacterial mat structures, the rates of primary productivity by intertidal smooth mats, the rates at which sulfate is reduced in sediments below the mats and the sulfur isotope composition of the sulfides and porewater sulfates within the mat sediments. These studies will be reported in detail in subsequent publications. Our study area in north-eastern Spencer Gulf (Figs. 1 and 2) was chosen not only because it was extensively colonized by cyanobacterial mats, but also because it was subjected to regular tidal inundation and, hence, predictable biological activity.

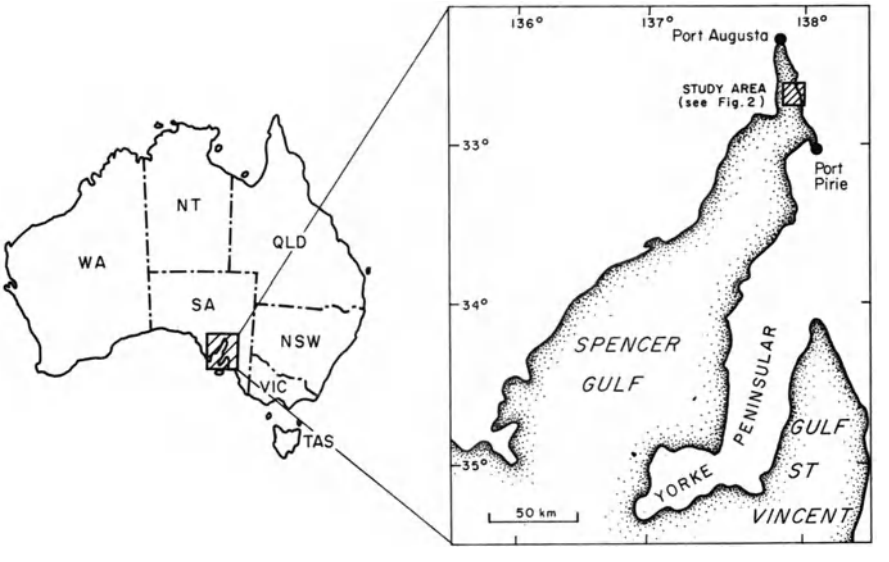

Figure 1: Location of study area, north-eastern Spencer Gulf, South Australia.

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* *This paper is a synopsis of four papers presented at the ISEB meeting and has no senior author. R.V.B. and J.F. publish with the permission of the Director, Bureau of Mineral Resources, Geology and Geophysics, Canberra.*

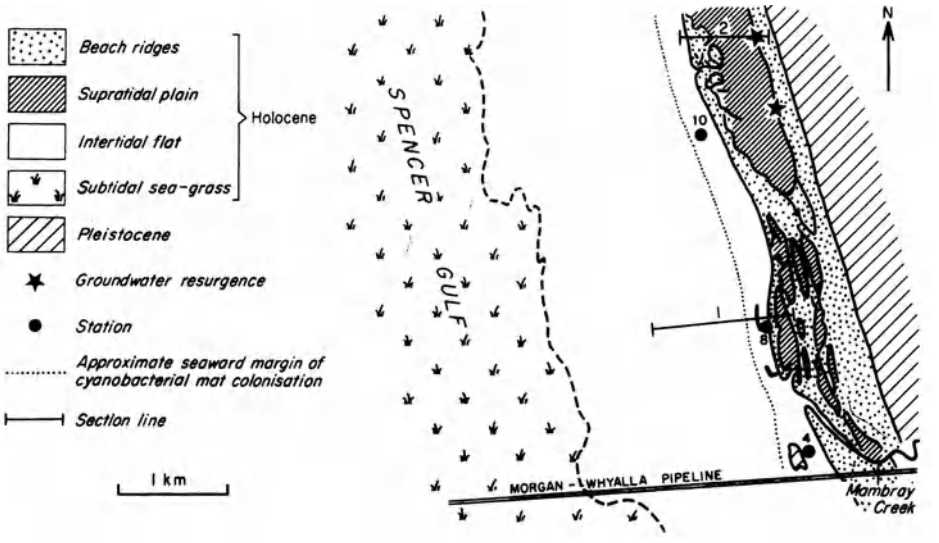


Figure 2: Location of stations and sections referred to in text, and the distribution of Holocene environments.

DESCRIPTION OF THE ENVIRONMENT

Spencer Gulf is a large funnel shaped incursion of the Southern Ocean into the heart of the Australian continent (Fig. 1). It is an area of carbonate sedimentation strongly influenced by tidal currents and lies within a non-tropical, semi-arid climatic zone. Areas of prograding shoreline are found on the north-eastern shores of the Gulf. Progradation has resulted from the accumulation of a series of beach ridges and associated environments. These are composed mainly of carbonate sediments which were formed in sub-tidal sea grass beds, and which were transported shoreward by wave action and tidal currents. The sediments principally consist of molluscan, foraminiferal and bryozoan debris. The coarser sediments tend to be concentrated on prograding windward shores as shelly beach ridges while the finer material accumulates on protected shorelines.

There are two situations where cyanobacterial mats colonize areas within the high intertidal and supratidal zones of these sedimentary complexes:

1. the sheltered coasts and tidal creeks associated with mangroves or halophytes, and
2. the high intertidal plain of exposed coastlines.

This study concerns cyanobacterial mats in the latter setting (Fig. 2).

Two accurately surveyed transects across the intertidal and supratidal zones together with tidal inundation and salinity data are shown in Fig. 3. Dense beds of seagrass occur seawards of the extreme low tide level. Landward of the seagrass beds are sand-bars composed of well stratified alternating layers of terrigenous and carbonate sand. Discharge from Mambray Creek introduces terrigenous sediments to the area (Fig. 4a). Landward of these sand-bars the broad intertidal flat is composed of a monotonous association of ridges and channels comprising rippled, silty and sandy sediments with shells scattered on the surface. There is a gradual sorting of shells in the surface until, in the higher areas, the shell material becomes concentrated in incipient bars (Fig. 4b).

Accretion of beach ridges against a coastline extending north-northwest results from south-southwesterly wave attack and northerly longshore drift (Fig. 4c). Behind the beach ridges, there is a supratidal plain which is inundated only at the highest tides or after heavy rain (Fig. 4d). In the latter instance, groundwaters emerge at the landward margin of the plain.

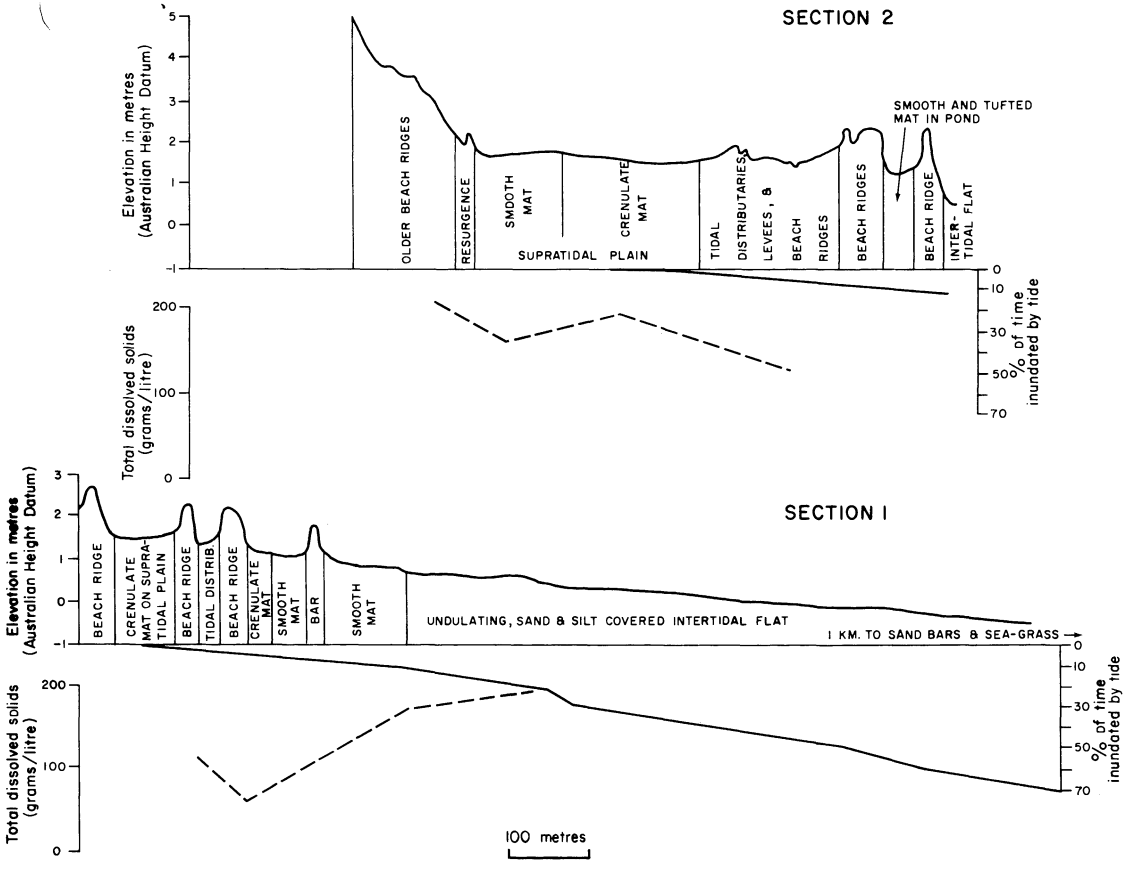
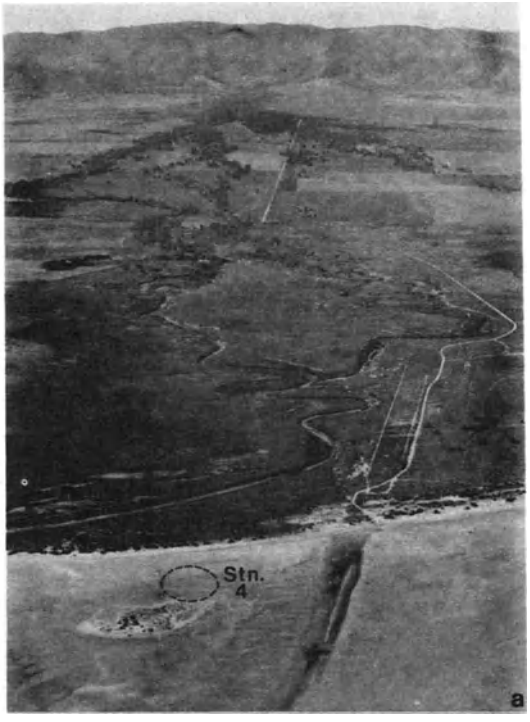


Figure 3: Sections showing -

(1) elevation relative to Australian Height Datum,

(2) percentage of time for which the section is inundated by tidal waters (computed for minimum heights of section and ignoring local topography), and

(3) salinity of pore waters, expressed as total dissolved solids (dashed line).



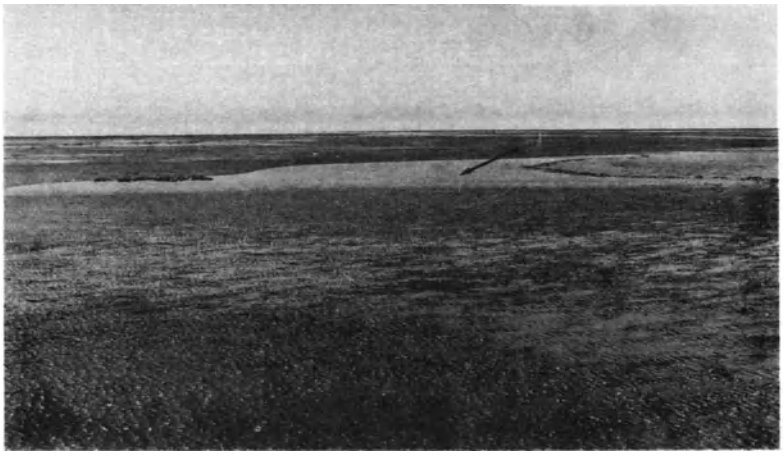
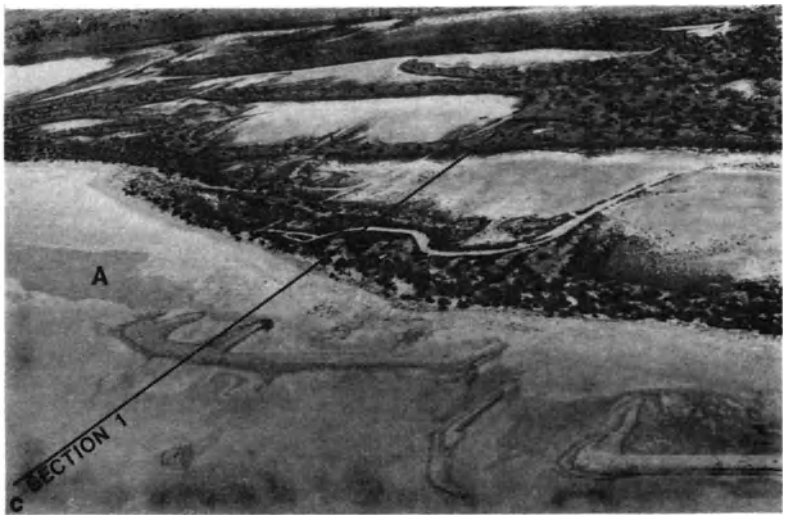


Figure 4: a. Setting of station 4, shoreward of small Pleistocene inlier on intertidal flat. Alluvial plain of Mambray Creek, and Flinders Ranges in background.

b. View southwards across intertidal plain near seaward limit of cyanobacterial colonisation along section 1. Note rippled sand flat and low shelly bar (seaward face to the right). Post (arrowed) is 1 m high.

c. Aerial view of shoreward margin of section 1. Note water ponded on cyanobacterial mat at A.

d. Aerial view with section 2 at extreme right showing
(A) supratidal plain
(B) tidal distributary channel, and
(C) intertidal sand-bars.


A
SECTION 1
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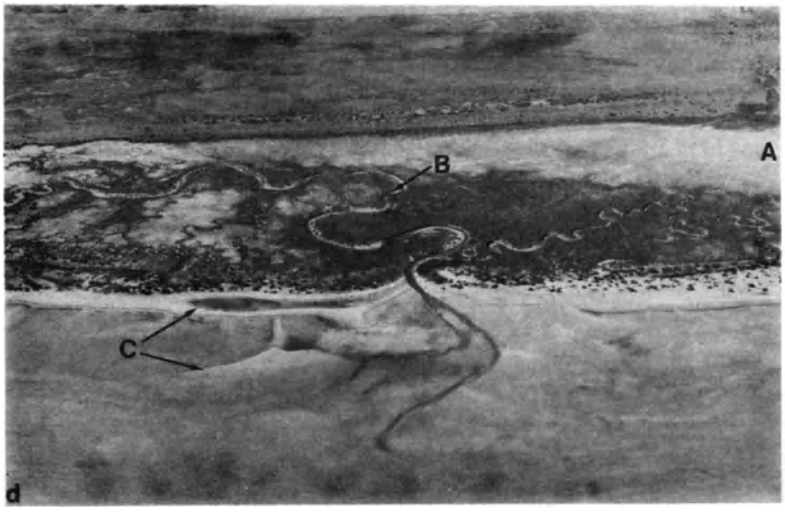
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A
C
d

Groundwater is derived from both marine and continental sources. Continental groundwater is provided mainly by a near-surface aquifer system associated with highly permeable surface sands which overlie clay sediments of much lower permeability (Chebotarev, 1958).

In contrast to coastal carbonate environments of arid zones (Levy, 1977), the relatively high rainfall, low temperatures and high hinterland relief have resulted in domination of the supratidal zones by continentally-derived groundwaters (Fig. 3a). Consequently, evaporative concentration of marine waters in the intertidal and supratidal areas has been constrained and seawater-derived brines are limited to a maximum concentration of about 5X seawater. In addition, a large number of continental groundwater resurgences occur in the supratidal and, to a lesser extent, the intertidal areas. Depending on the local topography, this groundwater is spread over the tidal flats or is retained and concentrated in depressions formed either by deflation, by spring erosion or as a result of enclosure by beach ridges.

Cyanobacterial mats are associated with intertidal areas influenced by a marine-dominated groundwater regime (Fig. 3; Section 1), and with supratidal areas which are sites for mixing of continental and marine waters (Fig 3; Section 2). Variations in the local topography strongly influence the salinity profile across the intertidal flat (Fig. 3; Section 1). Section 2 starts at a continental groundwater spring on the landward edge of a semi-enclosed supratidal area. Water from this spring forms small streams which discharge onto the supratidal flat where evaporation at the surface and in the capillary wetting zones usually results in the deposition of gypsum. Although this area appears to be almost completely isolated from the sea, there are tidal distributary channels through the seaward beach dunes which allow flooding of the area during spring tides (Figs. 4c and 4d). As a result, the groundwater regime is not dominated by continental influence but contains an appreciable marine component which increases towards the sea. The combination of intermittent marine flooding and the seasonal variation of continental groundwater discharge produces wide salinity variations, ranging from about 0.6X seawater for the spring source in winter, to about 6X seawater for the supratidal waters in summer.

CYANOBACTERIAL MATS

Three mat types are recognized and these are referred to as smooth, crenulate and tufted (Fig. 5). The mats colonize the high intertidal plain seaward of the beach, especially in areas afforded some protection from prevailing winds and wave action. The seaward limit of the mats is marked by a scarp formed as a consequence of the erosion of old, dead mats exhumed from beneath migratory sand-bars. The zone shoreward of this scarp is composed of mat-covered flats, silty rippled flats and migrating shell ridges which form bars and beaches near shore (Fig. 4). Between elevations of 0.8 and 1.06 m (Australian Height Datum) the sediments are colonized primarily by smooth mat (Fig. 3) whereas at higher elevations (1.06 to 1.5 m) the sediments are colonized primarily by crenulate mat (Fig. 3). Behind the beach the supratidal plain is covered by the dry relicts of crenulate mat up to extreme high tide level (1.6 m), and by relict smooth mat around the groundwater resurgences. Ponds formed at the resurgences contain thick, smooth mats. In general, the mats of the supratidal zone dry out rapidly and are eroded by the wind.

Intertidal mat development is most prolific in slight topographic lows where tidal waters are retained for the longest periods after coverage at high tide. The layer of sedimentation influenced by the cyanobacteria is thin and susceptible to erosion. In addition to the large-scale effect of tidal inundation (Fig. 3) it is clear that microtopography within the favourable inundation zone plays an important role in the distribution of the three mat types (Figs. 3, 4c and 5). For instance, tufted mat (Fig. 5b), which colonizes areas covered by water for the longest periods, generally occurs within very slight depressions which receive water draining from the surrounding smooth mat rather than seawards of smooth mat. Smooth mat (Fig. 5a,c), which covers the largest intertidal sediment surface area, comprises a 1 to 2 mm layer of living cyanobacteria overlain in places by a thin layer of sand or silt, some of which is trapped by the cyanobacterial filaments. The mat, in turn, overlies sediments containing a black zone of active sulfate reduction (Fig. 5c) which may be up to 5 cm thick.

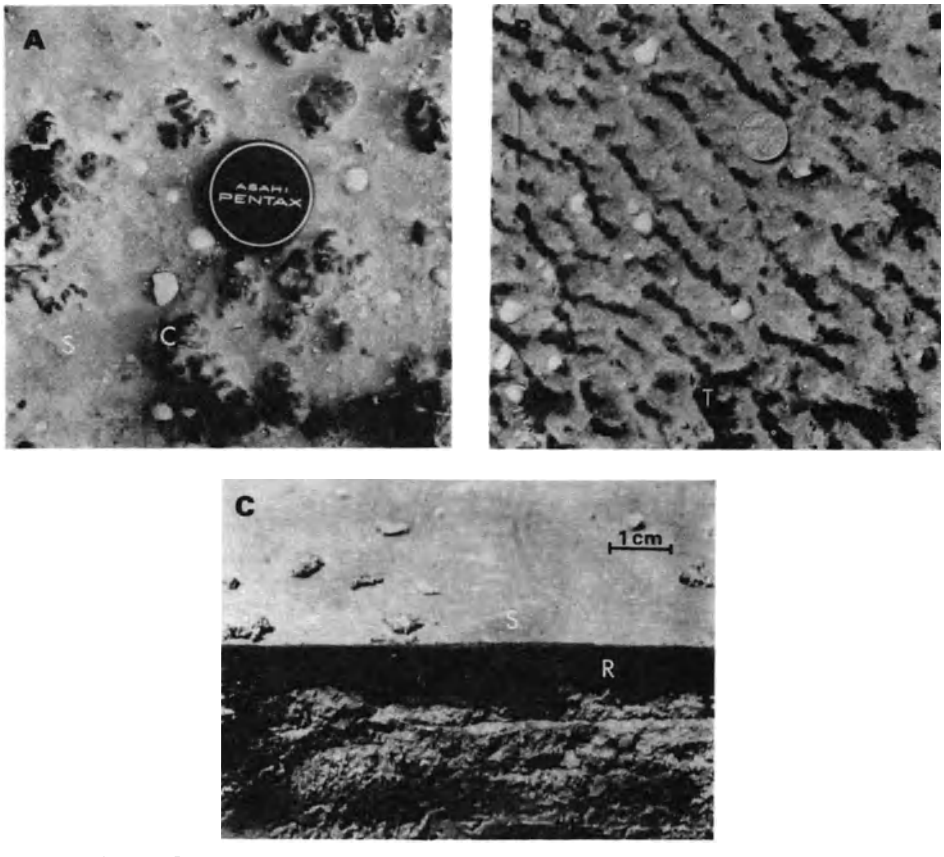

Figure 5: Intertidal cyanobacterial mats.

a. Surface view of smooth (S) and crenulate (C) mats. Diameter of lens cap is 5.2 cm.

b. Surface view of tufted mat (T). Diameter of coin is 2.8 cm.

c. Vertical section through smooth mat showing black sulfate-reducing zone (R) below living cyanobacterial layer (S).

CARBON AND SULFUR CYCLING

Methods for determining primary productivity (Bauld and Brock, 1973; Bauld *et al.*, 1979), sulfate reduction rates and porewater sulfate (Chambers *et al.*, 1975; Bauld *et al.*, 1979) have been described previously. Temperatures and conductivities were measured with a Townson and Mercer TPS Portable Pollution Monitor. Salinities as per mil NaCl were calculated from conductivity data. The experimental stations were at the southern (4) mid (8) and northern (10) areas of the 5 km strip of intertidal flat (Fig. 2). The samples of smooth mat for estimation of primary productivity, sulfate reduction rates and sulfur isotope distributions were taken from each station at monthly intervals during spring tides from November 1977 to November 1978.

Primary productivity rates were greater during late winter and spring than during the summer months. About 60% of the rates were found to be within the range 42 to 125 mmol

m^{-2} day^{-1} while the lowest and highest rates were 17 to 258 mmol m^{-2} day^{-1}, respectively. These rates are compared with other benthic cyanobacterial mats in Table 1. The cyanobacteria from smooth mats were tolerant of varying salinity. Smooth mat exhibited maximum photosynthetic activity at salinities between 70 and 105 per mil with activity remaining between 60 and 90% of maximum in the salinity range 17 to 140 per mil and being detectable even at 175 per mil.

TABLE 1

PRIMARY PRODUCTIVITY OF CYANOBACTERIAL MATS FROM VARIOUS ENVIRONMENTS

Location	Rate (mmol m^{-2} day^{-1})	Reference
Drakesbad Hot Springs, California	583-1000	Lenn (1966)
Solar Lake, Sinai, Israel	417- 833	Krumbein *et al.* (1977)
Antarctic freshwater [a] lakes	133- 300	Goldman *et al.* (1972)
Spencer Gulf	17- 258	This paper

[a] Algal and Skua Lakes.

The sulfate reduction rates obtained for 423 samples taken from the 0 to 5 cm layer of smooth mat at the three stations, ranged from 2 to 103 mmol SO_4^{2-} m^{-2} day^{-1} and the average was 30 mmol SO_4^{2-} m^{-2} day^{-1}. The highest rates were found during the winter and spring from May to November 1978. There was a significant but negative linear correlation between the salinity of porewaters and sulfate reduction rates ($r = -0.4$ and $p = 0.02$). Two separate experiments, in March 1978 and May 1978, respectively, showed that:

1. the sulfate reduction rates for sediments beneath photosynthetically active mat were much greater (17 mmol m^{-2} day^{-1}) than for inactive mats, and

2. 86% of the sulfate reduction activity occurred in the first 1.25 cm of sediments associated with active mat (Table 2).

TABLE 2

SULFATE REDUCTION RATES IN INTERTIDAL CYANOBACTERIAL MATS

Depth (cm below sediment surface)	Rate (mmol sulfate m^{-2} day^{-1})
0 to 1.25	45
1.25 to 2.50	5
2.50 to 3.75	1
3.75 to 5.00	1

When replicate samples were incubated at 10, 20, 30 and 40° there was a significant linear correlation between the logarithm of sulfate reduction rates and the reciprocal of the absolute temperature of incubation ($\log_{10}$ rate = $\frac{-2709}{T}$ + 10.2, r = -0.90 and p = .01) in accordance with the Arrhenius equation:

$$\log_{10} v = -\frac{\Delta H}{2.303RT} + C^{*}$$

The acid labile sulfide precipitated in the sediments had $\delta^{34}S$ values ranging from -30 to -48 per mil relative to seawater sulfate. This distribution is within the range reported for biogenic sulfide (Chambers and Trudinger, 1979). Some of the porewater sulfates in the Spencer Gulf sediments were slightly enriched in ^{34}S (2 per mil), indicating only occasional and minor depletion of the sulfate supply.

DISCUSSION

Distinctive environmental and hydrological conditions are associated with the establishment of cyanobacterial mats on wide intertidal sediments such as those occurring in north-eastern Spencer Gulf. Mat-forming cyanobacteria are the dominant primary producers within the high intertidal environment. The constructing organisms and mat morphologies, at any one location within the zone of colonization, are determined by the degree of tidal inundation and the microtopography. At their seaward edge, cyanobacterial mats are restricted by wave action and metazoan grazing (Garrett, 1970), whilst desiccation and subsequent deflation limit landward colonization across the supratidal zone. The preservation of intertidal mats in our study area appears generally poor (Fig. 5c) compared with the preservation of similar intertidal mats on the Trucial Coast (see Fig. 12 of Park, 1977).

Primary production of intertidal mats in north-eastern Spencer Gulf was less than those recorded for mats in Drakesbad Hot Springs or Solar Lake, but are comparable to those measured in Algal and Skua Lakes, Antarctica (Table 1). The latter environments may be considered extreme with respect to temperature and/or salinity, but the environmental stress is maintained at a relatively constant level. In our study area however, diurnal variation of temperature (e.g. 12-48°) and salinity (e.g. 50-200 per mil) in the intertidal environment may be considerable. In addition, these intertidal mats are inundated for only 5 to 10% of the year and, therefore, intermittent desiccation would be an additional stress.

The observations that photosynthetically active mat supported higher sulfate reduction rates than did inactive mat, and that almost all of the sulfate reduction occurred in a thin (about 1 cm) layer immediately below active mat, are indicative of the dependence of the sulfate reducers on cyanobacterial primary productivity. The ratios of moles CO_2 fixed: moles sulfate reduced, for contemporaneous daily rates, varied from 1.2 to 92.0. This result showed both apparent shortages and excesses of organic carbon relative to anticipated demands for sulfate reduction (Trudinger *et al.*, 1972). One interpretation of this result is that there was a considerable lag between the time of CO_2 fixation and the time at which the fixed carbon was used in the sulfate reduction processes. Studies are continuing to resolve these complexities. Of the environmental factors affecting sulfate reduction rates, the most obvious were those of salinity and temperature. From the temperature-effect experiments, it is expected that the in situ sulfate reduction rates would increase as the temperatures increase during the summer months. However, the potential for increased rates would be modified by the concomitantly increasing salinity of the porewaters. Despite the extreme variations in salinity, temperature and water regimes, the range of sulfate reduction rates found for these intertidal mat-associated sediments is within that reported for other marine environments (0.08 to 200 mmol m^{-2} day^{-1}; obtained by transforming the values in μmol l^{-1} day^{-1} given in Table 6.1.3 of Trudinger, 1979).

The sulfides produced and precipitated in these sediments exhibit isotope distribution patterns which are similar to sulfide in sediments beneath smooth mat at Shark Bay, Western Australia (Bauld *et al.*, 1979) and the sulfides from an estuarine intertidal environment at Newport Marsh, southern California (Kaplan *et al.*, 1963). These environments are considered to be essentially open with respect to sulfate.

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* v, *reaction velocity;* ΔH, *activation energy of the reaction;* R, *gas constant;* T, *absolute temperature.*

The frequent association of mineral deposits and stromatolites (Renfro, 1974; Mendelsohn, 1976) suggests that microbiological processes may have been involved in metal sulfide deposition. The area we have studied contains a regressive sedimentary sequence associated with cyanobacterial mat colonization analogous to the depositional environments in which some Proterozoic stromatolites formed (e.g. the regressive Rocknest Formation; Hoffman, 1976), and the sulfate reduction rates appear to meet some of the quantitative requirements for biologically influenced sulfide ore formation (Trudinger *et al.*, 1972).

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THE MICROBIAL GEOCHEMISTRY OF SOLAR LAKE, SINAI

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INTRODUCTION

Solar Lake is a stratified, hypersaline, heliothermal heated water body situated on the coast of Sinai of the Gulf of Aqaba. The lake is 140 x 70 m and its maximal depth is 5.5 m. It is fed by seawater seeping through a 60 m wide terrestrial barrier which separates it from the sea. The lake is strongly stratified for a period of nine to eleven months per year (Cohen *et al.*, 1977a,b). During this period a mesothermal profile develops with a maximum temperature of 65° measured at 2 m water depth. Previous papers have dealt with the limnological cycle of the lake (Cohen *et al.*, 1977a), the distribution of photosynthetic and nonphotosynthetic microorganisms and their activities in the water (Cohen *et al.*, 1977b,c), sulfur transformations in the chemocline of Solar Lake (Jørgensen *et al.*, 1979a), and the contribution of the cyanobacteria to the sulfur cycle in the water column (Padan and Cohen, 1980).

The lake is highly productive and is floored by a thick cyanobacterial mat. This well preserved mat, up to 1.5 m thick, represents a sediment record of 2,500 yr. The lake was initially an open lagoon as indicated by beachrock formation which contains *Pirenella conica* and *Brachidontes* sp. typical of present open lagoons near Solar Lake. The lagoonal stage prevailed from 5,000 to 2,500 yr ago. From 2,500 until about 1,940 yr B.P. the lagoon was isolated. The lagoonal fauna disappeared and cyanobacterial mats dominated the lake floor. During these 560 yr the shallow lake dried out periodically and gypsum precipitation replaced cyanobacterial mat growth. From 1,940 yr B.P. onwards the uniform cynaobacterial mat developed only in the boundaries of the lake and a mixture of gypsum and cyanobacterial mud dominated at the central part of the lake. This probably occurred due to a minor tectonic action at the time which caused the subsidence of the central part of the lake.

ALGAL MATS

Krumbein and Cohen (1974, 1977) and Cohen *et al.* (1977d) recognized four types of cyanobacterial mats in Solar Lake:

1. *Flat shallow-water mat* at the boundaries of the lake. This finely laminated mat occupies a 15 m belt around the shallow part of the lake and is partially exposed in summer. Similar finely laminated cyanobacterial mats (stromatolites) are found throughout the world, mostly in arid areas. These include stromatolites in Shark Bay, Western Australia (Logan *et al.*, 1964) and the Coorong Lagoon, South Australia, (Walter *et al.*, 1973), the cyanobacterial mats covering vast areas of the Persian Gulf (Kinsman and Park, 1976) and stromatolite formations in Baja California, Mexico (Javor, 1978);

2. *Pinnacle mat* appears at the shallower slope of the lake composed mostly of coccoid cyanobacteria and diatoms;

3. *Cyanobacterial and photosynthetic bacterial film*. This film occupies the slope of the lake and is exposed to high temperatures and salinities. It borders the chemocline and upper hypolimnion of the lake. Both carbonates and sulfate precipitate in this zone and are covered by a thin microbial film;

4. *Flocculose cyanobacterial mat*. This mat lies at the central deep part of the lake

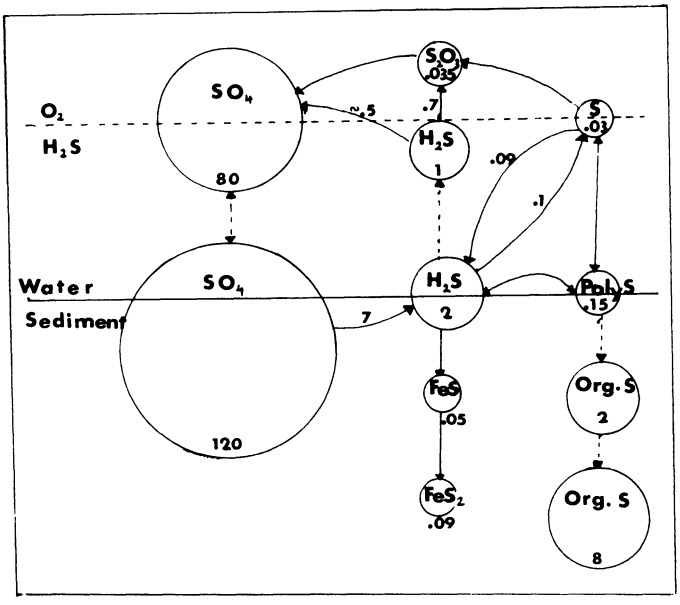


Figure 1: Sulfur cycle in the cyanobacterial mat of Solar Lake and overlying water column. Pools of various sulfur compounds are given in mmol l^{-1} for dissolved compounds and in mmol cm^{-3} x 100 for organic sulfur, FeS, and FeS_2. Numbers along the arrows indicate rates of transformation of the sulfur compounds in mmol l^{-1} day^{-1}.

and is composed mostly of the filamentous cyanobacterium, *Oscillatoria limnetica*, which prevails at the hypolimnion under up to 3 mmol l^{-1} sulfide.

PRIMARY PRODUCTIVITY AND SULFATE REDUCTION

Most of the analytical work was done on the flat shallow-water mat. Sulfate reduction and primary production in this mat were discussed by Jørgensen and Cohen (1977) and the diurnal cycle of oxygen and sulfide microgradients at the top of this mat, and the photosynthetic microbial activities in it, by Jørgensen *et al.* (1979b).

The shallow-water cyanobacterial mat of Solar Lake is rich in organic matter. At the uppermost layer it contains up to 15% dry weight of organic carbon (C_{org}). This high organic content is a result of a primary production, as measured by the ^{14}C method (Cohen *et al.*, 1977c), of 8.4-12 g (0.7-1.0 mmol) C_{org} m^{-2} day^{-1} which would result in a theoretical annual accretion of 3066-4380 g C m^{-2} yr^{-1}. The measured accretion rate, on the other hand, is much lower. Only 1.2 mm of cyanobacterial mat builds up annually corresponding to only 30-40 g C m^{-2} yr^{-1}. Thus, of the organic matter produced photosynthetically, only 1% is incorporated into sediment. The remaining organic matter undergoes a rapid mineralization at the expense of sulfate reduction. The rate of sulfate reduction in the mat was measured using $^{35}SO_4^{2-}$ as described by Jørgensen and Cohen (1977) and was found to be 70 mmol S m^{-2} day^{-1}. This would require the utilization of 140 mmol (1.68g) C_{org} m^{-2} day^{-1} which would be sufficient to mineralize all the organic carbon within a month. In order to support this high rate of sulfate reduction a continuous flow of dissolved organic matter from the uppermost layer of the cyanobacterial mat has to occur. The high rate of CO_2 photoassimilation measured can well account for this organic carbon requirement for sulfate reduction (Jørgensen and Cohen, 1977).

The dominant phototrophic microorganisms composing the mat are non-heterocystous filamentous cyanobacteria, *Oscillatoria limnetica, Oscillatoria salina, Phormidium* sp. and *Microcoleus* sp., and unicellular cyanobacteria, *Aphanotheca halophitica, Aphanocapsa* sp. and *Synechococcus* sp. *Flexibacterium* sp., and various Chromatiaceae and Chlorobacteriaceae, are also found in the mat. A morphological description of the mat is given in Krumbein and Cohen (1977) and Cohen *et al*, (1977d).

Diurnal variations in the microgradients of O_2, H_2S and Eh were studied by inserting oxygen, sulfide, and redox microelectrodes into the mat as described in Jørgensen *et al.* (1979b). Results were correlated with light penetration and CO_2 photoassimilation. Extreme diurnal changes were found, with an oxygen peak of 0.5 mM at 1-2 mm depth below the mat surface during the day and a sulfide peak of 2.5 mM at 2-3 mm depth at night. Oxygen and sulfide coexist over a depth interval of 0.2-1.0 mm and with turnover time of a few min. The photic zone in the mat ranges from 2.5 mm in summer to 1 mm in winter.

The sharp diurnal fluctuations of oxygen and sulfide require a daily shift in the mode of photosynthesis of the cyanobacteria. The cyanobacteria account for about 80% of the biomass of the top 3 mm of the mat. Anoxygenic photosynthesis (Cohen *et al.*, 1975a,b) prevails in the morning and late afternoon while oxygenic photosynthesis prevails at noon.

SULFUR CYCLE

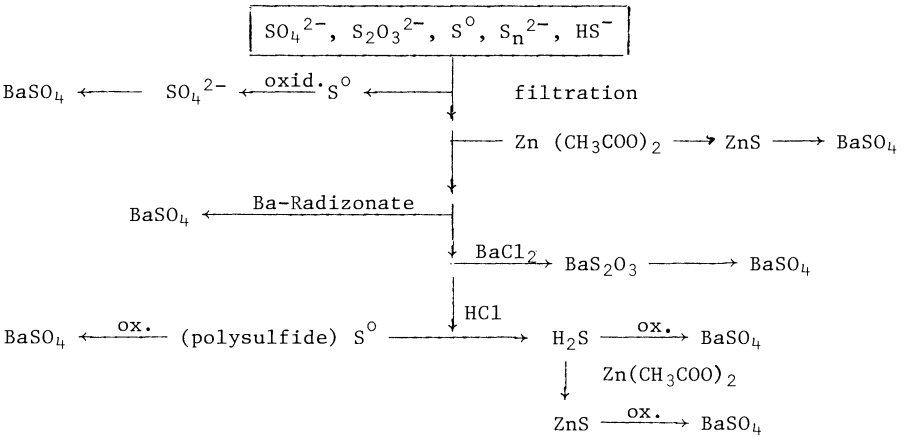
A long series of sulfur analyses was carried out on the cyanobacterial mat (Jørgensen and Cohen, 1977) and water column (Jørgensen, *et al.*, 1979a). The various sulfur transformation rates were measured using radioactive sulfur compounds (Jørgensen *et al.*, 1979a). A generalized sulfur cycle for the cyanobacterial mat is shown in Fig.1. The overall turnover rate of sulfur is very rapid, especially at the H_2S-O_2 coexistence layer (Jørgensen *et al*, 1979a), where turnover times of sulfide are a few min and those of elemental sulfur a few hours.

Polysulfide forms a steady-state pool of 150 mmol l^{-1} and is in equilibrium with sulfide and elemental sulfur. Ferrous sulfides comprise a minute fraction of the cyanobacterial mat and no framboidal structures were detected by scanning electron microscopy. Only traces of ferrous ions were detected as a slight colouration in the presence of thiocyanate. The major source of iron is clay particles of terrestrial origin which are brought to Solar Lake by desert floods. This supply is limited because of the arid environment where the mean annual rainfall is only 10 mm. Organic matter, on the other

hand, is practically unlimited and it reacts with polysulfide causing a dramatic increase in organically-bound sulfur in the cyanobacterial mat. In the upper layer of the mat only 1-2% of the organic matter contains bound sulfur but this rises to 8% at 80 cm depth - corresponding to a period of 1000 yr (Cohen *et al.*, 1977a).

The oxidation states of sulfur recorded in the water body vary from +6 (SO_4^{2-}) through +4 and +2 ($S_2O_3^{2-}$) to 0 (elemental sulfur) and -2 (HS^-), causing analytical difficulties. Quantitative identification of the sulfur species has been discussed previously by Jørgensen *et al.* (1979b). In the present study we used the separation procedures shown in Scheme 1:

Scheme 1



Sulfite is present in negligible amounts.

ORGANIC MATTER

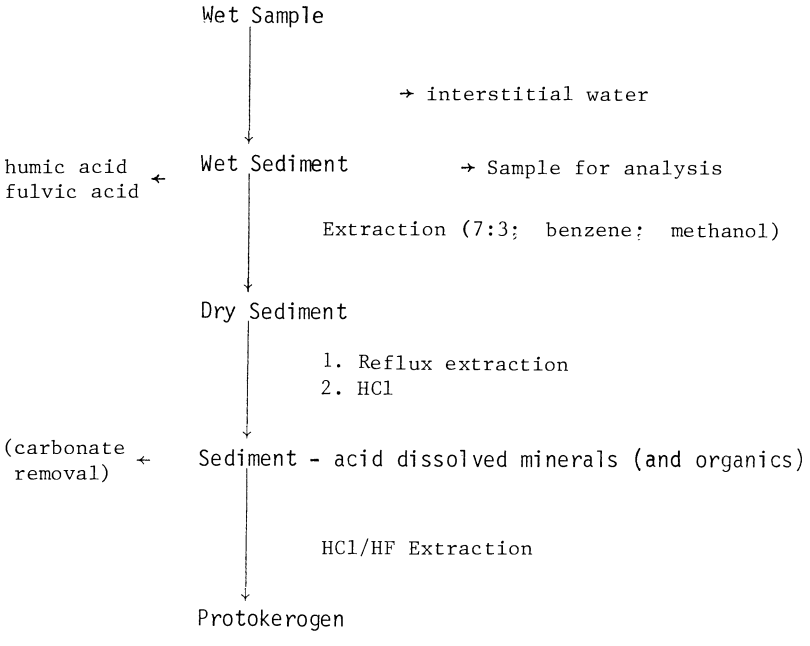
Pigments in the extractable fraction of the sediment were separated on silica gel G and examined by UV-visible spectrophotometry. Pheophytane, β-carotene, α-carotene and the blue band of phycocyanine typical for cyanobacteria, are well preserved down the sediment core indicating that the sediment is in a very early stage of diagenesis. The total pigment fraction covers almost the whole visible range from 780 nm to 398 nm.

Hydrocarbons in the algal mat arise from the different organisms in Solar Lake. No terrestrial contribution could be detected. The normal alkane distribution shows a maximum around C_{15}-C_{17} and there is no odd/even preference in the C_{23}-C_{31} range (CPI≈1). The C_{23}-C_{31} hydrocarbons are, as a rule, less abundant.

The distribution of organic matter (Scheme 2) within the sediment varies somewhat from the top of the core to the bottom. However, some water soluble organic matter (possibly amino-acids and fulvic acids) is lost in the analytical treatment: *no* humic matter was recorded in fresh cores. Total organic carbon makes up 7-16% of the sediment. This corresponds to 14-32% total organic matter when calculated on the basis of a protokerogen composition of C-45%, H-6%, S-5%, N-7%, O-35%. In the upper part of the core the total extractables (benzene 7: methanol 3) account for up to 1.2-1.5% of the organic matter whereas the protokerogen was 3%. At 80 cm depth extractables are about 3% and the protokerogen accounts for 22% of the total organic matter. The nitrogen content of protokerogen remains high (6-7%) along the core, while the organically-bound sulfur is low at the top and very high at the bottom.

Dinur *et al.* (1979) proposed a model for the enrichment of the organically-bound sulfur in sediments. It was suggested that the low availability of iron and the high

Scheme 2



Each fraction was analysed and separated according to Aizenshtat (1973) and Aizenshtat *et al.* (1973).

concentrations of HS^- in the presence of S° could lead to a free radical controlled reaction between sedimentary organic matter and polysulfides. This mechanism was based on ^{34}S data for pyritic and organically-bound sulfur of the Israeli "Oil Shales". Moreover, in at least one site of bituminous sediment (Nabi Musa), hypersaline and high bacterial reduction of sulfate was suggested to be associated with high organically-bound sulfur (Spiro and Aizenshtat, 1977).

The Solar Lake cyanobacterial mat allows an experimental test of this model. The continuous production of both H_2S and elemental sulfur results in a constant large pool of polysulfides in the interstitial waters of the upper part of the sediment (Fig. 1). These polysulfides are soluble in the pore water at pH values higher than 7.5. But, at neutral or lower pH values, the polysulfides may precipitate as elemental S°. In the present study, acidification of core material from 80 cm depth caused large amounts of H_2S to be released, and elemental sulfur was extracted in the benzene:methanol extract (up to 25% of this fraction) and was subsequently isolated as sublimed S°.

CONCLUSIONS

From these preliminary studies it is evident that the following conditions exist in the Solar Lake: (i) hypersalinity (ii) high microbial activity as a result of high primary production (iii) exceptionally rapid microbial sulfur transformations (iv) low availability of iron (v) somewhat elevated temperatures (vi) anoxic sediment (vii) organically rich sediment (viii) very low terrestrial contribution and (ix) high H/C values for the organic matter.

The sulfur enrichment of the protokerogen, formed from the interaction of polysulfides with the cyanobacterial organic matter in very young sediments, provides as a good test

case for the possible mechanisms of secondary sulfur enrichment processes.

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COMPARATIVE ORGANIC GEOCHEMICAL STUDIES OF RECENT ALGAL MATS AND SEDIMENTS OF ALGAL ORIGIN

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INTRODUCTION

Organic geochemical studies of contemporary algal mats and organisms play a very important role in furthering our understanding of the history of marine and freshwater sediments of predominantly algal and bacterial origin. Blue-green algae are of special interest because of their ability to survive under conditions, such as hypersaline and reducing environments, which favour the preservation of organic matter. Further interest in contemporary algae arises from the search for the origins of unicellular life on earth. Micro-structures referred to as exhibiting "algal-like" and "filamentous" morphologies occur in cherts from South Africa that are older than 3 Gyr (Schopf and Barghoorn, 1967).

Geochemical studies of recently-deposited algal mats should provide useful information which can be applied to ancient sediments and provide an insight into the distribution of organisms within these sediments. In this paper, results obtained from the studies of the soluble organic fractions of some Recent algal mats will be reviewed and their application in interpreting similar data from Precambrian samples will be discussed. Also, the use of pyrolysis in analysing the corresponding kerogens will be discussed, with particular emphasis being placed on Curie-point pyrolysis coupled with gas chromatography.

BACKGROUND

Extensive organic geochemical studies have been carried out over the past few years on the algal mats from Laguna Guerrero Negro and Laguna Mormona, Baja California (Cardoso *et al.*, 1975; Philp *et al.*, 1978a,b; Javor *et al.*, 1979), Shark Bay, Western Australia (Watts *et al.*, 1977), Abu Dhabi, Persian Gulf (Cardoso *et al.*, 1978) and the Texas lagoons (Parker and Leo, 1965; Behrens and Frishman, 1971). In general, these studies were designed to examine the fate of various classes of lipids after their deposition in the environment. However, Behrens and Frishman (1971) utilized carbon isotope measurements to demonstrate that stable carbon isotopes contained within stromatolitic structures in Recent sediments, as well as in ancient sedimentary rocks, may be useful in inferring the origin of these structures. Javor *et al.* (1979) developed a new technique for the introduction of radiolabelled substrates into algal mats or other microbial systems. This technique permits incubations with radiolabelled compounds to be performed at discrete horizons below the surface of the mat. From these extensive studies and earlier work on lipid composition of pure cultures of algae and bacteria (Han *et al.*, 1968; Winters *et al.*, 1969), much information has emerged to illustrate geochemical processes taking place in this type of environment. This information is potentially useful as an aid in determining the significance of chemical fossils (Eglinton, 1973) found in Precambrian samples.

By comparison with the Recent algal mat extracts, the extracts from Precambrian rocks are not as complex and, in particular, lack unsaturated compounds. Certain types of compounds, thought to be of biological origin, are widely distributed in the Precambrian; but it is difficult to make direct geochemical comparisons between extracts from samples whose ages differ by 1 Gyr. There were no higher animals or higher plants in the Precambrian, and thus present day environments are not strictly comparable to the Precambrian. Lipids in contemporary organisms and algal mat ecosystems should be examined in order to identify compounds characteristic of particular groups of microorganisms whose first representatives appeared during Precambrian times.

In an analogous fashion most of the identification and determinations of fossilized remains in palaeobiological studies have been based on a comparison with modern cyanophytes, assuming that primary silicification preserved the microorganisms well enough to compare them with healthy live cells. Awramik *et al.* (1972) recognised that post-mortem degradation of cellular structures in modern algal mats and their early diagenesis was a relevant key to

the interpretation of microbial fossils. Golubic and Campbell (1979) pointed out that there is a growing list of relationships between microfossils and their recent counterparts that bridges a time span of billions of years. These relationships raise such questions as: (i) are the paired morphologies linked to survival in particular environments after having convergently evolved in otherwise unrelated organisms, and (ii) have these morphologies, well suited to certain environments, been maintained through the entire course of time?

In comparative geochemical studies one is also concerned with recent and fossil counterparts trying to match pairs of compounds rather than pairs of organisms. The question of whether compounds can be directly related over such a great time span and the problem of their survival throughout the geological record from the Precambrian are both relevant to organic geochemical studies. In the following sections, the distribution of biological markers in Recent algal mats is reviewed and compared with the distributions of similar compounds in Precambrian sediments.

SOLUBLE LIPID FRACTIONS FROM RECENT ALGAL MATS AND PRECAMBRIAN SEDIMENTS

Recent Algal Mats

The methods used to isolate, fractionate and analyse the various classes of compounds from the algal mats can be found in the references given in Table 1. The classes of compounds which, from these studies, appear to have the greatest potential as biological markers in Recent algal mats, are hydrocarbons, fatty acids, and sterols. These three classes of compounds will be used to illustrate that some correlations can be made between the Recent algal mats and Precambrian sediments.

Hydrocarbons (Table 2)

The hydrocarbons which have been found in algal mats can be correlated, to a certain extent, with their source material, as summarised in Table 2. The dominant hydrocarbons found in all the algal mats which have been examined (Table 1) were a mixture of 7- and 8-methylheptadecanes and n-C_{17} alkane. Han *et al.* (1968) previously reported that these compounds are characteristic of cyanobacteria. The uppermost layers of the Laguna Guerrero Negro mats were also characterized by the presence of a C_{17}-branched alkene, 8-methylhexadecane, and various isomers of phytene. The presence of a higher plant contribution to the Laguna Guerrero mats was evident from the presence of normal alkanes in the range C_{24}-C_{30} (Brooks *et al.*, 1976).

Gelpi *et al.* (1970) showed that the triterpene hop-22(29)-ene was an abundant alkene constituent of *Lyngbya aestuarii*. The main mat-forming species in these environments (Table 1) is *L. aestuarii*, and hop-22(29)-ene was found as an abundant alkene constitutent in extracts from the mats. Hop-21(22)-ene, possibly formed by isomerization of hop-22(29)-ene, was also present in the Laguna Guerrero Negro and Abu Dhabi mats. C_{27}-, C_{28}-, and C_{29} -sterenes were found to be widely distributed in the lower layer of the mats from Laguna Mormona and Laguna Guerrero Negro and to a lesser extent the Abu Dhabi sample. The sterenes were absent from the living layers of the mats suggesting that they were products of diagenesis (Rubinstein *et al.*, 1975). Further diagenesis of cholest-2-ene could account for the presence of the C_{20}- and C_{24}-cyclic compounds found in the lower layer of the Laguna Guerrero Negro mats. Isoprenoid hydrocarbons were also found to be widely distributed throughout the algal mats. Phytane was slightly more abundant than pristane in the Abu Dhabi mats. The major isoprenoid hydrocarbon in the Laguna Guerrero Negro samples was phytane with varying amounts of phytenes and phytadiene also present. The presence of the phytenes and absence of pristane suggests that reductive processes are occurring in these mats. By comparison, the concentration of pristane was found to be much greater than phytane in the Laguna Mormona mats. The differences in distribution of these compounds could reflect differences in conditions of deposition and preservation of the organic matter (Didyk *et al.*, 1978). Han *et al.* (1968) found pristane and phytane in a photosynthetic bacterium *Rhodopseudomonas spheroides* and a non-photosynthetic bacterium *Micrococcus lysodeikticus*. The differences in the relative proportions of pristane and phytane in the algal mats may thus also reflect differences in bacterial contributions to the sample.

Fatty Acids (Table 3)

Parker and Leo (1965) observed a systematic change in the ratio of saturated to unsaturated acids with increasing depth of sample in the algal mats of the Texas lagoons. Oleic acid

TABLE 1

SUMMARY OF ALGAL MATS USED IN ORGANIC GEOCHEMICAL STUDIES

Locality	Environment	Major Organisms Identified		Major Classes of Compounds Studied				Reference
		Algae	Bacteria	Hydrocarbons	Fatty acids	Sterols	Carotenoids	
Laguna Mormona, Baja California	Shallow hypersaline ponds separated from ocean by sand dunes. Water level changes by movement under dunes and by surface run off and evaporation.	*M.chthonoplastes* *L.aestuarii*	*Chloroflexis*(?) Purple photosynthetics	+	+	+	+	Cardoso *et al.*, 1975 Tibbetts *et al.*, 1978
Laguna Guerrero Negro	Shallow hypersaline ponds	*M.chthonoplastes*	*Chromatium sp.*	+	+	-	-	Philp *et al.*, 1978a,b
Baja California	pH8; flooded by tidal movement	*L.aestuarii*	*Beggiatoa sp.*					
Baffin Bay, Texas	Tidal lagoon, flooded at regular intervals followed by evaporation.	*L.confervoides*		+	+	-	-	Parker and Leo, 1965 Winters *et al.*, 1969
Abu Dhabi, Persian Bay	Hypersaline pools, intertidal flats of sheltered tropical and arid coast associated with formation of evaporites and coastal sabkha plains.	*L.aestuarii* *M.chthonoplastes*	*Chromatium sp.* *Thiocystis sp.*	+	+	+	+	Cardoso *et al.*, *1978* Watts *et al.*, 1977

TABLE 2

DISTRIBUTION OF HYDROCARBONS IN RECENT ALGAL MATS

		Laguna Mormona		Laguna Guerrero[a]			Abu	Texas	Inferred source
		Mat	Ooze	U	M	L	Dhabi	Lagoons	
n-Hydrocarbons	Range	C_{15}-C_{33}	C_{17}-C_{35}	C_{17}	C_{17}-C_{35}	C_{17}-C_{35}	C_{17}-C_{31}(Minor	C_{15}-C_{18}	C_{15}&C_{17} - Algae
	Max	C_{17}	Bimodal		C_{17}&C_{29}	C_{17}&C_{27}	Amounts > C_{20})	C_{17}	C_{20}-C_{35}Higher plants
							C_{17}	$C_{17:1}$;$C_{19:1}$;$C_{19:2}$	
pristane		+	+				+		Bacteria
phytane		+	Minor		+	+	+		Algae
phytenes				+	+	+			Diagenetic products of phytol
phytadienes				+					
7&8-MeC_{17}		+	Minor	+	+		+	+	Cyanobacteria
8-MeC_{16}				+					
monocyclic-C_{17}							+		
C_{17}-alkene (branched)				+			+		
C_{20}-alkene (branched)							+		
C_{23}-alkene (branched)							+		
$C_{20}H_{32}$&$C_{24}H_{38}$-(cyclics)					+	+			
Δ^2-cholestene			+		+	+	+		
Δ^2-methylcholestene			+		+	+	+		
Δ^2-ethylcholestene			+			+	+		Diagenetic products of
C_{27}-diunsat.sterene			+						cyanobacterial sterols
C_{28}-diunsat.sterene			+						
C_{39}-diunsat.sterene			+						
hop-17(21)-ene							+		Diagnetic products of
hop-22-(29)-ene		+	+		+	+	+		triterpenoid indicative
hop-21(22)-ene						+	+		of bacterial contri-
									bution.

[a] U = Upper layer of mat; M = Middle layer of mat; L = Lower layer of mat.

TABLE 3

DISTRIBUTION OF FATTY ACIDS IN RECENT ALGAL MATS

Acid \ Mat	Laguna Mormona		Laguna Guerrero			Abu Dhabi	Inferred Source
	Mat	Ooze	Upper	Middle	Lower		
i-C_{14}	+	Not	+	+	+		Bacteria
i-C_{15}	+	analysed	+	+	+	+	"
a-C_{15}	+	in	+	+	+	+	"
i & *ai*C_{16}		detail				+	"
Δ-C_{17}	+		+		+		"
i & *ai*C_{17}						+	"
Phytanic acid	+		+			+	Phytol
Δ-C_{19}	Two isomers					Two isomers	Bacteria
17βH-bishomophopanoic acid	+	+				+	
2 Me-substituted isoprenoid (C_{16}-C_{20})				+			Phytol(?)
α,ω - dicarboxylic acids			+		+		Diagenetic oxid. of branched ω-hydroxy acids - higher plants
n-alkanoic acids	C_{14}-C_{24} $C_{16:1}$ $18:1$	C_{14}-C_{32} $C_{16:1}$ $18:1$	C_{15}-C_{18} $C_{16:1}$ $18:1$	C_{15}-C_{18} $C_{16:1}$ $18:1$	C_{12}-C_{18} $C_{16:1}$ $18:1$	C_{16}, C_{18} $C_{16:1}C_{18:1}$ $C_{19:1}$	Algae and higher plants

was the major acid in the living layer of mat but disappeared rapidly in the first mud layer underneath the mats. Similar trends were observed in the three layers of algal mats examined from Laguna Guerrero and Laguna Mormona. It was proposed by Parker and Leo (1965) that the disappearance of the unsaturated acids resulted from either their incorporation into the insoluble organic residues or their preferential removal as a result of increasing bacterial activity with depth within the mats. The higher plant contribution thought to be present in the mats of Baja California was confirmed by the presence of *n*-alkanoic acids in the range C_{20}-C_{29}. Saturated acids above *n*-C_{22} are not abundant in algae (Erwin, 1973), bacteria (Shaw, 1974) or algal detritus (Cranwell, 1976a,b). The absence of higher plant material from the Abu Dhabi mats was demonstrated by the absence of *n*-alkanoic acids above C_{20}. Bacterial activity in all of the mats was indicated by the presence of *iso*- and *anteiso*-C_{15}, C_{16} and C_{17} acids and C_{17}- and C_{19}-cyclopropanoid acids (Cranwell, 1973, 1978). Phytanic acid was tentatively characterized in the Abu Dhabi mat. A pentacyclic triterpanoic acid, 17βH-bishomohopanoic acid, was found in the Abu Dhabi and Laguna Mormona samples. The presumed precursor of this compound is a C_{35}-triterpenoid tetrol. Occurrence of the triterpenoid tetrol or related compound in algae has not been demonstrated but it has been identified from *Acetobacter xylinum* (Forster *et al.*, 1973; Rohmer and Ourisson, 1976), as well as from *Bacillus acidocaldarius*.

Sterols

The algal mats from all of the environments mentioned in Table 1, except Baffin Bay, contained complex mixtures of Δ^5-sterols, $\Delta^{5,22}$-sterols and 5α-stanols which would be expected to be derived from the major source organisms, *Microcoleus chthonoplastes* and *L. aestuarii* (de Souza and Nes, 1968; Reitz and Hamilton, 1968; Teshima and Kanazawa, 1972). These included 5β-cholestan-3β-ol; 24-methyl-5β-cholestan-3β-ol; 24-ethyl-5β-cholest-22-en-3β-ol; 24-ethyl-5β-cholestan-3β-ol. In a separate study on sterols from the algal mats at Baffin Bay, Texas, Huang and Meinschein (1978) observed the relatively rare C_{27}-,C_{28}-, and C_{29}-Δ^{22}-5α-stenols in addition to the Δ^5 and $\Delta^{5,22}$ sterols and 5α-stanols. The Δ^5-sterol and 5α-stanol distributions for Abu Dhabi and Laguna Mormona samples do not show the same parallelism between the distributions observed in a depth study of the sterols/stanols in the core of an English lake sediment (Gaskell and Eglinton, 1975, 1976). Cardoso *et al.* (1978) have suggested that the absence of any parallelism suggests: (1) that reduction proceeds at different rates for different Δ^5-sterols or (2) that preferential degradation of Δ^5-sterols in sediments proposed by Nishimura and Koyama (1976) proceeds at different rates for Δ^5-sterols in the algal mats.

Precambrian Sediments

The complexity and diversity of compounds found in algal mats has been demonstrated above by using three classes of compounds. Relatively few individual compounds survive diagenetic changes or remain structurally intact for a sufficiently long period of time to be of any use as chemical fossils for the Precambrian. Some compounds will be removed or transformed by bacterial action, others will undergo rearrangement or chemical reactions to lose functional groups. Thermally stable hydrocarbons, such as alkanes, isoprenoids and steranes, derived from fatty acids and sterols or unaltered hydrocarbons, are the compounds most likely to survive from the Precambrian. Other compounds possessing suitable structural characteristics such as double bonds, carboxyl or hydroxyl groups may be incorporated into the kerogen. The mode of preservation of organic matter is also important. Simple compacted organic matter often loses many of its original chemical characteristics. Clays may permit organic-inorganic interactions and, in quartz-sandstones, the porosity might allow oxygenated or reduced waters to percolate through the intersitial organic matter thus removing or altering the nature of the functional groups.

One of the major problems in examining the soluble organic extracts from Precambrian samples is determining whether or not the compounds are syngenetic or contaminants. Hoering (1965) compared $\delta^{13}C$ values of the soluble lipid fraction with that of the insoluble organic fraction from various Precambrian sediments and concluded that, in certain cases, it is difficult to say whether the soluble organic matter in a very ancient rock has the same origin as the kerogen in the rock although, in other cases, there is a good correlation between the $\delta^{13}C$ values of kerogen and extracts.

In spite of this problem, a comparison between the compounds in the Recent algal mats and those structures found in Precambrian sediments is a useful exercise since the algal mats

contain many groups of microorganisms whose first representatives appeared during Precambrian time. At the same time, it should be noted that for such a comparison to be more valuable, information on the temperature to which the Precambrian deposits have been exposed is really necessary to take into consideration the possible effects of thermal alteration on the chemical fossils.

McKirdy (1974) has given a fairly comprehensive description of chemical fossils which have been found in Precambrian sediments. Table 4, which has been adapted from that review, summarises the distribution of fossils relevant to this discussion. As with the palaeobiological studies, there are many problems in trying to relate the chemical fossils with their recent counterparts. Several compounds present in the algal mats could be regarded as precursors of the chemical fossils in the Precambrian. Such an association would in turn support the theory of a biological origin for the organic matter in these ancient sediments (McKirdy, 1974). Sterols, for example, in the algal mats, have already undergone the initial steps of diagenesis to form stanols and sterenes. Further hydrogenation would produce the sterane skeleton such as that found in the Onverwacht sediments. The normal and branched alkanes which have been found in Precambrian extracts either could have survived unaltered, or have been derived from the corresponding fatty acids, or have been formed from cracking of the kerogen. The distributions of these hydrocarbons correlate well with those found in the Recent algal mats. Whether the fatty acids in the Precambrian have survived intact or result from post-depositional contamination is questionable and hence their use as fossils for the Precambrian is also doubtful.

It can be seen, therefore, that some information as to the possible origin of organic matter in Precambrian sediments is available by comparison with the biolipid distribution of Recent algal mats, but that the picture is by no means complete. Schopf (1970) has suggested that one possible, but imperfect, recourse is to use the morphological fossil record of the Precambrian as a basis for evaluating the chemical record; this in turn relegates the extractable chemical fossils to a subsidiary position. An alternative and more promising geochemical solution is to pursue the examination of the bulk of the organic material which is to be found as the kerogen fraction in Precambrian sediments.

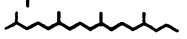
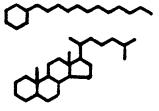
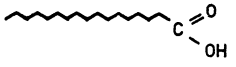
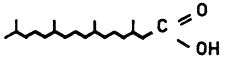
KEROGEN FROM RECENT ALGAL MATS AND PRECAMBRIAN SEDIMENTS

Kerogen is isolated from sediments by removal of the inorganic matter by acid treatment, and of soluble organic matter by exhaustive extraction with organic solvents (Saxby, 1976). The major soluble contaminants are also removed during this preparation step. Due to its lack of mobility, kerogen is more likely to be indigenous to the original rock than is the extractable fraction, hence examination of the kerogen has the potential to provide an accurate picture of the origin of organic matter in sediments. The major problem in utilizing the structural information potentially present in kerogen is the analytical inaccessibility of the material. Chemical degradation has been used by many investigators to obtain small fragments more readily amenable to analysis by techniques such as gas chromatography (GC) and mass spectrometry (MS) (Burlingame *et al.*, 1969; Philp *et al.*, 1978). However, most of the chemical degradation reagents are either specific and cleave particular types of bonds, resulting in low overall yields, or non-specific, leaving uncertainty as to whether the products truly reflect the structure of the original kerogen. These disadvantages can be eliminated to a large extent by using pyrolysis as the degradation method. Pyrolysis has become a powerful analytical tool in the past few years, especially in the study of macromolecular materials. Pyrograms, obtained by either pyrolysis-gas chromatography or pyrolysis-mass spectrometry, are found to be sufficiently reproducible and specific to be used for the characterization of technical polymers, biopolymers and geopolymers. From an organic geochemical viewpoint, pyrolysis techniques have been successfully employed in the study of biopolymers (Meuzelaar *et al.*, 1977), humic substances (Bracewell *et al.*, 1976) and kerogens (Maters *et al.*, 1977; Larter and Douglas, 1978).

Only a limited number of pyrolysis studies have been performed on Precambrian kerogens (Scott *et al.*, 1970; Dungworth and Schwartz, 1972). Scott *et al.* (1970) pyrolysed samples from the Onverwacht and Fig Tree series; the Onverwacht samples produced mainly aromatic products whilst the Fig Tree produced mainly *n*-alkanes. The origin of the aromatic products from Precambrian samples is unclear. Dungworth and Schwartz (1972) pyrolysed kerogen samples from the Fig Tree and Onverwacht series, the Skillogalee chert of South Australia and the Devonian Rhynie Chert. Zumberge (1975) pyrolysed a stromatolite from the Wolkberg Group, Transvaal sequence at 600^{o} and produced furan, among other compounds. It was proposed that, since this stromatolite contained fossilized filamentous cyanobacteria and modern analogues secrete

TABLE 4

DISTRIBUTION OF POSSIBLE CHEMICAL FOSSILS IN PRECAMBRIAN SEDIMENTS DERIVED FROM HYDROCARBONS, FATTY ACIDS AND STEROLS

Compound Type	Generalized Carbon Skeleton	Examples	Suggested Sources	Sediment	Reference
ALKANES - normal saturated		C_{10} to C_{35}	Algal, bacterial, fungal lipids	Onverwacht	Macleod, 1968; Han and Calvin, 1969
				Swaziland	Hoering, 1967; Oro and Nooner, 1970
mono-unsaturated		$C_{16}, C_{18}, C_{20}, C_{22}$	Biological alcohols; Catalytic condensation of ethylene	Fig Tree	Oro and Nooner, 1970
ALKANES - branched					
iso		C_{16}, C_{17}, C_{18}	Bacterial iso and anteiso fatty acids	Nonesuch seep oil and shale	Johns *et al.*, 1966
anteiso		C_{16}, C_{17}, C_{18}			
isoprenoid		C_{14} to C_{16} C_{18} to C_{21}	Isoprenoid alcohol side-chain of algal and bacterial chlorophylls. Phosphatidyl glycerophosphate of halophilic bacteria	Fig Tree	Oro and Nooner, 1970
ALKANES - cyclic					
cyclohexyl		C_{16} to C_{19}	Algal unsaturated fatty acids (via diagenetic intramolecular cyclization)	Nonesuch seep oil	Johns *et al.*, 1966
sterane		C_{27}, C_{28}, C_{29}	Algal sterols	Nonesuch shale	Barghoorn *et al.*, 1965
FATTY ACIDS - normal saturated		C_{11} to C_{24}	Algal bacterial lipids	Onverwacht	Han and Calvin, 1969
		C_{16}, C_{18}	Recent biological origin (if even-carbon-numbered species dominant) i.e. contamination.	Gunflint	Smith *et al.*, 1970
FATTY ACIDS - branched isoprenoid		C_{16}, C_{19}, C_{20}	Isoprenoid alcohol side-chain of algal, bacterial chlorophylls (via diagenetic oxidation). Zooplanktonic lipids (if host rock < 1,000 m.y. old).		

large quantities of polysaccharides in currently forming stromatolites, the cyclic ether could conceivably be derived from carbohydrates.

More recently, a series of kerogens from Precambrian and other ancient sediments along with proto-kerogens from recent algal mats have been subjected to Curie-point pyrolsis[a] combined with gas chromatography (van de Meent *et al.*, personal communication). Fig. 1 shows the pyrograms obtained from the proto-kerogens of the algal mat samples of Laguna Guerrero Negro

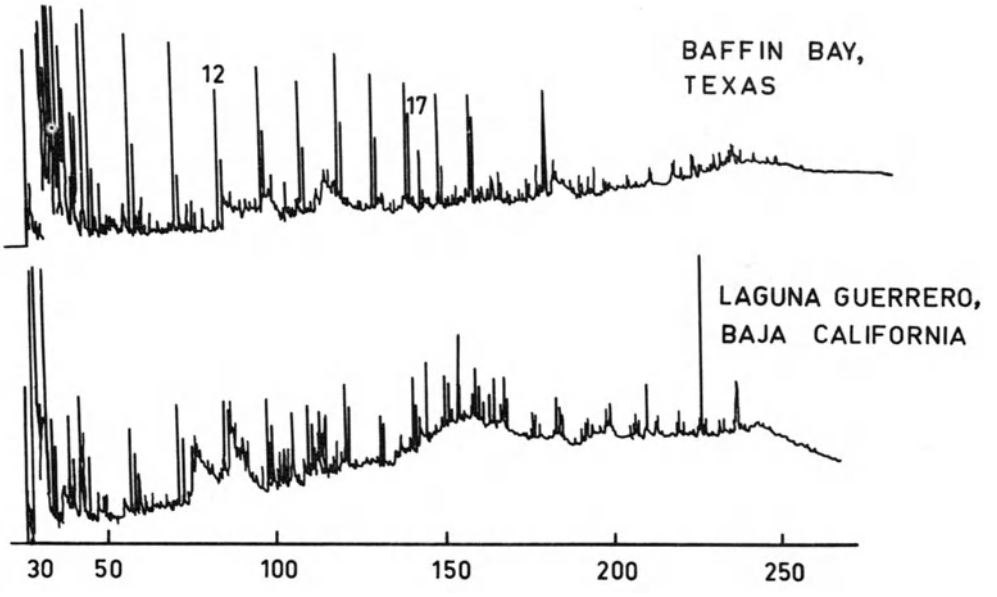


Figure 1: Pyrograms of protokerogens of algal mats from Baffin Bay Texas and Laguna Guerrero, Baja California (by courtesy of Meent *et al.*).

and Baffin Bay and pyrograms from the Fig Tree, Onverwacht and Kalgoorlie kerogens are shown in Fig. 2. The proto-kerogens of Baffin Bay and Laguna Guerrero Negro are characterized by series of alk-1-enes and n-alkanes in the range C_8-$C_{19}$and the presence of prist-1-ene. The presence of phenolic type compounds is indicated by the broad baseline humps in the C_{10}-C_{14} region. The main feature of these pyrograms is that most of the pyrolysis products are to be found in the region below C_{20}: this is a characteristic feature of programs from algal-type kerogens and proto-kerogens (van de Meent *et al.*, personal communication).

The Fig Tree pyrogram is dominated by a series of alk-1-enes in the range C_9-C_{15}, similar to the results obtained previously by Dungworth and Schwartz (1972). In addition, complex mixtures of alkyl benzenes and phenolic compounds appear to be present but their presence should be confirmed by GC-MS analysis. The Onverwacht and Kalgoorlie kerogens are dominated by series of alk-1-enes from C_8-C_{32}, with the Onverwacht showing some even/odd preference and

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[a] Curie point pyrolysis: a ferromagnetic wire, supporting the sample, is suspended in the work coil of a radio frequency (r.f.) oscillator and absorbs energy rapidly from the r.f. field until the temperature of the wire rises to the Curie point. At this temperature, which is dependent on the alloy composition of the wire, the magnetic permeability falls suddenly and further heating of the wire ceases: thus the temperature of the wire is maintained at the Curie point until the r.f. field is removed.

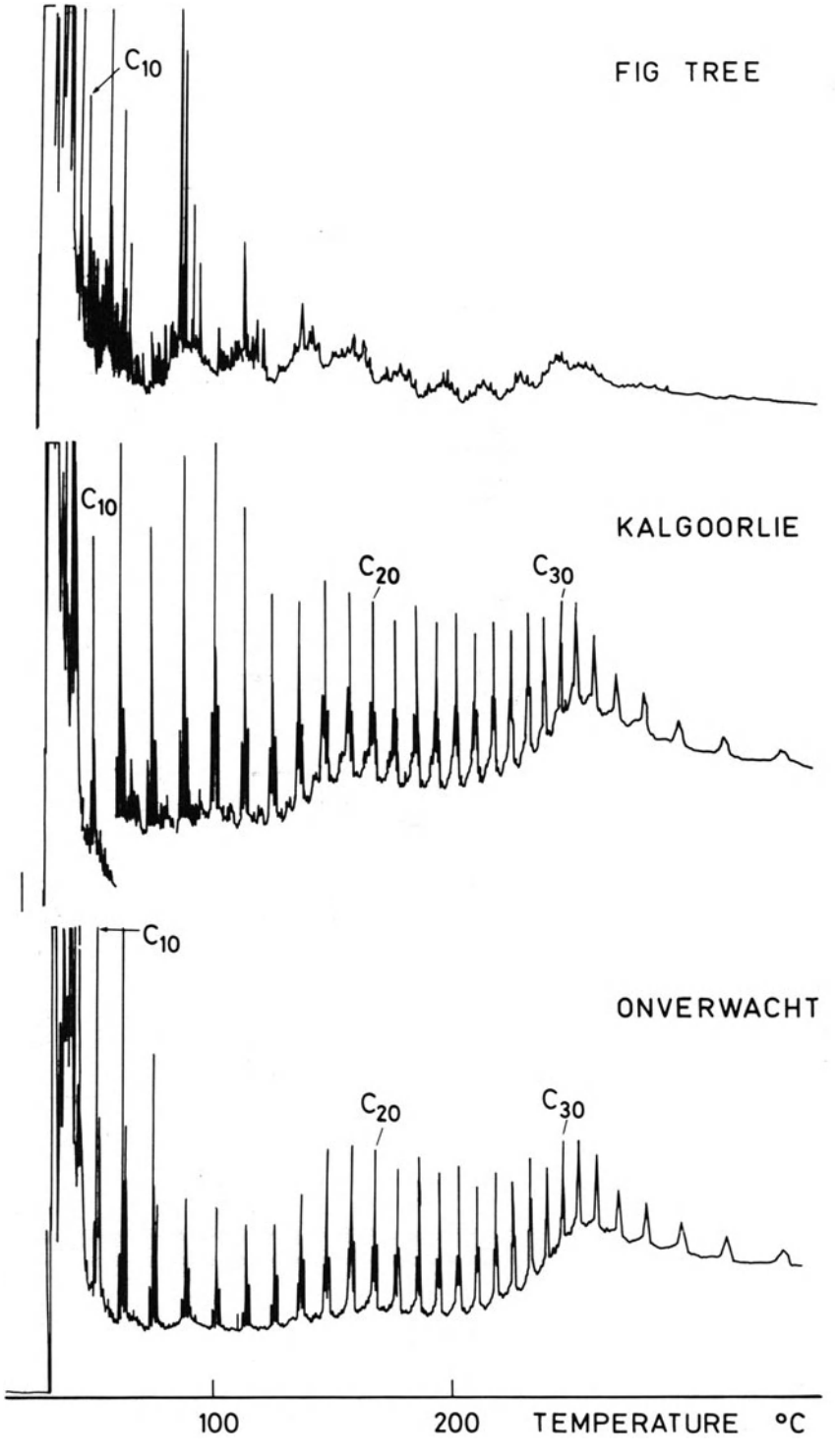

Figure 2: Pyrograms of kerogens from three Precambrian sediments (by courtesy of van de Meent *et al.*)

maximizing at C_{26} and the Kalgoorlie showing no preference and minimizing at around C_{14}. Alkanes and alkadienes in the same range are also present. The Fig Tree results show some similarities to the Recent algal mat results in that the products are dominated by compounds below C_{20}. The presence of alkenes in the Fig Tree demonstrates its partial aliphatic structure containing ether linkages which on pyrolysis cleave to form alkenes in preference to alkanes.

The results from the Onverwacht and Kalgoorlie are more enigmatic. An exclusively aliphatic structure for the kerogens is suggested by the absence of any aromatic pyrolysis products. The predominance of alkenes in the products again reflects the nature of the linkages within the kerogens but the presence of alkenes $>C_{20}$ is surprising. They could not have originated from higher plants and it is highly improbable that they result from contamination. Whether they reflect a difference in the structure of the material originally responsible for the formation of the kerogen is questionable. It is more probable that they reflect differences in the thermal history experienced by the kerogens during and after their formation.

CONCLUSIONS

In this paper an attempt has been made to show that studies of contemporary algal mats are of some use in interpreting geochemical data obtained from Precambrian sediments. The extractable organic material is the most widely studied fraction due to the relative ease with which it can be analysed. It is also the fraction most susceptible to contamination during sample collection, extraction and fractionation. However, it is clear that some of the chemical fossils found in Precambrian sediments have potential precursors in contemporary algae. The insoluble organic fraction of Precambrian sediments has the potential to provide information on the types of organisms present when these sediments were being deposited. The algal contribution in the Fig Tree sediment is suggested by comparing the pyrolysis products with those obtained for contemporary algal mats.

Comparative geochemical studies of algal mats and Precambrian samples appear to be of limited use. Many of the problems encountered by the palaeobiologist in this type of comparative study are similar to those encountered by the geochemist. The problem is complicated by the fact that even within the algal mat system there will be differences between the living matter and the degraded algae. Studies of Precambrian samples are complicated by contamination problems and also by the temperature and burial history of the samples. To get a more informative picture on the origin of organic matter in Precambrian sediments, additional samples known to be of algal origin, and of varying geological ages and modes of preservation, should be examined. Recent algal mat material also should be subjected to thermal maturation experiments and a study made of the changes that occur to both the soluble and insoluble organic fractions.

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COMPARATIVE ANALYSIS OF STROMATOLITIC AND OTHER MICROBIAL KEROGENS BY PYROLYSIS-HYDROGENATION-GAS CHROMATOGRAPHY (PHGC)

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INTRODUCTION

The technique of pyrolysis-hydrogenation-gas chromatography (PHGC: McHugh *et al.*, 1976, 1978) provides a convenient means of analysing the carbon-skeleton structures of coals and coal-derived kerogens. Catalytic hydrogenation of the pyrolysate has the advantage of simplifying its composition prior to analysis by GC (or gas chromatography-mass spectrometry, GC-MS) without significantly diminishing the amount of structural information available in the resulting pyrogram. Alkenes and heteroatomic compounds are converted to the corresponding alkane or aromatic hydrocarbon. In the present study we extend the application of PHGC to kerogens of microbial origin, i.e., insoluble sedimentary organic matter derived from algae and bacteria.

The use of pyrolysis-gas chromatography (PGC) to study microbial kerogens is not new. Scott *et al.* (1970) pyrolysed finely-ground samples of early Precambrian (>3 Gyr B.P.) carbonaceous sedimentary rocks from the Swaziland System, South Africa, and identified hydrocarbons containing up to 20 carbon atoms. Onverwacht Group samples yielded mainly aromatic hydrocarbons whereas a sample from the younger Fig Tree Group gave mostly normal and branched aliphatic hydrocarbons (but cf. Philp, 1980). Kerogens isolated by acid digestion from three Precambrian cherts (including the Skillogalee Dolomite: Table 1) were analysed in a similar manner by Dungworth and Schwartz (1972). Stepwise PGC of 30 organic-rich Precambrian sediments by Leventhal *et al.* (1975) yielded CO_2, CO and low molecular-weight hydrocarbons (C_1-C_8). The H/C atomic ratios of these kerogens, where reported, are low (generally <0.30) and typical of high-rank organic matter. Analysis by vacuum pyrolysis-GC-MS of kerogen (thucolite) from the 2.6 Gyr old Witwatersrand Vaal Reef gold-uranium deposit, demonstrated its highly aromatic character which is attributable to prolonged exposure of the kerogen to ionising radiation (Nagy *et al.*, 1977; Zumberge *et al.*, 1978).

Algae were major precursors of the organic matter in coorongite, oil shales and boghead coals (Hutton *et al.*, 1980), and in the most prolific petroleum source rocks (Tissot and Welte, 1978). Previous pyrolytic studies of oil-shale kerogen from the Green River Formation (Table 1) include those by Scrima *et al.* (1974), Gallegos (1975), Leventhal (1976) and Larter *et al.* (1978, 1979). In boghead coals and oil shales, algal remains are preserved morphologically as either discrete chlorophycean bodies (*alginite A*) or thin films of lipid-enriched algal (probably cyanophycean) mat material (*alginite B*) (Hutton *et al.*, 1980). PGC analyses of alginite concentrates from torbanites have been reported by Larter *et al.* (1977, 1978) and Larter and Douglas (1978). The pyrogram of a Silurian marine shale containing abundant *Tasmanites* (Combaz, 1971) shows that, like other varieties of alginite A, tasmanite kerogen is rich in long-chain aliphatic moeities. Hydrogen-rich organic matter of this kind (original atomic H/C > 1.5: Fig. 1) has been classified as Type I kerogen by Tissot and Welte (1978). However, both forms of alginite also may contribute to the more heterogenous liptinitic Type II kerogen (Table 2), exemplified in this study by the Cretaceous Toolebuc Formation oil shale (Table 1).

McKirdy (1976) showed that the kerogen preserved in Proterozoic and Palaeozoic stromatolitic carbonates and cherts commonly is depleted in hydrogen and enriched in oxygen (Fig. 2) consistent with its probable derivation largely from cyanophyte mucilage (mucopolysaccharide). Such organic matter herein is designated Type III or IV kerogen (Table 2). PHGC analysis of selected stromatolitic kerogens (McKirdy, McHugh and Tardif, unpublished results) provides additional geochemical evidence for the previously reported chemotaxonomic similarity of two examples of the stromatolite form genus *Tungussia*. Pyrolysis in vacuo of kerogen from the 2.7 Gyr old Rupemba-Belingwe stromatolite (Sklarew and Nagy, 1979)

TABLE 1

SAMPLES ANALYSED BY PHGC

Formation or Deposit	Basin and Location[a]	Sediment Type	Age	Kerogen			
				C % d.a.f.[b]	H/C atomic	O/C atomic	Type[d]
Coorongite	Murray, S.A.	algal peat	Recent	72.7	1.79	0.16	I
Green River	Piceance Creek, CO.	oil shale	Eocene	76.9	1.50	0.10[c]	I
Illawarra Coal Measures	Sydney, N.S.W.	torbanite	Permian				
- Newnes				83.9	1.60	0.03	I
- Joadja				82.1	1.33	0.06	I
Beetle Creek	Georgina, Q.	phosphorite	Cambrian	80.9	1.13	0.05	I
Un-named	Browse, W.A.	limestone	Eocene	61.2	1.29	0.29	II
Hibernia Beds	Browse, W.A.	limestone	Miocene	72.0	1.27	0.16	II
Toolebuc	Eromanga, Q.	oil shale	Cretaceous	68.6	1.26	0.14[c]	II
Horn Valley Siltstone	Amadeus, N.T.	shale	Ordovician	64.3	1.10		II
Horn Valley Siltstone	Amadeus, N.T.	shale	Ordovician	85.8	0.75	0.06	II
McMinn	McArthur, N.T.	shale	Proterozoic	73.6	1.17	0.13	II
McMinn	McArthur, N.T.	shale	Proterozoic	81.2	0.86	0.06	II
Phosphoria	Cordilleran, WY.	phosphorite	Permian	68.7	0.54	0.18	III
Stairway Sandstone	Amadeus, N.T.	shale	Ordovician	66.7	0.83	0.28	III
Pacoota Sandstone	Amadeus, N.T.	shale	Ordovician	70.7	0.63	0.23	III
Jay Creek Limestone	Amadeus, N.T.	siltstone	Cambrian	71.6	0.71	0.23[c]	III
Pertatataka	Amadeus, N.T.	shale	Proterozoic	78.0	0.66	0.15[c]	III
McMinn	McArthur, N.T.	shale	Proterozoic	81.9	0.65	0.08	III
McMinn	McArthur, N.T.	shale	Proterozoic	81.4	0.59	0.10[c]	III
McMinn	McArthur, N.T.	shale	Proterozoic	83.4	0.54	0.09[c]	III
Cavan Limestone	Taemas Platform, N.S.W.	stromatolite	Devonian	81.2	0.54	0.12[c]	IV
Wonoka	Adelaide Geosyncline, S.A.	stromatolite	Proterozoic	77.9	0.47	0.09[c]	IV
Skillogalee Dolomite	Adelaide Geosyncline, S.A.	stromatolite	Proterozoic	75.3	0.33	0.20[c]	IV
Skillogalee Dolomite	Adelaide Geosyncline, S.A.	cryptalgal chert	Proterozoic	85.2	0.31	0.08	IV
Bitter Springs	Amadeus, N.T.	dolomite	Proterozoic		0.28		IV
Bitter Springs	Amadeus, N.T.	gypsiferous dol.	Proterozoic		0.46		IV
McMinn	McArthur, N.T.	shale	Proterozoic	86.6	0.36	0.06	IV
Cavan Limestone	Taemas Platform, N.S.W.	stromatolite	Devonian	88.2	0.26	0.06	s/g
Tapley Hill	Adelaide Geosyncline, S.A.	stromatolite	Proterozoic	84.0	0.24	0.07	s/g
Skillogalee Dolomite	Adelaide Geosyncline, S.A.	cryptalgal chert	Proterozoic	92.2	0.19		s/g
Skillogalee Dolomite	Adelaide Geosyncline, S.A.	stromatolite	Proterozoic	90.9	0.10	0.03	s/g

[a] Details of sample localities are given in McKirdy (1977). [b] d.a.f. = dry, ash-free.

[c] [d]

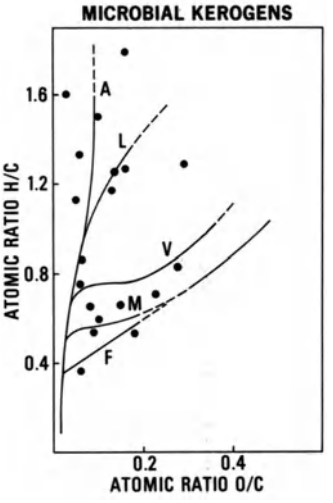

Figure 1: Van Krevelen diagram showing elemental composition of selected microbial kerogens. Lines are coalification tracks of coal macerals: A = alginite, L = liptinite (exinite), V = vitrinite, M = macrinite, F = fusinite.

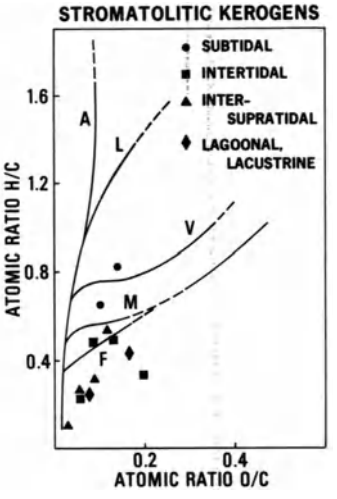

Figure 2: Van Krevelen diagram showing elemental composition of stromatolitic kerogens. See Fig. 1 for key.

yielded, among other compounds, 2, 5-dimethylfuran, 2-methylfuran, and C_9-C_{20} *n*-alkanes. These products were interpreted respectively as remnants of biological carbohydrates and fatty acids. Furan was identified in the pyrolysates of two other Precambrian stromatolitic kerogens analysed by the same technique (Nagy *et al.*, 1977).

Previous pyrolytic investigations of microbial kerogens may be categorised, according to their principal aim, as follows:

TABLE 2

POSSIBLE ORIGIN AND MACERAL EQUIVALENTS OF THE PRINCIPAL TYPES OF MICROBIAL KEROGEN (MODIFIED FROM McKIRDY, 1977)

Kerogen Type [a]	Maceral Group	Equivalent Individual Macerals	Source
Type I	Liptinite	alginite A	Lipid-rich eukaryotic algae, e.g. *Reinschia*, *Tasmanites*, *Gloecapsomorpha*
		alginite B	Bloom and mat-forming blue-green algae
Type II[b]	Liptinite	bituminite	Partially degraded (i.e. sapropelised algae and zooplankton; bacterial lipids
		liptodetrinite	Acritarchs
Type III	Huminite	humodetrinite detrogelinite	Biochemically humidified and gelified algal cellulose; Maillard condensation of algal amino acids and carbohydrates
		dopplerite	Calcium humate formed from algal humic acids in carbonate-depositing environments
	Vitrinite	desmocollinite	Geochemically gelified algal huminite incorporating some bacterial lipids; e.g. sub-tidal stromatolites
	(Inertinite)[c]	rank micrinite	Coalification residue of alginite B and/or bituminite
Type IV	Inertinite	degradofusinite	Partially oxidised algal cellulose and sheath mucilage; bacterial pectic tissue (cell walls); e.g. inter-supratidal stromatolites

[a] As defined by H/C and O/C atomic ratios, or H/C and %C d.a.f. where incomplete elemental analysis precluded determination of oxygen content.

[b] Alternatively, a mixture of lipid-rich (Type I) and humic (Type III) organic matter,

[c] Although micrinite has been traditionally classified as a member of the inertinite group, it "is neither inert nor especially carbon-rich, since it contains relatively large amounts of hydrogen and volatile matter" (Teichmuller, 1975, p. 228).

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1. characterisation of algal/bacterial organic matter using 'finger-print' pyrograms;
2. identification of specific 'biological marker' compounds in the pyrolysate; or
3. monitoring of the structural evolution of specific kerogen types during diagenesis and catagenesis.

The present study is based on earlier work (McKirdy, 1977) which showed that microbial kerogens are surprisingly diverse in composition (Figs. 1, 2; Table 2). PHGC was employed to investigate further this hitherto poorly documented compositional diversity and, in particular, its dependence on (i) the dominant precursor material (lipid, protein or carbohydrate) and (ii) the degree of thermal alteration (i.e., rank) of the kerogen.

SAMPLES AND METHODS

The 31 kerogens analysed in this study (Table 1) include eight from stromatolitic sediments and range in age from mid-Proterozoic (ca. 1.4 Gyr B.P.) to Recent. For many samples, microbial affinity was established simply on the basis of their pre-Devonian age. In the case of kerogens isolated from younger sediments, some of which were deposited in lacustrine or paralic environments, recognition of their source as predominantly microbial depended on micropalaeontological and other organic geochemical evidence.

Finely-ground sediments were extracted in Soxhlet apparatus for at least 48 h with a mixture of benzene and methanol (3:2) before isolation of the kerogen using the method of Powell *et al.* (1975). For coorongite and the two torbanites, PHGC analyses were performed on material which had not been extracted with solvent. The kerogen concentrates were analysed for C, H, N, S and ash by the Australian Microanalytical Service, Melbourne. Oxygen was determined by difference.

The basic apparatus and experimental parameters for PHGC analysis have been described by McHugh *et al.* (1976). For the present work, the equipment and operating conditions were modified as shown in Fig. 3. Upon completion of the pyrolysis, the cold trap was quickly removed from the end of the hydrogenator and placed (via Swagelok fittings) in the oven of the gas chromtograph between the injection port and SCOT column. Rapid heating of the trap by direct application of a low-voltage current volatilised the hydrogenated pyrolysis products and facilitated their flushing on to the column. The oven door was then closed and the chromatograph temperature-programmed from 50-300° at 10° min^{-1}, with a final heating time of 10 min. Major peaks in the C_4-C_{20} range of the resulting chromatogram were identified by comparison with a reference PHGC trace of the Greta coal. In this trace, obtained under the same experimental conditions, the major component peaks had been characterised by mass spectrometry. PHGC traces of the two phosphorite kerogens were obtained with a Finnegan 1015D GC-MS system, incorporating a 20 m x 0.5 mm i.d. glass SCOT column (stationary liquid phase SE-30), and interfaced to a Finnegan 6000 data system.

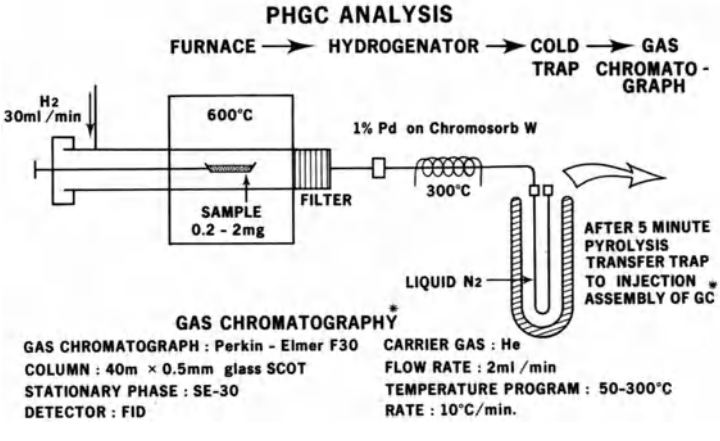

Figure 3: Schematic diagram of PHGC equipment and operating conditions.

RESULTS AND DISCUSSION

Microanalytical and PHGC data on examples of the four principal types of microbial kerogen (Table 2) are presented in Table 1 and Figs. 4 and 5. The spread in H/C values (1.79 - 0.10) reflects differences in both genetic type and thermal maturity. Kerogen maturation levels range from unaltered (early diagenesis) to subgraphitic (anchizone of metamorphism).

Type I Kerogen

This hydrogen-rich organic matter plots along the coalification track of alginite on a van Krevelen diagram (Fig. 1) and is characterised by PHGC traces in which *n*-alkanes are by far the dominant components (Figs. 6-8, 9a). Simple aromatic compounds (viz. benzene, toluene, ethylbenzene and the xylenes) occur in very low concentrations relative to aliphatic moeities (Figs. 4, 5) although the fact that aromatics are present at all in low to medium rank lipid-rich kerogens is significant. An homologous series of C_7-C_{19} *n*-alkylbenzenes (and the corresponding *n*-alkylcyclohexanes) was identified in the hydrogenated pyrolysates of coorongite and the torbanites (alginite A). Larter *et al.* (1978) found that the *n*-alkylbenzene homology in the 600° pyrolysate of a torbanite kerogen extended up to C_{31} and differed significantly from those of sporinite (Type II kerogen) and vitrinite (Type III kerogen).

Kerogen from the Green River Formation oil shale comprises predominantly alginite B. Inspection of its PHGC trace (Fig. 8) reveals the presence of peaks with the same Kovats retention indices on SE-30 as pristane (1710) and phytane (1814) (relative concentration pr>ph), in contrast to their apparent absence in alginite A-derived kerogens (Figs. 6, 7). This observation accords with the findings of Larter *et al.* (1979) who, however, reported only the C_{19} isoprenoid (as prist-1-ene) in the 600° pyrolysate of Green River kerogen.

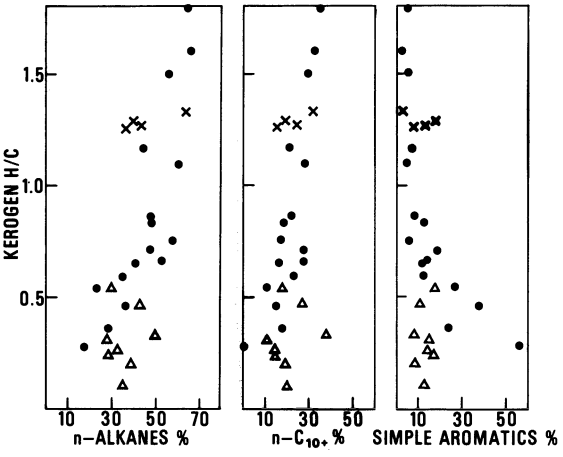

Figure 4: Plot of kerogen H/C atomic ratio against PHGC parameters % *n*-alkanes, % *n*-C_{10+}, and % simple aromatics. Percentage based on all peaks in range *n*-C_6 to *n*-C_{20}. • = microbial (non-stromatolitic) kerogen; x = microbial kerogen with significant land-plant component; Δ = stromatolitic kerogen. Data from phosphorite kerogens are not included.

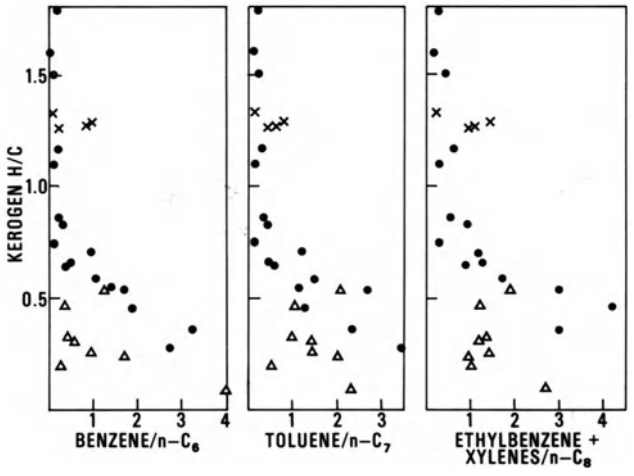

Figure 5: Plot of kerogen H/C atomic ratio against PHGC parameters benzene/n-C_6, toluene/n-C_7, and ethylbenzene + xylenes/n-C_8. See Figure 4 for key.

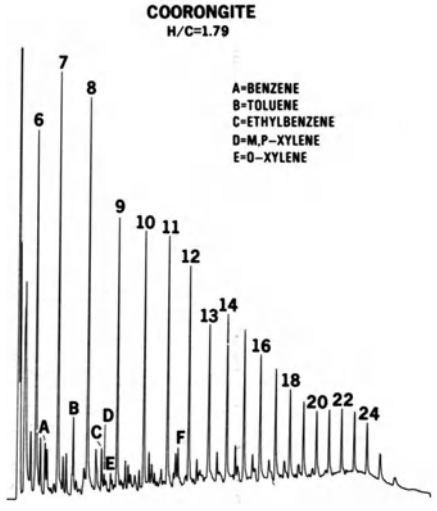

Figure 6: PHGC trace of coorongite (Recent). Peak F = n-pentylbenzene.

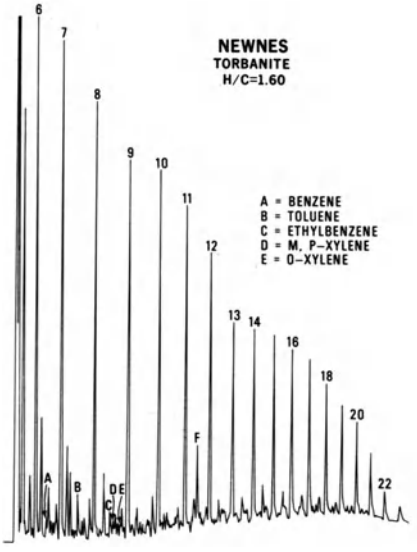

Figure 7: PHGC trace of Newnes torbanite (Permian). Peak F = *n*-pentylbenzene.

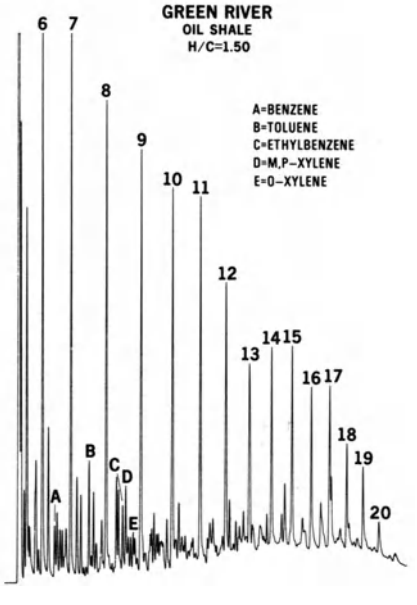

Figure 8: PHGC trace of Type I kerogen from Green River Formation oil shale (Eocene).

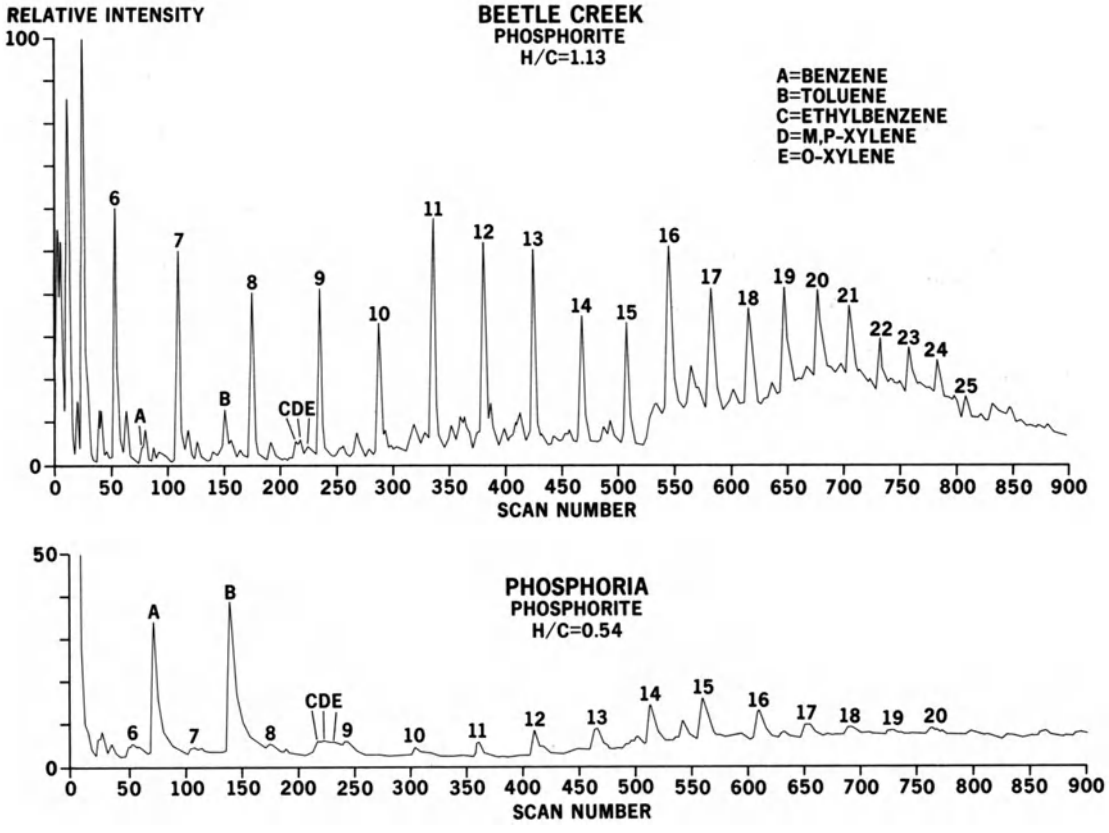

Figure 9: (a) PHGC trace of Type I kerogen from Beetle Creek Formation phosphorite (Cambrian).

(b) PHGC trace of secondary Type III kerogen from Phosphoria Formation (Meade Peak Member) phosphorite (Permian).

No systematic increase in aromaticity, as measured by the PHGC parameters percent simple aromatics in C_6-C_{20} hydrocarbons, benzene/*n*-C_6, toluene/*n*-C_7 and ethylbenzene + xylenes/*n*-C_8 (Figs. 4, 5), is evident for Type I kerogen over the atomic H/C range 1.70-1.13 (corresponding to ca. 0.2-0.8% R_{max} equivalent vitrinite reflectance). Larter *et al.* (1978) noted a similar constancy of the *m*-xylene/*n*-C_8 alkene ratio in alginite A pyrolysates over the same rank interval. It is noteworthy that even the appreciable humic, higher-plant contribution to the Joadja torbanite (vitrinite + inertinite = ca. 25% of total organic matter: A. J. Kantsler, personal communication) is not reflected in any of the above pyrolytic indices of aromaticity.

Comparison of the pyrograms of the medium rank (marginally mature) and high rank (mature) phosphorite kerogens shown in Fig. 9 throws light on the structural modification of an unusual variety of Type I microbial kerogen during the process of oil generation. Such kerogen, represented here by a sample from the Cambrian Beetle Creek Formation (Table 1), comprises a complex mixture of alginite B, acritarchs, and at least two vitrinite-like materials of which the major one has affinities with bituminite (A.J. Kantsler and A.C. Hutton, personal communication). By analogy with the genesis of micrinite from bituminite in high-volatile bituminous coals (Teichmuller, 1974), the Phosphoria kerogen may have formed via the rapid geochemical disproportionation of primary Type I organic matter similar to that preserved in the Beetle Creek phosphorite (or possibly a Type II kerogen),

resulting in liquid hydrocarbons (oil) and a secondary aromatic, oxygen-enriched kerogen. This would readily account for the appearance of its PHGC trace (Fig. 9b) which features toluene and benzene as major components, with residual amounts of *n*-alkanes up to C_{20}.

Type II Kerogen

Petrologically, Type II microbial kerogens consist of bituminite and liptodetrinite (e.g. McMinn Formation, Horn Valley Silstone: Table 1) or, more commonly, a mixture of lipid-rich (Type I) and humic (Type III) organic matter (e.g. Toolebuc Formation oil shale, Tertiary limestones: Table I). Acccordingly, on a van Krevelen diagram, they occupy an intermediate position which overlaps the liptinite coalification pathway (Fig. 1).

H/C values of the Type II kerogens analysed in this study extend over the range 1.29-0.75 (Table 1). The 'mixed' Type II kerogens are those to which land plant residues have contributed and in Figs. 4 and 5 they stand out as being more aromatic in composition than Type I and other Type II kerogens of similar H/C value. The PHGC trace of the Toolebuc 'mixed' Type II kerogen (Fig. 10) illustrates this enhanced concentration of aromatic hydrocarbons relative to *n*-alkanes. Also apparent are probable pristane and phytane (pr > ph), and a moderate odd/even predominance in the C_{10}-C_{20} *n*-alkanes.

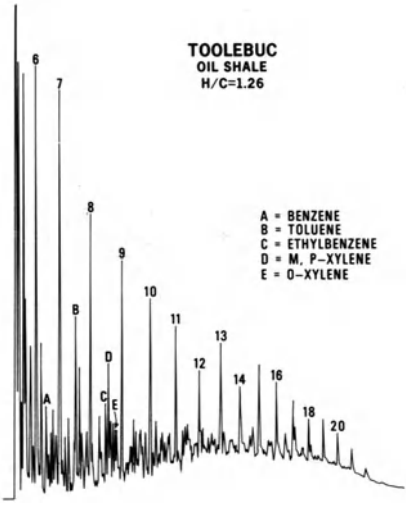

Figure 10: PHGC trace of Type II kerogen from Toolebuc Formation oil shale (Cretaceous).

Pyrolysates of Type II (bituminite, liptodetrinite) kerogens, with decreasing H/C atomic ratio, show a slight decrease in the proportion and chain-length of *n*-alkanes (Fig. 4) but otherwise differ little in aromaticity from Type I kerogens (Figs. 4, 5).

Type III Kerogen

Microbial organic matter of this kind (Table 2) is either a heteropolycondensate of algal humic substances or, as previously discussed, a by-product of the generation of oil from certain lipid-rich kerogens. The former, primary Type III kerogen, has a similar elemental composition to vitrinite (Fig. 1) and is exemplified by the humic organic matter in some

subtidal stromatolites (Fig. 2); whereas the latter is somewhat more depleted in hydrogen (cf. macrinite pathway, Fig. 1). The more aromatic composition of both primary and secondary Type III kerogens (relative to Types I and II) is reflected in their H/C values (0.83-0.54, Table 1) and PHGC parameters (Figs. 4,5). In the pyrolysates of half the samples analysed the C_{10+} *n*-alkanes display an odd carbon-number predominance. The significance of this feature is discussed below.

Type IV Kerogen

The majority of the stromatolite kerogens isolated by McKirdy (1976) have a hyrdogen-poor composition analogous to that of the coal maceral fusinite (Fig. 2, Table 2). The H/C atomic ratios of these and other Type IV kerogens selected for PHGC analysis fall within the range 0.54-0.28 (Table 1). Stromatolitic kerogens with H/C values $\leq$ ca. 0.25 appear to be of higher rank and are here described as subgraphitic (Table 1).

Comparison of the PHGC traces of stromatolitic and non-stromatolitic Type IV kerogens reveals that the former are considerably more aliphatic in character than the latter, although both contain more aromatic hydrocarbons than do pyrograms of Type III kerogens (Figs. 4,5). The aliphaticity of volatile carbon in the stromatolitic kerogens is illustrated by Fig. 11, and is still evident, although much less pronounced, in sub-graphitic samples (Fig. 12).

It should be emphasised that for Type IV kerogens the volatile carbon analysed by PHGC constitutes a very small proportion (ca. 20% or less) of the total organic carbon and, therefore, is far less representative of the carbon skeleton of the kerogen structure than are the pyrolysates of other kerogen types. PHGC of Type IV kerogens provides information on relatively minor structural moeities bonded to the periphery of what presumably is a highly condensed aromatic 'nucleus', itself largely inaccessible to pyrolytic analysis.

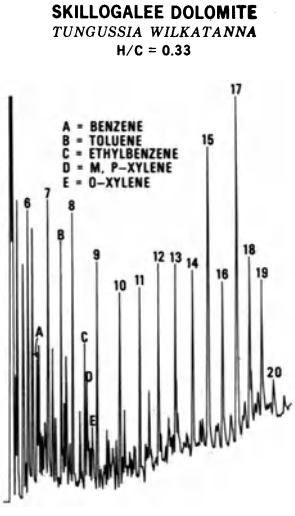

Figure 11: PHGC trace of Type IV kerogen from the stromatolite *Tungussia wilkatanna*, Skillogalee Dolomite (Late Proterozoic).

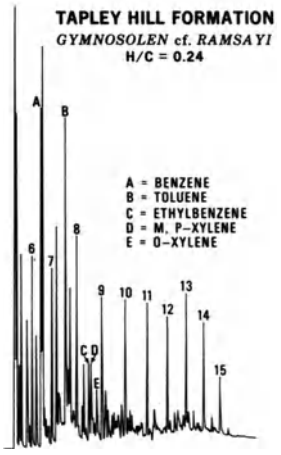

Figure 12: PHGC trace of (?) subgraphitic kerogen from the stromatolite *Gymnosolen cf. ramsayi*, Tapley Hill Formation (Late Proterozoic).

A predominance (which may be quite striking, as in Fig. 11) of odd carbon-numbered homologues in the C_{10}-C_{20} *n*-alkanes of many Type IV and Type III kerogen pyrolysates (Table 1) provides a clue to the origin of the straight-chain aliphatic component of this otherwise highly aromatic organic matter. A similar odd/even predominance has been noted in the *n*-alkane profiles of pre-Devonian oils and their source-rock extracts (McKirdy and Kantsler, 1978). Evidence from degradation studies of algal and bacterial cultures (Lijmbach, 1975; Philp and Calvin, 1976) suggests that, in both cases, this feature may be attributable to the cell-wall lipids of bacteria.

Rank Versus Type

PHGC traces of low to medium-rank kerogens (H/C > 0.80) show that the principal effect of early maturation is a steady decrease in the proportion and chain-length of the C_{6+} *n*-alkanes. The relative abundance in the pyrolysate of simple aromatic hydrocarbons (viz. benzene, toluene, ethylbenzene and the xylenes) increases significantly with decreasing H/C atomic ratio only at values less than 0.80. This may indicate a primary compositional difference between hydrogen-rich (Types I and II) and hydrogen-poor (Types III and IV) kerogens, or a major discontinuity in the structural evolution of microbial kerogen during catagenesis. Notable exceptions to the general pattern of maturation are Type IV stromatolitic kerogens which contain anomalously aliphatic volatile carbon.

Notwithstanding the arguments of Larter *et al.* (1978) against the use of benzene in any pyrolytic index of aromaticity, for the experimental conditions employed in this study, the ratio benzene/*n*-C_6 appears to be just as valid a measure of aromaticity as indicators based on other aromatic species such as toluene and the xylenes.

SUMMARY AND CONCLUSIONS

PHGC is an effective tool for the structural analysis of microbial kerogens which possess 'carbon skeletons' ranging from hydrogen-rich, highly aliphatic to hydrogen-poor, aromatic in character. Clearly, algal and bacterial precursors do not give rise only to aliphatic kerogens.

Oil generation from certain aliphatic (Type I and II) microbial kerogens may involve their rapid disproportionation into liquid hydrocarbons and a residual humic (secondary Type III) kerogen possibly analogous in composition and mode of origin to micrinite in bituminous

coals.

Stromatolitic Type IV kerogens contain small amounts of volatile carbon that is anomalously aliphatic for organic matter so deficient in hydrogen. The aliphatic component of these and other primary humic kerogens (Type III and IV) commonly is 'fingerprinted' by odd/even predominance in the C_{10+} *n*-alkanes and may be a biological marker of bacterial cell-wall lipids.

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LIPID PRODUCTIVITY OF A HIGH ANDEAN LAKE[a]

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INTRODUCTION

Various high altitude lakes are found on the inter-Andean plateau of Antofagasta province, Chile. Laguna Lejia is located in a caldera (23°30'S, 67°42'W) at an altitude of 4180 m. Its surface is approximately 1 x 2 km and the water depth is unknown. The water level appears to be maintained by active springs. A delicate algal mat (ca 0.5-1.0 cm thick, Fig. 1a) is found in the shallow water areas, followed by black anoxic mud (ca 1-5 cm) and below that, coarse volcanic rubble. Isolated flamingoes feed on the algal mats, and insects are common at the shore. The caldera is sparsely vegetated by coiron grass (closely related to *Stipa Venusta Phil.*), (areas on the distant mountain slopes, Fig. 1b). The afternoon westerly breezes generate a white foam on the lake surface, which accumulates on the eastern shore (Fig. 1b). This shore is covered by about 50 cm of dried foam and detritus.

Thus it was of interest to analyze the lipid and macromolecular carbon fractions of this simple, high altitude ecosystem, in order to assess the nature of the foam and the composition of the organic detritus preserved in the sediment.

EXPERIMENTAL

The samples of algal mat and sediment were obtained at the western shore,and the foam and dried foam detritus at the eastern shore. All samples (except the dry foam detritus, it was preserved without isopropanol) were placed into precleaned cans with 100 ml of isopropanol (to stop microbial activity) and sealed.

The samples were lyophilized and extracted repeatedly with aliquots of methylene chloride (CH_2Cl_2). The aliquots were combined and treated with BF_3 in methanol to esterify free fatty acids. The extracts were concentrated and subjected to thin layer chromatography (TLC) on silica gel plates with CH_2Cl_2 as eluant. Bands corresponding to hydrocarbons, esters and ketones were scraped off the TLC plate after visualization with iodine vapor, and eluted with CH_2Cl_2. The hydrocarbon and combined ester and ketone fractions were subjected to gas chromatographic (GC) and gas chromatography-mass spectrometric (GC/MS) analyses.

The GC analyses were carried out on a Hewlett-Packard Model 5830 gas chromatograph using a 30 m x 0.25 mm glass capillary column wall-coated with OV-101, which was programmed from 40-260° at 4° min^{-1} and then held isothermal for 60 min. The flow rate of He carrier gas was 2 ml min^{-1}.

The GC/MS analyses were carried out on a Finnigan Model 4000 quadrupole mass spectrometer interfaced directly with a Finnigan Model 9610 gas chromatograph, equipped with a 30 m x 0.25 mm glass capillary column and wall-coated with OV-101. The MS ion source temperature was 240° and the ionization voltage 70 eV. The GC conditions for the GC/MS analyses were the same as those for the analytical GC system. The mass spectrometric data were acquired and processed using a Finnigan-Incos Model 2300 data system.

Humic substances and protokerogen were isolated by the methods described by Stuermer *et al.* (1978). Elemental analyses were determined by Galbraith Laboratories, Knoxville, Tennessee, and the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley. Stable carbon isotope analyses were carried out by the method of Kaplan *et al.* (1970). The results are given in the "δ" notation versus Chicago PDB standard, where

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[a] Contribution No.1942 from the Institute of Geophysics and Planetary Physics, University of California, Los Angeles.

Figure 1a: Photograph of the algal mat at the Lake Lejia western shore (arrow indicates a spring).

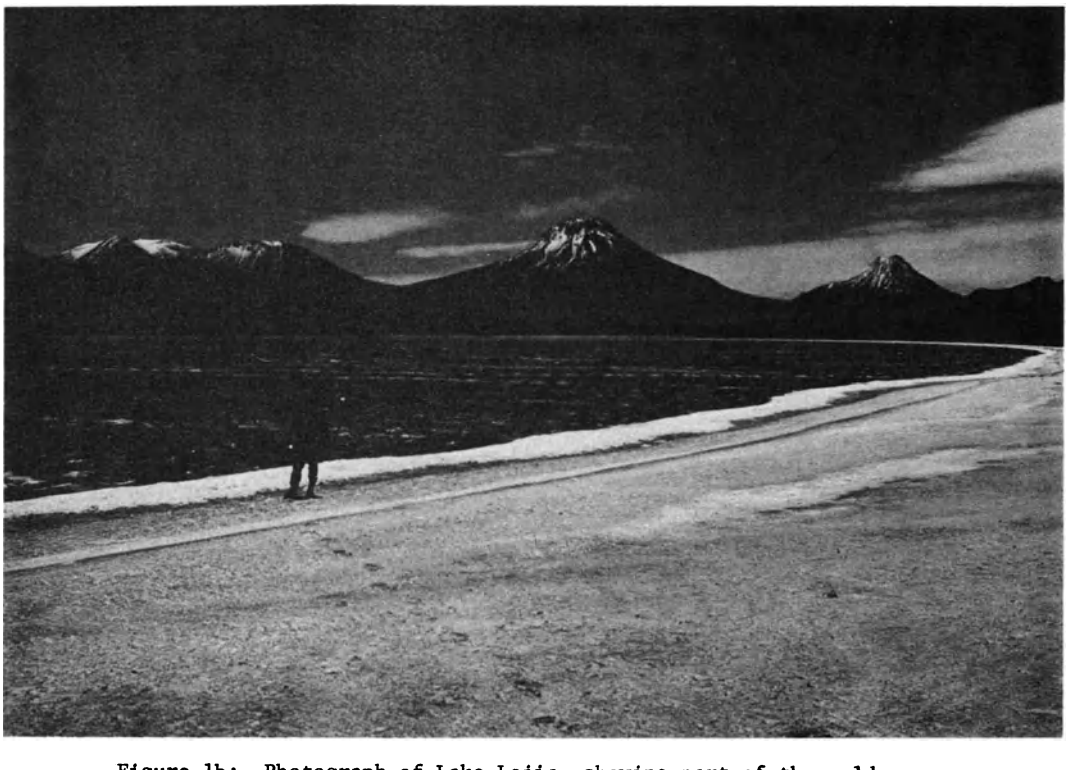

Figure 1b: Photograph of Lake Lejia, showing part of the caldera, the foam on the lake and dry foam on the eastern shore with a rubble and caliche type beach.

$$\delta^{13}C\ (^{\circ}/_{oo}) = \left| \frac{R_{sample} - R_{standard}}{R_{standard}} \right| \times 1000$$

and R is the $^{13}C/^{12}C$ ratio.

RESULTS AND DISCUSSION

The sample descriptions and results of the elemental and lipid analyses are described in Table 1. The organic carbon content for the dry foam and the sediments is significant at about 3%. The highest lipid yield is observed for the algal mat layers, then the dry foam and lowest for the anaerobic sediment. The total nitrogen and sulfur contents are greatest for the algal mat layer and lowest in the sediment below. These data indicate a rapid degradation and diagenesis of the algal detritus below the mat.

Lipids

The distributions of the various homologous compound series in the lipid fractions are given in Fig. 2. The lipids (wax) of coiron grass, which is the predominant vegetation in the caldera and throughout the Altiplano, were also analyzed (Table 1). The distributions of the *n*-alkanes and *n*-fatty acids from that epicuticular wax are shown in Figs. 2d and 2h, respectively. The *n*-alkanes for the dry foam exhibit a bimodal distribution (Fig. 2a), ranging from *n*-C_{15} to *n*-C_{33}, with maxima at *n*-C_{18} and *n*-C_{29} and a strong odd-to-even carbon number predominance >*n*-C_{22}. The homologs >*n*-C_{22} are derived from higher plant wax (Simoneit, 1979a). The source is the grass, since the *n*-alkanes of its epicuticular wax have the same distribution and maximum (cf. Fig. 2d). The dominant hydrocarbon in the dry foam is 8-methylhexadecane (Structure I).[a] Since a mixture (1:1) of 7- and 8-methylheptadecane has been found to be a major lipid component of blue-green algae (Han *et al.*, 1968) and has been characterized in Recent lagoonal sediments (Philp *et al.*, 1976; Cardoso *et al.*, 1976), the presence of 8-methylhexadecane may also be an indicator of algal lipids. The *n*-alkanes of the algal mat (Fig. 2b) and of the sediment below (Fig. 2c) exhibit similar distributions but very different concentrations. The homologs range from C_{14} to C_{33}, with a bimodal distribution and maxima at C_{23} and C_{29}. The homologs from C_{14} to C_{26} exhibit no carbon number predominance and are superimposed on an unresolved complex mixture (hump) of branched and cyclic hydrocarbons which maximizes at the GC retention time of *n*-C_{22}. This distribution is characteristic of lipids from anaerobically degraded algal detritus (eg. Jones, 1969; Johnson and Calder, 1973; Hatcher *et al.*, 1977). The hydrocarbons of the algal mat and surface sediment already show the effects of degradation, indicating that the living algae constitute only a delicate, thin layer. The homologs >*n*C_{26} exhibit a strong odd carbon number predominance, with a maximum at C_{29}, indicating an origin from the epicuticular wax of the grass (cf. Fig. 2d). The transport mechanism for this wax is via aeolian fall out (Simoneit, 1977a,b) since no rivers drain into the lake and the snow melt during the summer is nill. The 8-methylhexadecane is still present in lower amounts (mat > sediments). Pristane (Pr) and phytane (Ph) are found only in the sediments and the Pr/Ph ratio is 0.9 for both samples. This, coupled with the presence of elemental sulfur, indicates anaerobic conditions below the algal mat (Didyk *et al.*, 1978).

The *n*-fatty acids of the dry foam (Fig. 2e) range from C_{10} to C_{28}, with a strong even-to-odd carbon number predominance, and a bimodal distribution with a major maximum at *n*-C_{16} and a minor one at *n*-C_{24}. Mono-unsaturated fatty acids are also present in significant amounts, but poly-unsaturated fatty acids are found as minor components. This distribution is typical of Recent microbial lipids (Simoneit, 1978, 1979a). The homologs >C_{20} may be of an algal and/or higher plant origin. However, their distribution does not match that of the *n*-fatty acids from the grass wax (cf. Fig. 2h), where the maximum is at *n*-C_{28} and not at *n*-C_{24}. The *n*-fatty acids of the algal mat (with lake bed sediment) Fig. 2f) exhibit essentially the same range and distribution as is the case for the dry foam. However, the dominant fatty acids are the monounsaturated analogs, with $C_{16:1}$ as the maximum. This distribution is characteristic of lipids from lysed microbiota (eg. Klenck *et al.*, 1963; Kates, 1964; Jamieson and Reid, 1972). The *n*-fatty acids of the lower sediment (Fig. 2g) range from C_{12} to C_{32}, with a strong even carbon number predominance and a bimodal distribution with a major maximum at *n*-C_{16} and a minor one at *n*-C_{24}. Mono-unsaturated fatty acids could not be

[a] Footnote: Structures of chemical compounds are given in Appendix 1.

TABLE 1

LAKE LEJIA SAMPLES AND ANALYTICAL RESULTS

Sample		Carbon								*n*-alkanes		*n*-fatty acids		
No.	Description	Total (%)	Org. (%)	$CaCo_3$ (%)	Nitrogen Total (%)	Sulfur Total (%)	Phosphorus Total ($\mu g\ g^{-1}$)	Lipids Total ($\mu g\ g^{-1}$)	HC Total ($\mu g\ g^{-1}$)	CPI[a]	max.[b]	Total ($\mu g\ g^{-1}$)	CPI[a]	max.[b]
1	dry foam + salts	1.78	1.56	1.8	0.10	0.25	185	2,060	30	3.5	18,<u>29</u>	56	8.3	<u>16</u>,24
2	sediment (algal mat, top 1 cm)	4.26	3.08	8.0	0.34	0.22	n.d.	5,400	80	1.4	23,29	300	6.9	<u>16:1</u>,16,24
3	sediment (black mud, 2 to 5 cm)	2.33	1.19	8.6	0.05	0.16	n.d.	470	6	1.6	<u>23</u>,29	7	4.0	<u>16</u>,24
4	clump grass (predominant vegetation)	n.d.[c]	n.d.[c]	--	n.d.	n.d.	n.d.	21,000	890	9.2	<u>29</u>,31	360	7.4	16,<u>28</u>
5	lake foam (lyophylized)	2.04	1.95	0.7	<0.2	15.6	294	3,000	n.d.[c]	--	--	n.d.[c]	--	--

[a] CPI = Carbon Preference Index, summed from C_{10} to C_{35}

[b] The dominant homolog is underscored.

[c] n.d. = not determined.

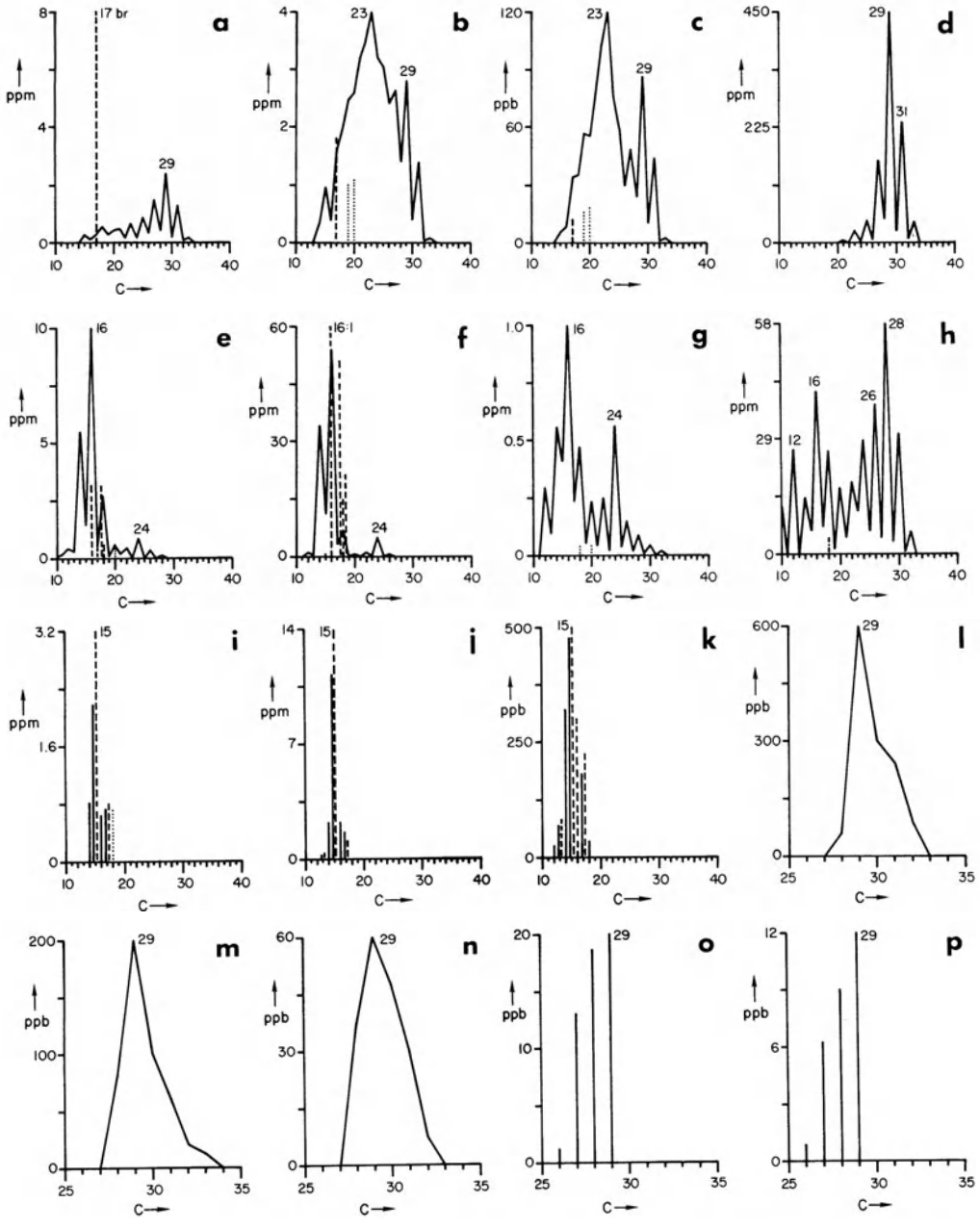

Figure 2: Distribution diagrams of homologous compound series in the lipids.
n-alkanes (...indicates isoprenoids, --- 8-methylhexadecane):
(a) dry foam; (b) mat (upper 1 cm); (c) sediment (2-5 cm subbottom); (d) clump grass;
n-fatty acids (...indicates isoprenoids, --- unsaturated analogs);
(e) dry foam; (f) mat (upper 1 cm); (g) sediment(2-5 cm); (h) clump grass;
iso- and *anteiso*-fatty acids (--- indicates *iso*- and —— *anteiso*-acids; ... isoprenoid ketone):
(i) dry foam; (j) mat (upper 1 cm); (k) sediment (2-5cm);
wax esters (RCO_2R'): (l) dry foam; (m) mat (upper 1 cm); (n) sediment (2-5 cm);
diasterenes: (o) mat (upper 1 cm); (p) sediment (2-5 cm).

detected. This is in agreement with the rapid diagenesis of unsaturated fatty acids to other products (Rhead *et al.*, 1971), indicating that this sediment is a few centuries old. This sediment also contains minor amounts of phytanic acid, pristanic acid and 6,10,14-trimethylpentadecan-2-one (Structure II), all degradation products from phytol (deLeeuw *et al.*, 1977). Phytanic acid, pristanic acid and 6,10,14-trimethylpentadecan-2-one are also present in the dry foam. The distribution of the *n*-fatty acids $<C_{20}$ is typical of a microbial origin and $>C_{20}$ of a probable dual origin from algae and higher plant wax.

Iso- and *anteiso*-fatty acids are present in all the samples (Figs.2 i,j,k) and range approximately from C_{12} to C_{18} with a maximum at C_{15} for both isomer series. These distributions are good indicators for an origin from bacterial lipids (eg. Kates, 1964; Kaneda, 1967). Wax esters (III) are present predominantly in the dry foam and the algal mat and they range from C_{28} to C_{32} only. The composition of the wax esters was determined from the following characteristic ions: molecular ion (R_FCOOR_A), fragment of the acid moiety (R_FCOOH_2) and fragments corresponding to the alcohol moiety (R_A-H) and CO_2R_A (Aasen *et al.*, 1971; Boon and deLeeuw, 1979). The distributions and approximate concentrations of the wax esters are shown in Figs.2 l,m,n. The dominant wax esters are C_{29} (14F-15A, 18F-11A), C_{30} (14F-16A), C_{31} (15F-16A) and C_{28} (14F-14A), with lesser amounts of C_{32} (14F-18A, 16F-16A), C_{29} (13F-16A, 15F-14A), C_{31} (16F-15A, 14F-17A) and C_{28} (13F-15A). A minor amount of mono-unsaturated wax esters is present in the dry foam (C_{24} and C_{28} as 9:1F-15A and 11:1F-15A, respectively). These distributions are unlike those found for diatomaceous ooze from Walvis Bay (Boon and deLeeuw, 1979) and they may be indicators of lipids from microbiota in the water and mat.

Molecular markers are present in these lipids and they can be correlated with their biogenic sources. Sesquiterpenoids are found in the dry foam and as traces in both the mat and underlying sediment. A 5,6,7,8-tetrahydrocadalene (Structure IV) is the major analog and a 1,2,3,4-tetrahydrocadalene (Structure V) and cadalene (Structure VI) are present in lesser amounts. In this case these sesquiterpenoids are indicators of an algal origin (Scheuer, 1973). Traces of diterpenoids are present in the mat and lower sediment. They consist of dehydroabietic acid (Structure VII) and a series of extended tricyclic diterpanes, C_nH_{2n-4} (Structure VIII), with *n* ranging from 20-25. The dehydroabietic acid is probably a marker derived from resinous higher plants (Simoneit, 1977b), whereas the extended diterpanes appear to be of an autochthonous origin from algae. A plant (Yareta) with a resinous odor grows at higher altitudes (>4500 m) and it may be a source of resin detritus. Steroidal compounds are found only as diasterenes (Structure IX) in the lipid hydrocarbons of the mat and lower sediment. They range from C_{25} to C_{29}, with a maximum at C_{29} (Fig.2 o,p) and only one stereoisomer predominates. Other sterenes, steranes and stanones could not be detected. The diasterenes are backbone rearrangement products from stanols or sterenes formed under acid-catalyzed environmental conditions (Rubinstein and Albrecht, 1975; Rubinstein *et al.*, 1975; Dastillung and Albrecht, 1977). They are of an autochthonous algal origin. Most of the steroidal residues are present probably as stanols and sterols in the alcohol fraction, which was not analyzed.

The dry foam and the mat contain small quantities of isoprenoidal γ-lactones which have been described by Brooks (1974). Two γ-lactones have identical mass spectra with a base peak at m/z 85 and prominent ions at m/z 95, 97, 109, 111, 123, 263, 278 (M-32), 295 (M-15) and 310(M), which is consistent with two diastereoisomers of phytenic acid γ-lactone (Structure X, Brooks 1974). A third γ-lactone has an additional major ion at m/z 99 due to a methyl group on the lactone ring. Its mass spectrum fits the fragmentation pattern of another phytenic acid γ-lactone (Structure XI, Brooks, 1974). These γ-lactones can be considered as molecular markers of the microbial degradation of phytol (deLeeuw *et al.*, 1977).

Minor amounts of triterpenoids are present in all samples and consist primarily of diploptene (Structure XII), 17β(H)-bishomohopanoic acid (Structure XIII) and traces of 17β(H)-hopanes ranging from C_{27} to C_{30} (Structure XIV). These are all of a recent microbial origin (eg., Bird *et al.*, 1971; Rohmer, 1974; Brooks *et al.*, 1977; Ourisson *et al.*, 1979), where the diploptene and hopane are primary biogenic markers and the bishomohopanoic acid, norhopane and trisnorhopane are degradation products.

Humic and Kerogenous Substances

The analytical results for the humic and kerogenous substances are given in Table 2. No humic substances could be isolated from the dry foam detritus, but the mat and underlying sediment contain significant amounts of humic material. The H/C ratio of this material (Table 2) is typical of lacustrine algal detritus, which is corroborated by the stable carbon

TABLE 2

ANALYTICAL RESULTS FOR THE HUMIC AND KEROGENOUS SUBSTANCES

Sample No.[b]	Humic Substances			Protokerogen		
	Total ($\mu g\ g^{-1}$)	H/C	$\delta^{13}C$ [a] (per mil)	Total ($\mu g\ g^{-1}$)	H/C	$\delta^{13}C$ [a] (per mil)
1	n.r.[c]	--	--	31,000	1.71	-18.7
2	8,300	1.67	-14.0	19,400	1.79	-15.0
3	670	1.90	-16.3	250	n.d.[d]	-20.4

[a] Versus Chicago PDB standard.
[b] As in Table 1.
[c] Non recoverable
[d] Not determined due to high ash content.

isotope values which range from -14 to -16 per mil (Table 2; Stuermer *et al.*, 1978; Kaplan, 1975; Pearson and Coplen, 1978). The residual carbon fraction after demineralization, the protokerogen, is largest for the dry foam detritus and lowest for the sediment (cf. Table 2). Again, the H/C ratio of this material is typical of lacustrine algal detritus and this is supported by the $\delta^{13}C$ values which range from -15 to -20 per mil (Table 2; Stuermer *et al.*, 1978; Kaplan, 1975; Pearson and Coplen, 1978). These results confirm an autochthonous microbial origin for the bulk of the organic matter. The $\delta^{13}C$ values of both the humates and protokerogen fit the range of microbial detritus (Smith and Epstein, 1971).

Other Biogenic Residues

The dry foam detritus was analyzed for total carbohydrate content by the method of Gerchakov and Hatcher (1972). The total carbohydrate content (ash-free) is 0.9% which corresponds approximately to 25% of the total organic carbon content.

The soluble sugars consist of glucose (22%), mannose (13%), galactose (11.6%), arabinose (10%), xylose (7.4%), rhamnose (6%) and fructose (1.6%), with about 28% unknown analogs. The low amount of glucose indicates essentially no contribution of cellulose, and the presence of mannose and xylose indicates a bacterial component (Swain, 1970).

The foam and dry foam detritus were analyzed for phosphorus content and the results are shown in Table 1. Assuming that the major proportion of the phosphorus is organic, the data indicate that about 25% of the total organic carbon could consist of phospholipids. The atomic C/P ratio for phospholipids is about 17, for algal protoplasm C/P = 41 and for the foam C/P = 178, with C/P = 197 for the dry foam detritus. These results corroborate the dilution of the organic carbon by non-phospholipids. Phospholipids are excellent surfactants and their presence in this foam greatly aids its stability.

Both foam and dry foam were hydrolyzed and analyzed for total amino acid content (protein hydrolysate). The yield for both samples was 26 ng mg^{-1} (average), which corresponds to about 3% of the dry samples and could amount up to 50% of their organic carbon content.

The dry foam detritus was leached with distilled water and the resultant solution had a pH of 8.8. Ion chromatography indicated the presence of sulfate and chloride in a ratio of 6:1 and ammonia was <1 ppm. The large amount of sulfate is derived from the endogenous $CaSO_4$ of the salars and SO_2 from volcanogenic and possibly copper refining sources.

CONCLUSIONS

The ecological environment of Lake Lejia is a simple system, consisting of the autochthonous algal mat and microbiota in the water, with a lesser allochthonous component from the surrounding sparse grass brought in by aeolian transport.

The foam and dry foam detritus consist primarily of lipids, carbohydrate, phospholipid and protein residues, macromolecular organic matter, salts and minerals. The lipids of the mat and underlying sediment are composed mainly of autochthonous components with minor constitu-

ents from grass wax. The dry foam and mat samples both contain microbial wax esters. The sources of the lipids were assessed from their homolog distributions and contents of molecular markers. The carbohydrate, protein and phospholipid residues of the foam material are of an autochthonous microbial origin.

The humic substances and protokerogen of the mat and sediment are also of an autochthonous origin and are of an aliphatic character.

The algal mat and water column of the lake represent the zone of organic matter production and the sediment below the mat represents the degraded residue of that organic matter. The foam is an annual by-product of the high primary productivity, which is not found preserved in the lake sediments. It is hoped that these preliminary data will stimulate further research in both basic and applied problems of such high altitude ecosystems.

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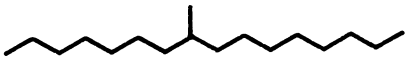
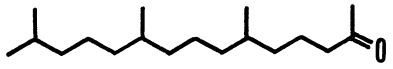
APPENDIX I

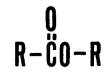
CHEMICAL STRUCTURES CITED



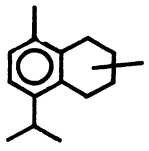
I. 8-Methylhexadecane



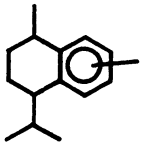
II. 6,10,14-Trimethylpentadecan-2-one



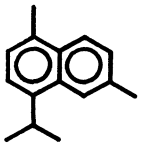
III. Wax esters



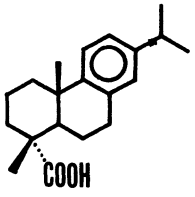
IV. 5,6,7,8-Tetrahydrocadalene



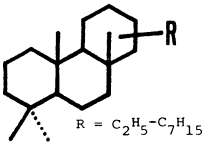
V. 1,2,3,4-Tetrahydrocadalene



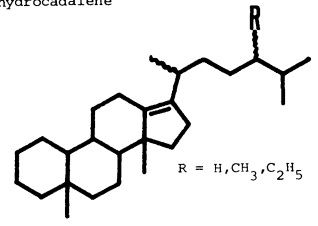
VI. Cadalene



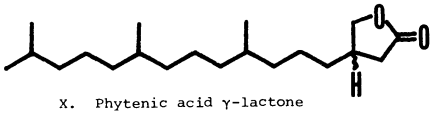
VII. Dehydroabietic acid



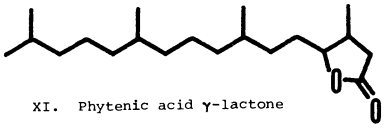
VIII. Extended diterpanes



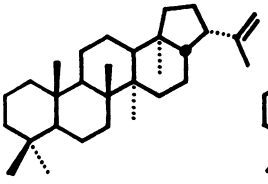
IX. Diasterenes



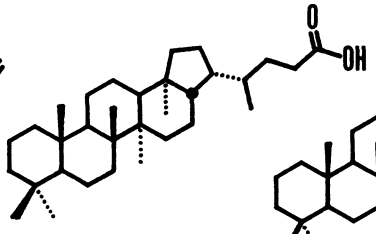
X. Phytenic acid γ-lactone



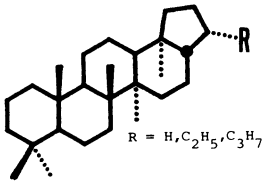
XI. Phytenic acid γ-lactone



XII. Diploptene



XIII. 17β(H)-Bishomohopanoic acid



XIV. Hopanes

THE CONTRIBUTION OF THE CHLOROPHYLL-CAROTENOID PROTEIN COMPLEXES OF BROWN SEAWEEDS TO THE PETROGENIC SILT OF THE OCEANS[a]

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INTRODUCTION

Micropalaeontological studies of carbonaceous cherts have revealed fossilized algae estimated to be 3.1 Gyr old (Schopf, 1970; Schopf and Barghoorn, 1967). R. Buick and J.S.R. Dunlop (1979, personal communication) have described microfossils occurring in a 3.5 Gyr old chert-barite bed. These fossilized organisms may have had affinities with cyanobacteria or sulfur-oxidizing bacteria. Algal fossils of the genus *Collenia* are similarly preserved in Precambrian black cherts of Central Australia. The alkanes, phytane and pristane, are present in these deposits, and C^{12}/C^{13} ratios confirm their photosynthetic origin. Organic molecules likely to survive lithification are those with greatest thermal stability - hydrocarbons and porphyrins. Chlorophyll-derived porphyrins and chlorins are widely found in Precambrian deposits through to the present-day sedimentary off-shore marine muds. The isoprenoid alkanes, phytane and pristane, occur in nearly all Precambrian rocks (McKirdy, 1974). Near-shore deposits have yielded 7-methyl- and 8-methyl-heptadecanes (Sever and Parker, 1970).

Some 30-50% of the world's photosynthesis occurs in the sea. Each year about 60 Pg of photosynthesis biomass is generated in the ocean. Greatest photosynthesis occurs in the shelf zones; here littoral seaweeds generate 16-21 kg C m^{-2} yr^{-1}. Organisms that contribute to this biomass range from the purple anaerobic photosynthetic bacteria to the oxygenic algae. The algae range in size from minute phytoplankton to the giant benthic seaweeds. The decay of the macrophytic vegetation and subsequent diagenesis of the lipids released, contribute to the world's future petroleum. Some of the biological and chemical factors which bear on this ultimate diagenesis are discussed here.

Our research has focussed on the isolation of the photosynthetic pigment-protein complexes of *Acrocarpia paniculata, Ecklonia radiata* and other brown algae. These pigment-proteins are of two classes - photoreaction centres and light-harvesting complexes.

ENVIRONMENTAL FACTORS

Light reaching marine algae has a narrower spectrum, due to selective absorption by water, than the light impinging on terrestrial plants. In the open ocean maximum transmission of the light flux is at 480 nm, but at about 520 nm in turbid coastal waters (Morris, 1974). This attenuation of the spectrum has led to various adaptations of the light-harvesting pigments in different marine photosynthesizers. Most of these organisms absorb strongly in the blue-green region of the spectrum (Fig. 1). Only the photosynthetic bacteria which inhabit shallow water have accessory pigments which absorb well into the red region of the spectrum.

The greatest density of carbon fixation (kg m^{-2} of water surface) - important in the context of petrogenesis - occurs with the benthic algae which cover littoral rock shelves with dense mats of vegetation. In arctic to temperate zones the greatest contributor to the biomass are the brown seaweeds, while in the tropics red seaweeds predominate. Both the brown and the red seaweeds occur in shallow, exposed rock pools down to waters of 80-120 m depth. Their growth extends from the shore-line out to three or so km into the ocean.

The cellular and molecular organisation of the seaweeds reflect their macro-environment.

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[a] *This paper is dedicated to the memory of Professor M. R. Lemberg.*

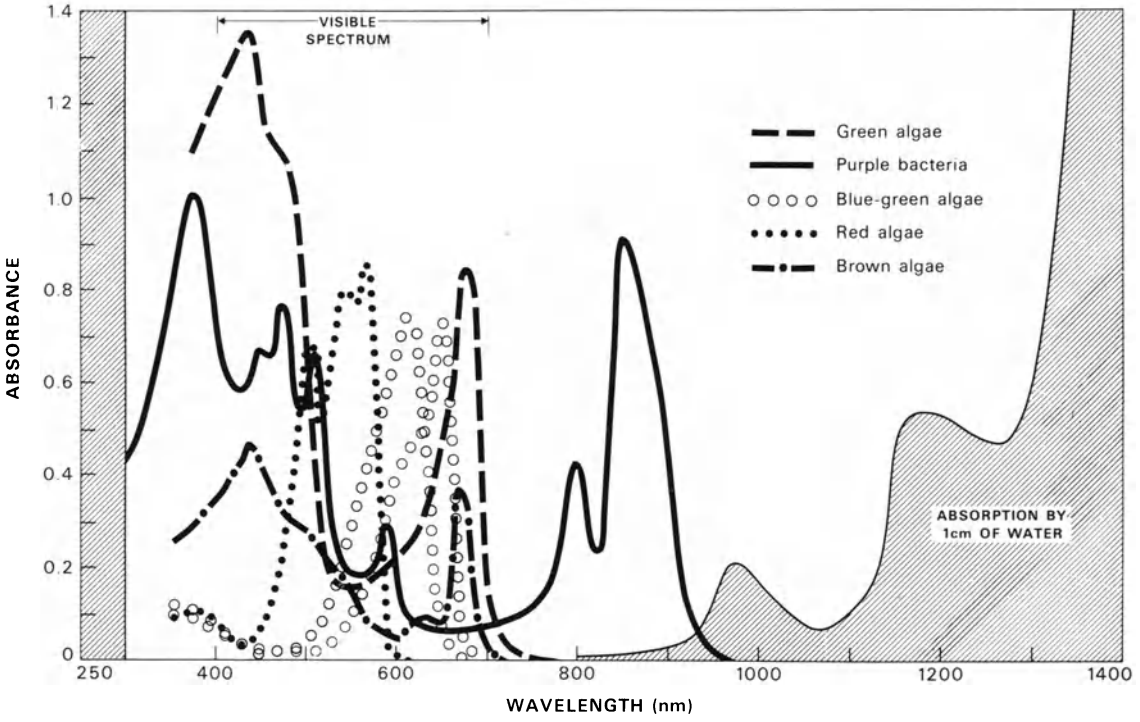


Figure 1: Comparison of the absorption spectra of green and brown algae with that of a purple photosynthetic bacterium. The absorption spectra of isolated phycocyanins, from cyanobacteria, and phycoerythrobilin, from red algae, are also included. The phycobiliproteins were in 0.1 M phosphate buffer (pH 6.8).

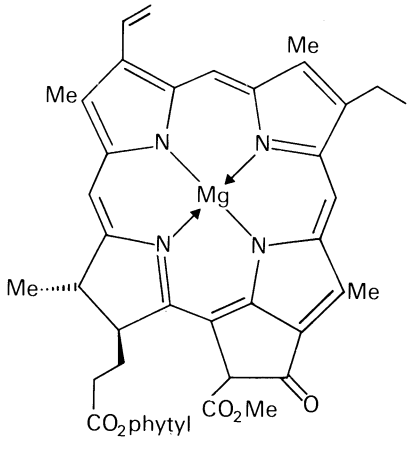
Seaweeds are subject to high osmolarity, intense sunlight having a high ultraviolet content, and undergo vigorous abrasion against rocks and sands. Some of the seaweeds become detatched and float in large mats in the seas; e.g., *Macrocystis* in the Tasman sea, or *Underra* in the northern waters of Japan. The form and structure of the brown-algae (*Phaeophyta*) vary from the tough, leathery fronds of *Ecklonia radiata*, covered with a heavy layer of complex mucopolysaccharides, to the fine fibrillar thalli of *Acrocarpia paniculata* and the planar thalli of *Padina commersonii*.

In contrast to the variation in structure and form of the brown seaweed thalli, there is a uniformity of pigments between the different classes. For light-harvesting, in addition to the ubiquitous chlorophyll *a*, the brown algae have chlorophylls *c* and, as the major carotenoid pigment, fucoxanthin. This allenic xanthophyll when complexed to its apoprotein in a specific conformation undergoes rearrangement of its chemical form so that it absorbs light well into the green region of the spectrum. In certain marine diatoms a similar allenic xanthophyll, peridinin, replaces fucoxanthin. The epoxide xanthophyll, violaxanthin, also contributes to the harvesting of blue light by *Phaeophyta*. In the red seaweeds the capture of blue-green is effected by the open chain tetrapyrrole proteins, phycocyanin and phycoerythrin, especially the latter (Fig. 1).

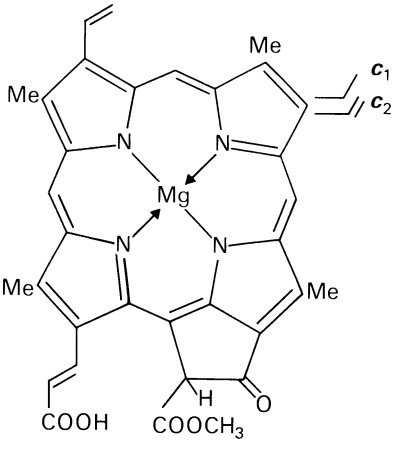
PIGMENT-PROTEIN COMPLEXES OF BROWN SEAWEEDS

Structural formulae of four chromophores of brown algae are given in Fig. 2. Chlorophylls c_1 and c_2 are not true chlorins but are magnesium-phaeoporphyrins, and more importantly in the context of petroleum formation, they have no long alkyl group, but instead a free acrylic acid.

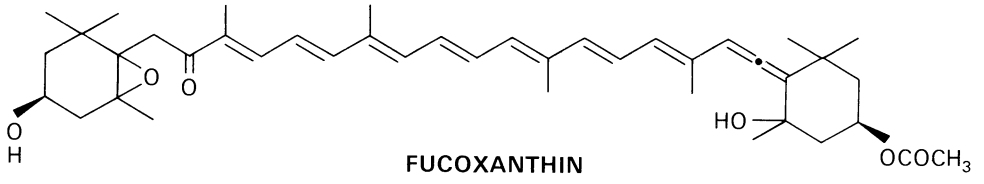
Current evidence encompassing electrophoresis (Anderson *et al.*, 1978; Henriques and Park, 1978; Thornber *et al.*, 1979), high speed density gradient centrifugation (Barrett and Anderson, 1977, 1980), Raman resonance spectroscopy (Lutz, 1977) and absorption and fluorescence spectroscopy (Brown, 1977) established that all chlorophylls and probably most of the carotenoids are bound non-covalently to proteins. These pigment-proteins are then further organized into large macromolecular complexes, often containing several pigment species; the whole being in a highly-ordered array in the inner chloroplast membranes (thylakoids).



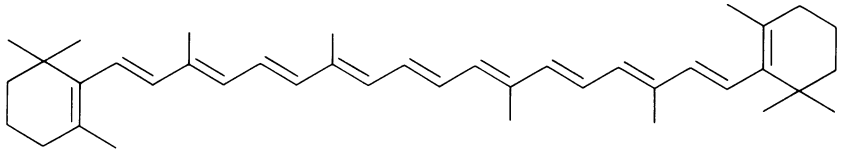
CHLOROPHYLL-*a*



CHLOROPHYLL-c_1 and c_2



FUCOXANTHIN



β-CAROTENE

Figure 2: Structure of chlorophylls *a*, c_1 and c_2, and of two carotenoids present in brown seaweeds.

The chloroplasts of *Acrocarpia paniculata* are located in the outer two layers of cells of the fibrillar thalli: a peripheral layer of mucilage protects these cells (Fig. 3). Each cell contains three to four elongated chloroplasts, and these are surrounded by thick cell-walls (Fig. 4) designed to withstand osmotic pressures (Nordhorn *et al.*, 1976) six times as great as found in spinach cells. The thylakoids have a tightly organized and reinforced structure (Fig. 5). The membranes of cell organelles are highly sophisticated structures. A simplistic schema (Fig. 6) shows a biological membrane having lipids in ordered array, with intrinsic proteins e.g., chlorophyll-proteins embedded in the matrix. In real membranes there would be different types of phospholipids occurring in the lipid domains. Interaction of different molecular species is likely. These membranes require quite strong detergents to disaggregate them, e.g., Triton X-100, sodium deoxycholate, sodium dodecyl-sulfate; certainly they cannot be dispersed by salt solutions.

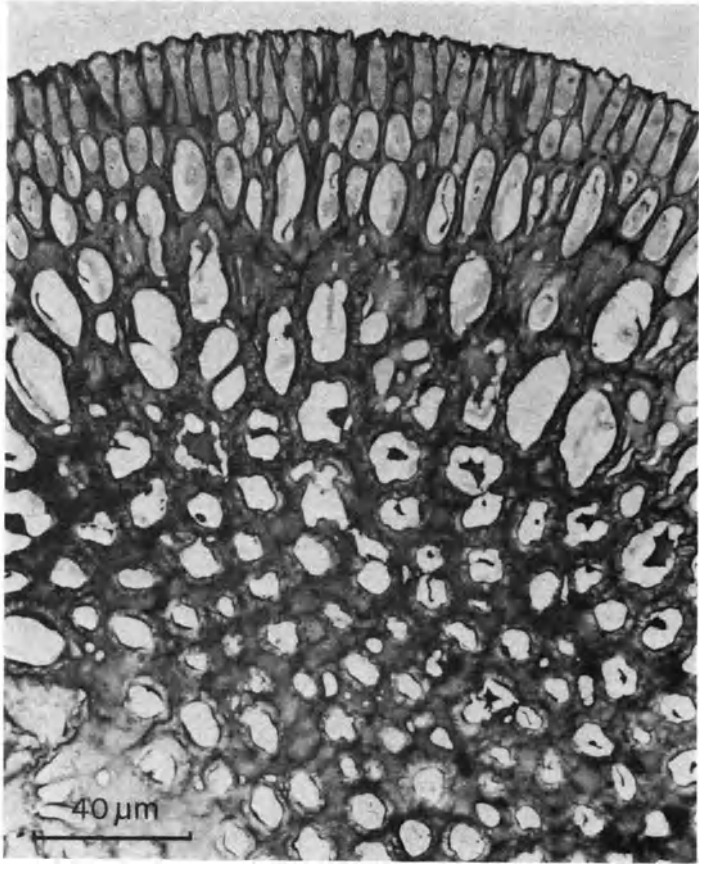


Figure 3: Section of circular thallus of ***Acrocarpia paniculata*** showing centre of thallus to peripheral cells containing chloroplasts. Stain: toluidine blue O. Light microscopy. (Courtesy S. Craig, CSIRO).

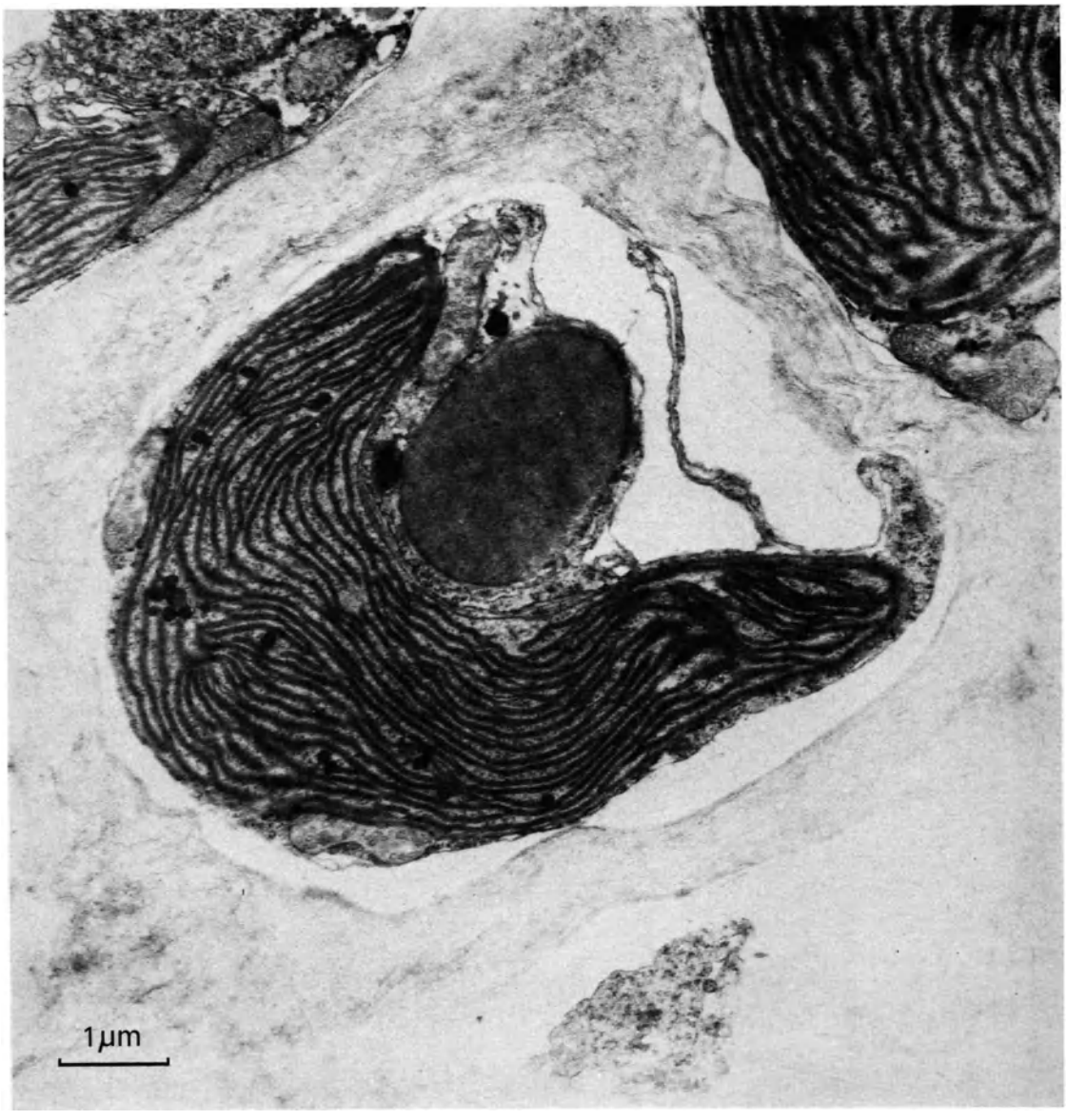


Figure 4: Electron micrograph of chloroplast containing cells of *Colpomenia sinuosa*. Fixative, 3% glutaraldehyde, with osmium tetroxide and lead acetate. (Courtesy, D.J. Goodchild, CSIRO).

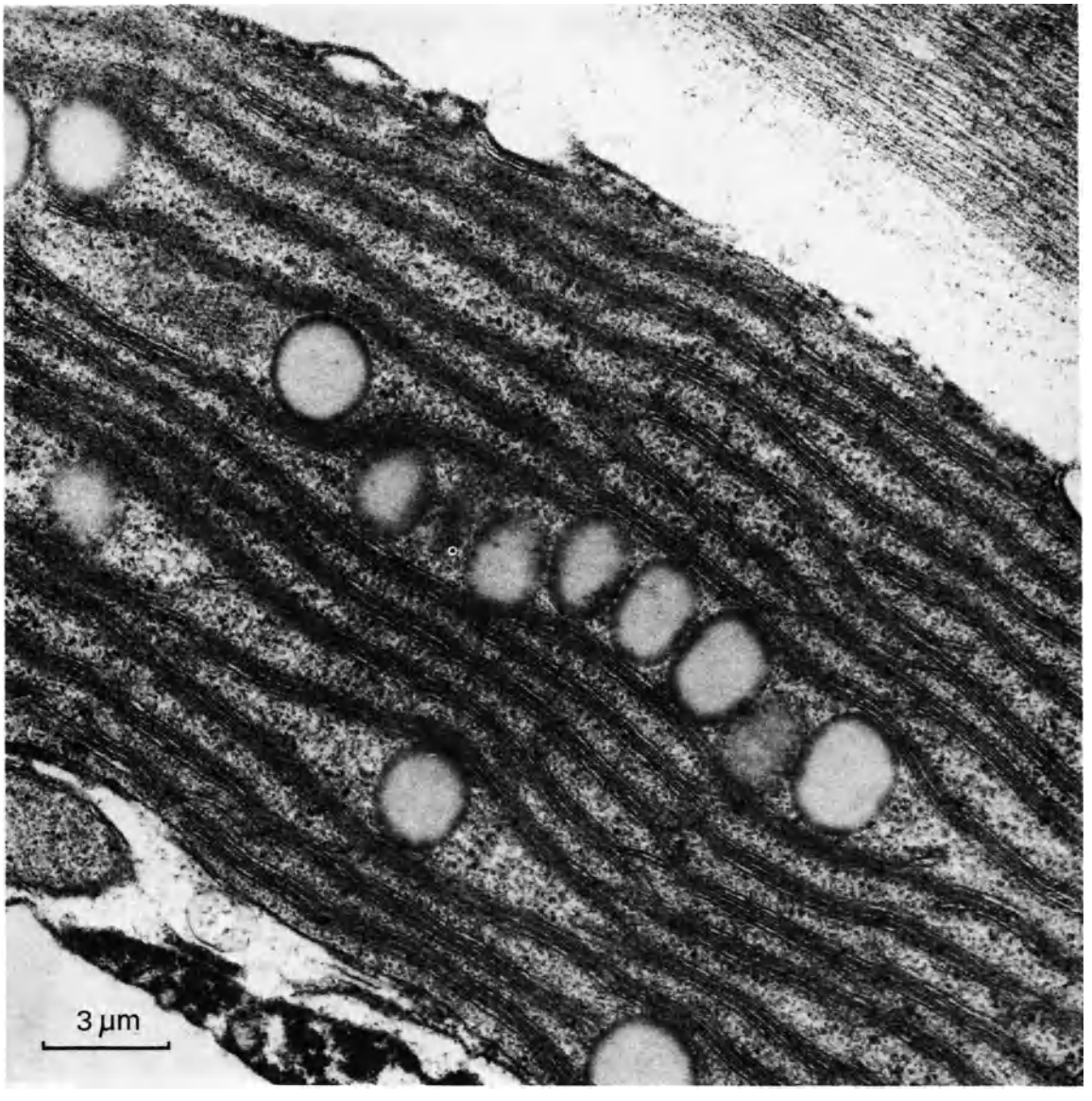


Figure 5: Section of chloroplast of *Sargassum* sp. Fixative 3% glutaraldehyde, with osmium tetroxide and lead acetate. (Courtesy D.J. Goodchild, CSIRO).

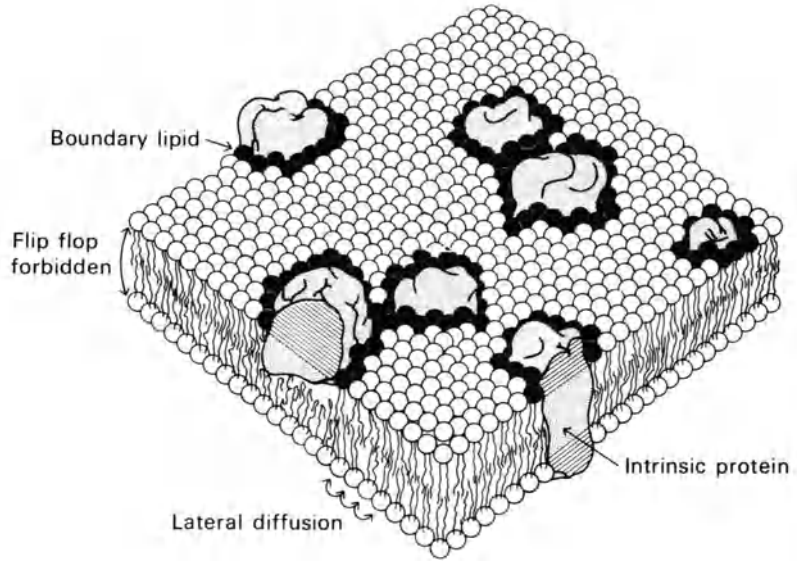


Figure 6: The fluid lipid-protein mosaic membrane model of Singer and Nicolson (1972).

The chlorophyll-carotenoid-proteins in Triton X-100 extracts of *Acrocarpia* and *Ecklonia* thylakoids have been separated into three main pigment-complexes by centrifugation in sucrose density gradients (Fig. 7). The fractions have been characterized by absorption and fluorescence spectroscopy and other analyses (Barrett and Anderson, 1977, 1980). The properties and distribution of the major components and two minor components are given in Table 1. At present we recognise at least seven isolatable pigment-protein entities. The fluorescence excitation (Fig. 8) and fluorescence emission spectra, show that the chlorophyll *a*-c_2-fucoxanthin-protein complex and chlorophyll *a*-c_1+c_2-violaxanthin protein complex both contribute to trapping of the blue-green light for transfer to the antennae chlorophyll *a* of the reaction centres of the two photosystems. The orange complex which contains fucoxanthin as well as chlorophylls *a* and c_2 is undoubtedly the principal light-gatherer in the green region of the spectrum. An approximate idea of their relative size and amounts is depicted in the schema (Fig. 9) which indicates the relative ease of extraction of the pigment complexes. This schema does not necessarily represent the molecular topographical relationship of the pigment complexes.

The manner of binding of chlorophyll *a*, chlorophyll *c* and the three carotenoids in these pigment-protein complexes is not known. Spectral evidence does suggest that the chlorophylls are liganded through the central magnesium to the protein, and in the case of chlorophyll *a* the phytyl chain could interact with hydrophobic amino acids of the polypeptide chain. The structure (Fig. 10) of a water-soluble bacteriochlorophyll-chlorophyll-protein from a green photosynthetic bacterium, established by X-ray analysis by Fenna and Matthews (1976), gives some insight into the complexity of the binding of chlorophyll *a*. The bacteriochlorophylls are in a highly ordered array within a hydrophobic cage formed by the polypeptide skein. In the alkaline seawater (pH 8.5) such a structure is expected to be quite stable. Chlorophylls *c*, which have no long alkyl chain to interact with hydrophobic amino acids,would have to depend on strong liganding by the protein, through perhaps histidine, to the central metal of the chlorophyll. Ionic binding to the polypeptide chain through the acrylic carboxyl is certain to occur. Structures of various cytochromes *b*, all intrinsic membrane proteins (cf. Adman, 1979) provide plausible models for a chlorophyll *c*-peptide.

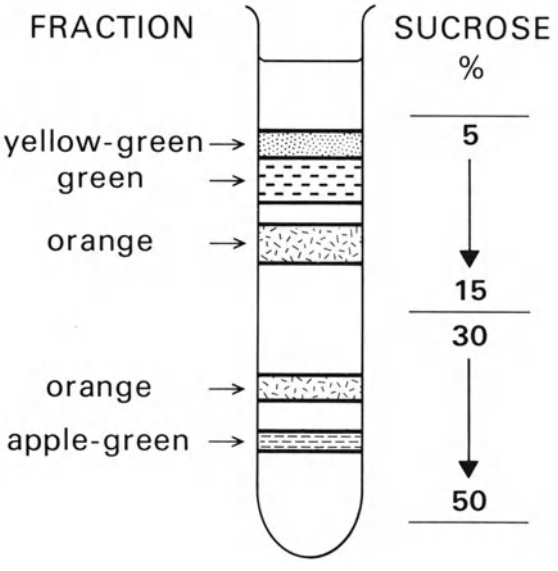


Figure 7: Sucrose density gradient centrifugation in 50 mM Tricine buffer (pH 8.0) of a 1% Triton X-100 extract of *Acrocarpia paniculata* chloroplasts; centrifugation in a Beckman Spinco SW 41 rotor at 270,000 x *g* for 60 h. Components: green fraction, chlorophyll *a* + c_1 + c_2-violaxanthin complex; orange fraction, chlorophyll *a* + c_2-fucoxanthin-protein complex; apple-green fraction, P700-chlorophyll *a*- β-carotene-protein complex.

TABLE 1

PIGMENT-PROTEIN COMPLEXES FROM *ACROCARPIA PANICULATA*

Major ratios of the pigments and absorption maxima of the pigments

Pigment	Complex			
	P700-Chl[a] protein	Heavy orange	Major green	Chl c_2-protein of low molecular weight
P700	1	0	0	0
Chl *a*	38	2	3	0
Chl c_1	0	0	0.5	0
Chl c_2	0	1	0.5	only c_2
Fucoxanthin	0	2	0.05	0
Violoxanthin	0	trace	0.5	0
β-Carotene	4	0	trace	0
Absorption maxima (nm). Tricine (50 mM), pH 8.0.	674 (includes 684, 700) 436, (sh[b]	673, 438 (sh)[b] 418 broad increase over range 470-540.	672,438 (sh) 418 sometimes (sh) at 480	640,583 452
Δ = peaks revealed by difference spectroscopy.		Δ: 639,463 530-540	Δ: 638, 458	Δ: 638, 582, 449-450

[a] Chl = Chlorophyll [b] sh = Shoulder

No information is available regarding the binding of carotenoids to any plant protein. The precise binding of rhodopsin - the visual pigment - to its apoprotein is not fully established. However, the structural elucidation (Ovchinnikov *et al.*, 1979) of bacteriorhodopsin protein implies that the carotenoid is held in a polypeptide cage, with many points of hydrophobic contact through the isoprenoid chain.

Having discussed the organization of the photosynthetic pigments at the cellular and molecular level let us consider how this affects degradation and subsequent diagenesis of the pigment-protein complexes.

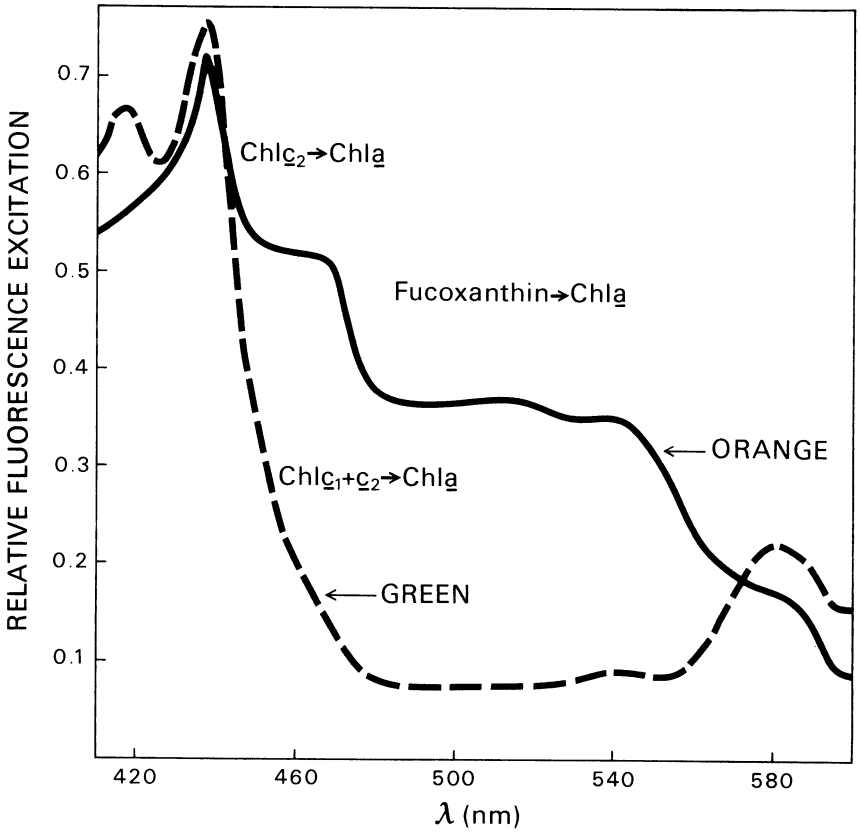


Figure 8: Fluorescence excitation spectra for chlorophyll *a* fluorescence emission at 683 nm of the two major light-harvesting complexes of *Acrocarpia paniculata*. (Courtesy, S.W. Thorne, CSIRO).

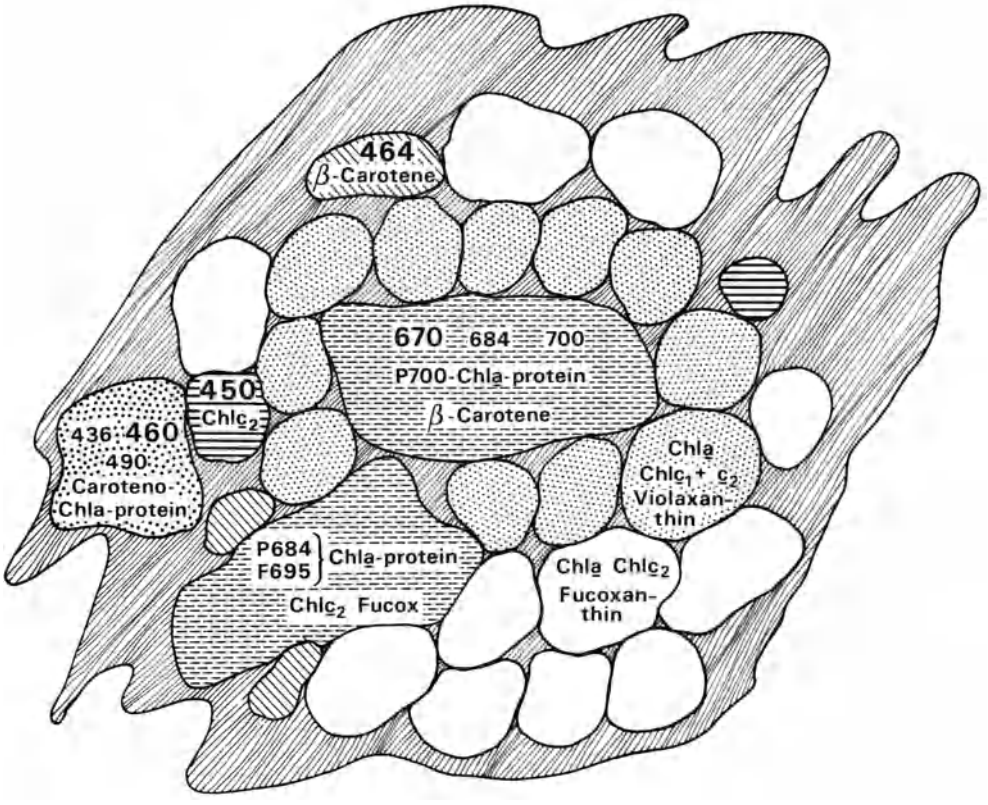


Figure 9: Scheme representing approximately the apparent relative size of the seven pigment-protein complexes isolated from *Acrocarpia*. Size is based on the evidence of sucrose density gradient centrifugation, SDS-polyacrylamide gradient gel electrophoresis and by filtration through Amicon molecular membranes. The position of the complexes depicted in the schema represent the ease and order of extraction using steroidal detergents and Triton X-100. This schema must not be construed to depict the actual molecular topography of the photosynthetic unit(s).

STRUCTURE OF
A BACTERIOCHLOROPHYLL a-PROTEIN COMPLEX

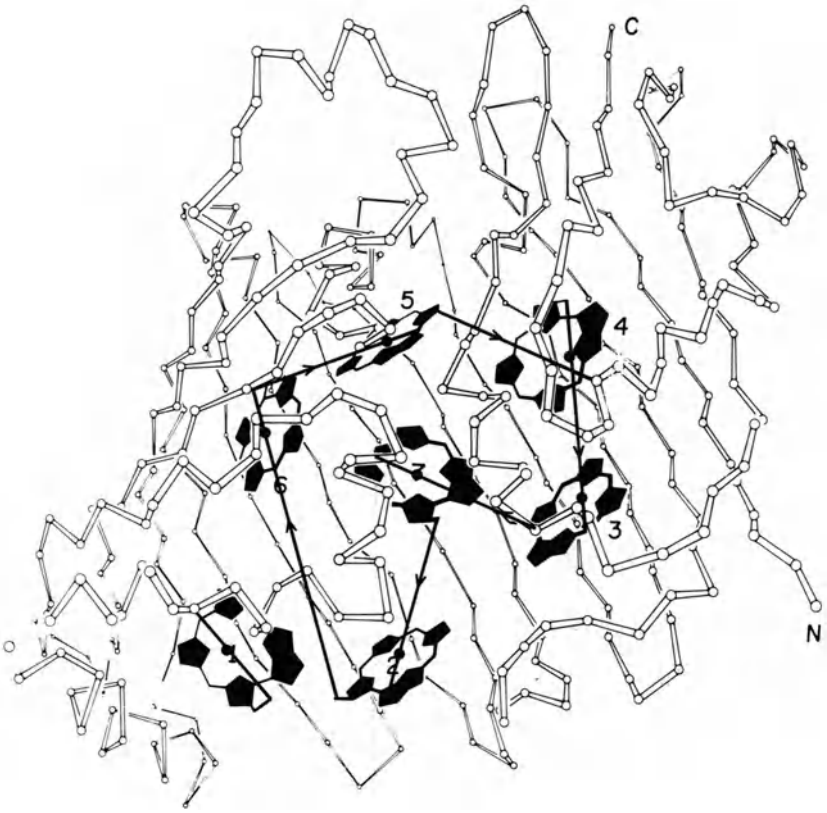


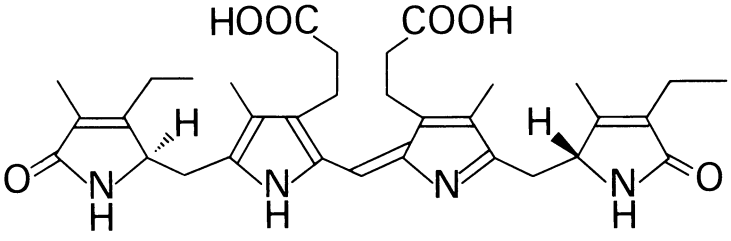
Figure 10: Diagram showing the polypeptide backbone and the arrangement of the seven bacteriochlorophyll molecules in a subunit of the trimeric bacteriochlorophyll-protein complex. The heavy block lines between and across the chlorophylls represent the orientation of the phytols (adapted from Fenna and Matthews, 1976).

DEGRADATION OF THE PIGMENT-PROTEIN COMPLEXES

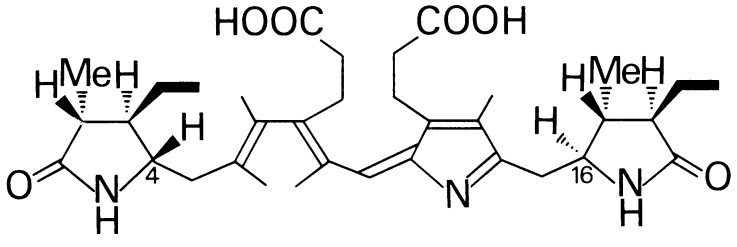
Degradation of the chlorophyll-carotenoid-proteins can occur through either of two pathways. In the context of petrogenesis we may ignore the haemproteins which are quantitatively much less important, and only through the farnesyl alkyl chain of the haem 'a' of cytochrome oxidase contribute to petrogenesis. Any porphyrins derived from chlorophylls, or from haem, will be of the type III β-position isomers. Other β position isomers presumably will have had a non-biological origin. However, as most modern analyses of porphyrins in geological material has relied extensively on mass spectrometry, which does not distinguish between positional isomers, as does proton magnetic resonance, the extent to which accumulated porphyrins in geological strata is due to a non-biological synthesis is not known.

The first degradative path is through the ingestion of abraided particles of thalli by zooplankton or by the grazing of the seaweed fronds by molluscs, crustaceans or fish. Catabolism of the haems is likely to be similar to that observed in animals where opening of the porphyrin ring leads eventually to the water-soluble, near colourless linear

tetrapyrroles, urobilin and stercobilin (Fig. 11). Less is known of the catabolism of chlorophyll in animals. Pigment analyses of faeces of mammals and reptiles show that the macrocycle of chlorophyll can survive ingestion and excretory processes. Phaeophorbides and chlorins may be found. The chlorophyll-derived porphyrin, phylloerythrin, which retains the pentanone ring of chlorophyll but not the phytol, and phylloporphyrin, in which both the phytol and pentanone ring carbons are eliminated, are both found in bile of ox and sheep (Rothemund, 1935; Lemberg and Legge, 1949). Phylloerythrin also occurs in the faeces of herbivores (Archibald *et al.*, 1966) and of man (Brugsch, 1936). The phytol of the ingested chlorophyll is split-off by esterases in the zooplankton, or gut of the herbivore, and may be incorporated into the lipid metabolism of the animal.



(+)-mesourobilin IXα



(−)-mesostercobilin

Figure 11: Formulae of the bilins obtained at the final stages of the catabolism of protohaem to bilins.

Carotenoids derived from seaweed may be precursors of animal lipids; e.g., vitamin *a*, (retinal) is a degradation product of β-carotene, which is ubiquitous in plants. Two allenes, fucozanthinol and paracentrone, occur in the coelemic epithelium of the sea urchin *Paracentrotus lividus*, arising from the metabolism of dietary fucoxanthin (Galasko *et al.*, 1969).

The second path is through destruction of the seaweed thalli by the shearing effect of

turbulent seas and by abrasive action of benthic particles. This fragmentation is followed by phytochemical and microbiological degradation of the thick-walled cells, with ultimate release of the chloroplast. The chlorophylls and carotenoids still associated with their complexing proteins will eventually sink down to the sedimentary silt, probably as macromolecular complexes still embedded in the thylakoid membranes.

Brown algal thylakoids are difficult to disperse. We have found that even after extensive repeated extractions of thylakoids with 1% Triton X-100 at pH 8.0 some membrane structure, in the form of strands of vesicles, is retained (Barrett and D.J. Goodchild, unpublished results). Release of chlorophyll from protein and lysis of the phytyl-ester bond may be mediated by proteases and esterases derived from bacteria in the silt. Some of the phytol could be attached to an imide, derived from ring 4 of chlorophyll, as found for some senescent leaves (Simpson *et al.*, 1976). This phytyl-imide would have greater solubility in seawater than would free phytol.

After dissociation of the chlorophyll from the protein, the magnesium of the macrocycle could easily be replaced by another metal such as Va, Ni, Cu, Fe, or Mn, all of which are abundant in seawater. With either Cu, Fe or Mn chelated to it the chlorin nucleus is unlikely to undergo further gross changes to its macrocycle structure. Many marine sediments are anaerobic and so chemical processes would be reductive. The chlorin nucleus could possibly be dehydrogenated to a phaeoporphyrin by transfer of the two hydrogens of ring 4 to an unsaturated fatty acid or to an alkane. In the absence of molecular oxygen the macrocycle is unlikely to be degraded to a linear tetrapyrrole, with subsequent rupture to give dipyrrolic fragments. Recently phycocyanobilin, the blue chromophore of phycocyanin, has been found in Precambrian sedimentary metamorphic rocks (Kolesnikov and Egorov, 1978). We may expect to find this pigment in the sedimentary mud under waters where red seaweeds occur. Since phycocyanobilin, and its tautomer, phycoerythrobilin, are covalently bound to protein by sulfur either and ester bonds (Fig. 12) they are likely to be present, albeit as colourless compounds in the mud of the sea bottom attached to polypeptide fragments; similarly this would be so for the haems of cytochromes *c* and *f*. Abundant as they are in red seaweeds and phytoplankton the phycobilins do not undergo diagenesis to alkanes to any extent, if at all.

Undoubtedly it is the long hydrocarbon chains of the chlorophylls *a* and *b* and of bacteriochlorophyll, which comprise around 36% of the number of the carbons of these pigments, and the carbons of the polyene skeletons of the major light-harvesting carotenoids, which are the most important progenitors of future liquid fuel. Other than as indicators of the presence of these hydrocarbons, it is doubtful whether the tetrapyrroles are of any importance in this context. For every 100 molecules of chlorophyll *a* in chloroplasts of *Acrocarpia paniculata* there are about 150 molecules of long chain hydrocarbons which can be derived from the phytol and carotenoids. To these sources of alkanes can be added the alkyl moieties of the quinones and phospholipids. The contribution of these last lipids to the hydrocarbon pool may be estimated from the lipid compositions of spinach chloroplasts given by Kirk and Tilney-Bassett (1978). For every 100 chlorophyll molecules (*a* + *b*) there are 34 molecules of quinones and related lipids, 50 molecules of phospholipids and 246 of glycolipids.

These lipids, which may still be associated with membranes - and indeed be integral components of these membranes, or have been dispersed in the form of vesicles - would have a high buoyancy and could not sediment, even slowly, through great depths of seawater which has a high specific gravity. So that it is probable that the chromophores and other lipids would be associated with biological detritus or be adsorbed onto benthic particles. Chlorophylls *a* and *b* have been reported to be present on particulate matter spreading over a broad zone at 150 m in the South Atlantic (Gieskes *et al.*, 1978). It must be borne in mind that sea-bottom sediments can resuspend due to various physical forces (Goldman *et al.*, 1979) and this resuspension may be an important factor in maintaining the benthic nephloid layer of particles which is wide spread throughout the oceans (Baker and Feeley, 1978). Sterols have been found down to depths of 1000 m in the Sargasso Sea; Cholesterol has been found in the entire legnth of a 4,500 m ocean water column (Gagosian, 1976). Vertical fluxes coupled with lateral ocean currents appear to control the movement of the petrogenic organis detritus, some of which is eventually distributed as silt over the continental shelves. The geochemical aspects of the diagenesis of the deposited lipids is extensively discussed by McKirdy (1974).

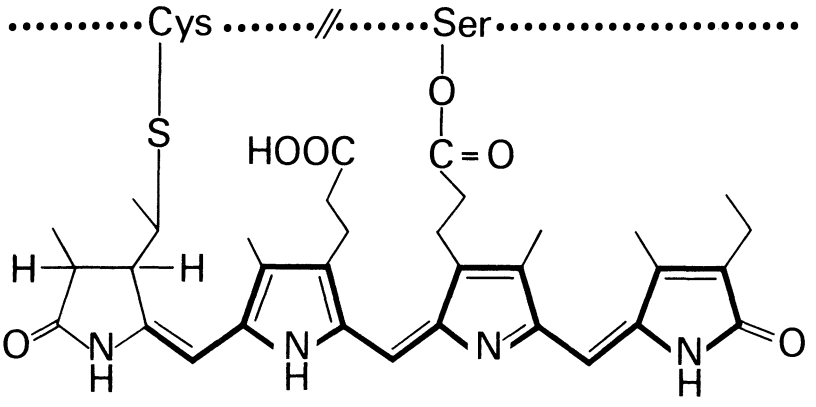


Figure 12: Phycocyanobilin: proposed structure of the chromophore as attached to the polypeptide backbone of phycocyanin, or allophycocyanin (according to O'Carra and O'hEocha, 1976).

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BIOLOGICAL METHANOGENESIS IN SEDIMENTS AND SANITARY LANDFILLS

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INTRODUCTION

Biological methanogensis, carried out by a mixed culture of bacteria, is an exceedingly common and widespread process in nature. Methane is produced in the soil, in peat bogs, in both fresh-water and marine sediments, in sanitary landfills, and in the anaerobic digesters used to dispose of sludge in sewage plants. Yet the process requires a rather specific set of environmental conditions, including the presence of suitable energy-yielding substrates, the usual nutrient elements, a pH near neutrality, a low Eh, and a sufficiently low concentration of inhibitory compounds. Substances found to be inhibitory include organic acids, ammonia, certain heavy metals, sulfide, sulfate and nitrate. Why, then, does the process appear to take place, sooner or later, whenever naturally occurring organic matter decays under anaerobic conditions? The answers appear to be in the realm of microbial ecology. Microorganisms widely distributed in nature alter the environment in such a way that the growth of methanogenic bacteria eventually is assured. Methanogenesis then proceeds until substrate exhaustion.

The biogas produced in anaerobic sewage digesters contains from 50 to 70% methane, the balance being carbon dioxide, with only traces of other constituents such as higher hydrocarbons, hydrogen sulfide, argon and nitrogen. The biogas may be easily purified to yield nearly pure methane, a pipeline quality gas with a heating value of 33-37 MJ m^{-3}, approximately the same as high-grade natural gas. Thus the manufacture of biogas from sewage sludge, animal wastes and plant biomass constitutes a potentially major source of energy for the next century when supplies of natural gas may become exhausted in many countries. This energy source has the advantage of being renewable as long as plants continue to grow on the earth. A study by Gaddy (1977) concluded that the U.S. could meet approximately 25% of its total energy requirements by growing biomass on idle lands and fermenting it to methane, and this could be accomplished without sacrificing our food and forestry needs. Methane is already being produced commercially from several large sanitary landfills in California, and this promises to be a growing source of energy for the future. Methane is also produced in the rumen of cattle and related ruminant animals, and in the heartwood of certain trees. The organisms responsible for the production of methane are members of a remarkable group of bacteria which are closely related biochemically but show wide differences in morphology. Twelve species have been described in the literature.

CHARACTERISTICS OF BIOLOGICAL METHANOGENESIS

Biological methanogenesis is capable of converting almost any organic substrate nearly quantitatively into a mixture of methane and carbon dioxide in accordance with the well known Buswell equation (Buswell, 1954):

$$C_nH_aO_b + (n - \frac{a}{4} - \frac{b}{2})\ H_2O \longrightarrow (\frac{n}{2} - \frac{a}{8} + \frac{b}{4})\ CO_2 + (\frac{n}{2} + \frac{a}{8} - \frac{b}{4})\ CH_4 \qquad 1$$

In the case of glucose Equation 1 becomes:

$$C_6H_{12}O_6 \longrightarrow 3CO_2 + 3CH_4 \qquad 2$$

The simplicity of these equations is deceptive. The conversion of glucose to methane and carbon dioxide requires more than a dozen different reactions, each catalysed by a separate enzyme, and many intermediate compounds may be formed. Ultimately, however, nearly all of them are converted to methane and carbon dioxide.

A few words about terminology are in order. The process of biological methanogenesis is often called the "methane fermentation" by applied microbiologists and bioengineers. Yet one of the major steps in methanogenesis, reduction of carbon dioxide by hydrogen, is not

a fermentation in the correct biochemical sense. Sanitary engineers call the process "anaerobic digestion".

According to Healy and Young (1979), carbohydrates, proteins, lipids, and even many aromatics including ferulic acid, cinnamic acid, 3-phenylpropionate, phenylacetate and benzoic acid, can be digested anaerobically to methane and carbon dioxide. Only hydrocarbons and lignin, out of many hundreds of compounds tested, proved to be refactory to anaerobic digestion (Buswell, 1956; Healy and Young, 1979).

Biological methanogenesis is a mixed culture process in which several groups of anaerobic bacteria act together to convert complex forms of organic matter into methane and carbon dioxide. Early workers, including Omelianski, believed that complex organic compounds such as cellulose could be converted directly to methane by methanogenic bacteria. More recent studies, critically reviewed by Zehnder (1978), have failed to support this idea, and it now appears probable that there are only a few reactions leading directly to the production of methane. The first of these reactions is carried out by all of the pure cultures of methanogenic bacteria isolated to date (Equation 3):

$$CO_2(aq) + 4H_2 \rightleftharpoons CH_4 + H_2O \quad \Delta G^\circ = -139 \text{ kJ (at pH 7)} \qquad 3$$

Barker (1956) postulated that this reaction takes place in four steps, with enzyme-bound forms of formate, formaldehyde and methanol as intermediates. The direct precursor of methane has been identified by McBride and Wolfe (1971) as methyl coenzyme M. It is the reduction of this compound which leads to the production of methane.

The second methanogenic reaction is the splitting of acetate. The mechanism of this reaction has been studied by several investigators using ^{14}C-labelled acetate and deuterium-labelled acetate, and it is clear that the methane comes from the methyl group of acetate and the fourth hydrogen from water. The reaction for *Methanosarcina barkeri* is the following (Equation 4; Zehnder, 1978):

$$CH_3COO^- + H_2O \rightleftharpoons CH_4 + HCO_3^- \quad \Delta G^\circ = -28.2 \text{ kJ (at pH 7)} \qquad 4$$

This reaction does not yield sufficient energy to form ATP from ADP under standard (all reactants 1M, 25°, 101 kPa) or natural (as in sediments, where concentrations of reactants are low) conditions, since the bicarbonate and methane concentrations are relatively high.

Nevertheless, Jeris and McCarty (1965) and Mountfort and Asher (1978) have shown that from 70 to 80% of the methane produced in anaerobic digesters comes from the methyl group of acetate. Smith and Mah (1978) showed that *Methanosarcina* strain 227 grows well on acetate, and suggested that 2 or more mol of acetate may be required to generate 1 mol of ATP. Mountfort (1978), using whole cells of *Methanosarcina barkeri*, showed that ATP was formed by a chemiosmotic mechanism in which a proton gradient across the cell membrane provided the driving force for ATP synthesis.

Since methane is formed only from carbon dioxide and acetate, the activities of other microorganisms must be invoked to supply these metabolites. It now appears clear that anaerobic digestion takes place in at least three stages, each stage mediated by a different group of bacteria. The first step, carried out by both facultative and obligate anaerobes, may be called liquefaction, since it is in this step that complex, largely insoluble, polymeric organic materials such as polysaccharides, proteins and lipids are hydrolyzed and fermented to low molecular weight products. Carbohydrates lead to fatty acids, alcohols, acetone, 2, 3-butanediol, hydrogen and carbon dioxide. Higher fatty acids are broken down to acetate and hydrogen. Proteins are broken down first to amino acids and these in turn to fatty acids, ammonia, carbon dioxide and hydrogen. The second stage involves reduction of protons to hydrogen with the concomitant production of acetate and carbon dioxide. Zehnder (1978) has described the organisms carrying out this stage as obligate proton-reducing acetogens, since they use only protons as an electron acceptor. The first such organism studied is the so-called S-organism, isolated by Bryant *et al.* (1971), from a culture of *Methanobacillus omelianskii*, which was previously believed to be a pure culture. This organism converts ethanol to acetate and hydrogen. More recently McInerney and Bryant (1978) have described an anaerobic bacterium which utilizes butyric, valeric, caproic, heptanoic and octanoic acids by β-oxidation with the production of hydrogen. This organism depends on the methanogens to maintain the low partial pressure of hydrogen required to

make the degradation of these compounds energetically favourable. No growth of this organism could be obtained on any of 63 compounds tested in the absence of the methanogen, and it may be an obligate syntroph. The third stage is methanogenesis, carried out by the two reactions described earlier, the reduction of carbon dioxide by hydrogen and the decarboxylation of acetate to yield methane and bicarbonate. Methane is also produced from formate and methanol by some species, but this process does not contribute appreciable amounts of methane under natural conditions.

ENVIRONMENTAL FACTORS

Methane is formed over the wide temperature range of 0 to 75^{o} but the reaction rate decreases with temperature, and is extremely slow below 10^{o}. No psychrophilic methane formers have been isolated, and only a single thermophilic species, *Methanobacterium thermoautotrophicum*, is known.

The optimum pH range for methane formation by either pure or mixed cultures is very narrow, from pH 6.8 to 8.0. Little or no methane will be produced below pH 6.0 or above pH 8.4.

The methane-producing bacteria are strict anaerobes which require a very low Eh before they can initiate growth. Thus all oxygen must be removed from the system before growth can take place, but oxygen removal alone cannot produce a sufficiently low Eh for growth. In the laboratory, strong reducing agents such as cysteine, sulfide, and titanium III citrate have been employed to cultivate methanogenic bacteria in pure culture (Zehnder and Wuhrmann, 1976). Under natural conditions the other facultative and obligate anaerobes present perform the function of consuming all available oxygen and then producing a strongly-reducing environment in order to permit growth of the methanogens.

Although the methanogens are incapable of growth in the presence of dissolved oxygen, Zehnder (1978) has shown that at least one pure strain, *Methanobacterium* strain A2, is not killed even by high oxygen concentrations. Thus methanogenesis can begin again soon after it is inhibited by oxygen, since facultative anaerobes in the environment are capable of rapidly depleting the dissolved oxygen if the supply rate is not excessive.

GEOCHEMICAL SIGNIFICANCE OF THE METHANE FERMENTATION

Methane occupies a unique position among hydrocarbons from both biochemical and geochemical points of view. It is the only hydrocarbon produced as a major extracellular fermentation product by living organisms. Other hydrocarbons are produced by organisms in relatively small amounts.

Higher hydrocarbons have been detected in biogas from anaerobic digesters and sanitary landfills, but only in trace amounts. Davis and Squires (1954) found ethane, ethylene, propane, propylene, and acetylene in gas produced by the anaerobic digestion of cellulose and glucose by bacteria from an inoculum of cow dung. When the experiment was repeated with ethanol as the substrate, ethane and ethylene were formed, but no propane, propylene or acetylene. Acetate as a substrate yielded ethane and ethylene, and traces of acetylene, but no propane or propylene. Butyrate gave ethane and ethylene but only traces of propylene and acetylene, and no propane. A fermentation of ethanol from a sewage-sludge inoculum yielded ethane, ethylene and acetylene. In every case the fermentation gas samples contained more than 50% of methane. The higher hydrocarbon gases were present in amounts varying from 0.1 to 6.8 $\mu l\ l^{-1}$.

The composition of natural gas is much more variable than that of gas from anaerobic digesters or sanitary landfills. Commercial quality gas contains 70% or more methane, but it also contains much larger amounts of the higher hydrocarbons, ethane, propane, and butane, than gas produced by fermentation. Tiratsoo (1972) lists the chemical composition of a large number of commercial high-energy natural gases. All contain from 3 to 8% ethane, 0.6 to 2% propane, 0.2 to 0.7% butane and 0.03 to 0.4% pentane. No unsaturated hydrocarbons were present. High-energy natural gas is usually found in large amounts associated with liquid petroleum. Other types of natural gas, not necessarily associated with petroleum reservoirs may contain up to 6% helium, and lesser amounts of hydrogen.

Claypool and Kaplan (1974) state that natural gas rich in methane is produced in marine sediments by anaerobic bacterial reduction of carbon dioxide. Their kinetic studies showed

that methane could also be formed at higher temperatures (>50°) by non-biological, thermocatalytic reactions. This type of non-biological reaction probably accounts for the much larger concentrations of higher paraffin hydrocarbons in natural gas than in anaerobic fermentation gas. These authors have studied the variation of methane content with depth of deep sea sediment cores, and the isotopic ratio $^{12}C/^{13}C$ in the methane. The methane is considerably enriched in the lighter isotope, ^{12}C, in agreement with a kinetic model of methane formation by biological reduction of bicarbonate by hydrogen. Claypool and Kaplan's data also support the idea that hydrogen is first used by sulfate-reducing bacteria, and methane formation only takes place at lower levels in the sediment after sulfate has been depleted. Martens and Berner (1974) analyzed cores from Long Island Sound for methane and sulfate, and found that methane was present only in the deeper layers of sediment, 20 to 60 cm below the sediment-water interface where sulfate was no longer present. The authors carried out a laboratory experiment with an organic-rich sample of marine sediment from close to the sediment-water interface. Samples were placed in glass jars, sealed, and incubated for a prolonged time period. At intervals, jars were opened and analyzed for sulfate and methane. The data showed conclusively that methane concentration remained constant, while sulfate disappeared with time. As soon as sulfate was depleted, methane increased regularly with further incubation. This experiment,and the results of Claypool and Kaplan (1974),indicate that sulfate reduction and methane production do not occur simultaneously in marine sediments. Only after sulfate is consumed does methane begin to be formed. The experiment also suggests that methane is not consumed by sulfate-reducing bacteria. On the other hand, Barnes and Goldberg (1976), on the basis of a diffusion model for methane in marine sediments, postulated that sulfate-reducing bacteria release acetate as an end product of metabolism, and that methanogenic bacteria then utilize the acetate, and ferment it to CO_2 and CH_4. They suggested that methane is utilized in turn as an energy source by the sulfate-reducing bacteria, and that sulfate reduction and methane formation may occur together in marine sediments. Davis and Yarbrough (1966) demonstrated, using ^{14}C labelled methane, that sulfate-reducing bacteria can oxidize traces of methane to CO_2 when growing in a rich nutrient medium containing lactate. Whether this reaction goes on at appreciable rates in marine sediments is open to question. In the absence of further information, the experiment of Martens and Berner (1974) renders this hypothesis doubtful. The results of Cappenberg and associates on lake sediments summarized by Cappenberg and Jongejan (1978), favour the interpretation of Martens and Berner. They showed, by bacterial counts, that sulfate-reducing bacteria occurred at a depth of 0-2 cm and the methanogens at a depth of 4-6 cm in the mud. Lactate was identified as the main energy source for sulfate reduction, and acetate for methane formation. A commensalism was identified between the sulfate-reducing bacteria and the methanogens. The sulfate reducers produced acetate from lactate, and the methanogens then consumed the acetate. Several workers have demonstrated that sulfate-reducing bacteria and methanogens often grow together in sewage sludge digesters, yet they do not seem to do so in sediments. This may be due to the much higher substrate levels in sewage sludge. Winfrey and Zeikus (1977), working with sediments from Lake Mendota, Wisconsin, found that methanogenesis was inhibited by the addition of as little as 19 μg SO_4^{2-} ml^{-1}. The inhibition was reversed by the addition of either hydrogen or acetate. This indicates that competition for available hydrogen and acetate between sulfate-reducing bacteria and the methanogens is responsible for the inhibitory effect of sulfate. In this connection it is important to recall the work of Widdel and Pfennig (1977) who isolated *Desulfotomaculum acetoxidans*, a new species of sulfate-reducing bacteria which oxidized acetate to CO_2 and H_2O. Other species of sulfate-reducing bacteria produce acetate from lactate, but *Desulfotomaculum acetoxidans* then competes with the methanogens for this substrate.

Belyaev and Laurinavichus (1978) developed an elegant means of studying the relative importance of carbon dioxide and acetate as precursors of methane formation by methanogens in marine sediments. Split samples of sediment cores were taken, and $NaH^{14}CO_3$ was added to one, $^{14}CH_3COONa$ to another,while a third was analyzed for methanogenic bacteria growing on hydrogen and carbon dioxide, and a fourth was analyzed for methanogens growing on acetate. A good correlation was found between the number of hydrogen-utilizing methanogens present in a sample, and the rate of methanogenesis from $^{14}CO_2$ in the presence of hydrogen. Most of the samples from the Gulf of California, the Arabian Sea, and the Persian and Oman Gulfs produced methane at a higher rate from $^{14}CO_2$ than from acetate, but a few samples from the continental slope of the Hindoustan Peninsula produced more methane from the methyl group of acetate than from CO_2.

Claypool and Kaplan (1974) have calculated that large amounts of methane may be present in

the cold depths of the sea in the form of crystalline hydrates. Such hydrates could be stable under a 3 km water column to depths of 600 m in the sediment provided that sufficient methane is present. Other very large deposits of gas are found in deep geopressured zones. Jones (1976) described the Cenozoic delta deposits of the Northern Gulf of Mexico basin. In this environment, at depths of 3,000 to 7,000 m in the sediment, temperatures of 100 to 200°, and pressures of 60-150 MPa, methane is generated from sedimentary organic matter, mostly kerogen, by thermal catagenesis. The author believes that most of the interstitial water in this vast sedimentary basin is saturated with methane. Further exploration is required to confirm this view but, if it is true, the amount of methane in this one basin is calculated to exceed 3.25×10^{10} m^3, more than the entire U.S. potential gas supply, estimated by the Potential Gas Committee in 1972. Studies are proceeding on how best to tap this enormous future energy reserve.

METHANE FROM SANITARY LANDFILLS

A sanitary landfill constitutes a convenient model for studying the sequence of events leading to the formation of methane in an organic-rich natural environment. The sequence of events was reconstructed by Updegraff (1979) along the following lines. The municipal waste which goes into a sanitary landfill contains ferrous and non-ferrous metals, glass, wood, paper, garbage and many other minor constituents. Paper, which is mostly cellulose, contributes approximately 50% by weight. When this highly porous mass is compacted and covered with soil, it contains a large volume of entrained air. Thus, the early history of a landfill is aerobic, and no methane is formed. The oxygen in the landfill will be consumed by aerobic and facultative microorganisms in a manner similar to aerobic composting systems. This process produces a great deal of heat from microbiological oxidations, so that the temperature within the landfill may rise from the average ambient temperature to from 35 to 60°. Once the oxygen is exhausted, anaerobic bacterial metabolism can begin and, in the presence of sufficient water and nutrients, the low Eh required for methanogenesis will soon be attained. Then the methane concentration of the landfill gas will begin to rise, and may eventually reach a level characteristic of anaerobic digester gas, i.e. from 50 to 65% methane. Table 1 shows the variation in composition of landfill gas over a period of three years after burial. Note that even after only three months some methane was present, but the major gas was carbon dioxide. Nitrogen was highest at this time, and declined with time. Methane concentration steadily increased from 5% after 3 months to 51% after 3 yr. The sum of nitrogen, carbon dioxide and methane accounted for 98 to 100% of the gas mixture.

TABLE 1

GAS COMPOSITION OF A TYPICAL LANDFILL

Time Interval	Volume %		
Months	N_2	CO_2	CH_4
0-3	5.2	88	5
3-6	3.8	76	21
6-12	0.4	65	29
12-18	1.1	52	40
18-24	0.4	53	47
24-30	0.2	52	48
30-36	1.3	46	51

Table 2 from Lott (1979) presents a detailed analysis of gas collected from within a landfill in Denver, Colorado, and of gas collected from the soil near the landfill which has migrated from the landfill to this site. Note the presence of ethane, ethylene, propane and propylene at levels of 5 to 50 $\mu l\ l^{-1}$ agreeing reasonably well with the analysis by Davis and Squires (1954) of fermentation gas produced from cellulose.

Large landfills produce a great deal of gas. The first commercial venture to tap this

energy source was established at the Palos Verdes landfill in California in 1975. According to Rice (1978) gas sales to a commercial company have increased then to a figure of 16×10^3 m^3 day^{-1} in 1978. The success of this venture on a renewable alternative energy source has led to the inauguration of several such projects at other landfills, and will probably provide an increasingly significant contribution of energy in the future.

TABLE 2

LANDFILL GAS COMPOSITION AT EAST 52nd AND DAHLIA [a]

Off Site, $\mu l\ l^{-1}$			On Site, $\mu l\ l^{-1}$		
Methane	61,306	(6.1%)	Methane	491,055	(49.1%)
Carbon dioxide	45,000	(4.5%)	Carbon dioxide	495,000	(49.5%)
Ethane	4.2		Ethane	26.4	
Ethylene	13.2		Ethylene	50.0	
Propylene	0.6		Propylene	24.4	
Propane	0.5		Propane	4.6	

[a] Adapted from Lott (1979).

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ROLE OF METHANE-PRODUCING AND SULFATE-REDUCING BACTERIA IN THE DESTRUCTION OF ORGANIC MATTER

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MICROBIOLOGICAL DESTRUCTION OF ORGANIC MATTER

The initial stages in the decomposition of organic matter in upper horizons of the lithosphere include aerobic processes carried out by representatives of various classes of microorganisms. Aerobic decomposition may be schematically described by Equation 1:

$$C_6H_{12}O_6 + 6O_2 \xrightarrow[\text{microorganisms}]{\text{aerobic}} 6CO_2 + 6H_2O \qquad 1$$

Only carbon dioxide and water are shown as products of this reaction, although in nature such a reaction results in the formation of microbial biomass as well as the production of a great variety of intermediate organic compounds.

Saprophytic bacteria play a leading role in aerobic mineralization of organic matter in contemporary sediments and their numbers generally correlate with the content of easily assimilated organic compounds (Kuznetsov, 1970). Numbers of saprophytic bacteria in the upper horizons of sediments in the Arabian Sea are shown in Table 1. The numbers of bacteria in sediments decrease when passing from shelf (stations 1960, 1961) to pelagic sediments (station 1954) - from a million cells to hundreds of bacterial cells per g of wet sediment. The most important consequence of the aerobic destruction of organic matter is the consumption of molecular oxygen, which finally leads to the replacement of an oxidative environment by a reductive one and the development of anaerobic decomposition.

TABLE 1

NUMBERS OF SAPROPHYTIC BACTERIA IN SEDIMENTS OF THE ARABIAN SEA

Station number	Location	Depth of sea (m)	Horizon (cm)	Eh (mv)	Number of bacteria (cells g^{-1})
1960	23°25'N 67°15'O	520	0-10	0	350000
1961	23°05'N 67°00'O	1040	0-5	+15	1080000
1959	21°26'N 67°02'O	1890	2-10	+135	120000
1958	19°01'N 67°02'O	3240	0-3	+500	2300
1956	14°02'N 67°04'O	4000	1-10	+550	770
1954	8°55'N 66°58'O	4524	0-10	+550	120

Anaerobic decomposition of organic matter is usually divided into two steps (Toerien and Hattingh, 1969). In the first step a heterogeneous group of anaerobic bacteria, the so-called "primary" anaerobes, convert proteins, lipids and polysaccharides to low molecular weight fatty acids, alcohols, aldehydes, ketones, CO_2, H_2 and H_2O (Fig. 1). In the second step these metabolites are used as substrates for "secondary" anaerobes; methane-producing and sulfate-reducing bacteria. Thus, in anaerobic natural ecosystems formation and consumption of low-molecular organic compounds takes place continuously although intermediates may accumulate. As shown in Table 2 lower fatty acids (C_1 to C_6) are permanently present in sediments of fresh-water and marine reservoirs. The sum of C_2-C_6 acids in fresh-water lakes in summer exceeded 50 mg kg^{-1} of wet silt. In winter the concentrations of these acids decreased which correlated with a depletion in numbers of microorganisms and with a lowering of the rates of anaerobic microbiological processes during this period (Ivanov, 1979; Belyaev *et al.*, 1979).

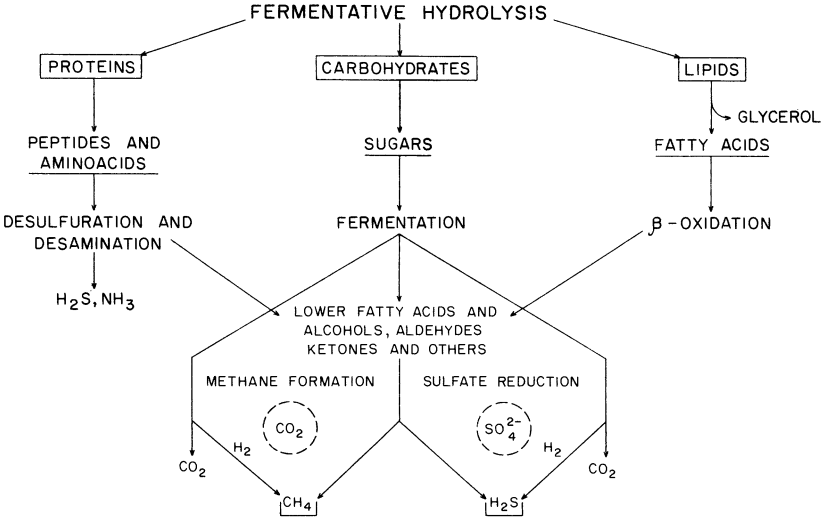

Figure 1: Scheme of anaerobic destruction of organic matter.

TABLE 2

CONTENT OF LOWER FATTY ACIDS (mg kg^{-1} WET SEDIMENT) IN FRESH-WATER AND MARINE SEDIMENTS

Acids	Fresh-water lakes of the middle zone of the USSR		The Gulf of California and Arabian Sea
	summer	winter	
formic	+	+	+
acetic	25	12.6	14
propionic	9	2.3	1.8
n-butyric	10	0.9	+
n-valeric	8	1	+
n-caproic	3.2	1.4	+

METHANOGENESIS AND SULFATE REDUCTION

The main purpose of the present report is to discuss our experimental data on the geochemical role of methane-producing and sulfate-reducing bacteria, whose activities are involved in destruction of organic matter, and accumulation of methane and reduced sulfur compounds in anaerobic ecosystems.

It has been established that sulfate reduction and methane production occur actively in a wide range of environments including sewage (Smith, 1966), soils (Barker, 1936; Omelyanski, 1953; Koyama, 1964), sediments of fresh-water (Kuznetsov *et al.*, 1962; Ivanov, 1964; Chebotarev, 1974; Cappenberg, 1975; Zeikus, 1977; Belyaev, 1979) and marine (Ivanov, 1978; Lein, 1978; Belyaev, Laurinavichus, 1978) reservoirs, and underground waters of oil-gas and sulfur deposits (Ivanov, 1964; Rosanova and Kuznetsov, 1974; Belyaev *et al.*, 1977). In the present work we shall only consider investigations concerning fresh-water and marine reservoirs.

Sulfate-reducing and methane-producing bacteria are wide-spread in reduced sediments of fresh-water lakes of the USSR (Table 3). Their numbers vary from hundreds to millions of cells per g of sediment, reaching maximum values in upper horizons.

The rates of sulfate reduction and methane formation were determined by radioisotopic methods. The rates of sulfate reduction (mg H_2S kg^{-1} wet sediment day^{-1}) in sediments of the lakes listed in Table 3 were similar: from 3 to 10.2 in upper horizons and 1.4 to 1.9 in deep zones. The rates of methane production varied within the range of 0.032 to 5.7 mg CH_4 per kg^{-1} sediment day^{-1}). Experiments with radiolabelled bicarbonate and acetate showed that various combinations of pathways of bacterial methanogenesis take place in the sediments of these lakes. For example, in Lake Kuznechikha more than 88% of methane was formed by reduction of CO_2 by hydrogen. However, in Lake Black Kichier and Lake Great Kichier only 46% and 33% of methane, respectively, was derived from CO_2.

TABLE 3

NUMBERS OF BACTERIA AND INTENSITY OF H_2S AND CH_4 FORMATION IN REDUCED SEDIMENTS OF FRESH-WATER LAKES

Horizon (cm)	Number of bacteria (cells g^{-1})		Rate of formation mg kg^{-1} day^{-1}		
	Sulfate reducers	Methane producers	H_2S	CH_4	At the expense of CO_2 reduction (%)
			Lake Kuznechikha		
0-5	20 000	60 000	3.6	5.4	97
5-10	20 000	25 000	3.0	5.7	96
10-15	10 000	60 000	1.9	4.1	88
15-20	3 000	6 000	1.4	3.5	98
			Lake Black Kichier		
0-10	1 600 000	6 000	10.2	0.38	46
10-20	2 000	500	1.4	-	-
			Lake Great Kichier		
0-10	50 000	500	4.7	0.032	33
10-20	500	100	1.6	-	

The remaining methane was produced from methyl groups of acetate. In the sediments of marine basins, such as the Gulf of California, and the Arabian and Caspian Seas (Table 4), sulfate-reducing and methane-producing bacteria were also wide-spread, although their numbers were notably lower than in sediments of fresh-water reservoirs (Table 3). The rates of H_2S formation, and especially of methane formation,were also lower than in sediments of fresh-water lakes. Maximum rates of sulfate reduction were a few orders of magnitude greater than the rates of methane formation in upper horizons of sediments from the Caspian and Arabian Seas, e.g. station 1960, located on the continental slope of the Hindustan Peninsula in the region of the Ind River estuary. Except for station 1960, methane production in marine sediments occurred mainly through CO_2 reduction.

Methane and reduced sulfur compounds were formed throughout the sediment columns. In contemporary and quarternary sediments of the Caspian Sea these processes were detected at depths of up to 90-110 m.

CONSUMPTION OF ORGANIC CARBON DURING SULFATE REDUCTION AND METHANE PRODUCTION

Microbiological H_2S and CH_4 formation is coupled with energy released during anaerobic oxidation of low-molecular organic compounds, or hydrogen formed during fermentation of organic matter. As seen from equation 2, the formation of one molecule of H_2S requires oxidation of two carbon atoms, i.e. 24 g of organic carbon (C_{org}) are oxidized and 32 g of reduced sulfur are formed.

$$\frac{2C_{org}}{24g} + SO_4^{2-} \xrightarrow[\text{bacteria}]{\text{sulfate-reducing}} \frac{S^{2-}}{32g} + 2CO_2 \qquad 2$$

$$\frac{4HCOOH}{48\ g\ C} \longrightarrow 4H_2 + 4CO_2 \xrightarrow[\text{bacteria}]{\text{methanogenic}} \frac{CH_4}{12\ g\ C} + 2H_2O + 3CO_2 \qquad 3$$

$$\frac{*CH_3COOH}{24g\ C} \xrightarrow[\text{bacteria}]{\text{methanogenic}} \frac{*CH_4}{12g\ C} + CO_2 \qquad 4$$

$$\frac{C_6H_{12}O_6}{72\ g\ C} + 2H_2O \longrightarrow 2CH_3COOH + 4H_2 + 2CO_2 \xrightarrow[\text{bacteria}]{\text{methanogenic}}$$

$$\longrightarrow \frac{3CH_4}{36g\ C} + 3CO_2 + 2H_2O \qquad 5$$

The bacterial reduction of one molecule of CO_2 to methane requires the oxidation of 4 molecules of H_2. Since both CO_2 and H_2 are derived from organic molecules (e.g. formate) this implies that 4 molecules of C_{org} are consumed by CO_2 reduction for the formation of 1 molecule of CH_4 (equation 3). Methanogenesis from acetate (equation 4) or glucose (equation 5) consumes 24 g of C_{org} per gmol of CH_4. These relationships can be used to calculate the amount of C_{org} utilized during sulfate reduction and methanogenesis in sediments.

Table 5 shows that from 0.5 to 3.7 g of H_2S and from 12mg to 2.07 g of CH_4 are formed annually in the sediments of the fresh-water lakes. The production of H_2S requires 0.4 to 2.8g of C_{org} annually (equation 2). The calculated consumption of C_{org} for CH_4 formation in sediments of the Kuznechikha Lake is one order of magnitude greater than that for the H_2S formation. In the other two lakes it is of 2-3 orders of magnitude lower.

Sulfate reduction rates in marine sediments (Table 6) are 2-3 orders of magnitude lower of those in continental reservoirs (Table 6). However, since sediment accumulation rates in marine basins are also 1-3 orders of magnitude lower than in lakes (Ivanov, 1979), the concentrations of reduced sulfur compounds in fresh-water and marine sediments do not differ significantly (Chebotarev, 1974).

Methane formation rates in marine sediments (Table 6) are 3-6 orders of magnitude lower compared to those in lake sediments. Calculated C_{org} consumption by marine sulfate reduction and methane formation was ≤ 65 mg C_{org} kg^{-1} sediment yr^{-1} (Table 6). In all ecological systems studied, except that of Lake Kuznechikha, the major part of anaerobic organic carbon oxidation was linked to sulfate reduction. It should be noted that,in the sediments under investigation ,sulfate reduction and methane formation were not spatially separated

TABLE 4

NUMBERS OF BACTERIA AND INTENSITY OF H_2S AND CH_4 FORMATION IN REDUCED MARINE SEDIMENTS

Station number and location	Horizon (m)	Number of bacteria (cells g^{-1})		Rate of formation ($\mu g\ kg^{-1}\ day^{-1}$)		
		sulfate reducers	methane producers	H_2S	CH_4	At the expense of CO_2 reduction (%)
			Gulf of California			
663	0.5-0.6	5	250	4.1	0.0019	86
27°09'N 111°08'W	1.6-1.7	13	60	0.8	0.0018	96
665	0.4-0.5	1000	150	112.7	0.0036	91
25°20'N 109°55'W	0.9-1.0	100	60	12.5	0.0029	98
			Arabian Sea			
1959	0-0.1	600	25	3.3	0.0076	89
21°26'N 67°02'W	0.5-0.6	120	25	7.0	0.0069	80
1960	0-0.1	6000	1000	162.4	0.0470	63
23°25'N 67°15'W	0.4-0.6	600	500	237.9	0.0930	4
	1.9-2.0	25	50	53.7	0.0160	21
			Caspian Sea			
562	0-0.5	25	10	232.5	0.0097	78
39°54'N 53°00'W	2.0-2.5	10	10	78.4	0.0390	96
	11-11.5	10	10	133.7	0.0033	76
	56-57	n.d.[a]	n.d.[a]	7.3	0.0005	80
	89-90	n.d.[a]	n.d.[a]	4.8	0.0005	80

[a] no growth detected with inoculum of 5 g silt.

TABLE 5

C_{org} CONSUMPTION DURING H_2S AND CH_4 FORMATION IN SEDIMENTS OF FRESH-WATER LAKES

Horizon (cm)	Intensity of formation ($g\ kg^{-1}\ yr^{-1}$)		C_{org} consumption in formation ($g\ kg^{-1}\ yr^{-1}$)		
	H_2S	CH_4 from CO_2/CH_3 (acetate)		H_2S	CH_4
			Lake Kuznechikha		
0-5	1.31	1.91	0.06	0.98	7.76
5-10	1.09	1.99	0.08	0.82	8.12
10-15	0.69	1.32	0.18	0.52	5.60
15-20	0.51	1.25	0.02	0.38	5.04
			Lake Black Kichier		
0-10	3.72	0.06	0.07	2.79	0.38
			Lake Great Kichier		
0-10	1.71	0.004	0.008	1.28	0.032

TABLE 6

C_{org} CONSUMPTION DURING H_2S AND CH_4 FORMATION IN MARINE SEDIMENTS

Station number	Horizon (m)	Intensity of formation ($mg\ kg^{-1}\ yr^{-1}$)		C_{org} consumption in formation ($mg\ kg^{-1}\ yr^{-1}$)	
		H_2S	CH_4	H_2S	CH_4
			Gulf of California		
663	0.5-0.6	1.5	0.0007	1.1	0.004
	1.6-1.7	0.3	0.0007	0.2	0.003
665	0.4-0.5	41.1	0.0013	30.8	0.005
	0.9-1.0	4.6	0.0011	3.4	0.004
			Arabian Sea		
1959	0-0.1	1.2	0.0028	0.9	0.011
	0.5-0.6	2.5	0.0025	1.9	0.009
1960	0-0.1	59.3	0.0172	44.5	0.056
	0.4-0.6	86.8	0.0339	65.1	0.071
	1.9-2.0	19.6	0.0058	14.7	0.014
			Caspian Sea		
562	0-05	84.8	0.0035	63.6	0.013
	2-2.5	28.6	0.0142	21.4	0.056
	11-11.5	48.8	0.0012	36.6	0.004
	56-57	2.6	0.0002	2.0	0.001
	89-90	1.7	0.0002	1.3	0.001

as reported by Claypool and Kaplan (1974). It is reasonable to assume that in natural ecosystems enriched in sulfate, the major part of energy released during anaerobic destruction of organic matter supports sulfate reduction, a reaction which is energetically more advantageous than methane formation (McCarty, 1972).

During the anaerobic decomposition of organic matter, and in particular during sulfate reduction, a considerable amount of CO_2 if formed. The isotopic composition ($\delta^{13}C$) of organic carbon from inland reservoirs varies within the range of -18 to 25 per mil (Galimov, 1973). The isotope composition of newly-formed mineral carbon in lake sediments should not, therefore, be heavier than -18 per mil. However, in some natural ecosystems (Table 7), where CH_4 formation is relatively intense and takes place largely at the expense of CO_2 reduction (e.g. in Lake Kuznechikha) the remaining CO_2 becomes heavier $\delta^{13}C$; -15 per mil. By contrast, in marine sediments where methanogenesis by CO_2 reduction is of minor importance in organic matter decomposition, the carbon of CO_2 was found to be lighter than the carbon of chemogenic carbonate or the carbonate of planktonic shells and benthic marine organisms (Lein, 1978). For instance, the disseminated diagenetic calcite from terrigenous sediments of the Californian Gulf and continental slope had $\delta^{13}C$ values of -17.6 to -23.0 per mil. On the other hand, the carbon isotope composition of carbonates from the Indian Ocean is characterized by $\delta^{13}C$ values from -4.5 to -12 per mil. Light CO_2 can be generated from organic matter during bacterial sulfate reduction (Kaplan and Rittenberg, 1964). As shown in Fig. 2, as the intensity of bacterial sulfate reduction in sediments increses, carbon of newly-formed CO_2 and HCO_3^- becomes increasingly enriched in ^{12}C.

In summary, information on rates of anaerobic processes such as sulfate reduction and methanogenesis can be used in the assessment of carbon turnover, and in the interpretation of carbon isotopic patterns, in anoxic sediments.

TABLE 7

ISOTOPE COMPOSITION OF METHANE AND CARBON DIOXIDE ($\delta^{13}C$, PER MIL) OF FRESH-WATER LAKES

Place of sample collection	$\delta^{13}C$		$\alpha(\frac{CO_2}{CH_4})$
	CO_2	CH_4	
Silt of Lake Kuznechikha	-15.5	-64.9	1.053
Silt of Lake Black Kichier	-18.1	-61.3	1.046
Silt of Lake Great Kichier	-18.9	-59.2	1.043

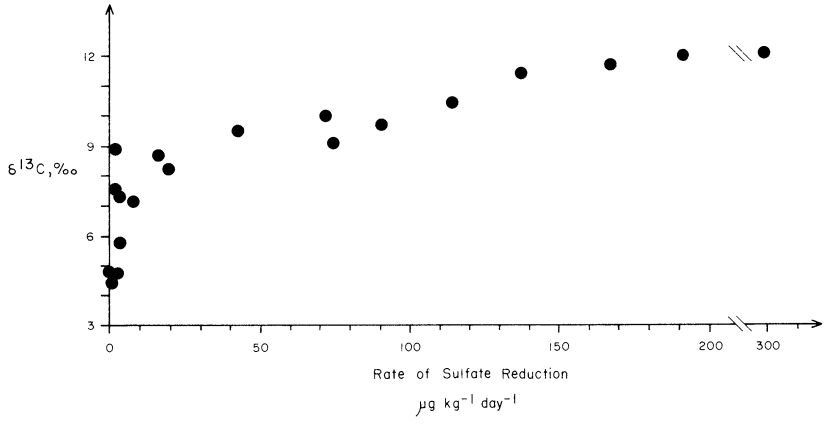

Figure 2: Intensity of bacterial sulfate reduction and carbon isotopic composition of carbonate in sediments of the Indian Ocean.

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METHANE IN A SALINE ANTARCTIC LAKE

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INTRODUCTION

The Lake

Ace Lake (Fig. 1) is a permanently stratified lake, 23 m in depth (Fig. 2), protected from wind driven mixing for at least 10 months of the year by ice-cover and supporting thick mats of benthic cyanobacteria, planktonic communities of algae, and a copepod crustacean. Evidence indicates that the lake had a marine origin and has undergone a complex evolution involving evaporation, replenishment with local freshwater and the loss of 76% of the original seawater sulfur content by sulfate reduction (Burton and Barker, 1979).

Figure 1: Ace Lake, free of surface ice on 25 January 1975. A permanent snow drift remains over summer in the north-eastern corner, the direction of the prevailing wind. Icebergs are visible out to sea.

Organic Matter Decomposition

Organic matter (proteins, carbohydrates and lipids) during its course of decomposition in anaerobic environments is progressively converted into increasingly smaller molecules, with the eventual formation of CO_2 and CH_4. However, an appreciable concentration of sulfate, acting as a preferred terminal electron acceptor, usually prevents CH_4 production

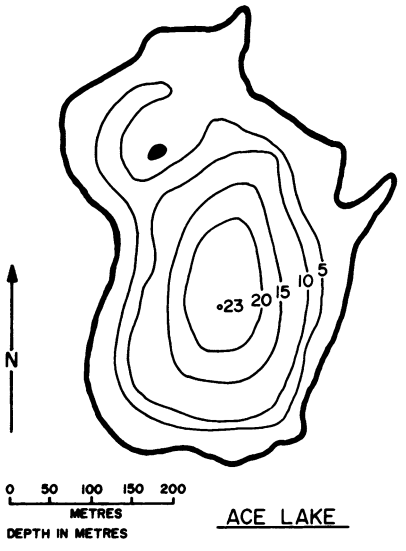

Figure 2: Bathymetric map of Ace Lake. Drawn from 22 soundings through ice. A small island is located in the northern bay.

even when methanogenic bacteria are present (Mah *et al.*, 1977). Bryant *et al.* (1977) reported that, in a mixed culture of *Desulfovibrio sp.* and strain MOH (a methanogenic bacterium), CH_4 production was stoichiometrically inhibited as sulfate was added. Even the addition of only 0.2 mM sulfate to sediments from Lake Mendota inhibited methanogenesis (Winfrey and Zeikus, 1977). The reason for this inhibition (e.g. inhibition of methanogenic bacteria by sulfide, competition for common substrates between rival bacteria and pathways) is still uncertain. However, the oxidation of H_2 when coupled to sulfate reduction makes more energy available to bacteria than when coupled to CO_2 reduction or the conversion of scetate to CH_4 and CO_2 (Mah *et al.*, 1977). Bacteria able to utilize sulfate, therefore, appear to have an adaptive advantage enabling them to be the dominant decomposers in anaerobic environments. Thus, in organic-rich anaerobic environments which have sulfate-free and sulfate-rich zones, CH_4 production could be expected to occur in the former but not in the latter zone. This is observed in marine sediments where the sediment acts as an obstacle to mixing and allows the two zones to co-exist in close proximity (Claypool and Kaplan, 1974).

Previous studies (Burton and Barker, 1979) have demonstrated that the bottom waters of Ace Lake are sulfate-free and the sulfur and oxygen isotopic patterns indicate that the lake is well stratified (unmixed) largely due to a salinity gradient. Hence, in Ace Lake the decomposition of organic matter could result in the production of H_2S in the upper part of the anoxic zone where sulfate is present, and in the production of CH_4 below 20 m where sulfate is absent.

This paper deals with changes in the concentrations of dissolved gases in Ace Lake with depth, and relates these to other variables including total organic carbon and nitrogen, numbers of bacterial cells, light, nutrients, conductivity and temperature. Equivalent data from Clear Lake, a nearby meromictic lake, are also presented.

METHODS

Samples were obtained through 2 m of surface ice using a 2 l transparent plastic kammerer

water sampler which passed easily through a 12.5 cm hole cut by a Sipre ice corer. Temperatures were taken from a mercury-in-glass thermometer clamped inside the sampler. Water samples were rapidly transferred to 250 ml gas-tight, heavy polyethylene reagent jars and placed in an insulated box to prevent freezing during the 2 h trip to the laboratory. However, when samples from depths greater than 15 m were brought to the surface, some gas usually bubbled out of solution and was lost. A 100 ml sample was taken from each depth and immediately fixed in formalin; aliquots were transferred to a haemocytometer and bacteria counted at a magnification of 500 X under phase contrast.

Samples for analysis of total organic carbon were preserved with mercuric chloride in the field and returned to Australia, There they were acidified with hydrochloric acid to pH2 and purged with N_2 to remove inorganic carbon. After this treatment, 5 ml of sample was sealed in a glass ampoule together with 0.25 ml of 6% phosphoric acid and 0.2 g of potassium persulfate and autoclaved for 24 h at 108^{o} to oxidise all organic matter to CO_2: CO_2 was measured using the Oceanographic International Corporation 0524B Total Carbon System comprising ampoule breaker, infra-red analyser unit and digital electronic integrator. Standards for comparison were prepared using sucrose as the source of organic carbon.

Total organic nitrogen was determined in Australia on deep-frozen water samples using a modification of the Kjeldahl method given in American Public Health Association (1971). An Orion ammonium electrode was used for the final ammonia measurement in place of a colorimetric or titration estimation.

CH_4, N_2 and H_2 were determined on a Beckman GCM universal instrument gas chromatograph using two 1220 mm x 3.2 mm 5A molecular sieve columns. He was used both as a carrier gas and as a stripping gas for CH_4 and N_2 determinations: N_2 was used similarly for H_2 determinations. The procedure was based on that developed by Rudd and Flett as described in Stainton *et al.* (1977). A small and reproducible trace of O_2 was present in all gas samples injected into the chromatograph and came from the boiled distilled water displaced by the stripped gas during sample treatment. These O_2 levels were used as a baseline to check for air leaks into samples.

Calculated values for maximum solubility of CH_4 were derived from the formula of Atkinson and Richards (1967) for water of 40 per mil salinity which is very close to the observed salinity at 22 m in Ace Lake. Equilibrium values for dissolved N_2 solubility in water of lake temperature and salinity in contact with the atmosphere were taken from Smith (1974).

O_2 was determined by the Winkler method as given in Major *et al.* (1972) and H_2S was determined according to Golterman and Clymo (1969). Sulfate was measured gravimetrically as barium sulfate using the picric acid modification of Vogel (1972).

CO_2 was determined by titration with hydrochloric acid; pH values were plotted to calculate the total alkalinity end point. At depths below 10 m where sulfide was a component of the total alkalinity, the contribution of sulfide was subtracted from the measured titration alkalinity to derive total alkalinity of the inorganic carbon system. Total CO_2 values were derived from these total alkalinity figures following Golterman and Clymo (1969), by making adjustments for pH and conductivity.

A Metrohm conductivity bridge with a cell constant of 0.745 cm^{-1} was used to measure conductivity at 25^{o}. The concentrations of reactive PO_4-P and ammonia plus amino acids were measured colorimetrically by the methods of Strickland and Parsons (1972). Light penetration was measured with a Kahl Submarine photometer by comparing transmitted light with incident light as determined by a deck cell.

RESULTS AND DISCUSSION

Meromixis

In meromictic lakes there is usually a narrow zone, called a chemocline, where salinity changes rapidly and which marks the boundary between the upper and lower waters (Bayly and Williams, 1973). Although Ace Lake is meromictic, i.e. perenially stratified, there is a relatively steady increase in conductivity with depth (Fig. 3) and there is no evidence for a permanent chemocline based on salinity. Thus the terms mixolimnion and

monimolimnion, which are usually associated with a conventional chemocline, may be misleading.

Ace Lake has an abrupt transition at about 9 m from oxygenated water above to anoxic conditions below (Table 1). I therefore use the words oxylimnion and anoxylimnion for the upper and lower waters in Ace Lake. The transition zone of oxygen depletion from 7 m to 9 m, I term the oxycline. However, Clear Lake (56 m maximum depth) follows the more usual pattern of meromictic lakes and a slight chemocline does exist between 28 and 30 m coincident with the change from oxic to anoxic conditions (Table 2).

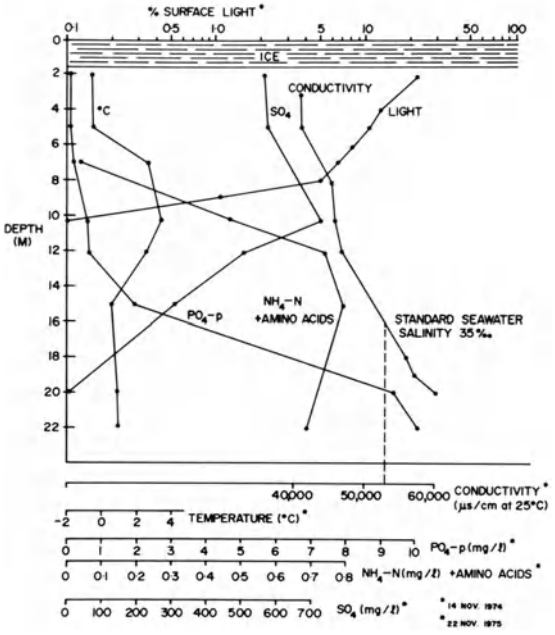

Figure 3: Depth profiles of light absorption, conductivity, temperature, PO_4-P, NH_4-N + amino acids, and sulfate from Ace Lake in November.

TABLE 1

DEPTH PROFILE OF BACTERIAL NUMBERS IN RELATION TO DISSOLVED GASES AND TEMPERATURE IN ACE LAKE, 21 OCTOBER 1978

DEPTH (m)	TEMP. (°C)	O_2 [a] (ml l^{-1})	H_2S [a] (ml l^{-1})	N_2 (ml l^{-1})	CO_2 (ml l^{-1})	CH_4 (ml l^{-1})	BACTERIAL CELLS (x10^{-6}) ml^{-1}
5	3.8	9.0	ND	16	298	BLD [b]	1.6
8	7.0	6.7	ND	15	338	BLD	3.05
9	6.8	ND [c]	BLD	15	337	BLD	0.95
10	6.2	BLD	6.5	15	339	.4	38.75
11	5.1	ND	15	15	ND	6	10.75 [d]
15	2.9	ND	75	14	486	44	ND
18	1.5	ND	ND	BLD	ND	104	ND
20	1.0	ND	143	BLD	1275	107	ND
22	1.1	ND	148	BLD	ND	139	ND

[a] Measured on 22 November 1975

[b] BLD = Below limit detection

[c] ND = Not determined

[d] Cells not estimated due to sulfur precipitate.

TABLE 2

DEPTH PROFILE OF BACTERIAL NUMBERS IN RELATION TO DISSOLVED GASES, TEMPERATURE, CONDUCTIVITY AND SULFATE CONCENTRATION IN CLEAR LAKE, 18 OCTOBER 1978

DEPTH (m)	TEMP. (°C)	K_{25} ($\mu S\ cm^{-1}$)	SO_4 ($mg\ l^{-1}$)	O_2 ($ml\ l^{-1}$)	H_2S ($ml\ l^{-1}$)	N_2 ($ml\ l^{-1}$)	CALCULATED N_2 [a] ($ml\ l^{-1}$)	BACTERIAL CELLS ($x10^{-6}$) ml^{-1}
5	1.2	20,300	ND [b]	10.4	ND	16	16	0.025
10	1.2	20,300	73	10.3	ND	ND	16	ND
20	1.2	20,200	ND	10.7	ND	ND	16	.05
25	1.6	20,200	73	10.2	ND	16	16	ND
28	2.3	20,200	73	6.3	BLD [c]	ND	16	0.15
30	3.9	21,400	68	BLD	17	16	15	32.5
35	3.9	22,000	67	ND	20	ND	15	4.75
40	3.9	22,200	68	ND	27	14.5	15	6.0
45	3.8	22,400	67	ND	31	14	15	7.0
50	3.6	22,300	ND	ND	34	ND	15	4.5
55	3.6	22,400	65	ND	36	12	15	7.25

[a] Calculated levels of N_2 in equilibrium with the atmosphere.

[b] ND = Not determined

[c] BLD = Below limit detection

Dissolved Gases

Concentrations of dissolved CH_4 in Ace Lake either exceed (22m), or lie close to (18, 20m) maximum solubility values, and there is no dissolved N_2 below 18 m (Fig. 4). I conclude that ebullition of CH_4 occurs from depths below 18 m, the bubbles of CH_4 stripping out dissolved N_2 as they rise through the lake. Reeburgh and Heggie (1974) advanced this explanation for low N_2 concentration in some Chesapeake Bay sediments. One difficulty with this explanation for Ace Lake is that H_2S, produced prior to the present methanogenesis at depths below 18 m, is still present in high concentration (148 ml l^{-1} at 22 m, Table 1) and has not been stripped out. The sulfur isotope-mass balances at all depths are also irreconcilable with upward transport of H_2S (Burton and Barker, 1979). It would seem, therefore, that CH_4 is removing N_2 but not H_2S. Published information on the speciation of sulfide forms (H_2S, HS^-, S^{2-}) under conditions similar to those of the bottom water environment of Ace Lake (temperature +1°, pH 6.8, and an ionic strength about 20% greater than seawater; Burton and Barker, 1979) is limited; the closest analogue being sea water, with a temperature of 5°, a pH of 7 and a salinity of 40 per mil, in which H_2S and HS^- comprise approximately 51% and 49% respectively of dissolved sulfide species (Almgren *et al.*, 1976). Therefore, the bottom waters of Ace Lake are likely to contain a considerable quantity of molecular H_2S which is firmly held in solution and apparently not removable by methane ebullition.

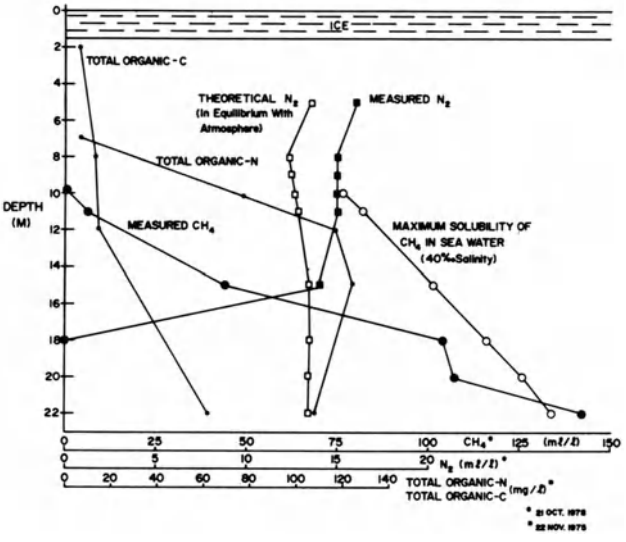

Figure 4: Depth profiles of total organic carbon, total organic nitrogen, dissolved nitrogen gas and methane from Ace Lake in October and November. Theoretical profiles are given for dissolved nitrogen gas if water from any depth, held at lake temperature, was in equilibrium with air at atmospheric pressure, and for maximum solubilities of methane in seawater of 40 per mil salinity.

Another possible mechanism of N_2 removal in an anaerobic environment is by bacterial fixation. This mechanism was suggested by Hammond (1974) to explain partial N_2 loss from some marine sediments, but these small N_2 reductions are not commensurate with the complete loss of N_2 from depths in Ace Lake. The small apparent loss of N_2 at 55 m in Clear Lake,

however (Table 2), could be due to N_2 fixation. The measured values of dissolved N_2 in the oxylimnion of Ace Lake are somewhat higher than calculated values. This excess N_2 could be partially derived from N_2 carried up from below by CH_4 bubbles and liberated as the CH_4 is removed by CH_4-oxidising bacteria. Lakes rich in CH_4, e.g. Lake Kivu in Africa, can have very large and diverse populations of CH_4 oxidisers (Degens *et al.*, 1973).

H_2 was not detected in either lake at a detection limit of 0.1 ml l^{-1}. Bryant *et al.* (1977) suggested that a lack of available H_2 is a major factor in suppressing methanogenesis when sulfate is present in natural ecosystems. Therefore, H_2 would not be expected to occur in sulfate-rich anaerobic environments such as Clear Lake and, between 10 m and 18 m, in Ace Lake. Even in Ace Lake at depths below 18 m, the high CO_2 concentrations (Table 1) and the presence of active methanogens (Hand, 1980) would be expected to keep H_2 at very low concentrations.

Although the profile of bacterial numbers in Ace Lake (Table 1) is incomplete, the peak at 10 m indicates that sulfur cycling is predominant at that depth (Hand, 1980). Hand also recorded a consistent increase in the population density of bacteria at 20 and 22 m particularly after summer; some of these numerous bacteria are no doubt involved in the production of ebullient CH_4. In contrast, no rise in bacterial numbers is observed in Clear Lake below the peak of sulfur cyclers at 30 m, and no CH_4 was detected in Clear Lake (Table 2).

Origin of Methane

Sedimenting organic matter from the productive waters above is the source both of carbon and energy for the complex of bacterial species which work together in the anoxylimnion and sediments of Ace Lake to produce CH_4. There is no evidence of allochthonous organic carbon inputs into Ace Lake, or of recent volcanic gas issues containing CO_2 which, in Lake Kivu for example (Degens *et al.*, 1973), are the precursors of high CH_4 concentrations. The large quantities of total organic carbon and nitrogen present in Ace Lake (Fig. 3) result from the growth of phytoplankton in the oxylimnion and the growth of photosynthetic bacteria at the upper surface of the anoxylimnion (Hand, 1980). Values for primary production from the oxylimnion of Ace Lake on February 9, 1979 were 0.16 and 0.47 mg C m^{-3} h^{-1} at 3 and 8 m, respectively (S. Wright, personal communication). These values were determined in late summer and would probably be exceeded in spring and early summer, at least at 3 m, when nutrients are likely to be more available following melt stream inputs. Even so, they are of the same order as Antarctic ocean values (1.22 mg C m^{-3} h^{-1} ± S.D. 1.69 for 656 observations: El-Sayed, 1968). No values are available for bacterial production immediately below the oxycline but in Lake Belovod (a Russian lake of similar depth but with fewer cells/ml at the oxycline) about 4 mg C m^{-3} h^{-1} was fixed through a depth of approximately 1 m at midsummer (Sorokin, 1970; Kuznetsov, 1968). Photosynthetic production by bacteria in Ace Lake is, therefore, likely to be significant.

Some favourable characteristics of Ace Lake which govern production are:

(1) temperature,
(2) light,
(3) salinity,
(4) nutrient concentrations, and
(5) available sulfate (Fig. 3).

(1) The temperature of Ace Lake below 5 m is always above zero and ranges up to 8° at the end of summer at some depths. An annual cycle of temperature is given by Hand (1980).

(2) The light available to lake organisms is controlled both by the seasonality of incident light due to the high latitude (0 J cm^{-2} in June, ca. 80.8 kJ cm^{-2} in December) and ice cover which can reach a maximum thickness of 1.9 m in October. However, strong summer solar radiation can inhibit primary production in Antarctic algae (Goldman *et al.*, 1963) and the ice cover persisting throughout this most intense radiation period may, therefore, at times be advantageous. Old white ice reduced incident surface light by 80% on 14 November 1974 (Fig. 2), but for most of the year Ace Lake has clear ice. The time when the ice completely melts varies greatly from year to year: in one of the five summers over which this study was made, ice persisted throughout.

(3) The salinity of the productive zones ranges from a seasonally variable salinity at 2 m of about 80% seawater to a value close to seawater itself at 11 m (Fig. 3). These salinities are favourable for algal growth (El-Sayed, 1968).

(4) Heightened levels of reactive phosphorus and ammonia exist below 7 m (Fig. 2). Below this depth nutrients are unlikely to be limiting to algal primary producers.

(5) Although only about 24% of the original seawater sulfate remains in Ace Lake in various forms of sulfur (Burton and Barker, 1979), sufficient sulfate (Fig. 3) is present to allow considerable quantities of sulfide to be produced (Table 1) and to be available for photosynthetic oxidation immediately below the oxycline (Hand, 1980). The peak in bacterial numbers at 10 m (Table 1) reflects this sulfur cycling activity.

In Clear Lake the oxycline is at 29 m, three times deeper than in Ace Lake, and consequently much less light reaches the bacterial sulfide oxidisers (0.04% of incident surface light, 18 October 1978). As well, the temperature of the water column is lower and increased levels of nutrients, which are likely to exist immediately above the anoxylimnion (in a similar manner to those in Ace Lake, Fig.3),are more distant from effective solar radiation, thus making it more difficult for algae to simultaneously exploit both these production requirements. On the three occasions Clear Lake was sampled, twice in January and once in October, the upper waters were very clear. A light profile from October demonstrated that less light was absorbed with depth than in Ace Lake a week later, although less light actually reached the oxycline of Clear Lake as previously mentioned. This indicates less numerous phytoplankton populations in Clear Lake.

CONCLUSIONS

As the data from initial studies (Burton and Barker, 1979) suggested, CH_4 is produced in Ace Lake at depths below 18 m. This present study suggests that the general reducing situation at 22 m in the water column of Ace Lake appears to be analogous to that found in certain anoxic marine and lacustrine sediments (Reeburgh and Heggie, 1974). Bacterial sulfate reduction has totally removed sulfate, CH_4 concentrations are high enough to cause ebullition which is likely to have brought about depleted N_2 concentrations, and CO_2 concentrations are greater than those in the oxylimnion. CH_4 ebullition in Ace Lake demonstrates that CH_4 can be vigorously produced in environments with very high sulfide concentrations. This situation suggests that, at least in some natural environments, sulfide is definitely not inhibitory to methanogenesis.

It seems that primary production is higher in Ace Lake than Clear Lake and that more organic matter is, therefore, made available to the decomposers at depth in Ace Lake. It is suggested that this has led to CH_4 production from the former and not the latter.

ACKNOWLEDGEMENTS

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THE PRODUCTION OF METHYLATED SULFUR COMPOUNDS BY MARINE PHYTOPLANKTON

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INTRODUCTION

Intensive study of the global cycle of sulfur in recent years has revealed the requirement for substantial natural inputs of sulfur into the atmosphere to balance the measured rates of deposition (Friend, 1973). Estimates of the oceanic contribution of reduced sulfur to the atmosphere, usually derived by difference rather than actual measurement, vary from 30 to 202 Tg yr^{-1}. The role of H_2S in this process has been debated (Slatt *et al.*, 1978), and methylated sulfur compounds have been commonly suggested as transfer agents (Rasmussen, 1974; Nguyen *et al.*, 1978). The evaluation of the true magnitude of these fluxes is not yet possible due to the scarcity of measurements of the concentrations of the volatile sulfur compounds in seawater and the marine atmosphere.

In view of the potential role of the biomethylation of sulfur in the global sulfur cycle it is surprising to find that very little research has been conducted on the biogeochemistry of reduced sulfur in the marine environment. The work of Challenger (1951), who demonstrated the presence of dimethylpropiothetin in a marine alga and found the release of dimethylsulfide (DMS) to occur due to enzymatic cleavage of this compound, is a notable exception. In later studies (Rasmussen, 1974, Nguyen *et al.*, 1978) a connection between algal activity and the release of DMS was established, but not thoroughly investigated. This paper reports the release of DMS and dimethylsulfoxide (DMSO) from axenic (i.e. bacteria-free) cultures of marine phytoplankton, and the distribution of DMSO in the surface ocean.

METHODS

Algal cultures were grown on a seawater-based medium either in batch cultures or in a chemostat. The cells were removed by gentle filtration at a low pressure differential. Seawater samples were collected from the research vessel A. AGASSIZ with specially cleaned Niskin samplers. They were immediately acidified for storage. This procedure has been shown to prevent changes in DMSO concentrations (Andreae, 1980).

DMSO was determined by reduction with sodium borohydride or chromium (II)-chloride to DMS, which was removed from solution by stripping with a stream of helium. The DMS was collected in a cold trap. After the reaction period the trapped gases were separated gas-chromatographically and the DMS measured by a flame ionization and/or a flame photometric detector. Details of the procedure can be found in Andreae (1980).

RESULTS

DMSO was found in all seawater samples from the euphotic zone. The results of a typical section are presented in Fig. 1. Stations 101, 103 and 106 were in the northeast Pacific off southern California and are located 0.5, 5 and 90 km from shore. Samples from all depths from the surface to a few metres above the sediment were analyzed, but no DMSO was found in the samples below 100 m depth. DMSO concentrations at these stations were generally between 1 and 5 $\mu g\, l^{-1}$, which is a typical range for unpolluted, non-eutrophic waters. In samples from eutrophic areas in Santa Monica Bay and the Salton Sea, values of up to an order of magnitude more have been observed (Andreae, 1980b).

Together with the DMSO concentrations, the values for dimethylarsinic acid (DMAA) and primary productivity measured by ^{14}C-uptake are shown in Fig. 1. DMAA recently has been shown to be an algal exudation product (Andreae, 1979; Andreae and Klumpp, 1979). For the deep-water stations the base of the euphotic zone as determined by the level of 1% light penetration is indicated by an arrow. The striking parallelism between the concentrations of DMSO and DMAA strongly suggests that DMSO, or a precursor substance, is

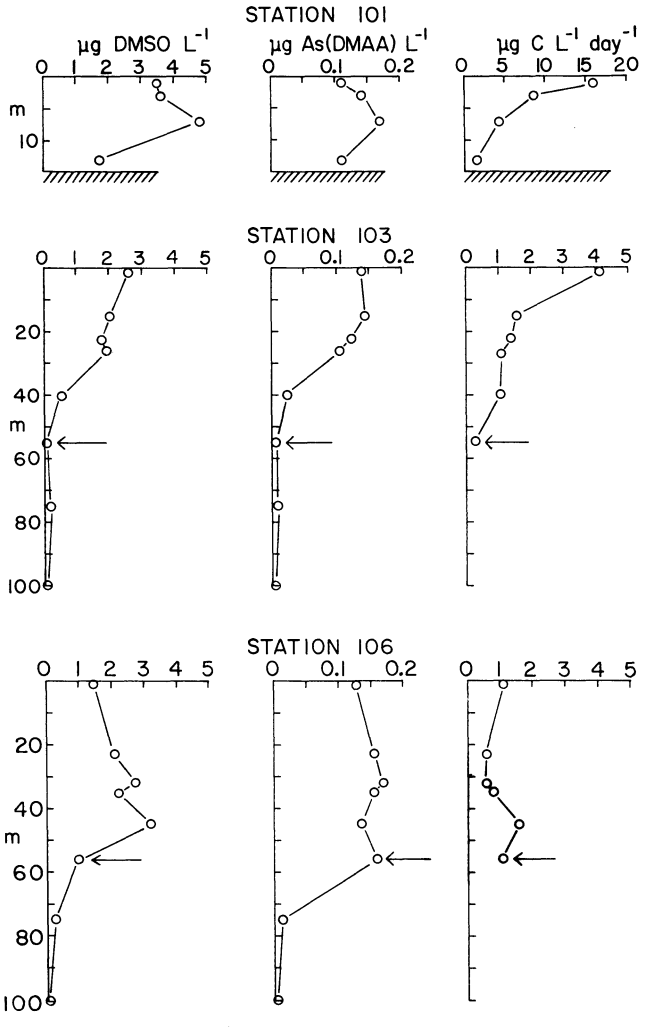

Figure 1: Distribution of dimethylsulfoxide, dimethylarsinic acid and primary productivity in the water column at three stations in the northeastern Pacific. The arrows indicate the level of 1% light penetration.

released by phytoplankton organisms. This is further supported by the fact that the presence of DMSO is restricted to the zone of photosynthetic activity.

Eight pure cultures of marine phytoplankters were investigated for the production of DMS and DMSO. Most of these cultures were axenic (others marked by *'a'* in Table 1). DMS was identified in the headspace above growing cultures of *Cricosphaera carteri*, *Coccolithus huxleyi* and *Skeletonema costatum*. Measurements of DMSO in the medium after the end of log-phase growth of these cultures are given in Table 1. It is evident that DMSO was present in concentrations above that found in the control (which is left over from the DMSO in the seawater used to prepare the medium), with the exception of *Thalassiosira fluviatilis*. The amounts of DMSO produced are quite variable; they range from values close to that of seawater to concentrations almost two orders of magnitude higher. The two species of coccolithophorids investigated, *Cricosphaera carteri* and *Coccolithus huxleyi*, stand out as the strongest producers of DMSO. This is in agreement with their high release of DMS, which can be clearly smelt above the cultures. A strong smell of DMS has also been

TABLE 1

CONCENTRATION OF DIMETHYLSULFOXIDE AND DIMETHYLSULFIDE IN THE MEDIUM AFTER THE GROWTH OF AXENIC ALGAL CULTURES

Species	µg DMSO l^{-1}	µg DMS l^{-1}
Diatoms		
Thalassiosira fluviatilis	0.39	0.24
Skeletonema costatum	24	0.70
Cylindrotheca closterium	4.9	n.d.
Coccolithophorids		
Cricosphaera carteri	94	1.3×10^3
Coccolithus huxleyi	30	n.d.
Dinoflagellates		
Gonodium cf. depressum [a]	2.6	n.d.
Gonyaulax polyedra [a]	14.4	n.d.
Prasinophyceae		
Platymonas cf. suecia	2.6	2.8
Sterile medium	0.59	0.00

[a] These cultures were unialgal, but not axenic.

n.d. : not determined.

observed from the Salton Sea and in the highly productive environments of the oceanic fronts in the Bering Sea (D. DiDomenico, personal communication). The productivity in these environments approaches that of laboratory cultures.

To verify that the release of methylated sulfur occurred during log phase growth and was not connected with the senescence of cells in the post-log phase stage, a chemostat experiment was run with a culture of *Thalassiosira pseudonana*. In Table 2 the concentration of DMSO and DMAA in the starting medium and the chemostat effluent are compared. It is clear that the release of both substances occurs also under conditions of log-phase growth.

TABLE 2

PRODUCTION OF DIMETHYLSULFOXIDE AND DIMETHYLARSINIC ACID BY *THALASSIOSIRA PSEUDONANA* IN A CHEMOSTAT

	µg DMSO l^{-1}	µg DMAA l^{-1}
Medium supply	0.0	0.002
Chemostat effluent	2	0.031

DISCUSSION

The presence of DMS has been observed in a number of macro-algae, e.g. *Ulva pertusa*, *Enteromorpha* sp., *Codium fragile* (Katayama, 1962), *Polysiphonia fastigata*, and *Acrosiphonia centralis* (Challenger *et al.*, 1957). Rasmussen (1974) was able to demonstrate the release of DMS and other volatile organosulfur compounds by cultures of freshwater blue-green algae. This paper presents for the first time evidence for the release of DMS and DMSO from axenic cultures of marine phytoplankton species, and for the occurrence of this process in the unpolluted marine environment.

The distribution of DMSO in the surface ocean closely parallels that of dimethylarsinic acid. Both compounds typically show a maximum near the surface, and disappear just below the level of 1% light penetration, below which phytoplankton activity also drops sharply. A similar distribution has been observed for DMS by Nguyen *et al.* (1978): this substance, which is present at concentrations typically 100-1000 times lower than DMSO, also shows a subsurface maximum, and with the exception of one data point, a sharp decrease below 30 to 40 m depth. Unfortunately, Nguyen *et al.* have not measured the depth of the euphotic zone and the rate of primary production, so that a direct comparison is not possible. The agreement of the distribution profiles of DMS and DMSO strongly supports that they are both released by algal excretion into the surface ocean.

Comparison of the detailed structure of the profiles of DMSO and primary productivity measured by ^{14}C-uptake usually shows significant differences, especially the absence in the DMSO profiles of the sharp peak just below the surface commonly observed in the productivity profiles. This peak usually is not observed in the methylarsenical distribution, either. Several factors may be responsible for this difference. Table 1 shows that different phytoplankton species excrete highly variable amounts of DMSO. Vertical stratification of the relative abundances of phytoplankton species and types could account for some of the differences observed. The persistent absence of a sharp sub-surface maximum of DMSO, however, suggests that a more systematic factor, e.g. a difference in the physiological state of the algal cells, may be responsible. Such differences have been found for the exudation of organic matter (Ignatiades and Fogg, 1973). If the release of organosulfur compounds is related to buoyancy control (R. L. Iverson, personal communication, 1979), it would have to be expected that most of it occurs by cells at some depth, rather than near the surface.

The fact that DMS is able to quench singlet oxygen, which is produced as a by-product of the photosensitization processes occurring during the early steps of photosynthetic energy transfer (Foote, 1976), has led to the suggestion that it acts as a protective agent in photosynthetic organisms. The product of singlet oxygen quenching by DMS is DMSO, and this process could offer a rationale for the occurrence of both compounds in algal exudates, and in the surface ocean. The widespread abundance of β-carotene, which is ca. 10,000 times more efficient in quenching singlet oxygen in photosynthetic organisms, and the lack of an increase of DMSO concentrations in the zone of maximal light intensity, however, do not support this argument.

With the exception of *Thalassiosira fluviatilis*, all phytoplankton species investigated released DMSO in significant quantities. The large differences in the amounts of DMSO produced have not yet been explained; as all cultures were harvested at the end of log-phase growth and all were grown up in media containing the same amounts of nutrients, they should represent comparable physiological states and biomass amounts. No systematic differences have been observed between DMSO concentrations in axenic cultures and cultures containing bacterial populations. In fact, of the two species of coccolithophorids studied, the axenic *Cricosphaera carteri* produced more DMSO than the agnotobiotic *Coccolithus huxleyi*. The production of methylated sulfur compounds is, therefore, not associated with bacterial degradation of organic matter in these systems. That this also applies to the production of these compounds in the ocean is supported by their presence only in the zone of primary productivity.

It is not yet completely clear if the DMSO is a direct excretion product of the algae, or if it results from the oxidation of DMS which has been excreted by the phytoplankters. In the absence of catalysts, the oxidation of DMS by molecular oxygen occurs only very slowly, on the time scale of months to years (Karchmer, 1972). The enzymatic oxidation of sulfides to sulfoxides has been observed in animals, but not in plants: the reverse reaction on the

other hand, the reduction of DMSO to DMS, has been found in plants, animals and bacteria (Jocelyn, 1972). Such biologically catalyzed redox reactions do not appear to be a major effect in the cultures investigated, as both bacteria-free and bacteria-containing cultures contained both DMS and DMSO; both substances appear to be directly released by the planktonic algae. The role of bioconversions of methylated sulfur species in the ocean with its more complex chemical ecology remains to be investigated, however.

Challenger (1951) identified the sulfonium compound, dimethylpropiothetin (Fig. 2), as the precursor of the DMS released by marine macroalgae. This substance may play a role in transfer of methyl groups in the algae; it is able to transfer one of its methyl groups to homocystine to form methionine, which then transfers this methyl group after S-adenosylation to its substrate. Why this process is shunted to produce DMS and acrylic acid instead is not clear. This reaction offers an interesting parallel to the biochemistry of arsenic, however. One of the most important metabolites of arsenic in algae is trimethylarsonium lactic acid, which is incorporated in phospholipids and replaces choline (Cooney *et al.*, 1978). This substance is thought to be the precursor of the methylated arsenicals, methylarsonic and dimethylarsinic acid, which are ubiquitous in photosynthetically active environments. The role of these onium intermediates promises to become an intriguing field of natural product chemistry.

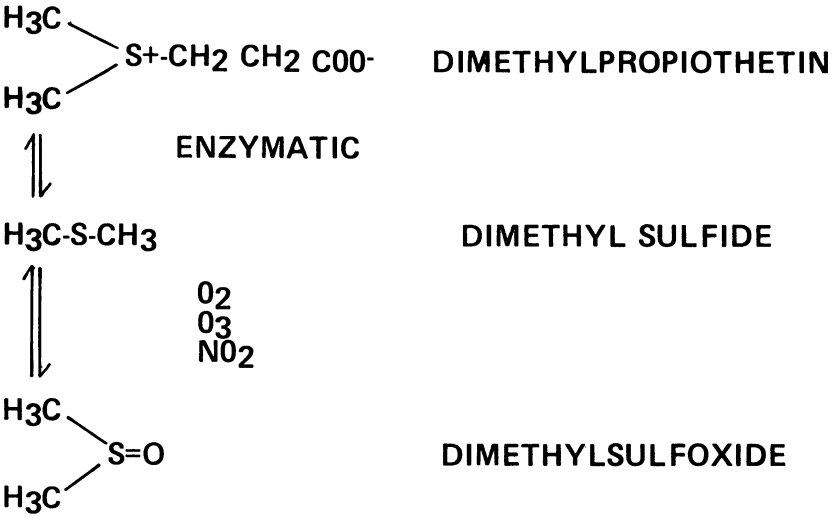

Figure 2: Dimethylpropiothetin is the biochemical precursor of dimethylsulfide, which in turn is the precursor of dimethylsulfoxide.

On the basis of the foregoing discussion, we can construct a hypothetical cycle for the methylated sulfur compounds in the ocean and the marine atmosphere (Fig. 3). In this cycle, DMS and DMSO are excreted by the marine phytoplankton. Once in the seawater, both compounds can be converted into each other by microbial activity. It is further possible that bacterioplankton and zooplankton also excrete either one or both of these substances. The fact that both compounds disappear rapidly at the base of the zone of primary productivity suggests. however, that the production by phytoplankton dominates, and that DMSO, which is quite stable in sterile solutions, is relatively rapidly metabolized by heterotrophic organisms, probably towards sulfate ion. Due to its high volatility and its enrichment in the microlayer at the air-sea interface, DMS can escape into the atmosphere. This flux has been estimated at 27 Tg yr^{-1} (Nguyen *et al.*, 1978). Evaporation of DMSO may

contribute to the flux of sulfur to the atmosphere. Once in the atmosphere, DMS is relatively rapidly oxidized either by photochemical processes or by NO_2 catalysis, at least in part to DMSO. This compound can then be either removed directly by precipitation, or oxidized further to SO_2 and H_2SO_4. DMSO has been found in several samples of rain from marine airmasses (Andreae, 1980a).

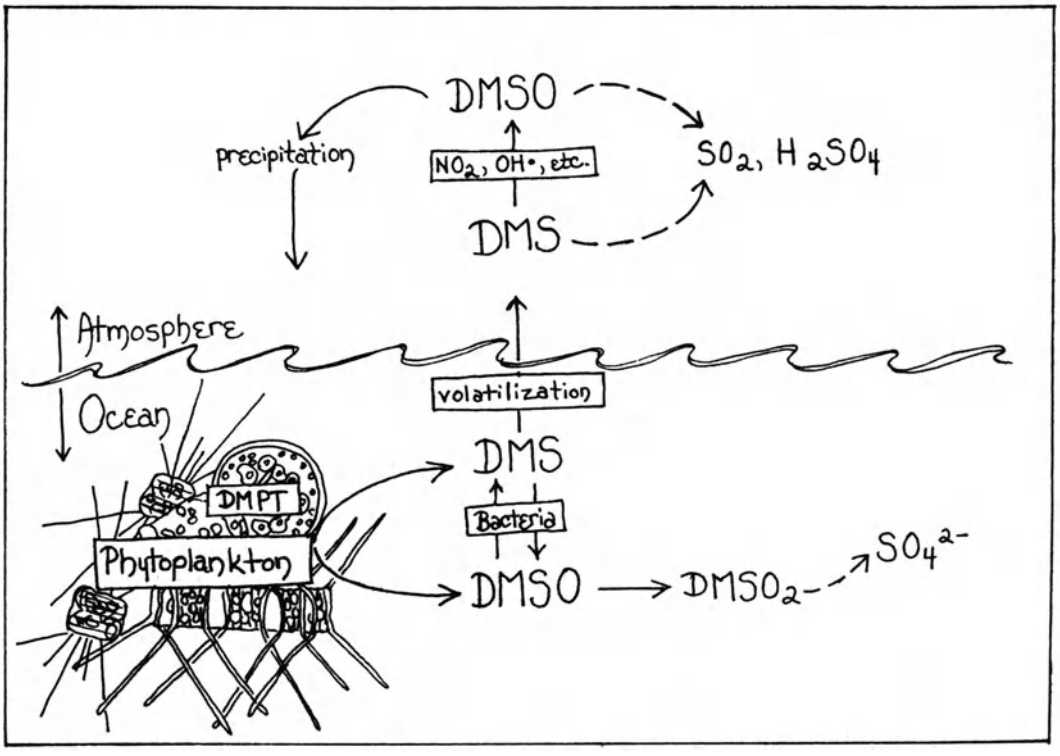

Figure 3: Hypothetical biogeochemical cycle of the methylated sulfur species (DMPT: Dimethylpropiothetin, DMS: dimethylsulfide, DMSO: dimethylsulfoxide).

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INFLUENCE OF SELENIUM COMPOUNDS ON REDUCTION OF SULFUR COMPOUNDS AND ASSOCIATED SULFUR ISOTOPE FRACTIONATION IN *CLOSTRIDIUM PASTEURIANUM*

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INTRODUCTION

From the viewpoint of biogeochemistry, *Clostridium pasteurianum* is a relevant organism to study for many reasons. It is involved in cycling of carbon, nitrogen, sulfur, and selenium and is capable of stable isotope fractionation of these elements (Laishley *et al.*, 1976; Rashid, 1978). An appreciation of its participation in the sulfur cycle has been overshadowed by the facts that this organism gained considerable importance in the elucidating of the mechanism of non-symbiotic N_2 fixation (Winter and Burris, 1976) and that SO_4^{2-} reduction has usually been associated with the "classical" reducers, *Desulfovibrio* spp. and *Desulfotomaculum* spp. In considering the early development of biochemical systems, however, ferredoxin and 5S ribosomal RNA sequence studies provide evidence that the clostridia evolved prior to the classical sulfate reducers (Schwartz and Dayhoff, 1978).

The interaction of element cycles is a complex and important subject from the viewpoint of their earlier geochemical development as well as current environmental considerations. Microorganisms can effect such interactions through the mechanisms of nutritional requirements, toxicity, and similar biochemical transformations.

If the sulfur and selenium cycles are considered, all three mechanisms cited above apply. Selenium is required in μM concentrations by bacteria for growth. However, because of its chemical similarity to sulfur, higher concentrations can affect cells adversely because the enzyme systems fail to differentiate the two elements, and the selenium analogues generally have greater reactivities and lower stabilities (Stadtman, 1974).

This report is concerned with the interaction of the selenium and sulfur cycles in *C. pasteurianum*. Such studies seemed appropriate because of the organisms wide-spread occurrence in nature and its antiquity. Preliminary data were presented previously (Harrison *et al.*, 1978).

METHODS

The techniques of culturing, monitoring growth and collection of H_2S for isotopic analyses were those described by Laishley and Krouse (1978). Filter-sterilized SeO_3^{2-} and SeO_4^{2-} additions were made during early log phase (ca. 100 Klett units). Cells for electron microscopy were prepared as described by Laishley *et al.* (1973).

RESULTS

Effects of SeO_4^{2-} and SeO_3^{2-} on Cellular Physiology

The effects of SeO_4^{2-} and SeO_3^{2-} additions on the generation times of *C. pasteurianum* growing on 1 mM SO_4^{2-} or 1 mM SO_3^{2-} are shown in Table 1. On 1 mM SO_4^{2-}, the addition of either 1 mM SeO_4^{2-} or SeO_3^{2-} increased the generation time by a factor of 3. 1 mM SeO_4^{2-} did not alter the growth rate of cells growing on 1 mM SO_3^{2-}. However, 0.05 mM SeO_3^{2-} was sufficient to dramatically increase the generation time of 1 mM SO_3^{2-} growing cells while higher SeO_3^{2-} concentrations depressed the growth rate even further.

1 mM SeO_4^{2-}, and concentrations of SeO_3^{2-} below 0.25 mM, had little effect on cell morphology provided additions were not made prior to early log phase growth. Within an hour after addition, at early log phase growth, 1 mM SeO_3^{2-} caused cell elongation and long chain formation. Two hours after the SeO_3^{2-} addition, the morphology of the cells was as shown in Fig. 1. Chain structures took on a "hooked" appearance when the contour and thickness of the cell walls were highly altered causing curvature of some cells. Similar morphological modifications were seen in *C. pasteurianum* grown on 10 mM SO_3^{2-} (McCready *et al.*,

TABLE 1

EFFECT OF SeO_3^{2-} AND SeO_4^{2-} CONCENTRATIONS ON *C. PASTEURIANUM* GROWTH AND H_2S PRODUCTION DURING SO_3^{2-} AND SO_4^{2-} REDUCTION

Growth Conditions	Generation [a] Time (min)	S^{2-} evolved (mg)		
		Growth Phase	Stationary Phase	Total
Control, 1 mM SO_3^{2-}	60	139.3	76.5	215.8
+ 0.05 mM SeO_3^{2-}	108	94.4	16.9	111.3
+ 0.1 mM SeO_3^{2-}	120	72.1	13.6	85.7
+ 0.25 mM SeO_3^{2-}	132	44.0	15.8	59.8
+ 1 mM SeO_3^{2-}	156	20.7	1.9	22.6
+ 1 mM SeO_4^{2-}	66	132.6	23.1	155.7
Control, 1 mM SO_4^{2-}	66	nil	15.8	15.8
+ 1 mM SeO_3^{2-}	216	nil	nil	nil
+ 1 mM SeO_4^{2-}	228	nil	nil	nil

[a] SeO_3^{2-} and SeO_4^{2-} were added to early log phase cultures growing on SO_3^{2-} or SO_4^{2-} at 37°. Generation times were determined after these additions.

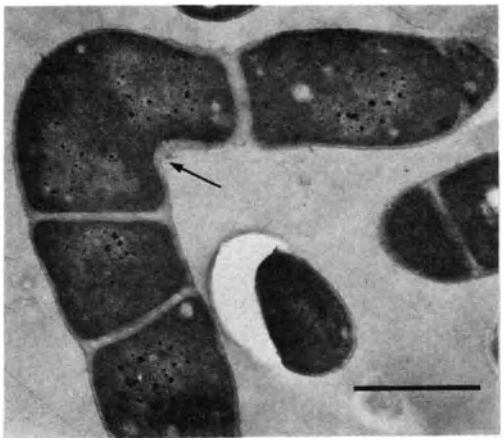

Figure 1: Thin section of *C. pasteurianum*, 2 hr after addition of 1.0 mM SeO_3^{2-} to a growing culture on 1.0 mM SO_3^{2-} showing cells in chains and irregular cell wall thickening. The bar in this electron micrograph indicates 1.0 μm.

1975). No Se^{o} granules were observed in the organism's cytoplasm. However *C. pasteurianum* metabolized SeO_3^{2-} to Se^{o}, excreting the latter into the growth medium causing it to turn brick red. As SeO_3^{2-} reduction proceeded, there was a tendency for cells to revert back to their normal shape (late log phase).

H_2S Production

The effects of SeO_4^{2-} and SeO_3^{2-} additions on H_2S production are shown in Table 1. H_2S is only evolved during stationary growth on 1 mM SO_4^{2-}. This production ceased when either 1 mM SeO_4^{2-} or SeO_3^{2-} was added. While 1 mM SeO_4^{2-} did not inhibit H_2S production during growth on 1 mM SO_3^{2-}, the production during the stationary phase was depressed substantially. All concentrations of SeO_3^{2-} inhibited H_2S production from 1 mM SO_3^{2-} in both the growth and stationary phases as shown in Table 1. The H_2S production decreased with increasing SeO_3^{2-} concentration (Table 1) and exhibited the characteristics summarized in Table 2.

TABLE 2

EFFECTS OF SeO_3^{2-} CONCENTRATION ON H_2S PRODUCTION DURING THE GROWTH PHASE OF *C. PASTEURIANUM* ON 1 mM SO_3^{2-}

SeO_3^{2-} concentration (mM)	H_2S Production Behaviour
1.0	Stopped immediately
0.25	Stopped for 90 min; resumed at lower rate
0.1	Stopped for 40 min; resumed at lower rate
0.05	Production rate depressed but not stopped

Sulfur Isotope Fractionation During SO_3^{2-} Reduction

The normal and inverse sulfur isotope effects observed previously with this organism growing on SO_3^{2-} (McCready *et al.*, 1975) were again found for 1 mM SO_3^{2-} reduction in the presence of SeO_3^{2-} (Fig. 2). The effects of SeO_3^{2-} and SeO_4^{2-} additions were to decrease the percentage reaction at which the maximum $\delta^{34}S$ value occurred. Table 3 shows that this downward shift increased with increasing SeO_3^{2-} concentration while Fig. 2 illustrates the curve obtained with 0.25 mM SeO_3^{2-}. Another pertinent observation is that the $\delta^{34}S$ maximum always occurred at the end of log growth irrespective of the amount of H_2S production.

DISCUSSION

These observations are relevant to our understanding of SO_4^{2-} and SO_3^{2-} metabolism by *C. pasteurianum* and the roles which this organism played in the evolution of the biogeochemical sulfur cycle.

In addition to the accepted assimilatory SO_3^{2-} reductase, Laishley and Krouse (1978) showed the presence of an inducible dissimilatory type SO_3^{2-} reductase in *C. pasteurianum*. Since 1 mM SeO_3^{2-} stopped the cellular production of H_2S immediately, SeO_3^{2-} was presumed to affect the enzyme(s) activity in the inducible dissimilatory pathway. $S_2O_3^{2-}$ and $S_3O_6^{2-}$ have been proposed as intermediates in the dissimilatory SO_3^{2-} reduction pathway in *Desulfovibrio vulgaris* (Drake and Akagi, 1978) and we have recently conducted experiments with cell free extracts of *C. pasteurianum* using a variety of oxidized sulfur and selenium compounds as substrates. In all enzyme experiments using SO_3^{2-} (10 mM) or SO_3^{2-} plus SeO_4^{2-} (10 mM), H_2S was formed but no $S_2O_3^{2-}$ was detected. However, using SO_3^{2-} plus SeO_3^{2-} (10 mM) no H_2S or $S_2O_3^{2-}$ was produced but over 75% of the available sulfur was converted to $S_3O_6^{2-}$ before the experiment was terminated. Thus it appears that SeO_3^{2-} did not affect the inducible SO_3^{2-} reductase in the cell free extract experiments but inhibited the action of

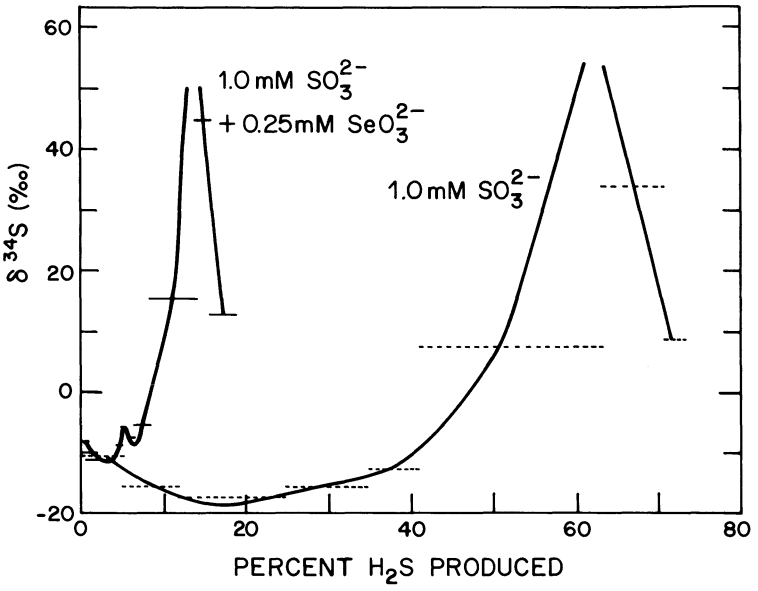

Figure 2: Effect of SeO_3^{2-} addition on the sulfur isotope composition of H_2S released during SO_3^{2-} reduction by *C. pasteurianum*.

TABLE 3

EFFECT OF SeO_4^{2-} AND SeO_3^{2-} ON POSITION OF THE $\delta^{34}S$ MAXIMUM FOR H_2S EVOLVED DURING REDUCTION OF 1 mM SO_3^{2-} BY *C. PASTEURIANUM*

Growth Conditions	Position of $\delta^{34}S$ maximum (% H_2S production)[a]
Control, 1 mM SO_3^{2-}	60
+ 1 mM SeO_4^{2-}	40
+ 0.05 mM SeO_3^{2-}	27
+ 0.1 mM SeO_3^{2-}	20
+ 0.25 mM SeO_3^{2-}	16
+ 1.0 mM SeO_3^{2-}	7

[a] See Fig. 2.

an enzyme involved in $S_3O_6^{2-}$ reduction. Such a mechanism would be consistent with the rapid termination of H_2S production by whole cells when 1 mM SeO_3^{2-} is added. The role of SeO_4^{2-} in inhibiting H_2S production seems to be more subtle. SeO_4^{2-} affects the synthesis of the electron carrier, ferredoxin, which in turn is involved in the inducible sulfite reductase pathway (Harrison and Laishley, 1979).

The question of $S_2O_3^{2-}$ formation is interesting from the viewpoint of elucidating the SO_3^{2-} reduction pathway and its role in the biogeochemical sulfur cycle. $S_2O_3^{2-}$ has been detected in thermal springs and ocean sediments (Volkov *et al.*, 1972) and it has usually been ascribed to S^{2-} oxidation. However, it might also be associated with SO_3^{2-} reduction, either as an intermediate (Drake and Akagi, 1978) or as a chemical side reaction between SO_3^{2-} and H_2S as proposed by Chambers and Trudinger (1979). In contrast to the latter authors, we have failed to detect $S_2O_3^{2-}$ either in the growth medium of whole cell experiments or in the cell free experiments cited above. It must be noted that our growth experiments were conducted at 1 mM SO_3^{2-} in contrast to the 5 mM SO_3^{2-} experiments of Chambers and Trudinger (1979). A point of issue is whether $S_2O_3^{2-}$ formation provides an explanation for the inverse sulfur isotope effects observed with *C. pasteurianum* during SO_3^{2-} reduction, and this in turn bears on the interpretation of the current work. The decreasing $\delta^{34}S$ values for H_2S released after the $\delta^{34}S$ maximum requires metabolism of the postulated $S_2O_3^{2-}$. Whole cells of *C. pasteurianum* do not grow on $S_2O_3^{2-}$ as the sole sulfur source (Laishley *et al.*, 1976; Chambers and Trudinger, 1979) whereas cell extracts can split off the sulfane group and reduce the sulfonate group (Laishley *et al.*, 1976). The sulfur isotope fractionations obtained with such crude extracts reducing $S_3O_6^{2-}$, $S_2O_3^{2-}$ or SO_3^{2-} do not show large inverse isotope effects, and it is therefore concluded that the integrity of the whole cell is necessary for the latter to be expressed (unpublished data, 1979).

In the current work, increased SeO_3^{2-} concentrations essentially compressed the isotopic pattern, i.e. shifted the position of the $\delta^{34}S$ maximum to lower percentages of H_2S production. This must be reconciled with the facts that the $\delta^{34}S$ maxima occurred near the end of growth and SeO_3^{2-} apparently can inhibit metabolism of $S_3O_6^{2-}$ within the cell. It is difficult to explain all of these observations in terms of an external chemical reaction.

It appears that the inducible SO_3^{2-} reduction pathway discovered in *C. pasteurianum* may also exist in many of the other species in this genus. *C. bifermentans* and *C. sartagoforum* isolated from soil surrounding buried sulfur-concrete cylinders were found capable of this conversion (Laishley *et al.*, 1979).

This report stressed the effects of SeO_3^{2-} and SeO_4^{2-} on SO_4^{2-} and SO_3^{2-} reduction by *C. pasteurianum*. The data are not only of interest for elucidating the metabolism of oxidized sulfur compounds but can serve in interpreting ecosystems where both cycles function e.g. seleniferous soils (Lakin, 1972).

A complementary study is how sulfur compounds influence selenium metabolism. For example, $S_2O_3^{2-}$ stimulates SeO_3^{2-} reduction to Se^0 by cell free extract (unpublished data, 1979). Selenium isotope selectivity has also been demonstrated during SeO_3^{2-} reduction by cell free extracts of *C. pasteurianum* (Rashid, 1978).

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MICROBIOLOGICAL NITROUS-OXIDE PRODUCTION: IMPLICATIONS FOR THE GLOBAL NITROGEN CYCLE

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INTRODUCTION

In 1972, Lovelock coined the use of the Greek work Gaia to denote the globe encompassing the biosphere, pedosphere, hydrosphere and atmosphere as an entity which has developed a powerful homeostatic system to control the global environment. This is a very important concept and a necessary one if we wish to describe, quantify and understand the global biogeochemical cycles. In order to elucidate this self-regulating system with regard to the atmospheric composition of non-noble gases, it is necessary to develop an integration between the atmospheric sciences and biology, especially microbiology (Margulis and Lovelock, 1978).

The biogeochemical cycles can be viewed as a global life-support system (Tolba and White, 1979, Appendix 1), on which "spaceship Earth" depends for survival. A deeper understanding of the global biogeochemical cycles is therefore necessary. These cycles are the basis for life, which depends on the carbon, nutrient and water cycles. They are also important as they affect directly to the fate of pollutants in the environment.

There is no sharp distinction between vital elements or compounds and those that are considered to be health hazards with respect to either man specifically or to other segments of the biosphere. For example, nitrate is the main form by which plants obtain nitrogen for their growth, but nitrate is also a potential environmental toxin and high levels of it in drinking water may render the water unpotable. The concentration of a compound in relation to the particular reservoir where it occurs determines whether it should be regarded as a vital compound or a pollutant. Commercial nitrogen fertilizers, e.g., ammonium nitrate, are no more unnatural than are manure or plant litter added to soils as the major nitrogen input to the unmanaged soil system. If we could add commercial nitrogen fertilizers at the same rate as nitrogen is utilized by plant roots, we would have achieved an optimal solution to circumventing nitrogen limitation in agricultural production with little or no environmental consequences (e.g., Ingestad, 1977).

The necessity of integrating information from atmospheric chemistry and physics and microbiology, and the delicate balance which exists between natural concentrations of compounds and unnatural concentrations that become environmental hazards, are nicely exemplified by the recent concern that nitrogen oxides produced by supersonic aircraft or from nitrogen fertilizers might constitute an important threat to the global stratospheric ozone layer (Crutzen, 1970; Johnston, 1971). Recent reports, based on refined determinations of rate constants for certain key processes in the stratosphere, seem to indicate that instead of decreasing the ozone content of the stratosphere, especially at lower altitudes, the ozone might increase (Turco *et al.*, 1978). Reaction rates in the atmosphere are difficult to predict due to complex interlocking reactions. Further data on atmospheric chemistry are needed and may prove the earlier concern to be unsubstantiated.

However, the concern over the possible link between nitrogen-fertilizer use and stratospheric ozone content has focused attention on the lack of understanding of the microbiological processes leading to nitrous-oxide production in soils and waters. The revitalized interest in the inorganic nitrogen metabolism of microorganisms has led to a number of important discoveries. It is certain that the ozone debate has triggered off new microbiological research efforts which will help us understand improtant facets of the global nitrogen cycle.

MICROBIOLOGICAL NITROUS-OXIDE PRODUCTION

Although nitrous oxide (N_2O) can be formed abiotically during combustion (Weiss and Craig,

1976) or by electrical discharge in the atmosphere (Delwiche *et al.*, 1978), the largest source is as end- or by-products of microbiological transformations. The production of nitrous oxide was recently reviewed by Hahn and Junge (1977), Liu *et al.* (1977), Delwiche (1978) and Hahn (1979).

Denitrification

Although there is still some doubt whether N_2O is an obligate intermediate in denitrification (Delwiche and Bryan, 1976), recent investigations indicate that this is the case (St. John and Hollocher, 1977; Sidransky *et al.*,1978; Hollocher, 1978).

Nitrous oxide is, however, generally a minor end-product in denitrification, one reason being that N_2O reduction is more rapid than nitrite reduction, which results in complete denitrification to dinitrogen. Under saturating conditions, the N_2O reduction rate in *Pseudomonas aeruginosa* has been shown to be twice that of nitrite reduction (St. John and Hollocher, 1977). Some organisms, however, cannot reduce N_2O which becomes the terminal end-product in their denitrification (Renner and Becker, 1970; Payne and Balderston, 1978).

Our knowledge regarding the regulation of the relative proportions of N_2O and N_2 as end-products of denitrification is poor. The N_2/N_2O ratio is affected by environmental conditions such as pH, pO_2, energy and substrate concentrations. Low pH, relatively high oxygen concentrations and high nitrate concentrations seem to favour N_2O production relative to N_2. From an energetic standpoint, N_2O is never a preferred product over N_2, independent of whether the organic substrate or the electron acceptor is limiting (Delwiche, 1978).

From theoretical considerations, based on free energy values of the various reactions involved, Delwiche (1978) concluded that N_2O as a terminal electron acceptor would yield only 28% of the energy released during nitrate reduction. In an experiment using continuous cultures of *Pseudomonas denitrificans*, Koike and Hattori (1975) showed that the molar growth yield was directly proportional to the oxidation number of nitrogen in the compound used as electron acceptor, with oxidative phosphorylation occurring at three reaction steps, viz., $NO_3^- \rightarrow NO_2^-$, $NO_2^- \rightarrow N_2O$, $N_2O \rightarrow N_2$. The growth yield on N_2O was 35% of that on nitrate. Focht and Verstraete (1977), however, noted that, theoretically, NO_3^- should provide more energy than N_2O per electron equivalent. Under conditions of nitrate limitation, dinitrogen would thus be the preferred end-product. A relative increase of N_2 over N_2O under nitrate limitation has also been observed in the field (Focht *et al.*, 1979). The relatively higher production of N_2O over N_2 at high nitrate concentrations will also be augmented by the observed nitrate inhibition of N_2O reductase (Blackmer and Bremner, 1978). It should be noted, however, that although nitrate inhibits N_2O reductase activity, low concentrations seem to induce enzyme synthesis (Blackmer and Bremner, 1979).

The inhibition of N_2O reduction by high nitrate concentrations is also supported by field measurements, where more frequent irrigations led to increased nitrate concentrations and larger N_2O production relative to N_2 (Focht *et al.*, 1979). Nitrogen maxima were observed to occur later than N_2O maxima in soil columns to which nitrate was added (Rolston *et al*, 1976; Rolston, 1978).

Figure 1 shows the percentage N_2O of total gaseous loss from soil cores after nitrate addition. There is a strong dependence between the proportion of N_2O and the nitrate concentration with as much as 93% being evolved as N_2O after 48 h with an initial addition of 83 μg NO_3-N g^{-1} of soil (Ryden *et al.*, 1979a). This is also supported by field studies (Rolston *et al.*, 1978).

With regard to the effect of water content, or aerated pore space, Smith (1978) concluded that the increased N_2O production under microaerophilic conditions was a result of its more rapid diffusion to aerobic microsites before complete reduction could take place. However, as observed earlier, nitrite rather than N_2O reduction seems to be the rate-limiting step, at least for *Pseudomonas aeruginosa* (St. John and Hollocher, 1977), although such considerations may not be valid when considering a complex system like a soil column. In an analysis of N_2O concentrations in soil as a function of soil water content, Focht (1978) concluded that high production rates were found at intermediate moisture contents

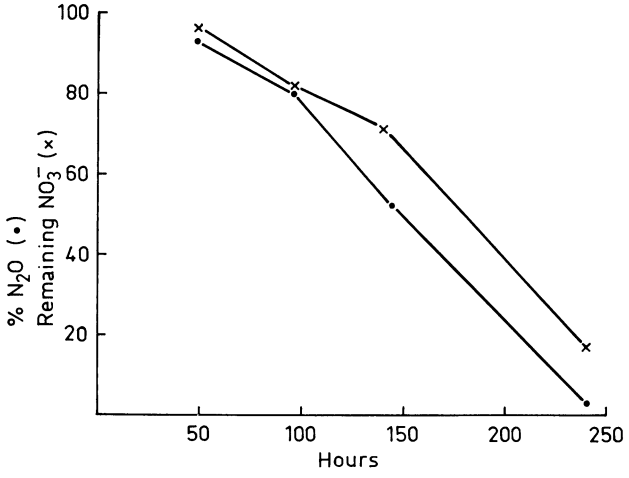

Figure 1: Percentage N_2O of total losses during denitrification after nitrate addition (data plotted from Ryden *et al.*, 1979a).

(5-15 kPa), while wetter and drier conditions led to low concentrations. The effect of water is complicated by the fact that it acts both directly, as a necessary physiological factor, and indirectly by limiting oxygen diffusion rates at high moisture contents. A further complication is the fact that N_2O is produced both aerobically and anaerobically, as shown by the data of Bremner and Blackmer (1980). It has also been concluded, on theoretical grounds, that the N_2/N_2O ratio should increase as the soil becomes more anaerobic (Focht, 1974).

Table 1 gives some examples of measured and estimated fractions of gaseous denitrification losses in the form of nitrous oxide. The values range from a low 6% given by CAST (1976) to a very high rate of 74% estimated by McElroy *et al.* (1977). The actual measured values show an even greater range (0.8-100%). The estimates were used in attempts to quantify global nitrogen cycles, especially as these cycles affect the ozone layer. These differences have been discussed in relation to the factors mentioned above, and the original references should be consulted for a fuller discussion.

Nitrification

Nitrous oxide has been shown to evolve during the oxidation of ammonium ion to nitrite by *Nitrosomonas europea* in pure cultures (Yoshida and Alexander, 1970; Ritchie and Nicholas, 1972). It was shown by the use of ^{15}N that *Nitrosomonas europea* could produce N_2O either through the oxidation of hydroxylamine or by reducing nitrite (Ritchie and Nicholas, 1972). Several authors have suggested that nitroxyl is the unidentified intermediate between NH_2OH and NO_2^-. Nitroxyl dimerizes and forms hyponitrous acid, which in turn decomposes to produce N_2O. For a more detailed discussion of the possibilities of N_2O production during nitrification, see Bremner (1980).

The fact that N_2O can be produced by *Nitrosomonas* was of purely academic interest until it was demonstrated that aerobic soils can produce significant amounts of N_2O (Bremner and Blackmer, 1978). This was shown to be a result, not of the existence of anaerobic micro-niches, where denitrification could occur, but of N_2O production during nitrification, as ammonium ion, not nitrate, stimulated the production of N_2O (Bremner and Blackmer, 1978). A summary of recent studies is given by Bremner and Blackmer (1980).

The possible pathways of N_2O production during nitrification and denitrification are outlined in Fig. 2.

For the oceans, N_2O production also seems to be formed primarily through nitrification (Cohen and Gordon, 1978, 1979).

TABLE 1

ESTIMATED AND MEASURED NITROUS OXIDE AS PERCENTAGE OF TOTAL GASEOUS DENITRIFICATION LOSSES

Reference	ESTIMATED $\%N_2O$
CAST (1976)	6
Crutzen (1976)	-8
Munn (1977)	7
Delwiche (1978)	-20
Söderlund and Svensson (1976)	25-44
Sze and Rice (1976)	9-50
McElroy *et al.* (1977)	74
	MEASURED
Stefanson (1972)	0.8-94
Ryden *et al.* (1979a)	0.9-93
Gilliam *et al.* (1978)	1-80
Rolston and Broadbent (1976)	2-67
Rolston *et al.* (1976)	5-26
Ryden *et al.* (1979b)	12-18 [a]
	2-100 [b]
Burford and Stefanson (1973)	14-94

[a] based on extrapolated losses

[b] based on given N_2/N_2O ratios

Other Possible Pathways for N_2O Production

Bollag and Tung (1972) reported that N_2O was released during nitrite reduction by two *Fusarium* spp., and they concluded that fungi may participate, at least indirectly, in N_2O production. Recently, it has also been shown that N_2O can be produced by facultative anaerobic bacteria during reduction of nitrate to ammonium ion (Tiedje *et al.*, 1979). The importance of this process in soils or natural waters is unknown. Further, Hahn and Junge (1977) suggested that N_2O may be produced as a by-product during nitrogen assimilation from nitrate and nitrite by phytoplankton and higher plants, but no evidence was presented to support this hypothesis.

Various hypotheses have also been advanced for the possibilities of chemical production of N_2O in soils, through chemical decomposition of hydroxylamine or nitrite. The reader is referred to Bremner and Blackmer (1980) for a discussion on recent studies of the role of chemical production of N_2O in soils.

ABIOTIC NITROGEN FIXATION

Nitrous oxide is produced during combustion (Pierotti and Rasmussen, 1976; Weiss and Craig, 1976), but the total production rate is probably less than 4 Tg N_2O-N yr^{-1} (Table 2). Lightning has been suggested to be a major cause of N_2O production in the atmosphere (Dubin and Zipf, 1977), but the rates are very uncertain. Hahn and Junge (1977) estimated a range of 15-90 Tg N_2O-N yr^{-1}.

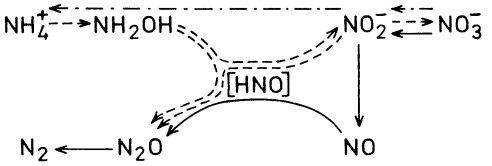

Figure 2: Proposed pathways for N_2O production assuming nitroxyl as the unidentified intermediate (Rosswall, 1978).

TABLE 2

ESTIMATES OF GLOBAL PRODUCTION RATES OF NITROUS OXIDE

Source	Tg N yr^{-1}	Reference
Combustion	3.5	Pierotti and Rasmussen (1976)
	1.6	Weiss and Craig (1976)
Lightning	15-90	Hahn and Junge (1977)
Denitrification	36-149	Söderlund and Svensson (1976)
	120	McElroy *et al.* (1977)
	130	Junge (1974)
	130	Sweeney *et al.* (1978)
	15-200	Cicerone *et al.* (1978)
	200	Hahn (1979)
Nitrification	4-10 (oceans only)	Cohen and Gordon (1979)

GLOBAL RATES OF N_2O PRODUCTION

Recent estimates of N_2O production rates are summarized in Table 2. The estimates are very crude, and the production rates from denitrification, considered to be the major source, range from 15 to 200 Tg yr^{-1}.

Sinks for N_2O are even less known. According to Hahn (1979), a likely value for the stratospheric sink is 20 Tg yr^{-1}, and a further 3 Tg yr^{-1} may be destroyed by homogeneous reactions in the troposphere. Biological reactions known to be possible sinks for N_2O include denitrification, as discussed above. Nitrogenase can reduce N_2O to N_2 (Hardy and Burns, 1973), but in view of the fact that ambient concentrations of dinitrogen are six orders of magnitude larger than that of N_2O, this process is probably of no importance in natural environments (Rosswall, 1978). Vedenina and Zavarzin (1977) have shown that N_2O can be removed by biological processes in soil and water under aerobic conditions. The nature of this process is not known, and it has only been observed at high N_2O concentrations (15%). Both soils (Blackmer and Bremner, 1976; Brice *et al.*, 1977; Delwiche *et al.*, 1978) and oceans (Cohen, 1978) have been shown to act as sinks for N_2O, presumably through the action of denitrifying bacteria, although the probability of soils acting as a sink has been questioned (Freney *et al.*, 1978). It seems unlikely, however, that soil and ocean denitrification represents the unknown sink which accounts for 180 Tg N_2O-N yr^{-1} (Hahn, 1979). Delwiche *et al.* (1978) have also suggested that an unknown biological sink exists.

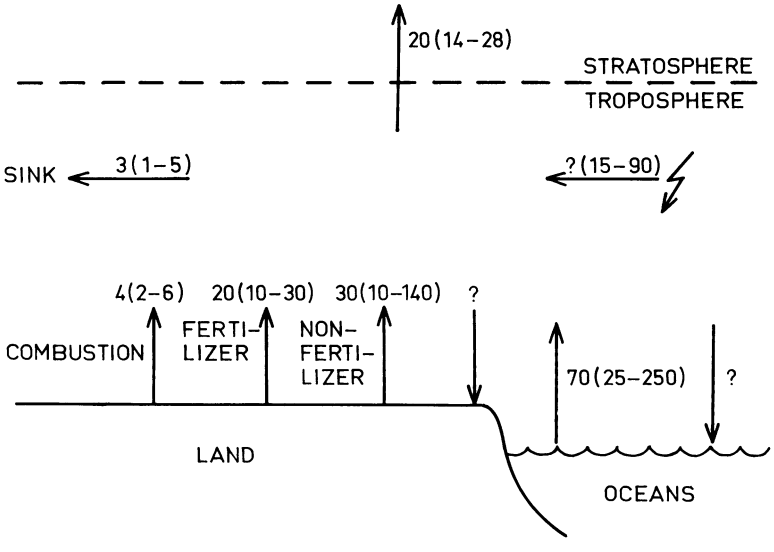

Figure 3: Proposed global budget for N_2O (data from Hahn, 1979).

Any attempt to construct a global budget for N_2O, such as the one proposed by Hahn (1979; Fig. 3), must take a number of factors into account, all of which are interdependent. Assuming that the biosphere is in an approximate steady state, denitrification losses should approximately equal global nitrogen-fixation rates, estimated at 220-320 Tg yr^{-1} (Söderlund and Svensson, 1976). It does not seem realistic from data vailable (Table 2) that N_2O accounts for more than 30% of total denitrification losses; probably the percentage is less. Taking possible nitrification losses into account, 30% of fixed nitrogen lost as N_2O may perhaps be a reasonable upper limit. There must, however, also be consistency in the data base with regard to probable residence times of N_2O in the atmosphere (Table 3), and the lower limit is probably 12-14 years. In Fig. 4, rates of denitrification are plotted versus turnover times for N_2O for different cases of N_2O losses as the percentage of total losses (calculated according to Johnston, 1977). If it is assumed that the global nitrogen cycle is in an approximate steady state, denitrification losses should equal nitrogen fixation. It can be seen that with a denitrification rate equal to nitrogen-fixation rate of 220-320 Tg yr^{-1} the residence time of N_2O in the atmosphere cannot be shorter than 12 years if the N_2O production is less than 30% of total N_2 and N_2O losses. If, on the other hand, the residence time is 30 years, a lower limit of ca. 12% for N_2O losses is obtained. As there is a need for consistency, by combining data from microbiological and meteorological observations, one can more effectively limit the possibilities for the three considered variables than by analyzing data for only one of them.

TABLE 3

ESTIMATED N_2O RESIDENCE TIMES

Reference	Residence time (yr)
Junge (1974)	8
Crutzen (1976)	10
Goldan *et al.* (1978)	11-16
Cicerone *et al.* (1978)	14
McElroy *et al.* (1977)	15
Sze and Rice (1976)	20
Rasmussen and Pierotti (1978)	25-30
Liu *et al.* (1977)	30-100
Bates and Hays (1967)	70
Johnston and Selwyn (1975)	170 [a]

[a] Based on known sinks.

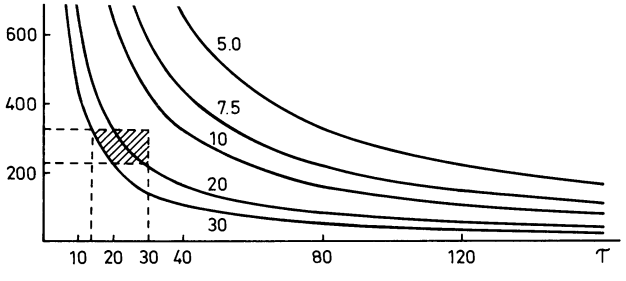

Figure 4: The atmospheric residence time (T, years) as a function of total denitrification loss to the atmosphere ($N_2O + N_2$) for different percentages (5.0-30%) of N_2O loss to total loss ($N_2O + N_2$) (0-650 Tg yr^{-1}).

CONCLUSIONS

Our knowledge of the processes leading to production and destruction of N_2O is poor. It seems that so far we have not even correctly assessed what the important production processes are, and nitrification may be a significant source of global N_2O in view of the extensive use of nitrogen fertilizers on aerobic soils. The possibilities for N_2O production from biological processes other than denitrification and nitrification should receive increased attention. The possibility of N_2O assimilation should be investigated further using different organisms and environmental conditions. Microbiological and meteorological observations, when combined, offer a powerful tool for assessing the probable limits for variables such as N_2/N_2O ratios in denitrification, and tropospheric residence times of N_2O.

This presentation has focused on N_2O production. Our knowledge of NO and NO_2 production from soils and waters is even scantier (e.g., Söderlund and Svensson, 1976; Galbally and Roy, 1978). Further attention should be given to their influence on the global nitrogen cycle.

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APPENDIX 1

Global Life Support System

A joint statement issued at the time of SCOPE General Assembly 1979 by Dr. Mostafa K. Tolba, Executive Director, United Nations Environment Programme, and Professor Gilbert F. White, President of the Scientific Committee on Problems of the Environment (SCOPE) of the International Council of Scientific Unions (ICSU).

The time is ripe to step up and expand current efforts to understand the great interlocking systems of air, water and minerals nourishing the earth. This is essential for a reliable assessment of the opportunities for enhancing food production on land and sea. Moreover, without vigorous action toward that goal, nations will be seriously handicapped in trying to cope with proven and suspected threats to ecosystems, and to human health and welfare, resulting from alterations in the cycles of carbon, nitrogen, phosphorus, sulfur and related materials.

Much has been learned over the years about these movements in and around the planet. However, only recently have scientists begun to see them in sufficient detail and perspective to permit establishing a base for prudent estimates of what may happen if people go on making major changes in energy and material flows.

The exchanges of these elements affect the productivity and quality of land, water and air. They depend upon many physical, chemical and biological processes, some of which are still unknown. It now is beginning to be possible to make preliminary estimates of global and regional budgets of the cycles. Scientists are striving to estimate the speed with which the exchanges and chemical transformations take place among the various components of the earth, ocean and atmosphere. Sometimes these processes operate rather rapidly and are effective in transforming material over great distances. For instance, the atmospheric carbon dioxide content continues to increase even in Antarctica, far from major sources of fossil fuel emissions. In contrast, the processes sometimes operate slowly or over small distances, as exemplified by the centuries that it may take before polluted groundwater is purified.

Society depends upon this life-support system of the planet Earth for sustained and increased production of food, fodder, fibre and fuel. Mankind also has a major influence on these cycles, as revealed by disturbances in the carbon, nitrogen and phosphorus flows. Vast amounts of carbon are transferred from the earth to the atmosphere through burning of fossil fuel. The input of carbon dioxide to the atmosphere is intensified by the clearing of tropical forests and cultivation of land. This increase in atmospheric carbon dioxide might eventually affect climatic patterns.

Nitrogen and phosphorus fertilizers are applied to increase agricultural production as a part of the attempt to meet the world food needs. Better understanding of the function of the nitrogen and phosphorus cycles will help to optimize the use of limited land resources. In the near future the industrial production of nitrogen in fertilizers will exceed the quantities produced by natural biological nitrogen fixation. The effects of these additions on the global nitrogen cycle are the object of searching speculation. For example, nitrogen oxides, of which nitrogen fertilizers are one source, may be important factors affecting the stability of the stratospheric ozone layer. Yet the rates of production, transfer and destruction of nitrogen oxides on the global scale are at present known only in broad outline.

Understanding the working of the biogeochemical cycles is made more difficult - and more necessary for policy purposes - by the evidence that each one of the cycles influences the others and may be related to trace elements and toxins. It is impossible to assess the capacity of biota to bind an atmospheric excess of carbon dioxide into biomass without considering other limiting elements such as nitrogen and phosphorus. The acidifying effect of sulfur in the atmosphere may affect the productivity of some land and aquatic ecosystems. These and other major dependencies must be appraised.

If in the next few years we achieve a better comprehension of these cycles we shall forge a powerful tool for identifying ways in which mankind can beneficially influence and utilize the resources of soil, vegetation, water, and atmosphere. We shall also possess a means of assessing many of the potentially serious risks to the global environment which so far have been studied without an adequate general frame of reference.

The work carried out so far is only a promising beginning. Based on results of such international scientific co-operation as the International Hydrological Decade (IHD), the Man and the Biosphere Programme (MAB), the Global Atmospheric Research Programme (GARP) and the Studies on Outer Limits to Biosphere Tolerance (UNEP) it is now possible to make crude estimates of the quantities and rates of exchange involved at the global level and reach preliminary indications of possible risks to the global environment. Nevertheless, these are insufficient to permit accurate prediction of how these cycles can be used to meet the increasing needs of mankind without causing undue deterioration of the environment. Presently, knowledge of the magnitude, rate and in some cases even the direction of the exchanges of basic elements between oceans and atmosphere is severely limited by lack of reliable data.

Over the next decade it should be possible to establish the essential basis for such understanding of the global life-support system. To do so will demand expanded co-operation and greater commitment to basic studies involving many individual scientists and new ventures such as the World Climate Programme, and will call for an integrated effort of many disciplines, including scientists from all regions of the world. Continuation of efforts at the present level will be too little, too late.

Accordingly, we draw attention to the fundamental scientific importance of understanding the biogeochemical cycles which link and unify the major chemical and biological processes of the earth's surface and the atmosphere. The results will have practical significance for all of us who inhabit an earth with limited resources and who, by our actions, increasingly affect the quality of the human environment. Further, we invite members of the scientific community in the various disciplines to contribute to the design and execution of a collective endeavour.

Stronger support by governments and closer co-operation among them and with intergovernmental agencies and individual scientists are essential if the opportunities are to be fully realised. Steps should be taken by the responsible agencies to stimulate a comprehensive research programme suited to the needs.

By 1982, ten years after the United Nations Conference on the Human Environment in Stockholm, we hope to see a major advance in understanding of the complex interactions among these cycles. If such advances were realized, it should be possible to present results promptly in a form useful to national policy-makers, and be a practical contribution to the requirements of environmental improvement in various parts of the world.

MECHANISMS OF NITROUS OXIDE PRODUCTION IN SOILS

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INTRODUCTION

Recent research by atmospheric scientists has created international concern that increased use of nitrogen fertilizers to aid world food production will increase emission of nitrous oxide (N_2O) from soils to the atmosphere via denitrification of fertilizer-derived nitrate and thereby promote destruction of the stratospheric ozone layer protecting the biosphere from biologically harmful ultraviolet radiation from the sun (see Council for Agricultural Science and Technology, 1976; McElroy *et al.*, 1977; Crutzen and Ehhalt, 1977; Liu *et al.*, 1977). This threat has stimulated extensive research on factors affecting N_2O emissions from soils and the effects of nitrogen fertilizers on these emissions. The purpose of this article is to report recent research in our laboratory relating to the mechanisms of N_2O production in soils.

Until very recently, it was generally assumed that N_2O is produced in soils only through denitrification of nitrate under anaerobic conditions and that detection of this gas in soil air or in gases evolved from soils is valid evidence for the occurrence of both denitrification and anaerobic conditions (see Flühler *et al.*, 1976a,b). The possibility that at least some of the N_2O evolved from soils may be produced by nitrifying microorganisms during oxidation of ammonium to nitrate has not been ignored by workers aware of evidence for N_2O production by pure cultures of *Nitrosomonas europaea* under certain conditions (Yoshida and Alexander, 1970, 1971; Ritchie and Nicholas, 1972, 1974) and has been mentioned in several recent articles (e.g. Blackmer and Bremner, 1977a; Hahn and Junge, 1977; Bremner, 1978; Bremner and Blackmer, 1978; Freney *et al.*, 1978, 1979). However the possibility that a significant amount of the N_2O evolved from soils is produced by nitrifying microorganisms has been generally regarded as very remote (see Focht, 1974). The first indication that this possibility merits serious attention was provided by studies showing that emission of N_2O can be detected when unamended soils are incubated under highly aerobic conditions and that N_2O emissions from well-aerated soils treated with nitrifiable forms of N greatly exceed those from soils treated with nitrate (Blackmer and Bremner, 1977a). These observations indicated that N_2O is produced in soils during nitrification of ammonium (NH_4^+) under aerobic conditions as well as by denitrification of nitrate under anaerobic conditions and that nitrifying microorganisms contribute significantly to N_2O emissions from soils treated with the NH_4^+ or NH_4^+-producing compounds generally used as N fertilizers. Subsequent work in our laboratory supported these conclusions. For example, we found that emissions of N_2O from well-aerated soils treated with nitrifiable forms of fertilizer N are greatly reduced by addition of nitrapyrin [2-chloro-6-(trichloromethyl)-pyridine], which inhibits nitrification of NH_4^+ in soils (Goring, 1962a,b; Bundy and Bremner, 1973), and that, whereas N_2O emissions from well-aerated soils are greatly increased by addition of any nitrifiable form of N (ammonium sulfate, urea, alanine, etc.), they are not significantly affected by addition of nitrate, glucose, or nitrate plus glucose (Bremner and Blackmer, 1978; Bremner *et al.*, 1978; Minami *et al.*, 1978). These observations indicated that most of the N_2O evolved from well-aerated soils treated with the N fertilizers used most extensively to increase crop production is generated during nitrification of the N in these materials and that very little of this gas is produced by denitrification of nitrate formed by nitrification of fertilizer N. We report here what would appear to be unequivocal evidence for these conclusions and for the further conclusion that, even when soils are not very well aerated, much of the fertilizer-derived N_2O evolved when they are treated with NH_4^+ or NH_4^+-producing fertilizers is generated by nitrifying microorganisms. This evidence was obtained by studying the effects of acetylene, nitrapyrin, and soil moisture content on emission of N_2O from NH_4^+- and nitrate-treated soils. The effect of acetylene was studied because recent work has shown that this gas inhibits reduction of N_2O to N_2 by denitrifying microorganisms in soils or pure cultures without affecting reduction of nitrate to N_2O by these microorganisms (Balderston *et al.*, 1976; Yoshinari and Knowles,

1976; Klemedtsson *et al.*, 1977; Yoshinari *et al.*, 1977; Smith *et al.*, 1978) and also inhibits oxidation of NH_4^+ by *Nitrosomonas europaea* (Hynes and Knowles, 1978) and the nitrifying microorganisms in soils (Walter *et al.*, 1979).

EXPERIMENTAL

The soils used to study production of N_2O by microbial processes in soils were surface (0 - 15 cm) samples of Iowa soils used for corn or soybean production. Unless otherwise specified, experiments were performed with sieved (<2 mm) samples of field-moist soils and involved determination of the N_2O produced when samples containing the equivalent of 30 g dry soil were incubated at 30°C in sealed 1,200-ml bottles after various treatments and adjustment of their moisture contents to 20-150% of their water-holding capacities (WHC). The bottles used were fitted with ground-glass joints and were sealed by glass stoppers fitted with ground-glass joints and glass stopcocks. Their atmospheres were sampled for gas analyses and renewed at 2-4 day intervals.

The soils used to study production of N_2O by chemical processes in soils were surface (0 - 15 cm) samples selected to obtain a wide range in properties, including pH (3.6 - 8.2), texture (2 - 94% sand, 1 - 64% clay), and organic-matter content (0.30 - 15.0% organic C). Before use, each sample was air-dried and crushed to pass a 2-mm screen. Unless otherwise specified, experiments were performed with 10 g samples of air-dried soil sterilized by autoclaving at 120° for 30 min after treatment with 3 ml water. Sterilization was performed in 125-ml Erlenmeyer flasks fitted with ground-glass joints. The sterilized soil samples in these flasks were treated with 5 ml water, and a glass vial containing 10 ml $NH_2OH.HCl$ or $NaNO_2$ solution was placed in each flask. The flasks were then sealed with glass stoppers fitted with a ground-glass joint and glass stopcock and, unless otherwise specified, were connected to a manifold system for replacement of air by He containing 20% (v/v) O_2. They were then tipped and swirled so that the contents of the vial were mixed thoroughly with the soil. They were subsequently placed in an incubator maintained at 30°C, and their atmospheres were sampled at intervals for N_2O analysis.

Where analyses for N_2O and N_2 are expressed as μg N_2O-N or N_2-N g^{-1} soil, they were performed as described by Blackmer and Bremner (1977b). Where analyses for N_2O are expressed as ng N_2O-N g^{-1} soil, they were performed as described by Blackmer and Bremner (1978a). Analyses of soils for pH, organic C, sand, silt, clay, nitrate-N, and nitrite-N were carried out as described by Blackmer and Bremner (1977c). Other soil analyses reported were performed as described by Cihacek (1979).

All experiments and analyses reported were carried out in duplicate or triplicate.

PRODUCTION OF N_2O BY MICROBIAL PROCESSES IN SOILS

The effects of acetylene on the amounts of N_2O-N and (nitrate + nitrite)-N produced on incubation of well-aerated samples of NH_4^+-treated soils are shown in Table 1. Besides confirming that acetylene is a potent inhibitor of nitrification in soils, the data reported show that this gas inhibited production of N_2O in NH_4^+-treated soils.

The effect of soil moisture content on emission of N_2O from samples of Canisteo soil treated with NH_4^+ or nitrate is shown in Table 2. Emission of N_2O from the NH_4^+-treated samples increased markedly with increase in soil moisture content and greatly exceeded emission of N_2O from nitrate-treated samples at the same moisture content, even when the samples were saturated with water.

The effects of acetylene and nitrapyrin on production of N_2O in samples of Storden soil incubated at different moisture contents after treatment with N as ammonium sulfate or potassium nitrate (200 μg N g^{-1} soil) are shown in Table 3. Besides confirming the results obtained in the work reported in Table 2, the data in Table 3 show that both acetylene and nitrapyrin almost completely inhibited production of N_2O in NH_4^+-treated samples.

Considered together, the results reported in Tables 1-3 leave little doubt that most of the N_2O evolved from soils treated with NH_4^+ or NH_4^+-producing fertilizers is generated by nitrifying microorganisms during oxidation of NH_4^+ to nitrate and that very little of this gas is produced through denitrification of nitrate produced by this oxidation. Further

TABLE 1

EFFECT OF ACETYLENE (C_2H_2) ON AMOUNTS OF N_2O-N AND (NITRITE + NITRATE)-N PRODUCED ON INCUBATION OF NH_4^+-TREATED SOILS UNDER AEROBIC CONDITIONS [a]

Soil	pH	Organic C(%)	N_2O-N produced in 12 days (ng g^{-1} soil)		(Nitrate + nitrite)-N produced in 12 days (μg g^{-1} soil)	
			Without C_2H_2	With C_2H_2	Without C_2H_2 [b]	With C_2H_2 [b]
Storden	8.2	0.4	170	<1	83(4)	<1(<1)
Harps	8.1	4.2	208	<1	105(11)	<1(<1)
Webster	6.8	3.8	30	<1	82(9)	<1(<1)
Nicollet	6.2	2.6	24	<1	67(6)	<1(<1)

[a] Soils treated with ammonium sulfate (100 μg N g^{-1} soil) were incubated (30°C; 50% WHC) under air or air containing 0.1% (v/v) C_2H_2.

[b] Figures in parentheses indicate (nitrate + nitrite)-N (μg g^{-1} soil) produced in control (no NH_4^+ added).

TABLE 2

EFFECT OF SOIL MOISTURE CONTENT ON EMISSION OF N_2O FROM SAMPLES OF CANISTEO SOIL TREATED WITH DIFFERENT FORMS OF N [a]

Form of N added [b]	N_2O-N evolved in 30 days (ng g^{-1} soil)						
	20% WHC	30% WHC	40% WHC	50% WHC	75% WHC	100% WHC	150% WHC
None	7	7	8	8	17	58	27
Nitrate	9	9	9	9	9	263	2673
NH_4^+	68	72	135	208	412	2199	3596

[a] Field-moist samples of soil (pH 8.2, 3.8% organic C, 13 μg nitrate-N g^{-1} soil) adjusted to different moisture contents were incubated at 30°C after treatments specified.

[b] N was added at rate of 100 μg g^{-1} soil (nitrate N as KNO_3, NH_4^+-N as $(NH_4)_2SO_4$).

TABLE 3

EFFECTS OF ACETYLENE AND NITRAPYRIN ON PRODUCTION OF N_2O IN SAMPLES OF STORDEN SOIL INCUBATED AT DIFFERENT MOISTURE CONTENTS AFTER TREATMENT WITH N AS AMMONIUM SULFATE OR POTASSIUM NITRATE (200 μg N g^{-1} soil) [a]

Form of N added	Soil moisture content (% WHC)	N_2O-N evolved in 14 days (ng g^{-1} soil)		
		Control[b]	Acetylene[c]	Nitrapyrin[d]
NH_4^+	70	419	<1	<1
	100	442	3	2
	130	3,160	1	1
Nitrate	70	<1	1	<1
	100	2	1	1
	130	4	4	2

[a] Soil used had a pH of 8.2 and contained 0.4% organic C and 5 μg nitrate-N g^{-1}.

[b] N-treated soil was incubated under air.

[c] N-treated soil was incubated under air containing 0.1% (v/v) C_2H_2.

[d] N-treated soil was treated with nitrapyrin (6 μg g^{-1} soil) and incubated under air.

evidence for this conclusion is presented in Table 4, which shows that nitrapyrin completely inhibited production of N_2O in samples of Storden soil treated with different amounts of NH_4^+, but had no effect on emission of N_2O from samples treated with different amounts of nitrate. It is also shown in Table 4 that, whereas emission of N_2O from NH_4^+-treated samples increased very markedly with the amount of NH_4^+ added, only trace amounts of N_2O were evolved from samples treated with different amounts of nitrate. It seems reasonable to conclude that very little, if any, of the N_2O evolved from the NH_4^+-treated samples was produced by denitrification of nitrate, particularly since recent work (Blackmer and Bremner, 1978b) showed that nitrate has an inhibitory effect on anaerobic reduction of N_2O to N_2 by soil microorganisms and that, because of this effect, the ratio of N_2O:N_2 in the gases evolved from soils through denitrification of nitrate increases markedly with the concentration of nitrate.

It is well established that nitrapyrin inhibits nitrification of NH_4^+ in soils, and work by Henninger and Bollag (1976) has indicated that nitrapyrin has very little, if any, effect on denitrification of nitrate in soils. However, for unequivocal interpretation of the effects of nitrapyrin in the studies reported in Tables 3 and 4, it is necessary to determine the effect of this compound on production of N_2O by nitrifying microorganisms during oxidation of NH_4^+ in soils and its effects on production and reduction of N_2O by denitrifying microorganisms during anaerobic denitrification of nitrate in soils.

Recent work (Bremner and Blackmer, 1979) in collaboration with Dr. E. L. Schmidt has shown that nitrapyrin completely inhibits nitrification and production of N_2O in sterilized (autoclaved) soils treated with ammonium sulfate and pure cultures of the chemoautotrophic nitrifying microorganisms believed to be responsible for oxidation of NH_4^+ to nitrite in soils (strains of *Nitrosomonas*, *Nitrosopira* and *Nitrosolobus*). This supports our conclusion that nitrapyrin decreases emission of N_2O from well-aerated soils treated with nitrifiable forms of N by inhibiting production of N_2O by chemoautotrophic nitrifiers. Shattuck and Alexander (1963) found that nitrapyrin was toxic to autotrophic nitrifiers (*Nitrosomonas*

TABLE 4

EFFECT OF NITRAPYRIN ON EMISSION OF N_2O FROM SAMPLES OF STORDEN SOIL TREATED WITH DIFFERENT AMOUNTS OF N AS AMMONIUM SULFATE OR POTASSIUM NITRATE [a]

Form of N added	Amount of N added ($\mu g\ g^{-1}$ soil)	Amount of N_2O-N evolved ($ng\ g^{-1}$ soil) [b]							
		2 days		7 days		14 days		21 days	
		-NP	+NP	-NP	+NP	-NP	+NP	-NP	+NP
None	0	1	<1	1	<1	1	<1	1	<1
NH_4^+	200	57	<1	382	<1	426	<1	428	<1
	500	118	<1	1,210	<1	1,680	<1	1,970	<1
	1,000	132	<1	2,350	<1	3,760	<1	4,940	<1
Nitrate	200	<1	<1	<1	<1	<1	<1	<1	<1
	500	<1	<1	<1	<1	<1	<1	<1	<1
	1,000	<1	<1	<1	<1	<1	<1	<1	<1

[a] Soil (30 g) was incubated (70% WHC, 30°C) after treatment with 0, 6, 15, or 30 mg N as $(NH_4)_2SO_4$ or KNO_3 and 0 or 180 µg nitrapyrin. Soil used had a pH of 8.2 and contained 0.4% organic C and 5 µg nitrate-N g^{-1}.

[b] NP, nitrapyrin (6 µg g^{-1} soil).

europaea and *Nitrobacter agilis*), but did not affect growth or activity of heterotrophic nitrifiers (strains of *Aspergillus flavus*).

Data obtained in studies of the effects of nitrapyrin on production and subsequent reduction of N_2O during denitrification of nitrate in soils under anaerobic conditions are given in Tables 5 and 6. The data reported show that nitrapyrin had a measurable effect on the amounts of N_2O-N, N_2-N and (N_2O + N_2)-N detected in the atmospheres of nitrate-treated soils incubated anaerobically for various times and that its effect increased with the amount added. It is evident from Tables 5 and 6, however, that the effect of adding nitrapyrin, at the rate of 6 µg g^{-1} soil, on production or reduction of N_2O by denitrifying microorganisms during any denitrification of nitrate that may have occurred in the experiments reported in Tables 3 and 4 must have been insignificant compared with its effect on production of N_2O through nitrification of NH_4^+.

Our finding that N_2O emissions from NH_4^+-treated soils increase markedly with increase in soil moisture content (i.e. with decrease in the availability of oxygen to soil microorganisms) is readily explained if nitrifying microorganisms are responsible for these emissions because Ritchie and Nicholas (1972, 1974) showed that *Nitrosomonas europaea* produces an enzyme (nitrite reductase) that allows it to reduce nitrite to N_2O during oxidation of NH_4^+ and thereby utilize nitrite as a terminal electron acceptor instead of oxygen when the supply of oxygen is limited.

Our conclusion that nitrifying microorganisms contribute substantially to N_2O emissions from soils fertilized with NH_4^+ or NH_4^+-producing fertilizers is supported by field studies carried out in collaboration with G.A. Breitenbeck. These studies have shown that N_2O emissions from plots fertilized with N as anhydrous ammonia, ammonium sulfate, or urea greatly exceed those from corresponding plots fertilized with the same amount of N as calcium nitrate (Bremner and Blackmer, 1979).

TABLE 5

EFFECTS OF DIFFERENT AMOUNTS OF NITRAPYRIN ON AMOUNTS OF N_2 AND N_2O DETECTED AT DIFFERENT TIMES DURING ANAEROBIC INCUBATION OF KILLDUFF SOIL TREATED WITH NITRATE (25 µg nitrate-N g^{-1} soil) [a]

Incubation time (h)	N_2O-N detected (µg g^{-1} soil)			N_2-N detected (µg g^{-1} soil)			$(N_2O + N_2)$-N detected (µg g^{-1} soil)		
	No NP	10 µg NP g^{-1} soil	30 µg NP g^{-1} soil	No NP	10 µg NP g^{-1} soil	30 µg NP g^{-1} soil	No NP	10 µg NP g^{-1} soil	30 µg NP g^{-1} soil
2	0	0	0	0	0	0	0	0	0
4	0	0	0	0.3	0	0	0.3	0	0
6	0.3	0.3	0.2	1.3	0	0	1.6	0.3	0.2
8	1.2	1.0	0.6	1.7	0.1	0.1	2.9	1.1	0.7
10	5.5	4.7	3.2	2.2	1.8	1.1	7.7	6.5	4.3
12	13.9	14.0	9.6	9.9	8.1	6.7	23.8	22.1	16.3
14	2.3	4.9	6.4	22.4	17.5	15.2	24.7	22.4	21.6
16	0.8	1.2	1.6	24.9	22.0	20.4	25.7	23.2	22.0
18	0	0.1	0.2	26.1	25.2	24.4	26.1	25.3	24.6
20	0	0	0	26.7	26.2	25.2	26.7	26.2	25.2

[a] 30-g samples of air-dried soil (pH 6.0, 2.3% organic C, 2.0 µg nitrate-N g^{-1}) treated with 10 ml water containing 0, 300 or 900 µg nitrapyrin (NP) and 5 ml water containing 750 µg N as KNO_3 were incubated (30°C) under He in 1.2-litre flasks for various times.

TABLE 6

EFFECTS OF DIFFERENT AMOUNTS OF NITRAPYRIN ON AMOUNTS OF N_2O DETECTED AT VARIOUS TIMES DURING ANAEROBIC INCUBATION OF NITRATE-TREATED SOILS [a]

Soil	Amount of nitrapyrin added ($\mu g\ g^{-1}$ soil)	Amount of N_2O-N detected ($\mu g\ g^{-1}$ soil)						
		2 h	6 h	10 h	14 h	18 h	22 h	36 h
Dickinson (pH 5.3, 1.2% organic C, 12 μg nitrate-N g^{-1})	0	0	0.5	3.0	7.9	7.6	6.0	0.7
	10	0	0.4	2.6	6.9	6.9	6.5	1.0
	30	0	0.1	1.9	6.2	6.6	6.9	1.9
Webster (pH 7.1, 3.3% organic C, 9 μg nitrate-N g^{-1})	0	0.1	0.4	7.2	7.6	0.5	0	0
	10	0	0.3	6.9	7.9	1.0	0	0
	30	0	0.3	6.6	9.4	1.3	0	0
Palms (pH 6.2, 15.0% organic C, 55 μg nitrate-N g^{-1})	0	1.1	7.9	27.0	0	0	0	0
	10	1.1	7.6	32.0	0	0	0	0
	30	1.0	7.5	26.7	0	0	0	0

[a] 30-g samples of air-dried soils treated with 10 ml water containing 0, 300 or 900 μg nitrapyrin and 5 ml water containing 750 μg N as KNO_3 were incubated (30°C) under He in 1.2-litre flasks for various times.

Our finding that nitrapyrin inhibits production of N_2O by nitrifying microorganisms and greatly decreases emission of N_2O from soils treated with NH_4^+ or NH_4^+-producing fertilizers indicates that this chemical has significant potential value for reduction of N_2O emissions from fertilized soils. Nitrapyrin is marketed as a soil nitrification inhibitor by Dow Chemical U.S.A. under the trade mark of N-Serve and has been registered for use as a fertilizer amendment to reduce losses of fertilizer nitrogen resulting from denitrification and leaching of fertilizer-derived nitrate. Besides inhibiting production of N_2O by nitrifying microorganisms, it retards oxidation of NH_4^+ to nitrate and should, therefore, decrease the amount of N_2O produced in soils through denitrification of nitrate by denitrifying microorganisms.

Although further research is needed to identify the various factors affecting production of N_2O by nitrifying microorganisms in soils, our work indicates that the amount of N_2O produced by these microorganisms increases with the amount of readily nitrifiable soil N (Table 4) and the moisture content (Tables 2 and 3), temperature (Table 7) and pH (Bremner and Blackmer, 1978) of the soil. These conclusions are in harmony with the results of microbiological studies in collaboration with Dr. E. L. Schmidt because, besides showing that N_2O is evolved when sterilized (autoclaved) soils are treated with ammonium sulfate and pure cultures of chemoautotrophic nitrifying microorganisms, these studies have shown that production of N_2O in sterilized soils treated with ammonium sulfate and *Nitrosomonas europaea* increases markedly with the amount of NH_4^+-N applied and the moisture content of the soil.

Work during the past year using a variety of soils has supported previous evidence (Bremner and Blackmer, 1978) that production of N_2O during nitrification in soils treated

TABLE 7

EFFECT OF SOIL TEMPERATURE ON EMISSION OF N_2O FROM WELL-AERATED SAMPLES OF UNAMENDED AND AMMONIUM-TREATED HARPS SOIL [a]

Temperature (°C)	N_2O-N evolved (ng g^{-1} soil)							
	Unamended soil				NH_4^+-treated soil			
	2 days	4 days	8 days	16 days	2 days	4 days	8 days	16 days
5	1	1	2	2	1	2	4	16
10	1	2	2	3	5	13	39	130
20	2	2	3	4	21	94	168	172
30	2	3	4	5	76	223	239	242

[a] Field-moist samples of unamended or NH_4^+-treated soil (pH 8.1, 4.2% organic C, 11 µg nitrate-N g^{-1}) were incubated (60% WHC) at different temperatures for various times. NH_4^+ was added as $(NH_4)_2SO_4$ (200 µg N g^{-1} soil).

with nitrifiable forms of N is more extensive in calcareous than in neutral soils and that production of N_2O in calcareous soils increases markedly with the concentration of NH_4^+. Since it is well established that a combination of high soil pH and high NH_4^+ concentration promotes accumulation of nitrite during nitrification in soil treated with NH_4^+ or NH_4^+-producing compounds (see Alexander, 1977), these observations are in harmony with Ritchie and Nicholas's (1972) conclusion that nitrifying microorganisms reduce nitrite to N_2O to prevent intracellular accumulation of toxic amounts of nitrite.

Since our work on N_2O production in soils by microbial processes has been confined to Iowa soils, it is noteworthy that Freney *et al.* (1978, 1979) have reported detection of significant N_2O emissions from Australian soils at low moisture content and under seemingly highly aerobic conditions.

Our conclusion that nitrification deserves much greater attention in research on sources of atmospheric N_2O is supported by recent work suggesting that nitrification may be the major source of N_2O in the oceans (Cohen and Gordon, 1978; Elkins *et al.*, 1978).

PRODUCTION OF N_2O BY CHEMICAL PROCESSES IN SOILS

Several investigations have shown that nitrite is decomposed chemically in neutral and acidic soils with formation of small amounts of N_2O and larger amounts of N_2 and NO or NO_2 (see Bremner and Nelson, 1968; Nelson and Bremner, 1970). The possibility that some of the N_2O evolved from soils may be formed by chemical decomposition of nitrite produced during oxidation of NH_4^+ to nitrate by nitrifying microorganisms or reduction of nitrate by denitrifying microorganisms (i.e. via chemodenitrification) cannot, therefore, be ignored. The possibility that some of the N_2O evolved from soils may be formed by chemical decomposition of hydroxylamine (NH_2OH) produced by nitrifying or nitrate-reducing microorganisms also deserves consideration because NH_2OH has been identified as an intermediate in oxidation of NH_4^+ to nitrite by *Nitrosomonas europaea* and postulated as an intermediate in microbial reduction of nitrate to NH_4^+ (see Alexander, 1977), and several investigations have shown that NH_2OH is decomposed in soils with formation of N_2O and N_2 by processes that appear to be largely chemical (see Arnold, 1954; Nömmik, 1956; Bremner and Shaw, 1958; Nelson, 1978).

We have found that production of N_2O by chemical decomposition of NH_2OH in diverse soils greatly exceeds N_2O production by chemical decomposition of nitrite. This is illustrated

by Table 8, which shows the amounts of N_2O produced by decomposition of NH_2OH and nitrite in sterilized samples of 19 soils selected to obtain a wide range in properties. Studies of the correlations between the amounts of N_2O produced by chemical decomposition of NH_2OH in these soils and various soil properties showed that production of N_2O was not significantly correlated with most of the soil properties studied, but was very highly correlated with exchangeable Mn and with Mn dissolved by dithionite-citrate buffer, which reduces and extracts oxidized forms of Mn (Table 9). This suggests that NH_2OH decomposes in soils to produce N_2O by reacting with MnO_2 and (or) other reducible forms of Mn ($Mn_{ox} + 2NH_2OH \longrightarrow Mn_{red} + N_2O$).

TABLE 8

PRODUCTION OF N_2O BY CHEMICAL DECOMPOSITION OF HYDROXYLAMINE AND NITRITE IN 19 DIVERSE SOILS [a]

Soil	pH	Organic C (%)	Sand (%)	Clay (%)	N_2O-N produced in 120 h ($\mu g\ g^{-1}$ soil)	
					Hydroxylamine	Nitrite
Paaloa	3.6	3.06	2	48	136	34
Kochi	4.1	4.86	26	18	184	48
Tillamook	4.8	9.08	13	28	211	51
Wahiawa	5.1	1.42	7	64	793	5
Buckner	5.4	0.30	94	3	135	1
Davidson	5.6	3.24	14	36	726	2
Floyd	5.7	2.78	30	24	266	7
Cresco	5.8	2.32	32	22	288	5
Thurman	5.8	0.64	90	1	91	2
Weller	6.0	3.69	2	21	414	4
Glencoe	6.0	5.86	19	41	235	6
Marshall	6.2	1.60	3	38	314	2
Dickinson	6.3	0.55	90	5	142	2
Webster	6.7	2.93	34	30	273	2
Sac	7.2	2.48	10	34	409	2
Ida	7.8	0.88	5	24	276	<1
Storden	8.1	0.30	51	18	151	<1
Rosebud	8.1	0.59	57	19	101	<1
Canyon	8.2	0.89	56	22	76	<1
Average					275	9

[a] Autoclaved soil (10 g) containing 5 ml water was treated with 10 ml water containing 10 mg N as $NH_2OH.HCl$ or $NaNO_2$ ($He-O_2$ atmosphere).

Mann and Quastel (1946) postulated that NH_2OH is decomposed by MnO_2 as indicated by the following equation: $2MnO_2 + 2NH_2OH \longrightarrow 2MnO + N_2O + 3H_2O$). We have confirmed by gas chromatography that N_2O is produced on treatment of NH_2OH with MnO_2 and have found that the rate of production of N_2O increases markedly with decrease in the particle size of the MnO_2 used.

TABLE 9

CORRELATIONS BETWEEN N_2O PRODUCTION BY CHEMICAL DECOMPOSITION OF HYDROXYLAMINE IN 19 DIVERSE SOILS AND VARIOUS SOIL PROPERTIES

Soil property [a]	Correlation coefficient (r)		
	N_2O produced in 2 h	N_2O produced in 72 h	N_2O produced in 120 h
pH	-0.17	-0.18	-0.19
Organic carbon	-0.06	0.12	0.11
CCE	-0.12	-0.21	-0.26
Sand	-0.35	-0.54*	-0.56*
Silt	0.07	0.32	0.34
Clay	0.61**	0.64**	0.65**
Surface Area	0.07	0.26	0.30
CEC	-0.10	0.11	0.14
Exchangeable Ca	-0.23	-0.26	-0.29
Exchangeable Mg	-0.15	0.09	0.15
Exchangeable K	0.35	0.37	0.33
Exchangeable Na	0.04	0.05	0.03
Exchangeable Mn	0.58**	0.74***	0.74***
DCB-soluble Mn	0.93***	0.96***	0.95***
Exchangeable Fe	-0.15	-0.04	-0.06
DCB-soluble Fe	0.11	0.18	0.15

[a] CCE, $CaCO_3$ equivalent; CEC, cation-exchange capacity; DCB, dithionite-citrate buffer.

*, **, *** Significant at 5%, 1%, and 0.1% level, respectively.

Studies of the rate of production of N_2O in sterilized soils treated with different amounts of NH_2OH showed that production of N_2O is rapid and increases with the amount of NH_2OH added (Table 10). Other studies showed that production of N_2O in soil treated with NH_2OH is not markedly affected if the soil sample is sterilized by autoclaving at 120° for 30 min or is treated with toluene (0.1 ml g^{-1} soil) to inhibit microbial activity. This indicates that most of the N_2O evolved from unsterilized soil treated with NH_2OH is produced by nonbiological reactions and that autoclaving does not markedly affect the soil constituents responsible for these reactions. Tests with several soils showed that O_2 has little effect on production of N_2O in sterilized soils treated with hydroxylamine.

Although there seems no doubt that NH_2OH is produced during oxidation of NH_4^+ to nitrite by the autotrophic nitrifying microorganisms believed to be responsible for NH_4^+ oxidation in soils, there is no evidence for extracellular release of NH_2OH during microbial oxidation of NH_4^+ in soils. It is evident from work reported here, however, that failure to obtain such evidence could be due to rapid decomposition of NH_2OH by MnO_2 and other soil constituents. Verstraete and Alexander (1973) detected formation of small amounts of NH_2OH during seemingly heterotrophic nitrification of NH_4^+ in soil suspensions amended with ammonium sulfate and sodium acetate.

Several workers (e.g., Corbet, 1935; Arnold, 1954; Wijler and Delwiche, 1954; Vine, 1962) have postulated that N_2O is produced in soils through interaction of NH_2OH and

TABLE 10

AMOUNTS OF N_2O EVOLVED FROM STERILIZED SOILS TREATED WITH DIFFERENT AMOUNTS OF HYDROXYLAMINE [a]

Soil	NH_2OH-N added (μg g^{-1} soil)	N_2O-N evolved (μg g^{-1} soil)			
		0.5 h	2 h	4 h	48 h
Davidson	10	3	5	6	7
	100	38	66	72	80
	1,000	219	360	431	690
Weller	10	1	2	2	3
	100	12	19	32	34
	1,000	33	100	161	368
Ida	10	4	6	7	8
	100	29	57	75	78
	1,000	39	86	101	220

[a] Autoclaved soil (10 g) containing 5 ml water was treated with 10 ml water containing 100, 1,000 or 10,000 μg N as $NH_2OH.HCl$ (He-O_2 atmosphere). For analyses of soils used, see Table 8.

nitrite produced by soil microorganisms ($NH_2OH + HNO_2 \longrightarrow N_2O + 2H_2O$). However, experiments reported in Table 11 show that production of N_2O in sterilized soils treated with NH_2OH is not greatly increased by addition of nitrite and is more rapid than N_2O formation through reaction of NH_2OH with nitrite in the absence of soil. This indicates that, if N_2O is formed in soils through nonbiological transformations of NH_2OH produced by soil microorganisms, very little of this gas is generated through reaction of NH_2OH with nitrite.

TABLE 11

EFFECT OF NITRITE ON PRODUCTION OF N_2O IN STERILIZED SOILS TREATED WITH HYDROXYLAMINE [a]

Soil	N added (μg)		N_2O-N produced (μg)	
	Hydroxylamine	Nitrite	2 h	24 h
None	500	0	0	25
	0	500	0	0
	500	500	102	679
Davidson	500	0	286	368
	0	500	0	0
	500	500	313	388
Weller	500	0	129	204
	0	500	0	0
	500	500	140	220
Ida	500	0	339	427
	0	500	0	0
	500	500	370	446

[a] Autoclaved soil (10 g) was treated with 500 μg N as $NH_2OH.HCl$ and (or) 500 μg N as $NaNO_2$ in He-O_2 atmosphere (15 ml water in all treatments). When soil was treated with both $NH_2OH.HCl$ and $NaNO_2$, $NaNO_2$ was added immediately before $NH_2OH.HCl$. For analyses of soils used, see Table 8.

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THE MEASUREMENT OF DENITRIFICATION IN SOIL, USING $^{13}NO_3$

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INTRODUCTION

^{13}N, a radionuclide with a half-life of 10 min, has been used for a variety of purposes in medical and agricultural research and in a variety of forms, e.g. as $^{13}N_2$ for study of nitrogen fixation and translocation in plants, $^{13}NH_4$ and $^{13}NO_3$ for studying plant nitrogen uptake, $^{13}NO_3$ for studying denitrification, and $^{13}N_2O$ in medical research (Straatmann, 1977). The advantages of ^{13}N are the ease of detection, measurement and identification of volatile products, and the short half-life that permits several consecutive experiments on the same material. The very small quantities of the label used ensure that there is no alternation of equilibrium reactions, but the short half-life does mean that experiments must be of simple design and must be conducted with minimum preparation of substrate and near the production source of the isotope. Nitrogen flux in soil due to dissimilatory denitrification, a form of anaerobic respiration, is a major component of the nitrogen cycle (Nielsen and MacDonald, 1978) and, in the experiments reported, ^{13}N was used to examine the movement of the major components of the cycle in a relatively undisturbed soil and the evolution of gases by different bacterial cultures and soil cores.

In cyclic processes the rate of a reaction is critical and this isotope technique provides a highly sensitive method of following the movement of the compound in the soil and the rate of release of volatile products. The principal gases released are dinitrogen and nitrous oxide, and methods for their discrimination have recently been reviewed by Tiedje *et al.* (1979). The production and relative concentration of these two gases in soil is considered to be commonly due to the activity of denitrifying bacteria and is affected by a number of factors including nutrient availability, oxygen tension and concentrations of nitrate and nitrite (Firestone *et al.*, 1979).

ISOTOPIC METHODS

Two sources of ^{13}N were employed; both using a Van de Graaff accelerator. A pellet of Li_2CO_3 was irradiated by a 2 MeV deuteron beam from the accelerator and used to prepare a $^{13}NO_3^-$ solution of about 3.7 MBq activity (McNaughton and More, 1979). The continual emission of $^{13}N_2$ and oxides of nitrogen was obtained by irradiation of graphite and $^{13}N_2$ was trapped on a molecular sieve (4A) in a liquid nitrogen trap, after the oxides had been removed by a liquid nitrogen trap.

The experimental procedure (Fig. 1) normally used helium as a carrier gas. Two NaI detectors connected to amplifiers, single channel analysers, scalers, and a teletype were used to record activity and were calibrated with a ^{22}Na source. The first trap, molecular sieve 13X, was for the $^{13}N_2O$ and the second, molecular sieve 13X in liquid nitrogen, for the $^{13}N_2$. The interference of $^{13}N_2$ on the $^{13}N_2O$ count was corrected on the basis of a pure $^{13}N_2$ experiment. Results were computed, correcting for decay, and for $^{13}N_2$ interference on the $^{13}N_2O$ count were necessary. The counting period was 50 s and a half-life of exactly 10 min was used.

EXPERIMENTAL MATERIALS

Bacterial Cultures

A number of bacteria were used, including three facultative and two obligate anaerobes. The facultative strains were a fluorescent *Pseudomonas, Enterobacter cloacae*, and the spore-forming *Bacillus polymyxa*, all of which are common in New Zealand soils, and the obligate anaerobes were *Clostridium subterminale* and *Clostridium septicum*, both isolated from the experimental soils, although *C. septicum* may be primarily an inhabitant of the animal gut. The facultative strains were normally maintained on glucose-tryptone-yeast

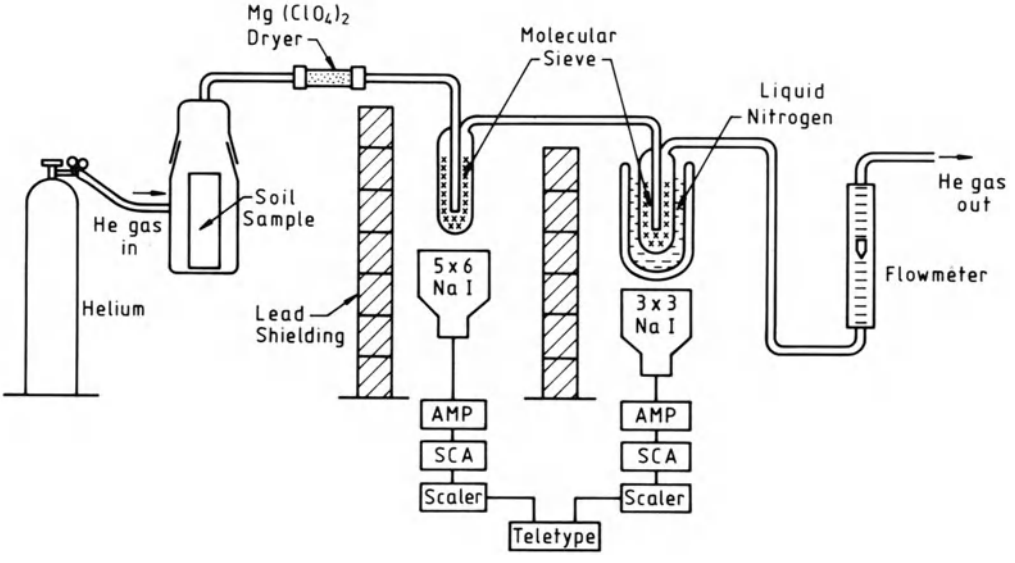

Figure 1: Experimental apparatus for measuring the cumulative production of $^{13}N_2O$ and $^{13}N_2$. 5 x 6 NaI detector for nitrous oxide, 3 x 3 NaI detector for dinitrogen; AMP, amplifier; SCA, single channel analyzer.

extract agar (Stout, 1958) and the obligate anaerobes on cooked meat medium or sterile soil, the soil used being one of the experimental soils. For denitrifying experiments, all strains were cultured in Difco nitrate broth, all having proved to be denitrifiers in this medium, or were tested in sterile soil.

Soils

The soils were collected in PVC tubes of 5 cm diameter and about 15 cm length, each sample being taken to a depth of about 10 cm with the aid of a drop-weight corer. By this means the physical integrity of soil was preserved (Ausmus and O'Neill, 1978). Cores representative of a wide range of New Zealand soils were examined (N.Z. Soil Bureau, 1968). Experiments reported here were carried out on the Tokomaru silt loam, a Typic Fragiaqualf, which is a well documented soil derived from loess (Goh, *et al.*, 1977).

EXPERIMENTAL METHODS

Samples were inserted into the experimental chamber and the tracer pipetted in 2.5 ml of water (McNaughton and More, 1979). The chamber was sealed, the flow of carrier gas commenced, and the cumulative ^{13}N in the traps counted and recorded. The initial counts were normally less than twice background. Activity of soil cores was either measured as the sample was collected in the field, after adjustment of moisture tension, or following the addition of amendments, including a suspension of bacterial culture (*Pseudomonas*). Cores were sterilized either by irradiation by a ^{60}Co source for 6 h, providing a dose of 50 kGy, twice the normal lethal dose, or by autoclaving at a pressure of 200 kPa for 30 min, twice the normal period. The experiments were carried out at room temperature (15-20°).

RESULTS

Bacterial Cultures

The fluorescent *Pseudomonas* strain was by far the most active of those tested and appeared to be the only strain with a nitrous oxide reductase. All other strains showed little activity, with only $^{13}N_2O$ evolved. With *Pseudomonas*, there was a similar evolution of $^{13}N_2O$, but mostly $^{13}N_2$ was produced. A typical experiment is illustrated in Fig. 2, which shows the activities of cultures of *Pseudomonas* and *Enterobacter cloacae*. The figure, showing the cumulative counts, indicates minimal evolution of the oxides by both organisms but an accelerating rate of $^{13}N_2$ formation by *Pseudomonas* and no $^{13}N_2$ formation by *Enterobacter cloacae*.

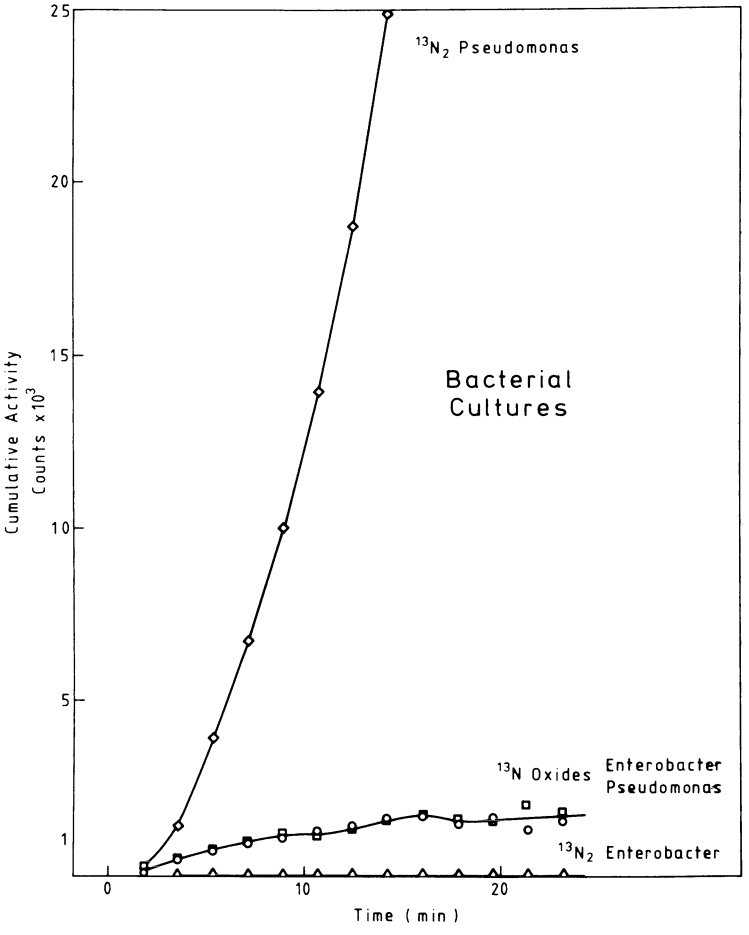

Figure 2: Production of $^{13}N_2O$ and $^{13}N_2$ by bacterial cultures.

Autoclaved Soil

Autoclaved soil showed no evolution of $^{13}N_2$ but evolved nitrous oxide, though at a diminishing rate (Fig. 3). When denitrifying bacteria (*Pseudomonas*) were added to the autoclaved soil, it evolved $^{13}N_2$ at an increasing rate (Fig. 3).

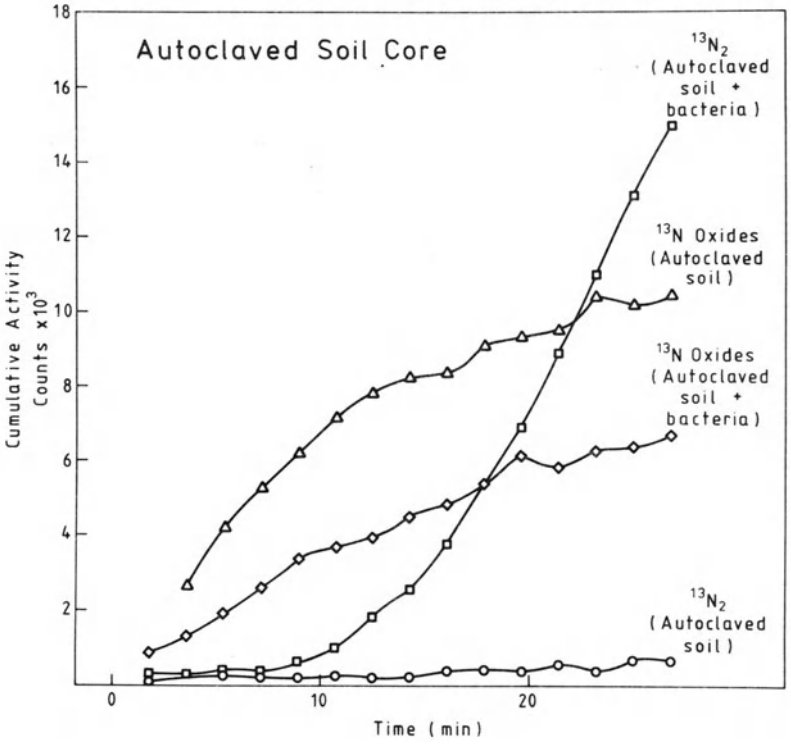

Figure 3: Production of $^{13}N_2O$ and $^{13}N_2$ by autoclaved soil, with and without denitrifying bacteria.

Irradiated Soil

Irradiation sometimes enhanced, and sometimes depressed denitrifying activity, but both $^{13}N_2O$ and $^{13}N_2$ were evolved. Differences in the evolution of $^{13}N_2O$ from an unamended soil, after the addition of denitrifying bacteris (*Pseudomonas*), and after irradiation, are shown in Fig. 4. This shows a marked enhancement of evolution of $^{13}N_2O$ by this irradiated soil. Evolution of $^{13}N_2$ (Fig. 5), was depressed, not enhanced, by irradiation of this soil.

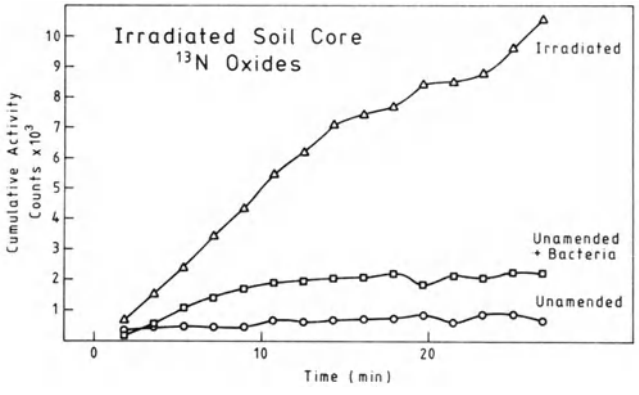

Figure 4: Production of $^{13}N_2O$ from unamended soil, with denitrifying bacteria, and after irradiation.

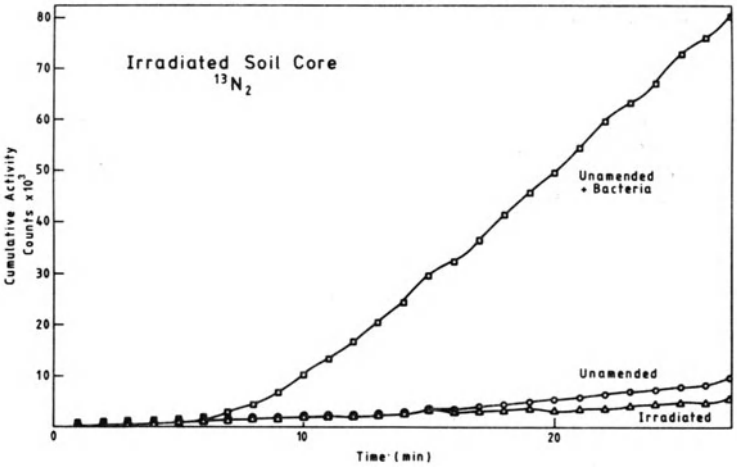

Figure 5: Production of $^{13}N_2$ from unamended soil, with denitrifying bacteria, and after irradiation.

DISCUSSION AND CONCLUSIONS

Unlike the agitated slurries used by Firestone *et al.* (1979), in which most of $^{13}NO_3$ was reduced, only about 5-10% of the activity in the cores studied was lost by the end of an hour. This means that the method is adequate to ensure comparable readings for different soil samples. It is assumed that only $^{13}N_2O$ and $^{13}N_2$ were evolved in any quantity, providing a relatively simple model system to evaluate soil uses and soil properties affecting denitrification.

The tests are carried out in an atmosphere devoid of oxygen but it was found that actively denitrifying cultures of *Pseudomonas* continued to reduce $^{13}NO_3$ even when oxygen was the carrier gas though the activity was less than with helium and there was a higher proportion of $^{13}N_2O$. This accords with bacteriological data suggesting the bacteria may use nitrate in preference to molecular oxygen (Focht and Verstraete, 1977). Thus conditions of complete anaerobiosis are not necessary for denitrification in soil. The dominant denitrifying bacteria in soil appear to be aerobic and capable of denitrification in an atmosphere of oxygen, provided both an energy source and nitrate are present (Gamble *et al.*, 1977). The co-incidence of three factors - organism, energy source and nitrate - is clearly the principal constraint on soil denitrification and this accounts for the contrast between the activity of the agitated slurry where there is active mixing of all three factors (Firestone *et al.*, 1979), and undisturbed soil where they may be separate.

Evaluation of enzyme activity is clearly fraught with difficulties in a natural system as complex as soil and the varying effects of irradiation indicate this. As indicated in Fig. 2, activity of the primary enzyme evolving N_2O was relatively constant while that of the nitrous oxide reductase increased with time. It seems reasonable to conclude that the evolution of N_2 and N_2O is normally due to microbial enzyme systems whether intracellular or extracellular, but the evolution of N_2O from autoclaved soil, in which enzymes should be destroyed, suggests that there may still be an abiotic capacity to reduce nitrate to nitrous oxide. The enhancement of both N_2O and N_2 production by the addition of denitrifying cultures to the soil, however, indicates that bacteria are the principal agents of denitrification and particularly N_2 evolution (Figs. 3, 4, 5).

Because of the very small injections of radionuclide and the accompanying addition of water, these experiments must be critically evaluated but the method provides a rapid, convenient and reproducible technique for comparative study of soil denitrification.

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NITROGEN DIAGENESIS IN MARINE SEDIMENTS: EVIDENCE FOR SUBOXIC AND ANOXIC CONVERSION OF ORGANIC-N TO N_2 GAS

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INTRODUCTION

In Fig. 1 are depicted the nitrogen species of known or presumed biochemical importance in each formal valence state, and the known or presumed reaction pathways for the major biochemical nitrogen transformations. Certain intermediate species such as hydroxylamine and nitrous oxide are common to more than one biochemical pathway and a possibility exists for "leakage" of these species between reaction pathways under appropriate environmental conditions. In such cases, analyses of stable subtrates or end products may not provide complete information about the nitrogen biochemistry of the system studied, especially if the analyses do not include all possible substrates and the end products.

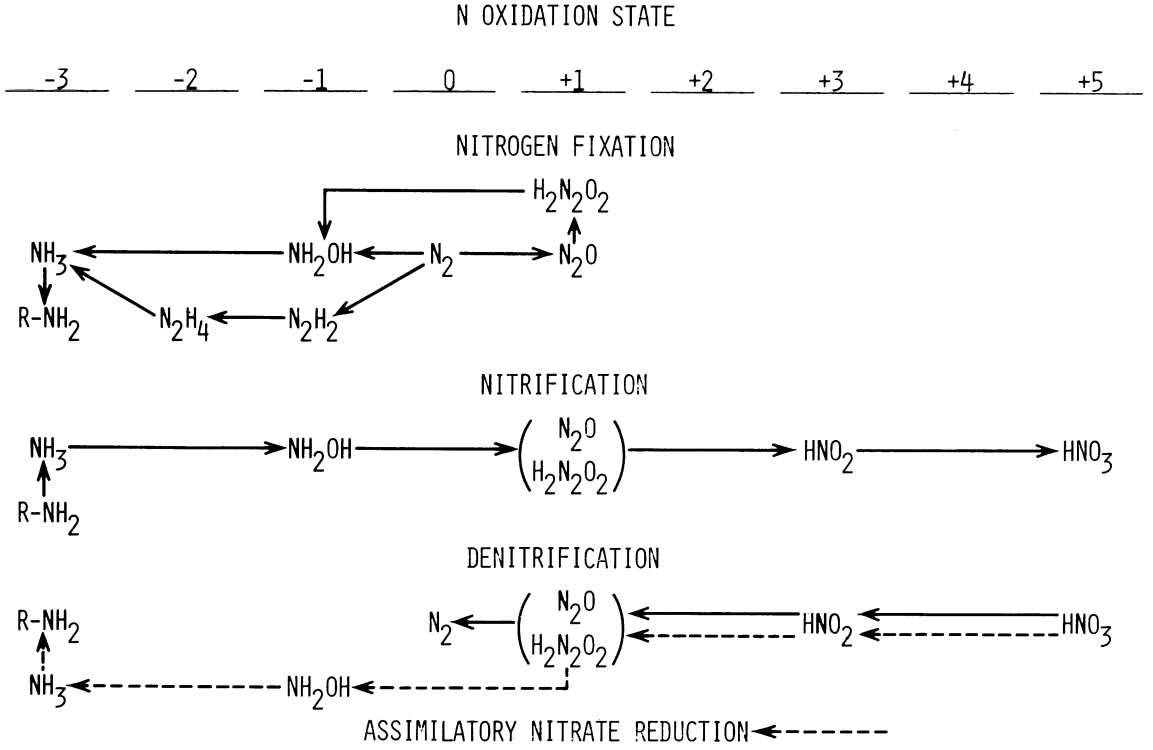

Figure 1: Oxidation state diagram for some nitrogen containing compounds of biochemical interest. Known or presumed pathways of important biochemical nitrogen transformations as given by Alexander (1965) and Fry (1955) are indicated.

Specifically, the usual nutrient analyses (NO_2^-, NO_3^- NH_4^+), even supplemented with organic-N determinations, will not provide sufficient information about denitrification systems if N_2 is produced from substrates other than NO_2^- and NO_3^-, or if nitrification and denitrification are coupled by leakage of common intermediates in sediments where these two processes occur in overlapping or contiguous zones. Analyses of N_2 and intermediates such as N_2O and hydroxylamine would be very useful in clarifying the nitrogen biochemistry of natural sedimentary environments.

Literature on dissolved N_2 in marine sedimentary environments is sparse. Reeburgh (1969) and Martens and Berner (1977) measured dissolved N_2, Ar and CH_4 in Chesapeake Bay and Long Island Sound sediments, respectively, and found no evidence of biological N_2 production within the precision of the sampling and analytical methods. Reeburgh and Heggie (1974) found excess N_2 concentrations and N_2/Ar ratios indicative of denitrification in shallow sediments from freshwater Ace Lake, Alaska. Thorstenson and Mackenzie (1974) reported excess N_2 concentrations in anoxic marine sediments from Devil's Hole, Harrington Sound, Bermuda, and suggested that there was a contribution from organic-N in the sediment via some unspecified reaction pathway. However, the lack of simultaneous dissolved Ar measurements (inert gas used as an internal standard) prevents an assessment of the reliability of their data. The excess N_2 could have resulted from atmospheric contamination of samples.

Hammond *et al.* (1973) and Hammond (1974) discussed measurements of dissolved N_2 and CH_4 in anoxic, sulfate-depleted sediments from 47 to 175 m sub-bottom depth at DSDP (Deep Sea Drilling Project) site 147, Leg 15, Cariaco Trench. They suggested that the thermodynamically favourable conversion of N_2 to NH_3 explained the apparently low pore water concentrations of N_2 relative to overlying sea waters. However, Ar was not measured, again preventing an assessment of the reliability of their data. Stripping of N_2 by methane bubbles could not be ruled out as an explanation of their data (Barnes *et al.*, 1975).

Barnes *et al.* (1975) measured dissolved Ar and N_2 in surface sediments of the Santa Barbara and Santa Cruz Basins and the San Diego Trough in the southern California continental borderland. The use of specialized in situ sampling equipment (Barnes, 1973) resulted in N_2 analyses with a claimed precision and accuracy of 1 to 2%. Increases in pore water N_2 concentration, and in N_2/Ar ratios of 4 to 15% over bottom water values, were attributed to denitrification, with a contribution from organic-N via closely coupled nitrification-denitrification processes.

This paper presents preliminary results of investigations of nitrogen biogeochemistry in near surface sediments of the eastern equatorial Pacific and in deep sediments probed by DSDP bore holes. The sampled depths range from 0 to 450 m sub-bottom and represent reaction time scales from weeks to tens of millions of years. Our studies have emphasized the analysis of dissolved N_2 in pore waters, with nutrient concentration measurements as appropriate.

SAMPLING LOCATIONS AND METHODS

Sedimentary pore waters were collected in March, 1979, at 5 stations in and around the hydrothermal mounds province several km south of the Galapagos Spreading Center (Williams *et al.*, 1979). The pore water samples were filtered and collected in situ using a sampler similar in general principle to that described by Sayles *et al.* (1976) but differing significantly in details and mode of operation (Barnes and M.O. Andreae, unpublished data).

Barnes (1979) describes the design and operation of the in situ pore water sampler used on the deep drilling ship *Glomar Challenger* operated by the Deep Sea Drilling Project. Pore waters are filtered and collected in situ from sediment depths of tens to hundreds of metres. Results from two sites are presented here (Table 1).

ANALYTICAL METHODS

Dissolved Ar and N_2 were measured using a thermal conductivity gas chromatograph with analytical precisions of about ± 1.5% for Ar and ± 0.5% for N_2 (Barnes and Will, 1980). Nitrate plus nitrite were measured on an Autoanalyzer II using the method described by Froelich *et al.* (1979).

TABLE 1

SAMPLING LOCATIONS

Station	Latitude	Longitude
Galapagos-126	$0^{\circ}36.2'N$	$86^{\circ}8.8'W$
DSDP site 410	$45^{\circ}30.51'N$	$29^{\circ}28.56'W$
DSDP site 438A	$40^{\circ}37.79'N$	$143^{\circ}14.15'E$

RESULTS

Galapagos

The pelagic surface sediments in the Galapagos area are calcereous oozes situated under the east equatorial Pacific high productivity zone. Pore water NO_3^- + NO_2^- concentrations at all 5 stations never exceed the bottom water nitrate concentration of 39-40 µM. Pore water concentration profiles of NO_3^- + NO_2^-, N_2 and N_2/Ar ratio were similar at all five stations. The only major difference was the depth of nitrate depletion which varied from 20 to 70 cm. For simplicity we show only one representative set of profiles from station 126 (Fig. 2). Nitrite was measured at two stations. It remains near or below detection limits (0.05 µM) at all depths. The maximum nitrite concentration was 0.34 µM at 6 cm sediment depth (P.N. Froelich, personal communication).

TABLE 2

AVERAGED Ar AND N_2 CONCENTRATIONS FOR PORE WATER PROFILES COMPARED TO BOTTOM WATER (BW) SOLUBILITIES OR MEASURED BW GAS CONCENTRATIONS

Station	Ar (ml STP kg^{-1})	ΔAr^a (%)	N_2 [b] (ml STP kg^{-1})	ΔN_2^a (%)	N_2/Ar	Equiv. NO_3^--N[c] (µM)
Galapagos						
BW-solubility	0.364	0	13.25	0	36.4	
BW-av. conc.	0.362	-1	13.26	0	36.6	
126	0.364	0	14.69	+11	40.4	132
Cruise av.	0.364	0	14.89	+12	40.9	150
DSDP site 410						
BW-solubility	0.355	0	12.96	0	36.5	0
Av. pore water 45-207 m	0.351	-1	14.04	+8	40.0	108
DSDP site 438A						
BW-solubility	0.359	0	13.10	0	36.5	0
Av. pore water 155-455 m	0.320	-11	55.63	+325	174	3930

a ΔAr, ΔN_2=% deviation from BW solubilities. Solubilities calculated from Weiss (1970).

b Only N_2 concentrations below the depth where N_2 increases above bottom water are included in these averages (Galapagos) or where N_2 concentration is apparently uniform with depth (site 438A).

c ΔN_2 excess relative to ΔAr, expressed as NO_3^- equivalents.

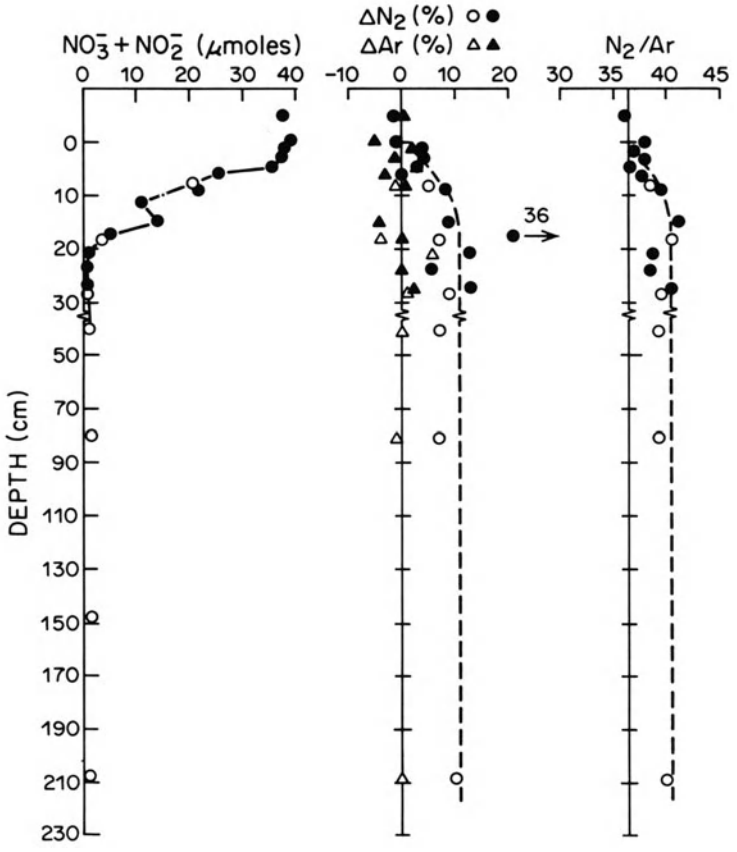

Figure 2: Nitrate plus nitrite and dissolved gas data for station 126, Galapagos mounds area. Solid symbols are for 0-30 cm surface probe, open symbols are for 0-205 cm main probe. The vertical axis on N_2/Ar profile is bottom water solubility ratio as listed in Table 2. Note vertical scale break at 30 cm.

Pore water Ar concentrations were indistinguishable from bottom water values within experimental precision. Pore water N_2 at all stations rose from bottom water concentration at the sediment surface to an average of 12% above bottom water concentration below the zone of nitrate reduction. All five profiles were within +2 and -1% of this cruise average (see Table 2). The precision of our N_2 analyses was not sufficient to resolve the actual shape of the N_2 concentration curve within the zone of nitrate reduction.

DSDP Site 410

The sediments at this site on the west flank of the Mid-Atlantic Ridge are also calcareous ooze. The presence of sedimentary sulfides at the sampling depths of 45 to 207 m indicates that nitrate reduction was completed higher in the core and sulfate reduction had commenced. The theoretical oxidation-reduction potential of the sediment would thus be similar to the lower parts of the Galapagos profiles. Fig. 3 shows the N_2 and Ar concentrations at this site relative to in situ temperature corrected solubilities. The Ar concentrations are above in situ solubilities because the ca. Myr relaxation time (see Appendix) prevents attainment of equilibrium with in situ conditions (Barnes and Will, 1980). Site 410 results are also listed in Table 2.

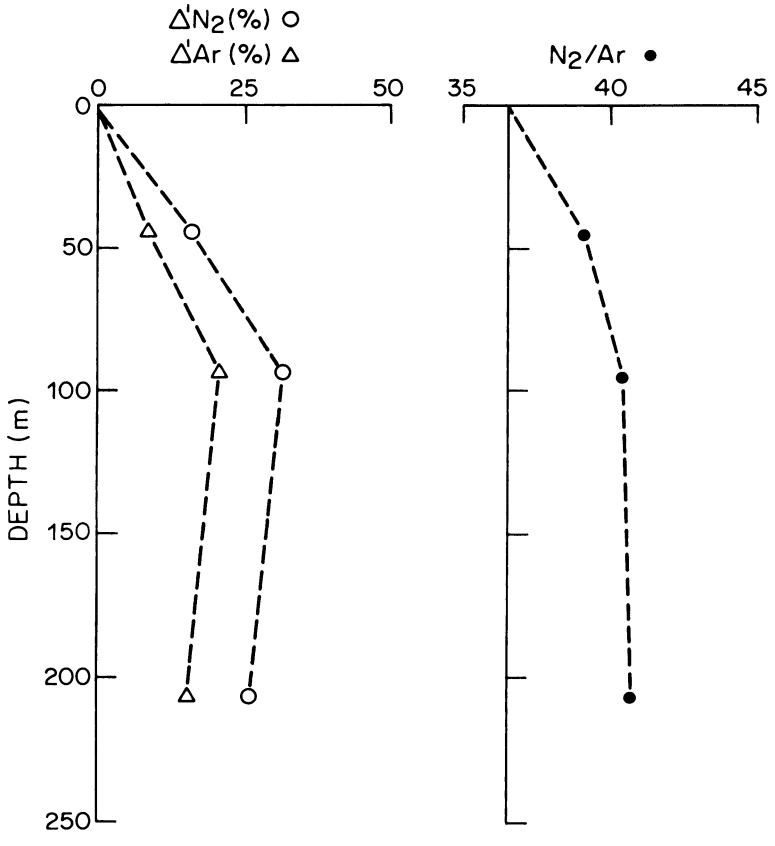

Figure 3: Dissolved gas data for DSDP, site 410. The $\Delta'Ar$ and $\Delta'N_2$ are relative to in situ temperature corrected solubilities taken from Barnes and Will (1980), not the ΔAr, ΔN_2 listed in Table 2. N_2/Ar vertical axis as in Fig. 2.

DSDP Site 438A

The sediments are largely hemi-pelagic, vitric, siliceous oozes and muds, turning to claystone near the base of the pore water profile at 455 m sub-bottom. A few sulfate measurements in the upper 100 m (Moore and Gieskes, 1980), combined with CH_4 concentration measurements that increase below 100 m (Fig. 4), suggest that sulfate approaches zero concentration near 100 m depth (Barnes and Goldberg, 1976; Reeburgh, 1976; Martens and Berner, 1977). Thus the sediments at this site are more reducing than the pelagic stations at Galapagos and DSDP site 410, in keeping with the hemi-pelagic setting on the upper landward wall of the Japan Trench.

Dissolved Ar, N_2 and CH_4 concentrations are shown in Fig. 4. The large scatter in the concentration data is the result of analytical difficulties posed by the very large CH_4 contents of the samples. The results should not be biased significantly in either a high or low direction; however, the individual measurements must be averaged to produce meaningful results. The mean pore water Ar concentration is statistically indistinguishable from the bottom water solubility value. The analytical problem did not apply to the upper two samples, and duplicate analyses agree within 5%, the estimated precision for normal DSDP samples (Barnes and Will, 1980).

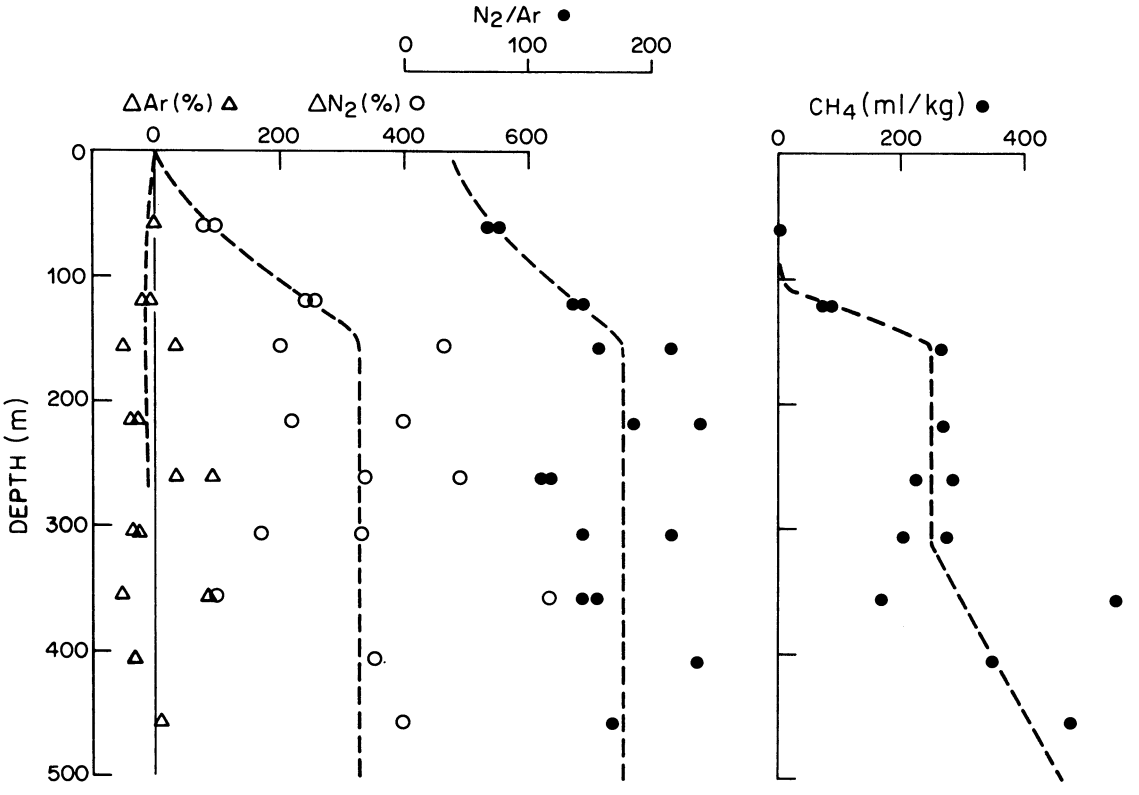

Figure 4: Dissolved gas data for DSDP, site 438A. Data taken from Barnes and Will (1980).

Pore water ammonia concentrations rise steadily to ca. 3.5 mM at 100 m depth and then remain at 3-4 mM to depths in excess of 500 m (Moore and Gieskes, 1980).

DISCUSSION

Galapagos

The dissolved N_2 profiles are the most interesting aspect of our data. Nitrogen increases within the nitrate reduction zone to an average of 12% above bottom water concentration and remains at this elevated level to the 200 cm depth limit of the profiles. The N_2 increase appears to result from nitrate reduction as expected, and N_2 behaves as an inert gas in the anoxic sediment below the depth of nitrate depletion (Barnes *et al.*, 1975). However, the 12% N_2 increase is equivalent to 150 μM of NO_3^- reduced (Table 2) which is almost 4 times the maximum NO_3^- content of the sediment. Let us briefly examine some possible ways in which the sedimentary N_2 content may be augmented above the 1:2 ratio to maximum sedimentary NO_3^- concentration that results from reaction stoichiometry.

1. The diffusion coefficients of NO_2^-, NO_3^-, NH_4^+, and N_2 are essentially equal in sea water at 2° (Li and Gregory, 1974; Wise and Houghton, 1966). Thus, there should be no significant enrichment or depletion of one of these species over another due to differential diffusion rates into or out of the sediment. Pore water concentrations should reflect reaction stoichiometry if all excess N_2 in pore waters results from a NO_2^- or NO_3^- precursor.

2. Ammonia released in the oxic zone near the sediment surface can be oxidized by

nitrification and then reduced to N_2 by denitrification. If increased pore water NO_3^- + NO_2^- is an indicator of nitrification, then it is not a significant source of excess N_2 since no detectable increase in NO_3^- + NO_2^- occurs in the surface sediment. However, intermediate species common to nitrification and denitrification may leak between the two processes, by-passing the more fully oxidized NO_2^- or NO_3^-.

A portion of sedimentary NH_4^+ is ion exchanged onto mineral surfaces. The adsorption constant is likely to be in the range 1-2 (Berner, 1977) so the available NH_4^+ would be 2 to 3 times the measured pore water concentration. Ammonium was measured in one pore water profile (C. Maris, personal communication). Concentration dropped from ca. 6 µM just below the sediment surface to ca. 1 µM at 5 cm depth and then increased slowly to ca. 20 µM at 200 cm. If this 5 µM concentration drop at the sediment surface is due to a coupled nitrification-denitrification reaction then the maximum N_2 increase from this source would be 7.5 µM.

3. Bender *et al.* (1978) and Froelich *et al.* (1979) have emphasized the importance of oxidized forms of sedimentary Fe and Mn as terminal electron acceptors in sub-oxic diagenetic reactions. Fe reduction occurs at an oxidation potential lower than nitrate reduction and, therefore, would not augment N_2 production within the nitrate reduction zone. However, the zone of Mn reduction appears to overlap that of nitrate reduction. Reactions of the overall form shown in Equation 1

$$3MnO_2 + 2NH_3 + 6H^+ \longrightarrow 3Mn^{2+} + N_2 + 6H_2O \; : \; \Delta G^\circ = -560 \text{ kJ mol}^{-1} \; N_2 \qquad 1$$

are thermodynamically possible, but not experimentally verified at present. Pore water Mn^{2+} concentrations are not yet available for our Galapagos profiles. However, concentrations up to 55 µM have been measured in near surface, eastern tropical Pacific sediments (Bender *et al.*, 1978). It is highly unlikely that a major portion of this divalent manganese is produced via Equation 1, but, if we assume all 55 µM *is* produced via Equation 1 then the maximum N_2 production is 18 µM. The resulting excess N_2 pore water concentration is reduced to ca. 6 µM because the diffusion coefficient of N_2 is >3 times that of Mn^{2+} ion (Li and Gregory, 1974; Wise and Houghton, 1966). This excess N_2 is further reduced by a factor of 10-17 if ammonia is only oxidized stoichiometrically with organic carbon having C:N ratios of 6.6 to 11.

4. Bender *et al.* (1977) conjectured that ammonia or amines released during diagenesis of organic matter by denitrification were also converted to N_2. Such a reaction is energetically possible in sub-oxic environments but has not been verified experimentally. If all nitrogen released by the denitrification process ends up as N_2 then 40 µM of nitrate reduced produces 23.4 µM of N_2 assuming a Redfield C:N ratio of 6.6. The combination of above items 1 to 4 produces a *maximum* excess N_2 concentration of 23.4 + 7.5 + 6 = 37 µM, approximately half the average measured excess of 75 µM.

5. The only other significant source of nitrogen in the sediment is organically bound. The ca. 1 µM concentrations of NH_4^+ in the zone of nitrate reduction suggest that NH_4^+ is readily converted to N_2. However, the mechanism of Bender *et al.* (1977) can supply less than 5% of the required excess N_2. We propose an organic-N → N_2 reaction that bypasses NH_3 formation and is not linked stoichiometrically to organic-C diagenesis. Nitrification, defined as production of NO_2^- or NO_3^-, is unlikely to occur deeper than 1 or 2 cm below the sediment surface. In terms of the known diagenetic pathways of ammonification, nitrification and denitrification, the proposed organic-N → N_2 reaction occurs by leakage of common intermediate species between the oxidizing pathway of ammonification-nitrification and the reducing pathway of denitrification. The intermediate species would have oxidation states of less than +3 (NO_2^-) because higher oxidation states are not discernable in the sediment pore waters.

One possible candidate is hydroxylamine. Bremner and Blackmer (1980) have shown that, in soils, hydroxylamine is rapidly converted to N_2O solely by chemical decomposition. The N_2O produced can be readily reduced to N_2 by denitrifying bacteria (see Fig. 1). Whether hydroxylamine is produced in sufficient quantity within the zone of sub-oxic diagenesis remains to be experimentally examined.

With no useful experimental data available on the abundances and turnover rates of possible

intermediate species, we can at least consider the mass balance implications of the proposed reaction. Organic-N can be considered fixed in the sediment and not subject to diffusive loss or replenishment like the N_2 dissolved in the pore water. About 25 µM of N_2 can be accounted for by reduction of sea water nitrate and oxidation of surface sediment ammonia released during oxic diagenesis (items 1 and 2 above), leaving ca. 50 µM of N_2 to be produced from organic-N below the zone of nitrification. If we assume that this 50 µM of excess N_2 forms a linear concentration gradient from 0 to 30 cm then the gradient is 470 µg N cm^{-4}. The corresponding flux is -5 µg N cm^{-2} yr^{-1} (see Appendix for calculations). If we assume a sedimentation rate of 6 x 10^{-3} cm yr^{-1}, then 5000 years are required for sediment to move below the zone of N_2 production. During this time 0.0835 g N cm^{-3} of sediment has been lost by upward diffusion of N_2 to overlying sea water. This lost nitrogen can be provided by completely stripping nitrogen from organic matter with an original C:N ratio of 6.6 (Redfield ratio) and a carbon content originally equal to 0.74% dry weight of sediment, assuming a solids content of 0.75 g cm^{-3} wet sediment. The 0.74% of nitrogen-depleted organic-C may appear on the high side of average organic-C values for this type of sediment. However, if we assume a modest error factor of 2 for our simple model calculations, the nitrogen loss is well within the realm of possibility.

It is well known that the C:N ratio of organic detritus commonly increases during early diagenesis from ca. 7 to as high as 13-14. The proposed organic-N $\longrightarrow$ N_2 conversion may be a mechanism for preferential oxidation of organic-N in sub-oxic environments where nitrification cannot proceed thermodynamically or biochemically and yet the sedimentary ammonia levels remain very low.

DSDP Site 410

The characteristic diffusion-limited residence time or effective age of the pore water solutes increases from ca. 1-100 yr at 10-100 cm depths in the Galapagos profiles to ca. 1 My at 100 m depth at site 410 (see Appendix for calculations). The excess N_2 profiles in Galapagos sediments suggested that N_2 was not significantly produced or consumed below the depth of nitrate depletion. The site 410 samples confirm this conclusion by extending the time scale a factor of 10^4 in sediments with similar redox potentials. The excess N_2 concentration at site 410 is in the same range but slightly less than that of the Galapagos samples (see Fig. 3, Table 2). Thus, in the sulfate reduction zone, N_2 appears to act as an inert gas.

DSDP Site 438

In contrast to the Galapagos region and DSDP site 410, the sediments below 100 m at site 438A are sulfate-depleted. Surprisingly, the N_2 concentrations in this hole are very high (Fig. 4). We concluded that N_2 acts as an inert gas within the zone of sulfate reduction. On the other hand CH_4 is consumed within the sulfate reduction zone (Barnes and Goldberg, 1976; Reeburgh, 1976; Martens and Berner, 1977). The profiles of N_2 and CH_4 above 150 m support these previous observations. The almost linear N_2 gradient suggests that concentrations in this zone are diffusion-controlled, with the N_2 source region below 100 m depth. Contrast this with the CH_4 profile which suggests a consumptive sink at about 100 m depth, the presumed depth of sulfate depletion.

Sulfate is never completely reduced in Galapagos or site 410 sediments. Thus, in contrast to site 438, there is no "methanogenic" zone below the depth of our pore water profiles at these sites that might influence N_2 concentrations by upward diffusion.

If the observed excess N_2 is generated in situ, then the pool or organic-N plus ammonia must be the source because other nitrogen species are absent or in too low abundance in this highly reducing environment. However, ammonia is the thermodynamically stable inorganic nitrogen species in the zone of methanogenesis (Hammond, 1974) so the proposed reaction is endothermic with respect to nitrogen and would require the existence of some higher energy intermediate reaction product. The fact that N_2 is inert in the zone of sulfate reduction but not in the zone of methanogenesis suggests that methane or some other low molecular weight hydrocarbon may be involved in the N_2 production mechanism. N_2 production is not a by-product of methanogenesis per se because methanogenesis also occurs in the sulfate reduction zone, but consumption of the methane produced maintains its concentration at low levels (Barnes and Goldberg, 1976; Warford *et al.*, 1979). Methylamine reacts with nitrosyl ion (NO^+) to produce molecular N_2 by dissociation of the $CH_3NH_2NO^+$

reaction complex (Dusenbury and Powell, 1951), but nitrosyl ion is unlikely to exist in these highly reducing sediments. Some more reduced but energetic nitrogen compound may exist in sufficient quantities to couple with amine nitrogen in an N_2-producing reaction similar to the nitrosyl ion reaction.

The reaction rates required to produce the observed excess N_2 are extremely low and unlikely to be observed in routine laboratory experiments. The N_2 profile suggests that the actual N_2 production occurs in a narrow interval near 150 m depth. If we assume that N_2 production occurs uniformly in a 50 m interval, and that this production balances the N_2 lost by diffusion to the sediment surface, then the production rate is 0.057 $\mu g\ N_2\ yr^{-1}\ kg^{-1}$ pore water (Barnes and Will, 1980). This rate is 2.3×10^{-4} times the proposed organic-N $\rightarrow$ N_2 reaction rate for the Galapagos samples and would produce an undetectable rise in pore water N_2 concentrations if the reaction were occurring in the upper metre of sediment.

Such a low reaction rate could be detected in a carefully controlled ^{15}N labelled culture experiment. A year's N_2 production in 1 kg of pore water would produce a 3% change in $^{15}N/^{14}N$ ratio when mixed with 0.4 ml of isotopically normal N_2. A culture experiment could probably be designed to meet these constraints.

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APPENDIX

1. Characteristic diffusion-limited solute residence time (γ_D) is defined as the time required for solute concentration at the depth of interest to reach 90% of the new equilibrium value when production or consumption rates are changed. It also represents the time span of sedimentary geochemical history that can significantly influence present pore water concentrations. $\gamma_D \simeq z^2/D'_s$, where z is the vertical distance from sediment-water interface to a depth of interest and D'_s is the apparent solute diffusion coefficient for bulk sediment. D'_s for N_2 is considered to be 5 x 10^{-6} cm^2 s^{-1}.

2. N_2 concentration gradient $= \dfrac{(50\ \mu mol\ N_2\ l^{-1}) \times (28\ g\ mol^{-1}\ N_2)}{(30\ cm) \times (10^3\ cm^3\ l^{-1})} = 470\ \mu g\ N_2\ cm^{-4}$

N_2 flux $= Q = -\dfrac{\Delta N_2}{\Delta z}\ \theta D'_s$, where θ = porosity

$= -(470\ \mu g\ N_2\ cm^{-4}) \times (0.7) \times (5 \times 10^{-6}\ cm^2\ s^{-1}) \times (31\ Ms\ yr^{-1})$

$= -5\ \mu g\ N_2\ cm^{-2}\ yr^{-1}$

$= -25\ mg\ N_2\ cm^{-2}\ (5000\ yr^{-1})$

$$\text{equivalent oranic-C} = \frac{(N_2\ \text{loss}) \times (\text{C:N ratio}) \times 100\%}{(30\ cm) \times (0.75\ g\ \text{solid}\ cm^{-3}\ \text{wet sediment})}$$

$$= \frac{(2.5\ mg\ N_2\ cm^{-2}) \times 6.6 \times 100\%}{(30\ cm) \times (0.75\ g\ cm^{-3})}$$

$= 0.74\%$

REGIONAL NITROGEN MASS BALANCE MODEL FOR THE FLORIDA PENINSULA

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INTRODUCTION

Prior to the industrial revolution, the biogeochemical cycling of nitrogen was powered by the diffuse flux of energy from the sun. Atmospheric N_2 was fixed into biomass using reduced compounds derived from photosynthesis, and some abiotic fixation occurred during electrical storms. The reduced products of photosynthesis also provided the means of returning fixed nitrogen to its less biologically active forms, N_2 and N_2O, through the process of denitrification. During the present century, these precultural fluxes of nitrogen have been greatly augmented on local and regional levels by the fixation of N_2 into NO_x during fossil fuel combustion and by the production of ammonia for fertilizer by the Haber process.

One has only to witness the cultural eutrophication of lakes or the increase in global CO_2 concentrations to appreciate that acceleration of fluxes of environmentally benign compounds may turn them into pollutants. Nitrogen is a component of many such compounds that act as oxidants, toxicants, mutagens, or carcinogens, that contribute to eutrophication, and that deplete stratospheric ozone (NRC, 1978). Although the ability to predict the effects of anthropogenic perturbations on the fluxes and storages of nitrogen species is essential to many facets of environmental management, the complexity of the cycle, together with incomplete data on fluxes and storages, precludes the use of dynamic models in complex, heterogeneous systems. However, an elemental nitrogen mass balance, while lacking the power of more complex models, can be used to gain valuable insight into fluxes occurring in such systems.

The Florida peninsula offers a unique opportunity for regional mass balance models of nitrogen fluxes. Because the peninsula is bounded mostly by water, both hydrologic and mesoscale atmospheric transport inputs are somewhat restricted, and outputs either are monitored or can be estimated using existing data. Agricultural shipments have been monitored since 1963, and a statewide precipitation monitoring network has been in place for over a year. Finally, although the remaining gaseous fluxes and most system storages are not well-quantified, information about their potential magnitudes can be gained through mass balance calculations. Previous N balances on large, culturally influenced systems (Ayers and Branson, 1973; Miller and Smith, 1976; Keeney, in NRC, 1978) have involved systems that are less well-isolated hydrologically, or atmospherically, or have neglected important fluxes.

In this paper we briefly summarize the construction and results of the nitrogen mass balance for the Florida peninsula, and evaluate the probable relative importance of system storage versus denitrification in accounting for the discrepancy between N inputs and outputs, a feature typical of most such balances (NRC, 1978, Messer, 1978). Earlier versions of this mass balance model (NRC, 1978, Appendix A; Messer, 1978) were based on less complete data. An improved atmospheric model (Messer and Brezonik, 1980) has resulted in a reduction (in this paper) of the original estimates of atmospheric nitrogen deposition. These sources should be consulted for detailed data and calculations.

METHODS

The mass balance model was constructed by combining the results of hydrologic, agricultural and atmospheric submodels. System boundaries are shown in Fig. 1. Upper and lower system boundaries include the plant canopy, and the deep limestone Floridan Aquifer, respectively. The model represents fixed nitrogen fluxes only.

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Figure 1: Map of Florida, showing the geographical system boundaries (surrounding crosshatched area) for the peninsula nitrogen mass balance model.

Hydrologic Submodel

Hydrologic N fluxes result from surface and groundwater flows across the system boundaries. Surface water inputs (e.g. USGS, 1976) are limited to the Suwannee River drainage basin; potentiometric contours (Healy, 1975) indicate minimal southerly groundwater movement. Slack and Goolsby (1976) found little variation in N concentration with streamflow in most Florida rivers, and thus the mean annual streamflow was multiplied by the mean N concentration to calculate N inputs and output. Although gauging stations close to the coast were chosen to estimate N output, ungauged areas still constituted 25% of the land area in the system. However, N export coefficients from high order streams varied only slightly (0.23 to 0.29 g N m^{-2} yr^{-1}) throughout the state, and runoff from ungauged areas was assumed to have an equal areal N loss rate. Groundwater seepage along coastlines and submarine spring discharge were calculated by assuming a runoff/precipitation ratio of 0.25 (south) to 0.35 (north) (Langbein *et al.*, 1949). Groundwater discharge was then multiplied by the N concentration of surface streams in each area to estimate groundwater flux. Losses of N resulting from wastewater discharges downstream from gauging sites were calculated on a per capita basis from census data.

Agricultural Submodel

Agricultural imports and exports are reported annually by the Florida Crop and Livestock Reporting Service. Livestock, citrus, dairy, vegetable, field crop, and forest product movements, when multiplied by appropriate N concentrations, provided flux data for plant- and animal-product minimodels that were combined to produce the agricultural submodel. Sales of nitrogen fertilizers are also monitored. Unmonitored agricultural fluxes

(processed food products and animal feeds) were calculated by mass balance on the human and animal compartments by subtracting instate production of food and feedstuffs from estimates of human and animal consumption. Animal consumption was calculated using waste production data (Lauer *et al.*, 1976).

Atmospheric Fluxes

Unlike the hydrologic and agricultural fluxes, atmospheric N fluxes (except for precipitation) are virtually unmonitored over the peninsula. These transfers thus had to be estimated by applying data on process rates obtained in other systems (admittedly not a desirable practice). However, an atmospheric fixed-nitrogen mass balance (Messer and Brezonik, 1980) for the troposphere above the peninsula has indicated that the fluxes estimated in this way are reasonable within the constraints of mass balance.

Inputs of nitrogen from bulk precipitation were primarily from Hendry *et al.* (1980). Dry gas deposition of NO_2 (Hill, 1971) was calculated by multiplying ambient gas concentrations (U.S. EPA, 1977) by a mean annual deposition velocity (v_D) of 0.75 (terrestrial surfaces) or 0.5 cm s^{-1} (open water). The values chosen for v_D should be considered averages of temporarily higher and lower values that depend on atmospheric stability, transpiration, and other factors (Wesley and Hicks, 1977). They were chosen as reasonable values in light of field experiments (Spedding, 1976) and mass balance constraints (Messer and Brezonik, 1980). Dry ammonia deposition was calculated by mass balance on the atmospheric submodel, and apparently represents primarily an internal cycle. Nitrogen fixation was calculated using data taken primarily from sources summarized in Burns and Hardy (1975) and Brezonik (1972), using values that most closely apply to Florida ecosystems.

Losses of nitrogen include NH_3 volatilization, chemidenitrification, and the suspension of organic N in dust and pollen. Ammonia volatilization was calculated from data on animal manures (e.g. Lauer *et al.*, 1976), fertilizer application (Volk, 1966), and primary producer decomposition (Dawson, 1977). Chemidenitrification estimates were derived from the data of Kim (1973). Organic N suspension was assumed to be closed cycle and set equal to organic N deposition in bulk precipitation. Although fossil fuel combustion does not represent a fixed N sink for the peninsular system, it was determined on the basis of fuel consumption data (Thompson, 1977) and EPA emissions factors (U.S. EPA, 1973) to contribute 190 Gg N yr^{-1} to the troposphere as NO_x and NH_3.

RESULTS

Hydrologic, agricultural, and atmospheric fluxes are summarized in Fig. 2. All outputs are given in Gg N yr^{-1}.

Submodels

Hydrologic outputs remove only 70 Gg N from the system annually. Neglecting sewage input to estuaries, runoff and seepage together remove only 50 g m^{-2} yr^{-1} of the 80 g m^{-2} yr^{-1} delivered to the system by precipitation. The export may be slightly higher if bedload transport of particulate N is significant.

Agricultural fluxes are dominated by the input of fertilizer (144 Gg N yr^{-1}), which accounts for 71% of the annual nitrogen inputs of the agricultural submodel. The internal cycle between soil nitrogen, plants, and grazing animals exhibits an apparent turnover time of only about 10 months, and much of this nitrogen (167 Gg N yr^{-1}) must either be stored or lost to the atmosphere or runoff. This loose cycle represents the high environmental and economic cost of producing the high-protein diet enjoyed by North Americans.

The atmospheric fluxes also show a large positive discrepancy between inputs and outputs. Although the volatilization and subsequent deposition of NH_3 and the suspension and deposition of organic N essentially operate as closed loops (Messer and Brezonik, 1980), only a small fraction of the nitrogen fixed by fossil fuel combustion and soil microorganisms is subsequently lost to the atmosphere in fixed form. Chemidenitrification, which may be overestimated (cf. Galbally and Roy, 1978), removes only 17 Gg N yr^{-1}, or at most 14% of the NO_2^- + NO_3^- deposited on the system annually. The result is an additional 158 Gg N yr^{-1} to be accounted for by denitrification or storage.

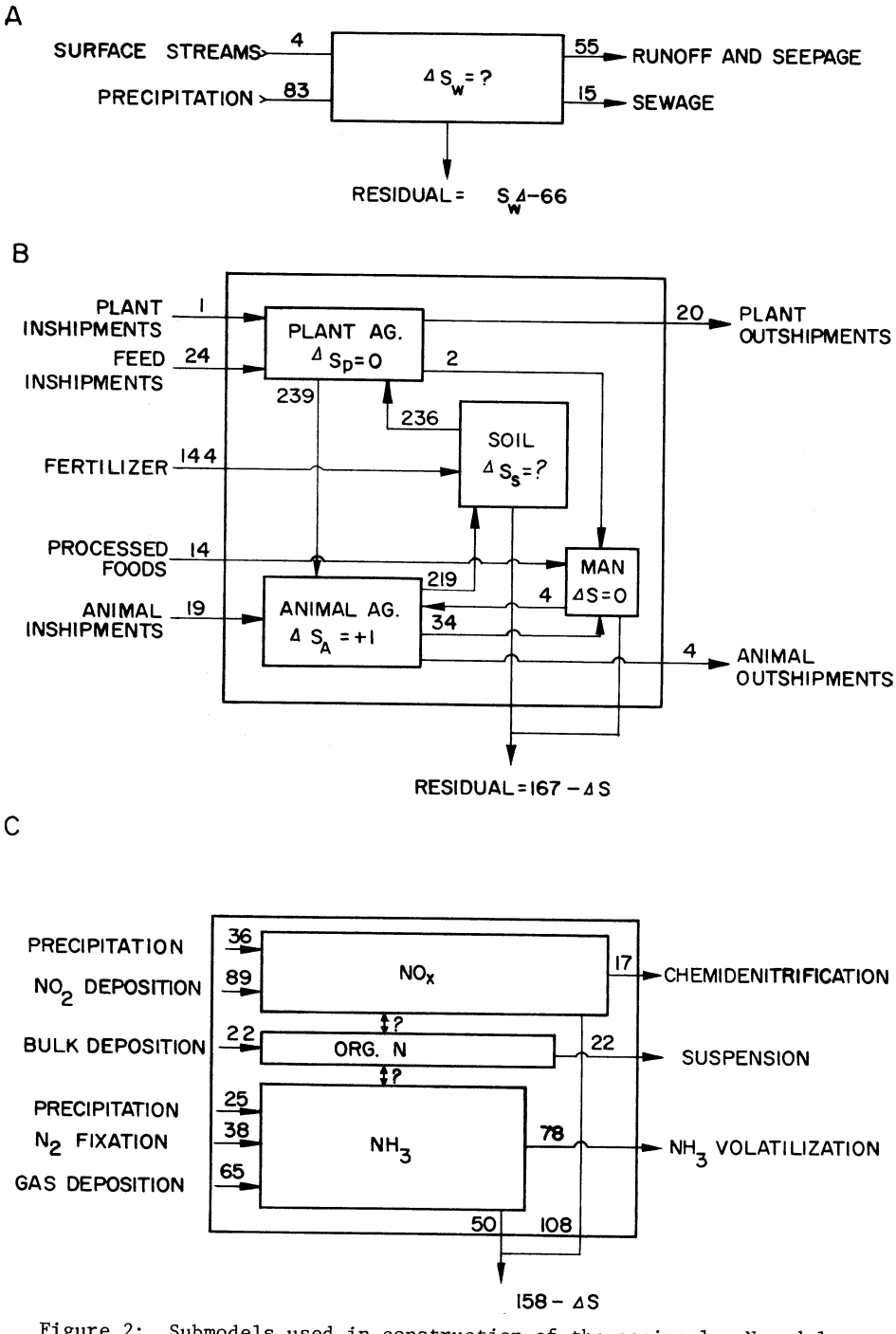

Figure 2: Submodels used in construction of the peninsular N model. A. Hydrologic submodel; B. Agricultural submodel; C. Atmospheric submodel. All fluxes in Gg N yr^{-1}.

Peninsular Mass Balance Model

Data from the submodels were combined to yield the annual nitrogen inputs and outputs for the peninsula (Table 1). The preponderance of anthropogenic nitrogen inputs, and the discrepancy between estimated inputs and outputs, are striking. The two principal sources of fixed nitrogen to the system are fuel combustion (190 Gg N yr^{-1}) and fertilizer import (144 Gg N yr^{-1}). Of the former source, 42 Gg N yr^{-1} is lost by horizontal advection to marine areas, and the rest is deposited on the peninsula. Of the total 478 Gg N deposited on the system annually, only 211 Gg is removed in the fixed form, and the remainder (267 Gg N yr^{-1}) must be stored or denitrified. Storage could be either beneficial (if it occurs in soil organic matter) or detrimental (if it occurs in groundwater). Alternatively, loss of this nitrogen by denitrification could contribute to the problem of stratospheric ozone depletion, depending on the relative fraction lost as N_2O.

TABLE 1

A MASS BALANCE SHEET FOR NITROGEN IN THE FLORIDA PENINSULA [a]

	Inputs (Gg)
Atmospheric	
N_2 Fixation	38
Bulk Precipitation	83
Dry Gas Deposition	154
Hydrologic	
Surface Inflows	4
Commercial	
Animal Product Inshipments	19
Plant Product Inshipments	36
Fertilizers	144
Total	478

	Outputs (Gg)
Atmospheric	
Ammonia Volatilization	78
Chemidenitrification	17
Windblown Solids	22
Hydrologic	
Surface Runoff and Seepage	70
Commercial	
Animal Product Outshipments	4
Plant Product Outshipments	20
Total	211

Unaccounted For Losses or Changes in Storage: 478 - 211 = 267 Gg

[a] Annual mean values for the period 1970-1975.

The scenario of nitrogen accretion can be explored using some simple calculations and observations. Accretion in surface water and shallow groundwater cannot be significant because of the relatively small size of these reservoirs. However, the deep limestone Floridan Aquifer, from which the state draws most of its potable and irrigation water, is much larger. The annual discharge from Florida's springs, about 6.3 Tl yr^{-1} (Rosenau and Faulkner, 1975), represents a likely minimum recharge of the annual aquifer. If the N concentration in recharge water is similar to the flow-weighted average N concentration in runoff (1.0 mg l^{-1}), and spring water has an N concentration of 0.5 mg l^{-1} (Odum, 1955), then the change in storage represents only 3 Gg N yr Although additional losses of "old", low-nitrogen water may occur through submarine springs, such losses would reduce the nitrogen export estimates in the hydrologic submodel, and thus maintain the discrepancy between imports and exports.

Although groundwater N storage could be higher if N concentrations in the recharge waters exceed 1 mg N l^{-1}, thus far such concentrations in well water are uncommon over more than local areas (USGS 1976). Nonetheless, only 63 Gg NO_3^--N yr^{-1} would be required to raise the mean groundwater nitrate concentration above the value presently deemed safe for human consumption (NRC 1978). This represents only 17% of the 361 Gg yr^{-1} of newly-fixed N (gross inputs minus atmospheric recycle of NH_3, NO_x, and organic N) entering the system annually. It would appear that only a slight decrease in the denitrification rate could result in rapid contamination of infiltrating groundwater, if indeed denitrification accounts for the mass-balance discrepancy in Table 1.

As an alternative to groundwater storage, nitrogen could be accumulating in the biomass or soils of aggrading ecosystems in the peninsula. Some Florida ecosystems accrete N at significant rates (Table 2), in response to heavy fertilization, to N_2 fixation, or to anoxic conditions that lead to low mineralization rates. Three of the ecosystems in Table 2 (pine flatwoods, legume pasture, and fertilized sod) show N accumulation rates in the same range as that obtained by dividing the N discrepancy (267 Gg N) by the ecosystem area (109 Mm), i.e. 2.5 g N m^{-2} yr^{-1}. However, opposing this accretion is the release of N whenever forests are cut or arable land is tilled. In the Florida peninsula, the demand for land by the rapidly expanding population has led to a conversion of agricultural acreage, commercial forests and wetlands to urban land (IFAS, 1975), i.e. from systems of high potential for storage of nitrogen to systems of low storage potential. Release of stored nitrogen resulting from changes in land use may actually exacerbate the mass balance discrepancy in Table 1.

TABLE 2

RATES OF NITROGEN ACCUMULATION AND DENITRIFICATION IN SEVERAL FLORIDA ECOSYSTEMS

System	N Accumulation [a]	Denitrification [a]	Reference
Lake Okeechobee	1.7	1	Messer and Brezonik (1978)
Everglades Agricultural Area	0.4-0.8[b]	12-25	Messer (1978)
Pine Flatwoods	2.2		Smith and Pritchett (1975)
Pine Flatwoods		0.4-2[c]	Boomsma and Pritchett (1979)
Legume Pastures	5.4		Blue [d]
Fertilized Sod	2.5		Blue [d]
Coastal Plain Cropland, Georgia		6	Gambrell *et al.* (1975)

[a] g N m^{-2} yr^{-1}.

[b] Prior to drainage and cultivation.

[c] N_2O only.

[d] W. Blue, Soils Dept., University of Florida (Personal Communication).

The scenario presented above assumes that primary productivity is a constant function of ecosystem age or development. We may hypothesize, however, that increased N inputs resulting from fertilization or fossil fuel combustion may increase primary productivity in some ecosystems. For example, N fertilization often increases standing crop N in Florida woodlands (Smith and Pritchett, 1975). Thus if old forests grown under low N inputs are harvested at the same rate that new forests are growing under higher N inputs, total N storage will increase until inputs to all forests are equivalent over their lifetimes (ca. 15 yr). Furthermore, N stored in soil humus or fixed on clays during steady-state may have long residence times, even after a natural system is tilled or urbanized. Under these circumstances, some N storage could occur during periods of increasing primary productivity, even though the overall area devoted to such aggrading systems could show a net decline. Such an explanation is likely to account for only a small fraction of the average N accumulation rate, however, and thus denitrification would appear to remove the majority of the anthropogenic N input to the system.

Denitrification rates have been determined in very few ecosystems in Florida. Boomsma and Pritchett (1979) have observed low rates of N_2O production in unfertilized pine flatwoods in the peninsula, but total denitrification was not estimated. Although anoxic lake sediments would appear to be an ideal location for the process, studies on Lake Okeechobee (Messer and Brezonik, 1978) revealed an annual loss of only 1.09 g N $m^{-2}yr^{-1}$ by denitrification. The high rates in EAA soils (Table 2) result from peat accumulated during the past 4,000 yr, and thus these rates do not account for recent N inputs. However, the high rates of denitrification found in nitrogen-rich EAA histosols, together with those in agroecosystems of the Georgia Coastal Plain (Gambrell *et al.*, 1975) suggest that a mosaic of high rates (>5 g N m^{-2} yr^{-1}) may be interspersed among a generally low background (<1 g N m^{-2} yr^{-1}).

Such a mosaic may have important consequences with respect to N_2O production. Although the ratio of N_2O:N_2 produced during denitrification has a wide range of reported values, high ratios are favoured by high soil nitrate, especially at low pH (Blackmer and Bremner, 1978). Addition of nitrogen fertilizers to Florida's acidic sands thus may provide ideal conditions for N_2O production. Boomsma and Pritchett (1979) observed N_2O production rates ranging from 0.1 to 5 mg N m^{-2} day^{-1} in fertilized pine flatwoods, and Messer (1978) found N_2O production rates up to 13 mg N m^{-2} day^{-1} from an EAA histosol, accounting for 20% of the total N denitrified. Inasmuch as the EAA histosols generally show a neutral pH, the high ratio must result primarily from the high nitrate concentration in the soil solution.

Although a strictly quantitative statement cannot be made with the existing data, the Florida nitrogen mass balance indicates the potential hazards that can derive from anthropogenic acceleration of the flux of a normally beneficial element. Presently, nitrogen concentrations in runoff and in groundwater supplies are not alarming. As NO_x emissions increase in concert with fossil fuel consumption, however, the effectiveness of ecosystems as scavengers of atmospheric nitrogen may decrease, if these systems become growth-limited by other elements or if they are replaced by systems less capable of storing nitrogen. These anthropogenic emissions of nitrogen may then result in impacts on drinking water supplies and in increasingly eutrophic estuarine ecosystems.

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PROCESSES CONTROLLING THE NITROGEN CYCLE IN THE ATMOSPHERE OVER AUSTRALIA

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INTRODUCTION

During the last few decades man has contaminated the earth's atmosphere with a large number of chemical species. His influence is difficult to quantify because for many species, the magnitude of the natural emissions is not known; nor do adequate records of their past or present atmospheric concentrations exist.

Most interest in nitrogenous species has been focussed on nitric oxide (NO) and nitrogen dioxide (NO_2) because of the prominent role they play in the generation of photochemical smog. However, increasing concern is now being expressed about ammonia (NH_3) because of its involvement in the contamination of water bodies and the formation of aerosols affecting health, visibility, corrosion, and precipitation processes, and nitrous oxide (N_2O) because of its possible role in the destruction of stratospheric ozone.

This paper presents information on the concentrations of these nitrogenous species in the atmosphere above Australia, and the processes controlling their production, transport and removal from the atmosphere.

CONCENTRATIONS OF NITROGEN COMPOUNDS IN THE ATMOSPHERE

The available data on atmospheric concentrations of nitrogen compounds over Australia are summarised in Table 1. Obviously the observed concentrations of these species depend on the strength and proximity of the source, the extent of mixing in the atmosphere and the effectiveness of removal mechanisms: eg. the concentration of NO and NO_2 in the urban atmosphere is high due to the dominating influence of combustion processes, but low in the rural atmosphere. Atmospheric NH_3 concentrations vary markedly and some of this variation is due to the dual propensities of the landscape to act as a net source or sink for NH_3 depending on the moisture status of the soil (Denmead *et al.*, 1978). Apparent variations may also occur as a result of different calibration standards. For instance, the discrepancy in Table 1 between the N_2O observations of Roy on the one hand and Denmead on the other has been found (by intercalibration tests) to be largely due to this cause.

SOURCES OF ATMOSPHERIC NITROGEN COMPOUNDS

Nitrous Oxide

An increasing amount of N_2O is produced by combustion processes (Pierotti and Rasmussen, 1976) but at present most appears to be produced in soils by the action of microorganisms on other nitrogenous compounds. There are a number of ways in which N_2O may be produced. It may be formed (a) under anaerobic conditions by reduction of nitrate (NO_3^-), (b) under aerobic conditions during the assimilation of NO_3^-, or (c) under aerobic conditions during the oxidation of ammonium ion (NH_4^+) to NO_3^- (Freney *et al.*, 1979).

Nitrate is not a preferred source of nitrogen for the growth of soil microorganisms and it is unlikely that much N_2O is generated by assimilatory NO_3^- reduction. The importance of NO_3^- or NH_4^+ as precursors of N_2O is probably determined by the moisture and oxygen content of the soil; under high moisture and low oxygen concentrations most of the N_2O seems to be produced from NO_3^- by the well known denitrification process:

$$NO_3^- \rightarrow NO_2^- \rightarrow [H_2N_2O_2] \rightarrow N_2O$$

However, at low moisture contents and under aerobic conditions much of the N_2O probably comes from the oxidation of ammonium ions:

$$NH_4^+ \rightarrow NH_2OH \rightarrow [H_2N_2O_2] \rightarrow N_2O$$

TABLE 1

CONCENTRATIONS OF NITROGEN COMPOUNDS (AS N) IN THE ATMOSPHERE OVER AUSTRALIA

Constituent	Urban	Rural Day	Rural Night	Oceanic	References
N_2O (ppb≡10^{-9}v/v)	340 ± 4[a]	338 ± 3[b]	338 ± 3[c]	338 ± 3[d]	Roy (1979)
	314 ± 12[e]				Denmead (unpublished)
NH_3 (ppb)	5 ± 4				Galbally (unpublished)
		1 - 3		~ 0.1	Ayers and Gras (1979)
		0 - 36[f]			Denmead *et al.* (1975, 1976, and unpublished)
				0.6 to 1.0	Tsunogai (1971)
NO + NO_2 (ppb)	0 - 500	1 - 4	0 - 20	0.1 to 0.2	Galbally and Roy (1979), Galbally (1975)
HNO_3 (ppb)				0.2	Huebert and Lazrus (1978)
Particulate NH_4^+ (μg m^{-3})		0.05 to 0.2			Ayers and Gras (unpublished)
NO_3^- (μg m^{-3})	0.03	0.02			Dixit *et al.* (1974)
Rainwater NH_4^+ (g m^{-3})		0.1		< 0.5	Wetselaar and Hutton (1963)
NO_3^- (g m^{-3})		0.6		~ 0.1	Baseline, 1977.

[a] Based on daily measurements at Aspendale (Vic), at 6 m above ground between October 1976 and May 1978.

[b,c] Based on 260 measurements at Rutherglen (Vic), at 1 m above ground between April 1978 and May 1978.

[d] Based on 5 measurements over the Southern Ocean between Latitudes 45 - 65°S in December 1977.

[e] Based on 780 hourly measurements by infra red gas analysis at Canberra (ACT) at 1.5 m above ground between April 1977 and November 1978.

[f] Based on 118 measurements of up to 2 h duration at approximately 1 m above ground.

Appropriate methods for measuring emissions of N_2O from soils in the field are still being developed (Roy, 1979; Denmead, 1979). As yet there is much uncertainty about the magnitude and seasonal patterns of emission. The most extensive Australian measurements have been made on an unfertilized grass-clover sward for 45 days at irregularly spaced intervals over a period of five months (Denmead *et al.*, 1979a). These measurements showed important influences of soil moisture and soil temperature on the production of N_2O. The surface soil moisture content on this sward ranged from air dry (8%) to near saturation (38%). Some N_2O emission was measured on every occasion even at very low soil moisture contents, but emissions increased markedly with increasing soil moisture. The average daily rate was 1.5 mg N m^{-2}, and the emissions ranged from 0.05 mg N m^{-2} (winter, dry soil), to 21.7 mg N m^{-2} (spring, wet soil). There was a diurnal cycle in the emission of N_2O which was closely related to the temperature of the surface 3 cm of soil.

Subsequent work by Roy, Denmead, Freney and J.R. Simpson (unpublished results) has emphasized the large variability in field N_2O emission rates. Working in a productive grass-clover pasture in late autumn with a dry soil, they found daily emissions of only about 0.3 mg N m^{-2}

and variations in emission rates within a few metres of more than 100%.

Australian studies of N_2O evolution from agricultural soils in the field by Burford and Stefanson (1973) and Denmead *et al.* (1979b) have indicated much larger emissions than those reported above (up to 28 mg N m^{-2} day^{-1}) but these have been short-lived, persisting for only a few days. Much more work is needed before a reliable estimate of N_2O production from Australian soils can be made.

Ammonia

The primary sources of atmospheric NH_3 appear to be animal urine and the microbial decomposition of organic material on the earth's surface (Söderlund and Svensson, 1976), but as with N_2O, large emissions of NH_3 can be expected to occur sporadically from applications of nitrogen fertilizers.

Given Australia's large areas of grazing land, it could be expected that pastures could be important contributors of NH_3 to the Australian atmosphere. Studies of NH_3 emissions from pastures grazed by sheep have been made by Denmead *et al.* (1974, 1976). They found that substantial fluxes of NH_3 into the atmosphere do occur. There is a diurnal cycle in the emission which appears to parallel the course of evaporation from the soil surface. Other factors which can be expected to have an influence are the nitrogen content of the herbage, pH, temperature and moisture content of the soil, animal water intake and animal stocking rate.

Denmead *et al.* (1974, 1976) measured an average daily emission rate of 13 mg N m^{-2} with 22.5 sheep ha^{-1}, and 26 mg N m^{-2} with 50 sheep ha^{-1}. Using the figures given by Healy *et al.* (1970) for urea excretion by sheep, these data indicate that about 26% of the nitrogen excreted in urine by the sheep escaped as NH_3 to the atmosphere. Applying the same percentage to the nitrogen excreted in urine by all the domestic animals in Australia (ABS, 1978 for numbers of animals; Healy *et al.*, 1970 for excretion rates), gives an annual input of approximately 500 Gg of NH_3-N from the continent.

The figure of 26% release of NH_3-N from urea-N which we have adopted is much higher than the 10% used by Healy *et al.* (1970) and Soderlund and Svensson (1976) in their estimates of atmospheric NH_3 inputs. The higher figure could account for a good part of the apparent imbalance in the global NH_3 budget calculated by the latter authors. More work is obviously needed.

Measurements of NH_3 emissions from an ungrazed grass-clover pasture were also made by Denmead *et al.* (1976). They found that, although NH_3 was released to the air at the soil surface at rates comparable with grazed pastures (presumably through decomposition of old plant material), almost all of it was absorbed by the growing plants above. Net emissions to the atmosphere measured intermittently over a period of 6 months, were only about 3 mg N $m^{-2}day^{-1}$. It is very difficult to arrive at an Australia-wide emission from decaying plant material.

Some of the NH_3 absorbed by plants from the air could subsequently be released at the end of the growing season. Farquhar *et al.* (1979) found that NH_3 can be released to the air by senescing plants. They measured rates equivalent to the release of 0.7 mg N m^{-2} $day^{-1}$for a leaf area index of 1. Using this figure for the NH_3 yield from senescing vegetation over the third of Australia which supports reasonably dense vegetation, and assuming that the senescence occurs over 30 days, we calculate an annual input to the atmosphere of approximately 50 Gg of NH_3-N.

Other Australian work has been concerned with NH_3 losses from fertilized, cropped soils. Emissions have varied from 1% of the nitrogen applied (Denmead *et al.*, 1977, for the injection of anhydrous NH_3) to 30% (V.R. Catchpoole and Denmead, unpublished, for the surface application of urea). Assuming an average loss, as NH_3-N, of 10% of the 200 Gg of nitrogen fertilizers applied annually in Australia, the total emission would be 20 Gg yr^{-1}.

Ammonia is also emitted to the atmosphere from combustion processes, most notably from the burning of coal. Using Robinson and Robbins (1972) emission factor of approximately 1 g NH_3-N kg^{-1} coal, an input of 67 Gg NH_3-N yr^{-1} occurs from this source in Australia.

These very tentative estimates suggest that 600 to 700 Gg of NH_3-N are emitted annually to the Australian atmosphere, most of it from rural sources, and perhaps more than 70% from

animal urine.

Nitric Oxide and Nitrogen Dioxide

Nitric oxide is produced naturally in soils by chemical and microbial reactions (Steen and Stojanovic, 1971) and, in the atmosphere, by oxidation of atmospheric NH_3 (Cox *et al.*, 1975), oxidation of molecular nitrogen by lightning (Chameides *et al.*, 1977) and meteoroid impact on the upper atmosphere. Of these processes, only lightning and meteoroid impact add to the pool of fixed nitrogen, the other reactions merely recycle fixed nitrogen from one reservoir to another.

Nitric oxide is formed in both waterlogged and aerobic soils and appears to be produced by the decomposition of nitrite (NO_2^-). Nitrite is produced as an intermediate in the denitrification of NO_3^- or during the nitrification of NH_4^+. However it normally accumulates in soil only under certain conditions which depend on fertilizer additions, the NH_4^+ level, pH and organic matter content of the soil, temperature, moisture, and soil - fertilizer geometry (Van Cleemput, *et al.*, 1976).

The NO flux from unamended upland soils has been shown to vary from 0.05 to 0.63 mg N m^{-2} day^{-1} (Galbally and Roy, 1978) but it is to be expected that this flux will increase as a result of NO_2^- accumulation after addition of NH_4^+ or NH_4^+-yielding fertilizers. Steen and Stojanovic (1971), for example, observed increased NO loss on addition of urea and ammonium sulphate to a calcareous soil and the loss was related to the amount of nitrogen applied.

The generation of NO in the atmosphere from the oxidation of NH_3 by OH radicals (Cox *et al.*, 1975), i.e.

$$NH_3 \rightarrow NH_2 \rightarrow NHO \rightarrow NO,$$

in the lower 2 km of the atmosphere is negligibly small compared with exhalation from the surface.

Nitric oxide is produced in the atmosphere by the heating of the air by the shock waves from lightning strokes. Using the lightning frequency data of Turman (1978) and the calculations of Tuck (1976) and Chameides *et al.*, (1977), we obtain a nitrogen fixation rate by lightning of 7 Gg N yr^{-1} for the Australian continent. Other estimates using different lightning flash rates (Chameides *et al.*, 1977) would suggest a fixation rate of as much as 70 Gg N yr^{-1}.

The extent to which bushfires fix atmospheric nitrogen, as well as release fixed nitrogen from within the fuel material, is presently not known although elevated nitrogen oxide concentrations are found in bushfire smoke plumes (Evans *et al.*, 1977).

Nitrogen oxides as NO or NO_2 are also emitted from automobiles, furnaces, etc. This source of fixed nitrogen was about 300 Gg N yr^{-1} in Australia in 1971 (Thompson and Strauss, 1973) and is rapidly growing. These emissions are predicted to increase by a factor of about three between the 1970's and 2000 AD, (Australian Transport Advisory Council 1974; I.C. McIlroy 1979, personal communication). At present they appear to be of the same magnitude as the emissions from natural sources.

TRANSPORT AND REMOVAL OF NITROGEN COMPOUNDS IN THE ATMOSPHERE

The distance of transport of nitrogen compounds in the atmosphere depends on the source location and the efficiency of removal mechanisms for these species.

Nitrous Oxide

There can be no doubt that soil has the capacity to act as a sink for N_2O as many soil organisms can reduce N_2O to N_2 (Payne, 1973). However the reduction of N_2O to N_2 by soil microorganisms is inhibited by NO_3^- (Blackmer and Bremner, 1978) and it is most unlikely that soils will act as a sink for N_2O whenever they contain NO_3^- or produce NO_3^- from NH_4^+ or soil organic matter (Freney *et al.*, 1978).

The only known sink for N_2O is removal by photodissociation and chemical reaction in the stratosphere (McElroy and McConnell, 1971; Nicolet and Peetermans, 1972). This removal gives an atmospheric lifetime of N_2O of the order of many decades. Thus if extra N_2O is produced in an area due to modification of soil processes we can expect that N_2O to **be evenly**

distributed throughout the global atmosphere.

Ammonia and Nitrate

There are very few published reports on the nitrogen content of rainwater over Australia and they come from scattered geographical regions. Nitrogen depositions in rainwater near Townsville (Probert, 1976) occurred predominantly as NH_4^+; the total depositions (170-310 mg N m^{-2} yr^{-1}, $NO_3^- + NH_4^+$) were similar to those reported for Cairns, Queensland (Brunnich, 1909) but higher than those at Katherine, NT, (Wetselaar and Hutton, 1963). The practice of burning native pastures in the area makes it most unlikely that the calculated deposition represents net accession to the soil. Similarly Wetselaar and Hutton (1963) found a strong correlation between NO_3^- and the ions, NH_4^+, K^+, Mg^{2+} and Cl^-, in rainwater at Katherine and concluded that most of the material in that rainwater was part of a local terrestrial cycle derived from a combination of dried vegetation and surface soil carried into the atmosphere in the form of dust. These figures come from northern Australia and can not be considered representative of the continent as a whole. Obviously, more measurements are needed, particularly in Southern Australia.

G.P. Ayers (personal communication) has examined particulate matter in the atmosphere and found other active removal processes of NH_3. He found that the plume from the Mt. Isa copper smelter in the north of the country consists, at emission, almost entirely of sulfuric acid particles. At large distances downwind these particles are found to have taken up NH_3 gas to form ammonium sulfate particles. He estimates (with considerable uncertainty) a fixation of NH_3 by the plume of 10 to 1000 Gg N yr^{-1}. His upper estimate is probably too high, since, by our previous calculations, it would account for all the NH_3 emitted from the continent. Nevertheless, this one industrial activity appears to be very important in determining the fate of atmospheric NH_3 over Australia.

Other mechanisms by which NH_4^+ and NO_3^- can appear in rainwater include the direct dissolution of gaseous NH_3 and gaseous HNO_3 into droplets. Gaseous HNO_3 is formed from the reaction:

$$NO_2 + OH\ (+M) \rightarrow HNO_3$$

The half life of this reaction for NO_2 removal under Australian conditions is probably of the order of 1 day (see Cox, 1977).

Nitric Oxide and Nitrogen Dioxide

Nitric oxide in the atmosphere is freely interconnected with NO_2 by the reactions:

$$NO + O_3 \rightarrow NO_2 + O_2$$

$$NO_2 + h\nu \rightarrow NO + O,\ \lambda < 410\ nm.$$

This gaseous NO_2 is continuously removed from the atmosphere by absorption at plant, soil and water surfaces. The usual way of describing gaseous uptake, U, (e.g. g N m^{-2} s^{-1}) is in terms of an uptake or "deposition" velocity, Vg, and the concentration of the gas within the air, C, where

$$U = Vg \times C.$$

In using this equation, we assume that for a given surface in a given condition, Vg is, to a first approximation, constant and U varies linearly with C. The equation is obviously an oversimplified description of gaseous transport and uptake in the real world, but in the present context, it provides some useful pointers to the fate of atmospheric nitrogen species. The uptake velocity for NO_2 of grassland and soil is of the order of 10^{-2} m s^{-1} (Galbally, 1974, and unpublished results).

We can calculate how far NO_2 travels in the lower atmosphere before 50% of it is re-absorbed at the soil surface using the above information and some simple assumptions about the atmospheric boundary layer (see Galbally, 1975a). A similar problem for SO_2 is discussed by Bolin *et al.*, (1973). We find that 50% of the NO/NO_2 is probably re-absorbed at the surface within 20 km at night and 100-200 km during the daytime.

Ammonia is absorbed directly on to soil and can be strongly absorbed by plants (Denmead *et al.*, 1976; Hutchinson *et al.*, 1972). The NH_3 may be absorbed through the stomata but other

mechanisms for plant absorption appear to be involved Denmead *et al.* (1976, 1978). It is probable that the uptake velocity for NH_3 is about 10^{-2} m s^{-1}, similar to that for NO_2. Thus the calculated distances of travel before 50% re-absorption will apply to both NO_2 and NH_3

SUMMARY - AN AUSTRALIAN PERSPECTIVE

There is insufficient data available to determine whether agricultural practice in Australia has resulted in increased N_2O release to the atmosphere. If, or when, such an increased release occurs, the N_2O will subsequently be homogeneously distributed globally (as would increased emissions from elsewhere). According to present knowledge (Nicolet and Peetermans, 1972) more than 90% of the N_2O will be transformed in the stratosphere to N_2. The remaining fraction is converted to NO and ultimately returned to the earth as NO_3^-.

The cycles of NH_3 and NH_4^+ in air over Australia must have been substantially changed by the introduction of grazing animals and industrial processes into Australia. A substantial proportion of the sheep and cattle in Australia are grazed in the south eastern region within 200 km of the coast at the latitudes where westerly winds are dominant. Given that perhaps one quarter of the nitrogen in urine is volatilized as NH_3 then it appears that a substantial amount of fixed nitrogen could be lost from the Australian continental region by atmospheric transport to the surrounding oceans.

Similarly with NO and NO_2, the emissions from automobiles and stationary combustion sources must have significantly perturbed the natural cycle. Also, the introduction of domestic animals most probably has increased nitrification rates within the soil causing increased NO emissions. Again, because a large proportion of the industrial, urban and agricultural activities are in south eastern Australia, much of this fixed nitrogen is probably lost to the surrounding oceanic regions by atmospheric transport also.

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DIFFERENT FORMS OF MICROBIAL MANGANESE OXIDATION AND REDUCTION AND THEIR ENVIRONMENTAL SIGNIFICANCE

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MANGANESE OXIDATION

A variety of bacteria have been found to be able to catalyze manganese oxidation. They can be divided into two major groups on a physiological basis. To one of these two groups are assigned those organisms that act on free Mn^{2+}, and to the other group are assigned those organisms that can act only on Mn^{2+} bound to Mn(IV) oxide. Of the group that attacks free Mn^{2+} ions, some members seem to be able to derive energy from the reaction while others cannot. So far, all members of a group of seven different organisms that attack only Mn^{2+} bound to Mn(IV) oxide are able to get energy from the oxidation (Ehrlich, 1976, 1978a; unpublished data). In the oxidation of the free ion according to the following reaction (Equation 1),

$$Mn^{2+} + 0.5\,O_2 + H_2O = MnO_2 + 2H^+ \qquad 1$$

the standard free energy change of reaction at pH 7.0 ($\Delta F'$) is -68.24 kJ (Ehrlich, 1978a). Thus, it is not unreasonable that, at neutral pH, oxidation of Mn(II) to Mn(IV) should, yield enough energy to be biologically useful. In Table 1 some manganese-oxidizing bacteria are classified according to their action on manganese in laboratory culture. *Leptothrix discophora* has been reported to grow autotrophically on Mn^{2+} (Ali and Stokes, 1971). *Hypomicrobium manganoxidans*, although not autotrophic, must oxidize Mn^{2+} in order to grow (Eleftheriadis, 1976). It has been implied that the latter organism is an obligate mixotroph, but this needs further confirmation. Manganese oxidation by these two organisms proceeds presumably according to Equation 1. *Arthrobacter siderocapsulatus*, *Metallogenium*, and *Leptothrix pseudoochracea* have recently been reported to oxidize Mn^{2+} with H_2O_2 which they produce, using catalase as catalyst (Dubinina, 1978a,b). In this reaction, catalase appears to act as a peroxidase (Equation 2),

$$Mn^{2+} + H_2O_2 = MnO_2 + 2H^+ \qquad 2$$

Presumably no useful energy is obtained in this reaction. Its function may be to detoxify the environment with respect to H_2O_2 (Dubinina, 1978b).

In the case of a number of organisms, e.g., *Arthrobacter* strain B (Bromfield, 1956; Bromfield and David, 1976), *Citrobacter freundii* E_4 and *Pseudomonas* sp. E_1 (Douka, 1977), it is not yet known whether useful energy is derived in the oxidation of Mn^{2+}. Interestingly, *Arthrobacter* strain B and some other bacteria do not oxidize Mn^{2+} until they have reached their stationary growth phase in batch culture (Bromfield, 1956; Jung and Schweisfurth, 1976). Oxidation of Mn^{2+} by *Arthrobacter* strain B is not enhanced by bacterially produced manganese oxide (Bromfield and David, 1976).

A number of unrelated bacteria from the marine environment have been found to oxidize Mn^{2+} only if it is bound to Mn(IV) oxide (Erhlich, 1968) or, as recently discovered, to an as yet unidentified substance found in certain marine sediments (Ehrlich, 1978b; unpublished data). The action of these bacteria on Mn^{2+} in the presence of Mn(IV) oxide is best summarized by the following two reactions (Equations 3 and 4).

$$Mn^{2+} + H_2MnO_3 = MnMnO_3 + 2H^+ \qquad 3$$

$$MnMnO_3 + 0.5\,O_2 + H_2O \xrightarrow{\text{bacteria}} 2H_2MnO_3 \qquad 4$$

With excess Mn^{2+}, the second reaction is rate limiting. The transfer of electrons to oxygen involves a cytochrome-containing system (Arcuri and Ehrlich, 1980). The components

TABLE 1

SOME BACTERIA WHICH OXIDIZE MANGANESE IN PURE CULTURE

Enzymic Oxidation				Non-Enzymic Oxidation
Free Mn^{2+} attacked			Mn^{2+} bound to Mn (IV) oxide attacked	
Derive energy	No energy derived	Not known if energy derived	Derive energy	No energy derived
H. manganoxidans[1]	*A. siderocapsulatus*[3]	*Arthrobacter*[4]	*Arthrobacter* 37[10]	*Pseudomonas manganoxidans*[12]
L. discophora[2]	*L. pseudoochracea*[3]	*A. citreus*[5]	*Oceanospirillum*[11]	*Streptomyces* sp.[13]
	Metallogenium[3]	*A. globiformis*[5]	*Vibrio*[11]	
		A. simplex[5]		
		Citrobacter freudii E_4[6]		
		Hyphomicrobium T37[7]		
		Pedomicrobium[8]		
		Pseudomonas E_1[6]		
		Pseudomonas spp.[9]		

1 Eleftheriadis, 1976 (Energy derivation presumptive).

2 Ali and Stokes, 1971.

3 Dubinina, 1978a.

4 Bromfield, 1956.

5 Dubinina and Zhdanov, 1975.

6 Douka, 1977.

7 Tyler and Marshall, 1967.

8 Aristovskaya, 1961.

9 Zavarzin, 1962.

10 Ehrlich, 1968; unpublished data.

11 Ehrlich, 1978; unpublished data.

12 Jung and Schweisfurth, 1979.

13 Bromfield, 1978.

of this system vary depending on the type of bacterium. The electron transfer system enables the cell to couple ATP synthesis to manganese oxidation via oxidative phosphorylation. Such ATP synthesis has been demonstrated with membrane vesicle preparations from these organisms (Ehrlich, 1976, 1978a).

Non-enzymic manganese oxidation by bacteria has been reported by several workers. Such oxidation may be readily demonstrated when the pH of a Mn^{2+}-containing culture medium exceeds 8, and is attributable to autoxidation. Many kinds of bacteria, given an appropriate medium, can promote such oxidation. These bacteria should not be considered true manganese-oxidizing bacteria, however, unless they can be shown to promote this reaction in nature. Soehngen (1914) reported non-enzymic manganese oxidation resulting from reactions with hydroxycarboxylic acids, such as lactic, citric, and malic acids, which may be produced metabolically. Bromfield (1978) has isolated a *Streptomyces* sp. which produces a water-soluble substance capable of oxidizing Mn^{2+} at pH 5.0. Jung and Schweisfurth (1979) reported the production of an intracellular protein by *Pseudomonas* MnB1 which acts as an oxidant of Mn^{2+} in the absence of air, cofactors and other low-molecular weight compounds. The protein is consumed in the reaction and is thus not an enzyme.

Some fungi oxidize manganese at least in laboratory media. Their action can be assumed to be non-enzymic (Ehrlich, 1978a). This is because their oxidases are intracellular (in mitochondria) and are not in their cell envelopes as in bacteria. Since Mn(IV) oxide accumulation inside manganese-oxidizing fungal cells has not been observed, it must be concluded that the oxidation is extracellular, very likely caused by such metabolic products as citric acid. Interestingly, recent isolates of *Metallogenium* were always found to develop in conjunction with a fungus (Schmidt, 1976; Schweisfurth and Hehn, 1972). This may indicate that these forms of *Metallogenium* are part of a microbial consortium. Indeed, the discrete deposits of manganese oxides at swollen sites of the fungal hyphae were attributed to the association with *Metallogenium* at these sites (Schmidt, 1976). Zavarzin (1964) concluded that a special form of *Metallogenium*, namely *M. symbioticum*, functions in this fungal association. Other workers, however, have questioned whether the structure corresponding to *Metallogenium* is actually an organism (e.g., Schweisfurth, 1969; Mulder, 1972; Klaveness, 1977).

MANGANESE REDUCTION

A variety of bacteria and fungi also reduce manganese oxides. The bacteria can be grouped as to whether they cause this reduction enzymically, non-enzymically, or by a combination of both mechanisms. Fungi reduce manganese oxides non-enzymically by producing, for instance, the reductant, oxalic acid. Enzymic manganese oxide reduction usually requires an organic electron donor, although hydrogen (H_2) with bacterial hydrogenase has been shown to be effective in reducing MnO_2 (Woolfolk and Whitely, 1962). The classification of MnO_2-reducing bacteria, and the physiology of MnO_2 reduction, is discussed in more detail in connection with leaching of manganese ores (Ehrlich, (1980). It should be stressed here that, in most cases, biological reduction of manganese oxides appears not to be very sensitive to oxygen.

MANGANESE TRANSFORMATIONS IN NATURE

Since a variety of bacteria and fungi appear to be active manganese oxidizers or reducers in pure culture, the question arises of their significance in nature. In soil, ample evidence indicates that the manganese reducers contribute to manganese mobilization while the oxidizers contribute to its immobilization (e.g., Mann and Quastel, 1946; Leeper and Swaby, 1940; Aristovskaya, 1961; Alexander, 1977). While it is probable that the latter organisms play a role in the formation of soil concretions (Aristovskaya and Zavarzin, 1971), this has not yet been clearly resolved.

The mobilization and immobilization of manganese by microbes also occurs in freshwater systems, such as lakes, where manganese reducers enrich the hypolimnion with dissolved manganese, and where oxidizers in the oxygenated water column or in shallow oxygenated waters, especially at the sediment-water interface, oxidize manganese (Klaveness, 1977; LaRock, 1969; Schmidt, 1976; Sokolova-Dubinina and Deryugina, 1967; Troshanov, 1968). The oxidations, in some cases, may lead to formation of a film on the sediment (LaRock, 1969) and, in other cases, to the formation of concretions (Kindle, 1932; Dean and Ghosh, 1978). To what extent manganese oxidizers have

been involved in manganese ore formation is in most cases unclear at present. It might be mentioned, however, that Crerar *et al.* (1980) found evidence of fossilized *Metallogenium* structures in manganese ore from the island of Timor. Although they view this ancient *Metallogenium* as a marine form, it seems not unlikely that it may have been a freshwater form active during diagenesis of the marine ore after uplift of the island from the ocean floor. Contemporary *Metallogenium* has so far not been reported from the marine environment (Sorokin, 1971).

In the marine environment, manganese is concentrated in concretions and pavements at the water-sediment interface of the ocean floor. The concretions have been shown to harbor significant populations of manganese-oxidizing, manganese-reducing, and manganese-inactive bacteria (Ehrlich *et al.*, 1972). They have also been shown to harbor foraminifera and other shelter-building protozoa (e.g., Dugolinsky *et al.*, 1977). These observations suggest that the nodule surface may be an ecosystem in which bound Mn^{2+} is a primary energy source. The nodules, through their constituent Mn(IV) oxide scavenge Mn^{2+} from seawater or the interstitial water of sediment. This manganese can then be oxidized by Mn(II)-oxidizing bacteria which gain useful energy from this process enabling them to assimilate scarce organic carbon (Ehrlich, 1975, 1976). Assimilable organic carbon, including that produced by the manganese oxidizers, would be kept scarce through the metabolism of Mn(IV)-reducing bacteria and manganese-inactive bacteria on the nodules. The protozoa can be assumed to feed on the bacteria and thus ensure continued bacterial multiplication, which insofar as manganese oxidizers are concerned, would ensure continued Mn(II) oxidation and thus nodule growth. As can be seen, a complicated food web may exist on the surface of the nodules.

Since microbial manganese oxidation can occur under conditions that range from aerobic (e.g., Bromfield, 1956; Douka, 1977; Ehrlich, 1968) to microaerophilic (Schmidt, 1976; Klaveness, 1977; Uren and Leeper, 1978) to anaerobic (Jung and Schweisfurth, 1979), evidence of biological manganese oxidation in the geologic record tells little of the prevailing redox conditions at the time the oxide was formed, without knowledge of the physiology of the organisms responsible. Similarly, evidence of microbial manganese oxide reduction is not a useful indicator of prevailing redox conditions during manganese oxide reduction because many bacteria are not particularly sensitive to oxygen (Trimble and Ehrlich, 1968; Troshanov, 1969). This is in contrast to iron as a redox indicator in ancient biological systems (see, e.g., Cloud, 1974).

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THE ROLE OF SURFACE ATTACHMENT IN MANGANESE OXIDATION BY FRESHWATER HYPHOMICROBIA

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INTRODUCTION

The existence of particular valency states of manganese is governed mainly by the pH and redox potential (Eh) of the appropriate system (Morgan and Stumm, 1965). In the oxidation of manganese from the Mn (II) to the Mn (IV) state, the overall reaction may be visualized as follows (Equations 1-3):

$$Mn(II) + O_2 \xrightarrow{slow} MnO_2(s) \quad 1$$

$$Mn(III) + MnO_2(s) \xrightarrow{fast} Mn(II).MnO_2(s) \quad 2$$

$$Mn(II).MnO_2(s) + O_2 \xrightarrow{slow} 2MnO_2(s) \quad 3$$

where $MnO_2(s)$ represents an approximation of the solid-phase Mn(IV) state. Morgan and Stumm (1965) determined that all of the Mn(II) removal cannot be accounted for as MnO_2. In fact, the product has varying compositions ranging from $MnO_{1.3}$ to $MnO_{1.9}$. These products can be accounted for by the rapid sorption of Mn(II) onto incipiently-formed hydrous MnO_2.

Microorganisms appear to catalyze faster rates in the reactions (Equations 1-3) given above (Ehrlich, 1968). A wide range of microorganisms are involved in manganese oxidation in soils, waters and in large scale manganese ore deposits (Marshall, 1979). These microorganisms deposit insoluble, higher valency manganese species on the outside of the microbial cells. Bromfield (1958) reported that *Corynebacterium (Arthrobacter)* deposited an amorphous material of variable composition in the range of $MnO_{1.76 - 1.88}$, whereas Ivarson and Heringa (1972) indicated that the fungus *Cephalosporium* deposited either hausmannite (Mn_3O_4) or birnessite (δ-MnO_2). Proposed mechanisms for the microbial oxidation of Mn(II) to higher valency forms include microbially-induced alterations to the pH and/or Eh of microhabitats, sorption of preformed oxides to cell surfaces, microbial degradation of organic-manganese complexes, the formation of insoluble protein-manganese complexes, and enzymic oxidation of Mn(II) (Marshall, 1979).

Microbial deposition of manganese from oligotrophic and mesotrophic waters occurs mainly at solid surfaces and involves the adhesion of manganese-oxidizing bacteria to the surfaces. The present paper describes the mode of adhesion by manganese-oxidizing hyphomicrobia to hydro-electric pipeline surfaces and the process of efficient manganese deposition by these prosthecate bacteria.

ATTACHMENT TO SURFACES

The levels of available organic and inorganic nutrients in oligotrophic waters are usually insufficient to support substantial microbial growth. Organic materials, especially macromolecules (Baier *et al.*, 1968), and nutrient cations tend to concentrate at the negatively-charged surfaces commonly found in natural habitats. These materials alter the wetting properties of the surface (Baier *et al.*, 1968) and provide a nutrient-rich environment for microorganisms in a normally nutrient-deficient aqueous phase. In high pressure hydro-electric pipeline systems, it is obviously imperative that microorganisms firmly adhere to the pipeline surfaces in order to take advantage of the continual nutrient replenishment occurring at the solid-liquid interfaces.

The transport to the surface of bacteria suspended in a turbulent flow fluid is dependent upon fluid velocity, turbulent eddy transport, Brownian motion, concentration, size and density of the bacteria, and motility generated by a chemotactic response in some bacteria (Characklis, 1979). Once near the surface, the bacteria can reversibly sorb because of a net attraction energy resulting from interactions between van der Waals attraction energies and diffuse-double layer repulsion energies near the surface (Marshall *et al.*, 1971).

Reversibly sorbed bacteria are held at a finite distance from the surface and can only establish a firm (irreversible) adhesion to the surface by the production of a suitable extracellular polymer, which serves to anchor the cell to the solid surface.

The colonization of hydro-electric pipeline surfaces by hyphomicrobia and the development of substantial manganese deposits have been described in detail by Tyler and Marshall (1967a,b). Subsequent studies (Marshall and Cruickshank, 1973) have revealed that hyphomicrobia exhibit a perpendicular orientation at solid-water, air-water and oil-water interfaces (Fig. 1A). It appears that the base of the larger mother cell is relatively hydrophobic, resulting in a

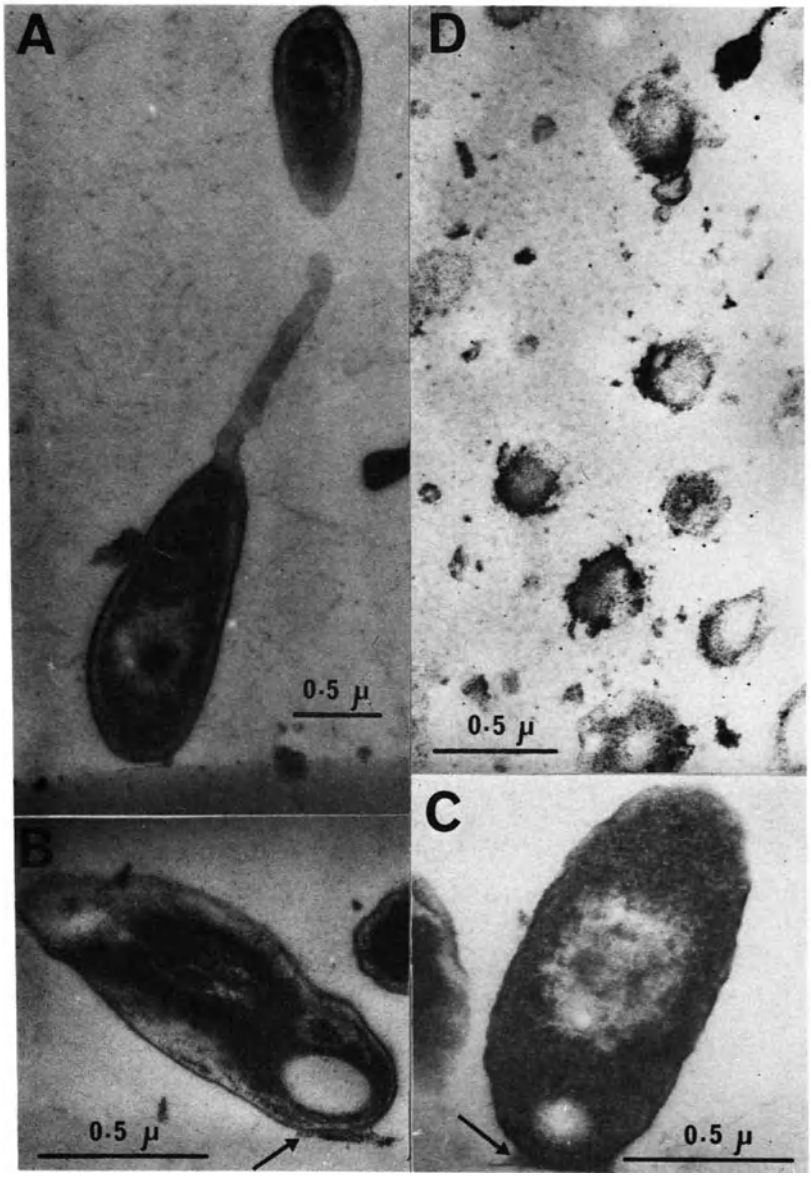

Figure 1: Attachment of hyphomicrobia to an araldite surface. After washing to remove superficially attached organisms, the block was reembedded in araldite and sections cut across or obliquely to the original solid-liquid interface. A, orientation at the interface with the base of the mother cell attached to the surface and the prostheca and bud facing away from the surface; B and C, showing the polymer anchoring the cells to the surface; and D, oblique section showing the amorphous nature of the polymer.

preferential attraction of this portion of the cell to the non-aqueous phase in any two-phase system. The hyphomicrobia produce an amorphous bridging polymer (Figs. 1B,C,D) which very firmly anchors the cells to the surface. Once firmly attached to the surface, the hyphomicrobia are able to take advantage of the nutrients and soluble manganous ions attracted to the solid-liquid interface.

DEVELOPMENT OF MANGANESE DEPOSITS

The early stage of manganese deposition from freshwater samples by hyphomicrobia was examined by regular microscopic examination of coverslips taken from a recirculation apparatus similar to that described by Tyler and Marshall (1967a). These authors had demonstrated that manganese deposition failed to occur in this system in the absence of microorganisms. The manganese-oxidizing hyphomicrobia were not the primary colonizing microorganisms, as adhesion of other conventional rod-shaped bacteria was observed prior to attachment of the hyphomicrobia (Fig. 2). This observation suggested that the hyphomicrobia depend on some

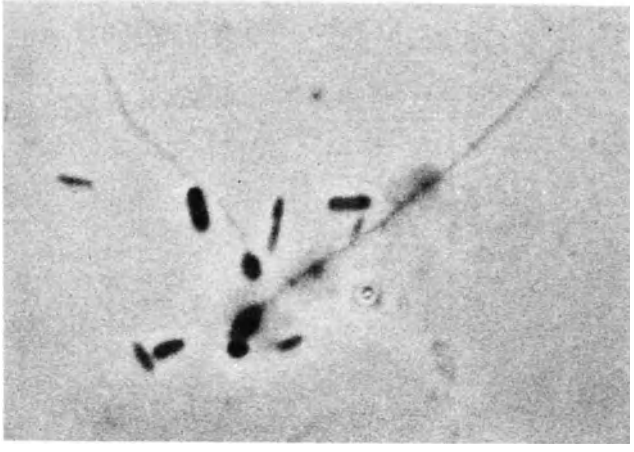

Figure 2: Colonization of a glass surface immersed in Lake King William, Tasmania, water for 48 h. Note manganese deposition on cell and prostheca of the hyphomicrobia.

prior modification of surfaces by the pioneer species (Marshall, 1979), yet these manganese-oxidizing bacteria ultimately become dominant on such surfaces. Manganese deposition was first observed after about two days as discrete zones of benzidine-reactive material on both the mother cells and on the prosthecae of the hyphomicrobia (Fig. 2), with no sign of manganese deposition by the other bacteria colonizing the surface. Such discrete zones suggest the occurrence of localized sites of activity resulting in modifications to the surrounding microhabitats. These modifications may include excretion of organic acids, direct enzymic oxidation of manganese or the production of insoluble manganese-protein complexes (van Veen, 1972). With continued recirculation of the water, extensive deposits developed progressively on the glass surfaces until the supply of soluble manganese in the water became limiting. As reported by Tyler and Marshall (1967a), further additions of Mn(II) to the water resulted in even greater Mn(IV) deposition on further recirculation of the water.

Although the bulk of the manganese appeared to be deposited on the glass surfaces, possible deposition of manganese by hyphomicrobia in the bulk aqueous phase was examined by centrifuging 5 l aliquots of water from the apparatus after 2, 6 and 10 days recirculation and observing the deposited bacteria under the microscope. A wide variety of bacteria were obtained from the water after two days including single (swarmer) and budding hyphomicrobia. None of these organisms showed any signs of manganese deposition. At later samplings, the hyphomicrobia still constituted only a small proportion of the water phase bacteria and, of these hyphomicrobia, between 10 to 25% showed slight manganese deposition. It is likely

that some of these free-floating manganese depositing bacteria were dislodged from the glass surfaces by reentrainment processes (see below). It appears unlikely, at least in nutrient poor waters, that potential manganese-oxidizing hyphomicrobia can obtain sufficient nutrient and manganese from the bulk aqueous phase.

Pleomorphic hyphomicrobia from hydro-electric pipeline deposits (Tyler and Marshall, 1967c) seem ideally suited to the rapid development of large scale deposits of manganese at surfaces (Tyler and Marshall, 1967b). Once firmly sorbed to the pipeline surface, the bacteria begin to deposit manganese (Fig. 2) and, subsequently, form three-dimensional colonies by branching of the prosthecae and the formation of intercalary buds (Fig. 3). This process

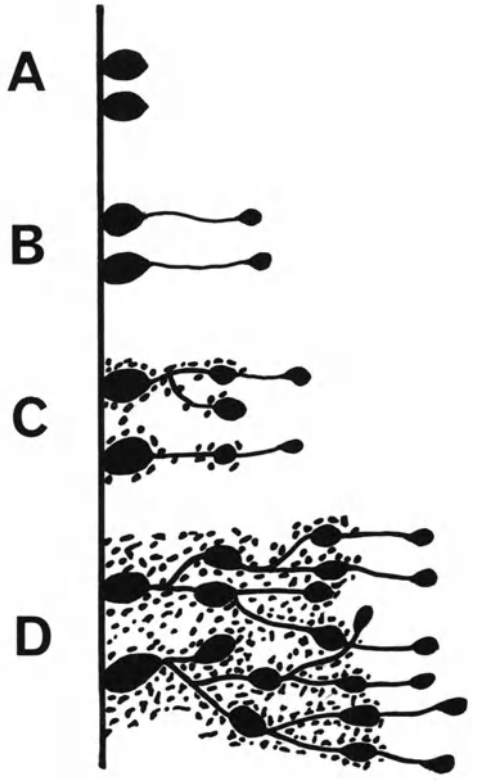

Figure 3: Diagrammatic representation of: A, attachment of hyphomicrobia to the surface; B, initial growth and bud formation; C, initial manganese deposition; and D, extensive branching and intercalary bud formation, with young buds not enveloped by manganese deposit.

has the advantage that, as manganese deposits develop around the cells and prosthecae, metabolically active buds are always present in the aqueous phase and maintain the colony by transporting absorbed nutrients along the connecting prosthecae. The efficiency of this process is shown by the fact that a deposit of 1 cm thickness, containing 30 to 50% manganese and only small amounts of iron on a dry weight basis, can develop on a freshly cleaned pipe within six months (Tyler and Marshall, 1967a). For a pipe of 1 m radius and 1 km length this represents about 60 m^3 of wet deposit (Marshall, 1979). The deposits do not develop beyond this maximum thickness because, once the microbial deposit penetrates the viscous sublayer of water and enters the region of turbulent flow, eddies and turbulent bursts

generated in the fluid stream result in reentrainment of further deposited material (Characklis, 1979). The manganese deposits develop a characteristic ripple pattern in high pressure pipelines (Tyler and Marshall, 1967a) and are of considerable nuisance value to hydro-electric authorities because of the increased fluid frictional resistance developed at the surface.

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ELECTRON TRANSFER COUPLED TO MN(II) OXIDATION IN TWO DEEP-SEA PACIFIC OCEAN ISOLATES[a]

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INTRODUCTION

The abilities of a number of different bacteria to couple the oxidation of manganous manganese to the generation of useful energy have been reviewed (Ehrlich, 1978). The oxidation of manganese is believed to occur via the action of a Mn(II)-oxidoreductase, which catalyzes the transfer of electrons from manganese to the electron transport system of the cell. The electron transport system involved in this transfer of electrons has not been thoroughly explained. However, in the case of the soil bacterium *Leptothrix discophora*, it was found that cell-free, manganese oxidizing particles contained both b-type and c-type cytochromes, as well as a cytochrome oxidase (Hogan, 1973). Manganese oxidation by these particles was completely inhibited by 10^{-5} M KCN and 10^{-4} M NaN_3. The inhibition by CN^- and N_3^- and the results of spectral studies indicated the involvement of a cytochrome oxidase in Mn(II) oxidation. The involvement of the b-type or c-type cytochromes was not shown.

In the case of the marine bacterium *Oceanospirillum* BIII 45, it has been demonstrated that dicumarol, antimycin A, 2-nonyl-4-hydroxyquinoline-N-oxide, cyanide, and azide inhibit manganese oxidation as catalyzed by cell-free preparations of the bacterium (Ehrlich, 1976) These results suggest the possible involvement of flavins, quinones, b-type cytochromes, c-type cytochromes, and cytochrome oxidase in the oxidation of Mn(II). The involvement of a c-type cytochrome in the oxidation of Mn(II), as catalyzed by *Oceanospirillum*, has been demonstrated spectrophotometrically (Arcuri and Ehrlich, 1979).

This report reviews some of the recent work that further elucidates the electron transport pathway involved in Mn(II) oxidation by *Oceanospirillum* strains BIII 45 and BIII 82, and it presents new information concerned with the cellular organization of the Mn(II)-oxidoreductase system in these organisms.

MATERIALS AND METHODS

The bacterial isolates, cultures BIII 45 and BIII 82, utilized in this study are gram-negative isolates that can catalyze the enzymic oxidation of Mn(II). These organisms are deep-sea, Pacific Ocean isolates that have been tentatively identified as members of the genus *Oceanospirillum* (E.P. White, unpublished results).

Cultures of BIII 45 and BIII 82 were grown for 48 h at 15° on sea-water nutrient agar slants. These cells were then fractionated into membrane, periplasmic, and intracellular fractions by an EDTA-lysozyme treatment (Arcuri and Ehrlich, 1979). The cytochrome content of the various fractions was determined by difference spectroscopy, and the ability of Mn(II) to reduce the cytochrome content of the various cell fractions alone and in combination was determined by spectrophotometric methods already described (Arcuri and Ehrlich, 1979).

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b *Operated by the Union Carbide Corporation under contract W-7405-eng-26 for the U.S. Department of Energy.*
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The ability of the various cell fractions, alone and in combination, to catalyze the oxidation of Mn(II) was also studied in terms of Mn(II) consumption as follows: to one set of duplicate flasks containing 0.1 g MnO_2, 10 ml of a mineral salts solution (2.7% NaCl, 0.115 M $NaHCO_3$, 0.004 M $MnSO_4$, pH 7.5) were added. To another set of duplicate flasks containing 0.1 g MnO_2, 9.0 ml of the mineral salts solution and 1.0 ml of cell fraction(s) were added. The contents of the flasks were then sampled by removing 3.0 ml of supernatant from the flasks, and centrifuging the sample at 2500 x g for 7.0 min at room temperature (23°). Duplicate 1.0 ml aliquots of the resulting supernatant were assayed for residual dissolved manganese by persulfate oxidation (Ehrlich, 1962).

RESULTS

The locations of the major cytochrome peaks observed in the dithionite-reduced minus air-oxidized difference spectra of the membrane, periplasmic, and intracellular fractions are given in Table 1. Peaks characteristic of b-type and c-type cytochromes were observed in the membrane fractions of cultures BIII 45 and BIII 82. No peaks characteristic of cytochromes were observed in the dithionite-reduced minus air-oxidized difference spectra of the intracellular fractions of cultures BIII 45 and BIII 82. It should be noted that no peaks characteristic of a-type cytochromes were observed in difference spectra of any of the cell fractions prepared from either cultures BIII 45 or BIII 82.

TABLE 1

LOCATION OF THE MAJOR CYTOCHROME PEAKS IN DITHIONITE-REDUCED MINUS AIR-OXIDIZED DIFFERENCE SPECTRA OF CELL FRACTIONS PREPARED FROM *OCEANOSPIRILLUM* STRAINS BIII 45 AND BIII 82 [a]

STRAIN	FRACTION TESTED	LOCATION OF MAJOR CYTOCHROME PEAKS (nm)					
		b-type			c-type		
		α	β	γ	α	β	γ
BIII 45	Membrane	561	532	429	554	526	429
	Periplasmic [b]	561	-	429	-	-	-
	Intracellular	-	-	-	-	-	-
BIII 82	Membrane	561	532	429	555	525	429
	Periplasmic [b]	-	-	-	555	-	427
	Intracellular	-	-	-	-	-	-

[a] The peak locations are those found in previously published difference spectra (Arcuri and Ehrlich, 1979).

[b] Although the peak locations observed with the periplasmic fractions of cultures BIII 45 and BIII 82 are listed as representing b-type and c-type cytochromes, respectively, it should be mentioned that the peaks observed in both cases were quite broad and probably represent mixtures of b-type and c-type cytochromes.

The ability of Mn(II) to reduce the cytochrome components of the various cell fractions alone and in combination was determined by difference spectroscopy. The results of these studies are summarized in Table 2. When the membrane fraction or the periplasmic fraction prepared from either culture BIII 82 or a starved cell suspension of culture BIII 45 was tested separately, no spectrophotometrically detectable reduction by Mn(II) of the cytochromes contained in these fractions was observed. However, when certain fractions were tested in combination, reduction of cytochromes was observed upon the addition of Mn(II). When Mn(II) was added to the combined membrane and periplasmic fractions of *Oceanospirillum* BIII 45, a significant reduction of a c-type cytochrome was observed. Similarly,

when either the combined membrane and periplasmic fractions, or the combined membrane and, intracellular fractions of strain BIII 82 were tested, a significant reduction of the c-type cytochrome was observed upon addition of Mn(II).

TABLE 2

LOCATION OF MAJOR CYTOCHROME PEAKS IN Mn(II)-REDUCED + AZIDE MINUS Mn(II)-REDUCED DIFFERENCE SPECTRA OF PERIPLASMIC AND INTRACELLULAR FRACTIONS SEPARATELY AND IN COMBINATION WITH MEMBRANE [a]

STRAIN	FRACTION TESTED	LOCATION OF MAJOR CYTOCHROME PEAKS (nm)		
		α	β	γ
BIII 45	Membrane	-	-	-
	Periplasmic	-	-	-
	Intracellular	-	-	-
	Membrane + Periplasmic	554	-	425
	Membrane + Intracellular	-	-	-
BIII 82	Membrane	-	-	-
	Periplasmic	-	-	-
	Intracellular	-	-	-
	Membrane + Periplasmic	553	-	425
	Membrane + Intracellular	553	-	424

[a] Azide (1 mM) was added to only the sample cuvettes; $MnSO_4$ (3.5 mM) was added to both the sample cuvettes and the reference cuvettes to circumvent a problem with precipitation that occurred when $MnSO_4$ was added to the cuvettes. The peak locations are those found in previously published difference spectra (Arcuri and Ehrlich, 1979).

The ability of the various cell fractions alone and in combination to remove Mn(II) from solution was also determined. The results of this study are presented in Table 3. The trends observed are quite similar to those seen in cytochrome reduction (Table 2). In the cases of both *Oceanospirillum* BIII 45 and BIII 82, neither the membrane, periplasmic, nor intracellular fractions alone exhibited any Mn(II)-oxidizing activity. On the other hand, when the combined membrane and periplasmic fractions, or the combined membrane and intracellular fractions of either *Oceanospirillum* BIII 45 or BIII 82 were tested, significant Mn(II)-oxidizing activity was observed.

A probable reason why cell membranes prepared from *Oceanospirillum* BIII 45 when mixed with either periplasmic or the intracellular fraction were active in the manganese oxidation test, while only cell membranes mixed with the periplasmic fraction were active in the spectrophotometric test, is the proportion of cell membrane to complementary fraction used. As shown in Fig. 1, manganese-oxidizing activity with a fixed amount of periplasmic or intracellular factor is optimal only over a narrow range of membrane concentration.

DISCUSSION

The results presented indicate the presence of b-type and c-type cytochromes in cultures BIII 45 and BIII 82. No spectrophotometric evidence for the presence of a-type cytochromes was obtained. The cytochromes in these cells were predominantly associated with the membrane fraction. A small amount of cytochrome was associated with the periplasmic fraction, but no cytochromes were located in the intracellular fraction.

TABLE 3

Mn(II)-OXIDIZING ACTIVITY OF PERIPLASMIC AND INTRACELLULAR FRACTIONS SEPARATELY AND IN COMBINATION WITH MEMBRANE

STRAIN	FRACTION TESTED	TOTAL PROTEIN (μg)	Mn(II)-OXIDIZING ACTIVITY [μmol Mn(II) removed in 4 h]
BIII 45	Membrane	275	0
	Periplasmic	500	0
	Intracellular	300	0
	Membrane + Periplasmic	525	700
	Membrane + Intracellular	450	1000
BIII 82	Membrane	125	0
	Periplasmic	1200	0
	Intracellular	175	0
	Membrane + Periplasmic	1250	1300
	Membrane + Intracellular	200	900

The ability of Mn(II) to reduce a c-type cytochrome in *Oceanospirillum* strains BIII 45 and BIII 82 was demonstrated. However, Mn(II) was able to reduce the c-type cytochrome contained in the individual cell fractions only when membranes were combined with either periplasmic or intracellular fractions. The reasons for this observed dependence are not yet clear. One question which arises concerns the function of the periplasmic and intracellular factors. If they are supplying something required by the membrane preparation, are they normal constituents of the periplasmic and intracellular fractions, or are they readily dissociable cell membrane components which, due to the fractionation procedure employed, become associated with either the periplasmic or intracellular fraction? Another question concerns the identity of the soluble constituents. Are the periplasmic and intracellular factors identical or different substances? The answers to these questions and the mechanism involved in Mn(II) oxidation await further experimentation. However, the available information makes it tempting to hypothesize a mechanism for Mn(II) oxidation similar to that proposed for Fe(II) oxidation as catalyzed by *Thiobacillus ferrooxidans* (Ingledew *et al.*, 1977). In this mechanism, a "blue" copper protein called rusticyanin, located in the periplasmic space (Cobley and Haddock, 1975), is the primary electron acceptor in Fe(II) oxidation. The oxidized rusticyanin reduces a c-type cytochrome also found in the periplasmic space. The reduced cytochrome *c* then reduces a membrane bound a-type cytochrome oxidase. The two previously reported c-type cytochromes found in *T. ferrooxidans* (Tikhonova *et al.*, 1967; Cobley and Haddock, 1975) would actually only be one cytochrome that could exist either in the free (periplasmic) state or bound to the membrane. If a mechanism of this type is involved in Mn(II) oxidation, it would explain the need to combine fractions to obtain cytochrome reduction when Mn(II) is added.

Thus it appears that in the cases of cultures BIII 45 and BIII 82, the reduction of a c-type cytochrome by Mn(II) involves the interaction of components of the cell membrane with components of the periplasmic space or intracellular fraction. If this actually occurs then one would expect to find a similar dependence when Mn(II) oxidation is measured in terms of Mn(II) consumption. As the results presented in Table 3 indicate, this is the case [i.e., Mn(II)-oxidizing activity in the cases of cultures BIII 45 and BIII 82 is only exhibited when the membrane fraction is combined with either the periplasmic fraction or the intracellular fraction. None of the cell fractions exhibited any Mn(II)-oxidizing activity when tested individually].

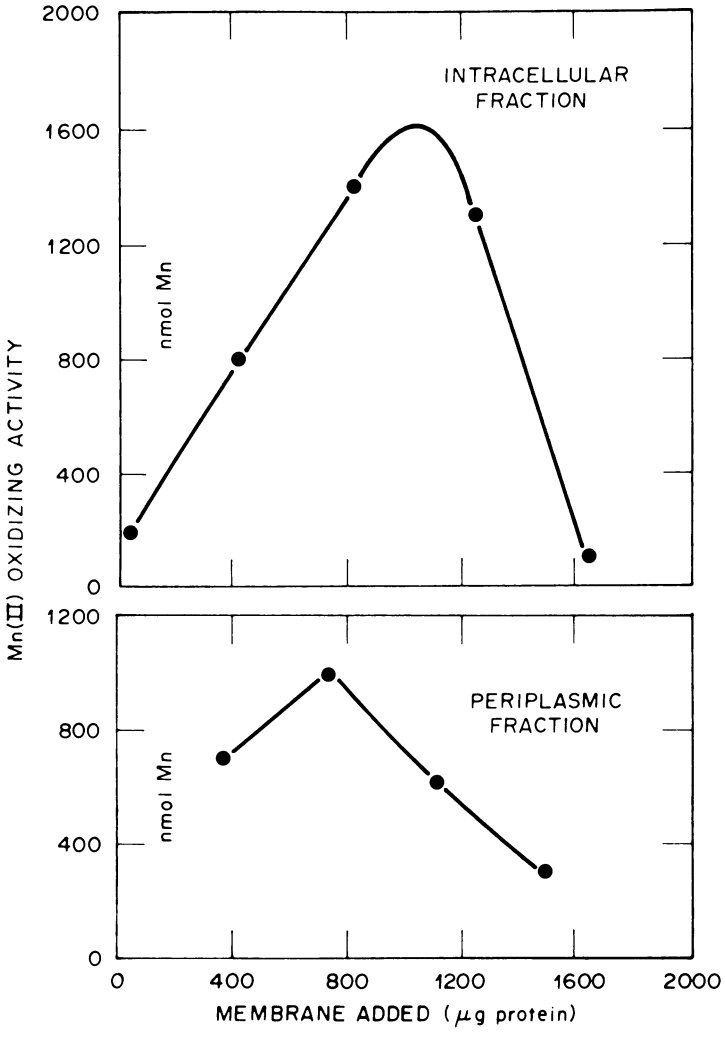

Figure 1: Effect of varying membrane concentration on the activity of constant amounts of periplasmic and intracellular fractions of BIII 45.

The results of our investigations indicate the involvement of cytochromes in the enzymic oxidation of Mn(II). The required interaction of soluble cellular constituents with the cell membrane in order for Mn(II) oxidation and cytochrome reduction to occur is also indicated.

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ELECTRON MICROSCOPIC ANALYSIS OF METAL-DEPOSITING MICROORGANISMS IN SURFACE LAYERS OF BALTIC SEA FERROMANGANESE CONCRETIONS

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INTRODUCTION

Electron microscopic analyses of ferromanganese concretions from the deep-sea have revealed in them a myriad of surface and internal structures, some of which have been identified or interpreted as being of microbiological origin (Dugolinsky *et al.*, 1977; Greenslate, 1974; Harada and Nishida, 1976; LaRock and Ehrlich, 1975; Wendt, 1974; Xavier, 1976). These results, along with work showing the presence of manganese-oxidizing bacteria (Ehrlich, 1975; Nealson, 1978; Schütt and Ottow, 1978), indicate that microorganisms may greatly influence the growth of deep-sea concretions.

Concretions found in shallow waters often resemble deep-sea concretions, although they are highly variable chemically, being formed under a greater variety of environmental conditions (Calvert and Price, 1977). For example, the concretions found in the western Baltic Sea are similar mineralogically, have similar iron and manganese contents, and have accumulations of trace metals similar to deep-sea concretions (Djafari, 1977). However, like most other shallow-water concretions (Manheim, 1965), the Baltic concretions grow much faster than their deep-sea counterparts, increasing in diameter on the order of 0.1 to 1.0 mm yr^{-} [deep-sea concretions grow at rates of 1 to 100 mm per million years (Hammond, 1974)]. These rapid growth rates along with higher organic carbon contents (Calvert and Price, 1977) suggest that microorganisms may also influence the growth of shallow-water concretions. Until recently, however, no investigations of microbial participation in the growth of shallow-water concretions have been reported.

Cultural evidence (Ghiorse, 1979), has indicated that numerous manganese-oxidizing bacteria inhabit the surfaces of Baltic concretions. In the present work, the surface layers of these concretions have been examined by scanning (SEM) and transmission (TEM) electron microscopy, and by light microscopy (LM), to determine the location of the manganese-oxidizing populations and the nature of the microbial involvement in the accumulation of metals.

MATERIALS AND METHODS

Ferromanganese concretions, which consisted mainly of encrustations on mussell shells and small stones, were collected at a depth of 23 m by SCUBA divers at a location (10° 5' E, 54° 46' N) in the western Baltic Sea [see Djafari (1977) for chemical analyses and further descriptions of the concretions and location]. Divers sampled the same location in February, July and August, 1977, each time gathering concretions, along with surrounding sediment and water, in sterile plastic bags.

For microscopic examination, freshly collected concretions were immersed within 1-2 h in a biological fixative solution consisting of 4% (v/v) glutaraldehyde in artificial seawater (ASW) (MacLeod, *et al.*, 1954), adjusted to pH 6.7 with NaOH. Fixed concretions were then stored in fixative until they could be processed further.

For SEM analyses, pieces of surface material removed from fixed concretions were washed several times with ASW, then dehydrated in a graded series of ethanol solutions. After several hours in absolute ethanol, the samples were air dried. Small portions of the dried surface material were mounted on SEM stubs in such a way that the outermost layers could be distinguished from layers just beneath the surface. This was facilitated by the fact that, after dehydration, the outermost layer (usually <0.2 mm thick) appeared much lighter in colour than the dark-brown inner layers (Fig. 1-A). Mounted samples were coated with platinum and examined in a Cambridge Steroscan electron microscope.

For TEM analysis, other pieces of surface material from the fixed concretions were washed in veronal-acetate buffer, pH 6.1, post-fixed for 4 h in a 1% (w/v) OsO_4 solution in the

same buffer (Ryter *et al.*, 1958), soaked for 1 h in 0.5% (w/v) uranyl acetate solution, dehydrated in ethanol and embedded in epoxy resin (Spurr, 1969). Ultrathin sections were obtained using a diamond knife on an LKB III ultramicrotome. After double staining with lead citrate and uranyl acetate (Venable and Coggeshall, 1965), the sections were examined in a Philips 300 electron microscope operated at 80 kV. For selected area diffraction of crystalline material, evaporated gold or platinum films were used as standards to determine the camera constant for the microscope.

Some pieces of the fixed surface material were washed in ASW, then treated overnight with 5% oxalic acid (OA) to extract iron and manganese oxides. These pieces were washed in the veronal-acetate buffer and processed for ultrathin sectioning as described above. Some of the pieces that had been extracted with OA were treated with ruthenium red (RR) in a post-fixative solution containing: 2% OsO_4 in distilled water (1 part), veronal-acetate buffer, pH 6.1 (1 part), and 0.15% ruthenium red in distilled water (1 part).

For LM a Zeiss Photomicroscope II equipped with either phase contrast or incident light objectives was used.

Spot tests were used to demonstrate the presence of iron and manganese oxides in samples of the concretions. The Prussian blue reaction for iron and acidified leucoberbelin blue for manganese were employed as described elsewhere (Ghiorse and Hirsch, 1978).

RESULTS

SEM of the outermost layer of the Baltic concretions (i.e. light-coloured area, Fig. 1-A) revealed numerous particles and filamentous structures on their surfaces (Fig.1-B). At higher magnifications, many of the particles appeared to be of detrital origin (Fig. 1-C). Considering their dimensions (3-5 µm diam.), the filamentous structures, which were themselves often covered with particles, could be fungal hyphae or cyanobacteria (Fig. 1-C). Manganese-depositing, filamentous fungi have been isolated from these conretions (unpublished results) suggesting that fungi may participate in the deposition of metals on surfaces of shallow-water concretions as they apparently do in fresh water and soil (Schweisfurth, 1971). Supporting evidence for the presence of filamentous cyanobacteria was not obtained.

Numerous unidentified structures were observed by SEM in the internal regions of the light-coloured surface layer; however, occasionally, bacteria-like forms could be identified. In these instances, rods and cocci (ca. 1 µm diam.) were always observed to be embedded in a matrix of polymer-like material (Fig. 1-D).

Dark-coloured material beneath the outer layer (i.e. dark-coloured area, Fig. 1-A) contained various mineral-like forms (Fig. 1-E). These resembled structures described previously in deep-sea concretions as "granular crystals" (Harada, 1978) and "Netzstrucuren" (Xavier, 1976). Bacteria-like forms (1.5 - 2.0 µm) were also seen in this material (Fig. 1-F). Similar small spheroids and cylindrical forms were described by Xavier (1976), who noted their likeness to bacteria and suggested that they could reflect the microbial origin of manganese nodules. However, such forms observed in these preparations were probably mineral structures (compare Fig. 1-F with Fig. 3).

TEM (ultrathin sections) of material from the light-coloured, outermost layers (Figs. 2-5) revealed the internal structures of some of the unidentified particles observed by SEM. Many of the particles contained bacterial cells which, by virtue of their ultrastructure (e.g. well preserved membranes, presence of ribosomes and nuclear material), must have been viable at the time of fixation (Figs. 2, 4, 5-A). Frequently, cells were partly or completely encrusted with material which closely resembled the manganese oxides observed previously to be deposited on bacterial cells in pure cultures (Ghiorse and Hirsch, 1979). In the surface layers, encrusted bacterial cells occurred in microcolonies or aggregates (Figs. 2-A, 2-B, 5-A) or as individual cells (Figs. 2-C, 4-A). Less frequently, sheathed bacteria were discerned (Fig. 2-D). In addition to common rod- and coccus-shaped cells, bacteria of a variety of other morphological types and ultrastructural diversity were observed infrequently. They included hyphal budding bacteria, star-shaped bacteria and bacteria containing intracytoplasmic membranes. Some of the membrane-containing cells resembled blue-green or purple photosynthetic bacteria, while others (Fig. 4-B) resembled methane-oxidizing bacteria.

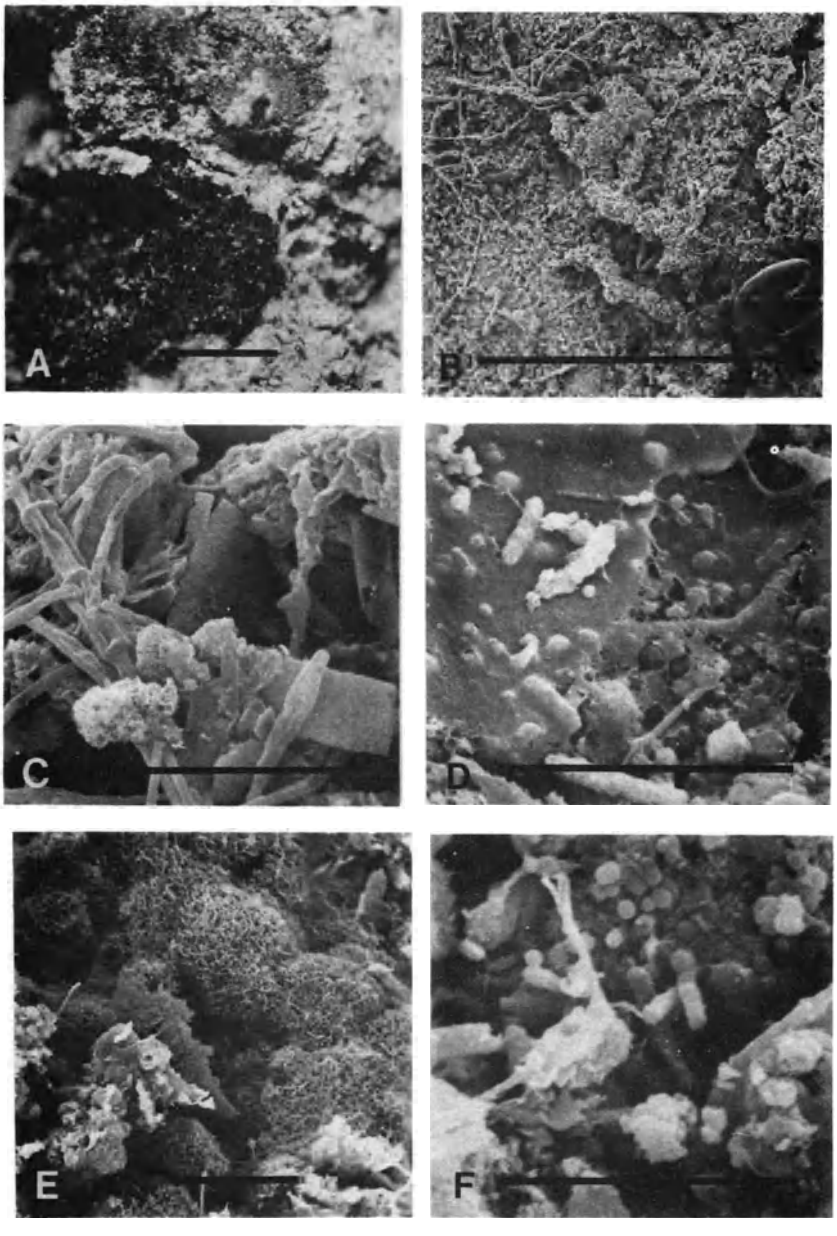


Figure 1: Incident light (A) and SEM (B-F) photomicrographs of Baltic Sea ferromanganese concretions. (A) After fixation, dehydration and air drying, the outermost surface layers (upper right in A) appeared lighter in colour than the underlying material (lower left in A). (B) SEM of the surfaces of the lighter-coloured material revealed on their surfaces a myriad of particulate and filamentous structures some of which appeared to be of detrital origin. (C) Structures resembling fungal hyphae or cyanobacteria were often observed with mineral particles attached. (D) Occasionally, bacterial forms enmeshed in polymer-like matrices could be identified in the interior of the light-coloured surface layers. (E) Structures which could be manganese-containing minerals were observed in the darker material beneath the light-coloured layers. (F) Cylindrical and spherical structures of various sizes, which might be interpreted as microorganisms, were also observed in areas containing the darker material. Bars = 0.5 mm (A, B); 25 µm (C); 10 µm (D-F).

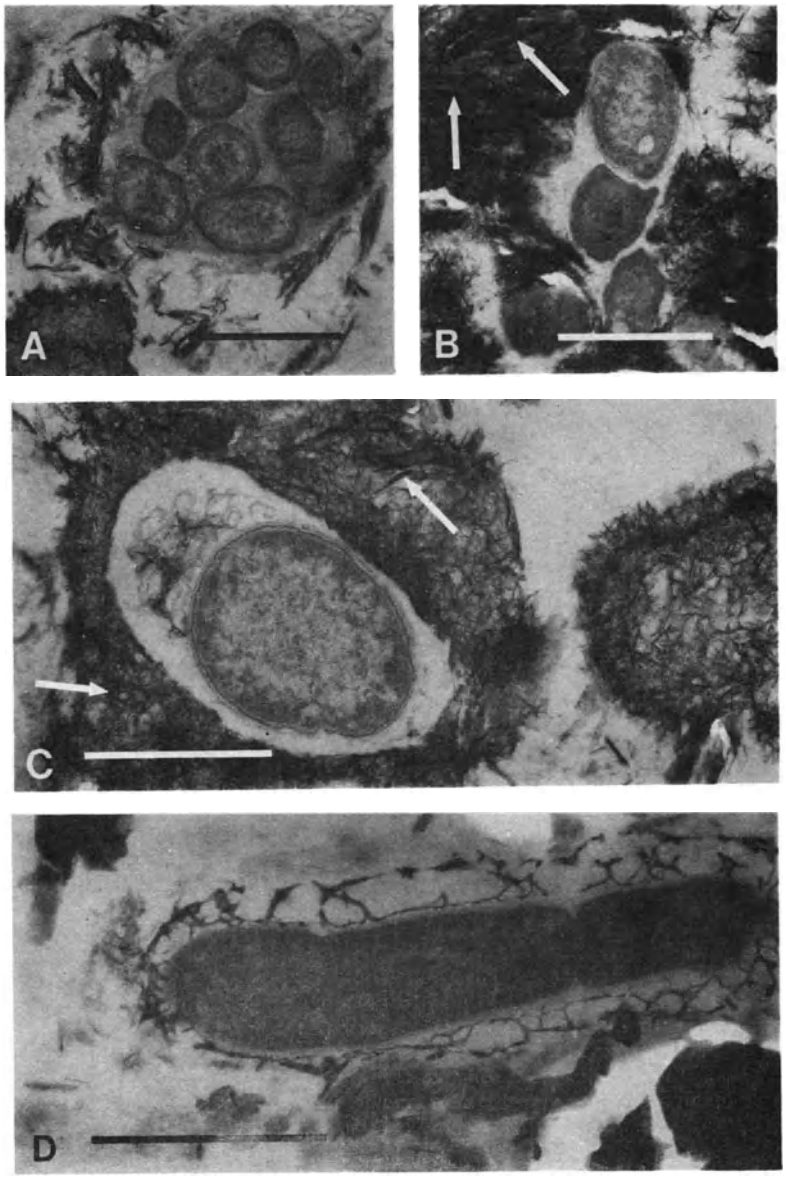


Figure 2: TEM photomicrographs (A-D) of ultrathin sections of material from the outermost light-coloured layers (see Fig. 1-A). These micrographs illustrate profiles of living bacterial cells, partly or completely encrusted with material resembling amorphous manganese and/or iron oxides, which were always observed in these layers. Microcrystals, (arrows, B, C), thought to be composed of iron oxide hydroxides, were frequently seen. Depicted are examples of (A) microcolonies, (B) aggregates and (C) individual bacterial cells which were encountered in these samples. Heavily encrusted cell aggregates (B) would probably escape detection in SEM. (D) Sheathed bacteria, as well as other morphologically and ultrastructurally distinguishable bacteria (e.g. Fig. 4-B), were found in the surface layers when ultrathin sections were examined. Bars = 1.0 µm.

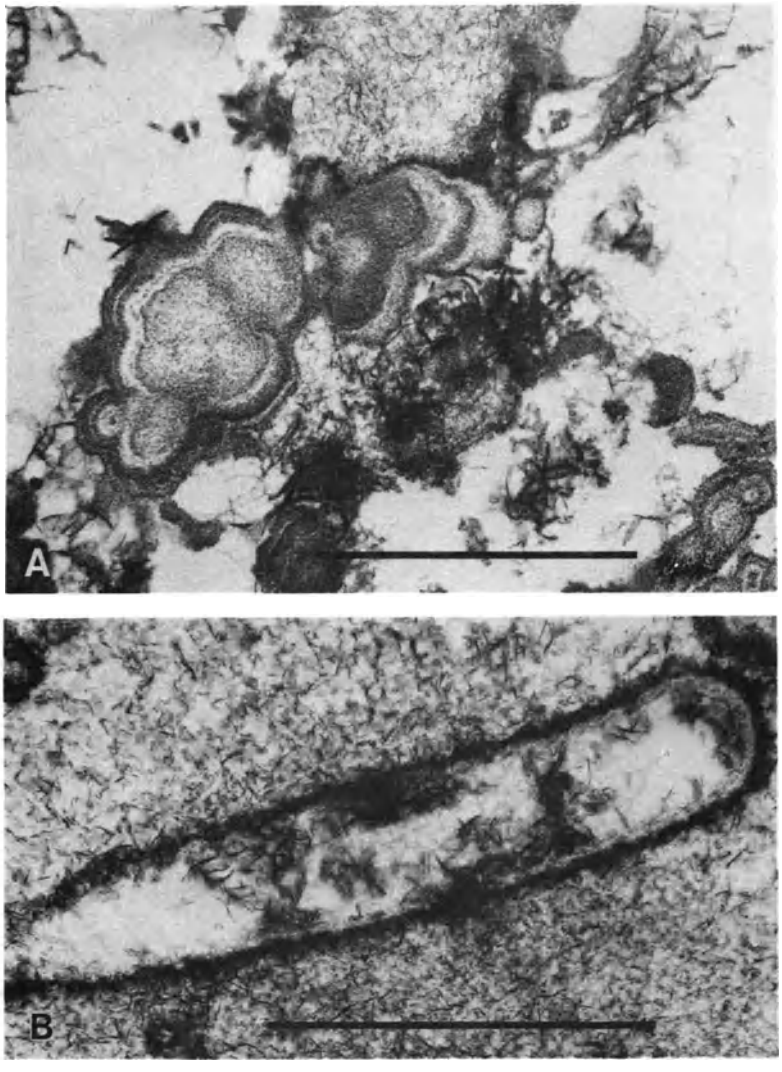


Figure 3: Ultrathin sections of the outermost layers also revealed mineralized structures such as (A) lamellar spheroids and (B) fossilized bacterial cells-walls which might have been interpreted as living microorganisms in SEM (but compare Fig. 3 with Fig. 1-F). These micrographs illustrate that some of the bacteria-like structures seen in SEM may completely lack features (e.g. membranes, ribosomes) which are characteristic of living cells. Bars = 1.0 µm.

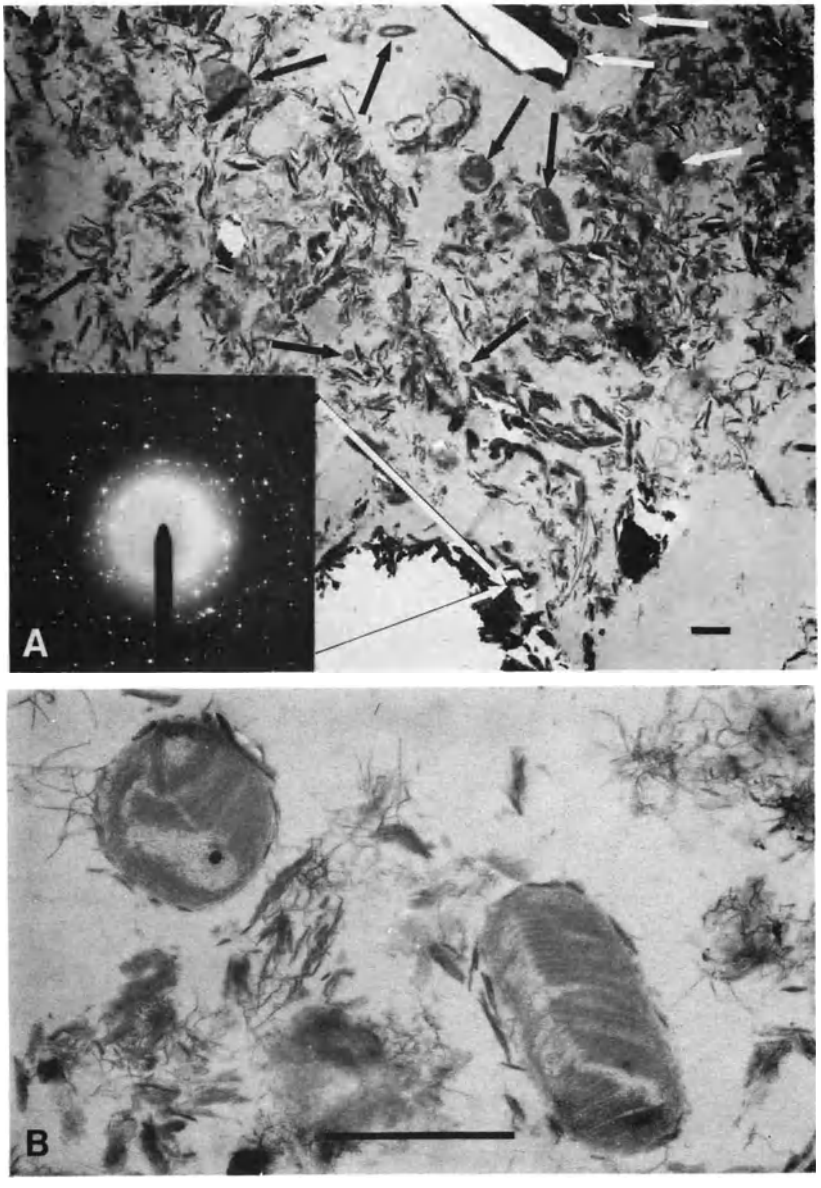
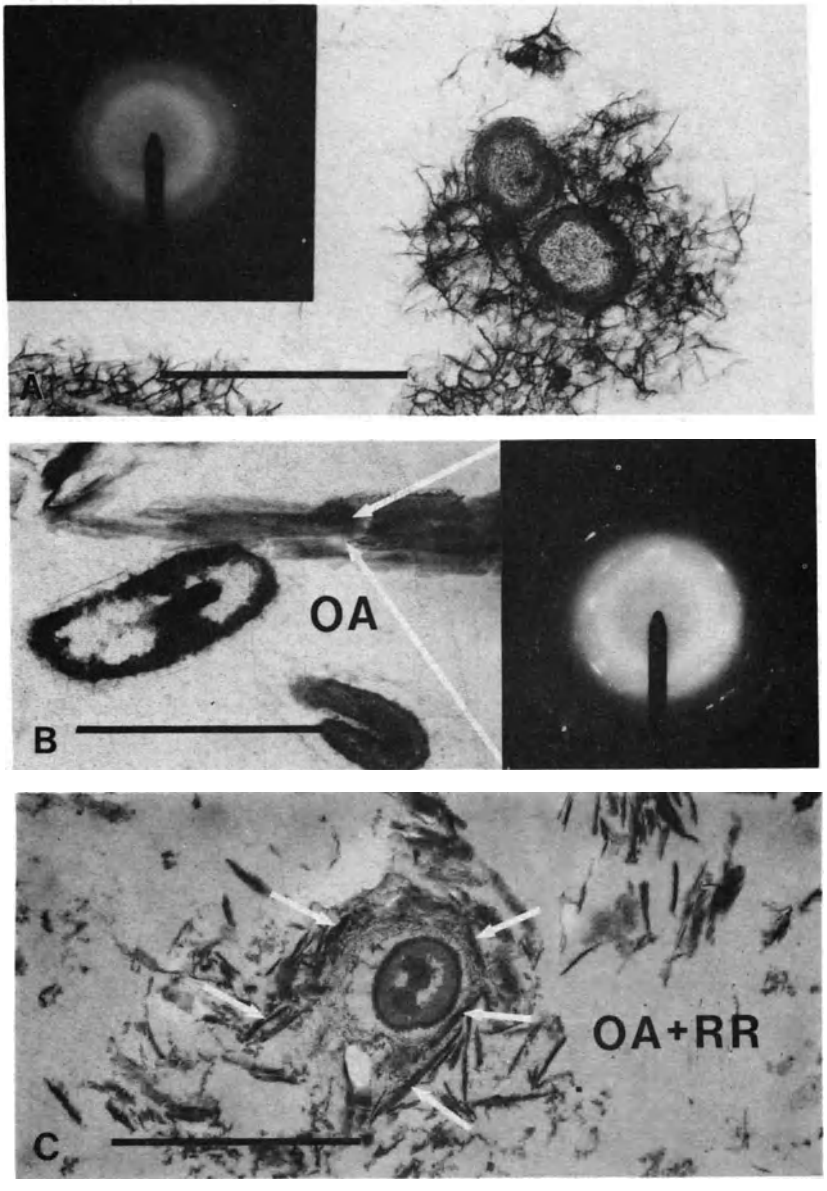


Figure 4: Ultrathin sections of the outermost layers (A) at low magnification showed the arrangement of bacterial cells (black arrows) and crystalline particles (white arrows). Selected area diffraction (inset in A) revealed that most of the crystalline particles were composed of iron oxide/hydroxides, some closely resembling goethite. (B) At higher magnifications bacterial cells were often distinguished by ultrastructural features such as internal membranes. These cells, like most of the bacteria observed in the surface layers, have metal oxides deposited on their surfaces, Bars = 1.0 μm.


A
OA
B
OA+RR
C

Often, structures were observed in ultrathin sections which were of the same size and shape as bacterial cells, but which did not contain any of the ultrastructural features usually associated with living cells (Fig. 3). Lamellar spheroids (Fig. 3-A) and forms resembling fossilized bacterial cell walls (Fig. 3-B) were often seen in these preparations. Such structures, when viewed by SEM, could be mistaken for microorganisms (cf. Fig. 1-D with Fig. 3).

Selected area diffraction of crystalline particles in ultrathin sections revealed the presence of iron oxide hydroxides in the outermost surface layer (Figs. 4-A, 5-B). Some diffraction patterns (Fig. 4-A) resembled those of goethite which has been reported to be present in Baltic concretions (Calvert and Price, 1977). Few, if any, particles gave diffraction patterns resembling those of crystalline manganese oxides. Most of the material immediately surrounding bacterial cells was amorphous to electrons (Fig. 5-A). Based on the ultrastructural similarity of this material to amorphous manganese observed on cells in pure cultures (Ghiorse and Hirsch, 1979), it seemed likely that the amorphous material coating bacterial cells in the surface layers also contained manganese oxides.

Further support for this idea was obtained when pieces of surface material, which were shown by spot tests to contain iron and manganese oxides, were extracted with OA. After extraction, spot tests revealed that no detectable manganese, but traces of iron oxides, remained. Subsequently, selected area diffraction on ultrathin sections of OA-extracted samples revealed that crystalline iron oxide hydroxides were present, but all of the amorphous material, which previously had been seen surrounding cells, was removed (Fig. 5-B). It is likely that the majority of this material was composed of manganese oxides. However, since the OA-extraction would be expected to remove both iron and manganese oxides, it is also possible that amorphous oxides of both metals were present in the material surrounding the cells.

In sections of OA-extracted samples which had been subsequently stained with ruthenium red (OA + RR), a matrix of electron-dense (i.e. RR-stained) material was often observed surrounding bacterial cells in place of the extracted metal oxides (Fig. 5-C). Based on its reaction with RR, this residual material was interpreted to be composed of polyanionic polymers, perhaps acidic polysaccharides of microbial origin (see Ghiorse and Hirsch, 1979). Associated with the polymer matrix were miniscule crystalline particles (iron oxides?) (arrows, Fig. 5-C). Microcrystals similar to these were also associated with amorphous metal oxides surrounding cells in non-extracted preparations (arrows, Figs. 2-B, 2-C).

DISCUSSION

TEM analyses of ultrathin sections have clearly demonstrated the presence of a variety of bacteria in the outermost surface layers of ferromanganese concretions taken from the Baltic Sea. The majority of these bacteria were encrusted with amorphous material which was probably composed of manganese and/or iron oxides. The encrustation of the cells made identification of bacteria in SEM preparations difficult. Furthermore, observations of many bacteria-like forms, which did not contain membranes or other features which typify living structures in ultrathin section, raises serious doubts concerning the interpretation of rod- and coccus-shaped forms as living bacterial cells when seen by SEM (see, e.g., Xavier, 1976).

In a parallel study, manganese-oxidizing bacteria were found to occur in significant numbers in surface material from these concretions (Ghiorse, 1979). These results suggest that, in this investigation, the bacteria observed in the surface layers probably participate in the growth of the concretions by oxidizing manganese and depositing amorphous manganese oxides on their surfaces.

The finding that the material which encrusted bacterial cells was associated with polyanionic (i.e. RR-staining) polymers, allows a microbiologically based mechanism for the accumulation of metals in the concretions to be proposed. In the proposed mechanism, negatively-charged, extracellular polymers produced by bacteria inhabiting the surfaces of the concretions would serve as cation exchange agents. The polymers, in an aerobic or partially aerobic environment such as exists at the sediment water interface, could scavenge divalent manganese and other metal cations which originate in the sediments [see Hartmann (1964) for analyses of Baltic sediments], causing their accumulation on surfaces where the polymer-producing bacteria grow. Bacteria in the polymer-producing population,

which were capable of manganese oxidation, could then oxidize divalent manganese resulting in the deposition of manganese oxides in the polymer matrix. Once deposited, the oxides themselves would act as cation exchange agents, thus scavenging more oxidizable manganese as well as iron oxides and trace metals. Such a scheme could explain how manganese-oxidizing bacteria can accelerate the accumulation of metals in these and other shallow-water concretions.

The results presented in this work raise questions concerning the location and possible encrustment of manganese-oxidizing bacteria which have been isolated from deep-sea ferromanganese concretions (Ehrlich, 1975; Nealson, 1978; Schütt and Ottow, 1978). Although bacterial microcolonies and polymers have been observed by SEM on the surfaces of manganese nodules from the Blake plateau (LaRock and Ehrlich, 1975), no direct associations of bacteria with metal oxides have been noted. In view of the results presented above, it seems likely that examination of deep-sea concretions by ultrathin sectioning methods could help to answer these questions and, perhaps, others (see Greenslate, 1974) concerning bacterial participation relative to other microorganisms in the formation of the deep-sea concretions.

ACKNOWLEDGEMENTS

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MINERALOGICAL, GEOCHEMICAL, AND MICROBIOLOGICAL ASPECTS OF IRON DEPOSITION FROM GROUNDWATER

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INTRODUCTION

Iron is readily soluble constituent in groundwater and the solubility is further increased by its chelation with organic matter. Iron, and minor elements such as Mn, are undesirable in water supply systems as they cause problems of taste, odour, and discolouration, and clogging and deposition in the screens and pipelines which eventually leads to corrosive conditions. The removal of iron can be accomplished by a sequential treatment involving oxidation, settling, and filtration with various modifications but, as well as the high cost of the treatment, incomplete iron removal and associated problems are frequently encountered.

The present work describes investigations carried out in a groundwater plant which employed natural aeration to oxidize Fe- and Mn-rich water without involving additional chemical treatment. The water from a groundwater well was pumped up and allowed to run down a slope during which reducing conditions were abolished and iron oxidation took place, causing iron and other metals to deposit on the slope. The slope was covered by natural vegetation most of which, including trees, was healthy and survived unless directly under a major route of the water runoff. The natural vegetation conveniently improved the sedimentation mainly by preventing the re-mobilization of the deposit once iron precipitated.

During years of operation, a layer of iron sediment, sometimes 30-40 cm deep, accumulated on the ground. The sediments were not removed but their moisture content was depleted by maintaining one half of the slope unused for periods of 12-18 months. The drying further increased the compactness of the iron deposit. The sediments on the slope provided an opportunity, with an easy access, to examine in detail geochemical and mineralogical phenomena associated with steady, continuing iron oxidation and deposition from the groundwater. Iron oxides are known to react as sorbents for minor and trace elements and, therefore, the patterns of distribution of other cations as well as that of organic matter were examined in the solution and solid phases. Attention was also given to bacteria which mediate iron transformations, in order to elucidate the biogeochemical nature of the uniquely iron-rich environment.

EXPERIMENTAL

General Description of the Location and Sampling

The groundwater plant at Pullassuo, city of Salo, SW-Finland, was constructed in 1969-1970. In the plant the iron-rich groundwater is pumped from sand and gravel deposits overlain by clay beds, and discharged to the forested soil of the upper part of a slope covered by natural vegetation. During runoff down the slope, most of the iron is precipitated on the ground and some water is infiltrated into shallow till and poorly sorted sand deposits overlying the Precambrian basement. The rest of the water is infiltrated on the lower part of the slope into recharge basins located on both sides of the pumping station (Fig. 1). The water is finally collected in wells which are screened with sand and gravel and located horizontally underneath the basins. It is then pumped (42 m^3 h^{-1}) into the main water supply pipeline. The chemical composition of water in the plant during 1974-1978 is briefly summarized in Table 1.

In the present work, samples were collected over four seasons (in July and October 1977 and in February and May 1978). The sampling sites on the slope are shown in Fig. 1. One half of the slope (the right-hand side on the map in Fig. 1) was not in use during the experimental period.

Water Samples

Standard methods (Standard Methods for the Examination of Water and Wastewater, 1975) were

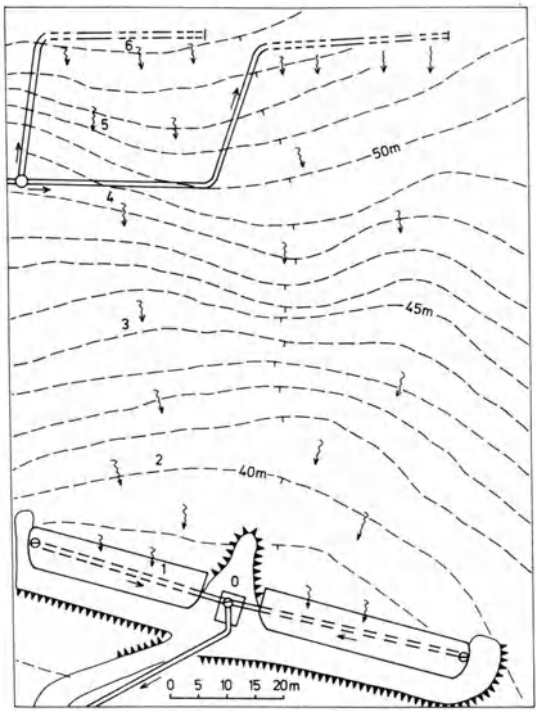

Figure 1: Sampling sites in the groundwater plant. Water pumped from a well is discharged at site 6 and runs down the slope (sampling sites 5-2). The water is collected at a basin (1) and after infiltration through gravel and sand, the water is pumped (0) to the main pipeline.

TABLE 1

WATER QUALITY IN THE GROUNDWATER PLANT IN 1974-1978.
EXCERPTS OF DATA PROVIDED BY THE WATER BOARD OF THE CITY OF SALO.[a]

Determination	March 1974		March 1975		April 1976		March 1977		March 1978	
	A	B	A	B	A	B	A	B	A	B
dH^0	5.4	5.3	8.1	6.4	5.0	4.8	4.8	4.5	5.1	4.9
Fe(mg l^{-1})	23.0	0.18	19.0	1.2	12.0	0.20	14.0	0.37	13.0	0.34
Mn(mg l^{-1})	0.45	<0.005	0.28	0.048	0.58	0.006	0.67	0.016	0.62	0.011
NO_3^-(mg l^{-1})	–[b]	–	–	–	0.17	0.23	0.11	0.20	0.004	0.011
NO_2^-(mg l^{-1})	–	–	0.011	0.010	0.20	0.006	0.23	<0.005	–	–
NH_4^+(mg l^{-1})	0.13	0.08	<0.01	<0.01	0.16	0.01	0.053	0.017	0.12	<0.005
SO_4^{2-}(mg l^{-1})	–	–	37	33	–	–	–	–	–	–
Cl^-(mg l^{-1})	8.7	6.5	8.7	8.4	13	11	9.3	8.8	12	11

[a] A and B columns represent samples of the influent ground water and water after the final infiltration, respectively.

[b] –, Not determined.

used for chemical and physical determinations unless otherwise indicated. The following measurements were carried out in the field: temperature, pH, Eh, electrical conductivity (EC), O_2 content (DO; with an O_2-probe), and CO_2 content. In the laboratory, the samples

were analyzed for total hardness and Ca (by titration with EDTA), and Mg was calculated from these data. Na and K were determined by atomic absorption spectrometry (AAS) and SiO_2 colorimetrically. With metallic elements, the water samples were filtered (0.45 µm) and preserved with HNO_3 until analyzed with AAS. The chemical oxygen demand (COD) was determined by oxidation with $KMnO_4$. The use of this method in water analysis has been discussed by Gjessing (1970, 1976). Sulfate was determined indirectly by AAS after precipitation with barium chloride.

Precipitates

Samples were collected in glass jars and, after a few days, the aqueous phase was separated from the sediment by decanting. The decanted water was filtered (0.45 µm), concentrated by evaporation, and the residue was dissolved in HCl. The sediment samples were sieved to remove coarse organic detritus. The fine Fe-containing fraction was isolated and concentrated by sedimentation in distilled H_2O without any pretreatment. The concentrated samples were dissolved in HCl for AAS determination of Fe, Mn, Ca, Mg, Na, K, and Zn. The residual organic matter was destroyed by oxidation with $HClO_4$ before the determination of Co, Ni, Cu, Sr, Ba, and Cd by AAS. A cold-vapour method was employed for determination of Hg.

The Fe-oxide minerals in the concentrated samples were identified by X-ray diffraction (XRD) using a Philips PW1130 instrument with $CoK\alpha$ radiation and a graphite reflected beam monochromator. Samples were gently pressed against filter paper to minimize orientation. Total carbon was determined with a Carmograph carbon analyzer. Iron was extracted with sodium dithionite-citrate-bicarbonate (Fe_d) (Mehra and Jackson, 1960) and acid ammonium oxalate (Fe_o) in the dark (Schwertmann, 1964) and determined by AAS. Specific surface areas were measured by the BET single point method using an Areatron automatic instrument with Ar as adsorption gas. Infrared spectrograms were recorded with a Beckman IR-20A instrument using 0.3% KBr-disks.

Bacteria

Water and sediment samples were collected aseptically in sterile test tubes on ice and analyzed within 3-4 h. Viable counts were determined by a Most Probable Number (MPN) technique (5 tubes of each decimal dilution) in the following medium designed to select for chemolithotrophic iron-oxidizing bacteria (Tuovinen and Kelly, 1973): $FeSO_4 \cdot 7H_2O$, 120 mM; K_2HPO_4, 2.3 mM; $(NH_4)_2SO_4$, 3.0 mM; $MgSO_4 \cdot 7H_2O$, 1.6 mM; in 0.005 N H_2SO_4 (pH 2.5). This medium allows the growth of iron-oxidizers from sediment samples of acidic nature (Fjerdingstad *et al.*, 1976). The tubes were incubated at 28° for 4 weeks before recording the results. Further incubation for up to 7 weeks did not alter the MPN results. Microscopic examination of the sediment samples has been described elsewhere (Tuovinen and Nurmiaho, 1979).

RESULTS

Water Chemistry

With the movement of water down the slope (from site 6 to site 0) there was a decrease in the CO_2 concentration and an increase in the pH of up to 0.7 units (Table 2). The groundwater was initially reduced as indicated by the low redox potential and oxygen (DO) saturation level but became oxidized during passage down the slope (Table 2). The slight decrease in the electric conductivity (EC) may be the result of iron precipitation; the total hardness remained more or less constant throughout. The groundwater temperature at site 6 (influent) remained within 6.1-6.9°, whereas at site 1 (basin) the temperature showed seasonal fluctuations between 0.7° (February) and 8.9° (July).

The concentration of iron, initially in the range of 12 to 15 mg l^{-1} fell by more than 75% in the upper part of the slope (Table 3). Manganese precipitated at a slower rate but was also efficiently removed from the water during the runoff. Ca, Mg, Na, K, and SiO_2 levels in the water samples remained constant (Table 3); similar concentrations were found in water samples decanted from the sediments in the laboratory (Table 4) indicating that no significant precipitation of these elements occurred. No great variations in the levels of trace elements (Zn, Cu, Ni, Pb, Cd) in the water samples were observed (Table 3). The level of Zn was elevated in the decanted water (Table 4) indicating that some particulate Zn dissolved during storage. In contrast the concentrations of Fe and Mn in the decanted water (Table 4) indicated that these metals continued to precipitate during storage. Aluminium

TABLE 2

WATER QUALITY CHANGES IN THE RUNOFF DOWN THE SLOPE. THE SAMPLING SITES ARE INDICATED IN FIG. 1. THE RESULTS HAVE BEEN COMBINED TO REPRESENT THE OVERALL AVERAGE OF THE FOUR SAMPLINGS (JULY, OCTOBER, FEBRUARY, MAY).

Determination	Sampling Sites									
	6		4		3		1		0	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
pH (field)	7.2	0.2	7.4	0.3	7.7	0.3	7.9	0.2	7.6	0.2
pH (laboratory)	6.8	0.2	7.1	0.3	7.4	0.3	7.6	0.2	7.6	0.1
Eh (mV) (field)	170	57	253	21	315	5	305	28	325	5
EC ($\mu s\ cm^{-1}$)(field)	257	-[a]	214	2	221	21	200	12	198	10
EC (laboratory)	203	21	190	16	182	2	182	6	178	7
DO (% of saturation)	13	9	81	11	83	13	97	13	98	18
CO_2 ($mg\ l^{-1}$)	62	-	16	-	9	-	13	-	5	-
Hardness (dh^o)	5.4	0.1	5.4	0.1	5.3	0.1	5.3	0.1	5.3	0.1
COD ($mg\ l^{-1}$)	7.9	0.7	7.0	0.4	6.5	0.7	7.1	0.7	7.6	1.0

[a] -, Insufficient data to calculate the standard deviation.

TABLE 3

CHEMICAL COMPOSITION OF THE WATER SAMPLES. THE DATA REPRESENT THE COMBINED AVERAGES OF THE FOUR SAMPLINGS

Determination	Sampling Sites									
	6		4		3		1		0	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Fe ($mg\ l^{-1}$)	15.1	2.3	3.4	1.0	1.1	1.2	0.28	0.33	0.07	1.10
Mn ($mg\ l^{-1}$)	0.49	0.14	0.32	0.11	0.31	0.15	0.04	0.04	<0.01	
Ca ($mg\ l^{-1}$)	23.7	0.5	23.4	0.3	23.7	0.9	23.5	1.3	23.7	0.7
Mg ($mg\ l^{-1}$)	8.9	0.7	9.0	0.3	8.1	1.3	8.7	0.5	8.5	1.0
Na ($mg\ l^{-1}$)	7.4	0.4	7.2	0.2	7.4	0.2	7.5	0.5	7.4	0.4
K ($mg\ l^{-1}$)	2.7	0.2	2.7	0.2	2.8	0.2	2.8	0.2	3.0	0.7
SiO_2 ($mg\ l^{-1}$)	25.0	0.5	25.1	0.5	24.6	2.1	24.3	0.8	24.7	1.3
SO_4^{2-} ($mg\ l^{-1}$)	20.9	4.5	21.8	4.6	22.0	4.2	23.9	8.8	17.1	12.7
Zn ($\mu g\ l^{-1}$)	4.5	2.1	3.2	1.9	2.8	1.8	3.7	2.2	12.9	4.4
Cu ($\mu g\ l^{-1}$)	3.4	2.9	2.5	0.4	2.7	1.1	2.7	1.4	7.0	
Ni ($\mu g\ l^{-1}$)	<2		<2		<2					
Pb ($\mu g\ l^{-1}$)	<1		<1		<1		<1			
Cd ($\mu g\ l^{-1}$)	<0.5		<0.5		<0.5		<0.5		<0.5	

was a major cation in the decanted water sample (Table 4).

Precipitate Geochemistry

The iron content of the precipitates (Table 5) decreased down the slope while the total carbon content increased (Table 6). The concentrations of K, Na, and Al were highest in the uppermost and lowermost samples (Table 5; Fig. 2). The minor and trace element content in the sediment generally increased down the slope. Mn, Ni, Co, and Ba had similar distribution patterns in the sediment samples as did Ca, Mg, and Sr. Similarities in distributions were also noted for Hg and Cd and for Zn and Cu (Table 5; Fig. 2).

Mineralogical Characterization

Ferrihydrite ($Fe_2O_3 \cdot 2FeOOH \cdot 2.6H_2O$) was the main mineral form of the precipitate. This mineral is a poorly ordered iron-oxide which crystallizes near the surface of Fe-bearing waters (Chukhrov *et al.*, 1973; Schwertmann and Fischer, 1973; Carlson and Schwertmann, 1980a). Ferrihydrite is readily converted to more stable iron-oxides, particularly goethite, in a temperate or cool humid climate. The crystallization of ferrihydrite, and its transforma-

TABLE 4

CHEMICAL COMPOSITION OF THE WATER DECANTED AND FILTERED FROM THE SEDIMENT SAMPLES. SAMPLING SITES ARE INDICATED IN FIG.1. SAMPLE 6p WAS TAKEN FROM THE OUTER SURFACE OF THE PIPE AND SAMPLE 6 FROM THE GROUND BENEATH IT. SAMPLES 5, 4, 3, AND 2 WERE SEDIMENT SAMPLES FROM THE SLOPE; SAMPLE 1 WAS COLLECTED FROM THE BASIN AT THE FOOT OF THE SLOPE

Determination	Sampling Sites						
	6p	6	5	4	3	2	1
Fe ($\mu g\ l^{-1}$)	73	21	4	20	90	19	8
Mn ($\mu g\ l^{-1}$)	0.5	4.2	1.3	1.0	0.7	1.6	1.7
Al ($\mu g\ l^{-1}$)	150	60	100	70	50	60	70
Ca ($mg\ l^{-1}$)	13.9	22.2	23.1	15.4	18.5	22.2	18.1
Mg ($mg\ l^{-1}$)	7.50	8.33	8.90	4.80	7.50	8.50	7.08
Na ($mg\ l^{-1}$)	8.33	10.67	9.03	8.33	10.42	8.83	7.50
K ($mg\ l^{-1}$)	3.06	2.64	3.19	3.54	3.19	2.22	2.36
Zn ($\mu g\ l^{-1}$)	80	20	30	70	30	75	50

TABLE 5

CHEMICAL COMPOSITION OF THE PRECIPITATE SAMPLES. SAMPLING SITES ARE INDICATED IN FIG. 1 AND THE LEGEND TO TABLE 4. SAMPLE 2y WAS COLLECTED FROM THE TOP LAYER CLOSE TO SITE 2; SAMPLE 1p REPRESENTS MATERIAL FLOATING ON THE BASIN NEAR SITE 1

Composition	Sampling Sites								
	6p	6	5	4	3	2y	2	1p	1
Fe (%)	54.5	51.3	48.9	49.5	46.7	47.2	45.1	39.3	42.8
Mn (%)	0.04	0.11	0.19	0.16	0.39	0.67	1.39	1.93	2.38
Al (%)	0.19	0.13	0.11	0.09	0.08	0.14	0.12	0.15	0.16
Ca (%)	0.69	0.65	1.07	0.82	1.01	1.36	1.37	1.60	1.49
Mg (%)	0.02	0.04	0.04	0.05	0.07	0.08	0.12	0.15	0.12
Na (ppm)	2190	880	1140	1050	160	290	720	1400	840
K (ppm)	820	330	545	480	75	200	370	680	400
Zn (ppm)	70	65	80	73	70	100	100	120	110
Co (ppm)	9	10	8	9	9	17	21	27	36
Ni (ppm)	8	11	9	11	12	23	29	33	54
Cu (ppm)	6	7	7	6	7	9	9	10	14
Sr (ppm)	16	17	21	28	37	47	54	55	63
Ba (ppm)	38	38	33	45	58	90	105	140	170
Cd (ppm)	0.80	0.75	0.80	0.85	1.05	1.40	1.50	1.30	1.80
Hg (ppb)	-	-	-	175	270	290	-	410	650

tion via solution phase, is retarded or prevented by other compounds, in particular by organic matter (Schwertmann *et al.*, 1968).

The XRD pattern of sample 6p showed all five lines typical of reasonably well-ordered ferrihydrite. The number of reflections decreased in samples down the slope and in sample 2 only the two strongest reflections were present (Table 6). At the same time the top of the asymmetric 100 line shifted from 2.57 to 2.74 Å and its width at half height increased from 10.2° to 16° 2θ. Thus the unit cell grows when the mineral becomes less ordered. Down the slope the Fe-content (Fe_d) decreased and the total carbon content increased, as did oxalate-soluble iron (Fe_o) (Table 6), in good agreement with the decreasing order of the ferrihydrite structure. Goethite was identified in sample 6p and in the lowermost sample (1) taken from the sand through which the water infiltrated. In this case the mineral composition was affected less by the organic matter and more by the selective dissolution of less-ordered mineral compounds.

The specific surface area decreased when the ordering of the crystal structure, and thus the crystallite size, decreased for iron-oxides with $Fe_{o/d}$ values over 0.8 (Table 6; Carlson

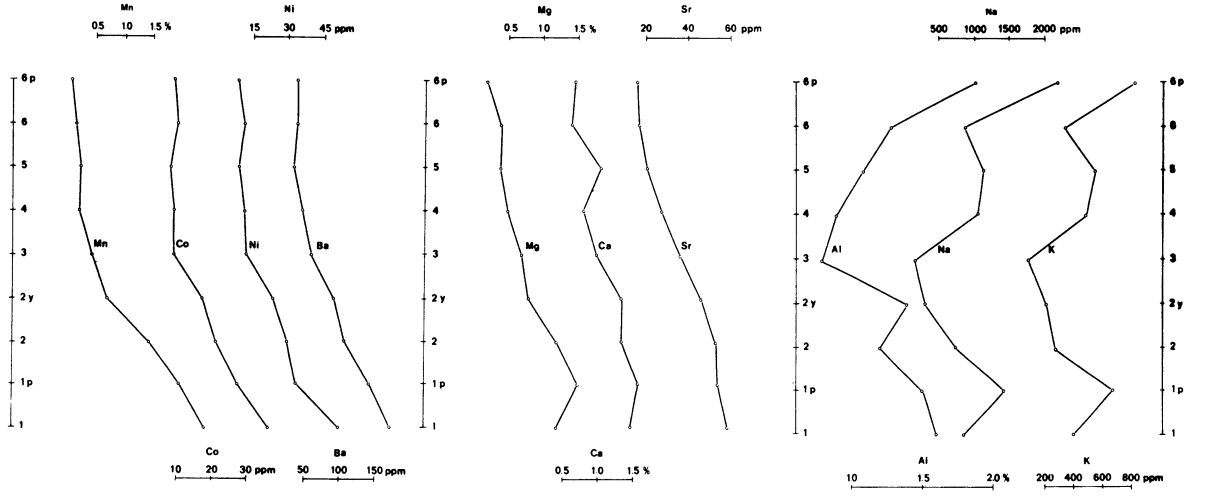

Figure 2: Distribution of cationic elements in the precipitates. The levels of metals (abscissa) are indicated at each sampling site (y-coordinate). See Table 5 for further details.

and Schwertmann, 1980a,b). This is likely to result from the strong aggregation of very poorly ordered material, as seen in electron micrographs (Carlson and Schwertmann, 1980a), which renders part of the surface inaccessible to the adsorption gas.

TABLE 6

MINERALOGICAL CHARACTERIZATION OF THE SEDIMENT SAMPLES. SAMPLE 6v REPRESENTS A SITE SIMILAR TO THAT OF SAMPLE 6 BUT COLLECTED FROM THE OTHER SIDE OF THE SLOPE (THE RIGHT-HAND SIDE IN FIG. 1)

Sample No.	Colour[a]	Fe_o %	Fe_d %	$Fe_{o/d}$	C_t %	Minerals[b] (Fh refl.)	d(100) Fh, Å	WHH[c] $^o2\theta$	SS[d] m^2g^{-1}	Si-O-Fe IR cm^{-1}	930/1080
6p	7.5YR 6/8	35.6	44.4	0.80	2.1	Fh (5), G	2.57	10.2	423	960	2.30
6v	7.5YR 6/8	33.1	39.8	0.83	2.2	Fh (4)	2.58	11.0	398	-	-
6	7.5YR 6/6	35.7	43.0	0.83	2.1	Fh (4)	2.62	11.6	425	965	1.90
5	8YR 6/6	33.1	39.5	0.84	2.8	Fh (4)	2.65	12.4	274	950	2.19
4	7.5YR 6/6	34.2	39.7	0.86	2.7	Fh (4)	2.67	12.6	200	965	1.97
3	10YR 6/6	36.8	39.1	0.94	4.2	Fh (3)	2.71	12.8	154	965	1.98
2	10YR 6/6	30.8	31.7	0.97	5.5	Fh (2)	2.74	16.0	(38)	970	1.36
1	10YR 6/6	31.5	36.0	0.88	4.1	Gt, Fh (4)	2.57	5.4	-	1005	1.17

[a] Munsell Soil Color Chart, 1975 edition. Munsell Color Company, Baltimore, MD.
[b] Fh=ferrihydrite, Gt=goethite and the number of Fh reflections on the X-ray diffractograms.
[c] WHH=width of half height.
[d] SS=specific surface area.

Si was found to be an essential part of the ferrihydrite structure. All natural ferrihydrite samples, and those synthesized with Si, show an IR band in the range of 900 to 1050 cm^{-1} which is assigned to Si-O-Fe bonds (Schwertmann and Fischer, 1973; Schwertmann and Thalmann, 1976; Carlson and Schwertmann, 1980a). The shift of the IR band from 950-965 cm^{-1}, typical of natural ferrihydrite, to 970-1005 cm^{-1}, and the change in the intensity ratio of 930 cm^{-1} (pure Si-O-Fe band) to 1080 cm^{-1} (pure Si-O-Si band), from values of about 2.0 to approximately 1.2 in the two lowermost samples (Table 6), is probably due to an increased content of amorphous silica in the latter samples.

Iron-oxidizing Bacteria

Table 7 lists the viable counts of iron-oxidizing bacteria found in the groundwater samples from the slope. Their density remained relatively low through the seasons, including the summer (July samples) during which the maximum counts of 660 cells ml^{-1} were recorded. These

TABLE 7

VIABLE COUNTS OF *THIOBACILLUS FERROOXIDANS* IN THE WATER

Sampling site	Viable counts (cells ml^{-1}) July	October	February	May
6	660	340	220	480
4	260	136	98	9
3	340	46	158	42
1	280	34	46	<0.02
0	a	16	16	14

[a] Not determined

bacteria are classified as *Thiobacillus ferrooxidans* which is a group of chemolithotrophic and acidophilic organisms capable of utilizing ferrous-iron as the sole source of energy for growth and carbon dioxide fixation (Lundgren *et al.*, 1974). Table 7 also shows that the bacterial counts fell as the distance of the runoff down the slope increased; very few bacteria were detected in samples after the final infiltration of the water. Irrespective

of the season, a 55-98% reduction in the bacterial counts was observed after the first 25 m of the runoff down the slope. The low bacterial counts may have resulted from (a) deposition of the organisms along with iron precipitation, and/or (b) inactivation or death of the organisms by exposure to surface conditions (temperature, pH, and related effects). The viable counts of the bacteria in the precipitate varied considerably from a few hundred to 3.2×10^5 cells g^{-1} (Table 8).

TABLE 8

VIABLE COUNTS OF *THIOBACILLUS FERROOXIDANS* IN SEDIMENT SAMPLES

Sampling site	Date	Approximate depth in the deposit (cm)	Viable counts (cells g^{-1}) $\times 10^{-3}$
6	July	*a*	320
3	July	*a*	70
4	October	2	2.2
4	October	10	1.5
4	October	20	1.4
6v	October	2	6.6
6v	October	7	9.8
6v	October	15	70
6	May	*a*	52
4	May	*a*	0.4
2	May	*a*	9

[a] Samples taken from the top surface and analyzed as a slurry; these results are indicated as cell counts per ml slurry.

The groundwater well, from which the water was pumped on to the slope, was also sampled in the summer and contained over 3.2×10^5 iron-oxidizing bacteria ml^{-1}. A flaky iron precipitate was commonly seen in these samples. This well was not investigated further, but the conditions prevailing in the well water (low Eh, low DO) could support the development of sheathed iron bacteria such as *Gallionella*, *Leptothrix* and *Crenothrix* (Glathe and Ottow, 1972; Hässelbarth and Lüdemann, 1972, 1973; Cullimore and McCann, 1977). These bacteria grow typically in filaments and often occur in iron-rich groundwater wells. In fact, microscopic examination of sediment samples from the slope revealed many different bacterial structures, most of them resembling the above-mentioned filamentous iron bacteria and ranging from single rod-shaped bacterial cells to filaments and associations of several cells with an extracellular sheath (Tuovinen and Nurmiaho, 1979).

DISCUSSION

On exposure to air, the groundwater became rapidly oxidized as indicated by the increasing Eh values and dissolved oxygen concentrations, and by data provided by the city Water Board showing that the ammonium and nitrate levels decreased with a parallel increase in the nitrate concentration. Oxidation was accompanied by rapid iron deposition and by a slower rate of manganese deposition. These kinetics can be explained by the different concentrations of Mn and Fe in the water, combined with their respective redox potentials which are higher for Mn- than for Fe-compounds. Stability constants of Mn and Fe indicate that manganese tends to be in natural waters in the form of hydrated Mn^{2+} (Alberts *et al.*, 1976), whereas iron is commonly chelated to colloidal compexes with organic ligands in aquatic systems as well as with humic and fulvic acids in the podsolic environment. In this respect, the iron precipitation resulted from the instability of the chelates under the oxidizing surface conditions. Filamentous iron bacteria such as those described in the sediment (Tuovinen and Nurmiaho, 1979) are known to utilize organic matter, and ferrihydrite and more poorly ordered Fe-oxides have been determined to be associated in environmental samples with iron bacteria such as *Gallionella*, *Leptothrix* and *Toxothrix* (Chukhrov *et al.*, 1973).

Chemolithotrophic iron-oxidizing organisms were ubiquitously present in the water and in the sediments. Their development in the well water and adjacent soil was undoubtedly supported by ferrous-iron and reduced sulfur compounds. The pH was outside the limits of permitt-

ing growth but the possibility cannot be ruled out that acidic micro-environments were established in association of iron precipitates and colloidal matter thereby providing protection for the bacteria. The bacterial distribution in the water on the slope indicated that the organisms were partly deposited with Fe-oxides and perhaps also inactivated owing to their rapid exposure to oxidized surface conditions at an adverse pH. Calculations based on the decrease in bacterial numbers in the runoff and their respective counts in the precipitate indicated that less than 0.1% of the bacteria survived in the sediment. Although these considerations indicate poor survival of the organisms, the acidophilic iron-oxidizers need to be re-evaluated in their environmental perspective as hitherto their role has been totally discounted in environments which do not fall within the limits (e.g., pH) determined in the laboratory.

Ferrihydrite is a Fe-oxide mineral frequently found in rusty precipitates formed where groundwater high in dissolved iron and organic compounds discharges to the surface (Schwertmann and Fischer, 1973; Carlson and Schwertmann, 1980a). When the precipitation occurs as cement in sand or gravel, one or more of the FeOOH polymorphs are formed: goethite, lepidocrocite, and feroxyhite (Carlson and Schwertmann, 1980b). Ferrihydrite often occurs with goethite which can be due to either their simultaneous crystallization or the transformation of ferrihydrite via solution to the stable form goethite. Soil organic matter tends to inhibit this transformation which, however, proceeds when the organic ligands are oxidized (Schwertmann *et al.*, 1974).

Goethite was identified in the uppermost sample (6p) as well as in the basin (site 1). On the surface of the pipe the precipitate was exposed to the influent water with low Eh and DO and high in iron, and the water easily washed and the dissolved poorly ordered components from the precipitate thereby enriching goethite and allowing its crystallization. The sample 6p had a minimum content of Mn and trace metals and a maximum of Al, Na, and K. The high content of Al may be due to its ability to substitute for Fe in goethite (Norrish and Taylor, 1961) and possibly also in ferrihydrite (Taylor and Schwertmann, 1978).

Al, Na, and K were also enriched in sample 1 where goethite was the predominant mineral phase. Sample 1 also contained the maximum levels of Mn and most trace elements. Previously it has been shown that the order of sorption on goethite follows the pattern Cd>Co>Zn>Cu (Forbes *et al.*, 1976) and that Mn-oxides become relatively enriched with Zn and Fe-oxides with Cu, Co, and Ni, respectively (Carpenter *et al.*, 1978). Organic matter accumulated and decomposed in the basin; this was evident by the presence of rusty, floating material with concomitant gas evolution. The precipitate accumulated on gravel at the bottom was loose and continually washed by the water infiltrating through the gravel. In this environment ferrihydrite dissolved and goethite crystallized, the latter process possibly being catalyzed by gravel surface.

On the slope the concentration of Fe in the water decreased and the concentration of organic compounds washed from the decomposing vegetation increased. Simultaneously with an increase in the total carbon content, ferrihydrite became less ordered and the $Fe_{o/d}$ increased. Goethite was not identified in samples 6-2. Thus, the organic matter may not only have inhibited the transformation of ferrihydrite to goethite but may also have retarded the crystallization of ferrihydrite.

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CONSEQUENCES OF ANAEROBIOSIS ON METAL-ORGANIC INTERACTIONS IN TWO PONDS OF THE SOUTHEASTERN UNITED STATES

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INTRODUCTION

Naturally occurring organic matter is known to complex cations (Schnitzer and Khan, 1972). However, there are differing opinions as to the importance of organic matter binding of metals compared to inorganic ligand binding (Stumm and Morgan, 1970). Ramamoorthy and Kushner (1975a,b) have suggested that organic matter is an important component of the binding capacity of certain rivers for Hg^{2+}, Cu^{2+}, Pb^{2+}. Sunda and Hanson (1979) have demonstrated that organic complexes are the principal forms of soluble copper in rivers. Giesy *et al.* (1978) showed a large effect of organics on Cu^{2+} and Pb^{2+} in the binding capacities of several surface waters in Maine, but no significant effect of organics in the binding capacities of European waters (Giesy and Briese, 1980). Finally, Schindler and Alberts (1975) in a study of Par Pond determined that humic type organics were relatively unimportant in this system for the removal or burial of cations, but may play a role in redox reactions of certain metals.

Scientists who have determined the binding capacities of surface water for Cu^{2+} and Pb^{2+} in an attempt to quantify the role of natural organic matter in the speciation of these elements (Ramamoorthy and Kushner, 1975a,b; Giesy *et al.*, 1978; Giesy and Briese, 1980), have worked primarily in aerated waters of northern latitudes. However, waters of the southeastern coastal plain of the United States are soft and highly coloured by organic matter. The importance of this organic carbon in association with trace metals has been documented (Giesy and Briese, 1977, 1978). It has also been shown that organic-metal associations vary in magnitude and distribution between aerobic and anoxic waters (Schindler *et al.*, 1972; Schindler and Alberts, 1974).

In this study, we attempt to determine the effects of aerobic and anoxic conditions on the Cu^{2+}- and Pb^{2+}-binding capacities of some southeastern United States waters and to investigate changes in the organic pools under these conditions. The results of these studies may be compared to previous work in other locales to help define the general applicability of current models for chemical complexing.

METHODS

Samples were collected from two pond systems on the Savannah River Project (Barnwell Co., S.C., Fig. 1). Par Pond is a 11 km^2, man-made impoundment with maximum depth of 16 m which receives water inputs from the Savannah River. Pond B is a man-made impoundment also, (1.5 km^2, max. depth 8 m) but has no river input and receives water only from rainfall. Water samples were collected above and below the thermocline in both ponds by pumping ("Masterflex" Model 7570 Portable Sampling Pump, Cole-Parmer Inst. Co., Oak Park, IL .). Water quality parameters (pH, dissolved O_2, temperature, conductivity and redox potential) were determined with a Surveyor Model 8000 *In Situ* Water Quality Analyser (Hydrolab Corp., Austin, Texas).

Water samples were filtered (0.45 μm membrane filter, Millipore Corp.) and an aliquot of the filtered water was retained for binding capacity determinations. Soluble components of the remaining water were concentrated by factors of 20-100 fold using a Diaflo H10P5 Hollow Fiber Ultrafilter (Amicon Corp., Lexington, Mass.; pore diameter ≃ 1.3 nm, nominal molecular weight ≃ 5000). Aliquots of the filtered but unconcentrated samples were aerated with an aquarium pump overnight. Additional aliquots of both the unconcentrated and concentrated samples were photo-oxidized in the presence of hydrogen peroxide for 5 h in an Ultra-Violet Photo Oxidation Unit (La Jolla Scientific Co., CA.).

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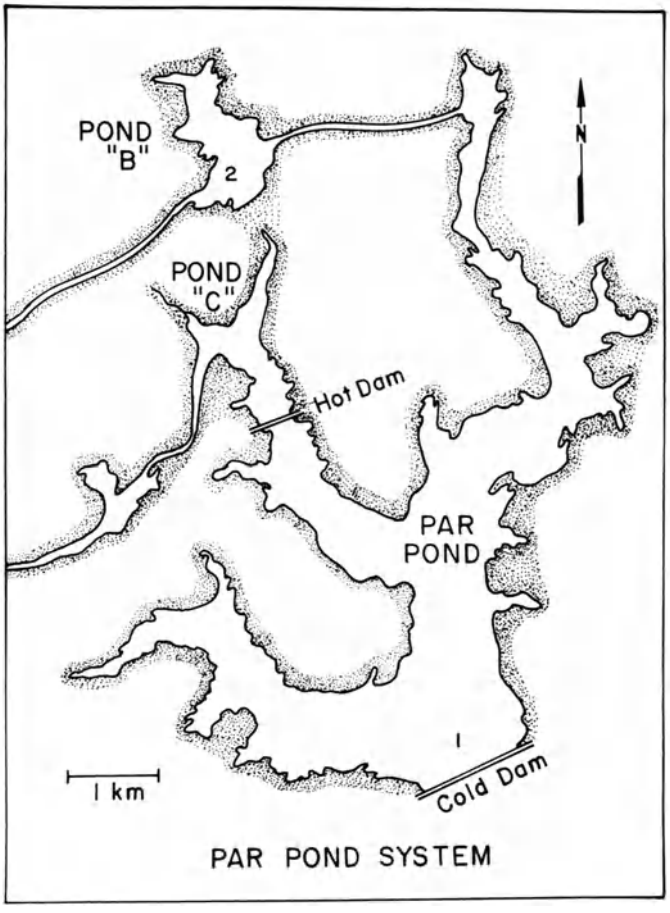

Figure 1: Par Pond system with sampling locations:

1. Par Pond station, 16 m depth.
2. Pond B station, 6 m depth.

Organic carbon determinations were performed on a Beckman Model 915 Total Carbon Analyser. The ratios of extinction (E_4/E_6) at 465 nm and 665 nm, respectively, were determined in a 1 cm path length cuvette (Beckman Acta CIII UV-Visible spectrophotometer).

Binding capacities were determined using the technique of Giesy *et al.* (1978). Specific ion electrodes (Orion Models 94-29 and 94-82; Cu^{2+} and Pb^{2+}, respectively) were interfaced to a Brinkman Model pH-102 Metrohm pH meter. Standard solutions were prepared from 1000 mg l^{-1} stock solutions (Fisher Scientific, Fairlawn, N.J.) and standard curves were linear throughout the range of concentrations used. For complete details regarding the technique and calculations see Giesy *et al.* (1978).

Total acidity values were determined directly on the concentrated fractions by the $Ba(OH)_2$ equilibration method (Schnitzer and Khan, 1972). Weights of material per ml were determined gravimetrically on dried samples of concentrates.

Sedimentary humic acids from Par Pond and Pond B were isolated by standard base extraction of sediments followed by repeated precipitation, resolubilization and dialysis (Alberts *et al.*, 1974). However, the extracted humic acids were neither treated with HF/HCl nor freeze-dried.

Dissolved sulfate concentrations were determined by nephelometry (Bauman, 1976).

RESULTS AND DISCUSSION

Par Pond has been studied for many years and has a well documented chemical and physical structure (Tilly, 1975). The pond is considered moderately productive, approximately 400 mg C m^{-2} day^{-1} (Tilly, 1973), and undergoes thermal stratification annually with attendant increases of Fe, Mn, Co, (Marshall and LeRoy, 1971) and ^{137}Cs (Alberts *et al.*, 1979). Evaporation exceeds input in the pond and hence it receives "make up" water from the Savannah River, which drains the Piedmont areas of North and South Carolina. Due to this frequent addition of river water and subsequent evaporation, Par Pond has increased in dissolved solids concentration over the years with an increase in pH. Hence, the pond is primarily a carbonate-bicarbonate system (HCO_3^- concentration approximately 20 mg l^{-1}; Tilly, 1975).

Pond B was connected into the Par Pond system until 1964, at which time the connection was closed, stopping exchange between the two ponds. Since that time, Pond B has received water only from rainfall. The limnology of the pond is not as well documented as that of Par Pond. Although both ponds are thermally stratified, Pond B does not appear to develop the intense anoxic conditions in its hypolimnion that are observed in Par Pond (Alberts, unpub. data). The pH of Pond B is generally lower than Par Pond (about 6.5 in the surface waters) with lower bicarbonate concentrations (about 6 mg l^{-1}; Tilly, 1975).

The two water bodies differed considerably in most physical and chemical characteristics at the time of sampling (Table 1), but were both stratified and anoxic. The probe employed in this study does not record below 0.1 mg l^{-1}. The elevated value recorded at 13 m in Par Pond is believed to be an interference due to the low REDOX potential of the water, as the DO values passed through 0.1 mg l^{-1} at about 7 m and began to rise after 11 m.

Schindler and Alberts (1974) have shown in four lake systems in the southeastern United States that greater than 90% of the dissolved organic carbon (DOC) in surface waters was larger than 2.4 nm in diameter. Their results, and those of other studies (Schindler *et al.*, 1972), indicated that less DOC was larger than 2.4 nm in diameter in anoxic, hypolimnetic waters than in oxic, epilimnetic waters. The data reported here (Table 2) show more DOC retained by ultrafilters (pore diameter about 1.3 nm) in the hypolimnetic waters than in the epilimnetic waters. The explanation for the differences between these results and those of Schindler and Alberts is not known. Due to the smaller pore size used in this study the difference may be the result of a large concentration of organic matter less than 2.4 nm but greater than 1.3 nm diameter. Alternatively, the systems under study here may have different organic arrays than did those investigated earlier. In both of the previous studies (Schindler *et al.*, 1972; Schindler and Alberts, 1974) aquatic macrophytes played little or no part in the total production or biomass of the systems. In Par Pond, aquatic macrophytes cover a large area of the pond substrate (Grace, 1977) and the same appears to be true of Pond B (personal observation). The sampling period in this study was after the period of maximum growth of macrophytes and hence, the DOC distribution at that time was probably dominated, at least in deeper, water by released organic matter and degraded plant fragments (Giesy and Tessier, 1979). The surface water DOC may be more affected by planktonic algae and lead to a different array of organic compounds. This latter argument is supported by the E_4/E_6 ratios of the isolated fractions (Table 2), which show values two to three times higher in the hypolimnion than in the epilimnion. In general high E_4/E_6 ratios are indicative of less condensed or humified organic matter (Schnitzer and Khan, 1972). The organic pools in the epilimnion and hypolimnion appear to be being acted upon by different mechanisms, as simple settling of the surface material should lead to lower E_4/E_6 ratios through microbial activity. A similar argument may be advanced for the total acidity values of the isolates (Table 2). The increase in exchangeable protons per gram organic matter in hypolimnion waters indicates less condensation of those compounds than the material in the epilimnion or a different spectrum of organic compounds, again suggesting different sources or degradation mechanisms for the epilimnetic and hypolimnetic isolates. In both ponds, the sedimentary humic acid fractions appear more condensed than the hypolimnetic fraction indicating further condensation reactions of the organic material must occur, either in the water column or the sediments, to convert the large sized organic compounds isolated from the water into the refactory organic matter found in the sediments that are often implicated in metal binding.

Low binding capacities were observed for both Cu^{2+} and Pb^{2+} in the isolated fractions of both ponds (Table 3). In all cases, the binding could readily be explained by the

TABLE 1

COMPARISON OF PHYSICAL AND CHEMICAL CHARACTERISTICS
OF TWO STUDY SITES

	Par Pond		Pond B	
	Surface	13 m	Surface	5 m
C°	29.2	15.1	29.1	23.2
pH	8.91	6.78	6.16	5.30
DO (mg l^{-1})	9.34	0.51 *	7.88	0.17
Cond. (μmhos)	50	150	24.1 - 46.6	ND +
REDOX (mV)	170	-105	314	330

* Probably redox interference

\+ ND = Not determined

TABLE 2

CHEMICAL CHARACTERISTICS OF ISOLATED ORGANIC COMPONENTS *
FROM FRESHWATER SYSTEMS

	C_{org} (mg l^{-1})	E_4/E_6	Total Acidities	
			meq ($g\ C_{org}$)$^{-1}$	meq l^{-1}
Par Pond Surface	0.19	4.15	0.82 ± 0.07	0.71 x 10^{-3}
13 m	0.25	11.12	3.30 ± 0.06	6.32 x 10^{-3}
Pond B Surface	0.71	2.32	2.34 ± 0.05	3.90 x 10^{-3}
5 m	1.29	5.00	3.24 ± 0.07	12.5 x 10^{-3}
Par Sedimentary Humic Acid		4.33	2.35 ± 0.51	
Pond B Sedimentary Humic Acid		3.99	ND +	

* Retained by 1.3 nm filter

\+ ND = Not determined

exchangeable protons available in the isolated organic matter (meq l^{-1}; Table 2). However, photo-oxidation of the isolates did not remove the binding capacity for Pb^{2+} in any fraction and removed none or only a portion of the Cu^{2+}-binding capacity in Par Pond, indicating that much of the binding was inorganic (Table 3). All the Cu^{2+}-binding capacity of the Pond B isolate appeared to be organic. It appears that organic matter larger than 1.3 nm does not bind significant quantities of Cu^{2+} and Pb^{2+}. However, Ramamoorthy and Kushner (1975a) reported considerable binding of these metals by organic matter less than 1.3 nm in diameter, possibly fulvic acids. To investigate the possibility of such binding occurring in our systems, binding capacities of filtered water samples were determined (Table 4).

TABLE 3

BINDING CAPACITIES (mg atoms l^{-1}) FOR Cu^{2+} AND Pb^{2+} OF FRACTIONS ISOLATED FROM WATER

	Ultrafiltered Water		Photo-oxidized water	
	Cu^{2+}	Pb^{2+}	Cu^{2+}	Pb^{2+}
Par Pond Surface	0.08	0.23	0.19	0.22
13 m	0.45	1.2	0.27	0.99
Pond B Surface	0.12	0.17	0	0.05
5 m	0.23	0.55	0	0.69

TABLE 4

BINDING CAPACITIES (mg atoms l^{-1}) OF Cu^{2+} AND Pb^{2+} IN EPILIMNETIC AND HYPOLIMNETIC WATERS

	Par Pond		Pond B	
	Surface	13 m	Surface	5 m
Whole water				
Cu^{2+}	122	15.6	6.1	15.6
Pb^{2+}	235	29.6	0	32.0
Aerated Water				
Cu^{2+}	17.5	18.6	6.0	13.4
Pb^{2+}	52.1	35.8	11.7	30.8
Photo-oxidized water				
Cu^{2+}	8.1	21.3	8.0	12.6
Pb^{2+}	30.7	42.8	0	18.3

Surface water binding capacities for both Cu^{2+} and Pb^{2+} in the Par Pond sample are extremely high. The pH (8.91) was also quite high. Apparently, the sample was taken from an *Anabena* sp. bloom (J. P. Giesy, personal communication). Briand *et al.* (1978) have shown that binding of Cu^{2+}, Pb^{2+}, Hg^{2+} and Cd^{2+} in Canadian waters is affected by algal

blooms, and values of Cu^{2+}-binding capacities of 150-200 µg-atoms l^{-1} were common and correlated to *Anabena spiroides* concentrations. Therefore, the high binding capacities reported here are realistic for bloom conditions. Surface values in Pond B are much lower and in the case of Cu^{2+} agree with values reported by Giesy *et al.* (1978). Hypolimnetic waters in both systems have similar binding capacities for the two metals and, at least in the case of Pond B, show an increase over surface waters. Unfortunately, because of the bloom in Par Pond, a similar increase over epilimnetic waters could not be demonstrated.

The hypolimnetic waters of Par Pond were intensely reducing (Table 1). Hence, the samples were aerated overnight to oxidize sulfide which might affect the binding of these waters. The effect of this treatment was to greatly reduce the Cu^{2+}-and Pb^{2+}-binding capacities of the Par Pond surface waters without affecting the values for the other water samples (Table 4). Aliquots of all the waters were photo-oxidized to determine the amount of organic binding in the samples. The results of this experiment (Table 4) were confusing in that water-binding capacity values of the Par Pond epilimnion decreased for both Cu^{2+} and Pb^{2+} indicating organic binding, but hypolimnion water values increased for both metals suggesting more inorganic binding after oxidation than in the untreated waters.

Due to the nature of the treatments employed in our study and the variations in original pH of samples, the pH of individual samples varied considerably in our experiments (Par Pond surface water ranged from pH 8.76 to 6.65 for Cu^{2+} determinations and 8.04 to 6.36 for Pb^{2+}). Since OH^-, CO_3^{2-} and SO_4^{2-} ligands may be important complexers of both Cu^{2+} and Pb^{2+}, the equilibrium concentrations of complex species (Table 5) were calculated for our experimental conditions. The results of those calculations were then used to determine the binding capacities of Par Pond and Pond B waters that could not be accounted for by pH-dependent concentrations of CO_3^{2-} and OH^-, and the concentrations of sulfate (0.044 - 0.006 mg-atom l^{-1} for Par Pond; 0.036 - 0.017 mg-atom l^{-1} for Pond B; Table 6).

TABLE 5

EQUILIBRIUM CONSTANTS OF INORGANIC COMPLEXES OF Cu^{2+} AND Pb^{2+}

	$CuCO_3^o$	$Cu(OH)_2^o$	$CuSO_4^o$
log K	6.77[a]	-13.7[b]	2.37[c]
	$PbCO_3^o$	$PbOH^+$	$PbSO_4^o$
log K	6.24[d]	-6.57[d]	2.62[d]

[a] Stumm and Morgan (1970)

[b] Vuceta and Morgan (1977)

[c] Sillen and Martell (1964)

[d] Hem (1976)

The most striking feature of the data, after correction for inorganic binding, is the fact that no organic binding of either Cu^{2+} and Pb^{2+} remains in the Par Pond surface water taken from the bloom. These results show that the large binding capacities reported for these elements may be explained entirely by inorganic binding. Briand *et al.* (1978) have discussed the importance of organic binding of metals by plankton populations in Canadian waters. However, their conclusions do not appear to extrapolate to the conditions observed in southeastern U.S. systems.

TABLE 6

EXCESS BINDING CAPACITY ($\mu g\text{-atom } l^{-1}$) NOT ACCOUNTED FOR BY CO_3^{2-}, OH^- AND SO_4^{2-}

	Par Pond		Pond B	
	Surface	13m	Surface	5m
Cu^{2+}				
Filtered Water	0	13.8	5.0	10.6
Aerated Water	0	13.9	2.8	6.7
Photo-oxidized Water	4.7	12.5	7.3	11.1
Pb^{2+}				
Filtered Water	0	15.6	0	0
Aerated Water	8.3	16.8	0	8.9
Photo-oxidized Water	15.9	26.4	0	7.0

In all cases the photo-oxidized samples have as much or more binding capacity for the metals as either the filtered or aerated samples. Since the organic binding capacity should be destroyed by photo-oxidation, the binding capacity measured in these samples must be inorganic. Large concentrations of Fe are dissolved from the sediments of Par Pond during stratification (Marshall and LeRoy, 1971). This reduced Fe can be sequestered by organic matter. Oxidation of the organic matter can release and oxidize Fe which may in turn precipitate as amorphous hydrous oxides, with subsequent occlusion and/or adsorption of trace metals. A possible explanation of the excess binding capacity for Cu^{2+} and Pb^{2+} observed in photo-oxidized samples would be reaction of these metals with hydrous oxide particles of Fe.

In conclusion, it appears that organic matter in the epilimnion and hypolimnion of two southeastern United States ponds are different in their degree of complexity, perhaps representing differing degradation mechanisms in the two zones. Binding capacities for Cu^{2+} and Pb^{2+} in the epilimnion and hypolimnion of these systems appear to be controlled by inorganic complexing, even in algal blooms, with organic matter having only a small impact on the total binding capacity.

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PHYSICO-CHEMICAL MODELS FOR HEAVY METAL TRANSPORT IN STREAMS

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INTRODUCTION

In order to model the transport of heavy metals in rivers it is necessary to take account of many physical and chemical effects. Not only are these components carried along in the bulk of the flowing waters, a process known as *advection*, they are simultaneously mixed or spread by *dispersion*. In addition these and other chemical components will be injected at various points by *sources* along the river. *Sinks* occur where components leave the flowing waters (mobile phase) to attach themselves to, or be trapped by, the bed or banks of the river (stationary phase).

Sources are conveniently divided into active and passive inputs. Active inputs are those such as industrial effluent discharges where the rate of injection of chemical components at specified locations is independent of the chemical state of the river. These become the driving functions of the model. Passive inputs on the other hand, are inputs such as the dissolution of oxygen during river re-aeration where the rate of injection depends, amongst other things, on the amount of oxygen already dissolved.

Heavy metal sinks may occur where chemical components can participate in chemical reactions to form precipitates of suspended solids, e.g. hydrous oxides, basic sulfates and carbonates which can then sediment to the bed of the river. At a later time, these may re-dissolve. Other sinks occur where chemical solute species can adsorb on to reactive surfaces such as clays, sands, chemically precipitated solids, or organic debris and living plant tissue.

The reactive surfaces may be either in the mobile phase, (i.e. suspended in the flowing waters) or the stationary phase, being incorporated into or attached to the bed or banks of the river. Solids with reactive surfaces may, under appropriate conditions, transfer from the mobile phase to the stationary phase or vice versa.

The dispersion of non-reactive, conservative solutes (or suspensions) in rivers (Fisher, 1968) is described by the well known convective diffusion Equation 1:

$$A \frac{\partial c}{\partial t} = \frac{\partial}{\partial x} \left(AD \frac{\partial c}{\partial x} \right) - \frac{\partial}{\partial x} (AUc) \qquad 1$$

where c is the concentration of the solute.
x is the distance downstream.
t is the time.
A is the cross-sectional area of the river.
D is the dispersion coefficient of the river.
U is the river velocity.

A somewhat more convenient form of the convective diffusion equation which is similar but not equivalent to Equation 1 has been used by Chapman (1979a,b).

The one-dimensional co-ordinate system specifying the position of any point on the river is defined by the time-of-travel, y, required for an average (non-reactive) particle to reach that point from an upstream reference point, rather than by the distance between these locations (Chapman, 1979a,b). Furthermore the mole-(or mass-) flow rate m of a chemical component in any particular cross-section in the river is used instead of the concentration. Equation 2 is used:

$$\frac{\partial m}{\partial t} = \frac{\partial}{\partial y} \left(D' \frac{\partial m}{\partial y} \right) - \frac{\partial m}{\partial y} \qquad 2$$

where $D' = \frac{D}{U^2}$, the velocity-reduced dispersion coefficient, which along with y and t, has

the dimensions of time. In both Equations 1 and 2 the left hand side represents the accumulation of mass at any point and the terms on the other side represent its dispersed and advected contribution, respectively. Approximate analytical solutions to this equation for varying D' have been obtained for different boundary conditions.

For "non-conservative" or reactive chemical elements such as heavy metals this equation must be extended to allow for sources and sinks.

THE MODEL FOR REACTIVE SOLUTES

Because of the complexity necessarily involved in a full treatment of the effects of adsorption we will confine attention to sedimentation as the only sink. We will now more strictly define chemical components (Westall *et al.*, 1976) as the group of ions or molecules e.g. Fe^{2+}, SO_4^{2-}, H^+, O_2, CO_3^{2-} whose concentrations and reactivities (i.e. equilibrium constants) are needed to describe fully the chemical composition of the river and from which every species (e.g. soluble complex and solid) may be defined.

As above, the one-dimensional co-ordinate system used is time-of-travel y.

Any river subjected to pollution such as acid mine drainage will contain numerous chemical components and correspondingly an even greater number of possible reaction species. Let the set of mole flow-rates through any river cross-section (m_1, m_2, m_3...) associated with the set of components (X_1, X_2, X_3, ...) be represented by the vector m. This is related to the concentration c of the components in the mobile phase and the volumetric flow-rate, v (Equation 3):

$$m = v\,c \qquad 3$$

For the stationary phase let the set of mole densities (s_1, s_2, s_3 ...) of precipitated solids sedimented on the river bed and associated with the set of all possible precipitates (S_1, S_2, S_3 ...) be designated by the vector s. Mole density is defined by moles of sedimented solid per unit "y" of river (a measure analogous to moles per unit length of river). If {C} is the matrix of stoichiometric coefficients relating the set of components to the set of precipitates, then the transport of components can be described by a form of the convective diffusion Equation 4. Thus:

$$\frac{\partial m}{\partial t} = \frac{\partial}{\partial y}\left(D' \frac{\partial m}{\partial y}\right) - \frac{\partial m}{\partial y} - \{C\}\frac{\partial s}{\partial t} + f + g \qquad 4$$

where f and g are the vectors of active and passive inputs of components respectively, having the dimensions of mole flow-rates per unit "y" of river. The term on the left side represents the accumulation of mass in the mobile phase at any point and the first two terms on the right hand side are the dispersive and advective contributions to it, respectively. $\{C\}\frac{\partial s}{\partial t}$ represents the loss (or gain) of components in the mobile phase by precipitated solids sedimenting into (or re-dissolving from) the stationary phase.

The sedimentation rate $\frac{\partial s}{\partial t}$ will in general depend on, amongst other things, the moles of suspended solids per unit "y" in the mobile phase, σ, which in turn will depend on the total concentrations of components in the mobile phase. The simplest sedimentation law is employed where the rate of sedimentation is proportional to σ:

$$\frac{\partial s}{\partial t} = \{R_s\}\sigma \qquad 5$$

where $\{R_s\}$ is a diagonal matrix of the sedimentation rate constants.

Where applicable the re-aeration rate of the river is assumed to follow a simple first order rate process so:

$$\frac{\partial O_2}{\partial t} = R_A\,(DO_\infty - DO) \qquad 6$$

where O_2 is the cumulative number of moles of oxygen injected, DO and DO_∞ are the dissolved

oxygen content of the river and its saturation value respectively and R_A the re-aeration rate constant. Equations 3 - 6 form the basis of the hydrological sub-model.

Ideally all chemical reactions should be treated kinetically so that the rates of formation of precipitates are given directly and from this the rates of sedimentation can then be calculated. The possible reactions are numerous and unfortunately they are not well understood nor are the relevant rate constants available. Therefore at the present for multi-component systems, we are forced to calculate all chemical reactions on the basis of chemical equilibrium theory. For a known mixture of chemical components, solutions are sought for the distribution of these components among the soluble and solid species determined by an assumed set of reactions with known equilibrium constants.

SOLUTIONS

The general numerical solution of Equation 4 is difficult and computationally expensive, therefore three special cases were considered. The first is the steady-state solution, the second is the dynamic case for which there is no sedimentation and the third is the general solution itself. The existing versatile chemical equilibrium program MINEQL is used for the chemical sub-model. This program was developed by Westall *et al.* (1976), and subsequently extended by Davis *et al.* (1978), James *et al.* (1978) and Davis and Leckie (1978) for adsorption reactions in colloidal suspensions. It calculates the concentrations of the various dissolved species and amounts of solid phases formed when a number of chemical components are mixed at known concentrations. Calculations are carried out for simple ionic reactions including complex formation, hydrolysis reactions, oxidation-reduction reactions, precipitation and adsorption reactions. The data bank of the program lists the necessary thermodynamic data for over 80 components and 1,500 soluble species and precipitates.

It is the solution algorithm of the hydrological submodel, accounting for the physical transport processes, which differs between each of the three cases studied. These cases will now be considered in turn.

Case I: The Steady-State

In principle, a steady-state solution may be obtained by running a dynamic model for a sufficiently long time. Although this can be useful as an occasional check, for routine usage it is less extravagant to simplify and solve Equation 4 for steady-state conditions. Under these conditions, at any point, mole-flow rates of sources and sinks are constant with time. That is, rates of injection and rates of sedimentation are constant at any point. Furthermore, mole flow-rates m of components in the mobile phase are constant and Equation 4 reduces to

$$0 = \frac{d}{dy}\left(D\frac{dm}{dy}\right) - \frac{dm}{dy} - \{C\}\dot{s} + f + g$$

where $\dot{s}$, f and g are functions only of distance.

In the steady-state, for ease of computation, it is convenient to neglect dispersion which is important mainly for transient inputs. The final equation becomes 7:

$$\frac{dm}{dy} = -\{C\}\dot{s} + f + g \qquad 7$$

where $\dot{s}$ $\left(\equiv \frac{ds}{dt}\right)$, f and g are functions only of y.

A computer program we have called STDEQL uses a fourth-order Runge-Kutta technique to solve Equation 7 which represents a set of simultaneous ordinary differential equations. Injection at all point and distributed sources are represented by the vector f and re-aeration of the river by g.

It is noteworthy that Equation 7 would have also represented the steady-state condition if adsorption on to stationary surface areas had been considered. This is because under these conditions there would have been no change in surface concentrations on stationary surfaces with time and the relevant terms would have dropped out to again yield Equation 5.

Case II: No Sedimentation

A case of considerable interest is the simplest dynamic situation, where any precipitates formed are not allowed to sediment but are constrained to remain suspended, e.g., as stable colloids, and disperse in a similar manner to solutes. Because of the nature of the algorithm used, passive inputs such as river re-aeration are also not considered. The convective diffusion equation then becomes 8:

$$\frac{\partial m}{\partial t} = \frac{\partial}{\partial y}\left(D' \frac{\partial m}{\partial y}\right) - \frac{\partial m}{\partial y} + f \qquad 8$$

This represents a set of simultaneous but independent partial differential equations. This means that although the components can react with each other, they are transported in the same way as if they were independent of one another.

To solve Equation 8 use is made of an approximate analytic solution to Equation 8 where f has the form of a Dirac delta function.

For an instantaneous release of one mole of components injected at y = 0 and t = 0:

$$m = \frac{1}{\sqrt{4\pi \bar{D}'}} \exp^{(-(y-t)^2/4\bar{D}'t)}$$

where $\bar{D}' = \frac{1}{y}\int_o^y D'(y)dy.$

For general input function f (y,t) the solution is obtained by forming a convolution integral (Equation 9):

$$m(y,t) = \int_o^y\int_o^t \frac{1}{\sqrt{4\pi \bar{D}'(t-\tau)}} \exp(-((y-\zeta)-(t-\tau))^2/4\bar{D}'(t-\tau)).f.d\tau.d\zeta \qquad 9$$

where $\bar{D}'(y,\zeta) = \frac{1}{y-\zeta}\int_\zeta^y D'(y)dy.$

Here the convolution is performed over all previous time. This result is then integrated over upstream distance to include the effects of all point and distributed sources. From Equation 3, the concentrations of all components at any point and time can be found and the chemical submodel will calculate the concentrations of all soluble and particulate species.

The integration over ζ can be converted to a limited summation if all sources are point sources or if all distributed sources are reduced to a series of point sources. A program called RIVEQL has been written to solve this particular case.

Case III: General Solution

In the general case where precipitates are allowed to sediment (i.e. a passive sink), and passive sources such as re-aeration are considered, the general Equation 4 must be solved. This now represents a series of dependent simultaneous partial differential equations. Since the mole flow rates of components of passive inputs $g(y,t)$ and the function for cumulative mole density of sediment $s(y,t)$ depend on the state of the river at any one time, these functions must be generated for each point in the river as the solution progresses for each time step. These can be calculated knowing the total mole flow rates for each component which are given by

$$m(y,t) = \int_o^y\int_o^t \frac{1}{\sqrt{4\pi \bar{D}'(t-\tau)}} \exp((-(y-\zeta)-(t-\tau))^2/4\bar{D}'(t-\tau))\left(f+g-\{C\}\frac{\partial s(\zeta,\tau)}{\partial \tau}\right)d\tau d\zeta.$$

where g and s have been generated for all previous values of y and t. From the values of m so obtained, new values of g and s are obtained. Since g and s will, in general, be distributed over y they cannot be represented by a small number of point sources and the integration over y cannot be replaced by a limited summation as in Case II.

This type of solution has a large storage requirement as the functions g and s must be stored as functions of time and space as they generated. In addition, at longer times, integration becomes lengthy. A program called SEDEQL has been written to solve this general case.

This method of solution must be compared with the conventional type of direct solution of the partial differential equations using, say, finite difference discretization in space and Runge-Kutta integration in time. In such a solution, in order to obtain sufficient spacial resolution, it is necessary to have grid points more finely spaced than that for the convolution integral technique, where the solution is partially analytical. This in turn means a greater number of calls to the chemical submodel.

Input Data Required

In general, the following information is required as input data to the above programs. All hydrologic functions are entered in tabulated form.

Velocity reduced dispersion coefficient	$D'(y)$
Volumetric flow rate of river	$v(y)$
Injection functions	$f_i(t)$
Points of injection	y_i
Re-aeration rate constant	R_A
Sedimentation rate constant(s)	R_S

Chemical input data required include the identity and quantity of the components. Many of the reactions amongst the components and the respective stoichiometry and the equilibrium constants are contained within the existing data bank.

EXPERIMENTAL

An abandoned base metal mine at Sunny Corner near Lithgow, NSW, was chosen as a field site to gather data on the transport of soluble and particulate heavy metal forms. Several small streams in the vicinity of the mine show the typical characteristics of acid mine drainage, eg, low pH, high heavy metal concentrations, high concentrations of sulfate from sulfide mineral oxidation and coatings of various hydrous iron oxides on rocks and in stream beds. In one experiment a concentrated solution of sodium hydroxide was added to one of the streams. Subsequently at each of three observation stations, A, B, and C, at respectively 7m, 20m, and 42m downstream from the point of injection, the chemical state of the stream was monitored. The purpose was twofold. Firstly, measurement of the essentially non-reactive sodium ion concentration at various locations and times enabled determination of certain stream parameters including the volumetric flow rate, velocity, and velocity reduced dispersion coefficient D' or the related "characteristic time" ($t^* = 4D'$). Secondly, the reactive metals may form colloidal precipitates which could then be studied to yield information about the transport of both soluble and particulate species.

After the injection of the 20 moles of NaOH over a period of one minute, in situ measurements of pH and conductivity were made as a function of time and location at the three downstream stations. At each time interval 2 samples of water were simultaneously taken by automatic sampler, one being immediately acidified (2 ml conc.HNO_3 per 500 ml), the other being filtered using 0.45μm membrane filters and then acidified. Samples were later analysed for Cu, Pb, Zn, Cd, Fe, Al and Mn as well as major cations and anions. The principal analytical technique was atomic absorption spectrometry using a Varian AA775.

RESULTS AND DISCUSSION

The characteristics of the study stream are given in Table 1 which shows the low pH and elevated heavy metal concentrations present. At the time of measurement the flow rates and velocities were low. The stream flowing from a collapsed adit was generally clear with little turbidity although algal mats were present and yellow-brown iron precipitates were evident on the banks and stream bed.

The effect of neutralization treatment was simulated by the addition of 20 moles of NaOH in concentrated solution in the stream. Immediately changes were visible which included the formation of a colloid dispersion of iron, aluminium and zinc hydrous oxides. At Station A the pH rose suddenly with the arrival of the base from pH 2.8 up to a peak of 12.

TABLE 1

PROPERTIES OF STREAM UNDER STUDY

A. Hydrologic Characteristics	
Characteristic time	7 min
Flow rate	38ℓ min^{-1}
Station A, y = 13 min	(7m)
Station B, y = 20 min	(20m)
Station C, y = 39 min	(42m)
Average velocity 1.1m min^{-1}	

B. Chemical Characteristics		
pH	2.8	
κ	1,560 μS cm^{-1}	
Element	Concentration	
	mM	ppm
Fe^{2+}	.5	28
Fe^{3+}	1.1	62
Al	1.2	32
Na	.13	3
Ca	.25	10
Mn	.05	3
Cu	.05	3
Zn	2.0	130

This is shown in Fig. 1 together with the accompanying increase in specific conductivity which is principally due to Na^+ and OH^- ions.

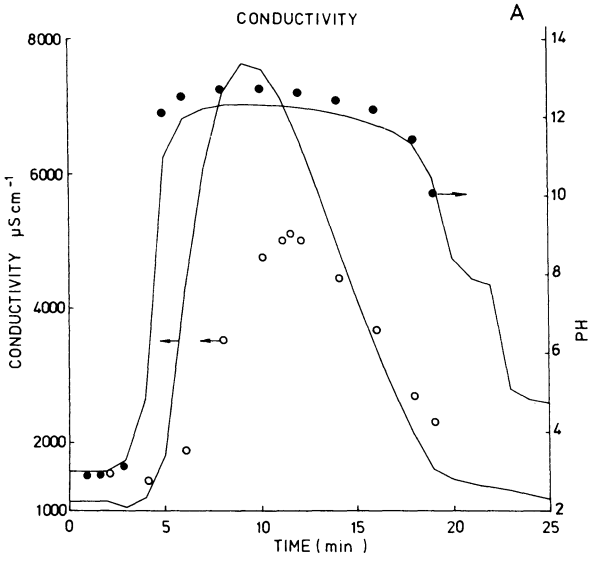

Figure 1: The pH (•) and specific conductivity (o), κ, as a function of time following an injection of NaOH into Daylight Creek, Sunny Corner, measured at station A. The points are experimental values and the lines the theoretical fit using the RIVEQL model.

The increase in Na^+ concentration as the added base reaches Station A is shown in Fig. 2. Integration of the concentration time profile shows that Na^+ is conservative and so this profile was used to determine the characteristic time and other hydrologic parameters of the stream. The solid line in Fig. 2 indicates the Na^+ concentration predicted by the physico-chemical model RIVEQL using these parameters. The lines in Fig. 1 also represent predicted values of pH and specific conductivity at Station A as a function of time using the same hydrologic parameters. The pH line gives reasonable but not exact agreement with the measured pH, while the predicted specific conductance is rather larger than the measured value. The reason for the lack of agreement for the specific conductivity will be discussed later.

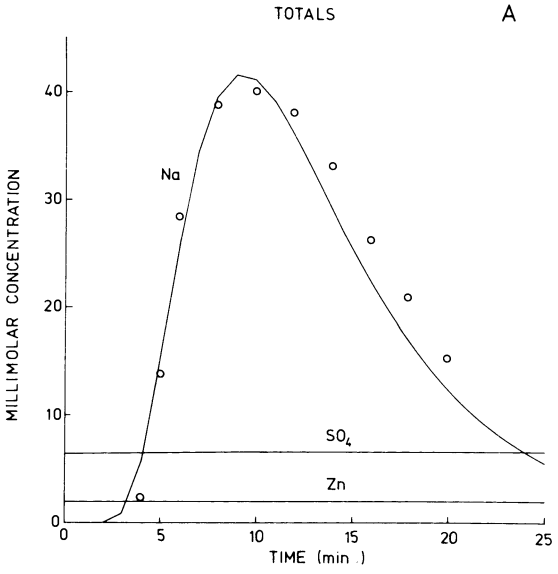

Figure 2: The concentration of sodium as a function of time after the injection of NaOH into Daylight Creek. The points are measured and the lines the theoretical fit using the RIVEQL model.

The effect of base addition on the concentration of soluble forms of the metals aluminium and zinc are shown in Fig. 3. The pattern of behaviour is quite similar as the base addition reaches Station A, the soluble zinc and aluminium concentrations immediately decrease as the pH increases through neutrality, however as the pH increases to its peak value, the soluble aluminium concentration increases approaching the initial level. Then as the peak passes the sampling station and the pH decreases, the soluble Al decreases.

This data is complemented by the results in Fig. 4, which shows the difference between the total soluble and suspended colloidal material and the soluble metal concentrations to yield the moles of metal hydroxides suspended per litre of solution as a function of time. As the added base solution reaches Station A both zinc and aluminium form filterable precipitates. As the excess base passes, less aluminium precipitates. This is due to the higher solubility of aluminium oxides in highly alkaline solutions. The solid lines in Figs. 3 and 4 represent the predicted concentrations of soluble species and amounts of solids species using the physico-chemical model RIVEQL outlined in the introduction. Although there are insufficient data to allow a detailed comparison, it is obvious that several of the major processes can be accounted for in the RIVEQL model.

However, because it does not include sedimentation of precipitates it cannot account for all of the data. For example, lack of agreement in specific conductance can be traced to the sedimentation of growing colloidal particles. As precipitates settle upstream of the observations stations, moles of ionic species are removed from the flowing water which are not observed but are calculated using the RIVEQL model. Thus the predicted specific conductance exceeds the observed values. In addition, if we compare the total metal at the various observation stations with the original analyses listed in Table 1, we find that the soluble plus suspended metal concentration has decreased. This is not due to any "non-

conservative" behaviour of the moles of metal but due to the sedimentation process that removes mass from the water body.

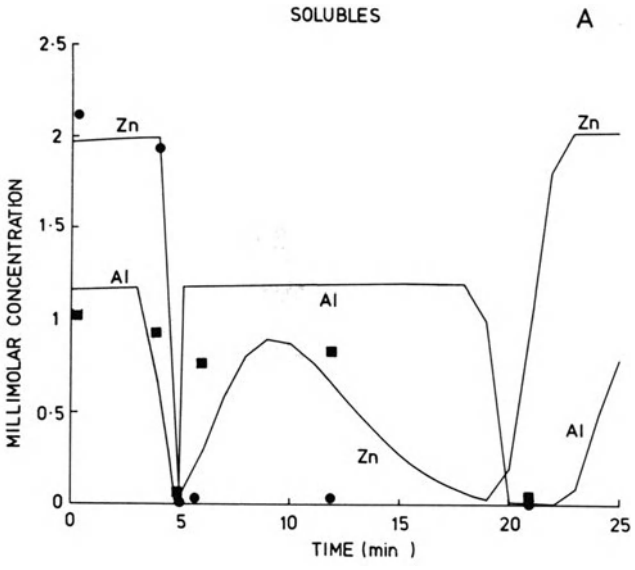

Figure 3: The variation in concentration of "soluble" forms of the reactive metals Zn (●) and Al ( ) with time in Daylight Creek at Station A after the injection of base. The points are measured data and the lines the theoretical prediction from the RIVEQL model.

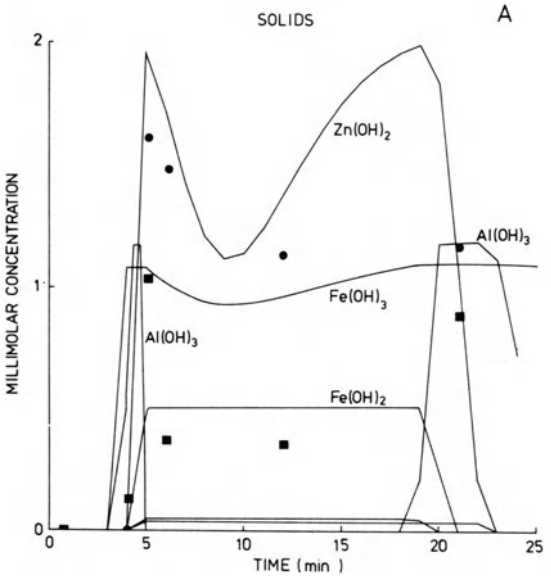

Figure 4: The variation of amounts of suspended precipitates or colloidal forms of Zn (●) and Al ( ) with time at Station A in Daylight Creek following the injection of NaOH. The points are measured data and the lines the theoretical predictions from the RIVEQL model.

While the model RIVEQL serves as a useful first approximation a more detailed account of the transport of heavy metals must consider the sedimentation processes. Work is therefore in progress to include these effects using the model SEDEQL.

ACKNOWLEDGEMENTS

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MAN'S EFFECT ON STRATOSPHERIC OZONE

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INTRODUCTION

Less than a decade has passed since McDonald in November 1970 (Dotto and Schiff, 1978) suggested to the U.S. Congress that H_2O released in the stratosphere in SST exhaust would, by destroying O_3, lead to an increase in skin cancer. Since then the list of potential modifiers of our UV-screen has grown to include NO_x (Crutzen, 1970; Johnston, 1971), Cl (Hoshizaki *et al.*,1973; Stolarski and Cicerone, 1974), Br (Wofsy *et al.*,1975), bremsstrahlung x-rays from relativistic electron precipitation events (Thorne, 1976) and modifiers of stratospheric temperature (Blake and Lindzen, 1973; Boughner and Ramanathan, 1975). While cosmic ray-produced negative ions (Ruderman *et al.*, 1976) and stratospheric particles (Cadle *et al.*, 1974) had been proposed as possible destroyers of O_3, these threats now appear to have been eliminated (Fehsenfeld *et al.*, 1976; Olszyna *et al.*, 1979).

Through these agents Man is now presumed capable of modifying his UV-screen by any of the following mechanisms:

1. Operation in the stratosphere or mesosphere of aircraft and rockets exhausting H_2O (Harrison, 1970), NO_x (Johnston, 1971) or Cl (Hoshizaki *et al.*, 1973).

2. Detonation of nuclear devices such that their debris clouds can produce or carry NO_x into the stratosphere or mesosphere (Foley and Ruderman, 1973).

3. Stimulation of N_2O production by addition of fixed nitrogen to the biosphere whether through nitrogen fertilizers, animal wastes, combustion-produced NO_x, expanded growth of legumes, infection of non-leguminous plants with nitrogen-fixing bacteria or by green mulching (Crutzen, 1974).

4. Atmospheric release of stable Cl-containing compounds such as chlorocarbons in general and chlorofluoromethanes in particular, which can penetrate the stratosphere before decomposing (Molina and Rowland, 1974).

5. Atmospheric release of stable Br-containing compounds such as the CH_3Br now used as a soil fumigant which can penetrate the stratosphere before decomposing (Wofsy *et al.*, 1975).

6. Stimulation of N_2O production by denitrifying bacteria through increased acidity of precipitation from atmospheric release of oxides of sulfur and nitrogen (McElroy, 1976).

7. Operation of the space shuttle or similar programs with frequent ballistic return of orbiting vehicles to the atmosphere where they produce NO_x by compressional heating (Park, 1976).

8. Atmospheric release of stable IR-absorbing gases such as CO_2, chlorofluoromethanes etc., which radiatively cool the stratosphere and thus shift the chemical equilibrium concentration of O_3. In contrast to the other mechanisms cited, this one is expected to lead to a thickening rather than a thinning of our UV-screen.

THE NO_x CYCLE

When efforts were first made to estimate the effect on O_3 of the NO_x exhausted by SST's in the stratosphere, there were many unknown and poorly known factors; the natural level of NO_x, its sources and sinks, with what other species did it react, how rapidly was it transported vertically and how should diurnal variations be accounted for. By field observations, laboratory measurements, model experiments and plain trial and error, carried out

principally under the auspices of CIAP (1974), COMESA (1975) and COVOS (1976), our knowledge of these factors slowly grew.

N_2O has been accepted as the major natural source of stratospheric NO_x. This stable gas is produced by soil and ocean bacteria and, being nearly uniformly mixed through the troposphere, ascends with the upwelling tropical Hadley flux into the stratosphere where over 90% of the net upward flux is photolyzed and the remainder reacts with $O(^1D)$. Somewhat more than half of these latter reactions lead to the production of NO.

NO_x was also found to interact with the HO_x and ClX cycles isolating significant fractions of all three catalysts as inactive compounds such as HNO_3, $ClONO_2$, etc., thus short circuiting the catalytic loops and even establishing new cycles which generated odd oxygen. In what Dotto and Schiff (1978, p.320) describe as a "peculiar anomaly ... like tossing fifty heads in a row" each new piece of the puzzle resulted in a progressive decline in the calculated O_3 depletion. Fig. 1 shows in chronological sequence the decrease in O_3 depletion for an NO_x injection estimated originally as equivalent to ca.500 SST's operating at 17 and 20 km calculated for significant evolutionary stages of the Lawrence Livermore Laboratory (LLL) one-dimensional model.

COMPUTED O_3 DEPLETION BY SST's

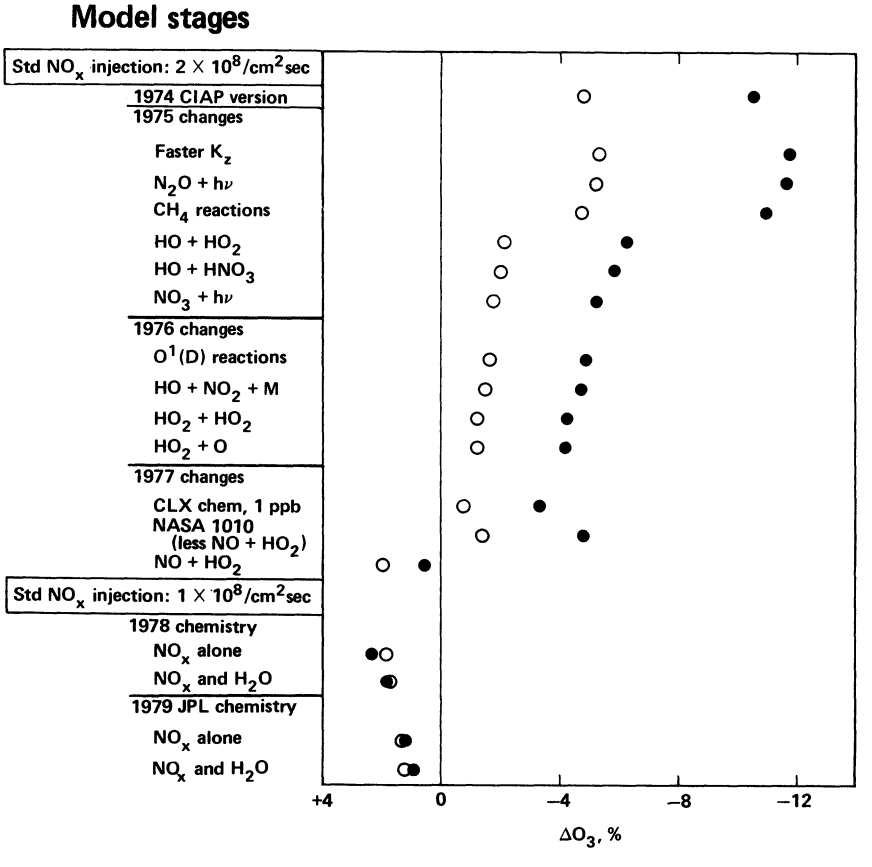

Figure 1: Percentage reductions in total O_3 computed for a standard NO_x injection (2 x 10^8 cm^{-2} s^{-1} reduced to 1 x 10^8 cm^{-2} s^{-1} after 1977 at 17 km (open circles); at 20 km (filled circles)) with different evolutionary stages of the LLL one-dimensional model. NASA 1010 and JPL chemistry refers to reaction rates in Hudson (1977) and NASA Panel for Data Evaluation (1979), respectively.

It is interesting, that after announcement of the aerosol spray threat to O_3 by Molina and Rowland (1974) and model estimates of the amount of O_3 destruction by chlorofluoromethanes began to appear, it was found that the rate constant being used for the $OH + HO_2$ reaction was up to an order of magnitude slower than had been used during CIAP. This slower rate increased the O_3 destruction by ClX but, as can be seen in Fig. 1, sharply reduced O_3 destruction by NO_x. The eventual reversal of the SST effect from O_3 destruction to augmentation occurred when Howard and Evenson (1977) measured the $NO + HO_2$ reaction to be about 40 times faster than previously accepted. This faster rate enhanced NO_x interference with the HO_x catalytic cycle, the dominant destroyer of O_3 in the lower stratosphere. Note that the almost monotonic progression in the calculated response of O_3 to NO_x appears to have reversed within the past year. (While Fig. 1 is now widely referred to as the Broderick Diagram, it was originally prepared by my colleague, Donald J. Wuebbles, who also helped with this updating).

O_3 augmentation due to NO_x injection is restricted to levels below about 25 km: with sufficient NO_x the effect saturates and the NO_x catalytic destruction cycle becomes dominant. Another development has been the disappearance of the scaling relationship between NO_x injection and O_3 destruction. This is because NO_x destruction of O_3 is now restricted to levels above about 25 km while, below this level, O_3 increases due to NO_x interference with the HO_x catalytic cycle. It is the net sum of these two different effects which determines the change in the O_3-column or total O_3.

Man can also put NO_x in the stratosphere by the detonation of nuclear bombs. Fig. 2 shows calculations with 1976, 1977 and 1979 versions of the LLL model for the atmospheric nuclear tests of 1956 to 1962. Again, the abrupt decline in NO_x destruction of O_3 is apparent in the 1977 chemistry. The 1978 calculations (not shown) showed even larger increases in O_3. The heights at which the nuclear clouds stabilized remains an additional uncertainty: the higher the cloud the greater the O_3 destruction, at least for yields up to about 25 MT. Note that the positive excursions in total O_3 are delayed several months after injection until the NO_x diffuses back to the lower stratosphere.

The fact that the chemical destruction of O_3 by NO_x is dependent on both the altitude and magnitude of the injection is shown graphically in Fig. 3. This shows time histories of total O_3 after NO_x injections corresponding to detonation of total nuclear yields of 100, 1000, 5000 and 10,000 megatons but made up of individual bombs of 0.25, 1 or 4 MT each. These sizes correspond to injection altitudes (nuclear cloud heights) of 9.5-16.5, 12.5-21.5 and 71.5-28.5 km, respectively. Note that the smallest bombs always cause O_3 increases (although 10,000 MT is enough to cause a small initial reduction) and that the largest bombs always cause O_3 decreases - over 55% in the 10,000 MT case.

Supplying additional fixed nitrogen to the biosphere in fertilizer or by the growing of legumes, etc. was also suggested by Crutzen (1974) as a threat to the O_3 layer. Until recently it was believed that soil bacteria, in the absence of oxygen, turned to nitrate as a source of oxygen and, in the process of denitrification, released a few percent of the nitrogen as N_2O. It was generally assumed that the natural N_2O production would be augmented in proportion to the amount of added fixed nitrogen. Since the fraction of the reduced nitrogen released as N_2O increases as pH is lowered, McElroy (1976) suggested that increased acidity of rain resulting from release of pollutant forms of sulfur and nitrogen would also cause increased production of N_2O and destruction of stratospheric O_3. These were rather simplistic views. According to Johnston's (1977) summary of our understanding of N_2O production, lowered pH increases the fraction of reduced nitrogen released as N_2O, but at the same time slows the overall rate of denitrification. In fact, denitrification is inhibited completely below a pH of about 5.5. Since this is the normal pH for raindrops in equilibrium with the atmospheric concnetration of CO_2, any additional lowering of the pH of rain would more likely prevent denitrification from occurring at all rather than increase the total output of N_2O. Also, soil scientists have been complaining for years about agricultural practices causing loss of top soil, humus and fixed nitrogen from the soil. Moreover, to prevent anaerobic conditions, we drain and aerate the soil, particularly that soil to which we add fertilizer. I have argued earlier (Ellsaesser, 1977) that these practices appeared more likely to decrease, rather than increase, N_2O output, in view of our understanding at that time of the factors favouring N_2O production (Johnston, 1977). However, it now appears that most N_2O is produced under oxidizing conditions as a product of nitrification and that denitrification is a minor source at most (Elkins *et al.*, 1978).

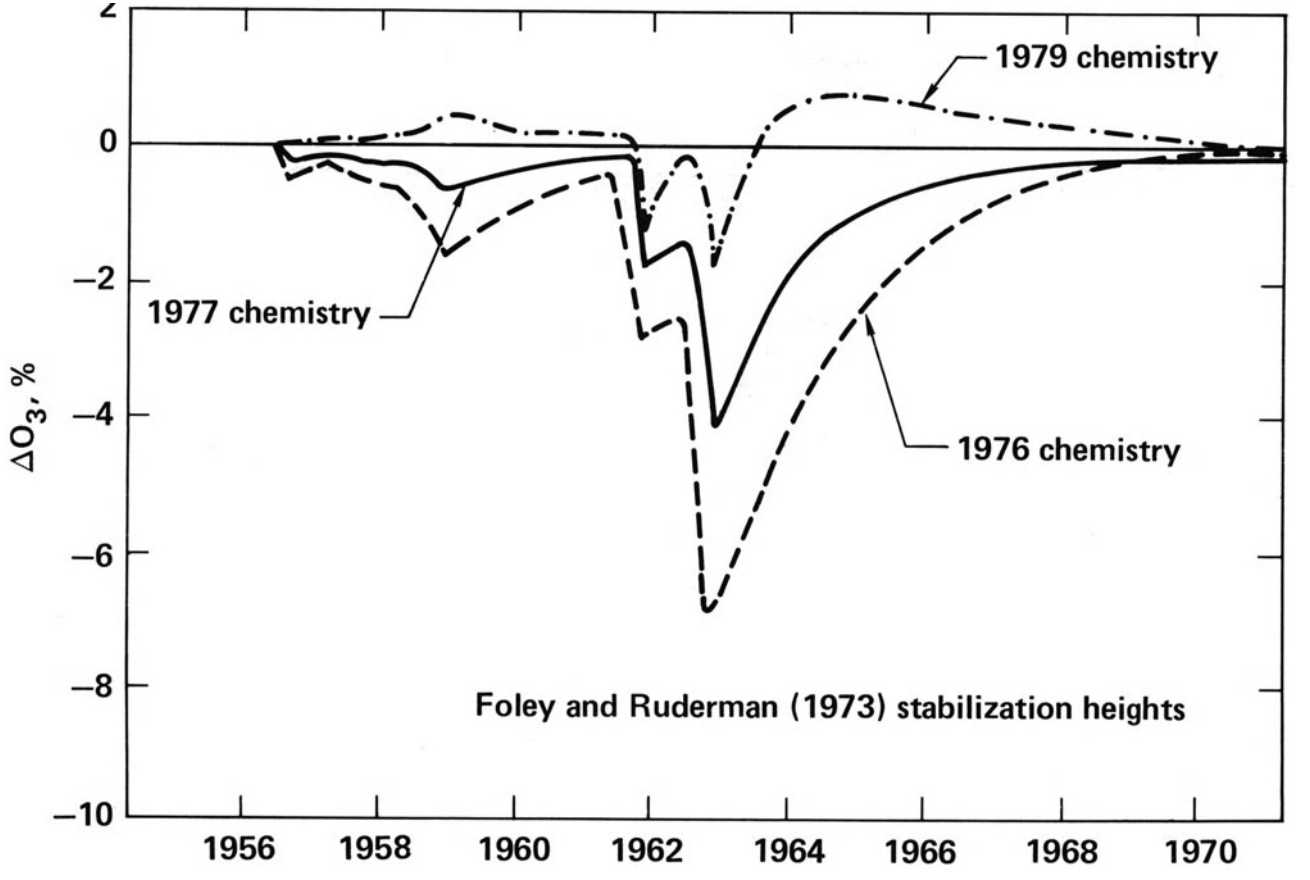

Figure 2: Time dependent changes in total O_3 due to NO_x from the 1956 to 1962 atmospheric nuclear tests computed by LLL one-dimensional model. 1979 results provided by William H. Duewer.

CHANGE IN OZONE COLUMN AT VARIOUS TIMES FOR VARIOUS TOTAL NUCLEAR YIELDS

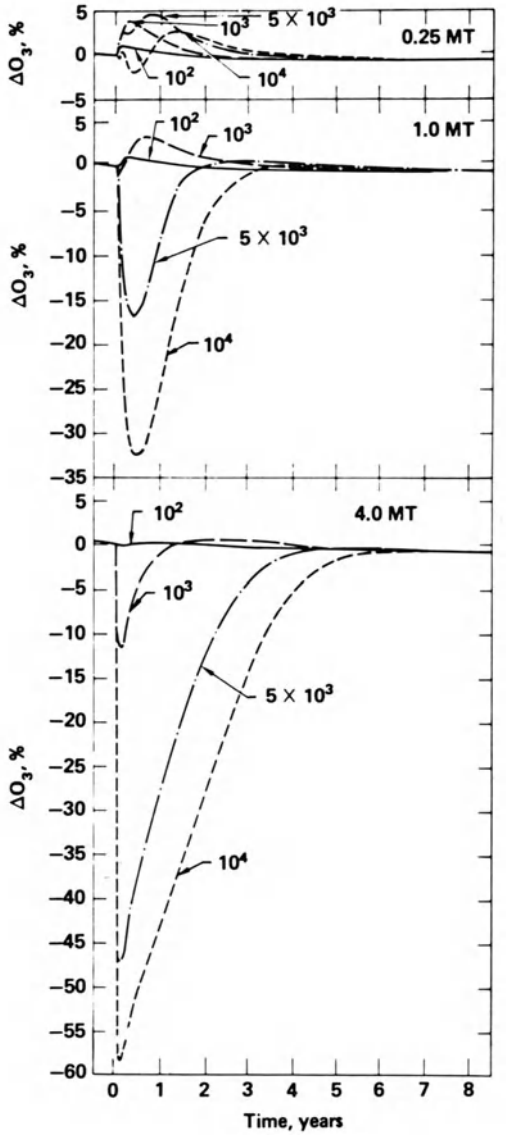

Figure 3: Time dependent calculations of change in total O_3 following nuclear detonations of 100, 1,000, 5,000 and 10,000 MT made up of individual bombs of 0.25, 1.0 or 4.0 MT each. From Luther (1978).

Presuming that atmospheric N_2O is increased by some mechanism, the resulting effect on the O_3-layer can be calculated. Fig. 4 shows changes in the O_3 layer computed by recent versions of the LLL one-dimensional model for large increases in the tropospheric concentration of N_2O. For small increases the layer was computed to thicken in the 1978 model, particularly for high concentrations of Cl, but the effect became saturated at about 2 times the present N_2O level. However, recent unpublished results of W. H. Duewer and D.J. Wuebbles again show a monotonic decline in O_3 with increasing N_2O.

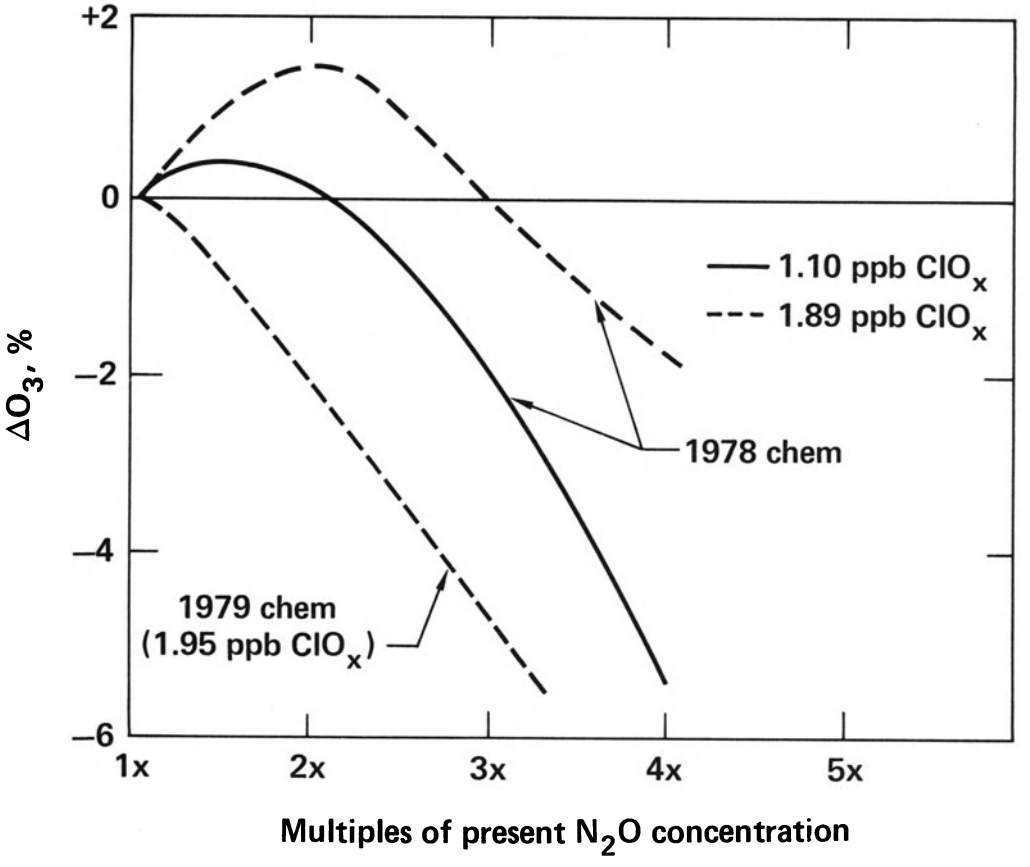

Figure 4: Computed steady state changes in total O_3 for multiples of present tropospheric concentration of N_2O. Solid line is for natural levels of stratospheric Cl, dashed lines are for computed Cl levels due to CFM's. 1979 results by William H. Duewer and Donald J. Wuebbles.

Operation of a continuing space shuttle program will involve releases of Cl and H_2O on the outbound flights. On return to the atmosphere, heating will produce an amount of NO_x equivalent to about 20% of the mass of the reentering vehicle, mostly between 50 and 70 km (Park, 1976 and private communication). Since the reentries are expected to occur at low latitude, most of the NO_x is expected to be photolyzed before it can reach the main O_3 layer. Accordingly, no currently foreseeable program is expected to have an appreciable

effect on O_3 due to NO_x so produced.

Similarly, future hypersonic aircraft may have a significant effect on O_3 but no calculations are likely until better information becomes available on engine design, operating parameters and fleet size.

RELEASE OF IR-ABSORBING GASES

According to current wisdom, Man can also influence the depth of his UV-screen by increasing the atmospheric concentration of stable IR-absorbing gases such as CO_2. By increasing the optical depth of the atmosphere in the so-called window region between 4 and 12 μm, such gases both warm the lower troposphere and cool the upper stratosphere, the latter temperature change being several times larger than the former as well as of opposite sign. Since the chemical equilibrium level of O_3 is negatively correlated with temperature (Blake and Lindzen, 1973), the stratospheric cooling will lead to an increase in O_3 concentration. Initial calculations by Boughner and Ramanathan (1975) of the effect of doubling the CO_2 concentration (or 600 ppm) gave an increase of about 2%. Later calculations have given increases in O_3 concentration near 40 km of 16 to 27% and increases in total O_3 of 2.9% (Luther *et al.*, 1977), 1.2-2.5% (Boughner, 1978), 4.7% (Groves *et al.*, 1978) and 7% (Haigh and Pyle, 1974). The last result was with a two-dimension dynamical model which gave a latitudinal variation of < 2 to > 14%, being greatest in high latitudes in winter. A recent unpublished recalculation by M. Luther, gave a decrease of 5%.

THE ClX CYCLE

Interest was initially focused on the effect of Cl on stratospheric O_3 because HCl is produced in the exhaust of the space shuttle. CIAP funded Hoshizaki *et al.* (1973) to investigate the possible effect of the space shuttle on stratospheric O_3. Meanwhile, NASA had funded Stolarski and Cicerone (1974) to look into this problem. According to Dotto and Schiff (1978), original NASA estimates in January 1974 were that 60 space shuttles a year might cause a 1 or 2% O_3 depletion, and as much as 5% in the launch corridor. Initial model calculations by several groups gave O_3 depletions of 0.15 to 0.2%. However, Howard and Evenson's (1977) 40-fold faster rate for the NO + HO_2 reaction (which had reversed the SST effect) also had the effect of doubling O_3 destruction by ClX. The most recent calculations for the effect of 60 shuttles a year is about 0.3% and the corridor effect has disappeared (R. C. Whitten, private communication).

As mentioned previously, at the time initial estimates of the O_3 destruction by chlorofluromethanes (CFM's) began to appear, modelers began to prefer the 10-fold lower estimates for the OH + HO_2 reaction rate. This increased the effect of CFM's and decreased that of NO_x. This and other developments led to a growing appreciation of the uncertainties in stratospheric model results, an appreciation which was all too lacking during CIAP.

The U.S. National Academy of Sciences study (NRCCISC, 1976; NRCPAC, 1976) put considerable effort into evaluating these uncertainties. They concluded that with continued release of CFM's at the 1973 rate the system would take hundreds of years to reach equilibrium, at which time the depth of the O_3 layer would have been reduced by 6 to 7.5%. The actual reduction was estimated, with 95% confidence, to lie between 2 and 20%. In addition to the quantifiable 8- to 10-fold uncertainty in this figure (kinetic rates 6-fold, transport 3-fold, alternate sinks 1.2-fold) several unquantifiable uncertainties were also listed. Issuance of the reports themselves were delayed several months by the uncertainties concerning the effects of $ClONO_2$.

The January 1977 NASA Workshop at Airlie House in Virginia (Hudson, 1977) estimated the uncertainties to be even greater. Nine groups with one-dimensional models and presumably using standardized input parameters, computed the steady state O_3 reduction from continued CFM release at the 1975 rate (essentially the same as the 1973 rate). Their results ranged from 5 to 9% reduction in O_3. At a smaller follow-up NASA Workshop at Goddard Space Flight Center in July 1977, the Howard and Evenson (1977) 40-fold faster rate for NO + HO_2 was the major of several new bits of information considered. Recomputation of the steady state effect of CFM's at the 1975 rate by eight modeling groups gave O_3 reductions of 10.8 to 16.5%.

Since July 1977, the calculated sensitivity of O_3 to ClX has continued to vary inversely

with that due to NO_x, as shown in Fig. 1. The calculated O_3 destruction by CFM's continued to rise until 1978 and has since declined. Interestingly, both the computed O_3 destruction and the estimates of the range of uncertainties are now essentially unchanged from the initial estimates of Molina and Rowland (1974).

My colleagues recently used the current LLL one-dimensional model to recalculate the CFM problem using the 1976 CFM release rates and the thermo-chemical reaction rates appropriate for 1976, 1977 and 1979. The results are listed in Table 1. Notice that the changes in chemistry result in different depths for the unperturbed O_3 column as well as different percentage reductions at steady state - which is only reached after about 300 yr. The Howard and Evenson (1977) 40-fold faster rate for NO + HO_2 increased the unperturbed O_3 column to 341 m atm-cm, which is at least 10% high for a global average. This computed O_3 column was 374 m atm-cm if diurnal variations in solar flux were not included. Subsequent changes in chemistry have alleviated this problem somewhat. Current chemistry differs from JPL 79-27 (NASA Panel for Data Evaluation, 1979) mainly in forming $ClONO_2$ about 4 times faster. It computes that CFM's have already (1978) reduced the O_3 column by about 1.5% and will reduce it about 5% by the year 2000. Steady state changes in O_3 concentration for continued CFM release at the 1976 rates are shown in Fig. 5. At 40 km, the level of maximum change, the reductions with the current LLL chemistry are 4.5, 8.2, and 11.2% in 1980, 1990 and 2000, respectively.

TABLE 1

RECENT LLL ONE-DIMENSIONAL MODEL CALCULATIONS
OF THE EFFECT OF CONTINUED RELEASE OF CFM'S
AT THE 1976 RATE. DATA SUPPLIED BY J.E. PENNER

Model Chemistry	Unperturbed Total O_3 ca, 1950 (m atm-cm)	ΔO_3 (%)	
		In 1978	At Steady State
1976 NBS 866	278	-0.4	-4.2
1976 Old NO Photolysis	285	-0.6	-5.8
1976 Non-Diurnal	316	-0.0	-1.3
1977 NASA 1010	341	-1.2	-11.2
1977 Non-Diurnal	374	-0.1	-4.7
1979 JPL 79-27	328	-1.5	-14.0
1979 JPL less OH + ClO			-19.4
1979 Current LLL	327	-1.3	-14.2

Since Angell and Korshover's (1978) analysis of the Umkehr data suggests a 6% increase in northern hemisphere O_3 between 1957 and 1976 (8% during 1962 to 1973) in the 32-46 km layer, the computed decline in O_3 at 40 km due to CFM's (ca. 4% in 1978) should give us cause for caution in interpreting current model results. If nothing else, such unexplained variations in the O_3 record will delay the point at which we can hope to observationally confirm the effect of CFM's on O_3.

THE BrX CYCLE

Aside from McElroy's "bromine bombs" (see Dotto and Schiff, 1978, p.189) Br has received little attention, principally because no one has suggested a credible Br source. Concentrations of Br detected in the stratosphere are about 20 pptv or 100-fold less than those

STEADY STATE O_3 CHANGES FOR CONTINUAL CFM RELEASE AT 1976 RATE

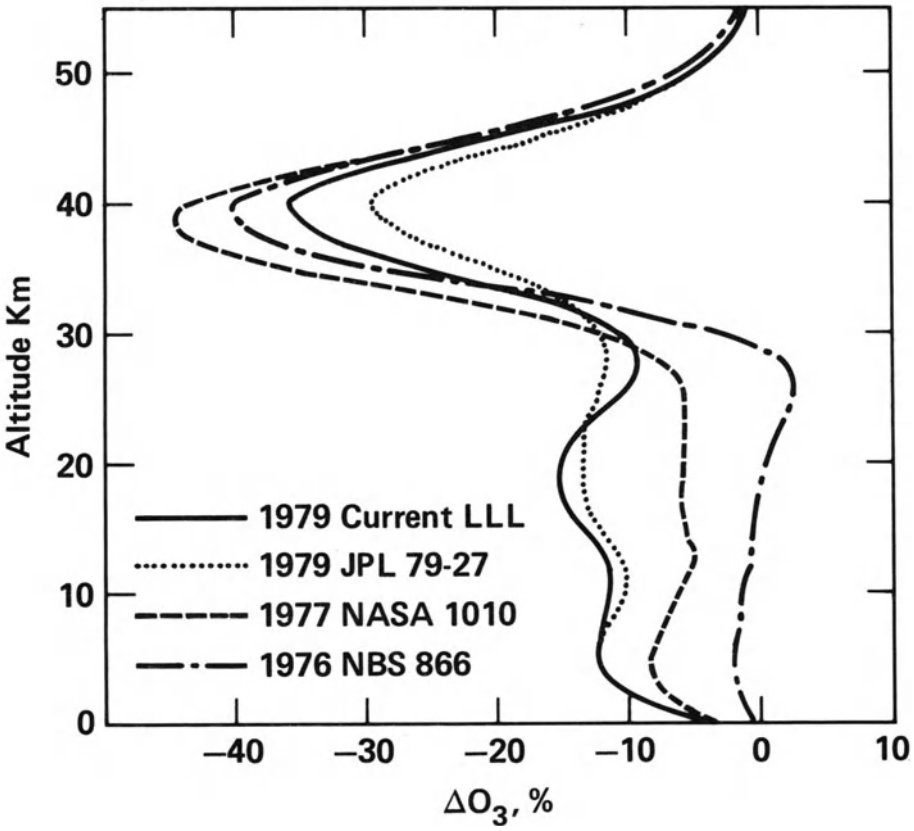

Figure 5: Computed percentage change in O_3 concentration as a function of altitude for continued CFM release at the 1976 rate. NBS 866, NASA 1010 and JPL 79-27 refer to reaction rates in Hampson and Garvin (1975), Hudson (1977), and NASA Panel for Data Evaluation (1979), respectively. 1976 production rates for CF_2Cl_2 and $CFCl_3$ differ from their 1973 rates by -3.2% and +2.9%, respectively. Computations performed by W. H. Duewer, J.E. Penner and D.J. Wuebbles.

of Cl. Thus, even though Br has been estimated to be about 10 times more effective than Cl in destroying O_3, its effect remains small. However, CH_3Br is used as a soil fumigant and other compounds of Br are used as fire retardants and in gasoline. If these uses grow, or additional uses of Br are introduced, Br may become cause for concern.

LESSONS OF "THE OZONE WAR"

Since MacDonald's dramatization of the importance of perturbations to the O_3 layer by tying them to skin cancer we have made rapid strides in increasing our knowledge of the stratosphere and of atmospheric chemistry in particular. However, there has been another result which may prove of greater consequence. The debate about O_3, or "The Ozone War" as it was called by Dotto and Schiff (1978), has again revealed for the world that scientists are mere humans - with sometimes only a thin veneer of objectivity. I would like to believe that it has served a larger role by also bringing this point home to all scientists, thereby causing each of us to quietly resolve to ourselves to strive that much harder to

retain our objectivity in the future.

Eventually it became clear that even in the role of the U.S. Government in funding and directing the CIAP study there were built-in, albeit subconscious, decision-determining biases (Ellsaesser, 1978). As an example, several projects were funded to study and quantify the relation between total O_3 and skin cancer, a clearly deleterious consequence; but I am aware of no comparable effort to study and quantify the relation between total O_3 and rickets where the presumed effect of the SST might well have been beneficial. Skin cancer afflicts primarily light-skinned humans while cutaneous generation of vitamin D via UV exposure appears to be a problem which must be surmounted by almost all terrestrial vertebrates.

To claim that the rickets problem can be solved by food additives is less justifiable in my opinion than to claim that the skin cancer problem can be solved by education and letting each individual control his exposure to sunlight through life style, sunshades, clothing and skin creams. I think this view will become acceptable to most when they consider the populations at risk and the principle of freedom of choice. But this is certainly not the impression conveyed by most of the news and literature generated by this problem to date. If the taxpayer is going to be asked to support the work of scientists, I believe he has the right to expect and to demand greater objectivity than he has so far received during "The Ozone War".

ACKNOWLEDGEMENTS

Advice, assistance and the sharing of unpublished results by my colleagues William H. Duewer, Frederick M. Luther, Joyce E. Penner and Donald J. Wuebbles are gratefully acknowledged. The author also profited from attendance and participation in the NASA Stratospheric Workshop at Harpers Ferry, WV, June 4-8, 1979. This work was performed in part under the auspices of the U.S. Department of Energy by the Lawrence Livermore Laboratory under contract No. W-7405-Eng-48, with support by the High Altitude Pollution Program of the Department of Transportation, Federal Aviation Administration and its delivery at ISEB4 was made possible by partial support by the Australian Academy of Science.

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THE TRACE COMPOSITION OF THE ATMOSPHERE AND THE GLOBAL ATMOSPHERIC OZONE CONTENT

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INTRODUCTION

Since Sydney Chapman first introduced the theory of photochemical production of O_3 in the stratosphere in 1930 there have been continuing efforts to reconcile theoretical models with the observational facts about atmospheric O_3. Recently this effort has intensified due to concern that humanity may inadvertently modify the ozone layer through various activities. In this paper we discuss the uncertain basis of current models of atmospheric ozone and then present some southern hemisphere measurements of trace gases that are involved in stratospheric chemistry.

O_3 is formed in the upper atmosphere, mainly above 30 km by the photodissociation of molecular oxygen by short wave ultraviolet solar radiation ($\lambda < 242$ nm). O_3 is also formed in the lower atmosphere, at heights up to about 20 km, during the oxidation of methane, carbon monoxide and hydrogen in the presence of nitrogen oxides.

Ozone is destroyed within the atmosphere by reactions involving atomic species and free radicals of oxygen, hydrogen, nitrogen and chlorine. Also there is a small contribution from heterogeneous decomposition of O_3 at the earth's surface. The atomic species and free radicals that destroy O_3 in the upper atmosphere are formed in situ in the stratosphere from water vapour, nitrous oxide and chlorocarbons. These molecules are carried from the earth's surface to the stratosphere by atmospheric motions.

The actual distribution of ozone in the atmosphere is determined by the combined effects of the production and destruction processes (including temperature and radiation induced variations) along with atmospheric transport processes. These processes vary diurnally, seasonally, with height and with latitude as well as on many other time and space scales within the atmosphere. Hence the only way the theory and observations of atmospheric O_3 can be compared is by use of complex numerical models.

ATMOSPHERIC CHLOROCARBONS

The major organochlorine compounds in the atmosphere are CCl_3F, CCl_2F_2, CCl_4, CH_3CCl_3 and CH_3Cl. Of these the former four are believed to be exclusively man-made while the latter is largely of natural origin. Data on the global release of CCl_3F, CCl_2F_2, CH_3CCl_3 and CCl_4 are shown in Fig. 1.

The chlorofluoromethanes (CFM) CCl_3F and CCl_2F_2 are used extensively as aerosol propellants, refrigerants and cellular plastic blowing agents. Through the period 1960 to 1974 their release increased consistently by 15 and 12% yr^{-1}, respectively. Since 1974 annual emissions have levelled off.

CH_3CCl_3 was introduced into general use in the 1950's in response to the need for a general solvent which was less toxic than those then in use. CH_3CCl_3 is used largely for metal degreasing. Its rate of release to the atmosphere is increasing rapidly (17% yr^{-1} since 1960).

The data for CCl_4 shown in Fig. 1 are those of Galbally (1976). Apart from its use as a chemical feedstock, CCl_4 was used extensively in the 1940's and 1950's as a solvent, degreasing agent and fire retardant, all of which resulted in extensive emissions to the atmosphere. Peak atmospheric emissions of CCl_4 occurred in the 1940's and the current growth rate of emissions (1960-1973) is approximately 4% yr^{-1}. The earliest routine measurements of atmospheric $CFCl_3$ were made at Bowerchalke (51°N, 1°W), England (Lovelock, 1974), commencing in early 1971 and continuing at a remote location (Adrigole, Ireland, 52°N, 10°W) from mid-1972 until 1977 (Pack *et al.*, 1977). During this period the $CFCl_3$ concentration increased at a rate of 22 pptv yr^{-1}. The concentration as of January 1978 was 192 (±12, 95%

conficence limits) pptv (parts per 10^{12} by volume).

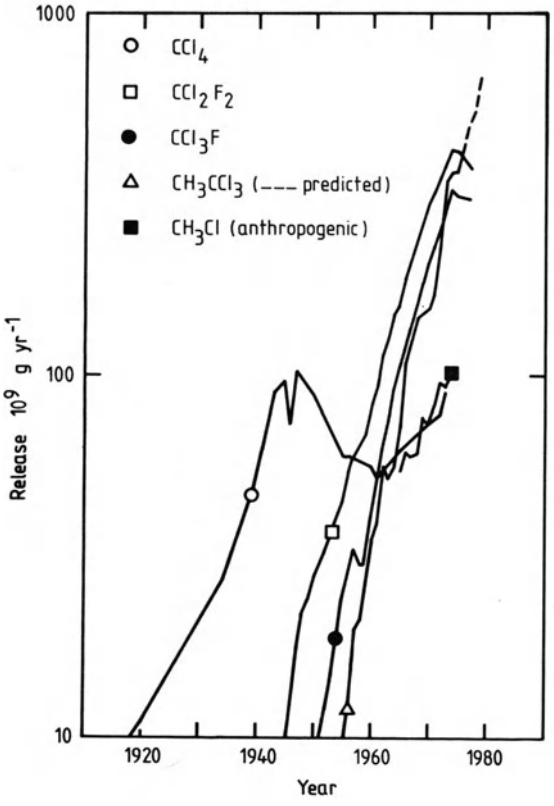

Figure 1: Global release data for CCl_3F and CCl_2F_2 (MCA, 1978), CH_3CCl_3 (Neely and Plonka, 1978; McConnell and Schiff, 1978), CCl_4 (Galbally, 1976) and anthropogenic CH_3Cl (see text).

Measurements of CCl_3F have been made at the NOAA Geophysical Monitoring for Climatic Change (GMCC) Programme baseline stations at Point Barrow, Alaska (71°N, 131°W), Mauna Loa, Hawaii (20°N, 156°W), and American Samoa (14°S, 171°W) since September 1973; at Niwat Ridge, Colorado (40°N, 106°W) since January 1976, and at the South Pole since January 1977 (W.D. Komhyr, E.G. Dutton, J.T. Peterson and T.M. Thompson, unpublished results).

A CSIRO programme to measure CCl_3F in the mid-latitudes of the Southern Hemisphere commenced in early 1976 using air samples collected in aircraft from the middle and upper troposphere, and in situ ground-based measurements at Cape Grim (41 S, 145 E) Tasmania, the site of the Australian Baseline Air Pollution Monitoring station (Fraser and Pearman, 1978). The results are shown in Fig. 2.

Results from the GMCC and CSIRO station for January 1978 and January 1979 are given in Fig. 3. The data for Mawson is from flask samples collected twice per month at the Australian Antarctic base (Fraser, unpublished results). Hemispherically averaged surface concentrations of 172.6 pptv and 158.4 pptv were obtained for the northern and southern hemispheres, respectively, from the January 1979 data in Fig. 3.

Data on the distribution of CCl_2F_2 are not so numerous. Measurements commenced at all GMCC stations in early 1977. Mean northern and southern hemisphere concentrations for January 1979 are 301.5 and 281.8 pptv. The average global increase from mid-1977 to the end of 1978 is 19 (±5) pptv.

Results from Bowerchalke and Adrigole (Lovelock and Pack, 1976; Pack *et al.*, 1977) and from

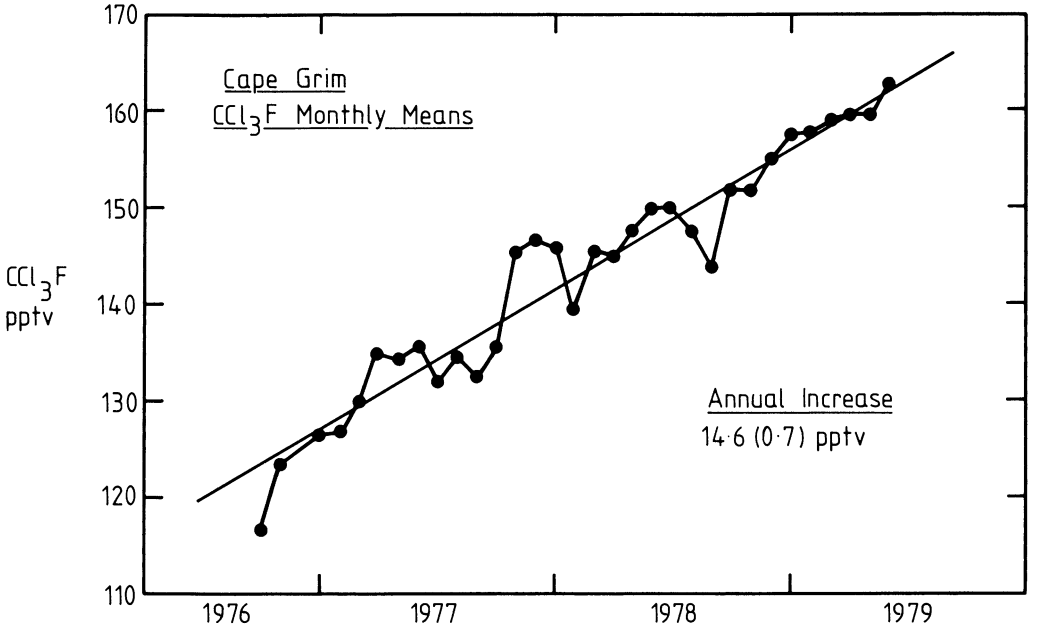

Figure 2: Monthly mean concentration of CCl_3F observed at Cape Grim, Tasmania.

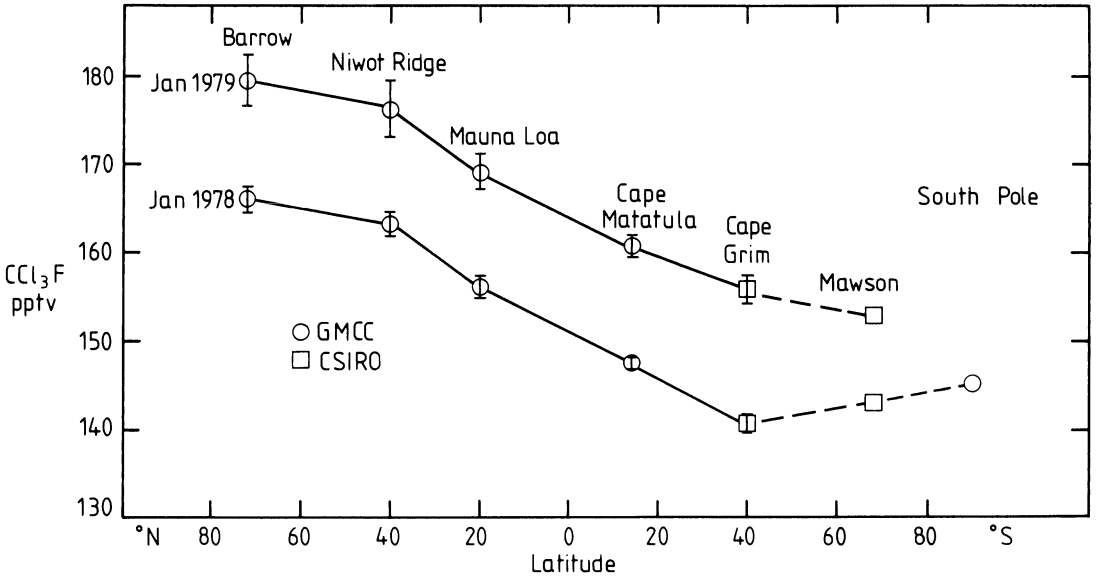

Figure 3: Global distribution of CCl_3F for January 1978 and January 1979 from the GMCC and CSIRO monitoring stations.

Harwell (Penkett *et al.*, 1979) represent a seven year record of CCl_4 measurements from the same region. The concentration at January 1978 was 159 (±14) pptv and was increasing by 8 (±2) pptv yr^{-1} or approximately 5% yr^{-1}.

CCl_4 has been monitored continuously at Cape Grim since late 1976. The concentration at January 1979 was 142 (±3) pptv and was increasing by approximately 1% yr^{-1}. These estimated

increases in atmospheric CCl_4 are consistent with Galbally's (1976) estimate of about 4% yr^{-1} during 1973.

For CH_3CCl_3 only limited data are available (Lovelock, 1977; R.A. Rasmussen, personal communication; Singh *et al.*, 1979). Northern and southern hemisphere concentrations for January 1978 and 140 (±8) and 85 (±18) pptv, respectively. Annual increases appear to be about 11-13%.

CH_3Cl in the atmosphere is largely of natural origin. It is believed to have a number of sources, the major one being the ocean, probably from the reaction of methyl iodide, produced by marine plant life, and chloride ion (Lovelock, 1975; Zafiriou, 1975). Other sources of CH_3Cl have been suggested such as microbial fermentation and smouldering combustion of vegetation (Lovelock, 1975). Rasmussen and Rasmussen (1977) measured CH_3Cl concentrations, over slash burning areas in Kenya, at three times background concentrations. Palmer (1976) calculated that forest fires in the USA generated approximately 0.13 Tg CH_3Cl in 1974. Lovelock (1975) calculated that a global source strength of 29 Tg yr^{-1} was required to maintain current atmospheric concentrations of CH_3Cl, assuming an atmospheric residence time of 0.37 yr based on reaction with hydroxyl radicals. Yung *et al.* (1975) and NAS (1976) deduce smaller global source strengths of 5.2 and 6.7 Tg yr^{-1}, based on larger residence times (0.89 and 0.94 yr, respectively). This would suggest that global forest and agricultural fires make a significant contribution to the annual global emission of CH_3Cl.

Measurements of CH_3Cl in the troposphere and lower stratosphere of the northern hemisphere and in the troposphere of the southern hemisphere show concentrations ranging from 500 to 800 pptv with no systematic variations (Cox *et al.*, 1976; Robinson *et al.*, 1977; Cronn *et al.*, 1977; Pierotti *et al.*, 1980; Singh *et al.*, 1979). These measurements are consistent with a large globally uniform source of CH_3Cl.

NITROUS OXIDE

Nitrous oxide is a ubiquitous constituent of the earth's atmosphere. It is predominantly of natural origin, being produced by microbiological action on fixed nitrogen at the earth's surface, and in seawater from nitrification.

Direct measurements of N_2O in the troposphere have been made since 1949 (see Pierotti and Rasmussen, 1977). However, the use of a variety of measurement techniques and the absence (until recently) of intercomparisons between groups means that very little information on long-term trends in atmospheric N_2O can be obtained. Measurement of atmospheric N_2O at Aspendale was commenced in late 1976. Results from an extensive gas sampling network covering Australia, the Southern Ocean and Tasman Sea show that N_2O is homogenously distributed throughout the troposphere in space and time.

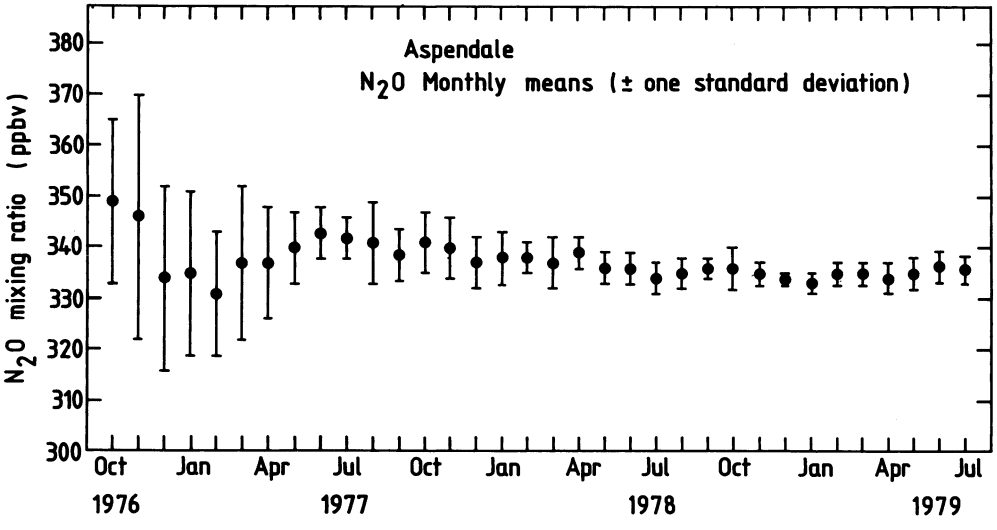

Figure 4: Monthly mean concentration of N_2O observed at Aspendale, Australia.

The data in Fig. 4 for Aspendale show the remarkable absence of variation of atmospheric N_2O mixing ratios indicative of the long atmospheric lifetime of this gas. Earlier measurements in the northern hemisphere by overseas groups showed changes in atmospheric N_2O annual average concentrations of 20% between two years (Goody, 1969; Schütz *et al.*, 1970). We now think that these changes were artifacts of the relatively insensitive measurement techniques then available.

The standard deviation of the monthly mean (see Fig. 4) is an indicator of the measurement precision and it can be seen that the precision improved considerably during the first few months of measurement, due to improved technique, and has remained relatively constant from that time.

The last year provides the most precise data of the study. A linear regression analysis of weekly means during this period yields an average mixing ratio of 335 ppb and a trend of +0.1 ppb yr^{-1} with 95% confidence limits of -0.2 and +0.4 ppb yr^{-1}. This trend is not significantly different from zero.

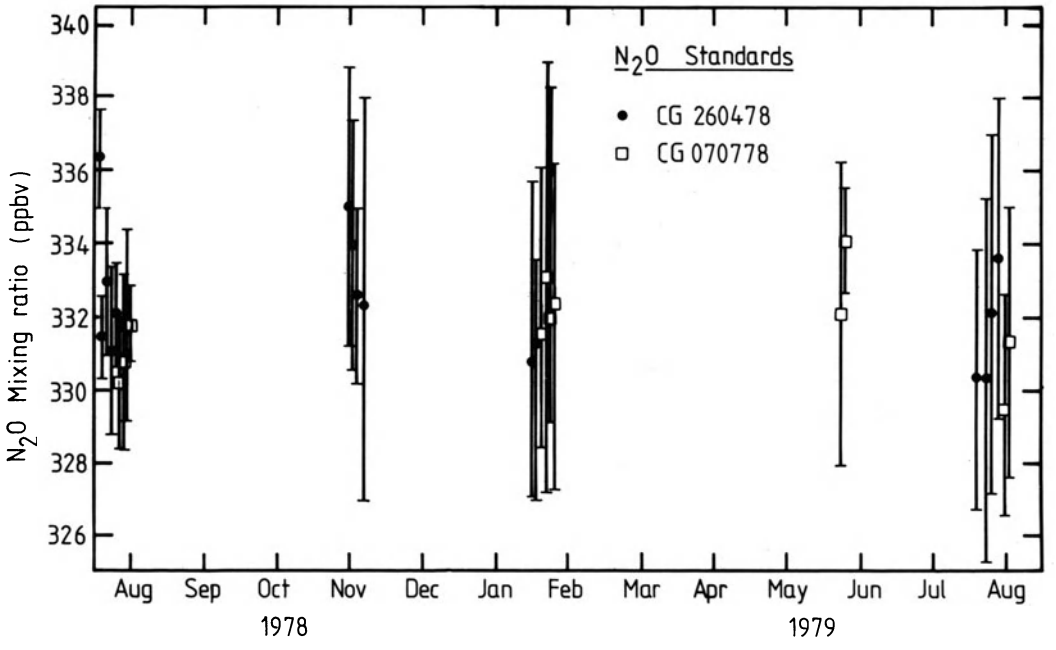

Figure 5: Calibration of two N_2O standards using commonly derived secondary standards.

The analysis does not consider the change in the gas standards used during this period. Intercomparison data for two standards cryogenically trapped at Cape Grim are shown in Fig. 5. The reference tanks used for this intercomparison were those provided by Rasmussen for the MCA programme at Cape Grim. The results would suggest that overall the two standards have remained relatively stable but the noise in the data makes it difficult to detect small (<1% yr^{-1}) trends in the N_2O mixing ratio.

Flask samples taken at Cape Grim during baseline conditions and analysed at Aspendale show a mixing ratio which is significantly lower (on average 2 ppb) than the in situ measurements at Aspendale. The difference has been attributed (Roy, 1979) to anthropogenic N_2O production.

The only widely accepted and quantified sink for N_2O is stratospheric oxidation and photolysis and this has been estimated at 13-15 Tg N_2O-N yr^{-1} (Crutzen, 1976; Schmeltekopf *et al.*, 1977).

If this is the only sink for N_2O then the atmospheric turnover time would be of the order of 100 yr.

Many estimates have been made of the global source strength of N_2O but much uncertainty persists. Cohen and Gordon (1979) calculate a maximum oceanic source of <10 Tg N_2O-N yr^{-1} and Hahn and Junge (1977) estimated a most likely value of 16 Tg N_2O-N yr^{-1} for the land source.

The production of atmospheric N_2O from the combustion of coal and fuel oil was estimated to be in the range 1.5 to 2.5 Tg for 1976 (Pierotti and Rasmussen, 1976; Weiss and Craig, 1976). Catalytic converters, in use for the reduction of NO in automobile and industrial exhausts, provide a potential global source of approximately 2 Tg N_2O-N yr^{-1}.

The increased use of artificial N fertilizers (together with changing agricultural patterns that involve increased planting of nitrogen-fixing species) could also result in an increased flux of N_2O to the atmosphere. In 1974, approximately 40 Tg N fertilizer was used and this was increasing at the rate of approximately 11% yr^{-1} (Pratt *et al.*, 1977). The fraction of this which is actually emitted to the atmosphere as N_2O, is the subject of considerable controversy (see Galbally *et al.*, 1980). It appears that through combustion and agriculture, man's activities are already influencing the atmospheric N_2O cycle. However the extent of this influence is more or less unknown.

ATMOSPHERIC MODELS

These models generally simulate either the height distribution alone or the height and latitudinal distribution of O_3 using inputs representing the incoming solar radiation, the density, temperature, height distribution of the relevant permanent constituents of the atmosphere and the chemical reaction rates of the variable species. A set of equations representing the chemical reactions and transport processes are numerically solved to yield concentration distributions of various species including O_3. Time varying inputs of pollutants including halocarbons and N_2O are included in some models for prediction of the effects of anthropogenic release of these pollutants on the O_3 layer. Examples of such models are presented in Crutzen (1970, 1971), Johnston (1971), Whitten *et al.*, (1978), Groves *et al.*, (1978), Luther and Duewer (1978), Logan *et al.*, (1978), Derwent and Eggleton (1978) and Sze *et al.*, (1978).

Areas where uncertainties, associated with these models, can arise are described:

(a) errors in the rate constants of reactions currently included in models. Smith (1978) suggests that the true uncertainties in model results from these reactions is probably much greater than a factor of five (95% confidence limits).
(b) problems of diurnal averaging (Turco and Whitten, 1978), feedback between radiation and chemistry (Luther and Duewer, 1978; Groves *et al.*, 1978) and multiple scattering (Hudson, 1977) are well recognised and many models now attempt to deal with them.
(c) the parameterisation of atmospheric dynamics by an eddy diffusion coefficient is adopted in most models. The uncertainty in model predictions due to uncertainty in the value of this coefficient is a factor of 2 (NAS 1976). Tuck (1978) shows that changes in transport cause differences in average reaction rates of ±40% between a 3D model and an appropriate 1D model. However, even the 3D model uses eddy diffusion coefficients to represent sub-grid scale effects, hence the total possible error due to crudely representing transport processes is larger again.
(d) the possibility that multiple real solutions exist for models of the chemistry of the atmosphere for fixed inputs under certain conditions (Prather *et al.*, 1979) has never been considered. This phenomena needs further investigation as there may be a requirement for considerable new complexity in models to obtain correct results.
(e) the existence in the atmosphere of important but presently unknown chemistry.

One method of testing models is to compare calculated concentrations or ratios of concentrations of species with those observed in the atmosphere. This process reveals some serious problems in the chemistry of current models (Derwent and Eggleton, 1978; Johnston and Podolske, 1978; Sze *et al.*, 1978; Murcray *et al.*, 1978). The observed ClO concentration at 40 km at 30°N in summer (Anderson *et al.*, 1980) exceeds the presently modelled total chlorine abundance by a factor of four, and should, according to current chemical understanding, be destroying ozone in this region more rapidly than it is being produced. This region of the upper stratosphere at low latitudes in summer is regarded as the source region for atmospheric ozone and the ClO reaction is only one of the O_3-destroying mechanisms, hence the serious problem. Other substantial discrepancies involve the ratio HF/HCl above 20 km and the absence of $ClONO_2$ and N_2O_5 at predicted concentrations in the atmosphere. Other com-

pounds, currently not included in models, including HOCl and HO_2NO_2 may have important presently unknown roles in stratospheric chemistry.

Thus, due to uncertainties in currently used reaction rates, methods of describing transport within the models, missing chemistry and perhaps the fundamental stability of the models, as listed in Table 1, model predictions of perturbations of atmospheric ozone by various pollutants are very uncertain.

TABLE 1

RANGE OF UNCERTAINTY IN PREDICTING A PERTURBATION TO ATMOSPHERIC OZONE FROM VARIOUS ASPECTS OF MODELLING

Source	Range expressed as a factor between ± 95% confidence limits
Reaction rates of reactions included	>5
Transport	>2
Multiple solutions of model	unknown
Missing chemistry	4?

ATMOSPHERIC OZONE MEASUREMENTS

The theoretical models described earlier are capable of simulating the average vertical and meridional distribution of O_3 and its variation with season. Also certain specific events including O_3 variations during a stratospheric warming and a solar proton event (Barnett *et al.*, 1975; Crutzen *et al.*, 1975) have been quantitatively explained. However, there are many features which remain unexplained, including the global long-term trends during the 1960's and 1970's.

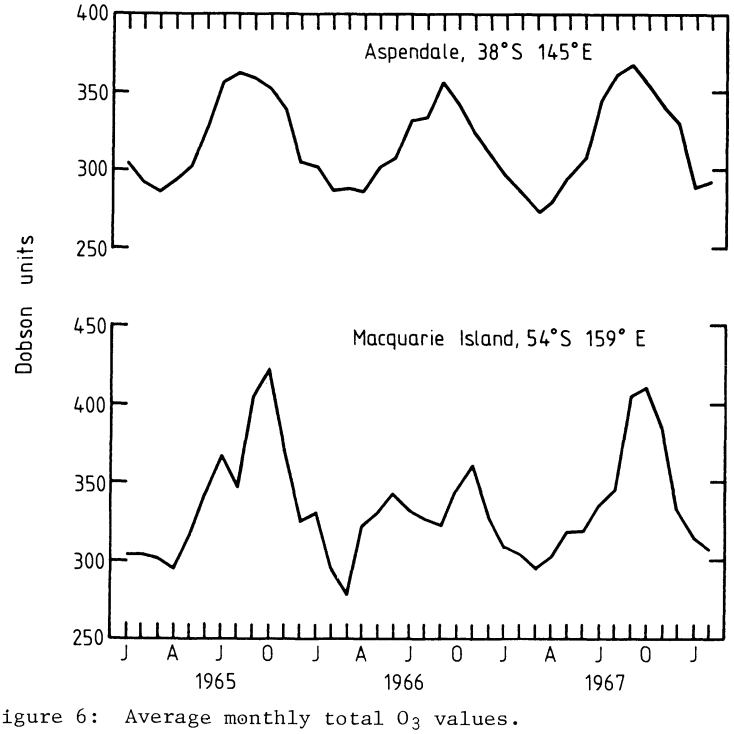

Figure 6: Average monthly total O_3 values.

There have been extensive measurements of atmospheric O_3 in the Australian sector of the southern hemisphere during the last twenty-five years (Kulkarni, 1976; Pittock, 1977; Galbally, 1971), as there have been throughout many other parts of the world, and many varied features are seen in these observations. Within the Australian sector, long-term trends during the 1960's and 1970's were certainly associated with circulation changes (Kulkarni, 1976). Another example of a specific event that remains inexplicable is the absence of a winter maximum in total O_3 at Macquarie Island in 1966, Fig. 6. If such events can be explained by a quantitative mechanism (eg. Pittock, 1973) then much of the apparent noise in O_3 records may be reduced and our ability to detect, from observations, a small anthropogenic perturbation to the atmospheric O_3 content greatly increased.

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EFFECTS ON THE CARBON CYCLE DUE TO HUMAN IMPACT ON FOREST ECOSYSTEMS

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INTRODUCTION

During the last decade the increase in atmospheric CO_2 concentrations has been approximately 1 μg g^{-1} yr^{-1} (Bischof, 1977). The concentration in 1977 was ca. 329 μg g^{-1} (Keeling and Bacastow, 1977). This increase was mainly from the accelerated combustion of fossil fuels and Man's increased disturbance of terrestrial ecosystems.

In 1977 the consumption of oil and condensed gas amounted to 3.11 Pg. This was an increase of about 5% compared with 1976 (Shell, 1978). The annual release of CO_2 from fossil fuels has been estimated at ca. 5 Pg C yr^{-1} (Keeling, 1973a).

In addition to combustion of fossil fuels, human activities in terrestrial ecosystems result in the release of large amounts of carbon dioxide. Different recent estimations of the annual carbon release range from 1.2 - 3.2 Pg (Baes *et al.*, 1976) to a maximum value of 18 Pg C yr^{-1} (Woodwell *et al.*, 1978). Between the years 1860 and 1970 up to 120 Pg C might have been released to the atmosphere due to combustion or net shift in the difference between biological storage and release (Olson *et al.*, 1978).

New agricultural methods involving use of artificial fertilizers and deeper ploughing have increased the mineralization of soil organic matter and decreased the net storage in soil organic matter (Bramryd, 1979a). Exploitation of peat for fuel, and the drainage of peatlands for forestry or agricultural purposes which rapidly increases the oxidation of matter, lead to release of CO_2 to the atmosphere. In addition to this, the carbon accumulating capacity of the area is lost.

Most products from agriculture and forestry are used by humans and are sooner or later turned into garbage. A more frequent use of incinerators for disposal of solid waste means a more rapid oxidation of organic material compared with landfills, where the mineralization rate is slow (Bramryd, 1977, 1979a). Human impact of greatest magnitude on the carbon cycle can probably be found in the forest ecosystems of the world. Deforestation of large areas, especially in the tropics, and new forestry methods have meant an increased release of CO_2 from the world's forests.

In terms of the global C budget, one must consider the possible net accumulation of organic matter in some ecosystems, especially in the temperate and boreal zones, which might reduce the net release of carbon to the atmosphere. The CO_2 concentration in the atmosphere has only increased by approximately 50% of that which could be expected from fossil fuel consumption (Baes *et al.*, 1976). Bearing in mind that other human activities produce extensive amounts of carbon dioxide it is evident that there must be mechanisms operating to counteract the build-up of atmospheric carbon dioxide. The oceans probably serve as large, but slow, sinks for the excess of carbon, but it is doubtful whether they alone could absorb these large quantities of atmospheric carbon at an appropriate rate.

POOLS OF CARBON IN FOREST ECOSYSTEMS

Plant Biomass

The total forest area of the world has been estimated to be about 3.8×10^7 km^2. This corresponds to ca. 22% of the global land area (Persson, 1974). According to Whittaker and Likens (1975), the total amount of carbon in the living biota in the 1950s was about 830 Pg. More than 90% could be present in the vegetation in forests and woodlands. About 60%, or 461 Pg, could be found in tropical forests.

Duvigneaud estimates a lower value (592 Pg) for carbon in the land phytomass (Bolin *et al.*,

1979). According to Duvigneaud, the humid tropical forests account for the highest concentrations of living biomass, ca. 35 kg m^{-2}. Tropical seasonal forests have only about half as much, ca. 18 kg m^{-2}. Boreal forests have ca. 20-25 kg m^{-2}. Temperate coniferous plantations have a remarkably low biomass-concentration, only 18 kg m^{-2} (Bolin *et al.*, 1979). The low figure for coniferous plantations might be explained by a shorter rotation time (younger trees) and a more sparse herb-layer compared with other forests.

There are differing recent estimates of the net primary production of the land biota. Wittaker and Likens (1975) give a value of 53 Pg C yr^{-1}, while Bazilevich *et al.* (1971) estimate 78 Pg C yr^{-1}. According to Duvigneaud, the total biospheric net primary production should be 62.7 Pg C yr^{-1} (Bolin *et al.*, 1979). The tropical forests account for at least 25% of the global terrestrial carbon fixation (Brünig, 1971).

Root biomass and root production vary considerably between different forest types. Production might be ca. 50-70% of the total annual tree production in dry boreal pine forests (Albrektsson, 1978), but only ca. 10-20% in fertile temperate deciduous forests (Nihlgård and Lindgren, 1977). The distribution between different parts of the trees depends, for example, on species, age, water supply and availability of nutrients. From a carbon viewpoint these relationships might be essential for calculations concerning inputs of organic carbon to the soil and the percentage of the forest biomass being removed from the forest when cutting the trees.

Soil Organic Matter

Estimates of the total amount of soil organic carbon are even more variable than those for the living land biota. Most papers indicate that the amounts of soil organic carbon are at least equal to the carbon in the living plant biomass. Keeling (1973b), for example, derived a global total of 1050 Pg C. Higher estimates are given by Schlesinger (1977; 1456 Pg C) and Bolin *et al.* (1979; 1672 Pg C; Table 1). The highest estimates for the world's soils can be found in Bohn (1976; 3000 Pg C).

Calculations based on data of Bramryd (1976, 1980) suggest that, on average, the organic layer (the upper ca. 5 cm) in pineforests in Sweden (podzols) contains ca. 4 kg C m^{-2}. The upper 10 cm of the mineral soil contains, on average, 2 kg C m^{-2}, the layer between 20-50 cm ca. 2 kg C m^{-2}, and that between 50-100 cm ca. 1.5 kg C m^{-2}. This gives a total value of 9.5 kg C m^{-2}. In more fertile spruce forests the soil organic carbon content can be calculated at ca. 15 kg C m^{-2}. According to Nihlgård (1971) and Nihlgård and Lindgren (1977) the upper 65 cm of a fertile acid brown forest soil in south Sweden contains ca. 10.3 kg C m^{-2}. Bohn (1976) gives a higher estimate (21 kg C m^{-2}) for the upper 100 cm in podzols, while Bolin *et al.* (1979) estimate the mean value for the taiga region to be 9 kg C m^{-2}. If we assume that the mean value for the whole taiga region is 13 kg C m^{-2} based on an area of ca. 15.4 x 10^6 km^2), the total amount of soil organic carbon in the taiga should be 200 Pg.

It is also important to consider the turn over time for the soil organic carbon. The carbon compounds in the mineral layer are generally much older and turn over more slowly than carbon in the litter and humus layers. Long term accumulation of carbon in the soil takes place mainly in the mineral soil layers. Tamm and Holmen (1967) dated the soil organic carbon in some podzol profiles in Sweden by means of the ^{14}C method and found that the carbon compounds in the organic layer were mainly derived from the latest forest generation, while, in the deeper mineral layers, the ages varied from ca. 300 to 1200 yr. Similar investigations in middle Europe have found 5000 yr old carbon compounds in 8000-10000 yr old forest localities (Perrin *et al.*, 1964; Lüders, 1961).

Forest soils might act as long term sinks for carbon, and storage may increase the accumulation if the net primary production should increase, for example, as a result of an increased CO_2 concentration in the atmosphere. However, large amounts of the long-lived soil organic carbon are probably washed out and transported to lake sediments and to the sea, where the final accumulation takes place. The annual river discharge of dissolved and particulate organic carbon into the ocean has been calculated to be 0.189 Pg yr^{-1}, 0.445 Pg C of which is discharged as HCO_3^- (Baumgartner and Reichel, 1975). Relatively large amounts of carbon are further stored in the standing dead trees in forests. This is released slowly by biological decay, but much more quickly by forest fires.

TABLE 1

SOIL ORGANIC MATTER IN MAJOR ECOSYSTEMS

(Modified after Bolin *et al.*, 1979)

Ecosystem type	Area ($m^2 \times 10^{-12}$)	Organic matter ($kg\ m^{-2}$)	Total Carbon (Pg)
Equatorial rain forest	11.0	8	40
Tropical seasonal forest	5.0	10	23
Savannas	19.0	42	180
Deserts	27.0	28	57
Temperate grasslands	8.7	105	147
Temperate forests	9.0	45	56
Peatlands	1.5	1000	882
Mixed forest	1.5	20	14
Taiga	15.4	20	140
Tundra	6.7	10	30
Mangroves	0.3	6	1
Swamps and marshes	2.0	30	27
Halophytes, lakes, streams	2.0	2	2
Cultivated land	16.0	10	73
Total biosphere			1672

The annual release of CO_2 from the decay of humus held within forests soils in the world has been estimated to be 2.0 Pg C on a global basis (Woodwell *et al.*, 1978). These kinds of estimations, however, must be regarded as very uncertain since the mineralization rate fluctuates widely from ecosystem to ecosystem depending on soil type, nutrient status, climate etc., and also on human disturbances in the form of, for example, air pollution or mechanical influences.

EFFECTS OF MODERN FORESTRY METHODS ON THE CARBON BALANCE

Human Influence on the Accumulation of Carbon in Forests

Man-made forests in the world today cover approximately 10^6 km^2 (Persson, 1974). It has been estimated that the forest area of western Europe has been reduced from about 90% to 18-23% of the total land area during the last thousand years (Darby, 1956). According to Persson (1974), the largest planted areas can be found in China (ca. 200 km^2) and North America (ca. 106 km^2). In Australia and New Zealand large areas have been planted with *Pinus radiata*.

In Africa about 1.4×10^6 km^2 of forest are relatively unexploited today. Of this area, it has been estimated that around 1.1×10^6 km^2 may be exploited over the next few years. About 10^6 km^2 of closed African forests have been cleared each year, and it is estimated that the total closed forest area in Africa might decrease from ca. 1.8×10^6 km^2 today to ca. 1.45×10^6 km^2 by the year 2000 (Persson, 1977).

Managed forests usually have a shorter turnover time, being harvested before reaching their maximum biomass. Large areas of intensively managed forests are continually being cleared and replaced by young forest plantations which have a low biomass density. Disturbance of forest sites often leads to increased mineralization of soil organic carbon. Areas subject to windthrow, and fire in virgin forests, are probably of much smaller magnitude.

Comparative studies on some virgin and managed forests in south Sweden (Bramryd, unpublished results) indicate that, in addition to lower tree biomass, man-made forests generally have a more sparse bush- and herb-layer compared with virgin forests. Based on these facts, and taking into account the younger average tree age in planted forests, it would be realistic to assume that intensively managed forests, on average, contain ca. 20% less carbon than virgin forests. The productivity in planted forests is often higher than in virgin forests. However, this only leads to a more rapid turnover time, and the effects on the carbon accumulating capacity of the forest are uncertain.

Assuming that a boreal forest contains 9 kg C m^{-2} in living biomass (Revelle and Munk, 1977) and ca. 20.6 kg C m^{-2} in the soil, including peatlands (Schlesinger, 1979), a total figure for virgin boreal forests would be 29.6 kg C m^{-2}. A 20% lower value for intensively managed forests would give ca. 23.6 kg C m^{-2}, a difference of 6 kg C m^{-2}. At the present time these calculations might be relevant for Scandinavia but probably only to a certain extent for the remaining countries of the boreal belt. However, there are trends for more intensified exploitation of the boreal forests in most parts of the world. Therefore, these maximum figures are of interest as an upper limit for the amount of CO_2 that might be released to the atmosphere due to cultivation of boreal forests. If 100% of the boreal forests (12×10^{12} m^2) were managed to the maximum extent, ca. 12×10^{12} m^2 x 6 kg C m^{-2} (or 72 Pg) less carbon would accumulate compared with virgin forests.

In temperate, deciduous forests used for timber production, the practice of clear-cutting is less common. However, the difference in tree biomass and herb-layer phytomass is pronounced between virgin and managed forests. Plantations of conifers will strongly reduce the herb-layer phytomass, but the larger number of trees per unit area might compensate to some extent. One may assume also that managed temperate forests might contain less carbon (20%) in vegetation and soil than a virgin forest (Bramryd, unpublished results). With ca. 16 kg C m^{-2} in the living biomass of 5×10^{12} m^2 of temperate evergreen forests, ca. 14 kg C m^{-2} in 7×10^{12} m^2 of temperate deciduous forests (Revelle and Munk, 1977), and ca. 13.4 kg C m^{-2} in the soil (Schlesinger, 1979) of virgin temperate forests, the net difference in carbon accumulation between 100% virgin temperate forests and 100% intensively managed temperate forests in the world could thus reach a maximum of 51 Pg C.

Effects of Increased Cutting

Clear-cutting of forest areas, especially in the tropics, leads to a significant release of carbon. Bolin (1977) has calculated the annual CO_2 release due to harvesting forests to be about 1.1 Pg C yr^{-1}. This is removed as timber, pulp or firewood. In the tropics only 10-20% of the cuttings are replaced (Adams *et al.*, 1977). Erosion of soil organic matter from cleared tropical rainforests can also be considerable.

Between 1950 and 1973 the volume of biomass removed annually from cuttings increased, on a global scale, by ca. 80%. The increase varied in different parts of the world and was most pronounced (a little over 200%) in Asia. The increase was only about 20-25% in Europe and North America (Skogsstyrelsen, 1976). These figures can probably be partly explained by the fact that clearing activities in several countries in the Far East are now much better reported.

The proportion of harvested biomass used as industrial wood is approximately 96% in North America, while in Africa and Asia (Except USSR) it is only 14% and 29%, respectively (Skogsstyrelsen, 1976). Most of the wood in Africa and Asia is used for fuel and will thus be rapidly converted to CO_2, while part of the industrial wood is preserved in buildings, paper etc., or is only slowly decomposed in landfills. There has been a small increase in the global firewood consumption from 1025×10^6 m^3 in 1961 to about 1120×10^6 m^3 in 1970. At the sare time the amounts of wood used for pulp and paper production have rapidly increased (Skogsstyrelsen, 1976). This means that an increasing amount of the world's forests is converted to CO_2 rather rapidly. Sioli (1973) calculated that the Amazonian

forest contains about 30 kg C m^{-2}. With an area of 4 x 10^6 km^2, the whole Amazonian forest would, therefore, contain about 120 Pg C, equivalent to ca. 15% of the atmospheric gaseous carbon.

In addition to firewood, logging slash is burned in many parts of the world. Forest lands are also burned to create new agricultural lands or for planting new tree species. This practice is of special importance in tropical regions but is also widespread in Australia, New Zealand and many other parts of the world. Slash burning has decreased in, for example, Scandinavia during the last decades.

Wild-fires play an essential role in many ecosystems. In France, for example, some 350 km^2 of forest- and shrub-lands are destroyed each year by fire (Counsil of Europe, 1979). Forest fires are also common in Canada covering approximately 0.5 - 1.5 x 10^4 km^2 yr^{-1}. In the U.S.A., the area subject to fire has decreased during the last 20 yr but still covers ca. 10^4 km^2 yr^{-1} (White, 1972).

Whole Tree Utilization

In some industrialized countries there are plans to use stumps, bark and other logging residues as fuel. This would markedly decrease the amount of organic material available for humus production. The rapid oxidation of compounds that otherwise would have formed long-lived humus substances will result in a strongly depleted accumulation of carbon in the soil.

Branches, needles or leaves, stumps and roots contain considerable amounts of carbon. In temperate deciduous forests about 20%, on average, of the biomass of trees becomes logging slash when the trees are cut (Nihlgård and Lindgren, 1977). In pine forests, for example, the biomass of branches and needles can equal the biomass of the stems (Tamm, 1979).

Whole tree utilization may lead to nutrient impoverishment and a less favourable soil-water holding capacity, factors which may lead to decreased productivity on some sites.

FOREST PLANTATIONS FOR FUEL PRODUCTION

The forest plantations planned for energy extraction will increase the flow of carbon into the forests in the short-term, but their total effects on carbon accumulation are difficult to predict. They will vary depending upon the type of ecosystem that would be in the area if the "energy forest" had not been planted. The turn over time for the fast growing energy plantations (for example, *Salix, Prunus* spp. and *Eucalyptus*) will be very short and the biomass per unit area will be small compared with an ordinary forest. On the other hand, an energy plantation might contain more carbon than the living biomass in an open woodland or grassland, while an energy plantation on a former peatland probably will strongly increase the oxidation of the peat and the output of CO_2 to the atmosphere. The accumulation rate of long-lived organic matter in the soil will markedly decrease as most parts of the trees will be removed and used for fuel, and the disturbance will increase mineralization of soil organic matter. Therefore, it can be concluded that energy plantations in probably all environments will have negative effects on the soil as a carbon accumulator, while the effects on the phytomass are uncertain. The biomass production will be faster but on the other hand the turnover time of the biomass will be strongly decreased.

DRAINAGE AND EXPLOITATION OF PEATLANDS

Many countries use peat for industrial purposes, or drain the peatlands to establish new forest and agricultural lands. This leads to a rapid oxidation of the accumulated organic matter and often the carbon-accumulating capacity of the peatland is destroyed.

The U.S.S.R., which has ca. 60% of the global peatland area (Gjaerevoll, 1973), dominates peat exploitation with a present consumption of ca. 200 Tg peat yr^{-1}. This is about 95% of the world's peat utilization (Moore and Bellamy, 1973). Ireland produces about 5 Tg yr^{-1} of fuel peat (Nämnden för Energiproduktionsforskning, 1977). About 0.2x10^4 km^2 of peatlands have been drained in Ireland for agricultural purposes (H. Holmen, personal communication). Finland has an annual peat production of ca. 1 Tg yr^{-1} (Nämden för Energiproduktionsforskning, 1977). At present, 10 to 20 km^2 of peatlands are brought into

production every year in Finland. It has been estimated that by 1980 the annual peat utilization will be ca. 10 x 10^6 m^3 (Suoninen, 1971), which would correspond to ca. 1.7 Tg C (Bramryd, 1979b).

In many countries there are plans for increased exploitation of peatlands for fuel extraction, or for drainage operations aimed at increasing production of wood or agricultural products. Conservation of peatlands is, therefore, urgently required not only from a nature conservancy viewpoint but also in respect to the global carbon balance.

COMPENSATING FACTORS AND FEED BACK MECHANISMS

The CO_2 in the atmosphere has only increased about 50% or less of that which could be expected from the fossil fuel consumption and increased outflow of CO_2 from other human activities. The oceans probably serve as the largest sink for the excess carbon, but it is uncertain whether they can store sufficient carbon to account for the difference observed.

The annual removal of carbon from the atmosphere by reafforestation might be between 70-200 Tg (Bolin, 1977). Reafforestation seems to have taken place mainly in China and North America but has also been applied in other parts of the world. In Sweden, for example, the wood supply has increased by ca. 40% during the last 50 yr (from ca. 1.76 x 10^9 m^3 in 1925 to ca. 2.45 x 10^9 m^3 today; Jordbruksdepartementet, 1978). The same trend would probably apply to many other north European countries.

Photosynthesis might increase as a result of the increased CO_2 concentration in the atmosphere. However, other factors, such as nutrients, water supply and temperature, are often limiting. Lemon (1977) concluded that the effects of increasing atmospheric CO_2 concentrations on global photosynthesis are probably very small. If we assume that net primary production of the biota is linearly proportional to the atmospheric CO_2 concentration, then the increased atmospheric CO_2 concentration might cause an annual increment in net primary production of about 0.2 Tg C (Woodwell *et al.*, 1978). This increment would be too small to compensate for the amount of carbon dioxide being released by, for example, forest clearing.

It has been suggested that an increase in atmospheric CO_2 concentration would cause climatic changes. For example, a doubling of the CO_2 concentration is predicted to raise the average surface temperature by about 2.5° (the so-called Greenhouse effect). There could also be associated changes in cloudiness and precipitation (Manabe and Wetherald, 1975). However, the increased cloudiness should reduce incoming solar energy and might thus, to a certain extent, counteract warming and work as a feed back mechanism (Barrie *et al.*, 1976). Increased productivity might occur in boreal forests due to higher temperatures and a longer growing season. Increased temperature could, on the other hand, decrease productivity in arid and dry parts of the world. An increased temperature might promote more microbial activity in soil and accelerate mineralization of soil organic matter.

After the glaciation period a net accumulation of organic matter began in the boreal ecosystems. There is some doubt whether a steady-state has been reached today. Some forest ecosystems probably still have the capacity for net accumulation of carbon.

Accumulation of soil organic matter and peat seems to be one of the more essential mechanisms in the terrestrial ecosystems that might compensate for the increase in atmospheric CO_2 concentration.

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SEWAGE SLUDGE FERTILIZATION IN PINE FORESTS: ECOLOGICAL EFFECTS ON SOIL AND VEGETATION

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INTRODUCTION

Efforts to increase forest productivity have resulted in a need for fertilizers. Mostly energy-consuming synthetic fertilizers are used. At the same time sewage plants produce an increasing amount of sludge with a high nutrient concentration, especially nitrogen. This sludge has become a disposal problem but it could instead be used as fertilizer in forests.

Every year more nitrogen is fixed from the atmosphere than is released through denitrification (Delwiche, 1970). However, increased amounts of nitrogen in a forest ecosystem, particularly in the nitrate form, could lead to increased leaching and subsequent increased eutrophication of streams and lakes. Thus it would be favourable to use nitrogen already fixed to the sludge. When sewage sludge is used as fertilizer, the leaching of nitrogen is probably less than if synthetic fertilizers, where nitrogen is applied mostly in a salt form, are used. However, in addition to leaching, other processes are responsible for losses of nitrogen from forest soils. Especially at pH values around 5 to 6, volatilization of ammonia and denitrification might be essential factors (Bramryd, 1976).

Many earlier investigators observed greatly increased tree production after sewage sludge fertilization (Sopper and Kardos, 1973). Compared with the situation in agricultural practice, nutrients taken up by forest vegetation are retained longer in the ecosystems, and are redeposited annually in leaf and needle litter rather than being removed, as in the case with harvested agronomic crops.

Some problems are associated with sludge utilization, e.g., the introduction of pathogenic organisms and heavy metals. Nevertheless, investigations made in Chicago revealed that no living viruses can survive an anaerobic sludge stabilization for 14 days. Many sewage plants treat the sludge for a longer time (Lynam *et al.*, 1972). One month after fertilization of a forest area in Washington with anaerobically digested dewatered sludge, faecal coliform bacteria were absent although the original sludge contained 1.8×10^5 cells per gram (Edmonds, 1976). Tierney *et al.* (1977) found that viruses in unstabilized sewage or sewage sludge can persist for 11 days during the summer, although the actual virus concentration declines rapidly after the first two days. Lance *et al.* (1976) have used column-studies to show that most viruses stay in the first 5 cm of soil flooded with sewage water. The time of flooding and the infiltration rate did not effect the result. Kirkham (1974) found that pathogens are not taken up by the root systems of plants.

It would be desirable to stabilize all sewage sludge. Thermal stabilization is a rather widely used method, especially in Switzerland where the sludge is heated to 70° for about 30 min (Suess and Lessel, 1977). Another method used is gamma irradiation, often with ^{60}Co (Süss, 1976). As a fertilizer, irradiated sewage sludge is as effective as untreated sludge (Suess and Lessel, 1977).

Sludge with high concentrations of heavy metals should not be used as a fertilizer although, at pH values around 5 to 6, most heavy metals are rather immobile and are not easily taken up by the plants (Griffin *et al.*, 1976). In connection with plans for energy extraction from fast growing forest plantations, sewage sludge seems to be an appropriate soil improver by supplying humus particles to the soil.

The main objectives of this study were to investigate the effects of sewage sludge fertilization on soil mineralization and nitrogen balance in spruce forest on sandy soil under different climatic regions.

MATERIAL AND METHODS

The research areas were situated in ca. 50-70 yr old pine forests of similar structure in

1. the nemoral area near Brösarp in the northeastern part of Scania,
2. the southern boreal area at Jädraås in Gästrikland in east central Sweden, and
3. the northern boreal area near Jukkasjärvi, ca. 40 km northeast of Kiruna, in the subarctic region.

Their climatic characteristics are listed in Table 1.

TABLE 1

APPROXIMATE CLIMATIC CHARACTERISTICS OF ACTUAL RESEARCH LOCALITIES

Phytogeographical regions	Nemoral	Southern Boreal	Northern Boreal
Mean temperature (°C)	+7	+3	-1.5
Relative humidity (mm)[a]	100	300	250
Mean precipitation (mm)	600	550	400
Snow cover (days yr^{-1})	50	130	220

[a] Difference between annual mean precipitation and annual mean evaporation (Tamm, 1959).

The soils in the three areas can be classified as well developed podzols on sand sediments. The tree and bush layers are dominated by *Pinus silvestris*. Young trees of *Picea abies*, *Betula pubescens* and *B. verrucosa* are relatively common. *Vaccinium vitis idaea*, *V. myrtillus* and *Calluna vulgaris* dominate the herblayer in the Jädraås and the Jukkasjärvi areas, while *Deschampsia flexuosa* is more common in the southern research area at Brösarp. The bottom layers are dominated mainly by *Pleurozium schreberi*.

In each locality six plots of 20 x 20 m were investigated. Three of these were fertilized with sewage sludge (20% dry wt at the rates of 3, 2 and 1,5 kg dry wt m^{-2}) while three plots were kept for reference. The sludge was from sewage plants with biological treatment of mainly domestic wastewater, and was spread evenly over the surface by hand.

Before fertilization, and then once a year, soil samples were analyzed for pH, NH_4^+ and nitrate, Kjeldahl-N, and total-C. From each plot, 2 x 20 soil cores were randomly sampled (using a borer of 5 cm diam.) and mixed together in four soil samples, two for the organic layer (ca. 5 cm thick) and two for the first 10 cm of the underlying mineral layer. To investigate increases in the nitrogen mineralization rate, soil samples were incubated for 10 days under aerobic conditions at constant temperature (20°) and moisture. The mineralization rate was calculated as the difference between the NH_4^+ and nitrate concentrations before and after incubation.

Ammonium and nitrate in soil extracts were detemined with an Orion digital ammonium electrode. Nitrate was reduced to NH_4^+ using Devarda's alloy powder. The pH of aqueous solutions was determined with a Metrohm digital pH-meter. Kjeldahl-N was determined with a distillation procedure after digestion with conc. H_2SO_4 (Balsberg, 1975). Total carbon was determined according to Nömmik and Popovic (1971).

RESULTS

Effects of Sludge Application on NH_4^+-N and NO_3^--N

During the first year after fertilization NH_4^+-N increased in the organic layer in all fertilized areas. The increase seemed to be related to the applied amount of fertilizer (Fig. 1). The effects were most pronounced in areas fertilized with 3 kg dry wt of sludge per square metre. The NH_4^+-N concentrations also seemed to increase in the first 10 cm of the mineral layer, but the effects were much less evident. Before fertilization, the nitrogen concentrations in the mineral soils were almost zero. During the second year, the NH_4^+ decreased compared with the first year after fertilization, but the levels were higher than before the sludge application. The effects were more pronounced in the Jädraås areas than at Brösarp, where the starting values for soil nitrogen were higher. The effects seem to be significantly lower in the subarctic area where the summer is short.

Nitrate increased significantly during the first year after fertilization of Brösarp and Jädraås soils, compared with the first year after fertilization. There were no significant effects on the nitrate concentrations in the mineral soils.

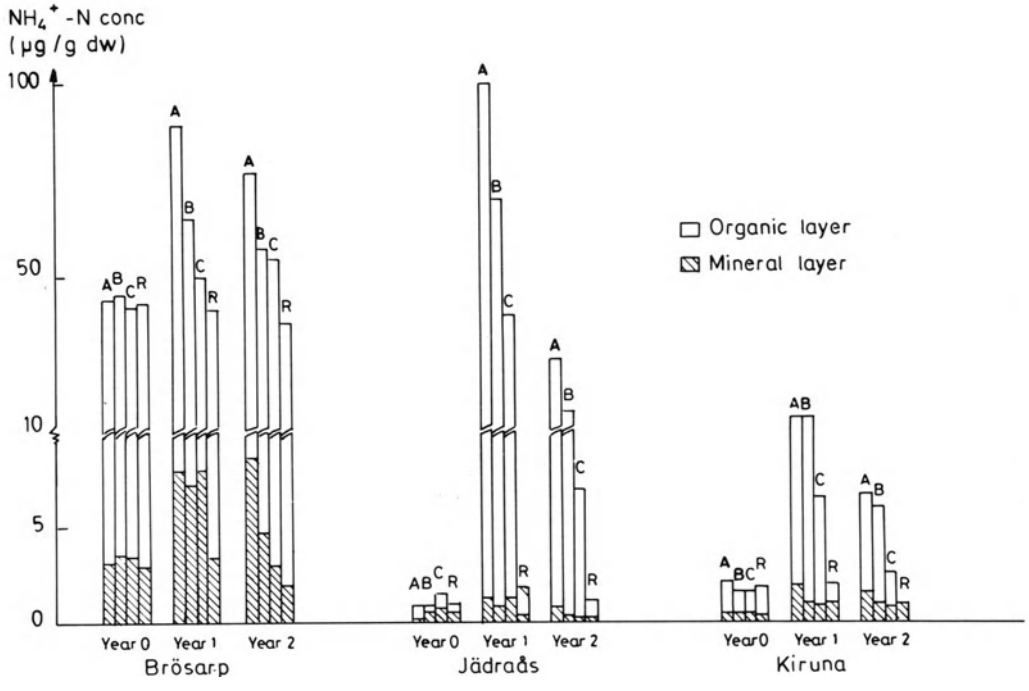

Figure 1: NH_4^+-N concentrations in the organic and mineral layers before sludge fertilization and 1 and 2 years after fertilization. Areas A, B and C were fertilized with sludge at rates of 3, 2 and 1,5 kg dry wt. m^{-2} respectively. Area R is a control.

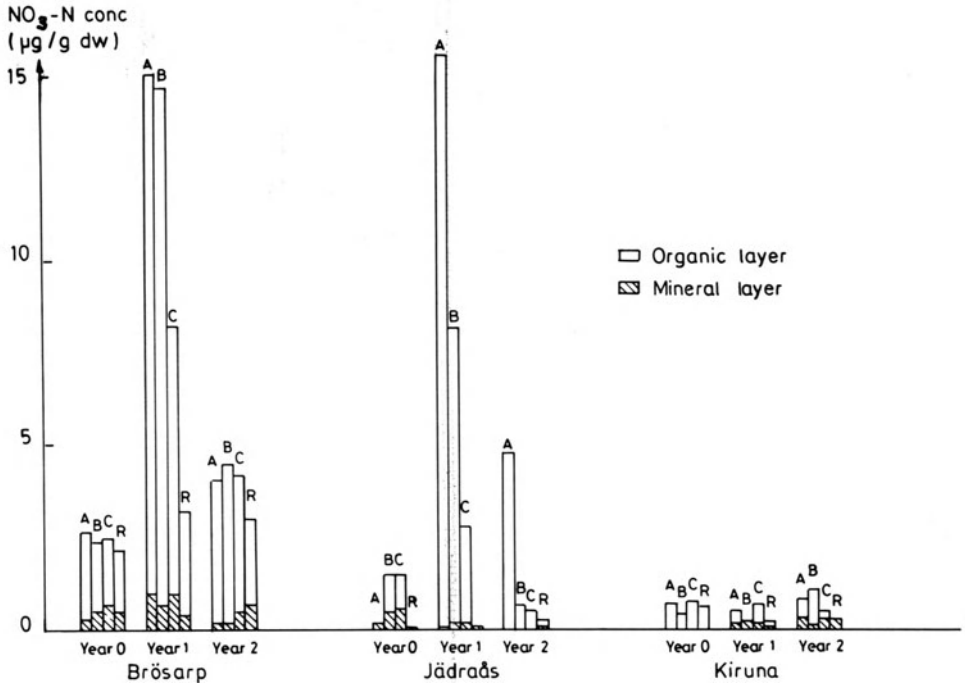

Figure 2: NO_3^--N concentrations in the organic and mineral layers before sludge fertilization and 1 and 2 years after fertilization. Areas as for Fig. 1.

Mineralization Rate

The incubated soil samples from the litter and humus layers, taken one year after sludge fertilization, showed an increase in NH_4^+ (Fig. 3). This was most pronounced in soil samples from Brösarp. In the second year soil samples from Brosärp and Jädraås maintained nearly the same nitrogen mineralization rate, while there was a decrease in samples from Kiruna. The mineralization rates in samples from the mineral soils did not change significantly during the incubation.

Nitrate decreased during incubation of litter and humus samples taken one year after fertilization from plots in Brösarp and Jädraås, fertilized with 3 and 2 kg dry wt m^{-2}, respectively (Fig. 4). This was probably as the result of denitrification. There was however, a clear increase in nitrate in samples from the less intensively fertilized plot (1.5 kg dry wt m^{-2}) and from the reference areas. Results of studies in the second year indicated an increase in nitrification during incubation.

Nitrate increased during incubation of soil samples from Kiruna, especially in samples from the intensively fertilized area (3 kg dry wt m^{-2}). After two years the effects had decreased but were still apparent compared with the reference areas. There were no significant effects in samples from the mineral soil.

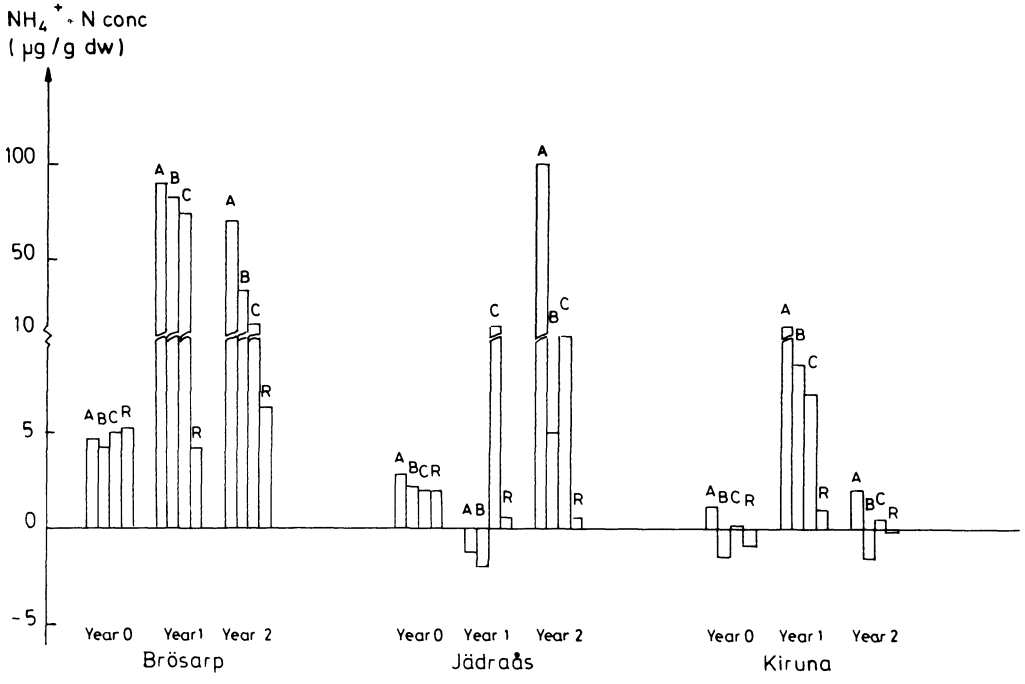

Figure 3: Changes in NH_4^+-N concentrations in the organic layer during incubation, before sludge fertilization and 1 and 2 years after fertilization. Areas as for Fig. 1.

Changes in pH and C/N Ratio

In fertilized areas the pH increased (Table 2). This increase was most pronounced in Jädraås and Kiruna samples which had, initially, very low nutrient status, and was also more pronounced in the organic layer than in the mineral soil. The pH of the applied sludge was around 7.

The C/N ratio (Table 3) decreased during the first two years in fertilized areas in all three localities due to the addition of nitrogen. In Kiruna samples, however, the ratio increased after the first year, probably due to the contribution of organic matter from the sludge.

The concentrations (per g dry wt.) of organic matter in soil, measured as total carbon, range from ca. 85 mg to ca. 275 mg in the organic layer, with a mean value around 170 mg. There were no significant differences between the three localities. In the first 10 cm of the mineral soil carbon generally increased after fertilization from ca. 5 mg to ca. 10 mg in the Brösarp soils, and from ca. 15 mg to ca. 20 mg in Jädraås and Kiruna soils.

DISCUSSION

The pronounced increase in NH_4^+ and NO_3^- concentrations during the first year after fertilization (Figs. 1 and 2) seems mainly to result from inorganic nitrogen applied with the sludge. Similar results were found in a spruce forest in south Sweden (Bramryd, 1976) and in localities in the U.S.A. (Sopper and Kardos, 1973). During the second year, NH_4^+ and NO_3^- decreased slightly in some areas probably due, to some extent, to volatilization of NH_3.

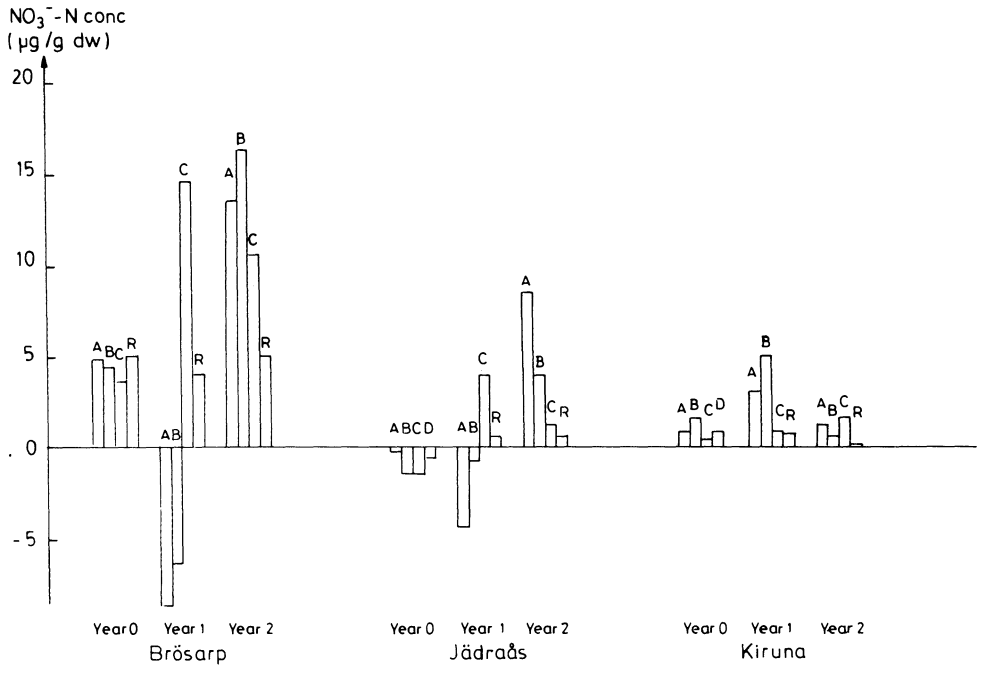

Figure 4: Changes in NO_3^--N concentrations in the organic layer during incubation, before sludge fertilization and 1 and 2 years after fertilization. Areas as for Fig. 1.

TABLE 2

pH VALUES IN THE ORGANIC AND MINERAL LAYERS OF FERTILIZED AND CONTROL AREAS IN RELATION TO TIME

Locality	Area [a]	Organic layer Year [b]			Mineral layer Year [b]		
		0	1	2	0	1	2
Brösarp	A	4.2	4.7	4.4	4.3	4.6	4.4
	B	4.3	4.6	4.5	4.4	4.4	4.4
	C	4.3	4.8	4.3	4.3	4.5	4.5
	R	4.2	4.3	4.2	4.4	4.5	4.3
Jädraås	A	4.1	4.6	4.9	4.7	4.8	4.8
	B	4.1	4.6	4.7	4.7	4.9	4.9
	C	4.1	4.3	4.7	4.8	4.7	4.8
	R	4.1	4.0	4.2	4.6	4.6	4.7
Kiruna	A	4.1	4.1	4.7	4.8	4.6	4.9
	B	4.1	4.2	4.4	4.6	4.8	4.9
	C	4.1	4.3	4.4	4.8	4.9	4.9
	R	4.1	4.1	4.2	4.7	4.7	4.9

[a] Areas as for Fig. 1.

[b] Year 0, 1 and 2 indicate sampling times; year 0 is before fertilization.

TABLE 3

C/N RATIO FOR ORGANIC AND MINERAL LAYERS IN RELATION TO TIME

Locality	Area [a]	Organic layer Year [b]			Mineral layer Year [b]		
		0	1	2	0	1	2
Brösarp	A	28	26	25	23	11	9
	B	30	24	25	18	12	14
	C	31	26	34	16	15	16
	R	34	31	30	13	15	18
Jädraås	A	40	34	26	35	10	7
	B	35	16	29	30	20	12
	C	44	27	25	33	24	17
	R	38	35	42	25	17	21
Kiruna	A	41	65	29	32	33	18
	B	55	61	38	31	57	24
	C	47	41	35	27	37	24
	R	47	55	41	28	37	34

[a], [b] See Table 2.

Volatilization of ammonia has been shown by Mitsui *et al.* (1954) to be directly dependent on the concentration of NH_4^+ in soil, and on pH. However, not more than a few percent of the applied nitrogen can be directly volatilized unless the soil has an extremely high pH value. Hinesly *et al.* (1971) claim that the thickness of the applied sludge layer only slightly affects volatilization of ammonia.

Due to a more favourable C/N ratio (Table 2) for decomposing microorganisms, especially in the intensively fertilized areas, the mineralization rate of organic matter is stimulated. Thus, probably part of the increase in NH_4^+ and NO_3^- can be explained by a slow release of nitrogen from organic matter in the sludge and from the original litter and humus material.

The mineralization rate in most areas increased one and two years after fertilization (Figs. 3 and 4). In the areas in Jädraås. fertilized with 3 and 2 kg dry wt of sludge m^{-2}, NH_4^+ and NO_3^- decreased during incubation. T. Sjökvist (personal communication) advocates that sludge fertilization often stimulates denitrification in soil. This, combined with the fact that the soil samples had a higher than usual water content due to heavy rains before sampling (leading to anaerobic conditions),might be one explanation for the decrease in inorganic nitrogen during incubation. Another contributory cause might be immobilization of NH_4^+ and NO_3^- by microorganisms. According to Bould (1948) and Jansson (1958),N-immobilization takes place at C/N values above 20. The decrease in NO_3^--N in incubated samples from Brösarp taken one year after fertilization, and the rapid increase in NH_4^+, might partly be explained by ammonification by microorganisms.

At the fairly high pH values, 4.5 - 5.0, prevailing in the intensively fertilized areas in Jädraås and Kiruna (Table 2), the conditions become favourable for nitrifying bacteria as can be seen from Fig. 4. In soils from Brösarp, which had, from the beginning, relatively high nitrate, there was probably an active flora of nitrifying bacteria at the start of the incubation. This might, to some extent, explain the distinct nitrification.

The incubation experiments were made in vitro in the laboratory, and it must be noted that nitrogen mineralization under these circumstances can be quite different from that in vivo. This can be due, for example, to disturbed soil structure and destruction of roots (Gerlach, 1973; Popovic, 1974).

The effects of sewage sludge fertilization seem to be less pronounced in Kiruna soils which are from the subarctic parts of Sweden, than in more temperate parts. This indicates that the effects on the nutrient balance in soil depend on climate. However, there is a much smaller difference between Brösarp and Jädraås than between Jädraås and Kiruna.

After ca. 2 yr the applied sludge organic matter is almost entirely incorporated in the ground vegetation or covered by moss-species, mostly *Dicranum* and *Mnium* spp. but, in Kiruna, also with *Splachnum luteum*. All plant species in the herb and bottom layers survive, but a few nitrogen-stimulated species like *Chamaenerion angustifolium*, *Ribes nigrum*, and some grasses, like *Poa nemoralis*, have colonized. Increased survival of young pine and spruce seedlings was noticed. Similar results have been found in other sludge application experiments (e.g. Urie, 1973). The annual increment of tree-shoots has, as indicated by other measurements, increased in all fertilized areas, as well as the productivity of some herb-layer species as,for example,*V. vitis idaea*.

It can be concluded from these investigations that sewage sludge fertilization increases the soil nitrogen supply and stimulates the mineralization rate in soil. This results in an increased growth of trees and herb-layer plants. The effects are most pronounced in the research areas in south and middle Sweden.

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POLYCHLORINATED BIPHENYLS IN SEDIMENTS AND MUSSELS FROM PORT PHILLIP BAY, VICTORIA, AUSTRALIA

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are highly-stable man-made compounds which have been used in a variety of applications since the 1930s. The accumulation of PCBs in nature was not known until 1966 (Jensen, 1972) but since then they have been found in waters, soils, sediments and living organisms from all parts of the world (Risebrough *et al.*, 1968; Peakall, 1972, 1975; USDA *et al.*, 1972; Finlay *et al.*, 1976; WHO, 1976). The toxicity of PCBs to living things has been well documented (Peakall, 1972; Higuchi, 1976; WHO, 1976) and evidence suggests that they are accumulated by organisms at all levels of the food chains in the same fashion as DDT and its metabolites (Jensen, 1972).

The presence of PCBs in the Australian environment and, for that matter, in the Southern Hemisphere, has not been widely documented (McMahon, 1975; Richardson and Waid, 1979). PCBs have been found in mullet caught in Sydney waters (Woollard and Settle, 1978) but little is known of contamination in other localities close to industrial activity. We present the results of a survey initiated by the Ministry for Conservation, Victoria, designed to detect the presence of PCBs in mussels and sediments from Port Phillip Bay, Melbourne.

EXPERIMENTAL

Sampling

Mussels (*Mytilus edulis*) were removed from rocks and piles at selected sites around Port Phillip Bay (Fig. 1). The shellfish collected were those being exposed principally to surface waters. All samples were wrapped in pre-washed aluminium foil, and stored at -30°.

Surficial sediment samples were removed with a grab sampler, and placed in PCB-free glass containers which had pre-washed aluminium liners in the cap. Sediments were stored at 4°.

All efforts were made to ensure that samples did not contact PCBs or PCB-containing substances during and after the sampling procedures (Hutzinger *et al.*, 1974).

Analyses

Mussels were removed from their shells, and ground up in a Waring Blender. Shellfish and sediment sub-samples (25-50g) were soxhlet extracted using 41:59 hexane:acetone for 48 h. The hexane phase was separated and the samples were cleaned up with conc. H_2SO_4 and concentrated in a Kuderna-Danish apparatus (Murphy, 1972; Hutzinger *et al.*, 1974). The PCBs were separated from other organochlorine residues by column chromatography using silica gel, and oxidation with acidified chromium trioxide (Collins *et al.*, 1972).

PCBs were detected and measured on a Hewlett Packard 5715A gas chromatograph, equipped with an electron capture detector and a Hewlett Packard 3380A Integrator. Chromatographic conditions were as follows: column, 90 cm x 4 mm i.d. borosilicate glass; packing, 1.5% OV-17 + 1.95% QF-1 on 80-100 Chromosorb W-HP; column temperature, 200°; E.C. detector temperature, 300°; injection port, on column unheated; carrier gas, 90:10 argon:methane; flow rate, 50 ml min^{-1}.

Known Aroclor mixtures (Monsanto Company, St. Louis, U.S.A.) were used as standards for qualitative and quantitative analyses. The limit of detection for Aroclors (1242-1260) was 10 pg. Blanks were run at frequent intervals. Recovery rates from spiked samples were better than 90% using this procedure.

Station	Location
	South East
1	No. 5 Beacon South Channel
2	No. 9 Beacon South Channel
3	South Channel Pile Light
4	Hovell Pile Light
5	Frankston Pier
6	3 km west, Mordialloc
	Hobsons Bay, Yarra River
7	1.5 km offshore, Brighton
8	Outer west St. Kilda Pier
9	Central east Station Pier
10	Central east Princes Pier
11	Offshore, Sandridge Beach
12	River Entrance Beacon
13	Gellibrand Pier
14	Offshore, Williamstown
15	North Wharf
16	Appleton Dock
17	Stony Creek mouth
18	Truganina Jetty
	South West
19	Point Wilson Pier Light
20	No. 13 Beacon, Wilsons Spit Channel
21	No. 8 Beacon, Point Richards Channel
22	Prince George Bank Beacon
23	West Channel Pile Light

Station	Location
	Corio Bay
	(a) Inshore
24	Shell Beach East
25	Shell Beach
26	Corio Shire Tip Drain, Station Road
27	100m from mouth, Tip Drain
28	50m from mouth, Tip Drain
29	Mouth, Corio Shire Tip Drain
30	North Shell Pier
31	Lascelles Wharf
32	Cowies Creek, Corio Quay
33	Rippleside Drain
	Corio Bay
	(b) Offshore
34	No. 10 Beacon, Corio Channel
35	No. 5 Beacon, Corio Channel
36	No. 3 Beacon, Corio Channel
37	No. 1 Beacon, Corio Channel
38	No. 13 Beacon, Hopetoun Channel
39	No. 8 Beacon, Hopetoun Channel
40	No. 1 Beacon, Hopetoun Channel

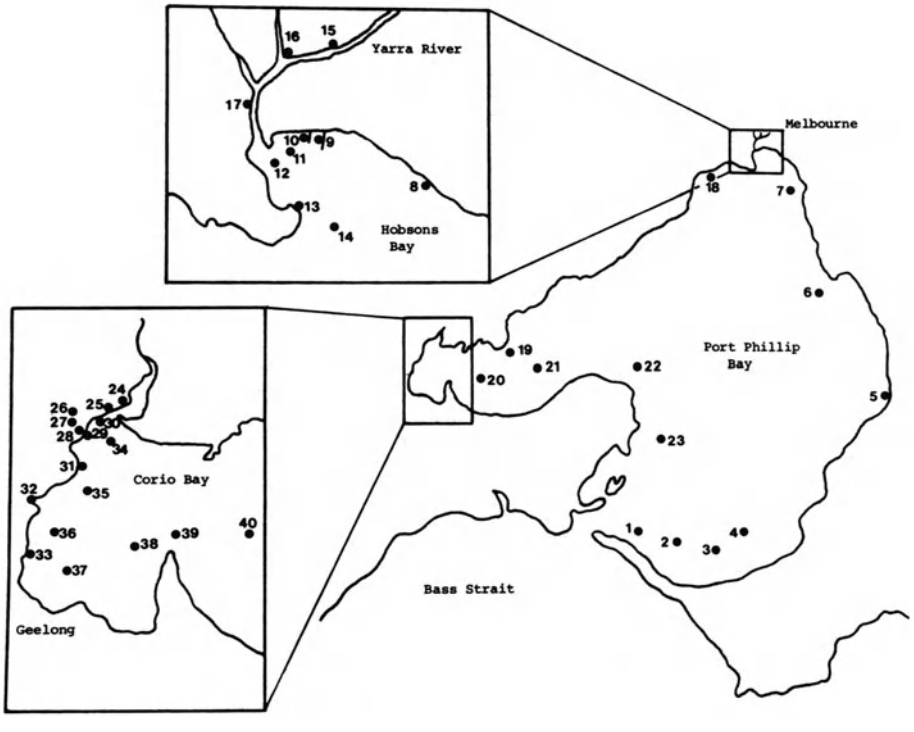

Figure 1: Sampling stations in Port Phillip Bay

RESULTS AND DISCUSSION

PCBs were found in all mussel samples analysed (Table 1). The locality of greatest interest was Corio Bay, Geelong, where mussels sampled from the inner shoreline contained the highest concentrations of PCBs encountered. It appeared that there may have been a number of sources of PCB input to Corio Bay, principally via sites of drainage. This presumption was further substantiated by the results of sediment sampling. Corio Shire Tip Drain sampling stations contained relatively high amounts of PCBs, as did sediments from Cowies Creek (behind Corio Quay) and Rippleside Drain.

The mussel samples collected from beacons further out in Corio Bay (more than 1 km offshore) contained less PCB. Concentrations in sediments were also noticably lower. Near Point Henry, however, concentrations were slightly higher.

The concentrations of PCBs in mussels dropped substantially in samples taken along the shipping channels leading into Corio Bay (Hopetoun and Point Richards Channels), although there was a rise near the mixing zone between Corio and Port Phillip Bays. Along the coastline on the South Western side of Port Phillip Bay, concentrations of PCBs in mussels were low.

The types of PCBs found differed according to the locality of sampling, and the type of sample removed. PCBs in sediments from inshore Corio Bay were of a high-chlorinated type (resembling Aroclors 1254 and 1260), while offshore sediments tended to be lower-chlorinated (resembling Aroclors 1242 to 1254). The reason for such variations may lie in the ability of PCBs to adsorb to surfaces; higher-chlorinated molecules are more strongly bound than the lower-chlorinated varieties, and will thus remain closer to their sources of input, often for extended periods (Haque *et al.*, 1974).

PCBs encountered in inshore mussels were of the Aroclor 1248-1254 type. PCBs of the Aroclor 1242-1248 type were found in mussels collected further offshore, and in the shipping channels. This indicated a lower chlorination than in the PCBs of the corresponding sediments, which was most likely due to the lower solubility and greater adsorption of higher-chlorinated biphenyls.

PCB concentrations were low in offshore samples from the South Eastern part of the Bay, which is distant from areas of industrial activity. Inshore stations close to populated areas were slightly higher. PCBs were found in high concentrations in the vicinity of the Yarra River Mouth.

The amounts of PCBs in mussels and sediments were similar to those recorded in comparable surveys undertaken elsewhere (Table 2). PCB concentrations in shoreline mussels from Corio Bay, and mussels from the Yarra River Mouth frequently fall in, or are close to the "polluted" category, by U.S. Mussel Watch standards. Offshore and shipping channel mussels fall into a moderately polluted to relatively unpolluted category. Like the U.S. Mussel Watch program, we acknowledge the difficulties associated in discriminating between relative levels of contamination (e.g. by accounting for variables such as seasonal fluctuations in accumulation, lipid content, age and size of the mussels and salinity of the water).

Our results cannot be compared to those obtained from waterways around Sydney (Woollard and Settle, 1978) where PCBs were detected in mullet, but they show that PCB concentrations in the drainage areas of the industrial Melbourne environment do not differ greatly from values recorded in Northern Hemisphere industrial areas. At present, the sources of PCB to Port Phillip Bay are under investigation (Richardson and Waid, 1979, unpublished results). We have not been sampling long enough to detect changes in PCB concentrations but it would seem probable that PCBs will persist in the region for some time.

ACKNOWLEDGEMENTS

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TABLE 1

CONCENTRATIONS OF PCBs IN MUSSEL AND SEDIMENT SAMPLES

Station Number and Location	PCB Concentration ng g^{-1} dry weight	
	Mussel	Sediment
South East		
1. No. 5 Beacon South Channel	160	-[a]
2. No. 9 Beacon South Channel	38	-
3. South Channel Pile Light	92	-
4. Hovell Pile Light	83	-
5. Frankston Pier	137	-
6. 3 km west, Mordialloc	69	-
Hobsons Bay, Yarra River		
7. 1.5 km offshore, Brighton	282	-
8. Outer west St. Kilda Pier	169	-
9. Central east Station Pier	339	-
10. Central east Princes Pier	362	-
11. Offshore, Sandridge Beach	-	< 10
12. River Entrance Beacon	350	10
13. Gellibrand Pier	593	-
14. Offshore, Williamstown	-	< 10
15. North Wharf	-	92
16. Appleton Dock	-	18
17. Stony Creek mouth	-	103
18. Truganina Jetty	656	-
South West		
19. Point Wilson Pier Light	109	-
20. No. 13 Beacon, Wilsons Spit Channel	34	-
21. No. 8 Beacon, Point Richards Channel	560	-
22. Prince George Bank Beacon	45	-
23. West Channel Pile Light	110	-
Corio Bay *(a) Inshore*		
24. Shell Beach East	20	-
25. Shell Beach	20	-
26. Corio Shire Tip Drain, Station Road	-	530
27. 100m from mouth, Tip Drain	-	230
28. 50m from mouth, Tip Drain	-	420
29. Mouth, Corio Shire Tip Drain	540-700	26
30. North Shell Pier	158	-
31. Lascelles Wharf	601-847	-
32. Cowies Creek, Corio Quay	290	270
33. Rippleside Drain	620-930	810
Corio Bay *(b) Offshore*		
34. No. 10 Beacon, Corio Channel	211	86
35. No. 5 Beacon, Corio Channel	139	< 10
36. No. 3 Beacon, Corio Channel	275	< 10
37. No. 1 Beacon, Corio Channel	313	< 10
38. No. 13 Beacon, Hopetoun Channel	455	19
39. No. 8 Beacon, Hopetoun Channel	477	-
40. No. 1 Beacon, Hopetoun Channel	238	-

[a] not done

TABLE 2

PCB CONCENTRATIONS (ng g^{-1}) IN MARINE BIVALVES AND SEDIMENTS

Location	Mussels (and other Bivalves)[a]	Sediments	Comment	Ref
Southern Puget Sound Washington	11 - 210 (ww)[b]	0.7 - 300 (dw)[c]	Largest amounts near areas of high population density, industrial and shipping activity	Mowrer *et al.*, 1977
Escambia Bay, Florida	< 10 - 6900 (ww)	< 10 - 30.000 (ww)	Source of PCB traced to accidental industrial leak	Wilson and Forester, 1978
West Coast USA (Mussel Watch)	< 3 - 8700 (dw)		Highest values (> 150 ppb) associated with areas of high industrial activity	Goldberg *et al.*, 1978 Bayne, 1978
East Coast USA (Mussel Watch)	15 - 838 (dw)		Concentrations <50 ppb considered to be from relatively unpolluted areas; 600-800 ppb from polluted areas	
Port Phillip Bay, Melbourne (This Study)	20 - 930 (dw)	< 10 - 530 (dw)		

[a] % water of Mussels 85 - 95

[b] ww = wet weight

[c] dw = dry weight

of samples, and Mrs C. Keith and Miss Oriella Lonni for typing the manuscript. This paper is publication No. 251 in the Ministry for Conservation, Environmental Studies Series.

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WATER POLLUTION AS A CONSEQUENCE OF LAND DISTURBANCE IN SOUTH-WEST OF WESTERN AUSTRALIA

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INTRODUCTION

The South West Coast Drainage Division, which extends over 3.14 x 10^9 km^2 (Anon, 1975), is economically the most important part of Western Australia. Sadler (1976) examines problems associated with increasing water demands and limited resources, and possible conflicts with development of land resources in the region. Of particular concern is the degradation of quality of surface water resources, which has occurred in the 150 years since European settlement (Wood, 1924; Peck and Hurle, 1973; Loh and Hewer, 1977). Thirteen of the nineteen river basins (over 50% of the surface water resources of the region) yield water containing total soluble solutes greater than 500 mg l^{-1}. Some of these streams were fresher before settlement but are now too saline for many potential uses (Mulcahy, 1978).

It is accepted that removal of perennial, deep rooted indigenous eucalypt forest and its replacement by annual, shallow rooted crops and pastures reduces evapotranspiration (Rutter, 1975; Sharma, 1978), thus providing excess water for leaching solutes from the deep lateritic profiles. Wood (1924) suggested that most of the excess water joins the existing groundwater in the deep clay sub-soils, and as a consequence, the groundwater level rises bringing solutes to the soil surface, thence into streams. On the other hand, Conacher (1975) proposed a throughflow hypothesis stating that the excess water moves primarily through the surface soil layer as a perched groundwater above a relatively impermeable hard pan or plough pan. Although these hypotheses have not been tested rigorously, observations showing rising water tables following land clearing (e.g. Williamson and Bettenay, 1979) appear to favour Wood's hypothesis. In fact models developed to predict stream salinity assume that the excess water joins streams at the same solute concentration as the groundwater (Peck *et al.*, 1977). Because of the complexity of lateritic terrain, it is difficult to determine with confidence the pathways, residence times and fluxes of water and solutes by direct measurement on a catchment scale. It is unlikely that either of the hypotheses (Wood or Conacher) could be shown to be exclusive, and in many catchments a flow model which includes the two pathways may be more realistic.

Because of large differences in solute concentrations in surface runoff, shallow perched groundwater and deeper groundwater, it is important to quantify the relative contributions from these sources to improve prediction of stream salinity. As an aid in the analysis of the hydrology and to evaluate stream pollution, we present data on the hydro-geochemistry of major ions in stream waters from forest and farmed catchments, and from various sources of solutes within the landscape. A simple analysis is applied to estimate the relative contributions of solutes from different sources.

ENVIRONMENT

The climate of the south-western region is predominantly Mediterranean with winter rainfall which can exceed potential evaporation by about 200 mm $month^{-1}$. The mean rainfall is as high as 1400 mm yr^{-1} and it decreases sharply with distance inland (Fig. 1).

The landscape has been deeply weathered and laterized. Soil profiles consist of a gravel to sandy clay surface layer of varying depth to 6 m, overlying a relatively less permeable deep (up to 30 m) kaolinitic pallid zone. Large amounts of solutes are stored in these profiles, particularly in unsaturated pallid zone clays (C.D. Johnston, personal communication). Solute storage increases with decreasing rainfall, and can be as high as 0.1 Tg km^{-2} in a 600 mm yr^{-1} area (Dimmock *et al.*, 1974). The soil materials in the lateritic profile have extremely low exchange capacity, and the exchangeable metal ions are dominated by sodium, the clays being strongly to mildly acid (Table 1).

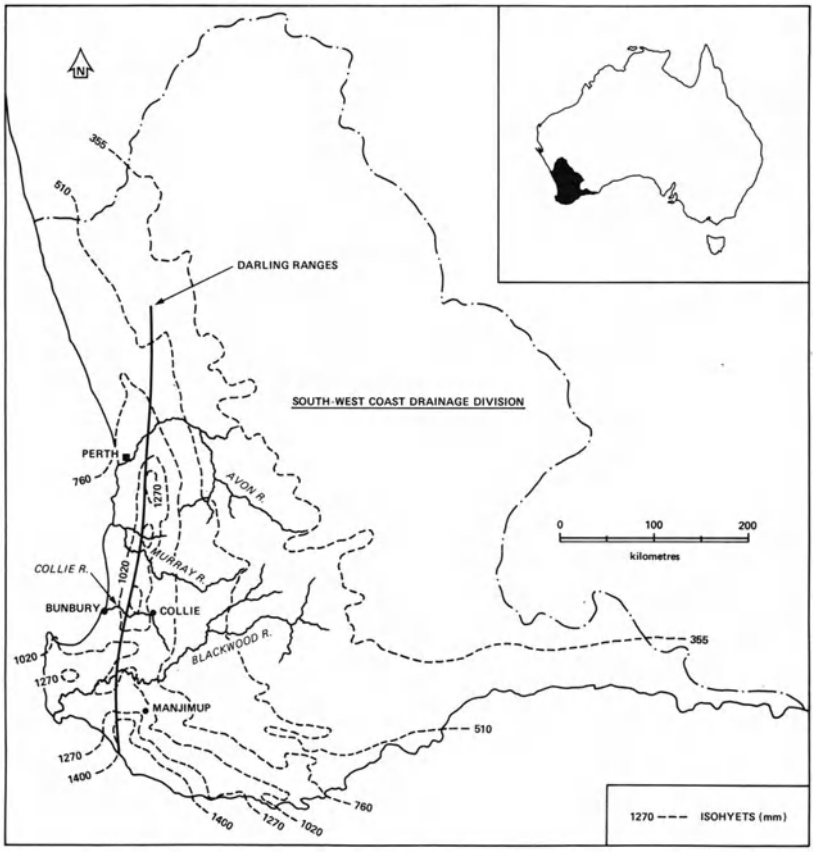

Figure 1: Location of South West Coast Drainage Division in Western Australia.

NATURE AND EXTENT OF CHANGES IN STREAM WATER QUALITY

Various aspect of Cl^- inputs in rain and outputs in streams for thirteen major catchments of the south-west were analysed by Peck and Hurle (1973). The output/input ratios for Cl^- were found to be close to unity for forest catchments, but were as high as 21 for catchments where between 30% and 70% of the area has been cleared for farming. To enable a better understanding of hydrogeochemistry in the region, ionic compositions of stream-water sampled from these catchments were determined in 1972 and 1973 (Table 2).

The rainfall in 1972 was 30% below the long term average and in 1973 it was about average. Comparison between these two years shows that the ionic compositions, as represented by the ratios of ions to Cl^-, differ only in Na^+/Cl^- and HCO_3^-/Cl^- and even for these ions the differences are small. The mean Cl^- concentrations in baseflows are closely similar for the two years and although the mean Cl^- concentrations in streamflow are lower in 1973 than in 1972, the differences between the years are not significant ($P<.05$).

Comparison of "forested" and "cleared" catchments (Table 2) shows that differences in ionic composition are small. Mean Cl^- concentration is significantly higher for cleared than for forested catchments. Ratios of Na^+, K^+ and SO_4^{2-} to Cl^- are significantly greater for forested than cleared catchments while the ratio of Mg^{2+} to Cl^- is greater for cleared than forested catchments. The concentration of Si is greater in the forest streams, and the B concentration is higher in the streams from cleared catchments. There are no

TABLE 1

PROPERTIES OF TWO TYPICAL LATERITIC PROFILES IN THE DARLING RANGE, WESTERN AUSTRALIA

Catchment Description	Mean Annual Rainfall (mm)	Profile Depth (m)	pH	Chloride in Saturation extracts (mg l^{-1})	Sodium[a] Adsorption Ratio	Exchangeable Metal Ions	
						Total (μ eq g^{-1})	Sodium Percentage (%)
High rainfall on granitic gneiss	1200	2.4	5.0	12	2	10.2	7
		3.4	3.9	166	14	4.0	59
		5.3	3.5	492	12	3.6	54
		7.4	3.6	255	10	3.5	47
		8.6	3.6	132	10	3.2	44
		14.5	4.5	37	2	4.7	9
		17.6	4.5	59	3	4.9	11
		21.8	4.8	302	5	48.3	9
Low rainfall on granite	750	4.7	3.6	1382	26	28.4	36
		7.4	3.6	1874	21	18.9	39
		10.6	4.6	1886	28	30.2	61
		13.8	4.4	1996	24	22.5	46
		16.5	4.6	1060	19	19.3	36
		19.0	4.3	1020	21	13.9	22
		22.3	5.0	403	8	17.7	19
		32.6	5.9	50	4	19.6	9

[a] Sodium adsorption ratio = $Na^{+} / \sqrt{(Ca^{2+} + Mg^{2+})/2}$ (ionic composition expressed as equivalents).

TABLE 2

STREAMWATER ANALYSIS FOR THIRTEEN CATCHMENTS IN THE DARLING RANGE, WESTERN AUSTRALIA

Catchment Type	Rainfall Mean (mm)	Stream-flow Mean (mm)	Concentrations of Chloride		Composition of Streamwater - Mean Ratios of Ions to Chloride						Minor Components ($\mu g\ l^{-1}$)			
			Mean[a] $mg\ l^{-1}$	Baseflow[b] $mg\ l^{-1}$	Na	K	Ca	Mg	SO_4	HCO_3	Si	B	Cu	Zn
						1972								
Forested[c] (<5% cleared)	765	97	170	299	.534	.017	.041	.077	.092	.104	3500	19	3	10
Cleared[d] (>30% cleared)	560	43	1543	2599	.464	.007	.045	.106	.066	.073	2100	63	2	15
Significance of difference		N.S.[e]	*[f]	*	*	*	N.S.	*	*	N.S.	*	*	N.S.	N.S.
						1973								
Forested (<5% cleared)	1150	201	99	277	.586	.016	.050	.081	.115	.167				
Ckeared (>30% cleared)	820	108	894	2459	.492	.006	.045	.107	.075	.105				
Significance of difference		N.S.	*	*	*	*	N.A.	*	*	N.S.				

[a] Mean concentration estimated from 12 monthly samples and weighted for flow.

[b] Concentrations in streamwater at lowest monthly flow.

[c] Estimated values for 7 catchments.

[d] Estimated values for 6 catchments.

[e] N.S. not significant.

[f] ($P<.05$).

significant differences in the concentrations of Cu and Zn.

During the last 10 yr, paired catchment studies have been carried out to examine the effects of clearing forest for agriculture. Five of these catchments are located in the Collie River basin. The ionic composition of waters for these catchments and the average composition of streamwaters for one of the high rainfall catchments (Wights) are presented in Table 3. Wights catchment was originally forested (a) and was cleared during the summer of 1977 and pastured (b).

TABLE 3

IONIC COMPOSITION OF SOLUBLE SOLUTES IN WATER AND SOILS IN SOUTH WESTERN AUSTRALIA

Source of Solutes	Ionic Composition (Ratios of Ions to Chloride)					
	Na^+	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	HCO_3^-
Seawater	.56	.020	.021	.067	.14	
Rainwater [a]	.50	.055	.077	.072	.25	
Soils at various depths (Saturation extracts) [a]						
(i) Zone A - above most saline zone	.57	.013	.014	.035	.065	
(ii) Zone B - most saline zone	.54	.003	.013	.053	.058	
(iii) Zone C - weathering zone	.54	.015	.031	.056	.117	
Perched groundwater [a]	.58	.028	.015	.069	.018	.445
Deep groundwater [a]	.49	.014	.044	.088	.060	.139
Streamwaters [b]						
(a) Forested	.50	.013	.041	.082	.051	.110
(b) Pasture for 2 yr	.50	.013	.045	.088	.042	.122

[a] Mean values for 5 catchments in the Collie area, W.A.

[b] Compositions for one of the high rainfall ($\simeq$ 1200 mm yr^{-1}) catchments (Wights) before (1974-76) and after (1977-78) clearing.

The ionic composition of seawater, rainwater, saturation extracts from lateritic material, groundwaters and streamwaters are remarkably similar. The compositions are consistent with the hypothesis that most of the solutes in the landscape have a common origin, having accumulated through accession of oceanic salt in rain. The reasons for the differences between ionic composition of seawater and rainwater have been discussed elsewhere (Hingston and Gailitis, 1976). However, there are small differences in ionic composition of other waters which may be due to biological processes and physico-chemical reactions with soils and

lateritic material. For example differential uptake of ions by forest vegetation may alter the ionic ratios, as illustrated by the elemental composition of the total above-ground biomass of the forest, in which the ratios of ions are, Na^+/Cl^- 0.38, K^+/Cl^- 0.87, Ca^{2+}/Cl^- 1.13, Mg^{2+}/Cl^- 0.56 and SO_4^{2-}/Cl^- 0.43. The residual solutes, after uptake of ions in these proportions from rainwater salts, would have a higher Na^+/Cl^- ratio and lower ratios of K^+, Ca^{2+}, Mg^{2+} and SO_4^{2-} to Cl^-. Ion exchange and adsorption in soils may alter ionic composition, for example by fixation of K^+ in some minerals and adsorption of SO_4^{2-} at low pH values of some lateritic materials. There is some evidence for sulfate adsorption shown by the variation with depth in the lateritic profiles of the SO_4^{2-}/Cl^- ratio for adsorbed plus soluble ions. The SO_4^{2-}/Cl^- ratio for the soil above the most saline zone is much higher than for the soil of the most saline zone, suggesting that SO_4^{2-} is separated from Cl^- by adsorption during leaching.

The concentrations of ions are not high enough to precipitate insoluble compounds such as $CaCO_3$ to alter ionic ratios of the soluble ions during leaching.

Small differences between the ionic composition of saturation extracts from weathering zones and those from the most saline zones are likely to be due to contributions from weathering of primary minerals in the material close to bedrock. We consider that most of the lateritic material is composed of secondary minerals and is not a significant contributor to the large amount of solutes stored in the profile. Further, the contribution of ions from the weathering zone over granite and granitic gneiss bedrock appears to be small relative to the total salt in the landscape.

Perched groundwater has low ratios of K^+, Ca^{2+} and SO_4^{2-} to Cl^- and deep groundwater has a low K^+/Cl^- ratio compared with rainwater. However, on the basis of ionic composition it is difficult to determine relative contributions of solutes from soil zones and rainfall. The streamwaters also have low ratios of K^+, Ca^{2+} and SO_4^{2-} to Cl^- compared with those in rainfall, and reflect the ionic composition of the groundwaters. The change in streamwater composition after 2 yr of pasture is small.

The results for streamwaters (Tables 2 and 3) can be compared with those from the Genoa River basin at the New South Wales-Victoria border (Reinson, 1976) and from Queensland and New South Wales (Douglas, 1968). In these studies, the regional differences in streamwater composition are readily related to the geology of the bedrock for catchments. By comparison, differences in ionic composition of streamwater from all the catchments studied in south-western Australia are small.

In the Hubbard Brook watershed in New Hampshire, U.S.A., the ionic composition of streamwaters from different catchments also varies with bedrock geology (Johnson and Reynolds, 1977). However in paired catchment studies, changes in ionic composition provide a useful analysis of the hydrologic response following land clearing (Likens *et al.*, 1970). In south-western Australia, the difference in ionic composition of groundwaters and of streams from forested and cleared catchments (Tables 2 and 3) may be helpful for interpreting the hydrogeochemistry of the region. However, the differences appear to be too small for quantitative analysis of the contributions of solutes from different sources within the landscape. Thus, the following analysis is based on differences in chloride concentrations in waters rather than on differences in composition of solutes.

ANALYSIS OF STREAM WATER QUALITY

Here we attempt to evaluate the contribution of solutes to a stream from various sources within the landscape. The proposed analysis is somewhat similar to the one presented by Pinder and Jones (1969). It is based on the principle of conservation of mass and ignores consideration of detailed mechanisms of water and solute transport.

Two frequently encountered lateritic profiles, schematically presented in Fig. 2, show a two-layered system consisting of surface soil (upper soil) and subsoil (pallid zone). Case (a) occurs frequently in relatively high rainfall (>1100 mm yr^{-1}) areas where the streams are incised to impermeable basement rock and intercept the permanent groundwater. Case (b) is characteristic of lower rainfall (<800 mm yr^{-1}) areas where the streams flow above a relatively impermeable clay sub-soil layer, the basement rock being several metres below ground level. It should be appreciated that these are the extreme cases; a regional catchment may encompass sub-catchments of both configurations.

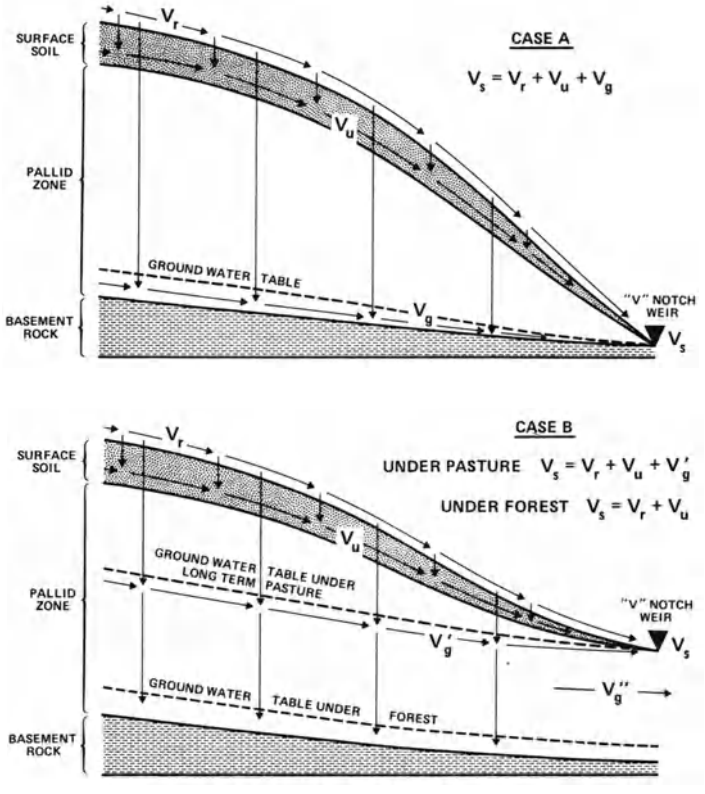

Figure 2: Generalised diagram showing two typical soil profile configurations and suggested pathways of water movement on a catchment scale. Case (a) is typical of high rainfall (>1100 mm yr^{-1}) regions while Case (b) is typical of low rainfall (<800 mm yr^{-1}) regions and for this $V_g = V'_g + V''_g$.

In this analysis of the outflow of water and solutes from the catchment in the streamflow, we consider a solute (e.g. Cl^-) which does not interact with lateritic materials (Table 1). The solute is primarily transported by convection and can take three paths to reach the stream (Fig. 2). Calculations are based on a long time interval (year) and therefore it is assumed that there is no lag between inflow and outflow.

The excess precipitation, defined as P - ET (where P and ET are precipitation and evapotranspiration in mm yr^{-1}) can be partitioned into surface runoff (V_r), through-flow from the surface soil layer (V_u), or groundwater flow (V_g). For case (a) groundwater recharge and discharge are assumed to be equal and therefore:

$$V_s = V_r + V_u + V_g \qquad 1$$

where V_s is streamflow in mm yr^{-1}.

For case (b) groundwater recharge, following changed land use, may cause a rise in the groundwater level. Before the water table intersects the stream bed, $V_s = V_r + V_u$. However when the groundwater rises sufficiently, a proportion of the total groundwater flow will pass to the stream, but this can be considered small (A.J. Peck, personal communication), and therefore Equation 1 holds. If V''_g (Fig. 2) is not small, it needs to be estimated since it is likely to make a significant contribution to the downstream salinity.

If the average Cl^- concentrations (mg l^{-1}) for V_r, V_u, V_g and V_s are respectively C_r, C_u, C_g and C_s, then by conservation of mass,

$$C_s V_s = C_r V_r + C_u V_u + C_g V_g \qquad 2$$

To partition streamflow into various components we define $\alpha = V_r/V_s$, $\beta = V_u/V_s$, $\gamma = V_g/V_s$ and therefore $\alpha + \beta + \gamma = 1$. It is reasonable to assume that $C_r = C_p$. The problem is to solve for α, β and γ from Equations 1 and 2, if we know C_p, C_u, C_g, C_s and V_s. It is not possible to solve for three unknowns from two equations unless either one of the components can be estimated independently or can be considered of minor importance. Estimates of α, β and γ were made for the following conditions:

1. By assuming that the contribution from surface runoff is negligible, therefore $\alpha = 0$ and

$$\beta = \frac{C_g - C_s}{C_g - C_u} \qquad 3a$$

$$\gamma = \frac{C_s - C_u}{C_g - C_u} \qquad 3b$$

This is applicable when the hydraulic conductivity of the surface soil is very high.

2. By assuming that the contribution from throughflow is negligible, therefore $\beta = 0$ and

$$\alpha = \frac{C_g - C_s}{C_g - C_p} \qquad 4a$$

$$\gamma = \frac{C_s - C_p}{C_g - C_p} \qquad 4b$$

This is applicable when surface soil is either not present or has been deliberately removed.

3. By assuming that the contribution from groundwater is negligible, therefore $\gamma = 0$ and

$$\alpha = \frac{C_u - C_s}{C_u - C_p} \qquad 5a$$

$$\beta = \frac{C_s - C_p}{C_u - C_p} \qquad 5b$$

This may be applicable under densely forested areas or in low rainfall areas with a subsoil of very low hydraulic conductivity.

In the absence of any long-term experimental data, the analysis is initially applied to a hypothetical catchment, the parameters for which were estimated from previous knowledge about the response of a range of catchments in this area (Table 3). The outflow/inflow ratios of Cl^- were selected to be 1.5 and 9.4 for the forested and pasture catchments respectively (Peck and Hurle, 1973); the differential streamflow between forested and

TABLE 4

ANALYSIS OF STREAM WATER QUALITY DATA
FROM THREE CATCHMENTS IN SOUTH WESTERN AUSTRALIA

Catchment	P (mm yr^{-1})	V_s (mm yr^{-1})	C_p	C_s (mg Cl^- l^{-1})	C_u	C_g	condition (1) $V_r = 0$		condition (2) $V_u = 0$		condition (3) $V_g = 0$	
							β	γ	α	γ	α	β
Hypothetical												
(a) Forest	1000	100	8	120	400	2000	unrealistic solutions		0.94	0.06	0.71	0.29
(b) Pasture	1000	300	8	250	400	2000	unrealistic solutions		0.88	0.12	0.38	0.62
Wights												
(a) Forest[a]												
1977	905	68	7	172	35	750	0.81	0.19	0.78	0.22	unrealistic solutions	
1978	979	70	7	148	35	750	0.84	0.16	0.81	0.19		
(b) Pasture												
1977	905	162	7	177	35	750	0.80	0.20	0.77	0.23	unrealistic solutions	
1978	979	217	7	216	35	750	0.75	0.25	0.72	0.28		
Lemon												
(a) Forest[a]												
1977	650	2.0	4	28	67	950	unrealistic solutions		0.97	0.03	0.62	0.38
1978	723	4.1	5	28	67	950			0.98	0.02	0.63	0.37
(b) Pasture												
1977	650	7.1	4	42	67	950	unrealistic solutions		0.96	0.04	0.40	0.60
1978	723	25.2	5	35	67	950			0.97	0.03	0.52	0.48

[a] Data calculated from regression analyses of paired catchments.

cleared land was also consistent with other observations in the region (Sharma, 1978). The C_u was estimated from Cl^- concentrations of saturation extracts usually found in surface soils under similar rainfall conditions.

The results in Table 4 show high α values for a forested catchment which is in accord with the general belief that the contribution of groundwater to streamflow is small (<6%), and β values suggest that contribution from the upper soil layer can be important. With a change from forest to agriculture, not only is there an increase in the contribution from groundwater and/or the upper soil layer, but there is also increased contribution from surface runoff.

The analysis was also applied to two experimental catchments, which have been cleared for pasture only for two years (Table 4). The Wights catchment comes under Case A while Lemon comes under Case B (Fig. 2). The analysis shows that it is not realistic to assume negligible contribution of groundwater to the stream for Wights catchment. Following clearing, the groundwater contribution increases while the relative contribution from surface runoff and/or the surface soil decreases. In the case of Lemon catchment, the analysis suggests that there has to be a substantial contribution from surface runoff. The most appropriate analysis for this catchment ($V_g = 0$), suggests that there is a significantly increased contribution from the surface soil as a result of land clearing. The extent and duration of contributions from various sources as a function of time, when available for these and other experimental catchments, will help further clarify their relative importance in salinity of streams.

Based on these analyses, it is suggested that the increase in stream salinity due to land clearing would be over-predicted if all the increased excess water (V_s (pasture) - V_s (forest)) were assumed to join the stream at a groundwater concentration, as in Peck's model (Peck *et al.*, 1977). The analysis also suggests that although the solute flow from the upper soil layer is increased in some cases, it is not always the main contributor of water and solutes as proposed by Conacher (1975). If we accept increased solute outflow solely from the upper soil layer, our calculations suggest that contribution of solutes from this layer will last for a relatively short period of time (10-20 yr), while in practice the effects of land clearing have been noted to persist for much longer periods. It is suggested that estimates of α, β and γ for different catchments may provide a technique for analysing the characteristics of catchments and their responses to clearing, taking into account known contributors to streamwater salinity.

SUMMARY AND CONCLUSIONS

Clearing of indigenous eucalypt forest has been directly responsible in increasing solute concentration of many streams and rivers of south-western Australia. Sodium and chloride are the major ions in streams. The composition of solutes, measured in terms of major ions (Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, HCO_3^-, SO_4^{2-}) for rainwater, soil water, and stream water is similar to that of ocean water. The differences in ionic composition of waters from various sources within the landscape and the streamwater are useful in interpreting the hydrogeochemistry of the catchments. However, these differences are too small to be useful for quantifying the annual contributions of solutes from various sources within the landscape. Ratios of Na^+, K^+ and SO_4^{2-} to Cl^- are significantly greater for cleared than forested catchments, while the ratio of Mg^{2+} to Cl^- is greater for cleared than forested catchments. Silicon concentration is higher and boron is lower in forested catchments. The concentrations of these and other minor elements (Zn, Cu) are too small for these elements to be considered pollutants.

A method, based on differential Cl^- concentration of waters, is presented to analyse streamflows so that contributions of solutes to stream from various sources within the landscape, can be partitioned. The analysis suggests that, with a change in land use from forest to agriculture, not only will there be a significant increase in the contribution from groundwater and/or the upper soil layer, there may also be increased surface runoff. Based on the analysis applied to a hypothetical catchment and two experimental catchments, it is suggested that land clearing may cause a significant increase in any of the three components depending on the configuration of the catchment, solute distribution in the profile and differential hydraulic properties of various sub-components of the catchment.

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INTERPRETATION OF SOIL WATER QUALITY FOR AGRICULTURAL PRODUCTION

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INTRODUCTION

Langelier (1936) described the state of saturation of natural waters with respect to $CaCO_3$ by Equation 1,

$$\text{Saturation Index} = pH_o - pH_c \qquad 1$$

where, pH_o is the observed pH of a solution and pH_c is the calculated pH that the same solution would have if it was saturated with $CaCO_3$, but with no alteration in ionic composition. In effect, positive values of the Saturation Index mean that solutions are supersaturated with respect to $CaCO_3$ and there will be a tendency for carbonate to be deposited. This tendency is aggravated by temperature increases since the solubility of $CaCO_3$ decreases at elevated temperatures, such as are experienced in hot water systems.

Bower *et al.* (1965) modified the expression slightly and verified that changes in HCO_3^- concentrations of synthetic waters were closely related to the calculated pH in systems both open and closed to CO_2. They further suggested that the Saturation Index (ΔpH) might be of practical significance in irrigation practices. Subsequently Bower and Wilcox (1965) showed that for upland crop conditions other factors, such as evapotranspiration, need to be considered also if accurate predictions of $CaCO_3$ accumulation or depletion are to be made.

Williams and Jakobsen (1974) examined the dynamics of $CaCO_3$ in a flooded calcareous soil and concluded that pH could form a rational basis for planning soil-irrigation water management practices. This paper describes the application of ΔpH as a survey test of the suitability of soils for rice production on the subcoastal plains of the Adelaide River in northern Australia.

EXPERIMENTAL METHOD

The field experiments were conducted at the Coastal Plains Research Station located near the Adelaide River, some 50 km south-east of Darwin. The soils consist of saline, heavy, cracking clays ranging in pH from 5.5 to 8.5. They have a complex pattern of distribution that makes generalization difficult at a farm planning level and soil testing on a close grid is required to obtain a real appreciation of the chemical variability present.

Two field trials were carried out in drill-sown rice crops; one in Field 5 during the wet season, the other in Field 1 during the dry season. Quadrats of 1 m x 1 m were located on 25 m x 25 m grids within the bulk crop and were hand harvested for yield estimates. Soil samples were collected from surface clods adjacent to the quadrats immediately after drill sowing and prior to the first irrigation. Saturation extracts of these soil samples were analysed for pH, electrical conductivity (EC), HCO_3^- and the major cations. Total carbonate, expressed as $CaCO_3$ was determined on the whole soil. Relevant chemical data are summarised in Table 1.

The $CaCO_3$ Saturation Index was calculated from Equations 2-4:

$$\frac{1}{[H^+]\gamma_{H^+}} = \frac{K_s}{K_2} \cdot \frac{1}{\gamma_{HCO_3^-}\,\gamma_{Ca^{2+}}} \cdot \frac{1}{[Ca^{++}]\,[HCO_3^-]} \qquad 2$$

or

$$pH_c = (pK_2 - pK_s) + pCa^{2+} \cdot pHCO_3^- \qquad 3$$

and

$$\Delta pH = pH_o - pH_c \qquad 4$$

TABLE 1

SATURATION EXTRACT DATA FOR SOILS

		Ca^{2+} (meq l^{-1})	HCO_3^-	EC[a] (mS cm^{-1})	pH	Saturation Index (ΔpH)
Field 1	Min.	3.50	0.49	1.41	6.35	-1.66
n = 34[b]	Max.	8.00	1.61	2.98	7.35	-0.46
	Mean	5.37	1.08	2.25	6.90	-1.02
Field 5	Min.	0.90	0.01	1.21	5.25	-4.52
n = 50[b]	Max	6.10	2.00	4.04	6.85	-0.74
	Mean	2.38	0.45	1.94	5.70	-2.80

[a] EC = electrical conductivity
[b] n refers to number of soil samples

K_s is the solubility product of $CaCO_3$, K_2 is the dissociation constant for the HCO_3/CO_3^{2-} system, γ is the activity coefficient and [] refer to molar concentrations.

The ion activity coefficients, $\gamma_{HCO_3^-}$ and $\gamma_{Ca^{2+}}$, are influenced by the ionic strength of the solution (μ) which can be calculated by empirical relationships such as those of Bower *et al.* (1965) or Ponnamperuma *et al.* (1966).

RESULTS

It is apparent from Fig. 1 that there were major differences between grain yield versus ΔpH relationships for the two experiments. The free $CaCO_3$ content of the surface soil in Field 1 ranged from 0.01 to 0.31% whilst $CaCO_3$ was not present in detectable amounts in Field 5. This is reflected in the restricted range of ΔpH values for Field 1. The non-calcereous soils of Field 5 had initial ΔpH values ranging from -0.75 to -4.52 and grain yields from 315 to 955 g m^{-2}. There was a marked trend for grain yield to increase at $CaCO_3$ Saturation Indices of less than about -2.5 for Field 5 but the trend was much weaker in the calcareous Field 1 soils.

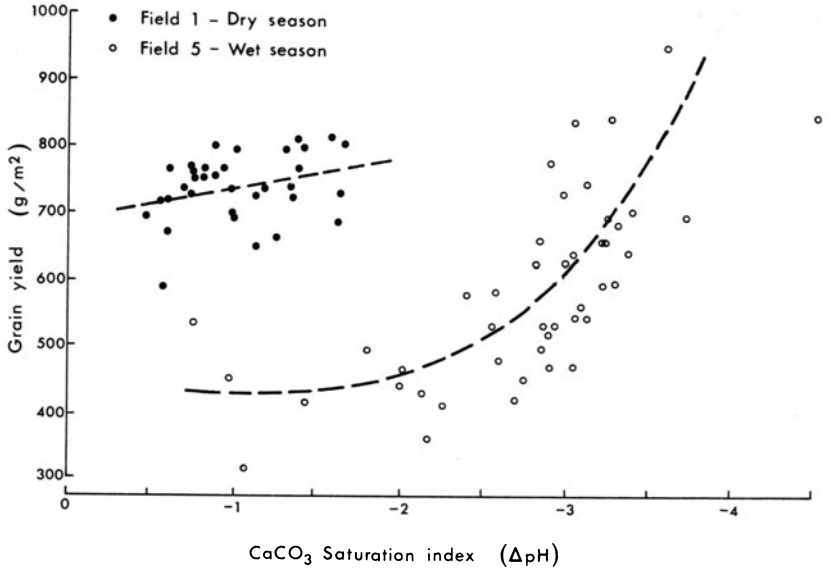

Figure 1: The effect of $CaCO_3$ Saturation Index (ΔpH) on the yield of rice.

The difference in overall yield levels between the two experiments can be explained in terms of seasonal and varietal effects and is not relevant to this present discussion.

DISCUSSION

From the definition of ΔpH, a negative value for the Saturation Index would indicate that the solution is undersaturated with respect to $CaCO_3$ and hence soil carbonates would come into solution. The ΔpH values of samples taken before irrigation in these experiments suggest that carbonate precipitation was not a factor in the variability of grain yields obtained. However, Williams and Jakobsen (1974) showed, in a pot-trial experiment, that the ΔpH values of a soil solution increase markedly with the length of time for which the soil is saturated with water. This is illustrated in Fig. 2 for a clay soil containing 0.2% $CaCO_3$ and flooded with either rain water or irrigation water containing 2.05 meq $HCO_3\ l^{-1}$ and 0.95 meq $Ca^{2+}\ l^{-1}$. The soil solution was sampled at a depth of 5 cm. It is apparent then that the initial low ΔpH values for these present experiments may well have increased to the stage where $CaCO_3$ precipitation could have taken place. This is further supported by recalculation of data presented by Williams (1972) for the chemistry of soil solution beneath a rice crop grown on the calcareous soil. In this instance ΔpH values within the root zone reached values of +0.7 within 6 to 8 weeks of flooding.

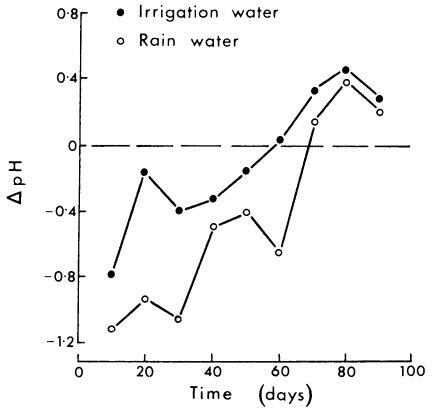

Figure 2: The change of $CaCO_3$ Saturation Index (ΔpH) with time of soil flooding.

Although this discussion has centred on the solubility of calcium carbonate the solubility of other metals of importance to the nutrition of rice plants, such as manganese, iron and zinc, may also be affected. All of these may be precipitated as carbonates and so become unavailable for plant growth. The pK_s values for $MnCO_3$, $FeCO_3$ and $ZnCO_3$, as calculated from the free energy of formation data of Garrels and Christ (1965), are 10.8, 10.7 and 9.8 respectively. Thus each of these would be expected to form solids before $CaCO_3$ (pK_s - 8.3).

It would seem, then, that Saturation Indices of other metal carbonates could be of considerable use in situations where biological activity is dependent on only trace amounts of metal ions. In the case described the Saturation Index is not a static value as it is largely controlled by the carbon dioxide pressure (P_{CO_2}) operative at the time (Ponnamperuma, 1967). Similarly, the redox potential of the solution is also of importance since it controls the valency status of many metal ions.

Invariably such Indices tend to be simplistic as they ignore ion-pair formation and co-precipitation phenomena (e.g. see Nakayama, 1969; Garrels *et al.*, 1960). However, even ignoring the value that their consideration might have, the use of the $CaCO_3$ Saturation Index in predicting yield responses of rice has been amply demonstrated in these experiments.

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FACULTATIVE-THERMOPHILIC *THIOBACILLUS*-LIKE BACTERIA IN METAL LEACHING

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INTRODUCTION

Thiobacillus-like microbes capable of oxidizing ferrous iron at pH 1.6 and a temperature of 55° have been found in thermal springs (Brock *et al.*, 1976; LeRoux *et al.*, 1977), a large-scale copper leaching test system (Brierley and Lockwood, 1977), commercial dump leaching operations at the Chino Mine, Hurley, New Mexico (Brierley, 1978), the Bingham Canyon Mine, Bingham, Utah, the Vlaidov Vrah Mine, Bulgaria (Groudev *et al.*, 1978), and ore deposits in the USSR (Golovacheva and Karavaiko, 1977). These microbes have a chemolithotrophic heterotroph or mixotrophic mode of metabolism. They oxidize ferrous iron, pyrite or other metallic sulfides for energy, but growth occurs only in media supplemented with a reduced carbon compound such as yeast extract or glutathione. These microbes do not appear to fix carbon dioxide (Brierley *et al.*, 1978) and may not reductively assimilate sulfate (Brierley *et al.*, 1979).

The facultative-thermophilic, *Thiobacillus*-like microbes catalyze reactions of importance for leaching of certain metal values from low-grade ores and mine wastes. They oxidize ferrous iron, pyrite (Brierley and LeRoux, 1977), and chalcopyrite concentrates (Brierley *et al.*, 1978). Other thermophilic microorganisms, specifically *Sulfolobus*, have been shown to more effectively leach copper than *Thiobacillus ferrooxidans* from some chalcopyrite concentrates (Brierley and Brierley, 1978). Additional study of the facultative-thermophilic *Thiobacillus*-like microbes was considered necessary in order to determine the relationship of this group with leaching, and whether they are more effective than other microbes in catalyzing the oxidative reactions necessary for leaching. Reported are the ability of these microbes to grow in leach-dump environments and their resistance to various metals.

MATERIALS AND METHODS

Microorganisms

Three strains of the facultative-thermophilic, *Thiobacillus*-like microbes were used. TH-1 was obtained from an Icelandic thermal spring sample (LeRoux *et al.*, 1977). TH-2 was found in samples from a copper-leaching, test system by Brierley and Lockwood (1977), and TH-3 was obtained from a commercial leach dump by Brierley (1978).

Culture Conditions

The microorganisms were grown at 50°. The medium used was a modification of the Tuovinen and Kelly (1973) medium containing (g l^{-1}): K_2HPO_4, 0.04; $MgSO_4.7H_2O$,0.4; $(NH_4)_2SO_4$, 0.4; $FeSO_4.7H_2O$, 27.8; yeast extract, 0.2; the pH was adjusted to 1.6 with H_2SO_4. Growth was followed by titration of residual ferrous iron with ceric sulfate using 1,10-phenanthroline-ferrous sulfate complex ('Ferroin') as indicator. Resting cell suspensions were prepared from 1.5 l Fernback flasks incubated in dry air with constant agitation at 200 rpm. Cells were harvested from early stationary phase growth using a refrigerated Sorvall RC2-B centrifuge and a Sorvall KSB continuous flow system. Cells were washed three times using H_2SO_4 -acidified distilled water.

Respiration Studies

A Gilson Differential Respirometer was used to measure the effect of metals on cellular respiration. Single sidearm reaction vessels were employed; each contained 3.5 ml of medium and 0.5 ml cell suspension. Ferrous iron, at 120 mM, was the oxidizable substrate. Metals were added as $CuSO_4.5H_2O$, $NiSO_4.6H_2O$, $ZnSO_4.7H_2O$, $UO_2SO_4.3H_2O$ and $Na_2MoO_4.2H_2O$. All experiments were run at 50°.

Sampling

Samples for determination of relative abundance of the facultative-thermophilic, *Thiobacillus*-like microbes were collected from the surface of the leach dump of the Chino Mine, Kennecott Copper Corporation, Hurley, New Mexico USA. A Most-Probable-Number method was used to estimate the concentrations of *Thiobacillus ferrooxidans* and the facultative thermophiles.

Protein Concentration

Cellular protein was determined by the method of Lowry *et. al.* (1951).

RESULTS

Occurrence of Facultative-Thermophilic, *Thiobacillus*-Like Microbes in a Leach Dump

Enrichment cultures of surface samples from the Chino Mine leach dump were positive for th *Thiobacillus*-like bacteria capable of oxidizing iron at 50°, pH 1.6. The Most-Probable-Number counting procedure indicated the presence of about 2 facultative thermophiles for every 2.4×10^7 *T. ferrooxidans* per gram dry weight of dump material. Only two surface samples were used, and no samples were collected from the warmer interior regions of the dump..

Leach solution from the Chino Mine dump was collected and examined for its ability to support growth of the facultative thermophiles. The leach solution was supplemented with ferrous sulfate to increase the ferrous iron content to 100 mM; yeast extract, 0.02% (w/v), was added to one set of flasks, and the solutions filter sterilized and inoculated. The results are presented in Table 1. None of the strains of microbes could oxidize the ferrous iron unless the leach solution was supplemented with yeast extract. The spontaneous oxidation of ferrous iron in the control flask was accelerated by the incubation temperature.

TABLE 1

IRON OXIDATION BY THE FACULTATIVE-THERMOPHILIC *THIOBACILLUS*-LIKE MICROBES IN CHINO MINE LEACH SOLUTION

Strain	Addition of 0.02% Yeast Extract	Percent Ferrous Iron Oxidized[1]	
		Day 8	Day 14
TH-1	-	25	44
	+	28	67
TH-2	-	27	47
	+	96	ND[2]
TH-3	-	30	49
	+	97	ND
uninoculated control	-	18	40
	+	21	46

[1] Ferrous iron at initial concentration of 100 mM

[2] ND = not determined

Effect of Metals on Iron Oxidation

Table 2 presents data for the effect of metals on cellular respiration of the three strains of facultative-thermophiles. Copper and nickel decreased cellular respiration at the highest concentrations tested, 363 mM and 382 mM respectively. The rate of iron oxidation was decreased by 25-30% at these concentrations. Zinc had little effect. Iron oxidation by strain TH-1 was not inhibited by zinc at 382 mM, but fell by 26 and 27% for strains TH-2 and TH-3, respectively. Iron oxidation in the presence of uranyl ion decreased 33% for TH-1 at 150 mM uranium. Slight decrease in iron oxidation was noted for strains TH-2 and TH-3 at this concentration. However, the microbes' iron oxidation system was apparently much more sensitive to molybdate with an 84% reduction noted at 5.2 mM molybdenum for TH-1. The rate of iron oxidation by the TH-2 and TH-3 strains was also decreased at this concentration, but these strains appeared to be more resistant, as iron oxidation persisted at 104 mM molybdenum.

Respiration rates were linear over the course of these experiments. Variations noted for respiration rates for a given strain in the absence of added metals was believed to be a result of ageing of resting cell suspensions.

TABLE 2

EFFECT OF METALS ON IRON-OXIDATION BY THE FACULTATIVE-THERMOPHILIC *THIOBACILLUS*-LIKE MICROBES

Metal	Concentration mM	Respiration: μ O_2 min^{-1} $mg\text{-}protein^{-1}$ TH-1	TH-2	TH-3
Cu^{2+}	0	7.2	22.4	35.4
	363	5.3	17.0	26.4
Ni^{2+}	0	5.2	22.7	33.6
	425	3.6	17.4	23.3
Zn^{2+}	0	4.7	24.2	39.3
	382	4.7	17.8	28.6
UO_2^{2+}	0	7.9	6.3	14.2
	150	5.3	5.9	13.2
MoO_4^{2-}	0	6.3	7.5	13.8
	5.2	1.0	5.3	7.9
	52	0.1	6.3	6.2
	104	0.7	3.1	4.4

Growth of Facultative-Thermophilic, *Thiobacillus*-Like Microbes in the Presence of Metals

The growth of the three strains of microbes in the presence of cupric and uranyl ion was evaluated by monitoring oxidation of ferrous iron. These metals were selected for growth study as they are of concern for current metal-leaching operations. Table 3 presents the data for microbial oxidation of iron in the presence of copper. Strain TH-1 was the most sensitive to this metal as 15.7 mM inhibited iron oxidation and growth. Some biological iron oxidation was presumed to occur as the percent iron oxidized at 24 and 48 hours exceeded that for the uninoculated controls. The inoculum may have remained viable and able to oxidize the iron without substantial increase in cell number. However, from 48 to 72 hours, the increase in percent of iron oxidized was similar for both the TH-1 inoculated flasks and the sterile controls. Strain TH-2 was also sensitive to cupric ion at 15.7 mM. TH-2 oxidized more iron than TH-1 but the trend of increase in percent iron oxidized with time was similar to that of the uninoculated controls after 48 hours incubation. Strain TH-3 was more resistant to cupric-iron; 87% of the ferrous was oxidized after 48 hours incubation at 157.4 mM copper. Iron oxidation and apparent growth was inhibited by 236.1 mM cupric ion.

TABLE 3

EFFECT OF CUPRIC ION ON GROWTH OF THE FACULTATIVE THERMOPHILIC *THIOBACILLUS*-LIKE MICROBES

Time hours	Strain	Copper concentration, mM				
		0	15.6	157.4	236.1	393.4
		Percent ferrous iron oxidized[1]				
24	TH-1	10	20	23	27	25
	TH-2	50	39	40	41	41
	TH-3	86	66	47	47	42
	control	1	13	17	13	14
48	TH-1	30	22	26	31	28
	TH-2	68	54	52	57	59
	TH-3	97	98	87	57	58
	control	2	9	18	13	16
72	TH-1	98	30	31	36	34
	TH-2	89	67	68	67	67
	TH-3	ND[2]	ND	ND	ND	ND
	control	13	29	35	29	23

[1] Ferrous iron at initial concentration of 120 mM
[2] ND = not determined

TABLE 4

EFFECT OF URANIUM ON GROWTH OF THE FACULTATIVE THERMOPHILIC *THIOBACILLUS*-LIKE MICROBES

Time hours	Strain	Uranium concentration, mM				
		0	2.0	4.2	21.0	42.0
		Percent Ferrous iron oxidised				
24	TH-1	98	19	17	7	11
	TH-2	26	15	14	10	10
	TH-3	87	13	14	8	12
	control	0	0	0	0	0
48	TH-1	99	40	23	15	15
	TH-2	97	97	10	14	14
	TH-3	98	74	28	13	13
	control	0	0	0	0	6
72	TH-1	ND[2]	79	31	25	22
	TH-2	ND	ND	53	22	22
	TH-3	ND	92	45	26	21
	control	ND	9	9	10	6
96	TH-1	ND	97	32	24	20
	TH-2	ND	ND	62	20	20
	TH-3	ND	96	59	27	23
	control	ND	4	12	11	8

[1] Ferrous iron at initial concentration of 120 mM
[2] ND = not determined

The influence of uranium on iron oxidation and growth of the facultative thermophiles is shown by the data in Table 4. Uranium at 2 mM caused a lag in growth and iron oxidation for all three strains of microbes with TH-1 exhibiting the most sensitivity. Some growth of TH-1 still occurred at 4.2 mM uranium, but this appeared to be near the limit of tolerance. TH-2 and TH-3 were similarly affected, but growth and iron oxidation were increased at 4.2 mM. The iron oxidation values at 21 and 42 mM uranium were low and similar in increase with the uninoculated controls. The limit of growth for strains TH-2 and TH-3 appeared to occur near 4.2 mM uranium concentration. The morphology of strain TH-3 changes from the typical rod shape to granulated, irregular, and sometimes twisted and swollen rods at 4.2 mM uranium concentration.

DISCUSSION

The occurrence of facultative-thermophilic, *Thiobacillus*-like microbes, designated TH strains, in copper leach dump environments was not unexpected. Within the dumps there have been reported regions with temperatures of 60 to 80°C (Beck, 1967; Brierley, 1978), and dump-surface temperatures may exceed 50°C during hot summers. This group requires organic matter be supplied by the leach-dump ecosystem. Heterotrophic microorganisms have previously been found in leach dump environments by Ehrlich (1963), indirectly indicating supply of various organic compounds. The very high ratio of *T. ferrooxidans* to the facultative-thermophilic, *Thiobacillus*-like microbes present in a leach dump sample suggested that the autotrophic *T. ferrooxidans* may provide the organic requirement of the TH series microbes through cellular leakage. The numbers of the TH series microbes would be expectedly much less than *T. ferrooxidans*. Data, to be reported elsewhere, demonstrates this possibility. The availability of the required compound or compounds for the TH-microbes must be very limited. Leach-dump solution cannot support the growth of these microbes unless it is supplemented with yeast extract (Table 1), indicating limited availability of the required organic compounds. The association among the consortium of various microbes in the leach dump must be very tightly knit. This is considered to an area for extensive further research.

Of the various TH strains of microbes examined, the strain TH-3 appeared to have the most resistance to cupric and uranyl ions when evaluated by respiration and growth using ferrous iron as the substrate (Tables 2, 3 and 4). The TH-3 microbe was the only strain obtained directly from an operating leach dump. Although other TH series microbes are widely distributed, not all possess the characteristics which would allow their use as leaching catalysts.

The iron-oxidation system of the various TH strains was not greatly sensitive to copper, nickel, zinc or uranyl ions (Table 2). This system was sensitive to low concentrations of molybdate, which was similar to *T. ferrooxidans* (Tuovinen *et al.*, 1971). However, iron-oxidation as determined by respiration studies with resting cell suspensions in the presence of increasing concentrations of metals was not a good indicator of tolerance of microbes to metals.

Long-term growth studies with TH strains using ferrous iron as an energy source with copper or uranyl ions present indicated much greater sensitivity of the microbes to these ions (Tables 3 and 4). Growth of TH-3 can occur at a copper concentration of 157.4 mM, but strains TH-1 and TH-2 were inhibited by 15.7 mM. Growth of all TH strains was inhibited by uranium concentrations exceeding 4.2 mM. *T. ferrooxidans* was affected similarly by uranium (Tuovinen and Kelly, 1974a; 1974b). None of the cultures used in these studies were adapted to the metals prior to the respiration and growth studies. Adaptation could possibly increase tolerance of the TH strains.

C.L. Brierley (1978) has discussed the possible use of thermophilic microorganisms in metal-leaching operation. It appears that some strains of facultative-thermophilic, *Thiobacillus*-like bacteria found in leach dump environment could have a role in metal recovery processes.

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THE LEACHING OF SULFIDE ORES BY A THERMOPHILIC BACTERIUM*

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INTRODUCTION

The leaching of sulfide minerals by *Thiobacillus ferrooxidans* involves a series of chemical and biochemical reactions. These have been set out in various publications (Tuovinen and Kelly, 1972: Brierley, C.L., 1978: Le Roux *et al.*, 1974). It seemed possible that thermophilic microbes might be contributing to metal leaching in sulfidic environments where the exothermic reactions gave rise to elevated temperatures. The presence of such microbes has since been demonstrated (Brierley and Lockwood, 1977; Brierley, J.A., 1978). In order to examine the possibility of enhancing metal extraction rates at elevated temperatures, a moderately thermophilic bacterial culture, isolated from a geothermal area in Iceland (Le Roux *et al.*, 1977), was examined using various sulfides as substrates. No attempt was made to purify this culture.

MATERIALS AND METHODS

Isolation and Growth of Organisms

The culture was isolated from a water sample collected from an Icelandic hot spring (Le Roux *et al.*, 1977). Stock cultures were maintained in 100 ml of basal salts medium (Silverman and Lundgren, 1959), supplemented with 0.02% yeast extract (Difco) and 0.2 ml trace element solution (Tuovinen and Kelly, 1973), and containing 0.5 g of pyrite or violarite as the sulfide substrate. The pH of this medium was 3.6. The stock cultures in 250 ml conical flasks were shaken on a rotary incubator at 200 rpm and incubated at 50°. Transfers were made every 3 or 4 days on pyrite or weekly on violarite by adding 4 ml of old culture to the fresh medium.

Experimental Procedure

Initially, tests were made batchwise using 100 ml cultures in 250 ml conical flasks, as described above, but using different quantities of ore. In later tests a semi-continuous technique was used, again with 100 ml cultures in 250 ml conical flasks. A proportion of the flask contents was removed each day after settlement of the ore, and replaced by fresh medium and ore. Unless otherwise stated, for all these tests a basal salts medium supplemented with yeast extract and trace elements as described above was used, and the cultures incubated and shaken at 50°. Media added to the cultures were preheated to 50°. Prior to removing spent culture each flask was weighed and the water lost by evaporation was replaced by the addition of distilled water.

In most of the semi-continuous culture tests iron was precipitated as potassium jarosite. In order to remove some of this precipitate each day while retaining unused ore, the following standardised procedure was adopted. Each flask, after removal from the shaker, was given a quick hand shake to distribute the jarosite and ore. The heavier ore was allowed to settle for 15 s and spent liquor containing suspended jarosite was removed by pipette from as close to the liquid surface as possible.

For metal analyses the withdrawn liquid samples were allowed to settle for 5 h at room temperature and samples of the supernatant were analysed for iron colorimetrically using a modified 2,2'-bipyridyl method and for nickel, copper and zinc by atomic absorption spectrophotometry. Filtration of the liquors through bacterial membrane filters was also used instead of settlement but no significant differences between metal values were found. Measurement of pH was done on liquid samples cooled to 25°.

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With nickel sulfides some comparative leaching tests were done at 30° using a mesophilic strain of *T.ferrooxidans*. The culture had been maintained on a basal salts medium with pyrite; then subcultured on pentlandite or violarite prior to use.

For control tests, sterility was achieved by adding 0.01% panacide (BDH Ltd, Poole, Dorset). These sterile control tests were put into separate orbital incubators to avoid contamination of the bacterial tests with vapours of panacide, particularly at 50°.

Minerals

Pyrite

A good commercial grade of Spanish lump pyrite with only 10% impurities. A complete chemical analysis has been published previously (Le Roux *et al.*, 1974).

Uranium ore

From the Elliot Lake region of Canada. It contained 0.12% U_3O_8 and pyrite.

Nickel-ore A

A Canadian ore containing Ni 1.24%, Cu 0.72%, S 12.7%, Fe 26.9% and SiO_2 36.4%. Nickel occurred as pentlandite.

Nickel-ore B

An Australian ore containing Ni 3.8%, Fe 26.7%, Cu 0.2%, Co 0.1% and trace amounts of Pb and Zn. Nickel occurred as violarite (about 8%).

Chalocopyrite

From the site of the old Parys and Mona mine workings as Anglesey, Wales. The sample contained Cu 0.68%, Zn 0.064%, Pb 0.12%. Copper occurred as chalcopyrite, zinc as sphalerite and lead as galena.

Lead-zinc ore A

A concentrate containing 36.0% Zn. The zinc occurred as sphalerite. Pyrite was also a major constituent.

Lead-zinc ore B

An Iranian ore containing 10.5% Zn and 33.3% Pb. Zinc occurred mainly as sphalerite with some smithsonite, and the lead mainly as galena.

RESULTS

Pyrite

No difficulty was experienced when semi-continuous cultures were placed on a 3 and then 2-day residence time with pulp densities of 4% pyrite. The maximum rate of pyrite oxidation attained without pyrite accumulating was about 3.3 g $l^{-1}d^{-1}$ when the culture was on a 3-day residence time with 1% pyrite. The pH remained between 1.3 and 1.5. Even at these low pH values iron was precipitated as potassium jarosite. Attempts to minimise jarosite precipitation by reducing the concentration of potassium present caused the bacterial cells to become elongated and form short chains. Also, bacterial numbers and the rate of pyrite oxidation were reduced.

Uranium

Very good growth in batch cultures was obtained using a basal salts medium containing mixtures of 0.5 g pyrite and up to 3 g uranium ore. Over 10^9 cells per ml were visible after 3 subcultures into flasks containing 0.5 g pyrite and 2 g uranium ore. Because of acid-consuming minerals in the ore, these cultures had final pH values of about 2, despite the presence of pyrite. This meant they could be stored for several weeks without loss of

viability. In contrast, cells grown on pure pyrite attained pH values of less than 1.3 if they were not subcultured in less than a week. Failure to subculture caused a loss in viability.

Pyrite medium supplemented with uranyl sulfate to give uranium concentrations of 0.05 to 2.0 g U l^{-1} supported good growth although there was a slight retardation at 2.0 g U l^{-1}. At this latter concentration and to a lesser extent at 1.0 g U l^{-1}, long chains of cells were observed.

Nickel

Pyrite-grown cells grew readily in batch cultures in 100 ml basal salts media containing either 5 g pentlandite or up to 2 g violarite. The cells often failed to grow with 3 g violarite and no growth was obtained with 4 g. However, a culture initially grown successfully on 2 or 3 g violarite continued to grow and leach when more ore was added, until the concentration of nickel in solution reached about 2 g Ni l^{-1}. Attempts to increase the nickel concentration above 2 g l^{-1} resulted in a rapid decrease in both cell numbers and leaching rate.

Using 5 g pentlandite about 90% nickel was leached in 35 days. With 3 g violarite about 95% nickel was leached in 4 days. In the sterile control 50% nickel was leached in 4 days. Using a mesophilic strain of *T.ferrooxidans* at 30°, comparable nickel extractions were only obtained after 55 days with pentlandite and 26 days with violarite. The results of the tests with violarite are plotted in Fig. 1.

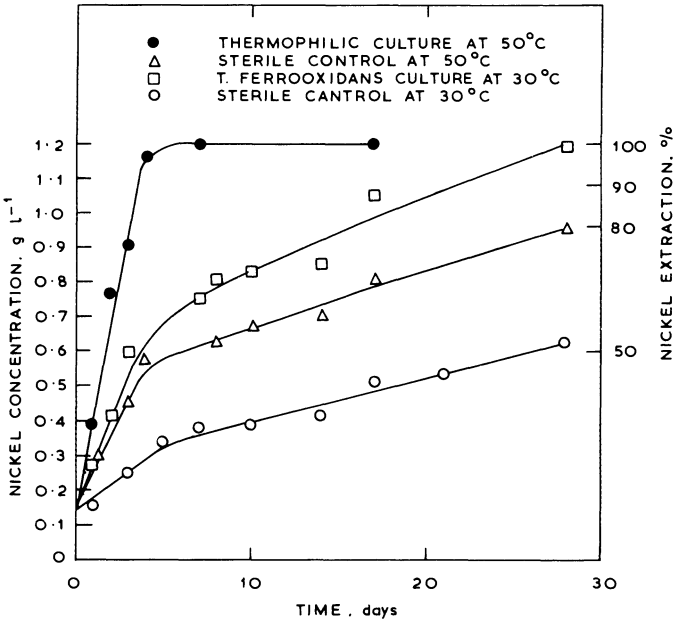

Figure 1: Leaching of violarite at 50° and 30°.

In a series of consecutive tests extending over 264 days, 100 ml cultures were operated at steadily increasing pulp densities of violarite to produce liquors containing increasingly higher nickel concentrations. For each pulp density studied, the residence time was adjusted to maintain a high extraction rate for nickel. During these tests the pH remained between 1.8 and 2.0, even when the added basal salts medium was at pH 7. The results are given in Table 1. Copious amounts of potassium jarosite were precipitated, being over 80% of the iron in the nickel ore which was added.

TABLE 1

THE INFLUENCE OF PULP DENSITY AND RESIDENCE TIME ON THE MICROBIAL LEACHING OF NICKEL ORE (VIOLARITE) AT 50°[a]

Pulp Density %	Residence time days	Nickel concentration in effluent liquor g l^{-1}	Extraction %	Nickel extraction rate g l^{-1} day^{-1}
3.00[b]	2	1.02	85.0	0.51
3.00[b]	3	1.17	97.5	0.39
4.50[b]	3	1.55	86.0	0.52
4.50[b]	4	1.70	94.5	0.43
5.26[b]	4	2.00	95.0	0.50
5.26[c]	4	2.15	99.5	0.54
6.58[b]	4	2.32	88.0	0.58
6.58[b,c]	5	2.40	96.5	0.48
9.21[c]	5	3.00	81.0	0.60
9.21[c]	7	3.40	92.0	0.49
10.50[c]	7	3.30	78.0	0.47
10.50[c]	8	3.70	88.0	0.46

[a] 100 ml semi-continuous cultures were maintained by daily additions of 9K basal salts medium + 0.02% yeast extract + trace elements + violarite. Only typical results during equilibrium conditions at each residence time are given.

[b] Test done with basal salts medium at pH 3.6

[c] Test done with basal salts medium at pH 7.0

Increasing the concentration of yeast extract to 0.03%, raising the pH and increasing the temperature to 55° and 60° had no beneficial effects on accelerating the rate of nickel extraction.

The mesophilic *T.ferrooxidans* was also grown in semi-continuous culture at 30° on violarite at 3.0% and 9.21% pulp density in tests lasting 107 days. The results for these mesophilic tests are presented in Table 2.

At the end of a semi-continuous bacterial leaching test which had been operating at 50°, the bacteria were deliberately killed by adding 1 ml of 1% panacide. The aim of this was to get information on the relative contributions from chemical and biological leaching. The withdrawal of liquor and addition of fresh medium was continued for a further 21 days after the addition of the biocide. The soluble nickel values were substantially reduced. Theoretical nickel values were also calculated for the decrease in nickel concentration which would have been expected by dilution alone, if neither chemical nor biological leaching was taking place. These results are plotted in Fig. 2. Similar results were obtained at 30° and 60°. The results indicate that at 50° the bacteria were responsible for about 75% of the leaching activity.

Chalcopyrite

The initial cultures had a poor tolerance to copper when grown in batch culture on chalcopyrite at 50°. Even mixing pyrite with the chalcopyrite did not help. The maximum soluble concentration of copper obtained was 20 mg l^{-1}.

TABLE 2

THE INFLUENCE OF PULP DENSITY AND RESIDENCE TIME ON THE LEACHING OF NICKEL ORE (VIOLARITE) BY *THIOBACILLUS FERROOXIDANS* AT 30°[a]

Pulp density %	Residence time days	Nickel Concentration in effluent liquor g l^{-1}	Extraction %	Nickel extraction rate g l^{-1} day^{-1}
3.0[b]	5	0.72	60	0.14
3.0	5	0.80	67	0.16
3.0[b]	7	0.97	81	0.14
3.0	7	1.03	86	0.15
9.21	10	2.48	67	0.25
9.21	14	3.55	96	0.25

[a] 100 ml semi-continuous cultures were maintained by daily additions of 9K basal salts *etc.* as per footnote to Table 1.

[b] Additions of 0.02% yeast extract and trace elements were omitted from these tests.

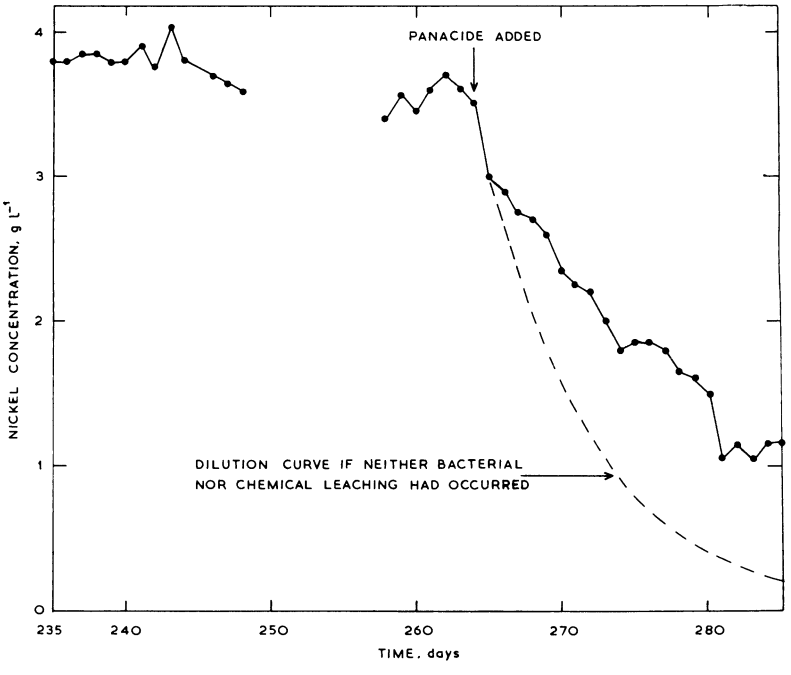

Figure 2: Effect of the addition of a biocide to the semi-continuous bacterial leaching of violarite at 50°.

The ability of mesophilic strains of *T.ferrooxidans* to adapt to increasing concentrations of heavy metals is well documented (Tuovinen and Kelly, 1972 ; Tuovinen *et al.*, 1971). Attempts were made, therefore, to increase the tolerance of this thermophilic culture to copper.

In one series of tests the bacteria were subcultured on a pyrite medium containing progressively increasing amounts of chalocopyrite. In another series, the bacteria were cultured on a pyrite medium to which was added increasing amounts of copper sulfate. In this way good growth was eventually obtained with 100 ml cultures containing 0.5 g chalcopyrite and 0.5 g pyrite. Some growth was obtained using higher concentrations of chalcopyrite but subcultures of these were unreliable. Eventually growth was obtained in pyritic media containing 2.5 g Cu l^{-1} but the rate of growth was again slow and the long chains of cells formed suggested a stress condition.

Lead-Zinc

In batch culture tests on media containing a mixture of 0.5 g pyrite and 0.5 g lead-zinc ore A, good cell growth was obtained in 5 days. When the amount of lead-zinc concentrate was increased to 1 g, equivalent bacterial growth was only obtained after 19 days.

In semi-continuous cultures using inocula from the batch tests, reasonably good growth was obtained on both lead-zinc ores A and B when sulfuric acid was used to maintain the pH at 2.5. However, less than 40% of the available zinc was leached in 6 days.

DISCUSSION

There was little difficulty in maintaining good growth of cultures on nickel sulfide and rates of nickel extraction were reasonable. These early studies indicated a need for mineral salts and yeast extract, a point to be noted for any commercial exploitation. Subsequent work has defined more clearly nutrient requirements for some of the strains present in this thermophilic culture. The precipitates of jarositic iron which occurred during the leaching of nickel were easily filtered and there was no significant loss of nickel with the precipitate. The thermophilic culture also showed good growth on the uranium ore, demonstrating a good tolerance to uranium. One method published for a bacterially assisted process for uranium recovery (Derry *et al.*, 1977), carried out the leaching of the uranium ore at 50° using an acidic sulfate lixiviant which was regenerated in a separate stage with a mesophilic strain of *T.ferrooxidans* at 30°. The possibility exists of maintaining the whole integrated circuit at 50° but attention would need to be given to the loss of iron as a jarosite precipitate.

It is relevant to consider the potential involvement of thermophilic strains of microbes in microbial leaching. "Hot" zones are known to exist in dumps containing sulfide minerals and recently thermophilic strains have been isolated from the dump environment (Brierley, J.A., 1978). However, their activity in situ and the magnitude of their involvement in the overall dump leaching process is unknown.

Underground solution mining carried out at depth will involve elevated temperatures. The potential contribution from thermophilic microbes is unknown but is being studied. With the bacterial leaching of metal sulfide concentrates having been demonstrated on a continuous basis (McElroy and Bruynesteyn, 1978), the exploitation of thermophiles needs to be considered for this process. The initially poor tolerance of this mixed culture towards copper was a disappointment but subsequent work with an isolated strain has given better results (Brierley *et al.*, 1978). Very little work was done using the Pb-Zn ores but the first response was a lack of tolerance. The possibility that traces of silver in the lead sulfide could have exerted biocidal effects was not studied.

This report has outlined briefly some of the initial work using a mixed moderately thermophilic culture containing thiobacillus-type bacteria. More detailed work has since been done. (Brierley and Le Roux, 1977; Brierley *et al.*, 1978). It is likely that other thermophiles, more tolerant of heavy metal concentrations and with better leaching characteristics, will be obtained from other natural environments.

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A SCANNING ELECTRON MICROSCOPY STUDY OF THE COLONY MORPHOLOGY OF *THIOBACILLUS FERROOXIDANS*

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INTRODUCTION

Colony formation is an important characteristic of a bacterial species. Using Scanning Electron Microscopy, (SEM), it is possible to examine detailed structures not observable by any other microscopic method (Roth, 1971). Such structures include the following: the inter-relationships between cells in a colony; the attachment of cells to the supporting surface and their modification of that surface as the colony grows; the way in which the bacterial cells are arranged within the colony as their numbers increase during growth; and the changes occurring in colony structure because of their growth.

Although several methods have been reported in the literature to obtain *Thiobacillus ferrooxidans* colonies on agar media (Tuovinen and Kelly, 1973; Manning, 1975), our attempts to isolate *T.ferrooxidans* populations from samples from the Rum Jungle mine area, Northern Territory, Australia, on plates containing agar as the gelling agent have been unsuccessful.

A method of preparing consistently high quality silica gel plates was devised by A. M. Khalid in our laboratory. These plates are now used routinely for the enumeration of *T.ferrooxidans* populations in samples from natural environments and in reactor studies; for the isolation of strains and subsequent purification of cultures by single colony selection methods; and for the study of the development of *T.ferrooxidans* colony types and their changes upon ageing. This paper presents the results of a study of the colony formation of a strain of *T.ferrooxidans* by SEM, and gives the method of preparation of silica gel plates (Khalid and Ralph, 1976).

MATERIALS AND METHODS

Organism

The strain of *T.ferrooxidans* used in this study was isolated from a rock sample from the Rum Jungle mine area, N.T., Australia. The culture was purified by repeated subculture of single colonies obtained by the method described in this paper.

Preparation of Silica Gel Plates

A glass, ion exchange resin column, 2" in diameter and 24" long, packed with Amberlite IR-120(H) ion exchange resin was prepared as per Skerman (1967). The column was regenerated by running through 500 ml of spent 2N HCl (from previous runs), followed by 1 litre of fresh, sterile 2N HCl. The column was then flushed with 1 litre of sterile distilled water until the effluent pH was neutral and chloride free when tested with $AgNO_3$ solution. The column was kept just covered by sterile distilled water.

A solution of sodium metasilicate containing 7% SiO_2 is prepared by dissolving 130 g of $Na_2SiO_3 \cdot 5H_2O$ (reagent grade) in 500 ml of distilled water. The turbid solution is autoclaved and becomes clarified without adversely affecting the setting quality of the silica gel.

The sterilised metasilicate solution is run through the charged column and the effluent collected in a sterile flask after the pH has dropped to 3.0, or less, usually after about 100 ml has passed. Collection of the silicic acid solution is continued until the pH of the effluent rises to above 3.5. Care must be taken as the pH rises rapidly and at higher pH the whole silicic acid solution can gel within seconds. A hot zone passes down the column and when this is near the bottom the run is almost complete. Sterile distilled water is added to the top of the column while the silicic acid solution is being collected, in order to maintain the liquid level above the resin. It is important that the resin does

not dry out. Approximately 450-500 ml of sterile silicic acid solution, pH 3, is normally collected.

The column is rinsed with 500 ml (or more if necessary) sterile distilled water immediately after use, until the pH of the effluent is neutral, and then the column is regenerated immediately after each run. Silicic acid solution is stored at 4° and may be kept up to 3 weeks without setting.

Silica gel plates were prepared by modifying the existing procedures (Leathen *et al.*, 1956; Pramer, 1957; Roslycky, 1972; Thatcher and Weaver, 1974). Basal 9K ingredients (Silverman and Lundgren, 1959) to make 1 litre, were dissolved in 400 ml distilled water and sterilised by autoclaving. 100 ml of 40% (w/v), $FeSO_4 \cdot 7H_2O$ was sterilised by filtration through a membrane filter (Sartorius - SM-11106, 0.45μ) and was mixed aseptically with the sterile basal 9K medium.

500 ml sterile silicic acid solution was mixed aseptically with the 9K medium and 25-30 ml aliquots were dispensed into sterilised plastic petri dishes. The medium was solidified by heating the plates at 60-70° in a hot air oven for 6-8 h (with the petri dish lids on). The plates were further sterilised by tyndalisation, by heating them at 70° for 4-6 h the following day. During this time the plates should be left undisturbed in the oven. The plates should not be dried as in conventional procedure for making microbiological plates, but rather if there is any liquid occurring on the surface it should be gently tipped off. The plates can then be stored at 4°, packed in sealed plastic bags and may be kept up to several weeks before use without any deleterious effects.

These plates were found to be very satisfactory and were superior in texture to those prepared by other procedures, where setting was achieved by autoclaving in glass petri dishes (Corrans, 1970). The pH of the plates was routinely checked and found to be between 2.5 and 2.0.

Growth of Single Colonies

Serial dilutions of *T.ferrooxidans* cultures, or prepared soil samples or water samples (Khalid and Ralph, 1976) were made using sterile distilled water pH 2.5 (using H_2SO_4 to adjust pH). The (appropriate) dilutions were filtered under vacuum by the standard microbiological method using a pyrex filter holder (millipore - XX 1004720) and Sartorius membrane filters (types SM 11407, SM 13756, SM 11456). Initially, membranes (type SM 11407) were boiled in distilled water for 15 minutes, to remove materials inhibitory to *T.ferrooxidans* (Tuovinen and Kelly, 1973), and then sterilised in distilled water by autoclaving. Later work showed, however, that the pre-sterilised packets of membrane filters (types SM 13756 and SM 11456) did not require boiling in distilled water before use and so these were then routinely used directly from their sterilised packets. The pyrex filter holder was wrapped in aluminium foil and sterilised by autoclaving. During filtration the bottom of the filter was transferred to a sterilised beaker containing cotton wool soaked in 70% ethanol, and then flamed before being put back on a new membrane. Membranes were transferred from their packets to the filter holder and subsequently from there to the silica gel plates by forceps flamed each time in 70% ethanol.

Each membrane was washed on the filter holder with 5 ml of sterile distilled water, pH 2.5. Then the vacuum was turned off and a further 5 ml was added to the filter holder. 1 ml of the appropriate dilution of the bacterial suspension was added to the distilled water, suction was re-applied, and the bacteria were distributed evenly over the membrane. The membrane containing bacteria was then rinsed with a further 3-5 ml of sterile distilled water pH 2.5 before being transferred to a silica gel plate.

Groups of nine plates were packed back into their plastic bags, sealed with tape, and incubated at 30°. This procedure ensures a relatively high humidity and growth may be monitored for several months without the plates drying out. Colony numbers were routinely counted after 2-3 weeks incubation by means of a binocular microscope (Olympus 251604). Counting is greatly facilitated by the use of the above types of Sartorius membranes which are marked with a grid pattern.

Scanning Electron Microscopy

Colonies of *T.ferrooxidans* grown on Sartorius membrane filters were cut out with a scalpel

blade (using a binocular microscope). The piece of membrane filter containing the colony was attached to a metal specimen stub with double-sided sticky-tape and allowed to air-dry, in a desiccator, for 24 h.

The specimens on the stubs were then gold-coated using a Polaron E 5000 Diode Sputtering System; the gold coating was between 200 and 500 Å thick. Specimens were examined in a Cambridge S4-10 Stereoscan at 4.5 - 10KV. A magnification of 5K on the SEM corresponds to a bar size of 2μm on the photomicrograph. Photomicrographs were taken using a Polaroid positive/negative film, type 105. Prints were developed on Agfa-Gevaert Rapiton P1-2 paper.

RESULTS AND DISCUSSION

The method of silica gel plate preparation, as standardised in our laboratory, has been found to be reliable and to consistently produce plates of a high quality and texture. These plates have been routinely used in the enumeration of *T.ferrooxidans* populations from hundreds of soil and water samples from the Rum Jungle mine area. We have found these plates selective for *T.ferrooxidans* colonies. Several strains of *T.ferrooxidans* from these samples have been purified by repeated single colony isolation from these plates. The colony morphology of strain number BJR-K3 is described below. Colony morphology has been found to differ amongst our isolated strains.

Fig. 1 shows the appearance of a colony after 4 weeks incubation. At this stage of growth the colony is fairly flat, completely round and chemical precipitation is just beginning. The diameter of the colony is approximately 0.45 mm. The square to rectangular-shaped crystals beginning to form in the centre of the colony were found to contain iron and sulfur by EDAX analysis (Energy Dispersive Analysis by X-rays).

Fig. 2 is an enlargement of the surface of the colony in Fig. 1. The rod-shaped cells grow in an ordered, parallel arrangement. The cells are not uniform in length, but have a fairly regular width. Most, but not all, of the cells collapsed during the vacuum-coating treatment. The few uncollapsed cells may be coated by a chemical precipitate which forms a hard, protective "shell". The surface of the colony, at this stage, is free from any covering of chemical precipitates obscuring the cells.

An 8 week-old colony is shown in Fig. 3. The colony now has a "lumpy, coated" appearance, caused by massive chemical precipitation, probably of jarosites and lipopolysaccharide complexes, but certainly of iron and sulfur complexes (found by EDAX analysis). The diameter of the colony is approximately 1 mm. Colony growth of these organisms tends to be more upward than spreading outward, as can be seen from the height of this colony compared to that in Fig. 1. (The cracking of the colony was caused by the vacuum-coating treatment).

The surface of the colony, seen in Fig. 4, is now completely covered by chemical precipitates. The composition of the large smooth particles, and the rough-looking material beneath these, is unkown, but it occurs all over the surface, obscuring bacterial cells and throughout the colony (see Fig. 5). The comparison with the appearance of the surface of the 4 week colony (Fig. 2) is instructive.

Fig. 5 shows a cross-section of an 11 week-old colony. It was cut using a sharp scalpel blade under a binocular microscope. A definite surface layer is clearly visible and chemical precipitation is seen throughout the colony. The height of the colony is approximately 0.2 mm. A magnification of a central area of this colony is shown in Fig. 6. The precipitation consists of large, smooth, mainly spherical particles with rod-shaped bacteria on their surfaces as well as irregularly-shaped chemical "crystals". These crystals appear extremely bright and probably contain iron, since bright spots are usually caused by dense material which reflects electrons from the beam more strongly than the surrounding area.

Fig. 7 shows another 11 week-old colony (diameter approximately 1.1 mm) which was immersed in drops of 10 N H_2SO_4 for 30 min, drained, rinsed three times with distilled water and air-dried. The centre of the colony beneath the surface has been exposed by the acid treatment. It is the surface crust, as seen in Fig. 5, which has been dissolved and the smooth patches at the top and lower edges are the undissolved remains of this surface layer. Beneath this layer there is considerable precipitation which is not soluble in acid.

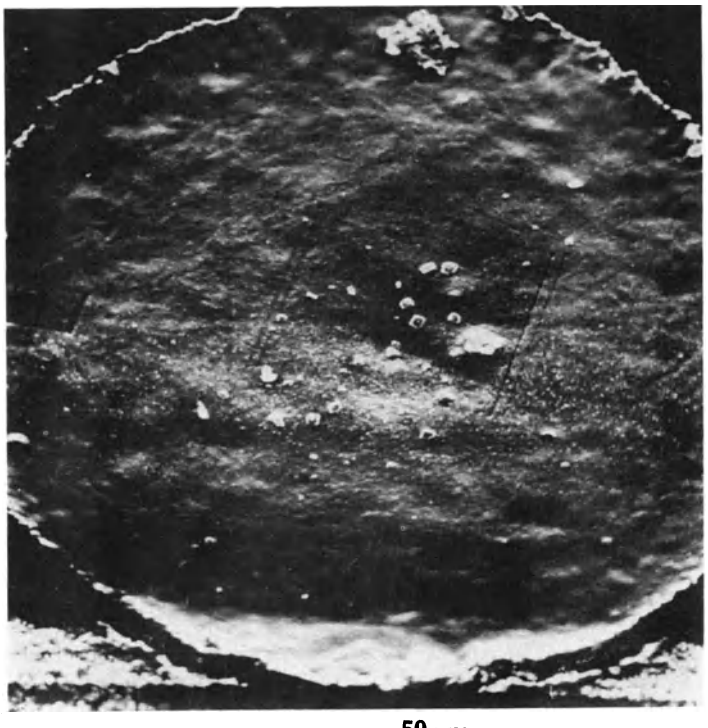

Figure 1: *Thiobacillus ferrooxidans* colony after 4 weeks incubation.

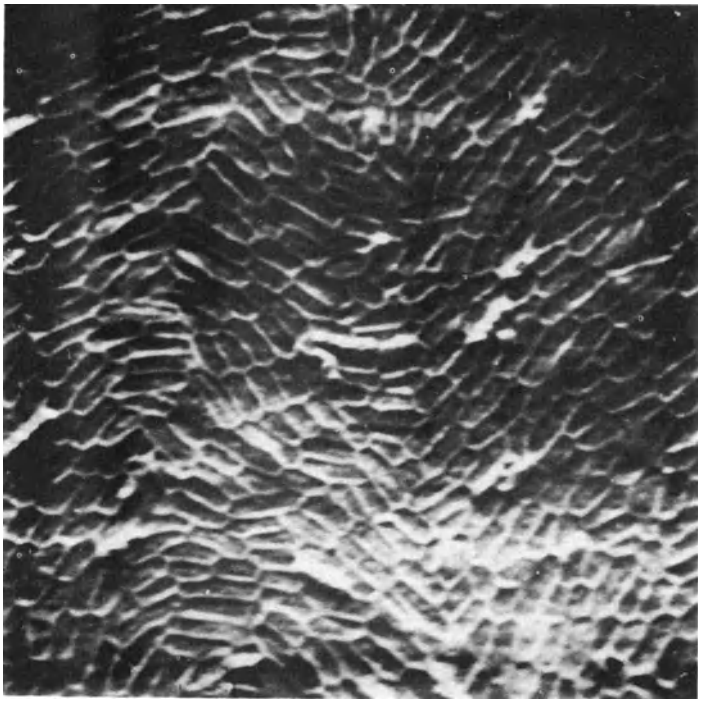

Figure 2: Enlarged view of centre of *T.ferrooxidans* colony after 4 weeks incubation.

Figure 3: *T.ferrooxidans* colony after 8 weeks incubation.

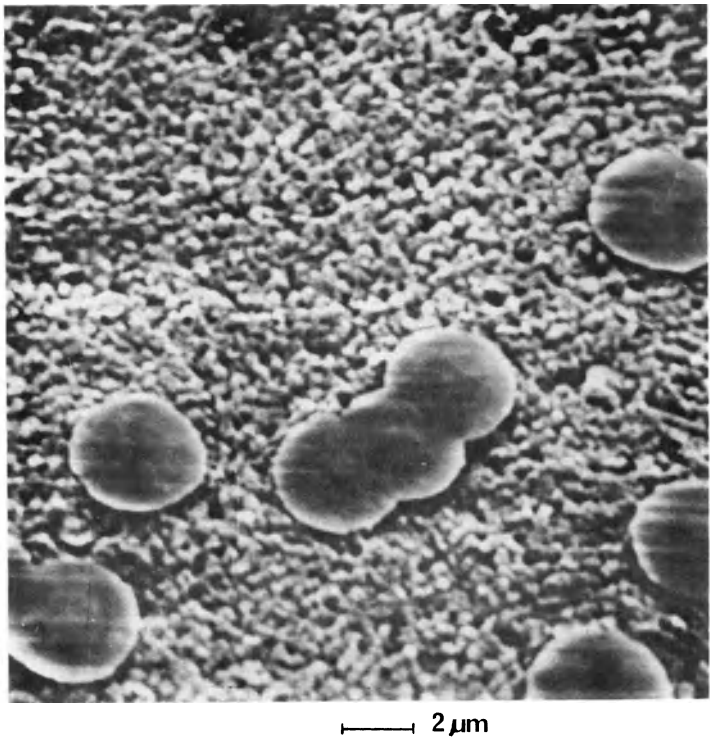

Figure 4: Enlarged view of surface of *T.ferrooxidans* colony after 8 weeks incubation.

Figure 5: Cross-section of an 11 weeks old colony of *T.ferrooxidans*.

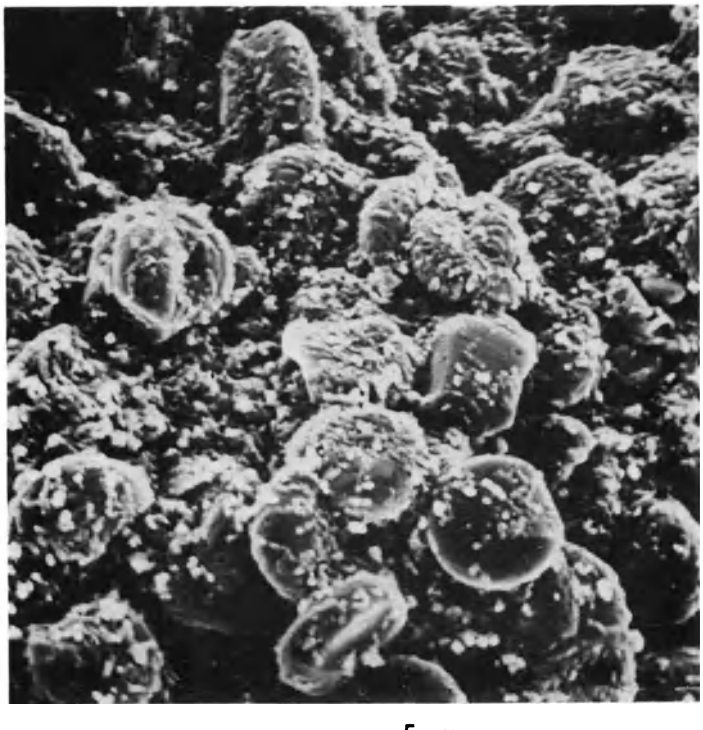

Figure 6: Enlarged view of centre of 11 weeks old colony of *T.ferrooxidans* shown in Fig. 5.

Figure 7: An 11 weeks old colony of *T.ferrooxidans* after treatment with 10 N H_2SO_4 for 30 min.

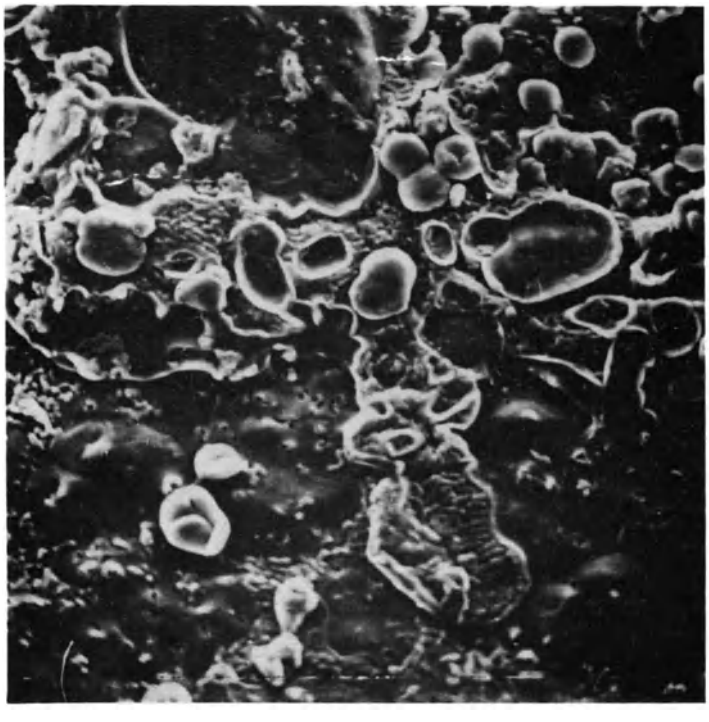

Figure 8: Enlarged view of edge of acid-treated 11 weeks old colony of *T.ferrooxidans* shown in Fig. 7.

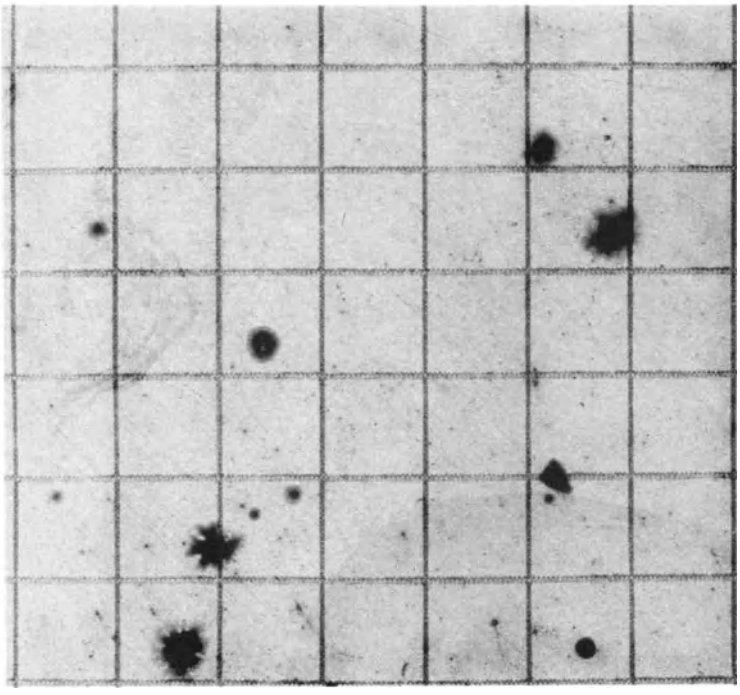

Figure 9: Photograph of portion of a membrane filter containing two colony types of *T.ferrooxidans*.

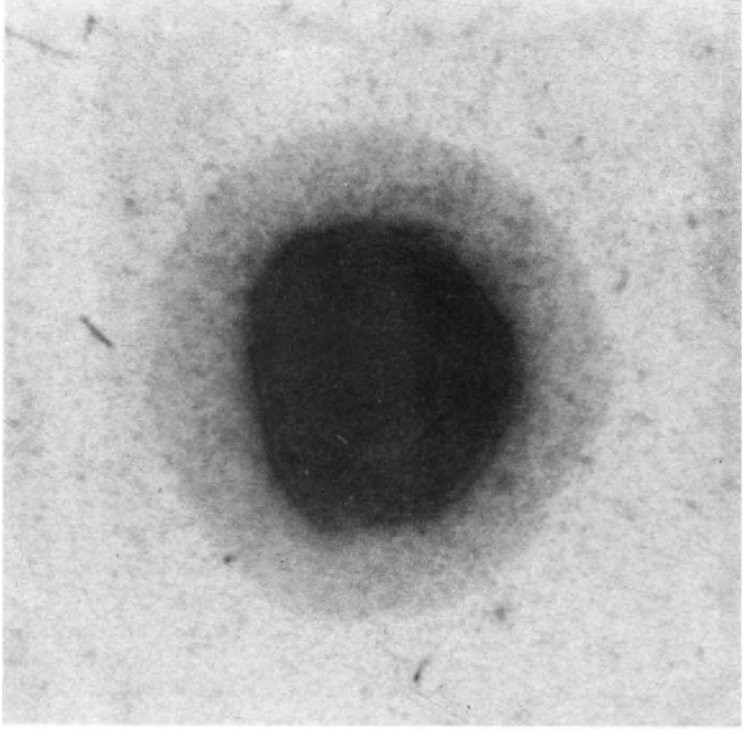

Figure 10: Enlarged view of small colony type of *T.ferrooxidans* shown in Fig. 8.

A magnification of an area at the edge of this colony is shown in Fig. 8. In some acid-exposed areas the regular, parallel arrangement of the bacterial cells is clear, but in other areas the cells are growing in random arrangements. Many cells occur beyond the edge of the colony and are lying on top of the membrane filter which appears flat, smooth and non-porous because it is also coated by a chemical precipitate. Again, large, smooth, spherical particles can be seen to be numerous and are insoluble in acid. They can be clearly seen to be many times larger than the rod-shaped bacterial cells. These spherical particles may be some kind of extra-cellular lipopolysaccharide complex, since *T.ferrooxidans* is known to excrete large amounts of such compounds when grown in liquid ferrous medium (Agate *et al.*, 1969).

Fig. 9 shows a portion of a membrane filter containing several colonies. The colonies range in size from 0.5 mm to 1.0 mm and have been incubated for four weeks. These colonies are from a water sample from White's Opencut and show two types of colony morphology. There are small colonies which are completely round and raised (one of these is shown magnified (Fig. 10) and others are larger (1.0 mm), irregularly shaped with lobed edges. These colonies are the same rusty-brown colour as strain-BJR-K3 examined by SEM.

By studying the development of *T.ferrooxidans* colonies, the different types and their changes upon ageing, by SEM, we hope to gain a better understanding of mechanisms of the development of these microorganisms in natural environments.

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A MICROCALORIMETRIC STUDY OF U(IV)-OXIDATION BY *THIOBACILLUS FERROOXIDANS* AND FERRIC-IRON

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INTRODUCTION

Uranium commonly occurs in the tetravalent form [U(IV)] in uranium-bearing rocks and the solubilization involves its oxidation to the hexavalent uranyl ion. For example, uraninite is solubilized by oxidation in dilute sulfuric acid and, more rapidly, in acidic ferric sulfate:

$$UO_2 + 2Fe^{3+} \longrightarrow UO_2^{2+} + 2Fe^{2+}$$

$$UO_2 + Fe_2(SO_4)_3 \longrightarrow UO_2SO_4 + 2FeSO_4$$

$$UO_2 + 2H^+ + 0.5O_2 \longrightarrow UO_2^{2+} + H_2O$$

$$UO_2 + H_2SO_4 + 0.5O_2 \longrightarrow UO_2SO_4 + H_2O$$

Iron- and sulfur-oxidizing thiobacilli enhance uranium leaching by regenerating acidic ferric sulfate from $FeSO_4$ produced in the chemical leaching and by oxidizing ore materials such as pyrite and other sulfide minerals to Fe^{3+} and sulfuric acid:

$$2Fe^{2+} \longrightarrow 2Fe^{3+} + 2e^-$$

$$2Fe^{2+} + 2H^+ + 0.5O_2 \longrightarrow 2Fe^{3+} + H_2O$$

$$2FeSO_4 + H_2SO_4 + 0.5O_2 \longrightarrow Fe_2(SO_4)_3 + H_2O$$

$$2FeS_2 + H_2O + 7.5O_2 \longrightarrow Fe_2(SO_4)_3 + H_2SO_4$$

Previous studies have shown that fast rates of uranium leaching, superior to those of the chemical leaching alone, are attained in the presence of iron-oxidizing thiobacilli (*Thiobacillus ferrooxidans*) (Tomizuka & Takahara, 1972; Guay *et al.*, 1975, 1976, 1977; Tomizuka *et al.*, 1976). Dissolved uranium is a potentially toxic cation which may act as an uncoupling agent and interfere with the binding of Fe^{2+} on the cell surface (Tuovinen and Kelly, 1973a, 1974a,b). Strain-related differences in the response to uranium by iron-oxidizing thiobacilli have been reported (Ebner and Schwartz, 1974). In general, these bacteria can be adapated to tolerate over 5 mM UO^{2+} by successive subcultures with increasing amounts of uranyl salts (Tuovinen and Kelly, 1974a).

The ferric iron-dependent U(IV) oxidation is essentially an electron-transfer reaction without the direct involvement of oxygen. However, oxygen is required as an electron acceptor in the acid leaching and in the bacterial ferrous iron oxidation. There is currently no evidence for direct U(IV) oxidation by thiobacilli (Kelly *et al.*, 1979) although the standard free energy change ($\Delta G'$) is theoretically sufficient to support bacterial respiration:

$$UO_2 \rightarrow UO_2^{2+} + 2e^- \qquad \Delta G' = -86.2 \text{ kJ mol}^{-1}$$

Similarly, the oxidation-reduction potential (E_h) of 446 mV for UO_2/UO_2^{2+} is within the range suitable for allowing the entry of electrons into the respiratory chain. The corresponding values for ferrous iron oxidation are as follows:

$$Fe^{2+} \rightarrow Fe^{3+} + e^- \qquad \Delta G' = -74.4 \text{ kJ mol}^{-1}$$

$$Fe^{2+}/Fe^{3+}\ E_h = 771 \text{ mV}$$

These values for uranium and iron are only relative guidelines as they do not include the effect of pH and the physiological concentrations of the compounds. In fact, $\Delta G'$ values of -32.80 to -24.64 kJ mol^{-1} at pH 1.5-3.0 have been presented for ferrous iron oxidation

(Lees *et al.*, 1969) but the effect of pH is complex since the intracellular pH is higher, at least pH 5.5 according to recent estimates (Ingledew *et al.*, 1978), thereby establishing a ΔpH across the cell membrane coupled with proton translocation (Ingledew *et al.*, 1977; Cox *et al.*, 1979). Calculations of the thermodynamic efficiency of growth indicate that the $\Delta G'$ values at acidic pH are probably underestimated (Kelly, 1978).

In the present study the bacterial oxidation of ferrous iron and UO_2 was investigated using a microcalorimeter to monitor heat generation. In the microcalorimeter system, the heat output is proportional to the enthalpy change and thus a measure of an exothermic reaction which may take place chemically or be mediated by bacteria. The results show that both substrates result in heat generation in suspensions of *T.ferrooxidans*. The data are appraised with a view to analyzing the experimental approach and the involvement of soluble iron in U(IV) oxidation.

MATERIALS AND METHODS

Bacteria

An iron-oxidizing strain of *Thiobacillus ferrooxidans* was previously adapted to grow at pH 1.5 (Tuovinen and Kelly, 1973b). At this pH, both Fe^{2+} and Fe^{3+} remained soluble throughout the growth of the bacteria. The bacteria were grown with 120 mM $FeSO_4$ at 28° in 13 l fermenters (12 l culture volume, aeration 13 l min^{-1}). The cells were harvested at the late exponential growth stage by centrifugation followed by two washes with pH 1.5 sulfuric acid. The cells were finally suspended in dilute sulfuric acid (pH 2.5) and the suspension were standardized by dry weight (105°) and protein (Lowry *et al.*, 1951; Herbert *et al.*, 1971) determinations. For the residual iron determination by atomic absorption spectrophotometry (Perkin-Elmer Model 403), the cell suspension was digested with concentrated HCl, HNO_3 and $HClO_4$ followed by reduction with hydroxylamine hydrochloride and chelation with 2,2'-bipyridyl before the measurement.

Chemicals

Powdered UO_2 (natural isotopic composition) was a generous gift from Dr J. D. Scott (Eldorado Nuclear Ltd., Ottawa, Ont.). The sample was 87.47% pure and had an O/U ratio of 2.045. The specific surface area of the UO_2 powder was 5.69 $m^2\ g^{-1}$ and the following impurities were determined ($\mu g\ g^{-1}$): Th (360), C (50), Fe (40), Ni (10), Al (<10), Si (<10), Ca (5), Cr (<5), F (<5), Mg (3), Cu (2), Mo (1.1), Mn (<1), B (<0.2), Cd (<0.2), Dy (<0.15) Gd (0.10). The sample contained not more than 0.3% U_3O_8. All the other reagents were from commercial sources and of analytical grade.

Microcalorimetry

A Model 2107 LKB Microbiological Flow Calorimeter System with an aeration vessel of 1.0 ml was used throughout the experiments. The calorimeter was thermostated at 28°. The system is schematically illustrated in Fig. 1. The suspension was kept in a 25 ml conical flask with a magnetic stirrer in a water bath at 28° and circulated with a peristaltic pump at a rate of 0.23-0.97 ml min^{-1} to the calorimeter. The heat effect was amplified and monitored with a recorder. After each experiment, the reaction cell was replaced and the system was rinsed with 1 N HCl, 1 N NaOH and distilled H_2O. The microcalorimetric monitoring system has been described in detail in various LKB Application Notes.

RESULTS

Heat generation was rapidly detected when washed cells of *T.ferrooxidans* were mixed with ferrous iron (Fig. 2). The rate of heat generation (i.e., the rate of iron oxidation) remained constant above 24 mM Fe^{2+} (Fig. 3). At lower Fe^{2+} concentrations the rate was dependent on the amount of the substrate. The amount of heat generated was proportional to the ferrous iron concentration and approached saturation level above 50 mM Fe^{2+}. Moreover, the aerobic nature of iron oxidation is evident from Fig. 2, which shows no heat effect when the aeration was turned off (arrows 1 and 2).

Rapid heat generation was recorded in the presence of UO_2 and *T.ferrooxidans* cells (Fig. 4) UO_2 alone, or UO_2 with boiled cells showed no heat effect. The irregular trace in Fig. 4B resulted from uneven flow of UO_2 the boiled cell suspension in the tubing. A similar problem of uneven flow was also encountered in the iron oxidation measurements (Fig. 2, the

first heat output) in which ferric iron partly precipitated causing disturbance. The chemical reaction between UO_2 and ferric sulfate is illustrated in Fig. 4C which shows an extremely rapid rate of heat generation during Fe^{3+} - dependent uranium(IV) oxidation.

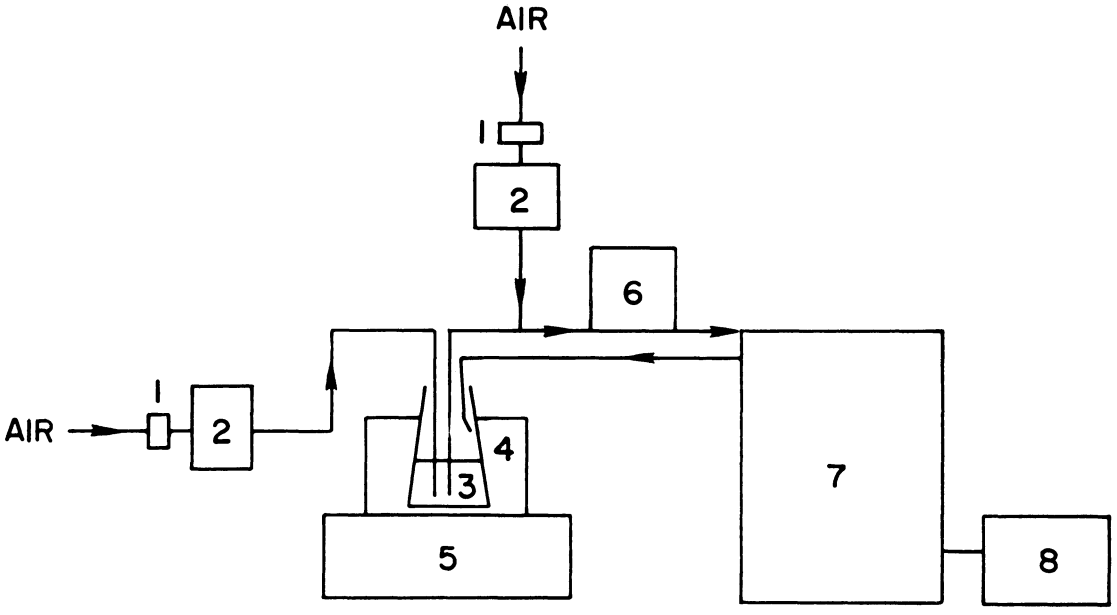

Figure 1: Schematic presentation of the microcalorimetric monitoring system. 1, Air filter (0.45 μm); 2, Air pump (LKB 12000 or LKB 2120 Varioperpex); 3, 25 ml flask with a magnetic stirrer; 4, Water bath; 5, Temperature and magnetic stirring control (LKB 1607 Polyferm); 6, Peristaltic pump (LKB 10200 Perpex or LKB 12000 Varioperpex); 7, Flow microcalorimetry system (LKB 2107-020: thermostat, control unit, and flow microcalorimeter unit); 8, Integrating recorder (LKB 2210).

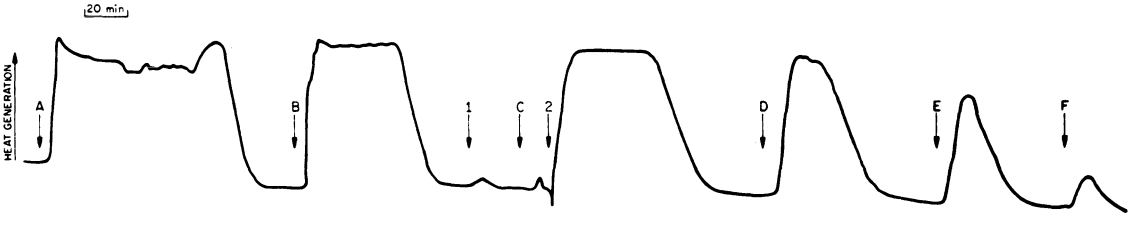

Figure 2: Heat generation during bacterial ferrous iron oxidation. 0.2 ml of washed cells of *Thiobacillus ferrooxidans* (4.9 mg dry weight of bacteria) were added (*arrow A*) to 10 ml of the mineral salts solution (Tuovinen and Kelly, 1973b) acidified to pH 2.5 with sulfuric acid and containing 1.2 mmol $FeSO_4$. Subsequently the following additions were made: *arrow B*, 0.6 mmol Fe^{2+}; *arrow C*, 0.6 mmol Fe^{2+}; *arrow D*, 0.3 mmol Fe^{2+}; *arrow E*, 0.18 mmol Fe^{2+}; *arrow F*, 0.06 mmol Fe^{2+}. Air was pumped into the tubing but was switched off between *arrow 1* and *2*.

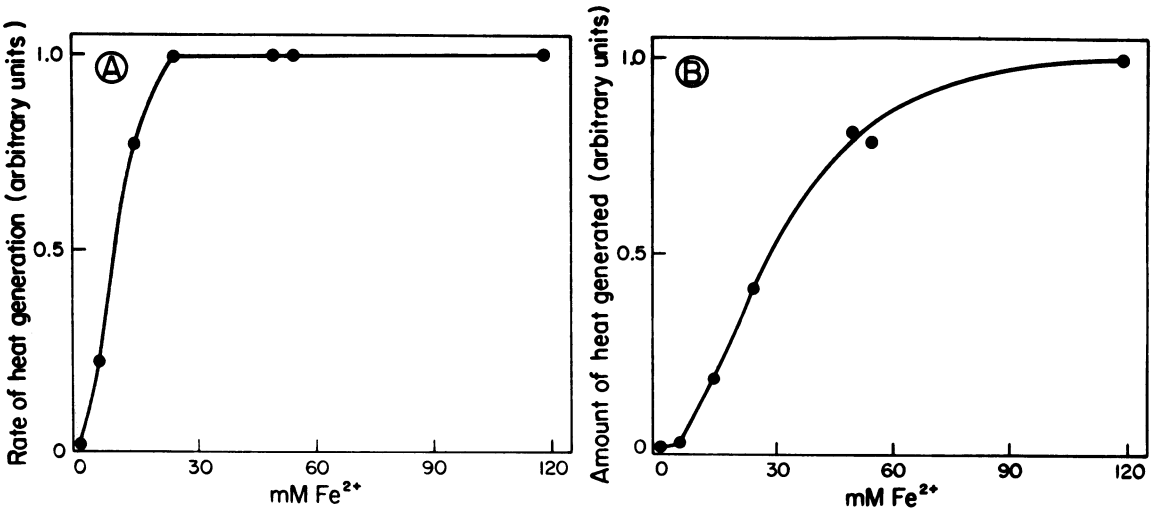

Figure 3: Relationship between heat generation and substrate concentration. *A*, Rate of heat generation in arbitrary units plotted against the initial substrate concentration at each addition. *B*, Amount of heat in arbitrary units plotted against the initial substrate concentration at each addition. Data calculated from the results presented in Fig. 2.

The iron determination of the cell suspension indicated that, in experiments illustrated in Fig. 4A and 4B, the residual iron in the washed cells was as follows (per reaction misture): Total iron: 28 µg = 0.5 µmol; Soluble iron: 4.4 µmol. Allowing for the iron impurity in UO_2, the total iron content in the reaction mixture amounted to 48 µg Fe (0.86 µmol). Thus, the U/Fe ratio was around 1880 in experiments involving UO_2 as the substrate, and the ratio is further increased two-fold if the calculations are based on the stoichiometry of the reaction between UO_2 and Fe^{3+}. Even this is likely to be a gross underestimate of the ratio of U and reactive iron since the soluble iron initially amounted to only 9% of the total iron content.

DISCUSSION

The microcalorimeter system was easily adapated to monitor *T.ferrooxidans* activity. The aerobic nature of this organism was evident in preliminary trials which showed that additional air was required to ensure rapid bacterial iron oxidation. The microbiological flow calorimeter unit was also suitable for monitoring solid substrate oxidation as long as additional air was provided to meet the oxygen demand and to prevent UO_2 deposition inside the tubing in the instrument. Temporary changes in aeration rates easily resulted in disturbance in the recording. Interference in the system was also brought about by ferric iron precipitation as well as by boiled cells which were not sufficiently homogenized after boiling. Thus, the stability of the recording system could be improved by (a) ensuring an even flow of suspensions with aeration; (b) using Fe^{2+} at a low pH to prevent ferric iron precipitation; and (c) vigorously homogenizing suspension before use. The technique could perhaps be extended to monitor heat effects with other solid minerals, subject to maintaining their even distribution in suspension and in the tubing by proper mixing and aeration. With appropriate standardization and calibration procedures, the present approach may be useful in initial evaluation of bacterial degradation of various types of ores; bioleaching studies presently rely heavily on metal extraction rates which do not directly indicate reactions coupled with bacterial respiration.

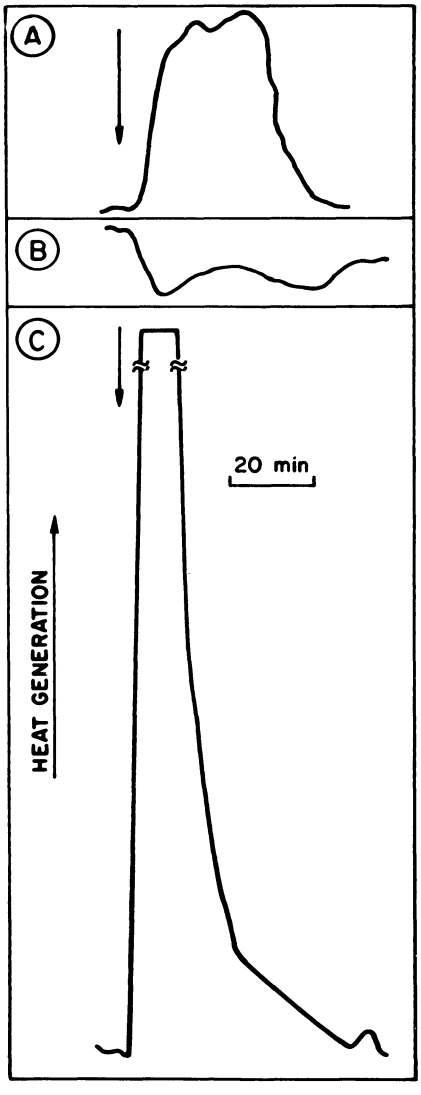

Figure 4: Heat generation during UO_2 oxidation. *A*, Washed cells of *Thiobacillus ferrooxidans* (0.4 ml: 10.3 mg dry weight of bacteria = 6.2 mg protein) were added (*arrow*) to a mixture containing 1.62 mmol powdered UO_2 (0.5 g samples) suspended in 20 ml of the mineral salts solution (pH 2.5). *B*, The above experiment was repeated using 0.4 ml of boiled cells. *C*, 2.4 mmol of Fe^{3+} (4 ml as ferric sulfate) were added (*arrow*) to a mixture containing 1.62 mmol UO_2 in 16 ml of the mineral salts solution. The calculated heat effects are: *A*, 306 μW; *B*, 0 μW; *C*, > 1.17 mW.

In the present work, heat generation was recorded in *T.ferrooxidans* suspensions with UO_2 as the substrate. The respective boiled cell control measurement though being an unstable trace, showed no heat output. The chemical reaction by ferric iron showed very rapid UO_2-oxidation. In view of only trace amounts of iron present in the cell suspension and in the UO_2 powder, the experiments indicated that uranium(IV) may have been directly oxidized by the bacteria. Alternatively, iron could have been recycled at extremely fast rates through ferrous and ferric states by bacterial oxidation and uranium-dependent reduction. However, most of the iron impurity was in the insoluble form in the bacterial suspension, and therefore the residual soluble iron (initially less than 0.08 µmol in the reaction mixture) was unlikely to be the major oxidizing agent for a 1900 to 20000-fold equimolar excess of uranium. In Fig. 5, a scheme is proposed in which UO_2 oxidation proceeds via two routes: coupling either directly with bacterial respiration or indirectly by first reducing Fe^{3+} to Fe^{2+}. The predominance of the route is determined by the ferric iron concentration and subsequent regeneration of Fe^{2+} to Fe^{3+}. Direct bacterial uranium(IV) oxidation may be limited owing to the toxicity of uranium ions but this problem is alleviated in leaching situations where the seepage of the leach solution ensures continuous removal o dissolved uranium in addition to the environmental pressure on selection of uranium-tolerant bacteria. The uranium toxicity may have caused the bacterial U(IV) oxidation to cease in the microcalorimetric experiments since a U-tolerant strain was not used. While the present work highlights the possibly direct transformation of uranium compounds by bacterial activity, further work is warranted to elucidate the mechanism of oxidation by cell suspensions completely free of inorganic iron salts. Moreover, the importance to uranium extraction of direct bacterial U(IV) oxidation remains to be evaluated as iron salt often exceed dissolved uranium levels in acid leaching circuits.

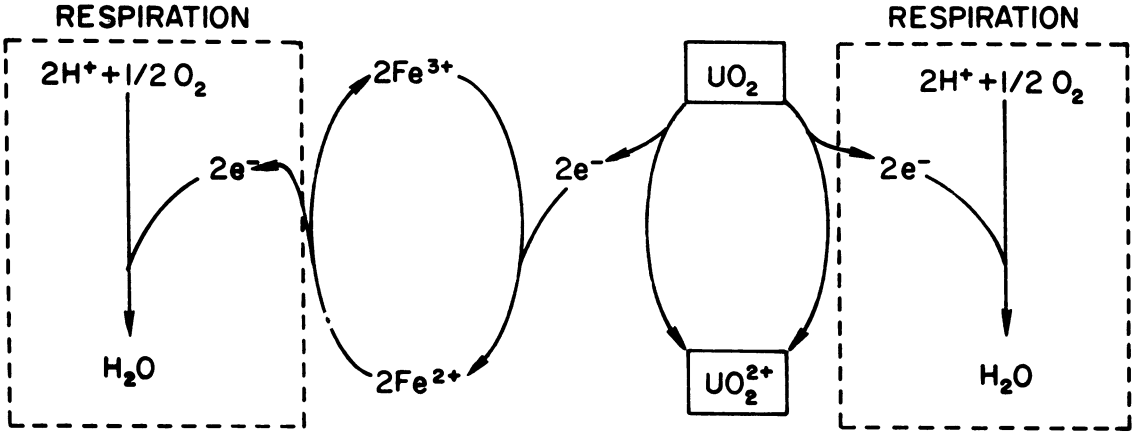

Figure 5: Path of electrons during UO_2 oxidation.

ACKNOWLEDGEMENTS

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A MICROCALORIMETRIC STUDY OF THE METABOLIC ACTIVITY OF TWO *THIOBACILLUS* SPECIES

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INTRODUCTION

The use of microcalorimetric techniques to investigate microbial processes has been increasing rapidly since easily-operable, high precision instruments became commercially available (Wadsö, 1970, 1973). Almost every process, chemical, physical or biological, occurs with either a gain or a loss of heat to the system. The heat quantities involved are related to the rate and extent of the process, and using microcalorimetry it is possible to measure heats of reaction in the order to microwatt quantities. Also, the process can be monitored for a considerable length of time without disturbing the system.

Microcalorimetry has been applied, with considerable success, to many areas of microbiology, including the following: the study of the antibacterial action of tetracyclines (Mårdh *et al.*, 1976); the bioassay of nystatin bulk material (Beezer *et al.*, 1977); the anaerobic growth of *E.coli* on various substrates (Belaich and Belaich, 1976); the detection of growth by mycoplasmatales (Ljunghold and Wadsö, 1976); the study of the activity of microorganisms in soil (Mortensen *et al.* 1972); the diagnosis of septicaemia and meningitis (Kallings 1976); and the identification and classification of bacterial species (Monk and Wadsö 1975, Russell *et al.*, 1975).

As yet microcalorimetry has not been applied to the field of mineral degradation, nor to the study of autotrophs. This paper presents some preliminary results of our work in this area.

We are attempting to obtain a "library" of thermogram patterns of each species of *Thiobacillus*, grown under controlled, standardised conditions. By matching a thermogram of an identified species to a thermogram of an isolate from the natural environment we may rapidly identify such isolates.

This initial study is also being used to investigate the mechanisms of thiosulfate oxidation by various species of *Thiobacilli*. The oxidation of inorganic sulfur compounds by *Thiobacilli* is extremely complex and many studies have been made on the mechanisms involved. At the present time the mechanisms of these oxidation processes have not been clarified and controversies over oxidation pathways abound. For a comprehensive review of this area, see Trudinger (1967) and Roy and Trudinger (1970).

MATERIALS AND METHODS

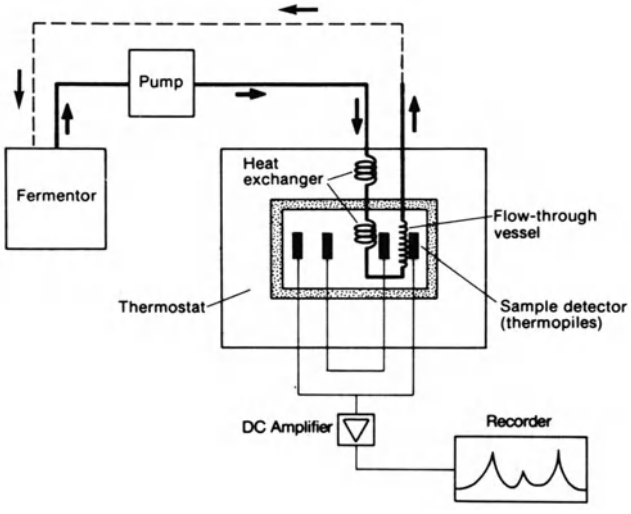
Calorimeter

The LKB Microcalorimeter (2107 - 021) was used with the microbiological flow module (2107 - 127). The calorimeter was thermostated at 30°. The voltage signal (amplified by an internal amplifier) was recorded by means of a chart recorder with a range of 100 mV. The measured microvolt output, the "released energy", as shown in the graphs, was converted to microwatts ml^{-1}.

The operation of the calorimeter is shown in Fig. 1.

The culture was pumped by means of a peristaltic pump from the fermenter vessel, through the calorimeter and recycled to the fermenter at 20 ml h^{-1}. A 20 ml h^{-1} flow of sterile air was pumped into the tubing containing the bacterial suspension (using a T-piece joint), to produce a total flow rate of 40 ml h^{-1} through the calorimeter. The addition of air in this manner, was essential to maintain aerobic conditions within the tubing, as 2 to 3 min elapsed from the time the cell suspension left the fermenter to the time it entered the flow cell where heat generation was measured. The volume of the flow cell was 1.1 ml and so the retention time in the flow cell was approximately 1.5 min. The tubing and the flow

cell of the calorimeter were sterilized by pumping 70% ethanol for 2 h and then rinsing with sterile distilled water for 1 h. The sulfur-oxidizing bacteria studied were *T.thioparus* and *T.thiooxidans*, which were isolated from a soil sample from northern N.S.W. and from a sulfur pad at Sulphide Corporation Ltd., N.S.W., respectively (Khalid, 1978). *T.thioparus* was grown on Vishniac and Santer medium (Vishniac and Santer, 1957), and *T.thio oxidans* on 9K medium (with thiosulfate as energy source) (Silverman and Lundgren, 1959).



After microcalorimetric analysis, samples may be collected or returned to the fermentor vessel.

Figure 1: General layout of equipment for microcalorimetric analyses.

Culture Procedure

The organisms were grown in 1 litre of the appropriate medium in a fermenter vessel designed and constructed in the School of Biological Technology. The culture was aerated using a "Flostat" flow controller, the air being sterilized by an in-line filter (Whatman, Gamma 12). The aeration flow rate was between zero and 900 ml min^{-1} depending upon the experiment. Agitation and air dispersal was achieved by the use of a "Chemap" Vibromix unit. The temperature was maintained at 30^{o} by use of a resistor sensor, controlled via an "Ether Mini" controller, and heat was supplied by use of an infra-red lamp.

Samples of 2 - 3 ml were taken daily to measure pH, using a Metrohm pH meter; cell numbers were measured by counting under phase microscopy (Olympus) using a "Thoma", (by Weber England), counting chamber with a depth of 0.02 mm; and thiosulfate concentration was estimated by the method of Roy and Trudinger (1970).

The fermenter vessel containing the growth medium was sterilized by autoclaving. A concentrated thiosulfate solution (20% $^{w}/_{v}$ $Na_2S_2O_3.5H_2O$) was sterilized by filtration through a membrane filter (Sartorius SM-11106, 0.45µ) and was added aseptically to the cooled fermenter to produce a final thiosulfate concentration of 1%.

The fermenter was inoculated with 5 ml of a stock, concentrated cell suspension, or with 15 ml of a log-phase shake flask culture. Inoculation procedure did not cause any variation to the thermograms produced, although the former method is the one preferred. Time "0" shown on the graphs refer to the point of inoculation.

RESULTS AND DISCUSSION

Oxidation of Thiosulfate by T.thioparus

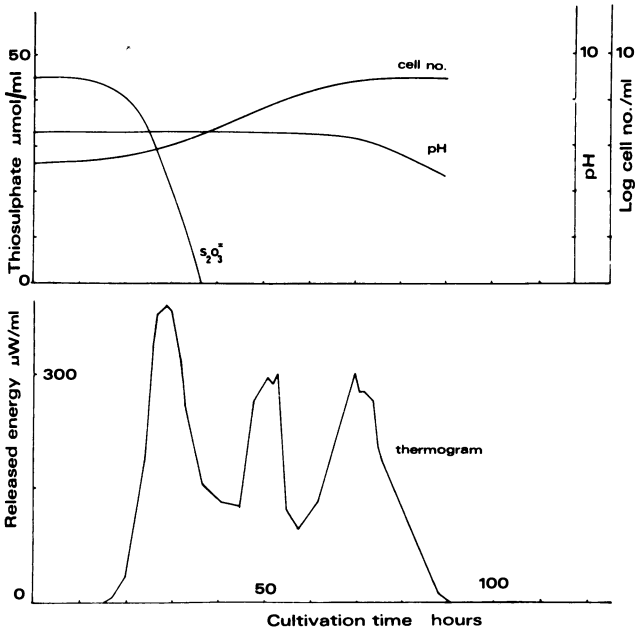


Figure 2: Growth of *Thiobacillus thioparus* on thiosulfate under fully aerobic conditions.

Fig. 2 shows the metabolic activity of *T.thioparus* grown on thiosulfate with an aeration flow rate of 900 ml min^{-1}. The thermogram obtained shows clearly that there are three steps in the oxidation of thiosulfate to sulfate. The first curve of the thermogram is the oxidation of thiosulfate to a polythionate, most probably tetrathionate (Trudinger, 1967; Sato *et al.*, 1976; Khalid, 1978), and by its completion the thiosulfate has been completely utilised.

The second product formed, that is, the oxidation of tetrathionate during the second curve, is unknown, although again, it is probably a polythionate (Khalid, 1978). The final oxidation product is sulfate, formed during the third growth period with a corresponding production of H^+ ions, causing the rapid fall in pH from 6.5 (initial) to 4.5.

It is important to note that the pH decreased only during the final growth phase and it is probably this increasing acidity which killed the bacteria. A return to baseline by the thermogram indicates cessation of metabolic activity. It is known that *T.thioparus* is not acid tolerant and that the bacteria are killed by low pH conditions (Parker and Prisk, 1953; Khalid, 1978). It is probable that the short lag phases between each growth curve result from the induction of the enzyme system required for the degradation of each new oxidation product.

Previous work in our laboratory (Khalid, 1978), has shown that the oxidation of thiosulfate by this strain of *T.thioparus*, under aerobic conditions, proceeds in at least two stages. By measuring thiosulfate decrease, polythionate production and dissolved oxygen tension, it

was shown that thiosulfate was initially converted to polythionates (mainly tetrathionate) and that during this phase cell numbers increased and the pH remained unchanged. This was followed by a short lag phase and then the polythionates were oxidised to sulfate with the production of acid, and a corresponding drop in the pH causing eventual cell death.

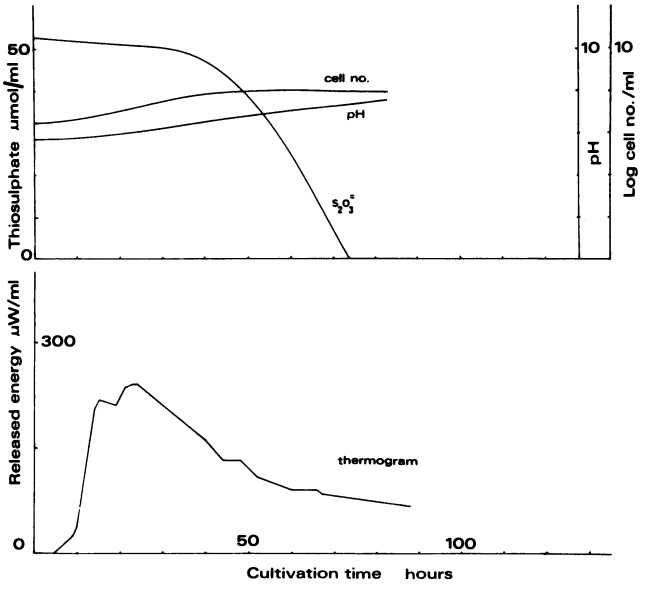


Figure 3: Growth of *Thiobacillus thioparus* without aeration.

Fig. 3 shows the growth of *T.thioparus* without aeration, but with just sufficient agitation to ensure thorough mixing. Under these conditions, the initial rapid growth peaks and then metabolic activity slowly declines over the next 65 h. The oxidation of thiosulfate is slow, but when it is completely utilised there is still low metabolic activity of the bacterial cells. However, without aeration, further oxidation of polythionates to sulfate does not occur.

It is thought that the thiosulfate has been oxidised to tetrathionate, as this reaction produces OH^- ions which would account for the pH rising from 5.5 (initial) to 7.5, throughout the entire growth curve. This reaction is given as:

$$2\ S_2O_3^{2-} + H_2O + \tfrac{1}{2}O_2 \longrightarrow S_4O_6^{2} + 2OH^-$$

(Trudinger, 1967).

Fig. 4 shows the oxidation of thiosulfate by *T.thioparus* during changing aeration rates and low agitation, again only to ensure mixing. Initially, no air was bubbled through the fermenter. Growth proceeded for 7 h (after an initial lag) and then the metabolic activity of the cells went into a stationary phase. At time 40 h, indicated by arrow 1 on the graph air was introduced at the rate of 150 ml min^{-1}, and metabolic activity increased for a further 28 h before declining. During this growth period the pH rose from 6.5 (initial) to 7.5, and it is probable that tetrathionate was the main oxidation product of thiosulfate.

At time 85 h (arrow 2 on the graph), the aeration rate was increased to 350 ml min^{-1} and metabolic activity increased for a further 30 h, during which time the pH decreased, indicating the formation of sulfate and acid production. After this final growth phase the metabolic activity declined rapidly until cell death occurred at pH 4.5.

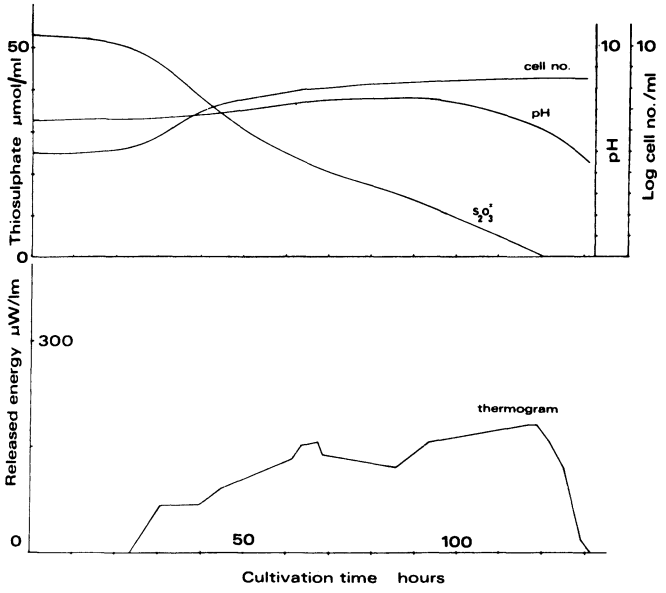


Figure 4: Growth of *Thiobacillus thioparus* with changing aeration rates.

Throughout the whole growth period, thiosulfate was utilised at a slow rate and it is probable that after the aeration rate was increased to 350 ml min^{-1}, tetrathionate and thiosulfate were being oxidised concurrently.

Oxidation of Thiosulfate by T.thiooxidans

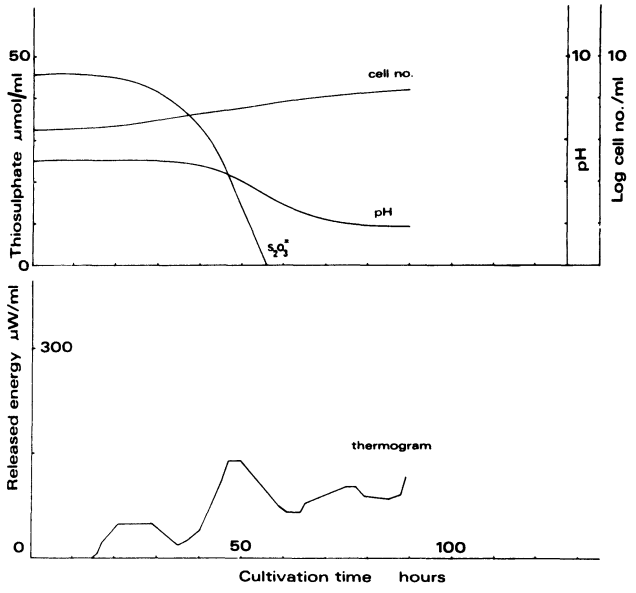


Figure 5: Growth of *Thiobacillus thiooxidans* on thiosulfate under aerobic conditions.

To allow a direct comparison of the thermograms produced by growth on thiosulfate by *T.thioparus* and *T.thiooxidans*, the latter was grown on Vishniac and Santer medium with an initial pH of 4.5. However, no metabolic activity was found after 70 h, so 9K (initial pH 4.5) was then used as the growth medium. The aeration was at a rate of 900 ml min^{-1}.

Fig. 5 shows that the oxidation of thiosulfate to sulfate by *T.thiooxidans* also occurs in more than one step. Thiosulfate was completely utilised by 55 h and the pH, which had remained at 4.5 during growth to this point, began to fall. Metabolic activity then increased for 13 h, during which time the pH fell to 2.5. This was followed by a slight decline in activity and a stationary phase and then metabolic activity began to increase rapidly. Because of technical difficulties the experiment was terminated at this point. Since low pH conditions are not lethal to *T.thiooxidans* it is thought that metabolic activity would have continued until the total oxidation of polythionates to sulfate had occurred.

In the present study the formation of elemental sulfate was not detected.

Further Work

We are currently examining the metabolic activity of *T.ferrooxidans* growing on various sulfide minerals using the fermenter system and the flow module of the microcalorimeter. Bacterial activity (as measured by the thermogram) is being correlated with oxygen uptake rate, acid production and the solubilisation of minerals. We are investigating whether or not the activity of *T.ferrooxidans* is related to counts of the viable numbers of cells in suspension, that is, to those cells which are not attached to mineral particles.

Preliminary work using the ampoule module has shown a promising correlation between heat generation and oxygen uptake. The main difficulties, at this stage, appear to be the need for high pulp density to maintain high bacterial numbers and also the need for the mineral to be very finely divided. Microcalorimetry is also being applied to the examination of mixed culture biodegradations.

ACKNOWLEDGEMENTS

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LEACHING OF COPPER-BEARING MINERAL SUBSTRATES WITH WILD MICROFLORA AND WITH LABORATORY-BRED STRAINS OF *THIOBACILLUS FERROOXIDANS*

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INTRODUCTION

At the present time the biological leaching of copper is applied on industrial scale in several ways: mainly as dump and heap leaching of mining wastes, and more rarely, as underground leaching of low-grade ore material in situ. Nevertheless, in all cases the leaching processes are carried out by the respective indigenous microflora. The latter includes wild strains of various microorganisms but the most important role in leaching is played by the chemolithotrophic bacterium *Thiobacillus ferrooxidans* (Trudinger, 1971; Corrans *et al.*, 1972; Rossi, 1974; Groudev *et al.*, 1978a). Its activity can be enhanced by an artificial improvement of some of the rate-limiting environmental factors, and, nowadays, this is the one and only applicable way to increase bioleaching under natural conditions. It must be noted, however, that a quite different way exists, at least theoretically: to "seed" leaching operations with laboratory-bred strains (or with wild strains taken from other ecosystems) possessing a leaching activity higher than that of the indigenous strains. That second way is closely connected with the problem of strain screening and improvement of *T. ferrooxidans*.

It is known that from a taxonomic point of view the species *T. ferrooxidans* can be regarded as a group of microorganisms characterized by marked physiological elasticity (Groudev, 1974; Silver, 1978). The various representatives of that group may differ greatly from one another with respect to many characteristics, including the level of their oxidizing activity towards different substrates (Groudev and Genchev, 1978a, 1978b; Norris and Kelly, 1977). This ferrobacilli special feature underlines the importance of preliminary strain screening of these bacteria for the purpose of leaching. Unfortunately, the strain screening is carried out under laboratory conditions (and only rarely under semi-industrial conditions) which are greatly different from the natural situations. This indicates that the most active among the screened strains may not be obligatory and the most active under natural conditions.

It is clear that the natural leaching environment will itself be the most potent selective system in the production of strains best suited to the prevailing conditions (Tuovinen and Kelly, 1972). For that reason the screening must cover obligatory ferrobacilli strains indigenous to the leaching ecosystem, and the most active of these strains must be used in experiments for strain improvement.

In a broad sense the strain improvement includes such routine interventions as adaptation to the particular mineral substrate being leached (Atkins and Pooley, 1977) and to high hydrogen and heavy metal ion concentrations (Tuovinen and Kelly, 1972; Groudev, 1974), as well as such potential genetic procedures as isolation of special mutants and plasmid introduction (Chakrabarty, 1978). Regardless of some recent progress (Chrabrova, 1972; Tuovinen and Kelly, 1974; Groudev, 1978) the genetic basis of these phenomena in respect of desired alterations of the cell qualities is not thoroughly clarified. Meanwhile, both natural and induced variability have been used successfully for breeding of strains possessing a high oxidizing activity towards different mineral substrates (Groudeva, 1978; Panova, 1972; Groudeva *et al.*, 1978a, 1978b). In these studies, UV rays and/or ethylenimine were used as mutagens and they were found to be efficacious only towards some strains. Such marked selectivity necessitates additional investigations in that field.

The present paper reports some data on the strain improvement of *T. ferrooxidans* as a result of a step-wise selection based on the combined use of UV rays, ethylenimine and natural variability. The leaching activity of some of these laboratory-bred strains is compared with that of the wild microflora.

MATERIALS AND METHODS

Microorganisms, Microbiological and Genetic Procedures

Eight strains of *T. ferrooxidans* isolated from acid mine drainage waters or ore lumps were used (Table 1). Each of these strains served as an initial breeding material for a step-wise selection based on the combined use of UV rays, ethylenimine and natural variability.

TABLE 1

STRAINS USED IN THE PRESENT STUDY

Strain	Substrate	
	Fe^{2+}	S^{o}
	maximum substrate oxidation rate, mg l^{-1} h^{-1}	
A-1	264	3.74
E-4	211	1.90
F-4	190	1.48
L	271	3.09
M-8	242	2.97
P	215	2.40
V-8	262	4.51
V-21	288	4.40

* - determined by shake-flask technique under conditions described elsewhere (Groudev and Genchev, 1978)

The mutagenic treatments were carried out by the procedures described by Groudeva (1978). The strains were grown on 9K medium (Silverman and Lundgren, 1959) with Fe^{2+} or S^{o} as an energy source and the cells were harvested by centrifugation at the end of the logarithmic growth phase. The cell pastes were washed three times in dilute H_2SO_4 (pH 2.0) and twice in distilled water to remove iron precipitates or precipitated sulfur. Cells were resuspended in water to a concentration of approximately 2×10^8 cells ml^{-1} (estimated by direct counting under phase-contrast microscope). For UV treatment, 10 ml of the cell suspensions were put into Petri dishes and exposed to a Phillips 15-W TUV germicidal lamp for different periods of time at a dose rate of 1 J m^{-2} s^{-1}. Ethylenimine treatment was carried out with various ethylenimine concentrations (from 0.02 up to 0.10%) for different periods of time (from 10 min up to 4 h) in phosphate buffer (pH 7.0). The ethylenimine action was stopped by dilution (up to 1 : 200) of the system with the same buffer, and the cells were removed by two centrifugations and washings in dilute H_2SO_4 (pH 2.0). After mutagenic treatments, 0.1 ml of the cell suspensions were plated on 9K solid medium or on AFS solid medium (Groudeva, 1978) with Fe^{2+} or S^{o} as an energy source. The viable cell numbers of some strains, whose growth on solid media was poor, were determined by the technique of Tuovinen and Kelly (1973), using Sartorius membrane filters. Dilutions of samples were prepared in 0.01 N H_2SO_4 and filtered within 1 h. The plates were incubated in a humidity jar at 35° for at least 18 days and then scored for colony-forming ability of the cells plated. The cell survival rate was determined by comparing with the survival rate of untreated cells. The mutants possessing high oxidizing activity towards Fe^{2+} and/or S^{o} were scored by the total selection method and the activity of each separate variant, i.e. of each single colony grown on the plates, was determined by the methods described by Groudeva (1978) and Groudeva *et al.* (1978c). The natural variability of the strains was determined by the same methods after plating of untreated cells.

The activity of each separate variant was estimated in comparison with that of the respective wild strain, which activity was accepted to be 100%. The distribution of the variants in separate classes was carried out at intervals of 20% (Groudeva, 1978).

The most active variants were subcultured (at least 3 - 4 times) into 9K liquid medium containing Fe^{2+} or S^{o} or $CuFeS_2$ as an energy source and their activity was tested before each subculturing. The subcultures in which oxidizing activity had been decreased in comparison with that of the previous subcultures were discarded. The stable mutants only were used as a breeding material for the following step of the step-wise selection.

The physiological and biochemical characterization of the strains, both wild and laboratory-bred, was carried out accordingly the methods described by Groudev (1974). The activity of the enzymes tested was determined by the methods used previously (Groudev and Genchev, 1978), and was expressed as nmoles of substrate transformed per minute per milligram of protein. Cell fractions S_{10} of crude extracts of $CuFeS_2$-grown cells were used.

The numbers of ferrobacilli in the solutions were estimated by direct counting under phase-contrast microscope and by the most probable number (MPN) method. The cells attached to the mineral substrates were estimated by the bacterial nitrogen determination (Gormely and Duncan, 1974).

The wild microflora of the mineral substrates was analyzed by the procedures described by Karavaiko *et al.* (1972), and the isolated microorganisms were identified by reference to taxonomic guides (Buchanan and Gibbons, 1974; Gilman, 1957; Lodder and Kreger, 1952).

Mineral Substrates

The following mineral substrates were used: two specimens of natural chalcopyrite, two chalcopyrite flotation concentrates, and three copper sulfide ores.

The two natural chalcopyrites contained only 1.5% and 1.3% impurities, respectively.

The concentrate No 1 contained 16.52% copper, 29.06% iron and 31.20% sulfur, and the concentrate No 2 contained 21.20% copper, 34.21% iron and 32.30% sulfur. The ore No 1 contained 0.53% copper, 7.34% iron and 3.14% sulfur. The main ore minerals were chalcopyrite and pyrite. The ore No 2 contained 0.50% copper, 6.19% iron and 8.06% sulfur. The main ore minerals were chalcopyrite, covellite, chalcocite and pyrite. The ore No 3 contained 0.68% copper, 3.81% iron and 4.17% sulfur. The main ore minerals were the same as those of ore No 2.

Some samples of the above-mentioned ores were taken from upper layers of dumps and were only slightly oxidized. Their chemical, phasic and mineralogical compositions were similar to those shown above. These partially oxidized ore samples possessed an abundant and varied microflora (referred as wild microflora A - Table 2), and were used as substrates in some of the column tests only.

Leaching Experiments

Shake flask tests were carried out in 300 ml Erlenmeyer flasks containing 95 ml iron-free 9K medium, 20 g finely ground substrate (size of particles less than 37 μ) and 5 ml of an active culture of *T. ferrooxidans*, previously adapted to the respective substrate or grown on Fe^{2+}, and containing 2 x 10 cells ml^{-1}. pH of the leach suspension was 2.3. The flasks were cultivated on a reciprocal shaker with 160 strokes per minute, in the dark, at 35°, and were aerated with air containing 0.2% CO_2 (Torma *et al.*, 1972). The water losses due to evaporation were compensated by addition of distilled water, and pH was readjusted with N sulfuric acid or N sodium hydroxide. In the sterile control flasks, 5 ml of a methanol solution containing 2% of thymol were added instead of the inoculum.

Percolator experiments were carried out in air-lift percolators containing 280 ml iron-free 9K medium, 600 g sulfide ore (crushed to 3 - 0 mm) and 20 ml of the inoculum above-mentioned. The initial pH of the leach solution was 2.3, and the incubation temperature was 35°.

TABLE 2

WILD MICROFLORA* OF THE SULFIDE ORES USED IN SOME OF THE COLUMN LEACHING TESTS

Microorganisms	Ore No 1	Ore No 2	Ore No 3
		cells g^{-1} ore	
T. ferrooxidans	2.10^8	2.10^8	3.10^8
T. thiooxidans	6.10^2	8.10^2	2.10^3
Sulfate-reducing bacteria (*Desulfovibrio, Desulfotomaculum*)	0	2.10^2	2.10^2
Heterotrophic bacteria (*Pseudomonas, Sarcina, Achromobacter, Aerobacter, Bacillus*, etc.)	1.10^2	4.10^2	2.10^2
Fungi (*Penicillium, Aspergillus*, etc.)	1.10^2	3.10^2	3.10^2
Yeasts (*Trichosporon, Rhodotorula*, etc.)	6.10^1	8.10^1	2.10^2
Algae (*Ulothrix, Hormidium, Dictyosphaerium*, etc.)	9.10^1	3.10^2	1.10^2
Protozoa (*Amoeba, Euglena, Eutrepia*, etc.)	5.10^1	2.10^2	9.10^1

* - referred as wild microflora A

Larger scale experiments were carried out in columns and tanks. The chalcopyrite flotation concentrates were leached in tank with a mechanical stirring and with 20 l effective (slurry) volume. These experiments were carried out at pH 2.3, temperature 35°, an initial concentration of ferrobacilli cells of approximately 10^7 cells ml^{-1}, 20% pulp density, and with air containing 0.2% CO_2.

The sulfide ores were leached in big percolation PVC columns with 1250 mm effective length and 200 mm internal diameter. Each column was charged with 50 kg of ore crushed to 20 - 2 mm and possessing its own indigenous microflora. Tap water was used as leach solution, and every day was pumped to the top of each column at a rate of 40 l ton^{-1} ore day^{-1}. Suitable acidity was achieved by the oxidation of pyrite contained in the ores. 100 ml of an active culture of *T. ferrooxidans* containing 5×10^8 cells ml^{-1} were added daily to some of the columns during the first ten days of leaching. The leach solutions of the control chemical leaching tests contained 50 mg l^{-1} panacide (2,2'- methylenebis (4 - chlorophenol)).

The copper extractions were determined by periodically removing a 1 ml sample from leach solutions and analyzing for its copper content with an atomic absorption spectrophotometer. The rate of copper extraction was estimated from the linear part of a plot representing the dissolved copper concentration as a function of time, using the linear method of least squares fitting.

RESULTS AND DISCUSSION

The survival of the different wild strains after UV irradiation treatment is show in Fig. 1. All strains had a similar shape of the survival curves but some strains differed greatly in their UV resistance.

The UV irradiation had an efficacious mutagenic action in relation to the induction of mutants possessing a high oxidizing activity towards Fe^{2+} and/or S^o but only on strain M-8 (Figs. 2 and 3) and on some mutant strains derived directly or indirectly from strain

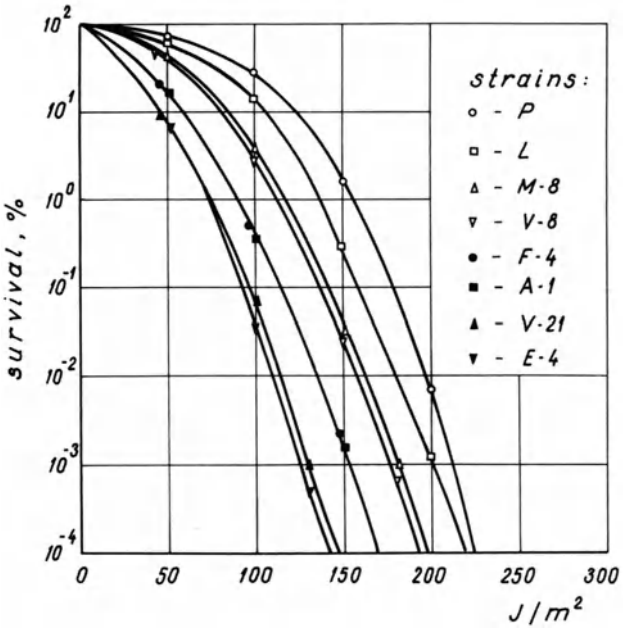

Figure 1: Survival of different strains of *Thiobacillus ferrooxidans* after UV - irradiation

M-8 or from strains P and V-8.

The histogram of the natural variability of strain M-8 in relation to the level of ferrous-oxidizing activity showed normal distribution with variability range from 40 to 140% and with a modal class possessing activity extent from 80 to 100% (the controls in Figs. 2 and 5). The histogram of the natural variability in relation to the level of sulfur-oxidizing activity also showed normal distribution with variability range from 40 to 160% and with a modal class possessing activity extent from 80 to 100% (the controls in Figs. 3 and 6).

Doses from 120 up to 160 J m^{-2} were most efficaceous for inducing mutants possessing high oxidizing activity. Such doses were most efficaceous and when mutant strains derived from strain were treated.

The histograms of the UV rays induced variability of the rest of the wild strains tested differed only slightly from those of their natural variability. Furthermore, the percentage of stable mutants among the more expressed plus-variants was low.

Similar results were achieved when the strains were treated by ethylenimine. All wild strains tested had survival curves similar to those of strain M-8 (Fig. 4). This mutagen was efficacious only towards strain M-8 (Figs. 5 and 6) and some mutant strains derived from it. Treatments with 0.04 - 0.06% ethylenimine concentrations for 1 - 3 hours were most efficacious. After treatments of the rest of the wild strains no expressed plus-variants were obtained.

Stable mutants characterized by their high oxidative activity towards Fe^{2+} and/or S^{0} were obtained as a result of a step-wise selection based on the natural and induced variability of strain M-8 and strains derived from it (Figs. 7 and 8). These mutant strains differed from their wild ancestor in many essential characteristics (Tables 3 and 4).

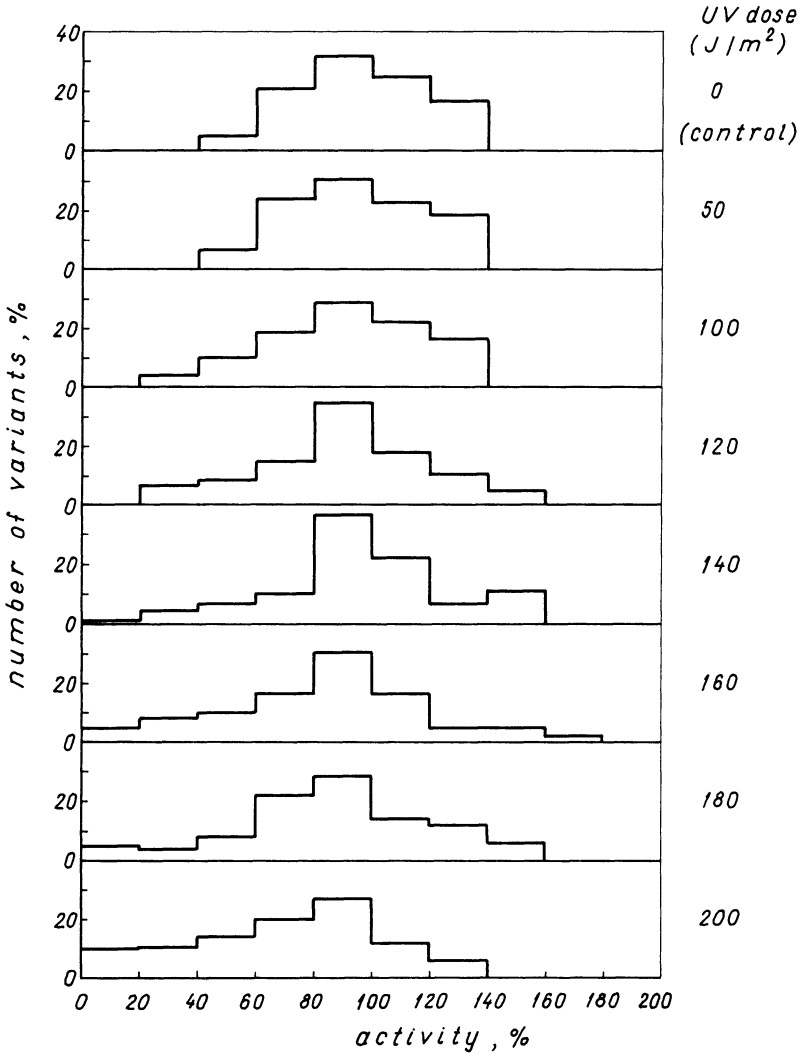

Figure 2: Histograms of UV-induced variability of *Thiobacillus ferrooxidans* M-8 in relation to the level of ferrous-oxidizing activity.

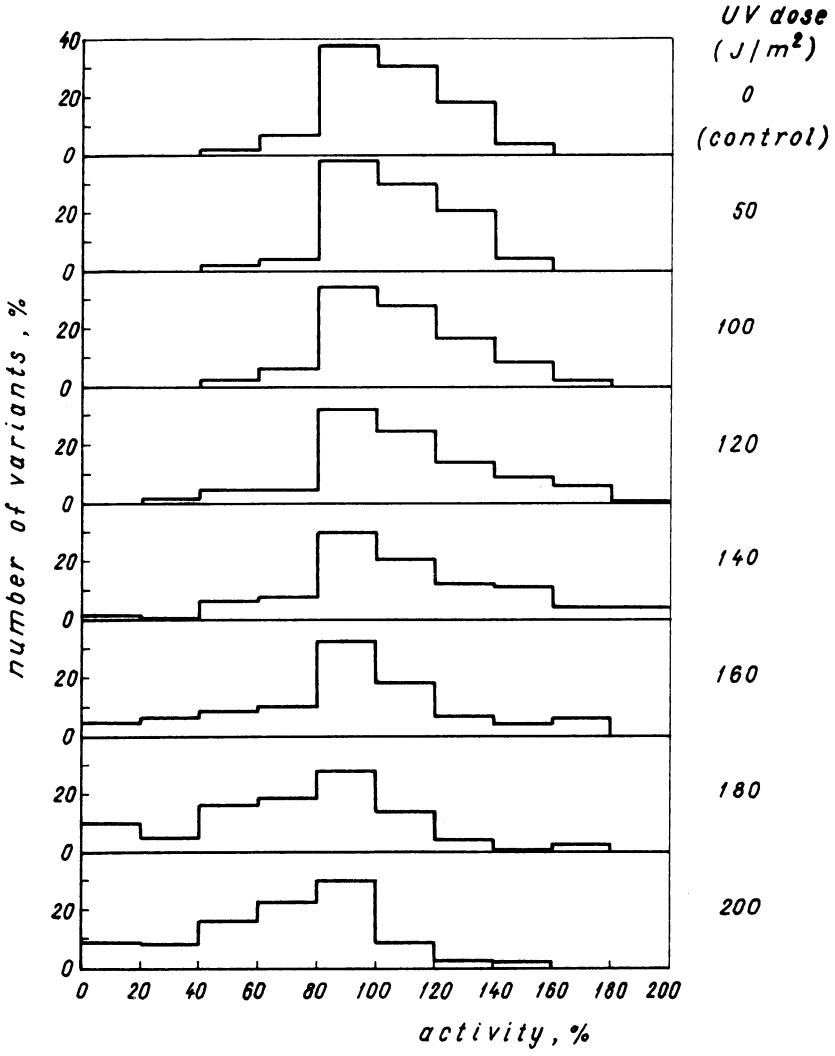

Figure 3: Histograms of UV-induced variability of *Thibacillus ferrooxidans* M-8 in relation to the level of sulfur-oxidizing activity.

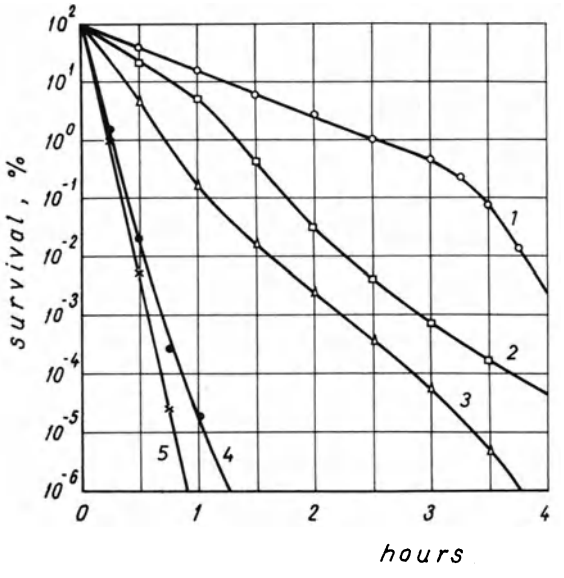

Figure 4: Survival of *Thiobacillus ferrooxidans* M-8 after ethylenimine treatment. Ethylenimine concentrations, %: 1 - 0.02; 2 - 0.04; 3 - 0.06; 4 - 0.08; 5 - 0.10.

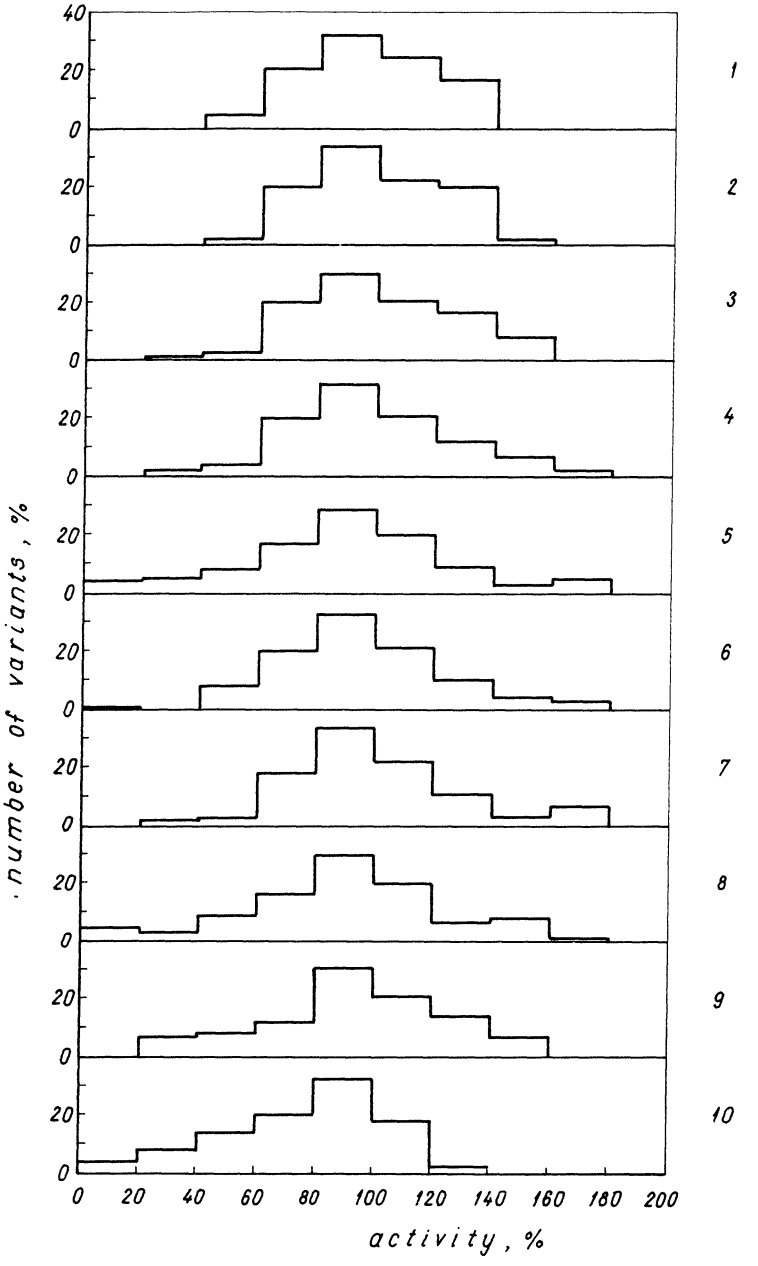

Figure 5: Histograms of ethylenimine-induced variability of *Thiobacillus ferrooxidans* M-8 in relation to the level of ferrous-oxidizing activity.

1 - control; 2 - 0.02% ethylenimine (EI) for 2 h; 3 - 0.02% EI for 4 h; 4 - 0.04% EI for 2 h; 5 - 0.04% EI for 3 h; 6 - 0.06% EI for 1 h; 7 - 0.06% EI for 2 h; 8 - 0.06% EI for 3 h; 9 - 0.08% EI for 30 min; 10 - 0.10% EI for 30 min.

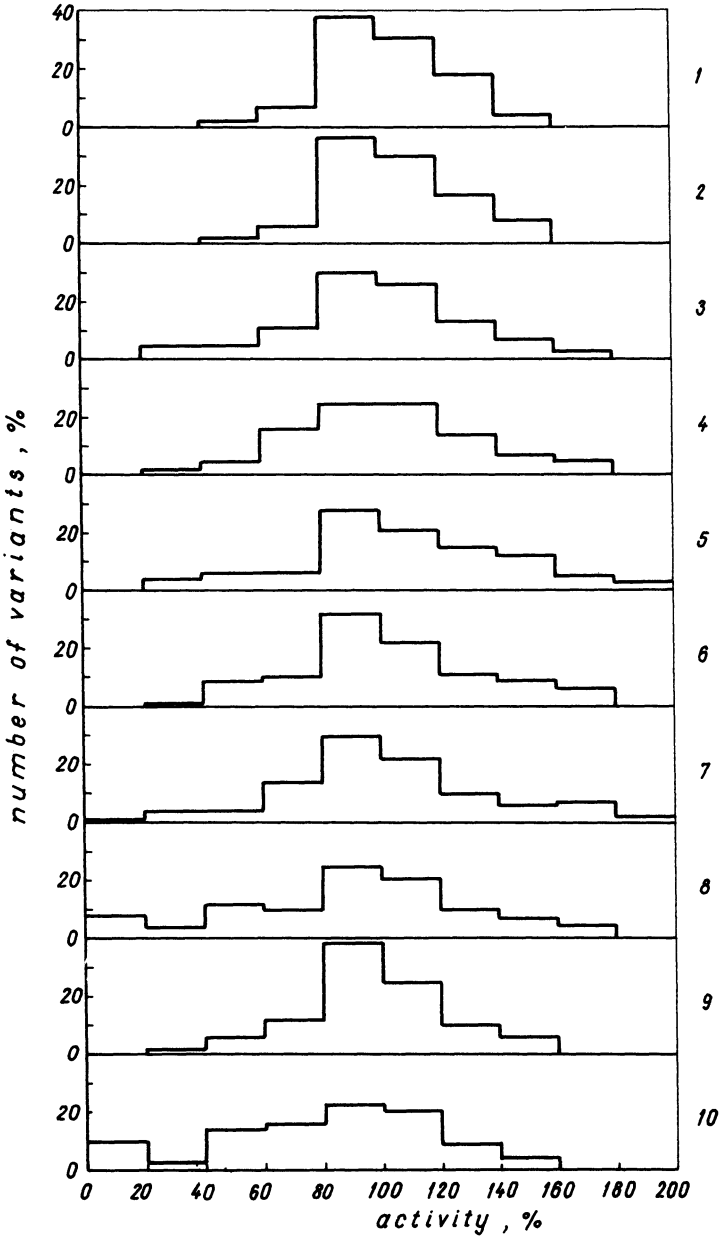

Figure 6: Histograms of ethylenimine-induced variability of *Thiobacillus ferrooxidans* M-8 in relation to the level of ferrous-oxidizing activity.
1 - 10, see Fig. 5.

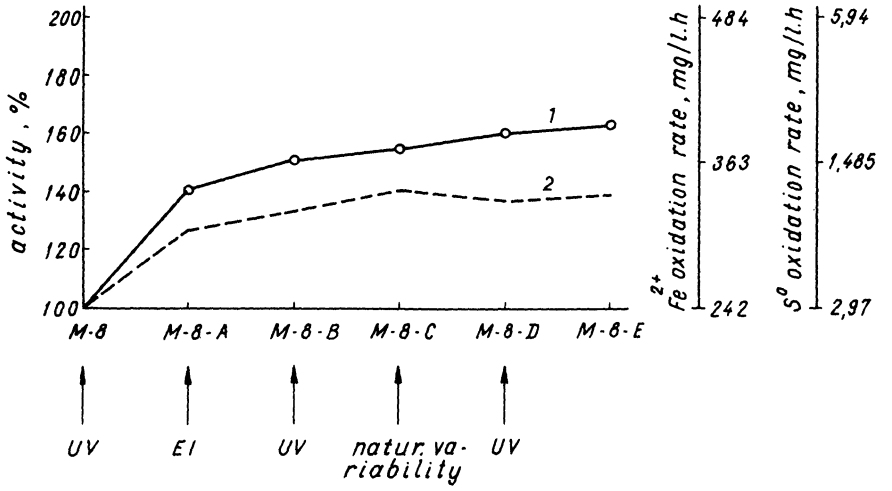

Figure 7: Step-wise selection of strains of *Thiobacillus ferroxidans* based on the level of ferrous-oxidizing activity.

1 - ferrous-oxidizing activity;
2 - sulfur-oxidizing activity.

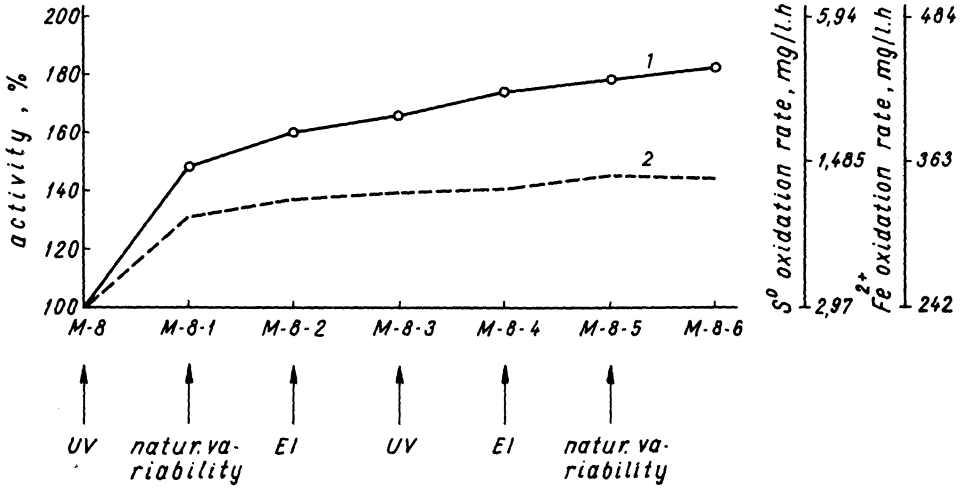

Figure 8: Step-wise selection of strains of *Thiobacillus ferrooxidans* based on the level of sulfur-oxidizing activity.

1 - sulfur-oxidizing activity;
2 - ferrous-oxidizing activity.

TABLE 3

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF *THIOBACILLUS FERROOXIDANS* STRAINS M-8 AND M 8-6

Enzyme	Strain M-8		Strain M-8-6	
	Fe^{2+}	S^o	Fe^{2+}	S^o
Minimum generation time, hours	6.41	18.10	4.47	9.95
Maximum specific growth rate (μ), h^{-1}	0.108	0.038	0.155	0.070
Maximum oxidation rate, mg l^{-1} h^{-1}	242	2.97	347	5.40
Apparent constant of Michaelis (K_m'), g l^{-1}	0.680	-	0.830	-
Economic coefficient (Y), g dry st g^{-1} substrate oxidized	0.0064	0.1181	0.0058	0.1070
Molar economic coefficient (Y_m), g dry wt mol^- substrate oxidiz.	0.357	3.780	0.325	3.425
Power coefficient (Y_{ATP}), g dry st mol^{-1} ATP formed	0.714	-	0.650	-
Effectiveness of CO_2 fixation, moles CO_2/100 mol O_2	5.60	9.86	4.99	8.92
Effectivenss of free energy utilization, %	23.60	14.82	21.05	13.41
Optimum pH	2.3	2.8	2.1	2.5
Optimum temperature, ^{o}	37	37	35	35
Tolerance to heavy metal ions, inhibitory level, g^{-1}				
Cu	30	23	25	20
Zn	12	10	10	8
Ni	10	8	10	8
Co	8	3	5	3
Mo	0.05	0.04	0.04	0.04

K_m' and Y_{ATP} are not determined during the growth on S^o: the former because of the substrate insolubility, and the latter because of the unknown quantity of ATP formed.

TABLE 4

ACTIVITY OF ENZYMES PARTICIPATING IN THE TRANSFORMATIONS OF INORGANIC SULFUR AND IRON COMPOUNDS BY *THIOBACILLUS FERROOXIDANS*

Enzyme	Specific activity (nmol min^{-1} mg^{-1} protein)	
	Strain M-8	Strain M-8-6
Sulfur-oxidizing enzyme	87	204
Sulfite oxidase	III	271
APS-reductase	3.05	3.81
ADP-sulfurylase	2.80	3.92
Adenylate kinase	7.43	12.65
Thiosulfate-oxidizing enzyme	1.86	6.04
Rhodanese	208	341
Fe^{2+}-cytochrome c reductase	3.14	4.08
Cytochrome oxidase	3.61	4.01

TABLE 5

SHAKE-FLASK LEACHING OF MINERAL SUBSTRATES WITH WILD AND WITH LABORATORY-BRED STRAINS OF *THIOBACILLUS FERROOXIDANS*

Mineral Substrate	Strains					
	M-8	M-8-6	P	Pmv	V-8	V-V
	Maximum copper extraction rate, mg l^{-1} h^{-1}					
Chalcopyrite No. 1	83	129	35	99	115	120
Chalcopyrite No. 2	1.1	1.5	0.6	0.9	1.2	1.3
Concentrate No. 1	114	201	48	170	194	217
Concentrate No. 2	78	122	39	81	107	115
Ore No. 1	18	27	12	23	26	27
Ore No. 2	24	32	19	26	30	30
Ore No. 3	26	38	21	31	38	40

TABLE 6

INTRODUCTORY EXPERIMENTS CARRIED OUT UNDER LABORATORY CONDITIONS

Test No.	Leaching Conditions	Strain used	Copper Dissolved, %		
			5th day	10th day	20th day
1	A	F-4	1.2	6.3	9.7
2	A	V-V	66.6	75.9	77.4
3	A	F-4 + V-V[1]	59.8	77.0	78.9
4	A	F-4 + V-V[2]	1.2	25.9	63.8
5	A	F-4 + V-V[3]	1.2	6.3	34.5
6	B	F-4	1.4	2.4	4.2
7	B	V-V	15.5	27.7	51.2
8	B	F-4 + V-V[1]	10.8	20.6	39.1
9	B	F-4 + V-V[2]	1.4	9.9	26.0
10	B	F-4 + V-V[3]	1.4	2.4	15.2

A - chalcopyrite concentrate No. 1 shake-flask leaching;

B - sulfide ore No. 1 percolator leaching;

1,2,3 - added at 0th, 5th and 10th day from the beginning of the experiments in 5 ml or 20 ml volume (for the Erlenmeyer flasks and the air-lift percolators, respectively) containing 2×10^8 cells ml^{-1} previously adapted to the substrate.

TABLE 7

COLUMN LEACHING OF SULFIDE ORES WITH WILD MICROFLORA AND WITH LABORATORY-BRED STRAINS OF *THIOBACILLUS FERROOXIDANS*

Biological leaching agent	Ore No. 1	Ore No. 2	Ore No. 3
	Copper dissolved for 280 days, %		
Wild microflora A[x]	8.7	20.2	19.4
Wild microflora B[xx]	6.1	12.7	12.0
Strain M-8-6 plus Wild microflora A	16.8	20.5	20.0
Strain M-8-6 plus Wild microflora B	15.0	18.9	18.3
Strain Pmv plus Wild microflora A	14.7	18.1	18.1
Strain Pmv plus Wild microflora B	13.5	16.0	16.2
Strain V-V plus Wild microflora A	16.1	20.1	19.5
Strain V-V plus Wild microflora B	15.4	18.4	17.8
Control chemical leaching (panacide added)			
- to wild microflora A	2.9	5.1	4.7
- to wild microflora B	1.9	3.2	2.9

[x] - wild microflora A - an abundant and varied microflora (see Table 2).

[xx] - wild microflora B - a poor microflora ($\lesssim 10^2$ cells g^{-1} ore before the leaching).

In general, the increase of ferrous-oxidizing activity was accompanied by an increase of the sulfur-oxidizing activity, and vice versa. The increase of the oxidizing activity wa connected also with alterations in cell size. The mutant cells were smaller than the cel of the wild strain and had larger surface-to-volume ratio. These alterations were revers ible and were probably due to the different growth rates since the growth of the mutant strains under conditions differing markedly from the optimal ones, e.g. in systems with insufficient aeration, resulted in cell size characteristic of the wild strain. Since th role of the cell surface in the ferrous iron, elemental sulfur and sulfide minerals oxidation is essential (Dugan and Lundgren, 1965; Wang *et al.*, 1970; Imai *et al.*, 1972; Bodo and Lundgren, 1974; Karavaiko and Pivovarova, 1977; Grouder *et al.*, 1978b), the existence of regulating mechanism adjusting the cell size in accordance with the growth conditions is not excluded. This mechanism, if it exists, is not ubiquitous.

On the other hand, reversible alterations in cell size but accompanied by morphological variations are observed during the growth of *T.ferrooxidans* in a response to excessively vigorous aeration (Silverman and Rogoff, 1971).

Strain Pmv was obtained as a result of step-wise selection described elsewhere (Groudev *et al.*, 1978, and strain V-V was obtained from the strain V-8 by two-stage selection: after plating of non-treated cells and after plating of UV-irradiated cells, respectively

Our studies on the mutation selection of *T.ferrooxidans* confirmed the finding of Groudev *et al.* (1978a; 1978b) that the UV rays and ethylenimine are efficacious for strain improvement of the thiobacilli (but only towards some strains).

Under laboratory conditions the active laboratory-bred mutant strains leached copper from various mineral substrates more rapidly than the wild strains (Table 5). Furthermore, the leaching with the mutant strains was characterized very often by a shortened lag-phase before the copper release as well as by a better final extraction. These data showed that at least in most cases, the increase of ferrous- and/or sulfur-oxidizing activity really resulted in an increased sulfide minerals oxidation ability. This finding makes the selection of mutants possessing the later characteristic very efficaceous since such mutants can be scored successfully among a large number of variants. It is necessary to note, however, that the relationships between the various oxidizing abilities are complex and need special attention (Groudev, 1974; Groudev and Genchev, 1978a, 1978b; Norris and Kelly, 1977).

The natural chalcopyrite No. 2 was very resistant to both chemical and biological leaching and even the most active laboratory-bred strains were inefficaceous towards it.

The leaching with laboratory-bred strains represented a more special interest in such cases, in which these strains were introduced in ecosystems already populated by other strains (wild or laboratory-bred) possessing a lower activity. Such introductions were carried out initially under shake-flask technique or air-lift percolator conditions. Most of the results obtained were contradictory but some introductions of strain V-V into systems populated by strain F-4 were very successful (Table 6). In these cases the leaching rate increase considerably after the introduction, although it never reached the level characteristic of the respective laboratory-bred strain (V-V) when the latter was used independently. The introductions were more efficaceous when they were accomplished during the initial phase of leaching.

The successful interventions of strain V-V in the systems inhabited by strain F-4 are connected probably with the great differences between the two strains. It was also found that before the inoculation of strain V-V, most of the cells in the leach systems were free-swimming . The amounts of cells attached on the solid substrate increased distinctly only after the inoculation of strain V-V. It is clear that the presence of significant free mineral surface permits to strain V-V to exhibit its high activity.

The results from the leaching of ores in percolation columns are shown in Table 7. In most cases, the leaching of fresh ores taken from the upper layers of the respective dumps immediately before the experiments, i.e. ores in which the indigenous microflora is abundant and almost unaffected, was more efficaceous when it was carried out without addition of exogenous bacteria. Exceptions were observed only when ore No. 1 was leached with additions of bacterial inoculum consisting of cells of each one of the laboratory-bred strains used, i.e. strain M-8-6 or strain V-V or strain Pmv, respectively. On the

other hand, the introduction of laboratory-bred strains into columns charged with ores taken from the deeper layers of the dumps was always useful. These ores contained no more than 10^2 ferrobacilli per gram.

The tank bioleaching of concentrates was efficaceous and leaching rates comparable with those observed under shake-flask technique conditions were readily obtainable. For industrial applications, however, continuous leaching is desirable to facilitate plant operation and eliminate the lag time associated with batch fermentation (McElroy and Bruynesteyn, 1978).

The data above-mentioned distinctly highlight the role of *T.ferrooxidans* in the leaching process. This bacterium is a powerful leaching agent but its relationships with the mineral substrates as well as with the rest of the members of the natural substrate microflora are complex and need further study. The isolation from the ores tested of different strains of *T.ferrooxidans* showed that some of them greatly differed from one another and that a large variety of strains cohabited even a single gram of ore. *T.thiooxidans* was always found in these systems but in smaller numbers. Like other workers (see Norris and Kelly, 1977) we were unable to reproduce rapid metal release by recombining isolated *T. ferrooxidans* and *T.thiooxidans*. We were also unable to do that by recombining the different isolated thiobacilli strains. The presence of heterotrophic microorganisms is not an obligatory condition for the leaching to proceed. However, their role in the mixed cultures should not be overlooked. Most of these microorganisms interfere directly and/or indirectly in both the growth and development of *T.ferrooxidans* (Groudev *et al.*, 1978), and some of them are able to leach copper in their own right (LeRoux *et al.*, 1978; Kusnezov, 1976).

At the present time, it seems that the laboratory-bred strains will be especially useful for leaching of sulfide concentrates which contain none of their own indigenous microflora as well as for in situ leaching of some ore sections in which autochthonic thiobacilli populations are not numerous.

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ISOLATION OF IRON- AND SULFUR-OXIDIZING BACTERIA FROM MINE WATER IN JAPAN AND SOME INVESTIGATIONS ON THE LEACHING OF SULFIDE ORES

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INTRODUCTION

The authors have studied the isolation of iron- and sulfur-oxidizing bacteria from mine water and carried out some investigations on the leaching of sulfide ores, in the hope that a technology of bacterial leaching of ores might be devised. The present paper is a brief summary of these results.

EXPERIMENTAL PROCEDURES

Isolation of Bacteria

The isolation of bacteria was achieved by the usual enrichment culture techniques followed by repeated streaking on thiosulfate-agar plates. Unless otherwise stated, the growth studies were carried out as follows: a loopful of cells grown on slants was transferred into 100 ml of the medium in a 500 ml shaking flask, and incubated at 30° on a reciprocating shaker. The media used in this study were *9K modified Colmer medium* (NH_4Cl 0.2g, $MgCl_2.6H_2O$ 0.1g, $CaCl_2.2H_2O$ 0.2g, KH_2PO_4 3g, $Na_2S_2O_3.5H_2O$ 5g and water 1 litre (pH 6.0 with NaOH)); *YE medium* ($NaH_2PO_4.2H_2O$ 7.5g, yeast extract 1g and water 1 litre (pH 6.0 with NaOH)); and *YES medium* $NaH_2PO_4.2H_2O$ 7.5g, yeast extract 1g, $Na_2S_2O_3.5H_2O$ 5g and water 1 litre (pH 6.0 with NaOH)).

Sulfuric Acid Leaching of Bismuth after Bacterial Pretreatment of Ores

100-120 litres of 9K medium was put in a 150 l polyvinylchloride tank and then oxidized by H_2O_2 (chemical pretreatment liquor) or *T.ferrooxidans* (bacterial pretreatment liquor). The desired amounts of ores were added to these solutions and then stirred mechanically at 370 rpm. Air was blown throughout in the bacterial pretreatment. A portion of the slurry was removed periodically and filtered. The residues, namely pretreated ores, were used for the subsequent acid leaching. In the bacterial pretreatment, the pretreatment liquor was repeatedly used 3 times under the conditions shown in Table 1.

TABLE 1

PRETREATMENT CONDITIONS

		Pulp density (kg wet ore[a] l^{-1} soln.)	Concn. of Fe^{3+}[b] (g kg^{-1} dry ore)
Chemical pretreatment		40/120	15.5
Bacterial pretreatment	1st	30/120	16.0
	2nd	40/120	9.6
	3rd	40/100	12.1

[a] H_2O content: 10 wt.%.

[b] Excludes the ferric iron in solid state.

The sulfuric acid leaching of ores was carried out by using a 300 ml three-necked flask equipped with a glass stirrer under the conditions: sample 10 g, 10% H_2SO_4 200 ml, temperature 30° and stirring velocity 400 rpm. At regular intervals, a small portion of the slurry was taken out, filtered and then analyzed for Bi, Cu and Fe.

Analysis

The amounts of $S_2O_3^{2-}$ and polythionates were determined colorimetrically, and SO_4^{2-} by gravimetry. Cell protein was determined by the method of Lowry *et al.* with bovine serum albumin as standard. Bi was determined colorimetrically with thiourea. Cu was determined by chelatometry by using PAN as indicator, and Fe by titrimetry with potassium dichromate.

RESULTS AND DISCUSSION

Isolation of Iron- and Sulfur-oxidizing Bacteria

Isolation of Acidophilic Bacteria

As for acidophilic thiobacilli, particular findings were not obtained. In the present paper, we point out the following aspects: all the iron-oxidizing bacteria we isolated oxidized elemental sulfur definitely in the presence of a surface active agent Tween 80; the activity of *T.thiooxidans* for oxidation of elemental sulfur was evidently higher than that of *T.ferrooxidans* (Fig. 1) (Mizoguchi *et al.*, 1970).

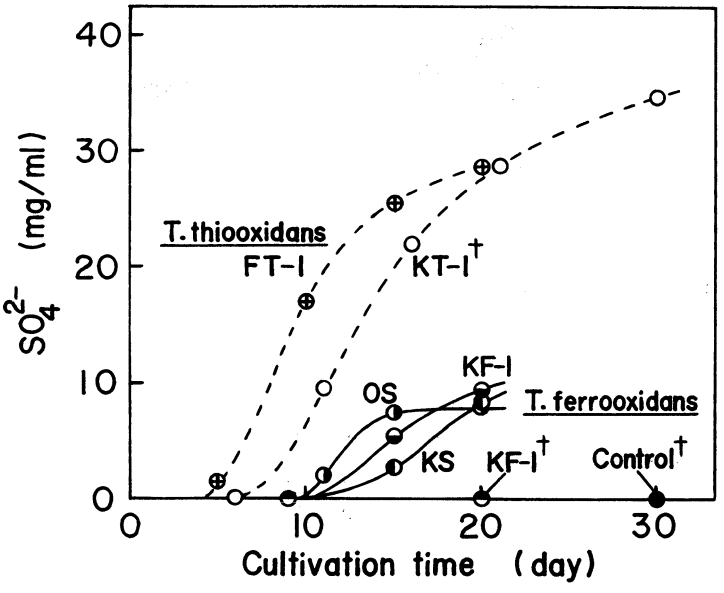

Figure 1: Oxidation of elemental sulfur by acidophilic thiobacilli. Medium: modified Colmer medium (1 g of elemental sulfur was added instead of thiosulfate), amount of Tween 80: 2.5 g. Without addition of Tween 80.

Isolation of New Sulfur-oxidizing Bacteria Capable of Growing Heterotrophically; Thiobacillus rubellus and Thiobacillus delicatus

Thiobacillus rubellus was isolated by chance from mine water of the Kosaka Mine of Dowa Mining Co., Ltd. This species gave reddish colonies and grew well only in the presence of suitable organic compounds.

Thiobacillus delicatus was isolated from mine water of the Tsuchihata Mine of Tanaka Mining Co., Ltd. The pure culture was obtained by depressing the growth of fungi with trichomycin. This bacterium perished within 2 weeks at 30°.

Under mixotrophic conditions, *T.rubellus* and *T.delicatus* oxidized $S_2O_3^{2-}$ to SO_4^{2-} through intermediate formation of $S_3O_6^{2-}$ and $S_4O_6^{2-}$, the results for *T.delicatus* being shown in Fig. 2. The effects of some other factors on the oxidation of $S_2O_3^{2-}$ and/or the growth of these two species are shown in Fig. 3 and Fig. 4. Taking other results into account, the characteristics of these species are summarized as follows:

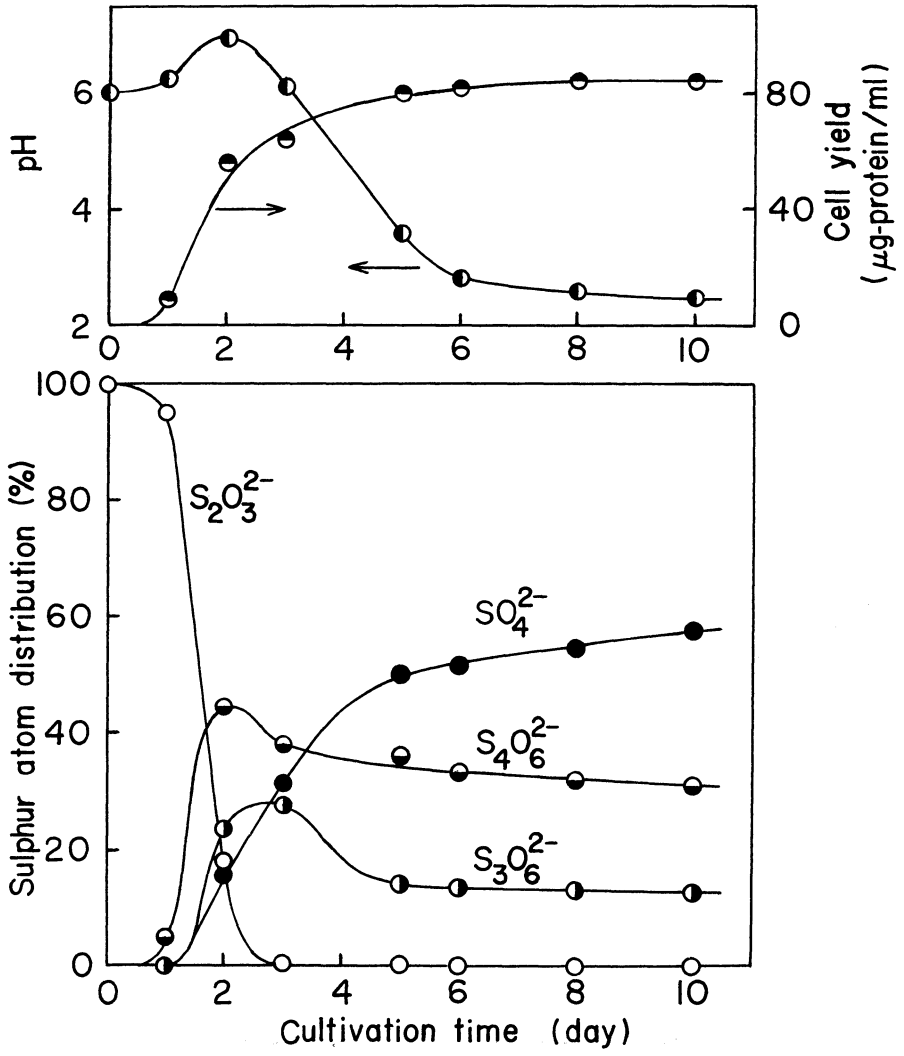

Figure 2: Time course of thiosulfate oxidation by *T.delicatus*. A loopful of cells grown on slants was transferred into 200 ml of YES medium and incubated at 30°.

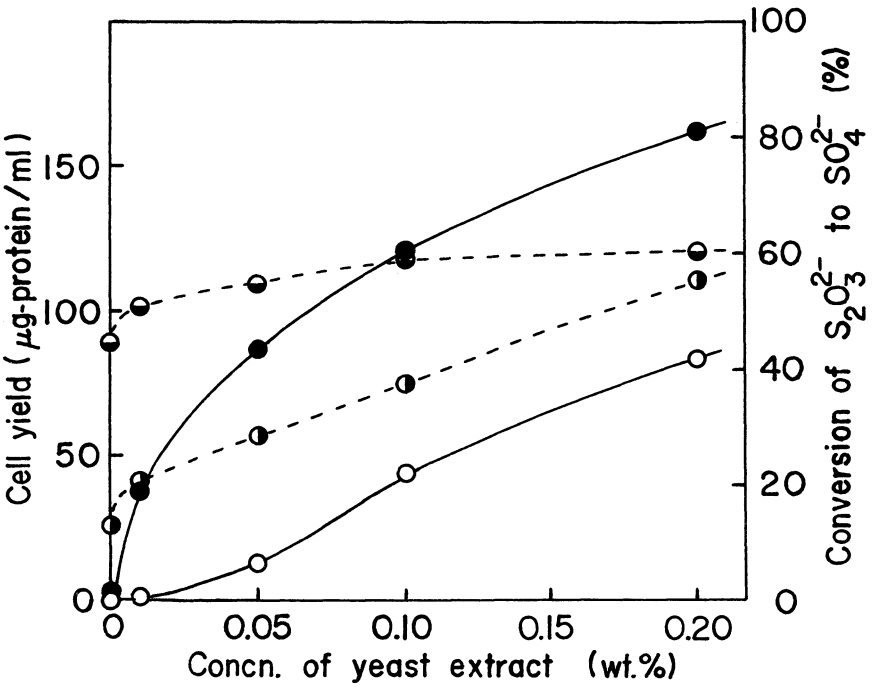

Figure 3: Effect of yeast extract on the growth of *T.rubellus* and *T. delicatus* and on thiosulfate oxidation. Cell yield: -O- *T.rubellus*, --●-- *T.delicatus*. Conversion of $S_2O_3^{2-}$ to SO_4^{2-}: -●- *T.rubellus*, --⊖-- *T.delicatus*. Medium: modified Colmer medium with various concentrations of yeast extract, cultivation time: 10 days.

Description of Thiobacillus Rubellus nov. sp.

Cells are rod-shaped, 1.0-1.6 μm by 1.7-3.0 μm, Gram-negative, and motile by a single polar flagellum. Colonies are tinged with red. Heterotrophic growth occurs in yeast extract medium, *etc.*, but autotrophic growth does not occur. This bacterium grows well an oxidizes thiosulfate under mixotrophic conditions. Its oxidation activity towards element sulfur is extremely low even if organic compounds are present. Optimum growth conditions are 30° and between pH 5 and 7. Marginal growth occurs at 35°. The G+C content of DNA is 65±3 mol%. According to the definition by Rittenberg (1969), this bacterium belongs to chemolithotrophic heterotrophs.

Description of Thiobacillus delicatus nov. sp.

Cells are rod-shaped, 0.5 μm by 0.7-1.3 μm, Gram-negative and non-motile. The colonies ar colourless and transparent at first, and then turn whitish yellow due to the deposition of elemental sulfur. Besides heterotrophic growth, autotrophic growth occurs in thiosulfate-mineral salts medium. This bacterium also grows well and oxidizes thiosulfate under mixotrophic conditions. The oxidation activity towards elemental sulfur is enhanced by organi compounds. Optimum growth conditions are 30° and between pH 5 and 7. Marginal growth occurs at 40°. The G+C content of DNA is 67±3 mol% (Mizoguchi *et al.*, 1976; Sato *et al.*, 1976).

LEACHING OF ORES BY IRON- AND SULFUR-OXIDIZING BACTERIA

Sulfuric Acid Leaching of Bismuth after Bacterial Pretreatment of Ores

Previously we studied the recovery of bismuth occurring to approximately 0.4% in a copper

concentrate (Mizoguchi *et al.*, 1970; Okabe and Mizoguchi, 1973). As a result, it became clear that bismuth in fresh ores is hardly leached, while bismuth in aged ores is very soluble in dilute sulfuric acid.

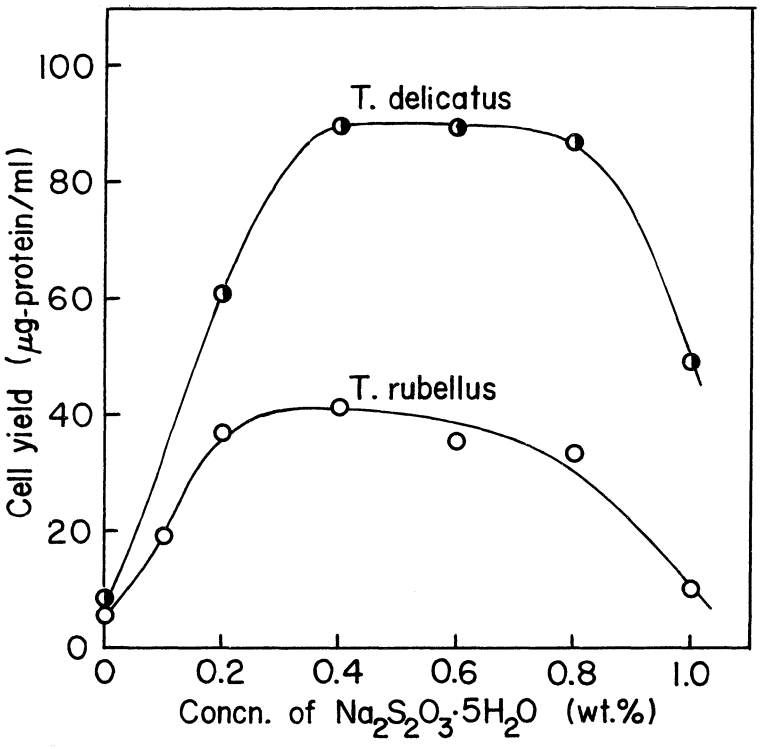

Figure 4: Effect of thiosulfate on the growth of *T.rubellus* and *T. delicatus*. Medium: YE medium with various concentrations of thiosulfate, cultivation time: 10 days.

In the hope that bismuth sulfide might be converted to non-sulfide state in the presence of ferric ions and iron-oxidizing bacteria, the authors investigated the two-step leaching method. The reason why sulfuric acid leaching must be carried out after the bacterial pretreatment is that bismuth is almost completely precipitated under the conditions which are suitable for the growth of iron-oxidizing bacteria.

The leaching rate of bismuth was extremely enhanced when the ores were subjected to the bacterial pretreatment prior to the acid leaching (Fig. 5). The results shown in Fig. 6 indicate that 80% or more of the bismuth is solubilized by the bacterial pretreatment for 6 days. On the other hand, chemical pretreatment is not so effective as bacterial pretreatment. Therefore, it may be considered that bismuth sulfide is hardly decomposed in the chemical pretreatment. Another reason for the superiority of bacterial pretreatment may be that ferric ion, which is an excellent decomposing agent for sulfide minerals, is continuously being supplied. The leaching rate of bismuth was somewhat enhanced by using the chemically pretreated ores. This result may be explained as follows: the basic iron (III) sulfate precipitated during the oxidation of 9K medium with hydrogen peroxide dissolves and attacks the bismuth sulfide in the stage of sulfuric acid leaching (Mizoguchi *et. al.*, 1970; Okabe and Mizoguch, 1973).

Effect of Metal Ions on the Leaching of a Copper Concentrate with Acidic Iron(III) Sulfate Solution

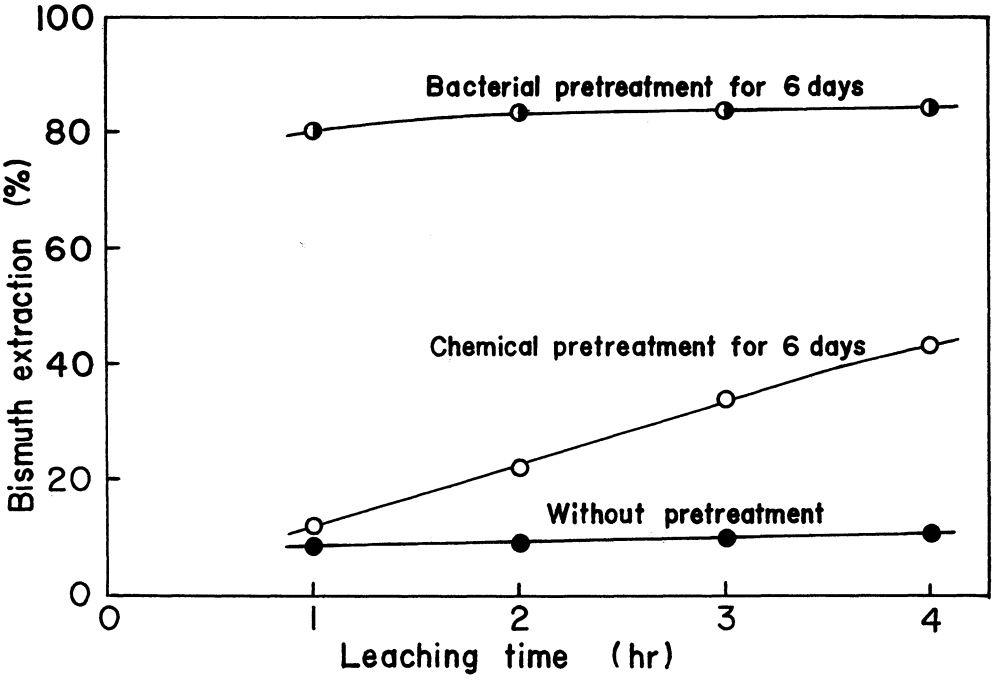

Figure 5: Effect of pretreatment on the sulfuric acid leaching of bismuth.

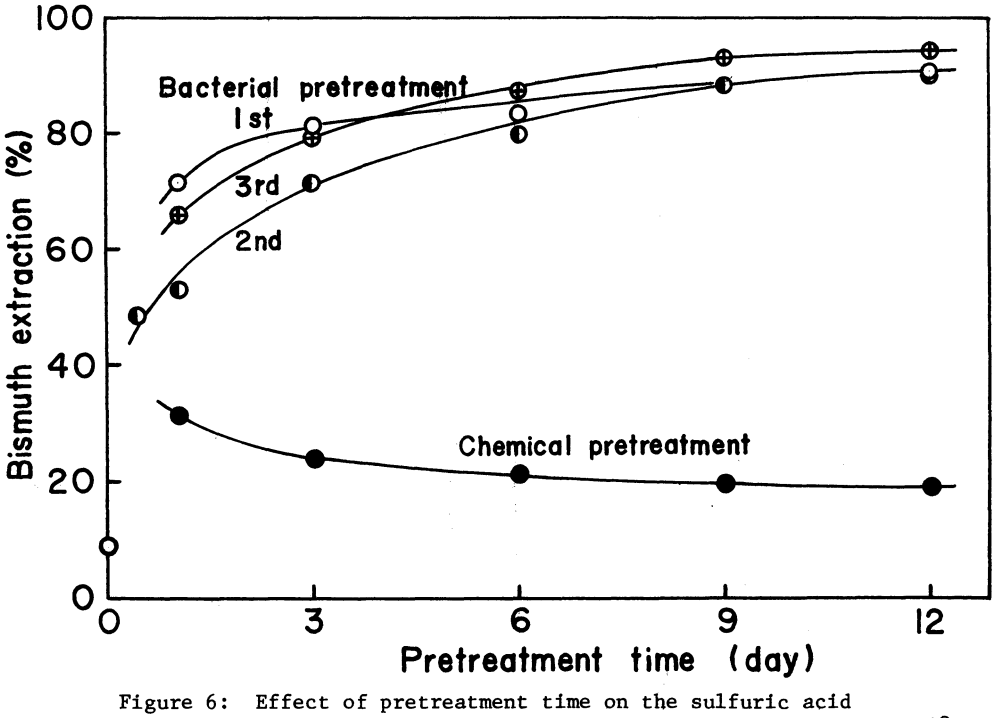

Figure 6: Effect of pretreatment time on the sulfuric acid leaching of bismuth. Sulfuric acid leaching: 2 h at 30°.

Noteworthy effects of metal ions on the leaching of a copper concentrate were observed in the experiments related to the recovery of bismuth. Table 2 shows clearly that ferric ion accelerates the dissolution reaction of bismuth, but this effect of ferric ion is completely diminished by Pd(II), Hg(II) and Ag(I). The dissolution of both iron and copper, that is, the decomposition of chalcopyrite with ferric ion, was accelerated by only Ag(I). These results indicate that the main reactions in the bacterial leaching are affected by trace amounts of metal ions. On the contrary, some investigators have shown that Ag(I) is detrimental to the growth of *T.ferrooxidans*, so further studies are necessary to clarify the roles of metal ions in the bacterial leaching of ores.

Leaching of Ores by T. Rubellus and T. Delicatus

Small amounts of sulfur were dissolved from "Kurokō" (complex ore) in the presence of *T.rubellus* and *T.delicatus* (Fig. 7), whereas copper was hardly dissolved regardless of leaching time or bacteria. Therefore, the activities of these microorganisms for solubilization of ores are considered to be extremely small, compared with those of such acidophilic bacteria as *T.thiooxidans* and *T.ferrooxidans*.

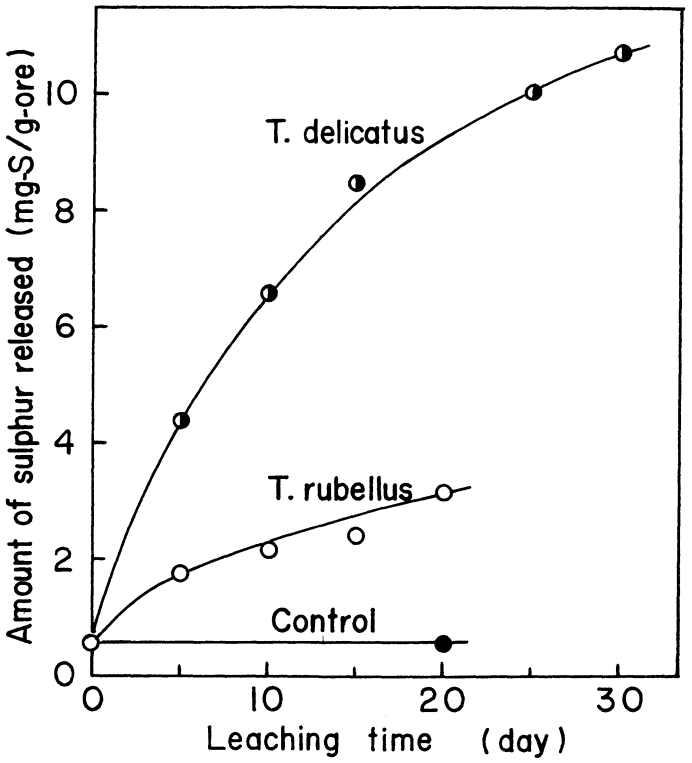

Figure 7: Leaching of "Kurokō" (complex ores) in the presence of *T.rubellus* and *T.delicatus*. 100 ml of YE medium was put into a 500-ml shaking flask together with 1 g of the ores (<100 mesh). After sterilization, the contents were inoculated with 5 ml of YE medium which had previously been incubated with the bacteria, and then shaken at 30°. Composition of ores (%): Cu 10.3, Fe 5.57, Pb 1.26, Zn 4.46, S 14.4, SiO_2 2.81, $BaSO_4$ 60.0.

TABLE 2

EFFECT OF METAL IONS ON THE LEACHING OF A COPPER CONCENTRATE WITH ACIDIC IRON(III) SULFATE SOLUTION

Metal ions added (mg g^{-1} ore)			None		Pd(II) 10	Hg(II) 10	Ag(I) 0.1	Ag(I) 0.5	Ag(I) 0.5	Cu(II) 10	Cd(II) 10	Ni(II) 10
Fe^{3+} added (mg g^{-1} ore)			0	30	30	30	30	0	30	30	30	30
Extraction (mg g^{-1} ore)	Bi	hr 1	0.38	0.60	0.61	0.26	0.64	0.27	0.58	0.81	0.56	0.66
		2	0.44	0.82	0.61	0.35	0.70	0.27	0.58	0.99	0.81	0.87
		3	0.51	1.05	0.64	0.42	0.74	0.27	0.60	1.13	0.99	1.12
		4	0.57	1.31	0.68	0.44	0.81	0.27	0.61	1.34	1.13	1.27
	Cu	1	1.24	1.37	1.05	0.95	1.40	1.38	4.51	1.51	1.27	1.04
		2	1.52	1.44	1.10	1.43	1.44	1.80	7.78	1.61	1.32	1.64
		3	1.88	1.51	1.16	1.63	1.48	2.16	9.41	1.66	1.34	1.67
		4	2.24	1.51	1.22	1.69	1.56	2.55	10.13	1.72	1.37	1.72
	Fe	1	4.47	4.67	5.03	3.03	4.46	3.96	7.14	4.97	4.67	5.06
		2	4.89	5.24	5.56	4.22	5.18	4.98	10.51	5.36	5.21	5.56
		3	6.14	5.65	5.68	4.61	5.32	5.87	12.45	5.53	5.56	5.65
		4	6.44	5.86	5.77	5.09	5.38	6.29	13.58	5.92	5.71	5.83

Sample (Bi 22.4%, Cu 22.4%, Fe 29.0%, S 28.7%): 10 g, leaching agent: 200 ml of 10% H_2SO_4 with or without iron(III) sulfate, temp.: 30^{o}.

If the reaction: $CuFeS_2 + 4\ Fe^{3+} = Cu^{2+} + 5\ Fe^{2+} + 2\ S^{0}$ occurs, 8.6 mg of Cu and 7.5 mg of Fe are solubilized theoretically with 30 mg of Fe^{3+}.

It has been demonstrated by many studies that cells harvested from media supplemented with organic compounds show a reduced rate of oxidation of inorganic compounds. However, the present results indicate the possibility of finding various types of bacteria having an enhanced growth rate and leaching activity even in the presence of organic compounds (Mizoguchi *et. al.*, 1976).

ACKNOWLEDGEMENT

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THE MICROBIAL ROLE IN PYRITE OXIDATION AT ALKALINE pH IN COAL MINE SPOIL*

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INTRODUCTION

The reclamation of land made derelict by coal mining waste involves a number of decisions and actions. Amongst these are the methods to be used in correcting the acidity resulting from past oxidation of pyrite in the spoil and in preventing the oxidation of the residual pyrite in the spoil. Neutralisation or 'liming' of the spoil (in particular with agricultural limestone) is currently practiced both as a corrective and preventative measure. However, there is not yet an adequate basis for predicting the efficacy of liming or the amount of 'lime' required.

One important step towards assessing the amount of lime required is to determine the Acid Generating Potential (AGP) of the spoil - that is, the amount of acidity that can be produced, under field conditions, from oxidation of the pyrite in the spoil. Since other spoil components besides pyrite are involved in acid production and consumption reactions, and since hydrogeological and other physical factors are involved, a simple analysis for total pyrite in the spoil is inadequate. The concept of reactive and unreactive pyrite (Caruccio, 1970, 1972) may be helpful in assessing the likelihood of acid production and a method (Pulford and Duncan, 1975) for determining the extent to which pyrite in a spoil has been oxidised can assist in characterising the material to be dealt with. However, in both cases, translation into lime additions would be difficult. Predictions based on total sulfur determinations plus bacterial oxidation experiments (Harrison, 1978) also have severe limitations, largely because of the variety of sulfur species present in spoil and the disparity between laboratory and field conditions. Development of an improved method for the determination of AGP, using laboratory and pilot scale leaching experiments, is part of the research programme at Warren Spring Laboratory.

Further difficulties arise when the estimated amount of lime required is to be incorporated into the spoil. Current reclamation practice typically involves grading, compaction, deep ripping to about 50 cm depth, liming, fertilising and disc harrowing to provide a seed bed. While compaction helps limit the zone of oxidation, vertical distribution of lime within this zone is almost invariably inhomogeneous, while even multiple passes of rippers and harrow fail to ensure horizontal mixing. Superimposed on an already uneven pyrite distribution, this inevitably results in localised imbalances of pyrite and lime (Table 1).

TABLE 1

PYRITE AND LIME DISTRIBUTIONS AFTER LIMING AND DEEP RIPPING (2 PASSES)

Site No.	Pyrite (mmol g^{-1} spoil)	Lime (mmol $CaCO_3$ g^{-1} spoil)
1	0.28	0.59
2	0.20	0.42
3	0.25	0.36
4	0.14	1.02
5	0.23	0.17
6	0.18	0.56
7	0.23	0.14
8	0.26	0.15
9	0.17	0.65

Note: Optimal stiochiometry is 1 mol pyrite : 2 mol lime

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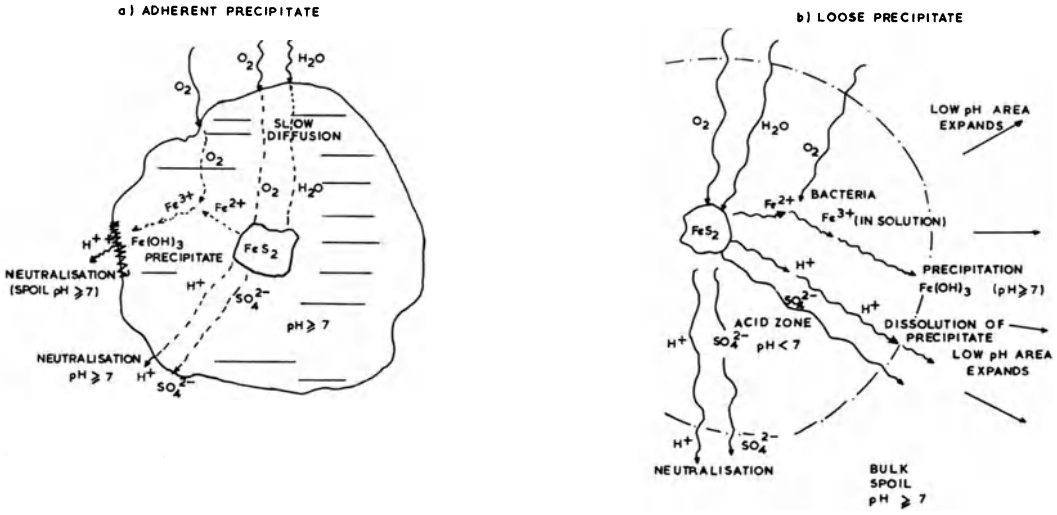

Figure 1: Development of hot spots. An adherent precipitate (a) will retard oxygen and water ingress but with a loose precipitate (b) more rapid pyrite oxidation is possible and a hot spot can form.

One would therefore anticipate 'hot spots' to develop in regions where the acid production from pyrite is in excess of the buffering capacity of lime and spoil minerals. The chemistry of the regions round hot spots may be highly complex (Fig. 1) and dependent on precipitation processes. Enlarged hot spots may appear as haloes of oxidised iron and acid surrounding masses of pyrite, or, in revegetated spoil, as areas of burnback. The mechanism of acid production from pyrite in the immediate vicinity of lime, or in fresh alkaline spoil is problematical. It seemed possible that neutrophilic thiobacilli could initiate pyrite oxidation but the rate and extent of this process and its overall importance under field conditions was uncertain especially since it has been generally assumed that an alkaline environment would prevent acid formation from becoming a practical problem in reclaimed spoils. Since the research at Warren Spring Laboratory is aiming to develop a strategy for understanding better the mechanisms involved in acid generation and its prevention, this seemed an important question to resolve. Thus the bacterially mediated production of acid in an artificial system, homogeneous in pH, was investigated and this paper outlines the resulting observations.

MATERIALS AND METHODS

Coal spoils, to be used as inocula, were obtained from the Higher Folds spoil reclamation site near Wigan, Greater Manchester, and from the Brodsworth reclamation site near Brodsworth, S. Yorks. The Higher Folds spoil is highly heterogeneous, accumulated from several mines over several decades with their changing practices in the separation of coal and spoil. It contains long-exposed oxidized pyrite and pyrite freshly exposed during landscaping. It is regarded as a highly acidic spoil, but our samples contained both acidic and neutral spoil. Pyrite is abundant and occurs in both coal and shale. The Brodsworth spoil is recent. Our samples were from recently mined material, freshly spread. It is regarded as a neutral to alkaline spoil and pyrite masses are rare. There is reported to be appreciable finely disseminated pyrite in the spoil. The Higher Folds spoil is limed as a part of the reclamation protocol and Brodsworth spoil is currently covered with top soil, but without liming, to allow revegetation.

Tharsis pyrite from Spain, ground to -200 mesh, was used as a substrate and provided the only energy source for the bacteria in most experiments. A few experiments used an unnamed Montana pyrite ground to -270 mesh. Elemental sulfur (flowers of S) and ferrous

sulfate were also used as energy substrates.

Bacterial cultures were raw, mixed cultures obtained from spoils by repeated subculturing using pyrite as the substrate, in a medium produced by dilution of Silverman and Lundgren's basal salts medium (Silverman and Lundgren, 1959). For manometric studies bacteria were spun down and washed with the same medium at the desired pH. Oxygen uptake was measured with a Gilson differential respirometer. The starting pH of manometric experiments was reached by adjusting Silverman and Lundgren's basal salts medium (Silverman and Lundgren, 1959), diluted fourfold to the required pH and by washing both cells and pyrite with this weakly buffered medium. The pyrite required washing immediately before addition to the Warburg vessels to achieve the desired initial pH.

Elemental sulfur oxidation was also monitored by adding 10 mg of flowers of sulfur containing ^{35}S to 80 ml of a one day old culture. Samples were withdrawn at intervals by pipette and filtered using a Millipore 0.45 mm Swinnex. A portion of the filtered medium was treated with $BaCl_2$ and refiltered to remove sulfate.

In a further series of tests, acid production was followed by recording pH versus time in erlenmeyer flasks incubated on a rotary shaker at 30^{o} and containing a mixed bacterial culture (derived from coal spoil), pyrite and Silverman and Lundgren's basal salts medium diluted fourfold. The starting pH was adjusted with NaOH solution after all other ingredients were present. No aseptic precautions were taken.

RESULTS AND DISCUSSION

Fresh samples of Higher Folds and of Brodsworth spoils, as well as old samples of these spoils stored outdoors in steel drums (and similar samples from other coal fields), when used as inocula, in 9K medium containing either pyrite or thiosulfate as the energy substrate, all produced a pH drop from about 7.5 to less than 3.0 in from 3 to 10 days.

The pH drop was accompanied by a proliferation of bacteria. No other microorganisms were seen. Some bacteria morphologically resembled typical thiobacilli but many large rods, short stout rods and occasional filaments were seen. Both gram positive and gram negative cells were present.

The starting pH was 7.5 in the first experiments on acid production. An initial pH of 8.0 to 8.5 was later adopted for routine subculturing. The highest pH tried, 9.0, was equally successful in growing bacteria and leading to high acidity.

While acid could be produced from both thiosulfate and pyrite as substrates, pyrite was chosen as the substrate for subsequent tests because of its particular relevance to coal spoil.

Subcultures at weekly to two weekly intervals using pyrite in 9K medium were continued for eleven months with unchanged results. Typical results are shown in Fig. 2, with a pH drop from 8.1 to 3.2 in four days, to pH 1.9 in eight days and to pH 1.3 in fifteen days. An inoculum taken from this acidic solution could then be used to reinoculate at pH 8 and the cycle repeated. Since the acidophilic thiobacilli are inactive above pH 4.5, this is strong evidence for the presence of a mixed culture of acidophiles and non-acidophiles. For comparison, Fig. 2 also shows the failure of this mixed culture to attack a resistant pyrite specimen. This exemplifies one of the factors (i.e. pyrite reactivity) which can be better evaluated by actual leaching tests rather than by purely chemical or physical analytical tests. Acid formation from susceptible pyrite samples was prevented by antimicrobial agents.

Plates of pyrite agar with added basal salts, streaked from liquid media which had become acid, yielded four distinct colonial types. One is a mixotrophic *Thiobacillus*; another may be a N_2-fixing acidophile. One is a *Nocardia*. Two others are gram positive heterophs. Identification of these bacteria is still incomplete. The medium contained no organic matter other than that transferred during inoculation or synthesized autotrophically. The persistence of these heterotrophs during prolonged subculturing is therefore an interesting ecological problem. Harrison (1978) has reported the presence of large numbers of heterotrophs during the initial stages of oxidation of an artificial coal spoil.

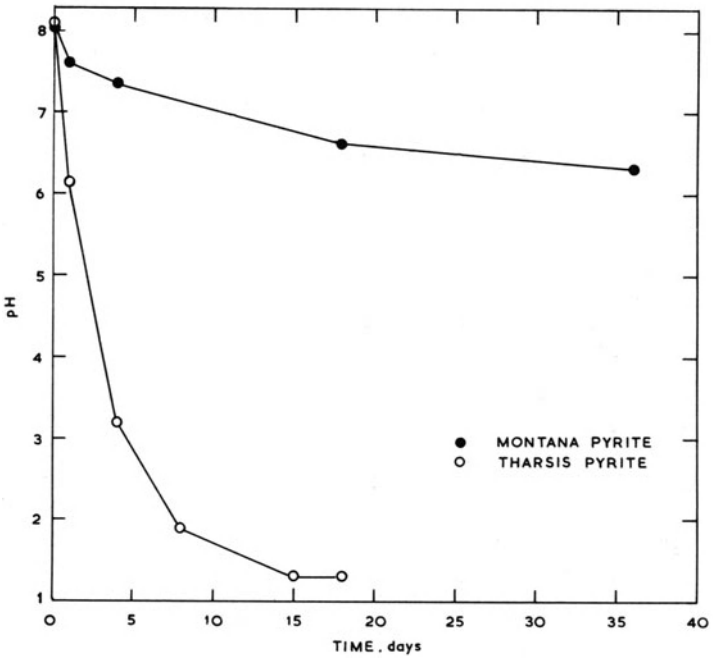

Figure 2: Development of acidity using two different pyrite specimens as substrate.

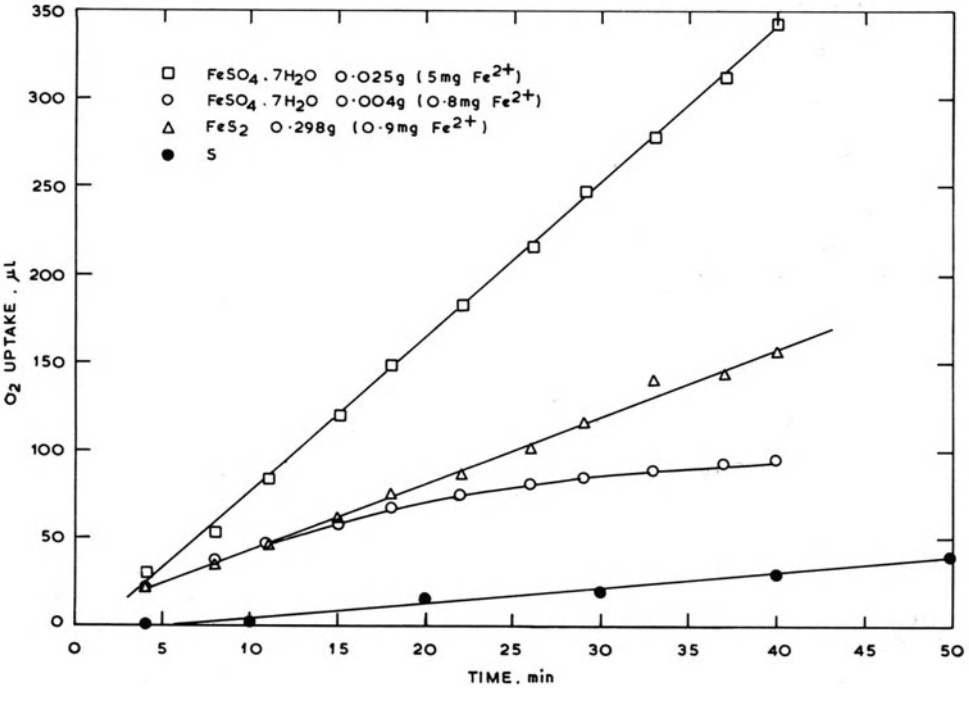

Figure 3: Oxygen uptake by pyrite-grown cells on FeS_2, $FeSO_4$ and S at pH3.

A water wash of the pyrite used for manometric experiments showed that a small amount of readily leachable ('free') ferrous iron (3.2 mg Fe^{2+} g^{-1} pyrite) was present. In manometric experiments, using pyrite-grown cultures (Fig. 3), initial oxygen uptake rates were the same on pyrite as on Fe^{2+} added as ferrous sulfate in amounts equivalent to this 'free' ferrous iron. However, while the ferrous sulfate was oxidised to completion, as judged by the stoichiometry of oxygen uptake, and the uptake rate consequently decreased, the initial O_2 uptake rate on pyrite was maintained long after all the 'free' ferrous iron would have been oxidised. However, the oxygen uptake rate on pyrite remained below that observed if much larger amounts of ferrous iron were used as substrate. Elemental sulfur gave a much slower O_2 uptake (Fig. 3).

Oxygen uptake rates on pyrite were highest at pH 2, identical at starting pH's of 4 and 6 and slower at pH 8. (Fig. 4). As the reaction proceeded, the pH in the Warburg flasks dropped and the uptake rate increased, provided the initial pH was above 4.0.

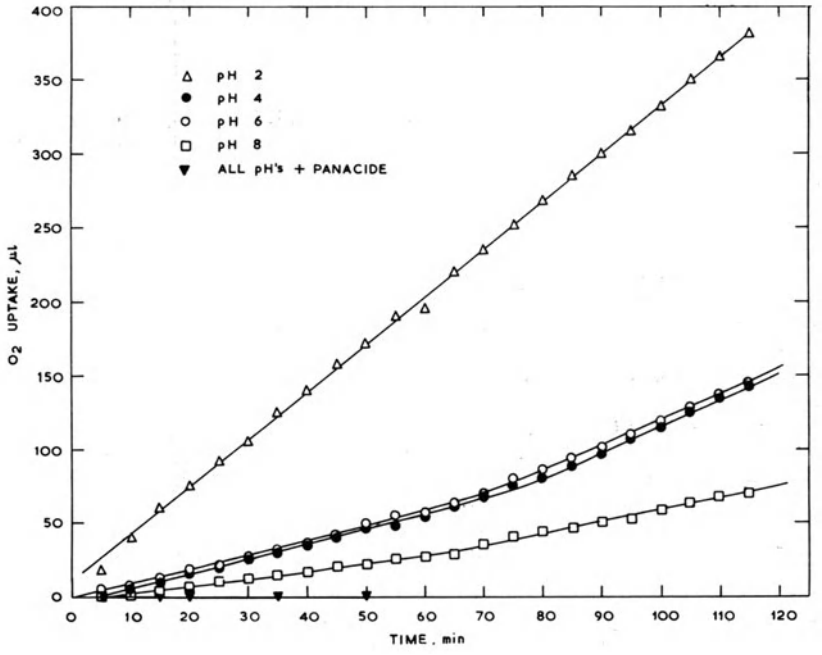

Figure 4: Oxygen uptake using pyrite as substrate at different starting pH values.

^{35}S elemental sulfur oxidation yielded small amounts of ^{35}S-sulfate and of other soluble products in 3 h at a pH above 7. This confirmed the slow oxidation of elemental sulfur in neutral to alkaline media by cells harvested from a strongly acid stage of cultures. No adaptation period was required. Oxidation at lower pH values was more rapid as would be expected.

Oxygen uptake did not occur in the presence of antimicrobial agents.

Spoils from very different types of coal reclamation sites thus contain a mixture of bacteria which can catalyse the oxidation of pyrite with the formation of acidic products. In a scenario for acid formation in alkaline or limed pyritic spoils, the initial oxidation of pyrite may be envisaged as occurring by two concurrent processes. In areas where buffering material is lacking, acidic zones can readily develop, with hot spots forming

and the involvement of acidophilic thiobacilli. In alkaline regions, pyrite oxidation could involve a mixed and presumably dynamically changing bacterial population and our results demonstrate the feasibility of this. However, the detailed mechanism of this alkaline sequence has yet to be investigated and a number of questions will require answers Sulfur oxidation may be a minor component of the system at all pH levels and ferrous iron oxidation an important component at low pH values. However, these reactions fail to explai the rate of pyrite oxidation at high pH.

TABLE 2

OXIDATION OF ELEMENTAL SULFUR AS DETERMINED USING ^{35}S

DAYS	1 (3 h)	2	3	7
pH	7.3	6.8	5.5	2.8
$^{35}SO_4^{2-}$[a]	100	324	683	13,459
	136	290	692	11,068
	130	248	509	13,127
other[a] soluble ^{35}S	107	163	125	80
	94	186	137	86
	67	138	131	84

[a]Counts per minute corrected for a blank

Since the results were obtained on pyrite, rather than pyritic coal spoil, their application to the field situation should be cautious. Further experiments are in progress to test the pyrite oxidation results in the presence of coal, lime and coal spoil, all of which could contain bacterially inhibitory components. The introduction of coal and spoil can also enlarge the range of possible substrates for acid production (Harrison, 1978).

However, until it is definitively proven whether or not pyrite in alkaline spoil is susceptible to bacterial attack, liming practice must be based on a reliable quantitative knowledge of the acid-generating potential under the actual spoils reclamation practice. Not only could uneven distribution of lime cause hot spots to occur, but the alkaline sequence above, if operative, will eventually merge into the hot spot sequence once the neutralising capacity of the lime and other buffers has been overcome.

The ecological characterisation of the system which develops from alkalinity to acidity provides a challenging problem which promises to cast light on the basic mechanisms of pyrite oxidation.

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THE EFFECT OF CHEMICAL AND BIOLOGICAL REDOX REACTIONS ON THE GROWTH OF *THIOBACILLUS THIOOXIDANS*

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INTRODUCTION

The interaction between bacteria and metals can vary considerably, depending on the level of oxygen and the pH of the system. By its limitation of the redox potential of a system, pH will consequently limit the species of bacteria existing under those conditions and will also constrain the redox reactions accessible to metals of variable oxidation states. Interest herein has been directed primarily towards determining the effect of reducible species such as vanadium(V) and to a lesser extent, chromium(VI) and molybdenum(VI), on the growth of *Thiobacillus thiooxidans*. We became interested in this topic through a study into the use of *T. thiooxidans* in the bioleaching of a petroleum coke derived from Athabasca oil sands bitumen (Zajic *et al.*, 1978). It was observed that leached vanadium and iron were almost entirely in their lower oxidation states (+4 and +2 resp.) and that small concentrations of the metals chromium and molybdenum in their +6 oxidation states could curtail growth of the bacteria.

Bacteria of the genus *Thiobacillus* exist over a wide pH range but in an acidic environment, the organisms *T. ferrooxidans* and *T. thiooxidans* predominate. These species can exhibit offsetting effects, e.g. *T. ferrooxidans* oxidizes V(IV) (Goren, 1966) and Fe(II) in an oxygenic environment, while *T. thiooxidans* reduces Fe(III) under usual growth conditions (Brock and Gustafson, 1976). It was of interest therefore, to examine the effect of *T. thiooxidans* on reducible metal species such as the isoelectronic ions of V(V), Cr(VI), and Mo(VI).

METHODS

The inhibition of growth of *T. thiooxidans* was investigated by the following methods:

Inhibition of Growth (Method 1)

Flasks containing standard medium* (100 ml) and sulfur (0.5 g) were inoculated with a log phase culture of *T. thiooxidans* ATCC 8085 (2 ml). Metal salts at various concentrations were added to the flasks which were then shaken at 24°. Growth was monitored by cell counts, protein production and pH.

Inhibition of Growth Rate (Method 2)

The culture was grown in standard medium (500 ml) with sulfur (2.5 g) in a shake flask at 24°. In mid-log phase growth, the culture was divided into 100 ml aliquots and various concentrations of metal salts were added. Growth was monitored by cell counts.

RESULTS AND DISCUSSION

The results for chromate (Method 2) are shown in Fig. 1. At concentrations above 0.2 mM (10 ppm), Cr(VI) prevents growth of *T. thiooxidans*; below this level, a lag in the growth curve is observed, followed by normal growth. Similar results were encountered with Mo(VI). Although not indicated in Fig. 2, Mo(VI) at a concentration of 20 ppm (0.2 mM) also suppresses growth.

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* Standard medium contains: KH_2PO_4, 3.0 g; $(NH_4)_2SO_4$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.25 g and $MgSO_4 \cdot 7H_2O$, 0.5 g in 1 litre of distilled water.

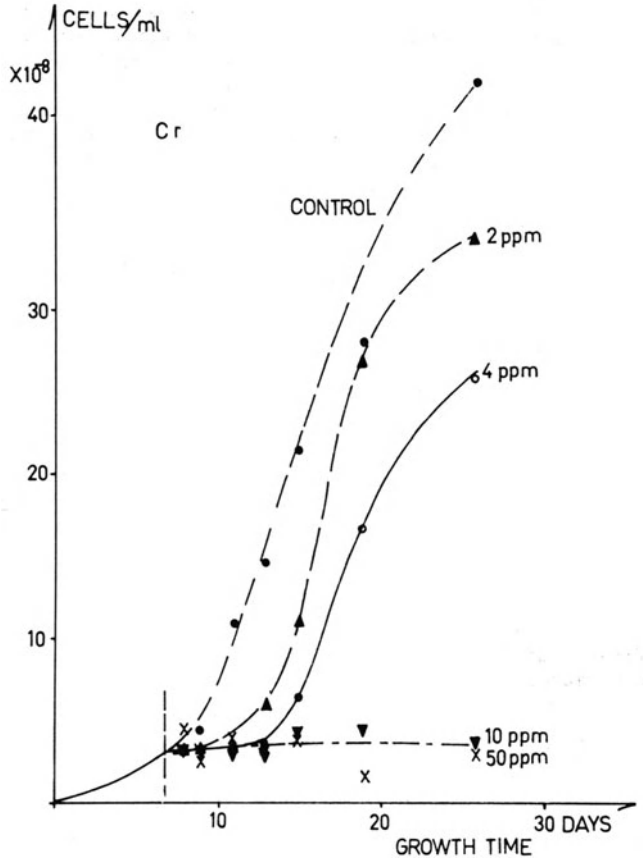

Figure 1: The concentration dependence of growth inhibition by chromium(VI) on *T. thiooxidans* ATCC 8085.

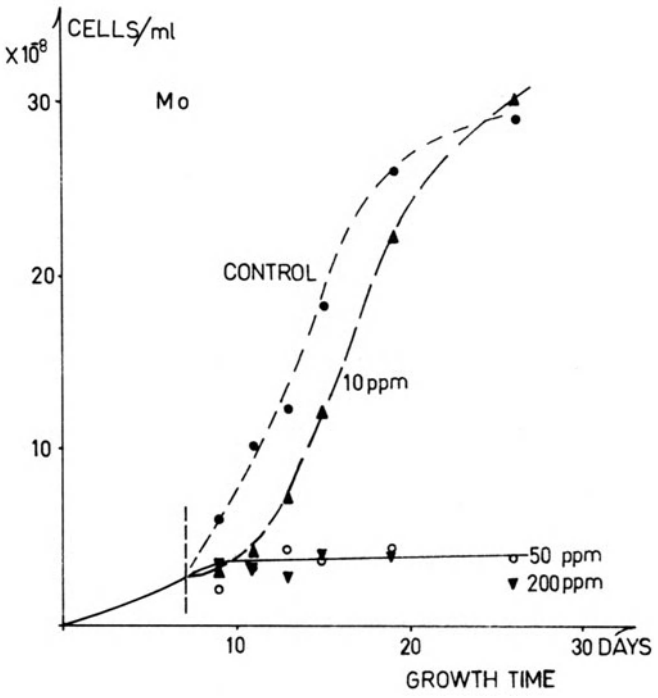

Figure 2: The concentration dependence of growth inhibition by molybdenum(VI) on *T. thiooxidans* ATCC 8085.

Upon examination of the vanadium(IV)/vanadium(V) system, it is seen (Fig. 3) that V(IV) does not adversely affect the growth of *T. thiooxidans*, up to a concentration of at least 1370 ppm by Method 1 and up to 2850 ppm (56 mM) by Method 2. Fig. 4 shows, however, that at concentrations of 3-600 ppm, V(V) causes a lag phase in the growth curve of *T. thiooxidans*. Eventually, growth is prevented at 1000 ppm. Solutions of V(V) at the lower concentrations turn from yellow to blue, indicating that V(V) is being reduced to V(IV) during the growth of the bacteria.

The bioreduction has been followed spectrophotometrically, by observing the formation of the blue vanadyl ion VO^{2+}, at a wavelength of 750 nm. The results shown in Fig. 5 indicate that the V(V) is almost completely reduced within a day. Since this is almost the same as the lag phase in the growth curve (Fig. 4), it appears that the growth of *T. thiooxidans* is curtailed until all the V(V) is reduced to the inocuous V(IV).

The reduction process is first-order in V(V) concentration (Fig. 6), with a half-life of about 10 h(Table 1). The deviation of the plots for the higher V(V) concentrations (600, 687 ppm) is probably due to the depletion of biologically-produced reducing agents. The concentration of available reducing agents depends on the number, viability and metabolism of the cells and this will be affected by the higher V(V) concentrations. The rate of reduction of V(V) ($t_{1/2}$ ca. 10 h) by *T. thiooxidans* is an order of magnitude faster than the corresponding rate of reduction for Fe(111) ($t_{1/2}$ ca. 9 days: Brock and Gustafson, 1976).

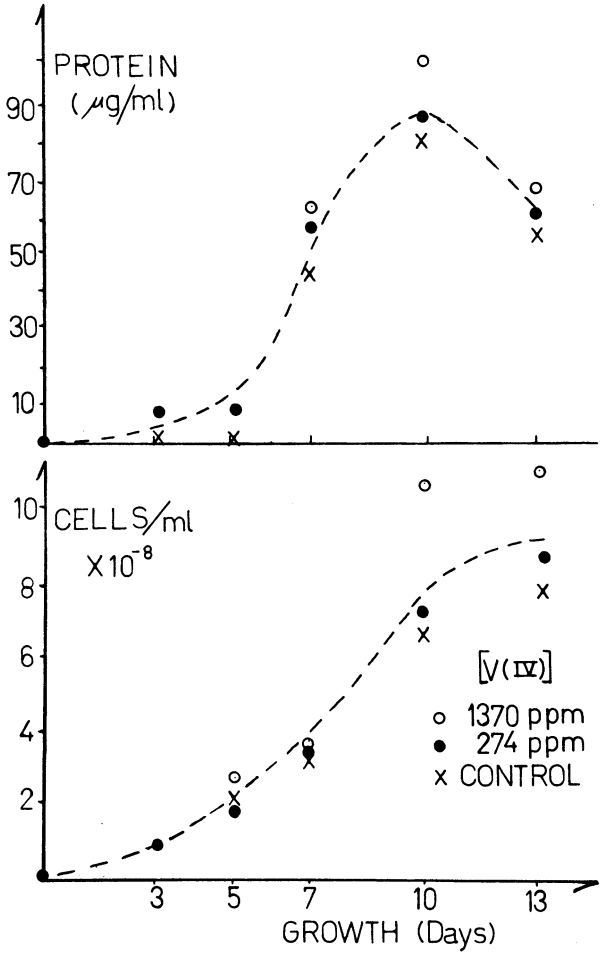

Figure 3: The effect of vanadium(IV) on the growth of *T. thiooxidans* ATCC 8085 determined by protein production and cell numbers.

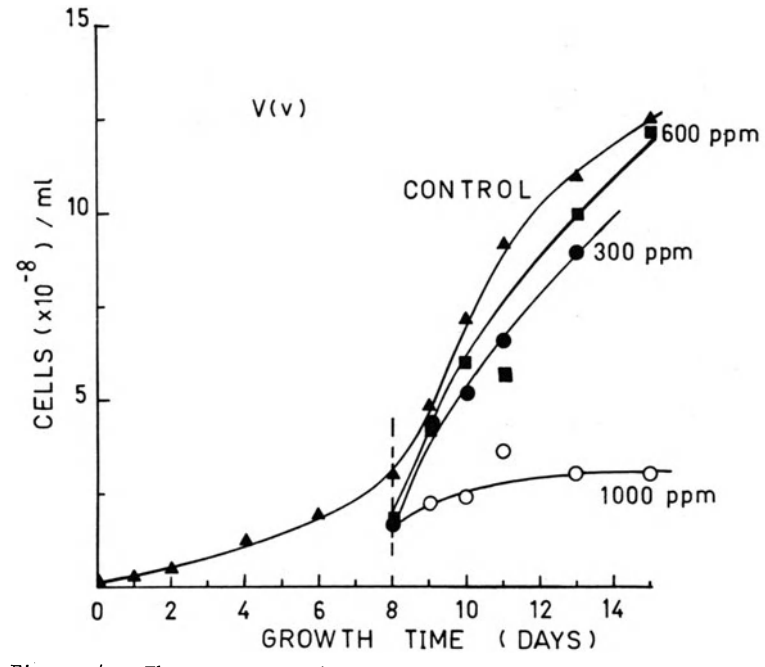

Figure 4: The concentration dependence of growth inhibition by vanadium(V) on *T. thiooxidans* ATCC 8085.

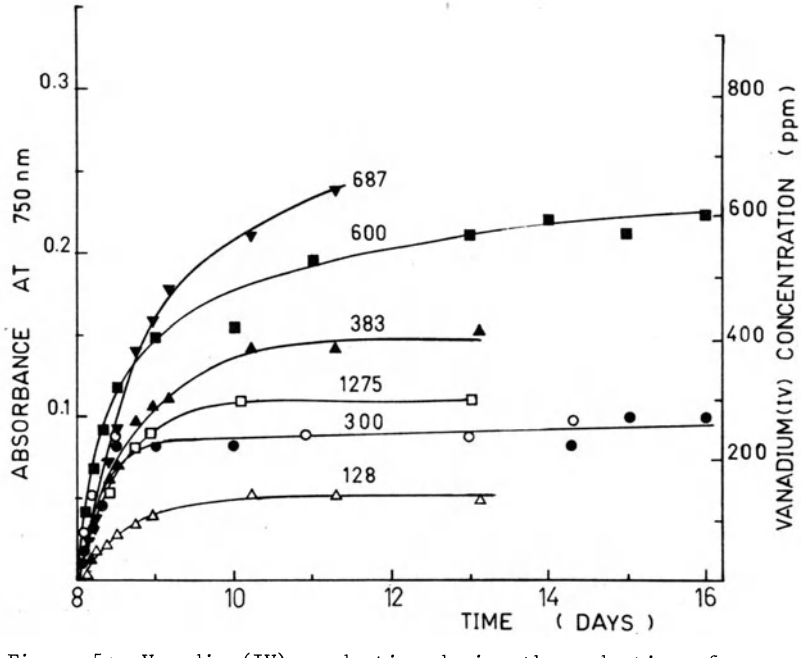

Figure 5: Vanadium(IV) production during the reduction of vanadium(V) by *T. thiooxidans* ATCC 8085.

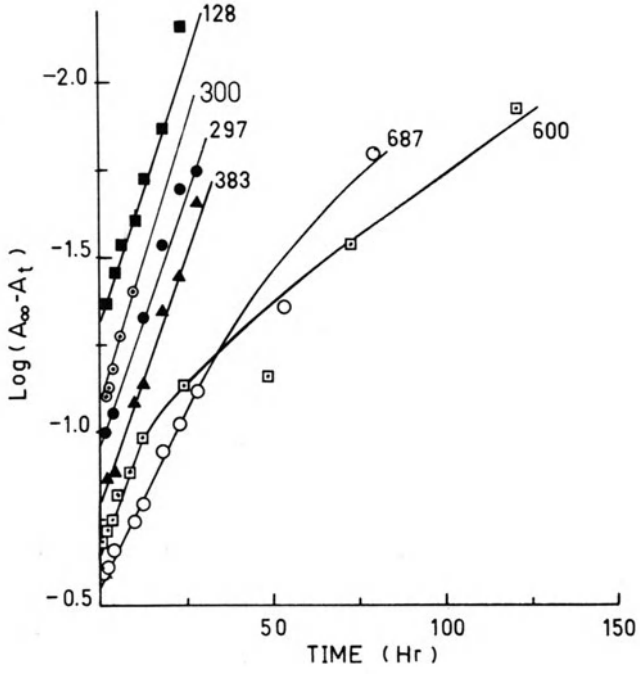

Figure 6: First-order rate plots for the reduction of vanadium(V) by *T. thiooxidans* ATCC 8085.

TABLE 1

RATE CONSTANTS FOR THE REDUCTION OF VANADIUM(V) BY *T. THIOOXIDANS* ATCC 8085

Vanadium(V) Concentration (ppm)	First-Order Rate Constant x100 (h^{-1})	Half-life $t_{1/2}$ (h)
128	7.02	9.9
300	8.90	7.8
296*	6.99	9.9
383	6.97	9.9
600	6.37	10.9
687	4.42	15.7

* Estimated from absorbance of V(IV): solution initially contained 1275 ppm V(V).

In the oxidation of elemental sulfur

$$S + H_2O + 1.5O_2 \rightarrow SO_4^{2-} + 2H^+$$

catalyzed by *T. thiooxidans*, sulfur compounds in intermediate oxidation states are produced. If these intermediates are polythionates, thiosulfate or sulfite (London and Rittenberg, 1964; Kelly, 1968), then they will react readily with chemical oxidizing agents such as Cr(VI) (Baldea and Niac, 1968). From a consideration of the reduction

potentials shown in Table 2, it would have been expected that sulfur intermediates would be able to reduce Cr(VI), V(V) and Fe(111). For such reactions to occur during the microbial oxidation of sulfur, it is necessary that either the oxidizing agent be taken up by the cells or that the oxidizable intermediates be released into the culture medium. There is, of course, a question of pH and chemical species here: the pH inside the cell and in the culture medium are likely quite different. In the current experiments, V(V) was only reduced in the presence of living cells; reduction was not observed if the bacteria were killed, either by autoclaving or with azide. This result suggests that the metal ions may be reduced by the products of bacterial growth rather than by oxidative interaction with the organic constituents of the cells.

TABLE 2

STANDARD REDUCTION POTENTIALS*

Half-Reaction	Reduction Potential (volts)
$14H^+ + Cr_2O_7^{2-} + 6e \rightarrow Cr^+ + 7H\ O$	1.33
$2H^+ + VO_2^+ + e \rightarrow VO^{2+} + H_2O$	1.00
$Fe^{3+} + e \rightarrow Fe^{2+}$	0.77
$2H^+ + H_3AsO_4 + 2e \rightarrow HAsO_2 + 2H_2O$	0.56
$2H^+ + H_2MoO_4(aq) + e \rightarrow MoO_2^+ + 2H_2O$	0.48
$2H^+ + SO_4^{2-} + 2e \rightarrow SO_2(aq) + H_2O$	0.17
$S_4O_6^{2-} + 2e \quad 2S_2O_3^{2-}$	0.17

* Decreasing power as oxidizing agents

The severity of the inhibition to growth is not related to the reduction potentials of the metals; Mo(VI) inhibits growth to a far greater extent (about 2 orders of magnitude) than does V(V) but the latter has the greater oxidizing ability. (See Table 2.) Vanadate, chromate and molybdate in their interactions with cells and enzymes under the appropriate conditions of pH can show a variety of effects; they inhibit thiosulfate and tetrathionate oxidation by thiobacilli (Kelly, 1968), V(V) and Mo(VI) compete with phosphate for certain enzyme sites (VanEtten *et al.*, 1974), V(V) inhibits the transport of Na^+ and K^+ across cell membranes (Cantley *et al.*, 1977) and Cr(VI) appears to lead to mutagenicity through its direct interaction with bacterial DNA (Petrilli and De Flora, 1977). A trace amount of molybdate (<0.3 ppm Mo(VI)) is beneficial to the growth of *T. thiooxidans* (Takakuwa *et al.*, 1977) but this stimulative effect was not observed with chromate and vanadate.

In the presence of oxygen, Mo(VI) and As(V) are the thermodynamically favoured species but all the metal reduction reactions show in Table 2 can be effected with an acidic solution of SO_2 (Cotton and Wilkinson, 1962; Palmer *et al.*, 1976). The reductions are rapid and even in an oxygenic environment, kinetic factors may probably allow the reduction of Mo(VI) and As(V) in the presence of *T. thiooxidans*. In the presence of molybdate, the cells acquired a faint blue coloration, indicating that at least some of the molybdate had been reduced. Mild reducing agents such as SO_2 are known to reduce Mo(VI) to "molybdenum blue". (Cotton and Wilkinson, 1962.)

Chromium, molybdenum and vanadium in their highest oxidation states curtail the growth of *T. thiooxidans* and in situations where these species are released from mineral ores as a consequence of microbial growth, the rate and extent of metal release may be significantly decreased. Lower oxidation states are readily accessible to these metals and the redox potential of the V(IV)/V(V) system allows vanadate at least, to be reduced to a relatively inocuous species.

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INHIBITION BY PYRITE OF BACTERIAL IRON OXIDATION ACTIVITY

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INTRODUCTION

Ores amenable to microbial leaching often contain pyritic material which contributes to the formation of leaching agents, i.e. acidic ferric sulfate. The bacterial attack on pyrite involves either a direct oxidation mechanism or acceleration of the parallel chemical degradation of pyrite by ferric iron and sulfuric acid (Silverman, 1967; Tuovinen and kelly, 1972, 1974d; Torma, 1977; Brierley, 1978a; Kelly *et al.*, 1979; Manchee, 1979). Thus, pyrite is an important source of leaching agents for the microbial solubilization of bres. Iron-oxidizing thiobacilli (*Thiobacillus ferrooxidans*) are able to grow on FeS_2 but a prior adaptation to this substrate as well as to other sulfide minerals is required (Silver and Torma, 1974; Norris and Kelly, 1978a). Previous studies have shown that fine-grained particles of different materials (glass beads, fluorapatite, sand, quartz, U-bearing rocks, and pyrite ore) inhibit the iron oxidation activity of *T.ferrooxidans* (Soljanto *et al.*, 1980). The inhibition of the bacteria was related to the shaking of the culture flasks and to the type, amount and particle size of the various materials studied. In the present work, high-grade pyrite was investigated in more detail with a view to determining its inhibitory nature for the iron-oxidizing thiobacilli. Other particles (coal, mixed copper-nickel ore) were also tested in the experiments to extend the previous bbservations.

MATERIALS AND METHODS

Bacteria

An iron-oxidizing culture of *Thiobacillus ferrooxidans* (strain 1; Tuovinen *et al.*, 1979) was routinely maintained in a liquid medium (pH 1.5) containing 120 mM $FeSO_4$. 100 ml cultures were grown in 300 ml shake flasks on a shaker at 75 strokes min^{-1} at 25° as described previously (Tuovinen *et al.*, 1979). The cultures were inoculated with 5 ml of 4-5 day old cultures (stationary phase) and the growth was monitored by titration with botassium permanganate of the residual ferrous-iron (Tuovinen *et al.*, 1979).

Particle Samples

Granular pyrite (85% min. -50 mesh) was purchased from Matheson, Coleman & Bell Manufacturing Chemists (norwood, OH). High-grade pyrite (99.9% pure; -100 mesh) was obtained from Cerac/Pure Inc. (Milwaukee, WI). Coal samples were obtained from Amax Coal Co. (Sun Spot Mine, Vermont, IL) and ground and sieved to different particle size fractions. The coal sample contained 3.09% Fe predominantly as FeS_2. The Duluth Gabbro ore concentrate was supplied by the U.S. Bureau of Mines, Twin Cities Research Center (St. Paul, MN) and concained chalcopyrite, millerite, pyrrhotite, pentlandite, and pyrite as the principal minerals. The ore was ground -270 mesh and contained 17.13% Fe, 7.17% S, 4.22% Cu, and 0.90% Ni (Tsuchiya, 1978). The particle samples were not sterilized unless otherwise indicated.

Inhibition of *T.ferrooxidans* by Pyrite

Pyrite particles (-50 mesh) above 1% (w/v) concentration were increasingly inhibitory to ferrous iron oxidation by *T.ferrooxidans* (Fig. 1). The inhibition was alleviated by increasing the pH from 1.5 to pH 2.5 (Fig. 1). High-grade pyrite (-100 mesh) was completely inhibitory even at 1% concentration but an increased pH slightly relieved the toxicity.

(Fig. 2). The following compounds were tested in the culture medium for alleviating pyrite toxicity but were without effect: Tween 20, Tween 40, Tween 80 (each at 0.1, 1.0, 10, 100, 500 and 1000 mg l^{-1}); NH_4^+, Na^+, K^+, Mg^{2+}, Cu^{2+} (each added as their respective sulfates at 100 and 200 mM); and 0.05% (w/v) yeast extract.

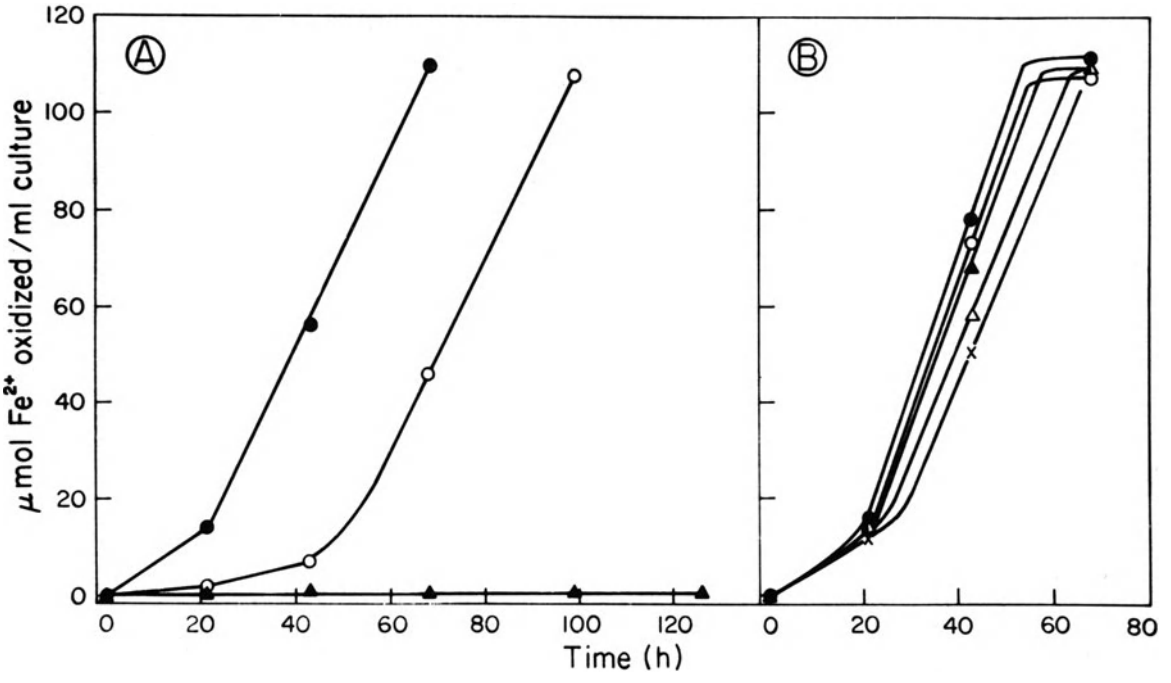

Figure 1: Inhibition by pyrite (-50 mesh) of bacterial iron oxidation. A. Cultures growing at pH 1.5 were supplied with 0 (●), 1% (○), or 2-4% w/v (▲) pyrite. B. Cultures growing at pH 2.5 were supplied with 0, (●), 1% (○), 2% (▲), 3% (△), or 4% (×) pyrite.

Inhibition of *T.ferrooxidans* by Coal and Ore Particles

The sulfide ore concentrate and high-sulfur coal were examined for comparison with pyrite toxicity. At pH 2.5 the sulfide ore was much more inhibitory than the -50 mesh pyrite sample (Fig. 3). The bacteria were also inhibited by the coal particles. As with pyrite, the inhibition showed pH-dependence and the iron oxidation rates were faster at the higher pH (Fig. 4 and Fig. 5). The tests with coal particles also demonstrated that the inhibitio was more pronounced with the smaller particle size fraction.

Effect of Washing, Autoclaving, and Filtration

Fig. 6 shows that the inhibitory effects by pyrite (-50 mesh) were reduced when the particles were first washed by soaking in distilled water. These results indicated that some of the toxicity was associated with a water-soluble component. To test this further, pyrite particles were suspended in the liquid medium for up to 24 h and filtered. The filtrates were inoculated with *T.ferrooxidans*. The results clearly indicated that a soluble toxic component could be leached from FeS_2 (Fig. 7). The toxicity of the filtrates was related to the amount of pyrite soaked as well as to the length of time of soaking. Moreover, autoclaving of pyrite or its filtrates at 120° for 15 min before inoculation greatly reduced the toxicity (Fig. 8). This indicated that the toxic agent was partly removed from the particle surface, or was oxidized during autoclaving, thereby causing less inhibition of the bacterial iron oxidation.

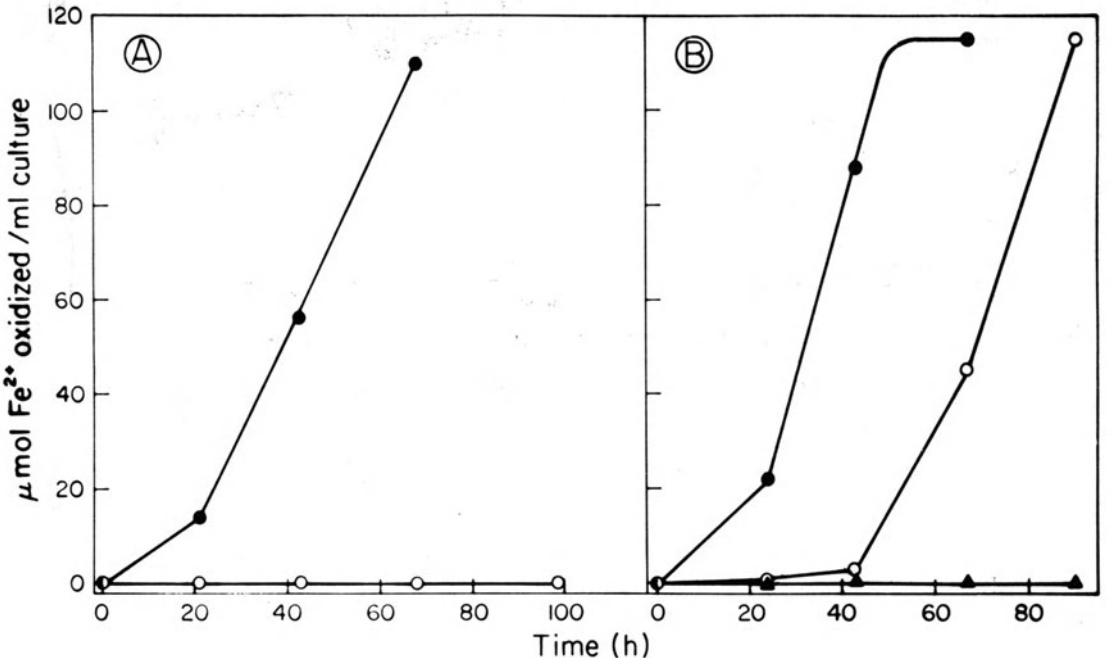

Figure 2: Inhibition by 99.9% pure pyrite (-100 mesh) of bacterial iron oxidation. A. Cultures growing at pH 1.5 received 0 (●), or 1-4% w/v (O) pyrite. B. Cultures growing at pH 2.5 received 0 (●), 1% (O), or 2-4% (▲) pyrite.

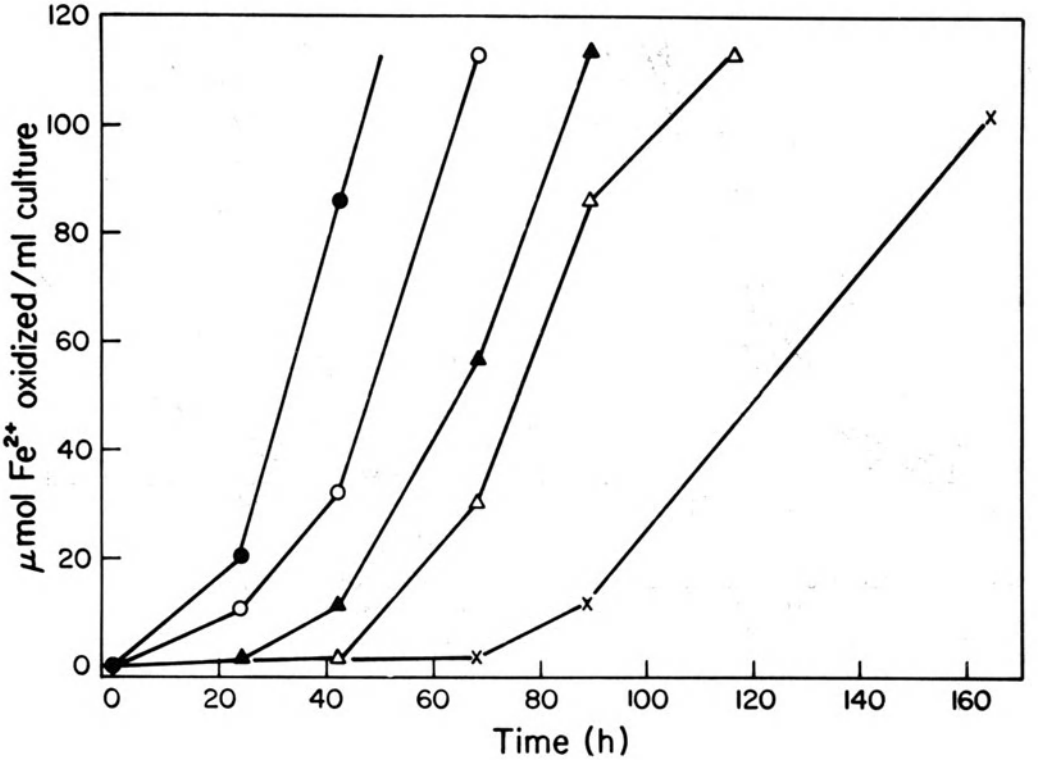

Figure 3: Inhibition of a sulfide ore concentrate of bacterial iron oxidation. Cultures growing at pH 2.5 received 0 (●), 1% (O), 2% (▲), 3% (Δ), and 4% (x) sulfide ore.

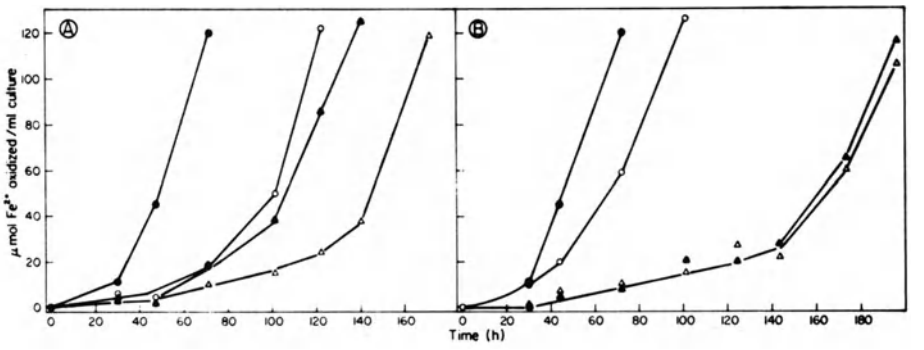

Figure 4: Effect of finely ground coal on bacterial iron oxidation at pH 1.5. Cultures received 0 (●), 1% (○), 3% (▲), and 5% (△) coal. Particle sizes: A, -200 mesh; B, -20 to + 40 mesh.

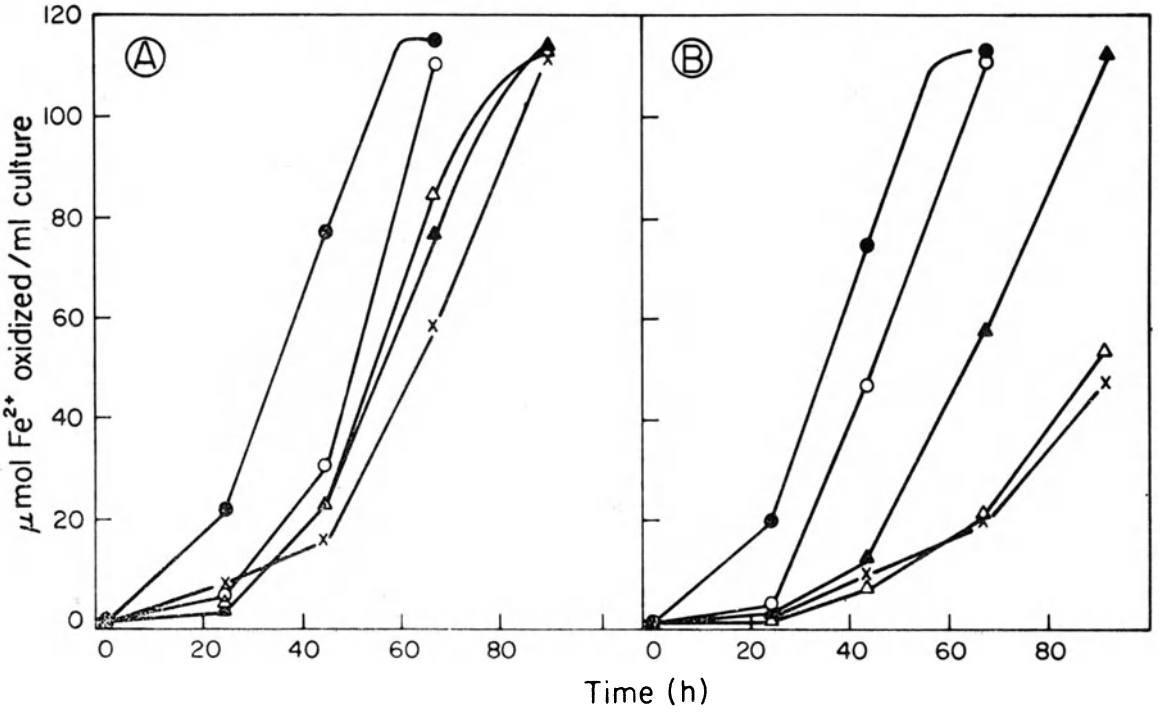

Figure 5: Effect of finely ground coal on bacterial iron oxidation at pH 2.5. Cultures received 0 (●), 1% (○), 2% (▲), 3% (△), and 4% (x) coal. Particle sizes: A, -200 mesh; B, -20 to + 40 mesh.

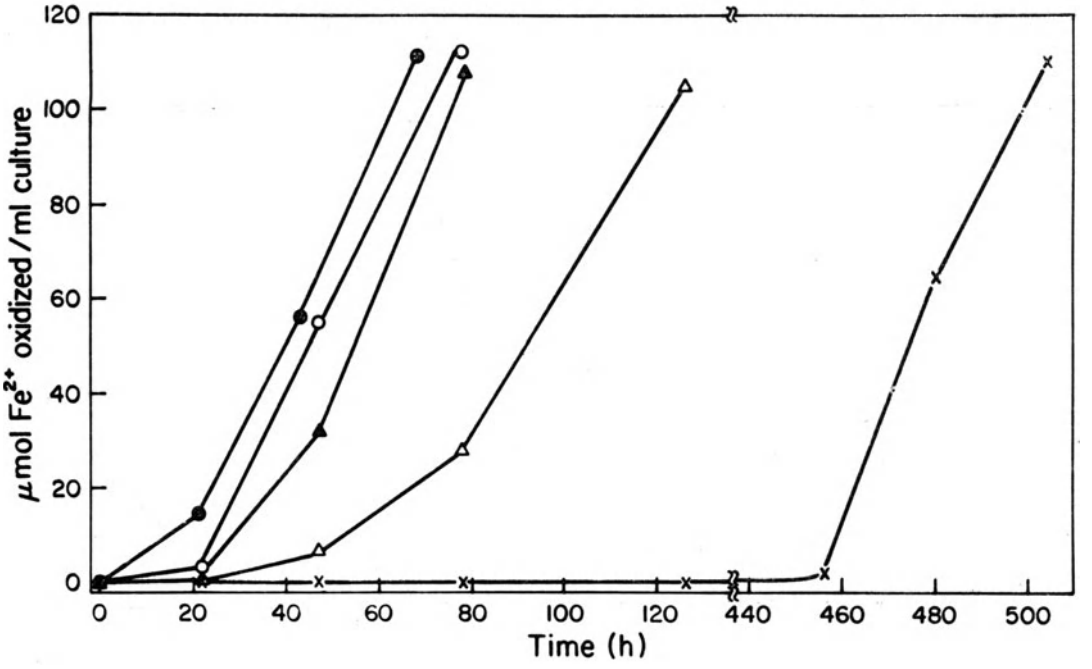

Figure 6: Effect of prewashed pyrite (-50 mesh) on bacterial iron oxidation at pH 1.5. Pyrite was washed by shaking in distilled water for 24 h and dried at 75° after filtration. Cultures received washed pyrite as follows: ●, 0%; ○, 1%; ▲, 2%; △, 3%; x, 4%.

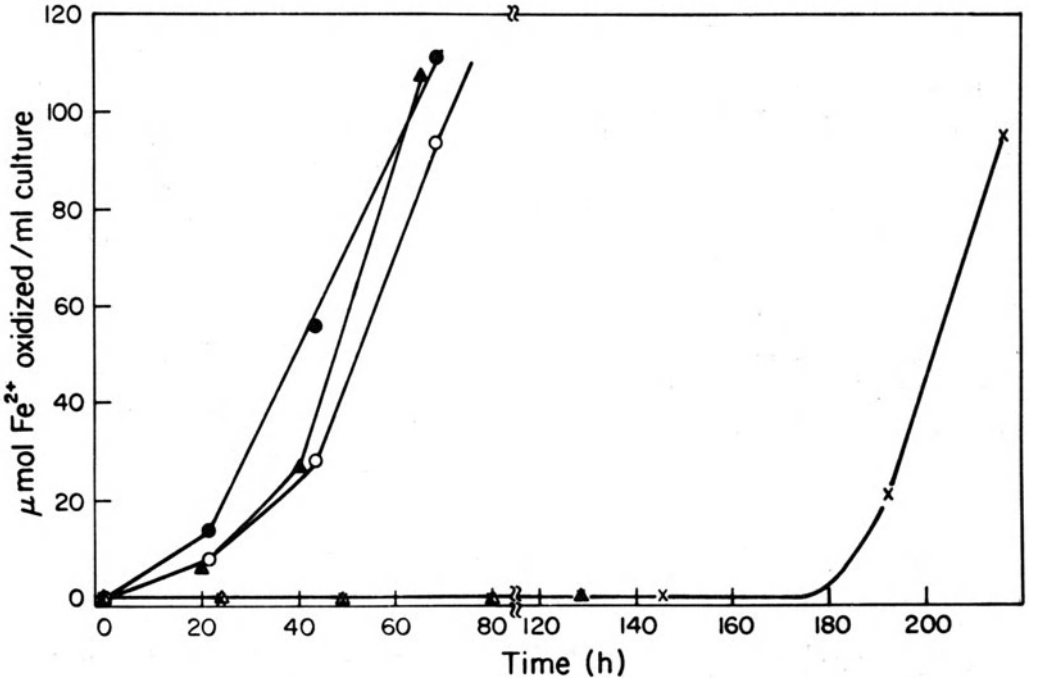

Figure 7: Inhibition of bacterial iron oxidation in pyrite filtrates at pH 1.5. Pyrite (-50 mesh) was suspended in the mineral salts solution containing ca.115 mM $FeSO_4$ and shaken for different lengths of time before filtration. The filtrates were then inoculated with *T.ferrooxidans*. Symbols: ●, control; ▲, 2% pyrite shaken for 8 h before filtration· ○, 2% pyrite shaken for 1 h; △, 4% pyrite shaken for 24 h; x, 4% pyrite shaken for 1 h.

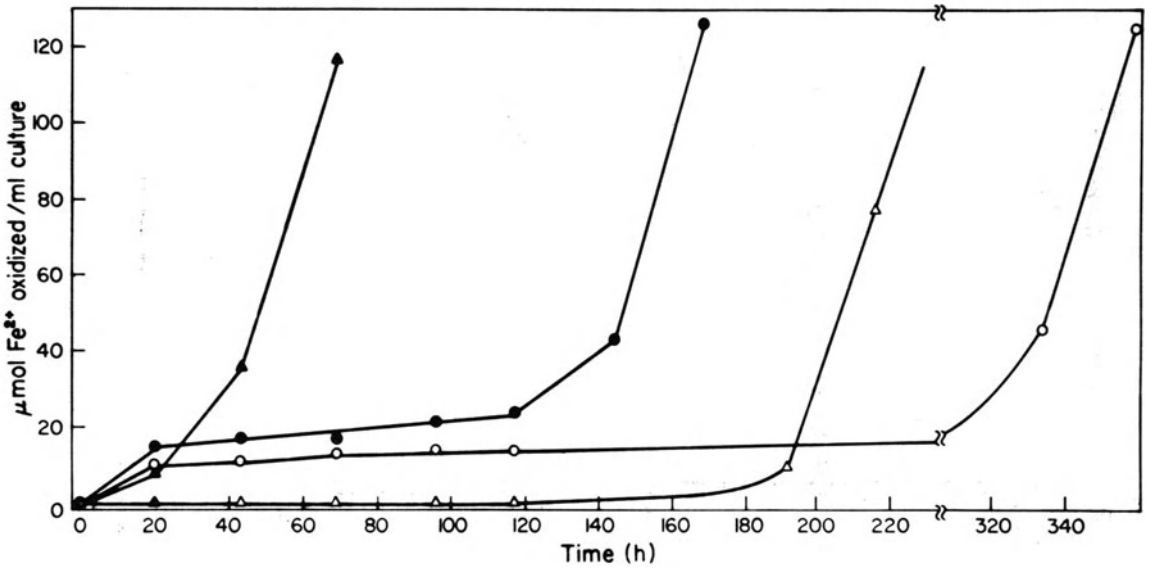

Figure 8: Effect of autoclaving on the toxicity of pyrite (-50 mesh) to bacterial iron oxidation at pH 1.5. Cultures received 1% pyrite which was (●) autoclaved in the mineral salts solution, or (○) not autoclaved before inoculation. Filtrates were tested by shaking 4% pyrite for 1 h in the mineral salts solution followed by filtration. The filtrates were (▲) autoclaved or (△) not autoclaved before inoculation.

DISCUSSION

Parallel chemical tests with acidic ferric sulfate employing a similar time course indicated that less than 7 µmol Fe^{3+} ml^{-1} was reduced by pyrite, coal and sulfide ore concentrate at the concentrations used in the bacterial iron oxidation tests. Therefore, the chemical reduction of ferric iron could not solely account for the apparent inhibition of the bacterial ferrous iron oxidation. It was noticeable that increased lag-periods commonly resulted from the addition of pyrite or its filtrates; the oxidation rates were much less affected after the bacterial oxidation ensued. This indicated that either the bacteria could initially adapt to tolerate such inhibitory interactions, thereby being les sensitive at the onset of iron oxidation, or the inhibitory constituents were oxidized or volatilized during the lag period, thereby reducing the subsequent toxicity.

Metal impurities in the materials studied may have partly accounted for the toxic effects. It is known that the iron-oxidizing bacteria are particularly sensitive to some metal ions such as silver (Hoffman and Hendrix, 1976), and the toxicity is substantially reduced at increasing pH values as demonstrated with UO_2^{2+} and Ag^{+} within the pH-range 1.4-2.0 (Tuovinen and Kelly, 1974c; Norris and Kelly, 1978b). The bacteria are relatively resistant or capable of building up resistance to Cu, Ni, Co, Zn, and even U (Tuovinen *et al.*, 1971; Tuovinen and Kelly, 1974a,b; Imai *et al.*, 1975). The coal sample contained a number of metallic elements which were relatively easily leached out as observed in our unpublished leaching experiments: for example, Cr, Ni, Pb, B, Cu and Zn. Other metals such as Ag may be present at trace levels in leachates of the coal and sulfide ore samples and the possibility of trace metal impurities in the pyrite cannot be ruled out.

Furthermore, the samples of coal and sulfide ore may have given rise to H_2S formation whic is rapidly volatilized and oxidized in acidic media. In preliminary experiments we have observed significant inhibition of the bacterial iron oxidation activity in sealed culture in which H_2S was generated (A. DiSpirito and O. H. Tuovinen, unpublished results). The chemical intermediates of sulfide oxidation include colloidal sulfur and various sulfur oxyions the effects of which remain to be elucidated in further work. In pyrite, traces o

FeS may have been present which dissolves evolving H_2S but more importantly, significant surface oxidation of pyrite particles and pyrite in coal may have taken place. A tarnished surface layer of Fe_3O_4, S^o, $FeSO_3$, and FeS_2O_3 may have formed inhibiting the bacteria attached on these surfaces. Extensive washing of the pyrite crystals or autoclaving may remove, for example, $FeSO_3$ from the surface or oxidize SO_3^{2-} to SO_4^{2-} thereby reducing the inhibition. In aqueous acidic solutions sulfite undergoes successive protonations:

$$H^+ + SO_3^{2-} \rightleftharpoons HSO_3^- \qquad pK = 7.1$$

$$HSO_3^- + H^+ \rightleftharpoons H_2O \cdot SO_2 \qquad pK = 1.9$$

HSO_3^- and SO_2, and any other intermediate sulfur oxyanion formed on the surface, are effective reductants and could rapidly reduce Fe^{3+} to Fe^{2+}. This may also explain the increased lag period before the bacterial iron oxidation commenced. The overall chemical reduction of the acidic ferric sulfate by the test materials was extremely slow in uninoculated tests but the bacteria may have initially (during the lag period) catalyzed surface-related reactions in such a manner that the bacterial series was not comparable with the chemical controls in this respect.

Nevertheless, the previous work with thiobacilli on the inhibition by relatively inert materials such as glass beads and quartz (Soljanto *et al.*, 1980) indicates that other factors are involved in addition to sulfur intermediates and toxic trace constituents. Particles may efficiently absorb organic compounds excreted by the bacteria. Many excretion products are known to be toxic to the bacterial growth (Tuttle and Dugan, 1976; Tuttle *et al.*, 1977). The inhibition of *T.ferrooxidans* activity has been briefly mentioned in some leaching studies (Ebner, 1978; Brierley, 1978b) but the data are insufficient to permit their careful examination. Restricted bacterial activity may also result from problems of mass balance of O_2 and CO_2, particle aggregation and cell attrition at high pulp densities (Torma *et al.*, 1972; Pinches *et al.*, 1976) but their role is mosly negligible at the particle concentrations tested in the present work.

In leaching operations iron-oxidizing bacteria are attached to the ore surface and they are also present in the leach liquor. The inhibition of the bacterial iron oxidation by pyrite and its filtrates suggests that these effects may be of importance in systems employing bacterial ferric-iron (re)generation for ore leaching. While the bacterial activity is inhibited, the Fe^{2+} oxidation rates remain low and thus the metal leaching rates are adversely affected. However, in leaching systems the microbial populations are quite heterogeneous (Groudev *et al.*, 1978) and likely to have evolved as a result of selection and adaptation which may have facilitated the bacteria to overcome toxic factors such as those reported in the present paper. While some controversy remains about inhibited or restricted bacterial activity in laboratory ore leaching tests (Atkins, 1978), more work is clearly warranted to resolve further considerations of the toxicity and inhibition associated with mineral materials.

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SULFUR AND HYDROGEN ION BUFFERING IN PYRITIC STRIP-MINE SPOIL

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INTRODUCTION

Accumulation of peat, the precursor of coal, requires a humid climate to support lush vegetation and a high water table to permit prolonged accumulation of organic material in a reduced environment (Averitt, 1973). Most of the eastern and central US coal measures were periodically inundated by sea water during their formation. Sea water contains approximately 2,700 mg l^{-1} sulfate (Garrels and Thompson, 1962) and when coupled with reducing conditions and readily oxidisable organic matter, as was the case during coal formation, significant sulfate reduction occurs. The sulfide produced may react rapidly with detrital ferrous ion (Fe^{2+}) to form ferrous sulfide which is ultimately transformed into pyrite (FeS_2) (Berner, 1971; Rickard, 1972).

Pyrite is the earth's most abundant and widespread sulfide mineral (Deer *et al.*, 1974) and the mechanism of pyrite oxidation has been the subject of a great deal of research. In spite of this research the exact kinetics and mechanics of the various steps are not fully understood. Complicating factors include the involvement of microorganisms and variations in pyrite crystal structure. For example, framboidal pyrite is significantly more reactive at room temperature than secondary pyrite (Caruccio *et al.*, 1976). Pyrite is thermodynamically stable only under strongly reducing conditions as shown by the stability region on the pe-pH diagram (Fig. 1) and oxidises in the presence of oxygen. The overall stoichiometry for complete oxidation of 1 mol of pyrite is:

$$FeS_2 + 3.5H_2O + 3.75O_2 \longrightarrow Fe(OH)_3 + 2SO_4^{2-} + 4H^+ \quad (1)$$

Singer and Stumm (1970) simplified the above reaction and identified two phases as follows:

Initiator reaction:

$$FeS_{2(s)} \longrightarrow Fe^{2+} + \text{S-compound} \quad (2)$$

Propagating cycle:

$$Fe^{2+} + O_2 \longrightarrow Fe^{3+} \quad (3)$$

$$Fe^{3+} + FeS_{2(s)} \longrightarrow SO_4^{2-} + Fe^{2+} \quad (4)$$

Ferric ion (Fe^{3+}) is extremely effective in oxidising pyrite (Equation 4) and in its absence Singer and Stumm (1970) observed a decrease in the rate of pyrite oxidation by a factor of 10^6. The chemical oxidation of Fe^{2+} to Fe^{3+} (Equation 3) is a function of pH and oxidation is rapid at pH greater than 5. However, the solubility of Fe^{3+} is also pH dependent and can be expressed by:

$$\log a_{Fe^{3+}} = 2.4^* - 3\ pH \quad (5)$$

* equilibrium solubility product for $Fe(OH)_3$ assumed to be $10^{-39.5}$ (Norvell, 1971).

According to Equation 5 the activity of Fe^{3+} at pH5 is only $10^{-12.6}$ and, therefore, at high pH there would not be sufficient Fe^{3+} in solution to effectively oxidise pyrite. If the pH is less than 3.5, abiotic chemical oxidation of Fe^{3+} is very slow and pH-independent (Zollinger, 1972; Singer and Stumm, 1970). However, evidence for bacterially-catalysed pyrite oxidation due to bacterially produced Fe^{3+} is documented in many studies.

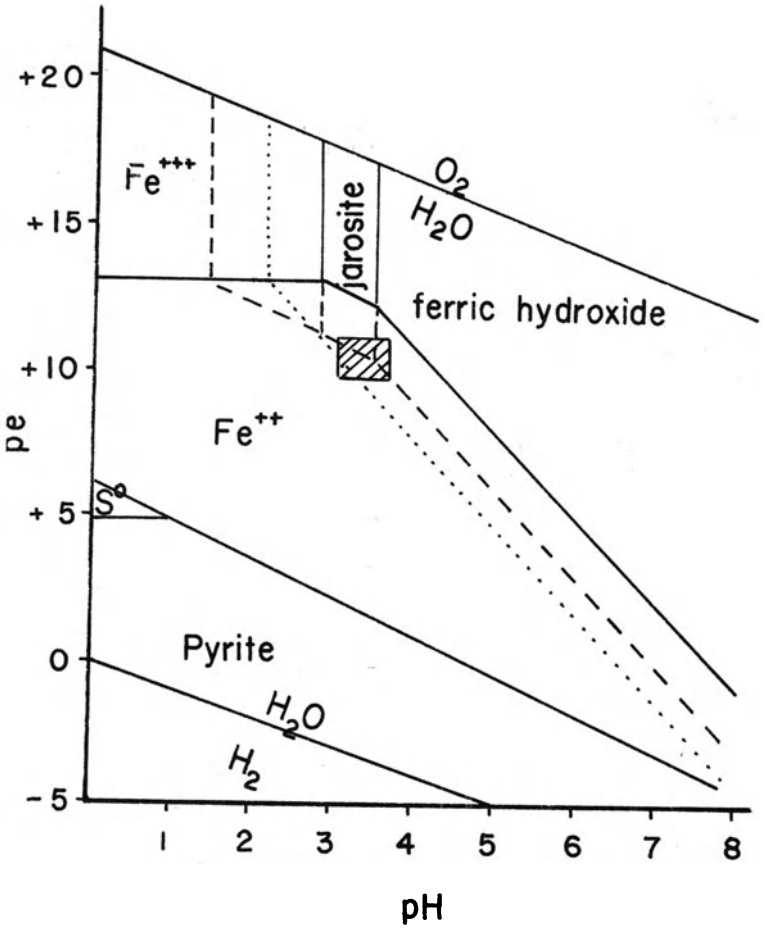

Figure 1: pe - pH diagram. Key: solid line (————) represents activity of iron equal to 10^{-7}, dashed line (----) 10^{-4}, and dotted line (.....) represents goethite stability boundary; values observed in leachates from spoil percolated with deoxygenated water and held in an argon atmosphere are enclosed by ▨. Given conditions: pSO_4^{2-} 3.5, pK^+ 4.6, pK (jarosite) 96.7, pK ($Fe(OH)_3$) 39.0.

The most conclusive evidence was presented by Zollinger (1972) and Smith and Shumate (1970) who observed that the rate of Fe^{3+} production and pyrite oxidation was proportional to the number of iron-oxidising bacteria present. Two bacteria, *Thiobacillus ferrooxidans* and *Ferrobacillus ferrooxidans*, are reported in the literature as being responsible for oxidising Fe^{2+}. However, there is some doubt concerning the taxonomic classification separating these two genera (Unz and Lundgren, 1961).

Reaction sites on pyrite have a much higher affinity for Fe^{2+} than Fe^{3+} and it is necessary to maintain an effective concentration of Fe^{3+} if pyrite oxidation is to be significant (Smith and Shumate, 1970; Garrels and Thompson, 1960). This is accomplished by rapid bacterial Fe^{2+} oxidation. The complete oxidation of 1 mol of pyrite produces 4 eq of hydrogen ion (H^+) as shown by Equation 1 and, because of the insolubility of Fe^{3+}, it is among the most acidic of all weathering reactions. Buffering reactions in the environment inactivate a significant portion of H^+ through acid attack on alumino-silicates, buffering by aluminium hydroxy-complexes and neutralization action of associated minerals such as limestone.

In the eastern and interior coal regions of the United States, spoils show a range of pH from 2.5 to 8 (Berg and Vogel, 1969). However, values between 3 and 4 are commonly observed even on sites that have lain idle for almost 50 yr (Byrnes and Miller, 1969; Einspahar *et al.*, 1955; Riley, 1969; Sutton, 1970). The solubility of many environmentally hazardous elements is pH-dependent having higher activity at lower pH. It is important to understand processes operating within acid spoil under aerobic and anaerobic conditions if environmentally sound and economically feasible rehabilitation programmes are to be initiated and maintained.

This paper examines these issues and identifies fundamental geochemical reactions responsible for the buffering of hydrogen and sulfate ions in an acidic strip-mine spoil.

MATERIALS AND METHODS

Spoil was collected from an abandoned strip-mine near Marion, Illinois. The spoil was leached under controlled atmospheric and moisture conditions in columns based on the design presented by Starr and Parlange (1975). The apparatus permitted unsaturated flow through adjustment of a constant pressure-head input and hanging water column output. Leachates were analysed for pH and the concentration of Fe^{2+}, Fe^{3+}, SO_4^{2-}, Al^{3+}, H_4SiO_4, K^+, Ca^{2+}, Mg^{2+}, Na, Mn^{2+}, Cu^{2+}, Zn^{2+}, and HCO_3^-. Standard colorimetric, gravimetric and atomic absorption techniques were used to determine concentrations. Ion activities in solution were computed from concentrations using the Debye-Hückel expression to correct for ion pairing.

The mineralogy was determined by standard chemical methods and by a Phillips x-ray unit and diffractometer using $CuK\alpha_1$ radiation. Since solution-mineral reactions depend on the composition of mineral and soil particle surfaces it was necessary to investigate the morphology and chemical composition of the surface material. The morphology was examined using an ETEC model U-1 Autoscan Scanning Electron Microscope (SEM). The composition of the near sub-surface (up to 20 Å) was determined using a Hewlett-Packard ESCA Spectrometer System 5950-A(XPS). Further details of methods are presented elsewhere (Miller, 1979).

RESULTS AND DISCUSSION

X-ray analysis of the soil-sized fraction showed quartz, kaolinite (7Å), illite (mica, 10Å), smectite (vermiculite, 14Å) and jarosite to dominate spoil mineralogy. Pyrite was identified in the larger size fraction following crushing and separation (Arora *et al.*, 1978) but no pattern was observed in the less-than 2 mm material. This was supported by chemical analysis which showed pyritic sulfur content, expressed as weight percent, to be 0.06 ± 0.02 in the less-than-2 mm fraction and 0.46 ± 0.03 in the 2 to 5 mm fraction. SEM photomicrographs showed the surface of spoil grains to be coated with an amorphous material which was confirmed to be $Fe(OH)_3$ by XPS analysis. In addition, SEM studies showed the presence of discrete crystals with a morphology consistent with jarosite. Details of these studies are reported elsewhere (Miller, 1979).

Jarosite and $Fe(OH)_3$ were shown to occupy only 4% by weight of the less-than 2 mm fraction. However, since these minerals were observed as discrete crystals and surface coatings they

would be significant in mineral solution reactions. Estimates of the equilibrium ion activity solubility products (pKso) for jarosite and ferric hydroxide vary widely. Recent determinations of pKso for $Fe(OH)_3$ by Norvell (1971) and Vlek *et al.* (1972) probably more accurately approximate true equilibrium than earlier values because of better accounting for ion-pairs and improved analytical procedures. The ion activity product (IAP) for $Fe(OH)_3$ in the soil-solution of spoil samples held at field capacity for six weeks was pIAP 39.0 to 39.6. These values agree with those determined by Norvell (1971). The IAP for jarosite $[KFe_3(SO_4)_2(OH)_6]$ in soil-solution indicated near equilibrium according to data presented by Brown (1970) and strong supersaturation according to Vlek's (1972) data. Vlek determined the pKso using soil jarosite nodules from an acid sulfate soil whereas Brown used freshly precipitated material. In this spoil, jarosite was observed to precipitate when held moist and aerobic for 15 months. In the less-than 2 mm fraction fresh spoil contained a mean jarosite content, expressed as weight percent, of 1.56. After 15 months weathering this increased to 2.35%. Therefore, it was assumed that the data presented by Brown was more applicable to this spoil than Vlek's data and the solubility product for jarosite was considered to be pKso 96.7 ± 0.5.

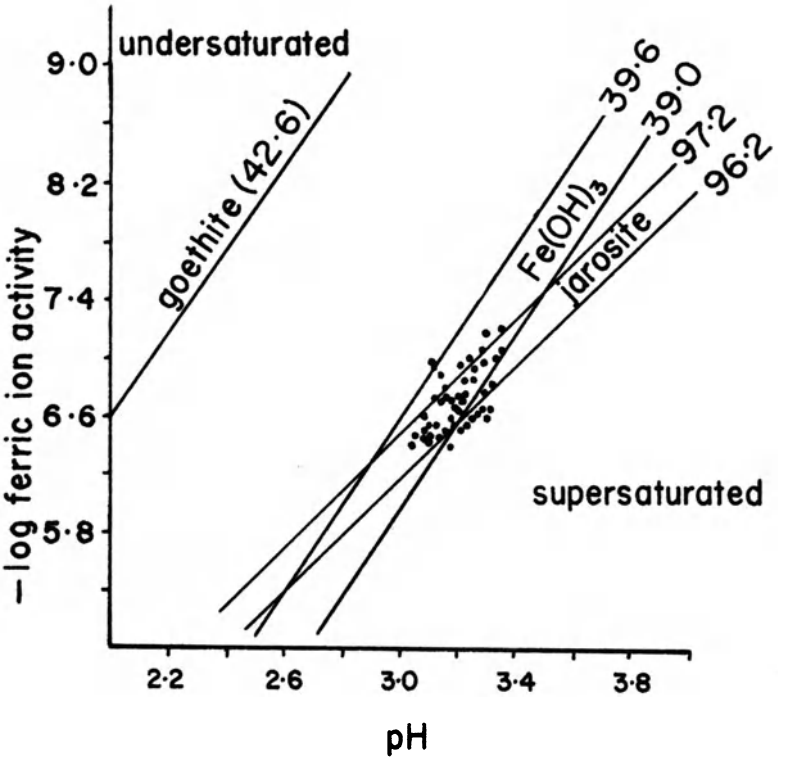

Figure 2: Iron-mineral stability diagram. Key: points represent observed values in leachates from spoil leached under aerobic conditions. Equilibrium solubility products for minerals are shown, the two values for jarosite and $Fe(OH)_3$ represent equilibrium limits.

Equilibrium lines for jarosite and ferric hydroxide as a function of pH and pFe^{3+} are shown on Fig. 2. Leachate values are plotted on this figure and show an accumulation of points near the intersection. This suggests the spoil-solution was close to equilibrium with both mineral phases which may be expressed by:

$$KFe_3(SO_4)_2(OH)_6 + 3H_2O \rightleftharpoons 3Fe(OH)_3 + K^+ + 2SO_4^{2-} + 3H^+ \quad (6)$$

At equilibrium and with both solid phases present the ion activity product $(K^+)(SO_4^{2-})^2(H^+)^3$ is a function of jarosite and ferric hydroxide solubility and expressed by:

$$pK^+ + 2pSO_4^{2-} + 3pH \rightleftharpoons pK\ (\text{Jarosite}) + pK(H_2O) - 3pK(Fe(OH)_3) \quad (7)$$

Substituting the appropriate values for terms on the right-hand side reduces Equation 7 to:

$$pK^+ + 2\ pSO_4^{2-} + 3pH = 20.8 \pm 1.4 \quad (8)$$

Under aerobic conditions and leaching at less than 8 ml h^{-1} IAP $(K^+)(SO_4^{2-})^2(H^+)^3$ ranged from 20.5 to 22.5 suggesting the existence of equilibrium. In addition, Equation 6 suggests that rapid leaching encourages jarosite hydrolysis to ferric hydroxide. This equation also suggests that accumulation of soluble components, as a result of pyrite oxidation and absence of leaching, will result in jarosite precipitation. These features were observed and it was concluded that quasi-equilibrium between jarosite and $Fe(OH)_3$ exists in this spoil and has a strong influence on the activity of H^+ and $SO_4^=$ which were buffered within the range pH 3.19 ± 0.17 and pSO_4 3.46 ± 0.14. These values were maintained even after the equivalent of 1200 cm of water percolated the spoil.

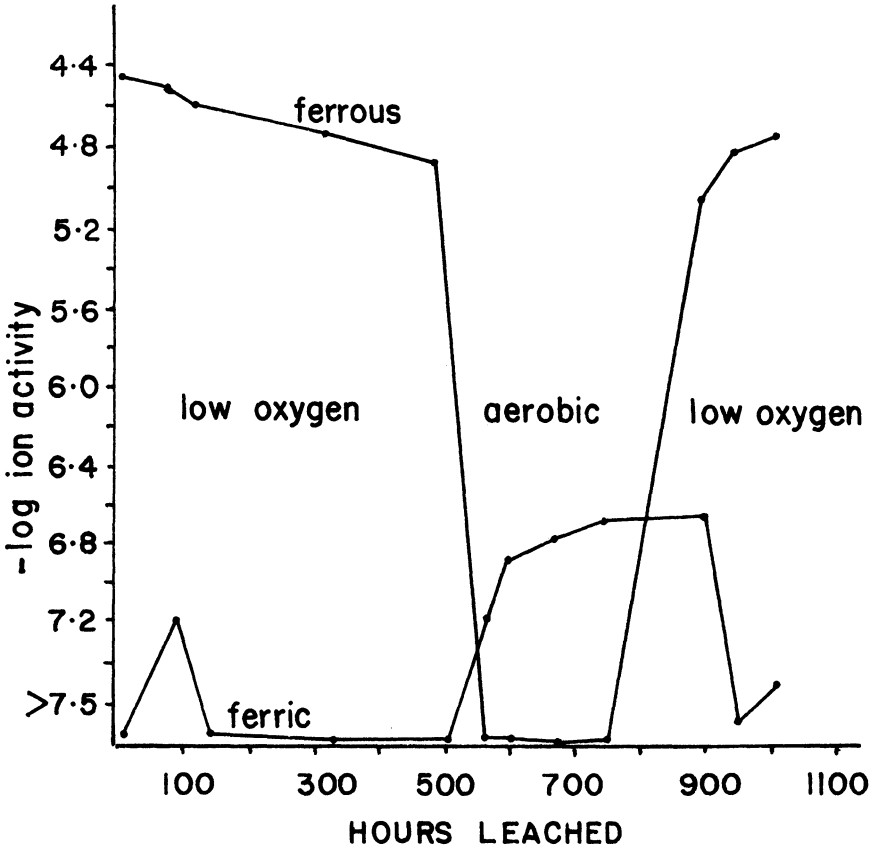

Figure 3: Change in dissolved iron composition with change in oxygen concentration. Key: points represent observed values in successive leachates. The leaching rate was maintained between 3 and 5 ml h^{-1}.

To represent the environmental conditions occurring deep in a spoil bank and at the site of buried acid material, spoil was leached with de-oxygenated water and held in an argon atmosphere. Fig. 3 shows that under an argon atmosphere with negligible oxygen the activity of Fe^{2+} was more than 1000 times greater than Fe^{3+}. In these conditions pyrite oxidation would cease since pyrite has a greater affinity for Fe^{2+} than Fe^{3+}, and absence of Fe^{3+} at the crystal surface results in negligible pyrite oxidation (Smith and Shumate, 1970). Therefore, it would be expected that solution-reduction of jarosite and ferric hydroxide would release Fe^{2+}, SO_4^{2-}, K^+ and OH^- ions as shown by Equations 9 and 10.

$$KFe_3(SO_4)_2(OH)_6 + 3e^- \longrightarrow K^+ + 3Fe^{2+} + 2SO_4^{2-} + 6OH^- \quad (9)$$

$$Fe(OH)_3 + e^- \longrightarrow Fe^{2+} + 3OH^- \quad (10)$$

If these reactions were significant the spoil-solution pH would increase. However, this was not observed. In fact, solution IAP values for the jarosite/$Fe(OH)_3$ equilibrium criterion shown by Equation 8 were maintained. To explain processes occurring under low oxygen conditions it was necessary to obtain an estimate of the redox potential. This was determined from the ratio of Fe^{3+} to Fe^{2+} activity and the range of values was pe 9.94 to 10.44. The location of this range is shown on Fig. 1 which indicates the existence of equilibrium with jarosite and $Fe(OH)_3$. Note that the stability zones for jarosite and $Fe(OH)_3$ are extended as the activity of soluble iron increases. This result suggests conditions were not sufficiently reducing to induce significant solution-reduction of these minerals.

Fig. 3 shows that, when the spoil atmosphere became aerobic, the activity of Fe^{2+} fell to zero rapidly and Fe^{3+} increased to normal aerobic levels. The pH of leachates during low oxygen leachings was maintained within the range observed under aerobic conditions. At low pH, abiotic chemical oxidation of Fe^{2+} is negligible. Therefore, the observed oxidation of Fe^{2+} could only be accomplished by microbial action which suggests low oxygen status did not destroy iron-oxidising bacteria. In addition, it appears that under these conditions there was sufficient oxygen present to enable some bacterial Fe^{2+} oxidation to occur. Kinsel (1960) and Razzell and Trussell (1963) also observed microaerophilic characteristics in iron-oxidising bacteria.

Under low oxygen conditions the SO_4^{2-} in leachates most probably was derived from jarosite hydrolysis since pyrite oxidation was negligible. Leaching under these conditions would eventually result in the hydrolysis of all jarosite causing the jarosite-$Fe(OH)_3$ buffering to break down and the pH to increase. However, since the activity of SO_4^{2-} in leachates was maintained at aerobic levels, pSO_4 approximately 3.5, and the jarosite content was 1.6%, it would take nearly 25 yr to completely hydrolyse jarosite from 15 cm depth of spoil subjected to continuous leaching.

CONCLUSIONS

Application of thermodynamic principles to the soil solution of this pyritic strip-mine spoil successfully identified the existence of quasi-equilibrium between jarosite and $Fe(OH)_3$. This equilibrium was significant in buffering the acidity and ionic composition of the soil-solution in a phytotoxic state which persisted under low oxygen conditions. Therefore, if pyrite oxidation has occurred and the jarosite - $Fe(OH)_3$ couple has become established, burial of acid material or covering with topsoil to establish vegetation and reduce the supply of oxygen to pyrite crystal surfaces may not prevent acid mine drainage or aquifer contamination.

It can also be concluded that a pyritic strip-mine spoil may remain acid for many years after reactive pyrite has been oxidised due to jarosite - $Fe(OH)_3$ buffering. This time will depend upon the jarosite content and the climatic and weathering characteristics the spoil is exposed to.

ACKNOWLEDGEMENTS

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LIQUID DISPERSION IN TRICKLE FLOW THROUGH ORE DUMPS

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INTRODUCTION

There have been two basic approaches to obtaining a description of liquid distribution in a packed bed. Tour and Lerman (1944) considered the flow through the packing to follow a random number of steps down to the left or right. The Gaussian Law of Errors was used to describe the spread of liquid in the packing at a given depth:

$$Q_x = k^2 e^{-h^2 R^2}$$

where, R = radius; k,h = constants; Q_x = flow/area.

The above approach was limited to an unconfined packing and other works (Cihla and Schmidt, 1957) recognised that the "diffusion equation" allowed a more general approach:

$$F = D \text{ grad } f(x,y,z)$$

where, F = horiz. flow/area time; D = spreading constant indep. of flow; f = flow function assumed constant.

For an axially symmetrical case:

$$\frac{\partial f(r,z)}{\partial z} = D \cdot \frac{1}{r} \cdot \frac{\partial}{\partial r} \left(r \cdot \frac{\partial f(r,z)}{\partial r} \right)$$

where, f = radius; z = depth of bed.

Both methods assume that the liquid distribution is independent of liquid flowrate. Experimental results obtained in the investigation show that for some flowrates this may not be valid.

Past observations of the effect of flow on liquid distribution have been qualitative in nature. Many workers such as Prchlik *et al.* (1975) have reported that liquid distribution becomes more regular with increasing flowrate. Mullin (1959) measured the change in liquid distribution with flowrate in grid packings. It was found that the liquid distribution was a function of the flowrate until a critical flowrate was reached where no further change in liquid distribution occurred. No similar work has to be carried out on random packings.

Two basic flow structures have been suggested as a basis to trickle flow. Porter and Stephens (1969) assumed that flow through the packing occurred in the form of narrow rivulets. It was claimed that the rivulet density varied proportionally with flowrate.

Other workers, such as Bennett and Goodridge (1970), claimed that the existing flowpaths in the packing were reinforced with increasing flow.

Experimental observations in this study support the latter theory. Existing flowpaths were found to become more established with increasing flowrate.

EXPERIMENTAL

As an experimental model for an ore dump, a triangular prism shaped bed was used to examine water distribution in such a bed of discrete particles (Fig. 1). The bed consisted of two triangular faces with 0.6m sides. The faces were 0.2m apart.

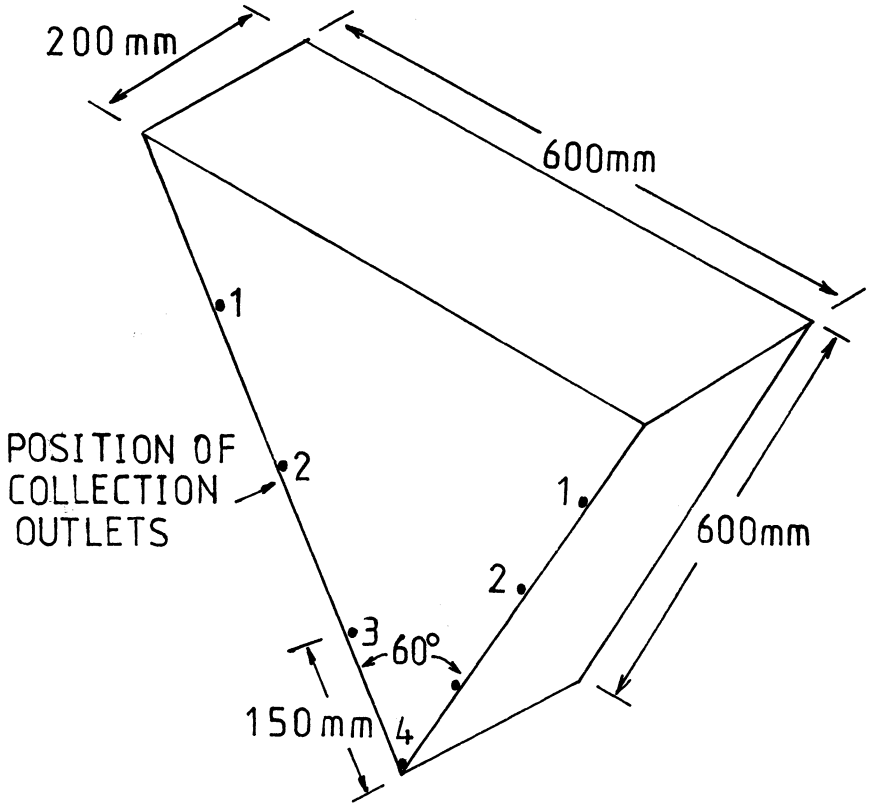

Figure 1: Packed Bed Container. Material - 10mm polycarbonate sheet. N.B. Ideally the troughs should have been spaced 150mm apart. The trough second from the bottom on the LHS was 6 mm vertically above that on the RHS which was positioned correctly.

Polycarbonate sheet was used for the container construction. This allowed observations of flow behaviour in the bed to be made. The non-wetting characteristics of the plastic also minimised the amount of water collecting on the vertical faces of the container.

Water reaching the inclined wall was collected in semicircular troughs placed flush to the wall. The 60° inclination of the wall reduced the tendency for the liquid to return to the bed. Four collection positions were used on each wall.

The bed was packed with crushed copper ore of 50mm average size made available by Bougainville Copper Ltd. A constant controllable flow of water was provided by a head tank through 6mm dia. plastic hose. The flow was adjusted using a clamp on the plastic hose.

THE EFFECT OF FLOW RATE ON WATER DISTRIBUTION

Water was supplied to the bed using a single, central feed source. Flowrates varied from 0.2 to 6 l min^{-1}. This corresponds to a loading of 0.03 l $s^{-1}m^{-2}$ to 0.8 l $s^{-1}m^{-2}$.

The flow from each outlet was recorded. Results were plotted as percentage of total flow from each outlet versus flowrate. The results can be seen in Fig. 2, Fig. 3, and Fig. 4.

Liquid distribution in the bed decreased linearly with flowrate in the lower flow region which extended from 0.1 l min^{-1} to 2 l min^{-1}. Data in this region were rather scattered.

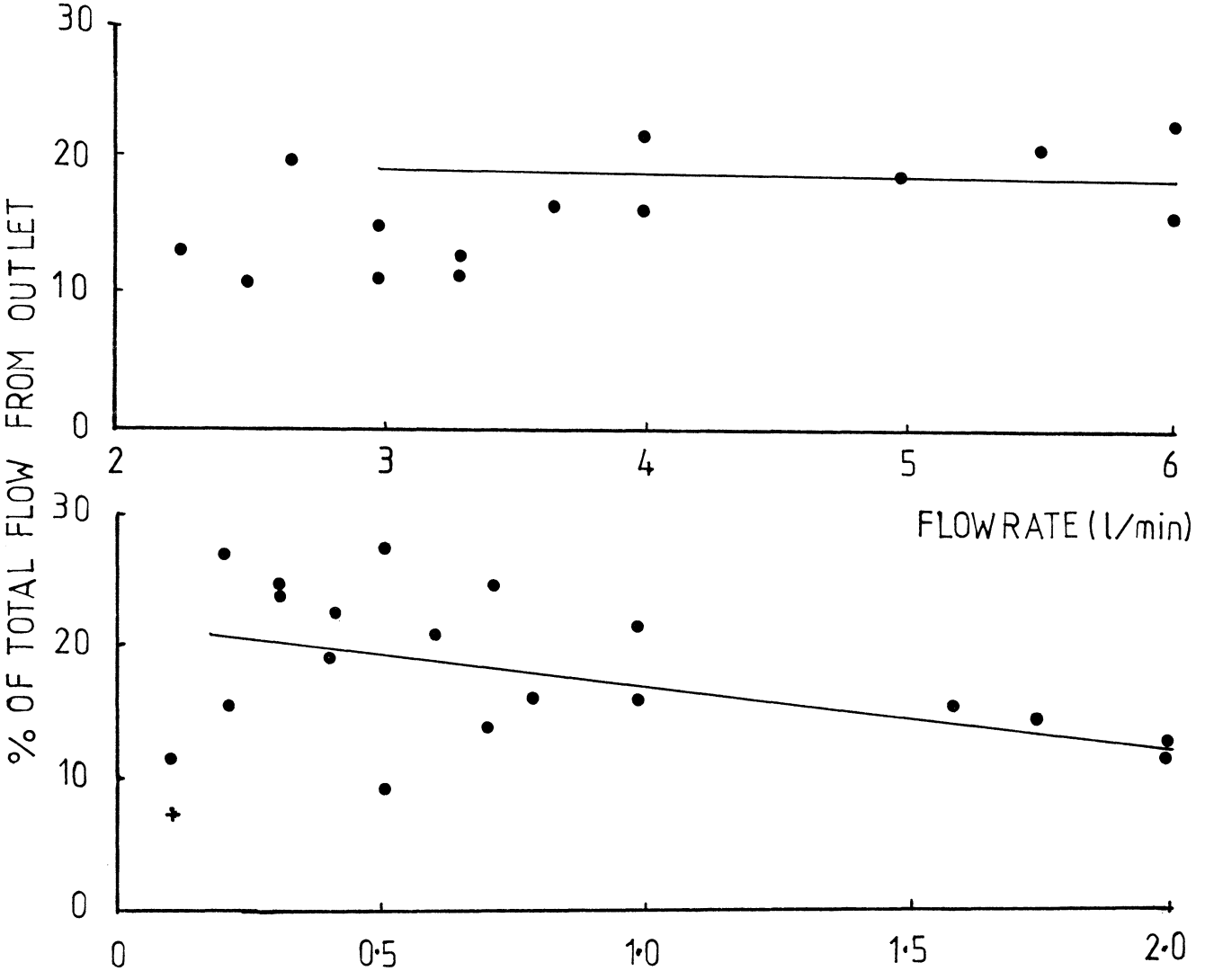

Figure 2: Experimental results - Outlet 3 LHS.

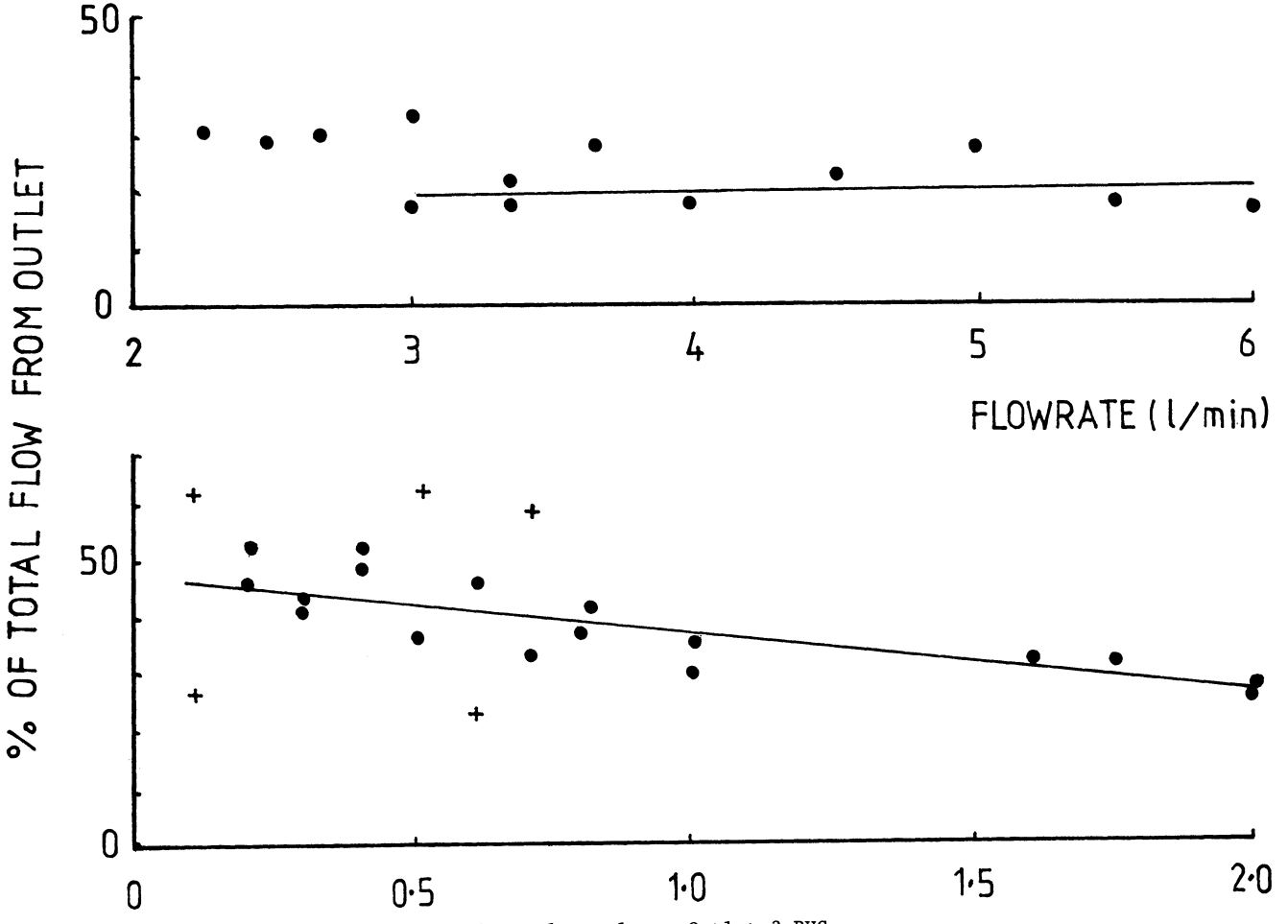

Figure 3: Experimental results - Outlet 3 RHS.

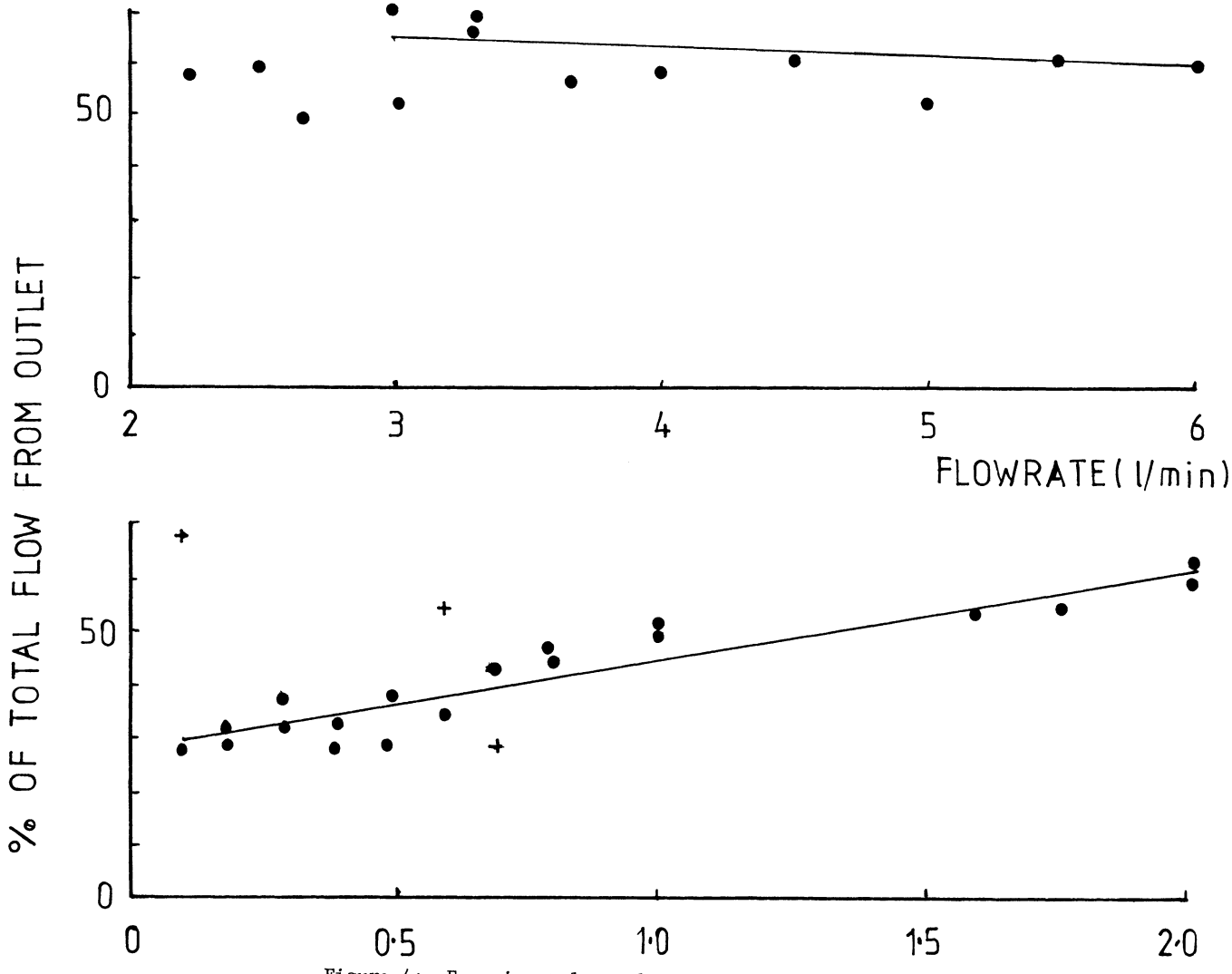

Figure 4: Experimental results - Outlet 4.

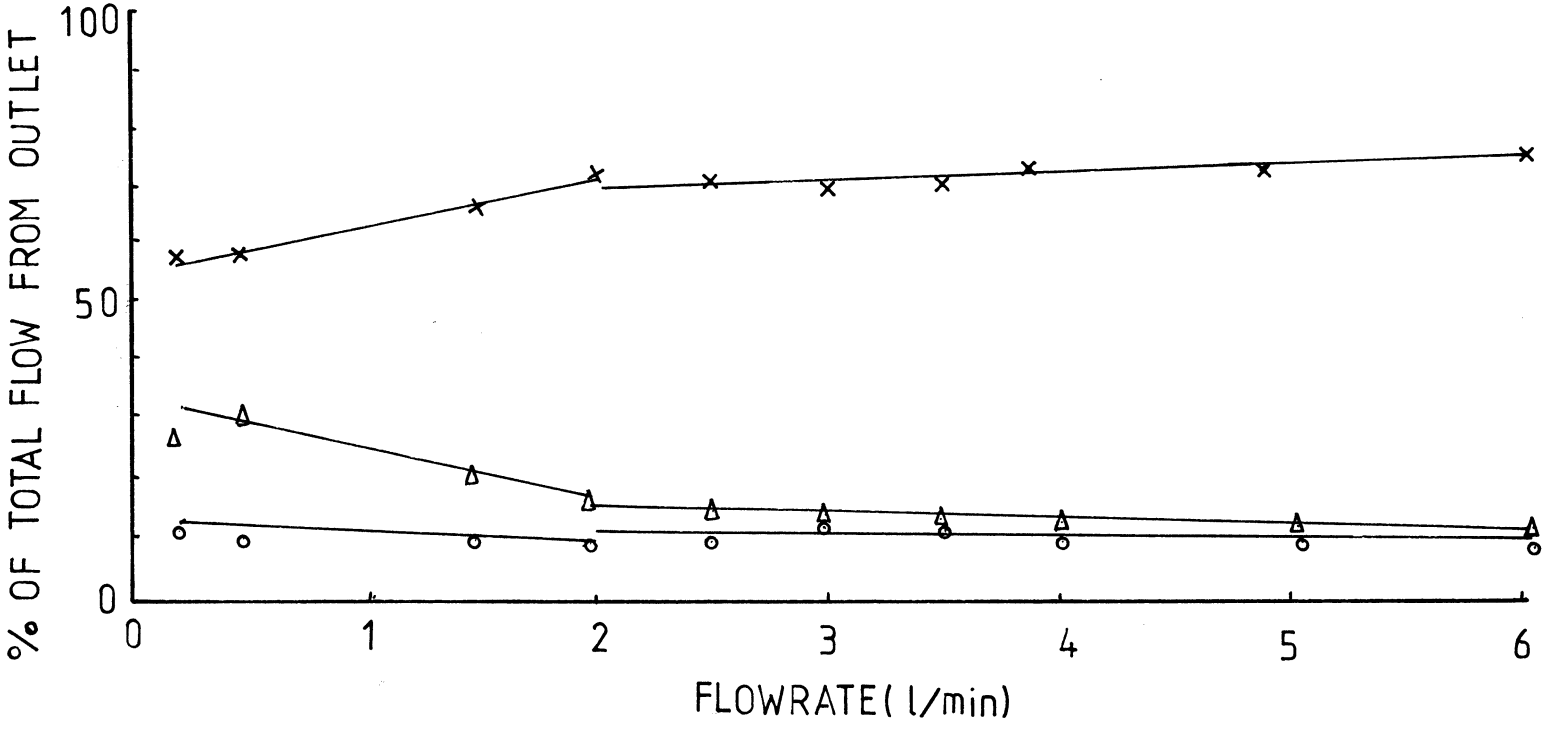

Figure 5: Experimental results. Several packing arrangements.

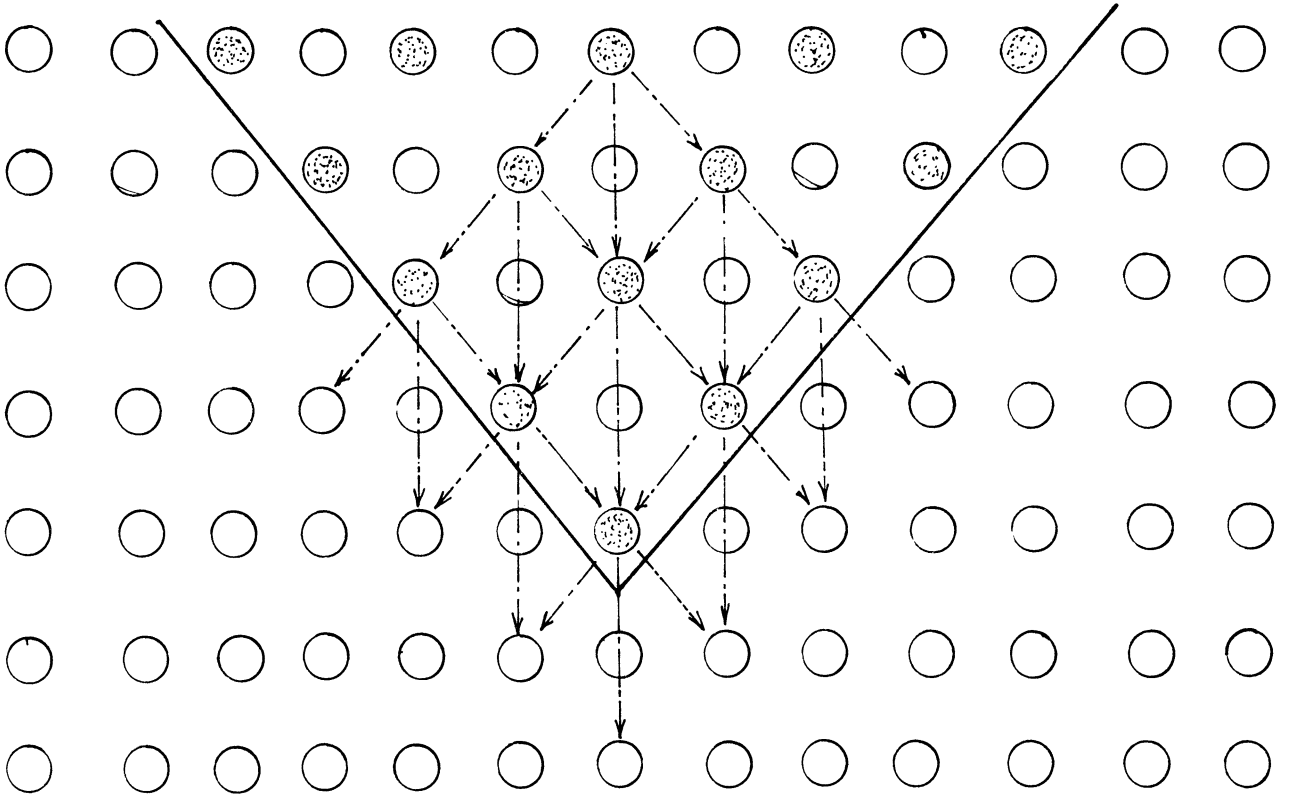

Figure 6: Computer model of bed - Triangular pitch. Program neglects unshaded elements. Broken lines represent possible liquid transfers.

In the higher flowrate region, from 3 to 6 1 min^{-1}, water distribution was found to be independent of flowrate. The results in the higher flowrate region were more regular than those at lower flowrates.

To give a more general result the exercise was repeated using several arrangements of the same packing. The results are shown in Fig. 5.

Results for the RHS and LSH outlet No. 3 differ. The RHS outlet was in fact 6mm below the LHS outlet. Results for the RHS outlet are higher because it is positioned lower in the bed in an area of higher liquid spread.

The results indicate that the current methods of determining liquid spread are valid for flowrates of at least (2-6 1 min^{-1}). This corresponds to typical loadings used in towers packed with dumped packing.

At lower flowrates or in large unconfined beds the liquid distribution may be a function of the flowrate. Current methods of determining liquid distribution are not valid for this situation.

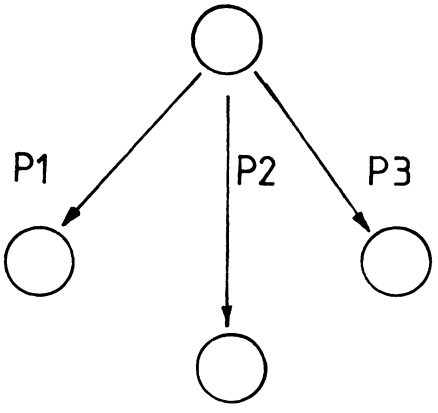

Figure 7: Computer model - Possible liquid transfers

$$P1 = \frac{\text{Range of LHS Transfer}}{\text{Range of Random Number}}$$

FLOW TYPES IN THE PACKING

Several different flow types were observed in the packing. For flows less than 2 1 min^{-1} liquid movement through the packing was in the form of narrow rivulets. Only a small fraction of the packing surface was wetted. The resulting distributions tended to be erratic, following the flowpaths initially formed.

At flowrates greater than 3 1 min^{-1} the flow was predominantly in the form of large sheets of liquid which covered a majority of the packing surface. Liquid distribution for the higher flowrate region was much more stable. Most of the packing surface was covered by a sheetlike flow.

In the sheet flow mode no formation of new flowpaths in the packing were observed with increasing flowrate, rather the existing flowpaths were gradually consolidated.

From the experimental observations it appears that the increase in sheetlike flow through the bed is associated with a decrease in liquid distribution. This may be because the

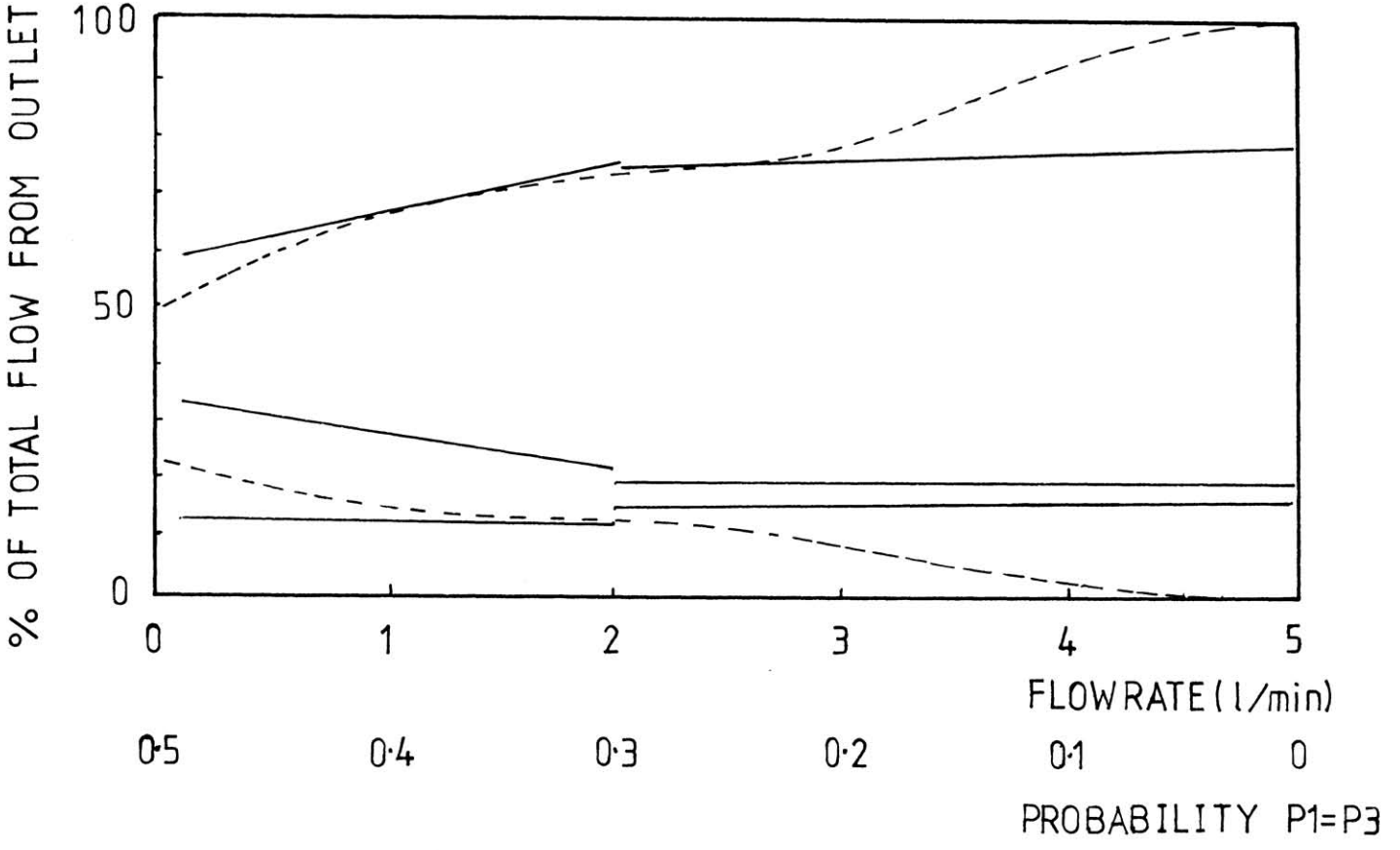

Figure 8: Experimental and computer results (square pitch).

sheetlike flow is a more kinetic flow loss dependent on the contact points within the packing. The rivulet type flow tends to be more dependent on existing flowpaths following a series of contact points throughout the packing.

A COMPUTER MODEL OF THE BED

Trickle flow through packed beds has been modelled by assuming that liquid movement through the bed is in the form of random steps down to the right or left. A computer allows this model to be examined.

A model of the bed was constructed using both a square pitched and triangular pitched matrix (Fig. 6) to represent elements in the bed. A triangular boundary representing the experimental bed wall was built into the matrix.

Liquid flow through the matrix was simulated using a random number generator (Fig. 7). The liquid transfer was passed down to the element to the left, right or directly below depending on the range of a random number generated. The probability of a transfer moving in any of the three directions could be controlled by varying the size of the controlling ranges.

The liquid transfer was passed down through the matrix until the boundary was reached, the position and number of liquid transfers crossing the boundary being recorded.

The results were recorded for a number of probabilities of the liquid transfer moving to one side as compared to directly below. Fig. 8 shows the results for a square pitched matrix. The results of the experimental work shown in Fig. 6 are superimposed on Fig. 8 so that some comparison can be made.

Fig. 8 shows that as the probability of liquid moving to the side decreases there is an initial rapid fall in liquid spread followed by a more gentle decrease. The trends resemble those observed in the experimental bed. At lower flowrates where channel type flow occurs the liquid distribution is a function of flowrate and the probability of a liquid transfer moving horizontally in the bed decreases with increasing flowrate. At higher flowrates the liquid distribution tends to be independent of flowrate. The predominance of the sheetlike flow is associated with a region where change in horizontal liquid movement in the bed has little effect on the net distribution of liquid.

CONCLUSIONS

Several types of flow behaviour may exist in a packed bed depending on the flowrate per unit area of liquid through the bed. At lower flowrates (<0.3 1 $s^{-1}m^{-2}$) the flow distribution is a function of the liquid flowrate. Flow in this region is in the form of rivulets and only a small fraction of the packing surface is wetted. Liquid distribution at low flowrates tends to be erratic.

Higher flowrates (>0.5 1 $s^{-1}m^{-2}$) comparable to those used in the commercial packed tower (0.8 1 $s^{-1}m^{-2}$), result in more stable liquid distribution. Flowrate had little effect on liquid distribution in the higher flowrate region. The majority of the packing surface was wetted in the high flowrate region by the sheetlike flow. Although liquid spread in the lower flowrate range was greater this would probably be offset by the small amount of packing surface wetted by the flow.

Higher flowrates allow a more stable operating range where a majority of the packing surface was wetted. This flow behaviour also allows the present models of predicting liquid distribution to be used.

ACKNOWLEDGEMENT

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COMPARISON OF BIOLOGICAL AND CHEMICAL LEACHING HEAP LEACHING USING *THIOBACILLUS THIOOXIDANS*

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INTRODUCTION

We have been investigating the possibility of recovering vanadium and other metals from petroleum cokes, using chemical and microbially-assisted leaching techniques (Zajic *et al.*, 1977, 1978). The cokes examined were derived from bitumen processed from the Athabasca oil sands in the province of Alberta Canada. These bituminous sands occupy a vast area and represent the largest deposit of vanadium in Canada. Vanadium is much in demand as an additive in high-strength low-alloy steels (Brown *et al.*, 1974).

Although the concentration of vanadium in the biumen is only 150-200 g^{-1} (Walker *et al.*, 1976), the total quantity is enormous: in the bitumen amenable to surface mining alone, there is approximately 2.4 T_g. During the upgrading of bitumen for oil recovery, the vanadium is concentrated in the coke at levels from 800-8000 $\mu g\ g^{-1}$ depending on the coking process employed (Table 1). Most of the elements of the periodic table are present also, but the elements of lower atomic number are the most abundant. Only vanadium, nickel, titanium and iron will henceforth be considered.

The two types of cokes currently produced in the Athabasca area, viz. delayed coker (Great Canadian Oil Sands Ltd.) and fluid coker (Syncrude Canada Ltd.) cokes are not amenable to leaching; the reason seems to be that the coke particles are impervious to the leaching solution. An experimental fluid coke produced by the Exxon "flexicoking" process (Matula *et al.*, 1975) is, however, amenable to leaching by acidic solutions and it is the leaching characteristics of this coke that will be discussed.

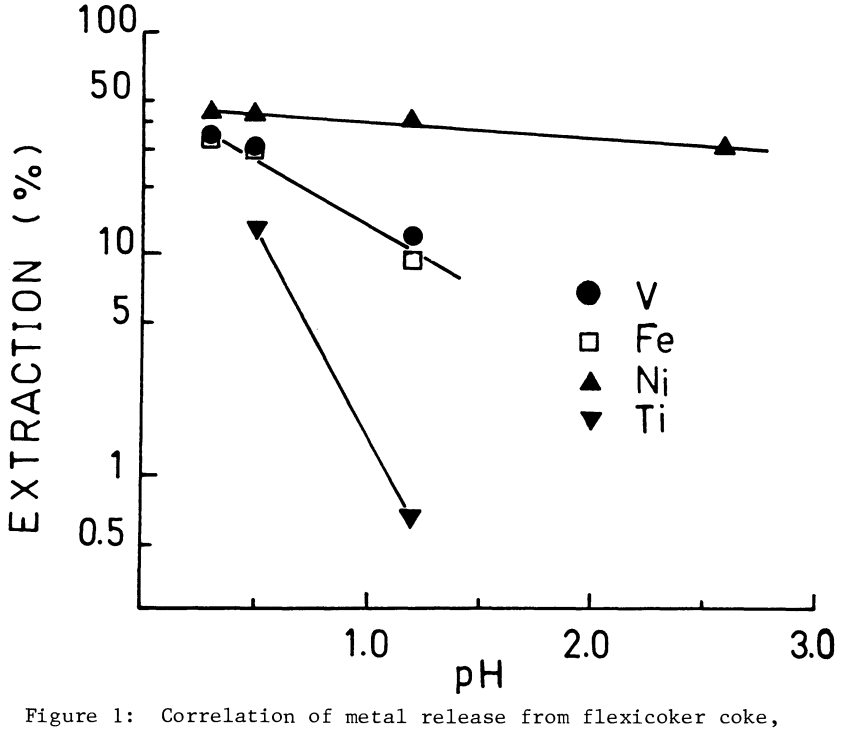

Figure 1: Correlation of metal release from flexicoker coke, as a function of pH. Duration of leach, 7 days.

TABLE 1

ANALYSIS[a] OF COKES FROM ATHABASCA BITUMEN

Element[b]	Concentration ($\mu g\ g^{-1}$)			
	GCOS Coke	Fluid Coke, Baton Rouge	Fluid Coke, Syncrude	Flexicoker Coke
Ag	0.5	0.4	0.3	2
Al	4720	6930	13300	7020
As	26	ND	c	90
Ba	30	30	c	80
Be	3	1.3	0.86	24
Ca	3950[d]	602	3540	1640
Co	8	10	14	18
Cr	9.5	51	25	59
Cu	4	11	8.8	20
Fe	2360	2570	5490	6990
K	2600	c	1410	5470
Mg	730	272	605	750
Mn	51	49	150	98
Mo	80	121	125	220
Na	260	370	730	960
Ni	356	660	695	1760
P	102	ND	69	188
Pb	5	20	10.6	6
Si	c	c	8300[e]	c
Sr	18	28.8	38	38
Th	6.1[f]	c	4.0	25[f]
Ti	828	1160	1495	2140
V	840	1590	1720	8010
W	20	c	c	40
Zn	6.4	13	17	33.4
Zr	ca28[f]	43	75	ca67[f]

[a] By radio frequency plasma emission spectrometry.

[b] Elements not detected: B, Cd, Eu, Au, Se, Te, Sn, U.

[c] Not determined.

[d] Value seems high; sample perhaps contaminated with $CaCO_3$.

[e] Analysis by Huffman Laboratories.

[f] Average value from analysis of size fractions.

ND: Not detected.

A limiting factor in the extraction of metals from flexicoker coke, is pH. Fig. 1 shows the extent of metal extraction by sulfuric acid solutions. The extent of vanadium extraction is seen to be significant only below pH 1. Thus, for microbially-assisted leaching to be feasible, the leaching organism must be capable of producing considerable amounts of acid. It would appear, therefore, that the most common species of leaching organism, *Thiobacillus ferrooxidans* would not be suitable for this case. However, *Thiobacillus thiooxidans* is able to produce the acidic conditions necessary for leaching.

The bacteria are not able to use the sulfur content of the coke (1.6%) as an energy source, so the coke must be supplemented with elemental sulfur. At the oil sands refinery sites, sulfur is readily available: over 300 Mg day^{-1} are produced at the GCOS plant and this is stockpiled.

Leaching is conducted in airlift percolator columns (Fig. 2) with 150 g coke, 6 g sulfur and 150 ml of a mineral salts medium, containing essentially phosphate and ammonium ions together with other trace elements (see below; Fig. 5). The sulfur was generally dispersed throughout the coke but growth proceeds equally as well if the sulfur is placed on top of the coke column, without uniform dispersal. The column is then inoculated with about 10 ml of a culture of *T. thiooxidans* ATCC 8085 in early log phase growth.

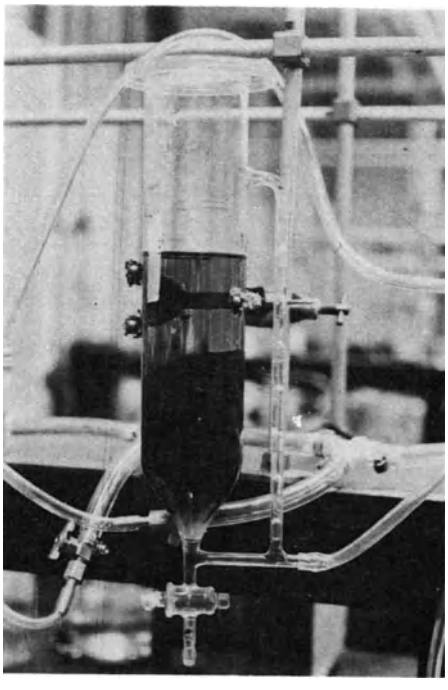

Figure 2: Leaching in an airlift percolator column.

During the course of leaching over a number of weeks, the leachate acquires a blue coloration indicative of the vanadyl ion VO^{2+}. This signifies that vanadium is being extracted in its +4 oxidation state. Furthermore, colorimetric analysis shows that iron is extracted in its lower-valent (ferrous) state.

In Fig. 3, a comparison between chemical and microbially-assisted leaching of flexicoker coke is shown. In the biological examples shown, the bacteria were only able to attain a minimum pH of 1.0; the extent of extraction is, however, comparable to that achieved by a chemical leach at the same pH, i.e. about 30% of the total metal content.

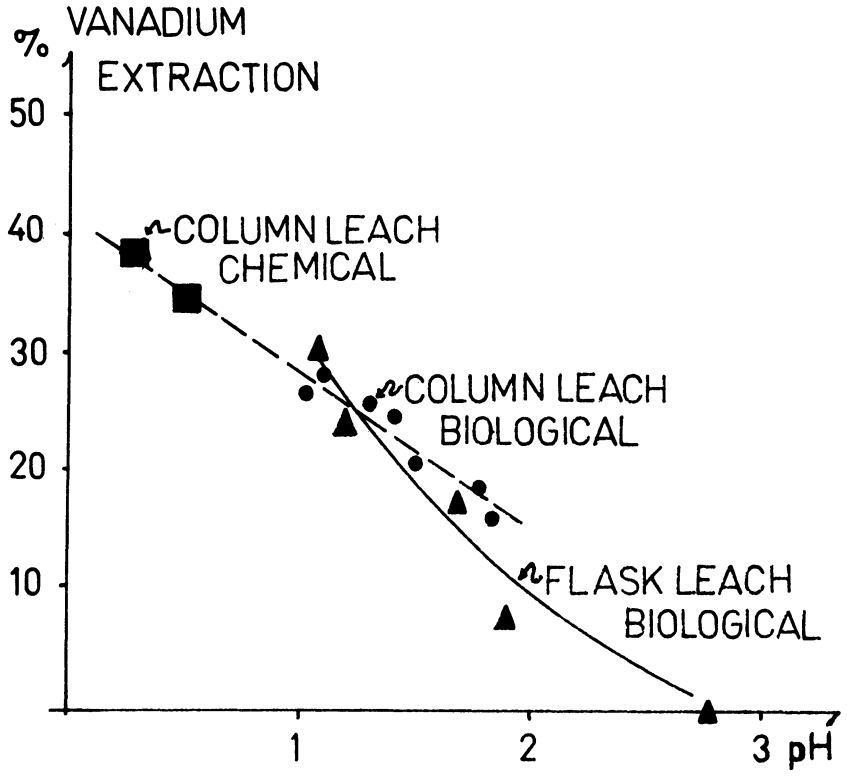

Figure 3: The release of vanadium in the leaching process carried out chemically in columns, biologically in flasks, and biologically in columns. Duration of leach: 3 weeks, 3 weeks, 7 weeks, resp.

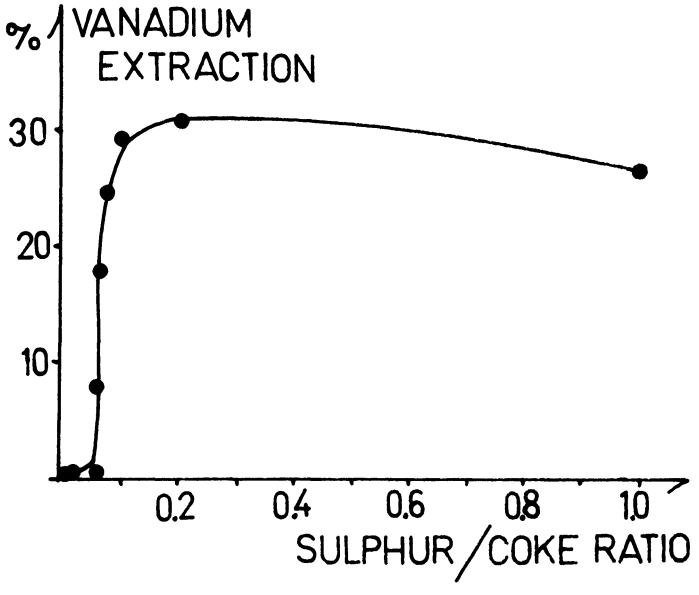

Figure 4: The dependence of vanadium release on the sulfur/coke ratio during the bioleaching process in flask experiments.

The final pH of the microbial leachate and hence the extent of metal extraction is somewhat dependent on the sulfur/coke ratio. Experiments with still flasks (Fig. 4) show that there is a minimum value of the sulfur/coke ratio necessary for the bacteria to become actively established. The minimum sulfur/coke ratio is about 0.1 for optimal vanadium release and the pH reaches a minimum value of about 1.0. For airlift columns, the minimum value of the sulfur/coke ratio is about 0.04 and scale-up from these experiments to a heap leaching operation may further alter the optimum value for this parameter.

Certain elements, such as Ca, Mg, K, P, N are generally necessary for growth of *T. thiooxidans*. The first three are extracted from the coke by acid leachates (Zajic *et al.*, 1977), in adequate amounts for bacterial growth but fixed nitrogen (ammonium) and phosphate must be added. The result of supplementing the leach solution with these ingredients is shown in Fig. 5. For the columns which contain phosphate, there is a lag of 2-10 days before the bacteria become established. The addition of a source of fixed nitrogen appears to be essential for significant vanadium release while the need for phosphate is less acute, at least for the amount of coke being leached here.

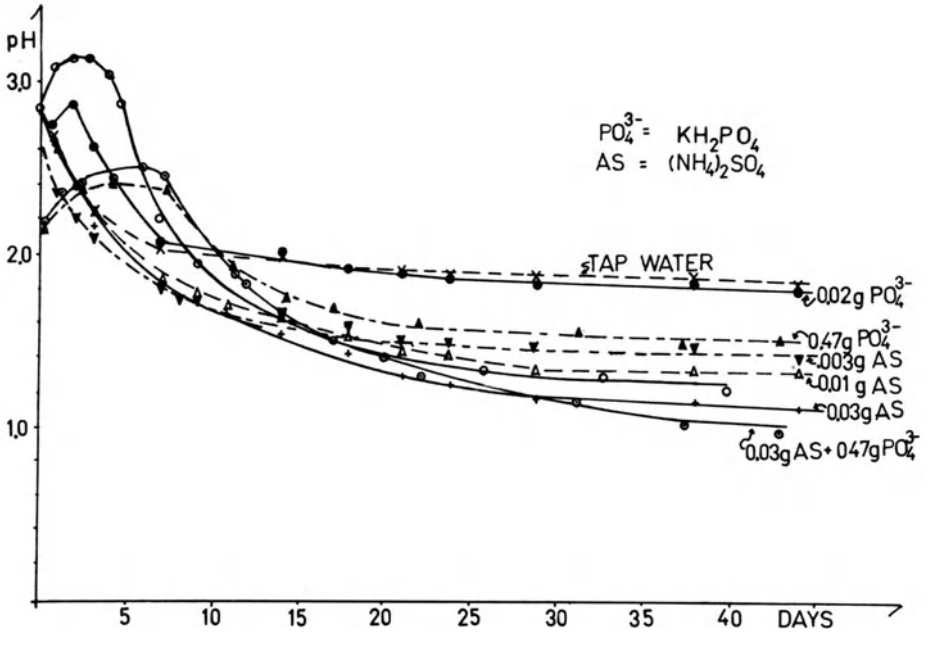

Figure 5: The effect of ammonium and phosphate ions as supplementary nutrients on the acidity arising from the growth of *T. thiooxidans* ATCC 8085 on sulfur-supplemented flexicoker coke in airlift percolator columns.

As mentioned previously (see Fig. 1), the extent of extraction of these metals depends markedly on pH. In particular, nickel removal can be effected at considerably higher values of pH than for the other metals. During microbially-assisted leaching, the pH is only gradually lowered as the bacteria become established and overcome the natural buffering action of the coke. Fig. 6 shows the kinetics of metal release for V, Ni, Ti and Fe and it can be seen that about 40% of the nickel content is selectively extracted in the early stages of the leach; vanadium and iron are subsequently extracted as the growth of *T. thiooxidans* decreases the pH. The lower the pH can be brought the better, since vanadium thereby becomes less susceptible to aerial oxidation and the consequent precipitation of insoluble vanadates. The bacteria play an important role, in keeping the metals (vanadium and iron) in their reduced forms (vanadyl and ferrous) thus further minimizing precipitation. Titanium is only poorly extracted at pH 1.

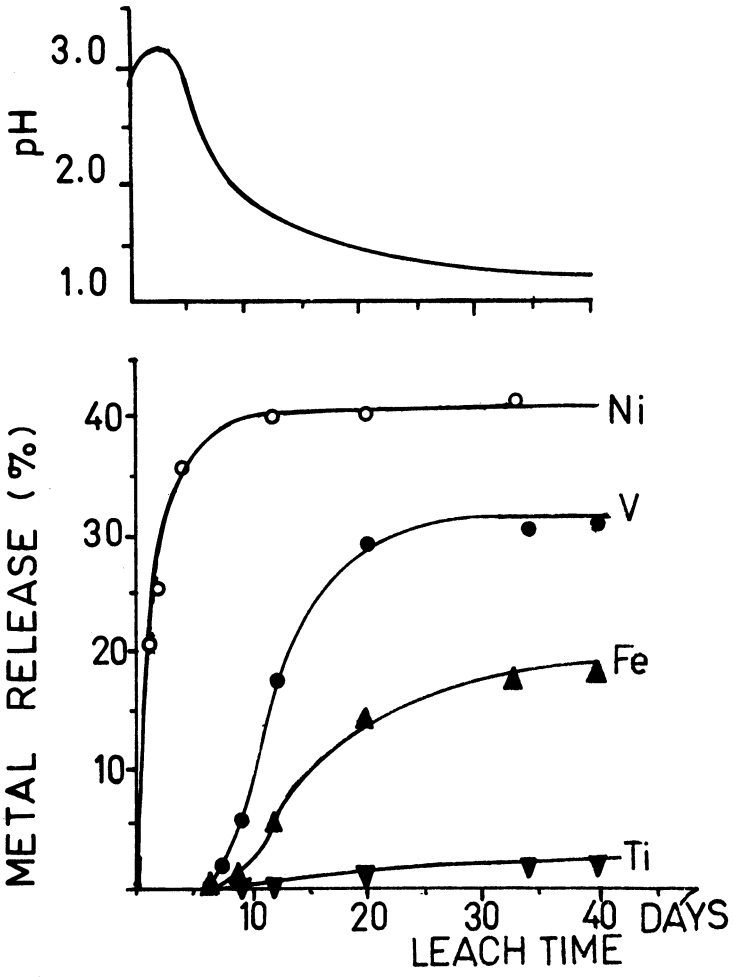

Figure 6: The metal release from flexicoker coke and the associated pH change during the microbially-assisted leaching process.

The presence of chemical species such as chromate and molybdate curtail the rate and extent of microbial growth and the release of these species may play an indirect role in determining the extent of metal release in our study. These effects do not preclude the feasibility of a microbially-assisted process for leaching coke produced by the flexicokir method.

ACKNOWLEDGEMENTS

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A REACTOR SYSTEM FOR MINERAL LEACHING INVESTIGATIONS

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INTRODUCTION

An early step in the assessment of the amenability of a mineral-bearing material to microbially-catalysed degradation or modification is the determination of its behaviour under optimised conditions. The results can give some idea of "ceiling" performance and indicate whether it is likely to be profitable to proceed with longer-term testwork in columns or other devices in which the constraints of real field situations can be more accurately simulated. This first objective can be secured by the use of the time-tried, accelerated shake-flask technique, which has the advantage of relative cheapness and simplicity of equipment and of the simultaneous handling of a larger number of samples. It has the disadvantages of rather large sampling errors, lack of precision in the range of operating conditions easily attainable, and is increasingly costly in terms of manpower.

A suitably-designed stirred reactor system enables larger samples to be handled, provides precise control over a wide range of operating conditions and with modern data logging equipment can substantially reduce manpower demands. While the initial capital cost is relatively high, banks of such stirred reactors provide an extremely versatile facility for the study of a wide range of mineral biodegradation phenomena. The reactor system to be described has been applied to the determination of the leaching behaviour of high and low-grade ores of a diversity of types, overburden materials, and process residues, under a variety of operating conditions; and to the assessment of processes for the microbial beneficiation of ores.

A number of modifications to standard reactor design have been made, the most important of which are those which ensure proper suspension of ore particles and minimisation of their abrasive effect, adequate aeration (or gasification) and the quantitative recovery of leached residues. Particular attention has been paid to arrangements for the initial sterilisation of equipment and materials and to the avoidance of fortuitous contamination over relatively long periods of operating time. It is desirable to be able to sample representative suspensions or particle-free leaching solution and this can be effected. To minimise manpower usage, the ractors are automatically controlled with respect to temperature and pH, and these and other variables can be continuously monitored.

DESCRIPTION OF EQUIPMENT

An individual 10 litre reactor (working volume, 8 litres) is illustrated in Fig. 1. The reactor body consists of QVF P6/18 pyrex glass pipe clamped to a stainless steel top plate via a neoprene rubber spacer. The top plate carries the seals and bearings of the stirrer shaft, acid or alkali input port, and the inoculation and gas exit ports. This whole assembly is bolted to the reactor-carrying frame.

The bottom section consists of a standard, high density PVC adapter which is clamped to the body of the reactor by means of a standard clamp with a neoprene gasket. The adapter clamp is held in place by adjustable steel guides to provide reactor stability. This removeable lower section of the reactor is an important part of the design. The bottom adapter section carries a teflon gas inlet tube, a resister temperature sensor, and a sintered-glass-tipped sampling tube. A bottom plug is provided for complete drainage of the reactor. The advantages of this design lie in the locating of the sampling, temperature sensor, gas-feed and other inlets in the lower section rather than the upper body of the reactor where, in earlier designs they were found to interfere with stirring and aeration. The current design also permits the upper section of the reactor and the impellor drive system to be left undisturbed while the bottom section is removed for loading with the material to be leached and for the recovery of the leach residue. The easy removeability of the bottom section very greatly facilitates the cleaning and servicing of the interior of the reactor.

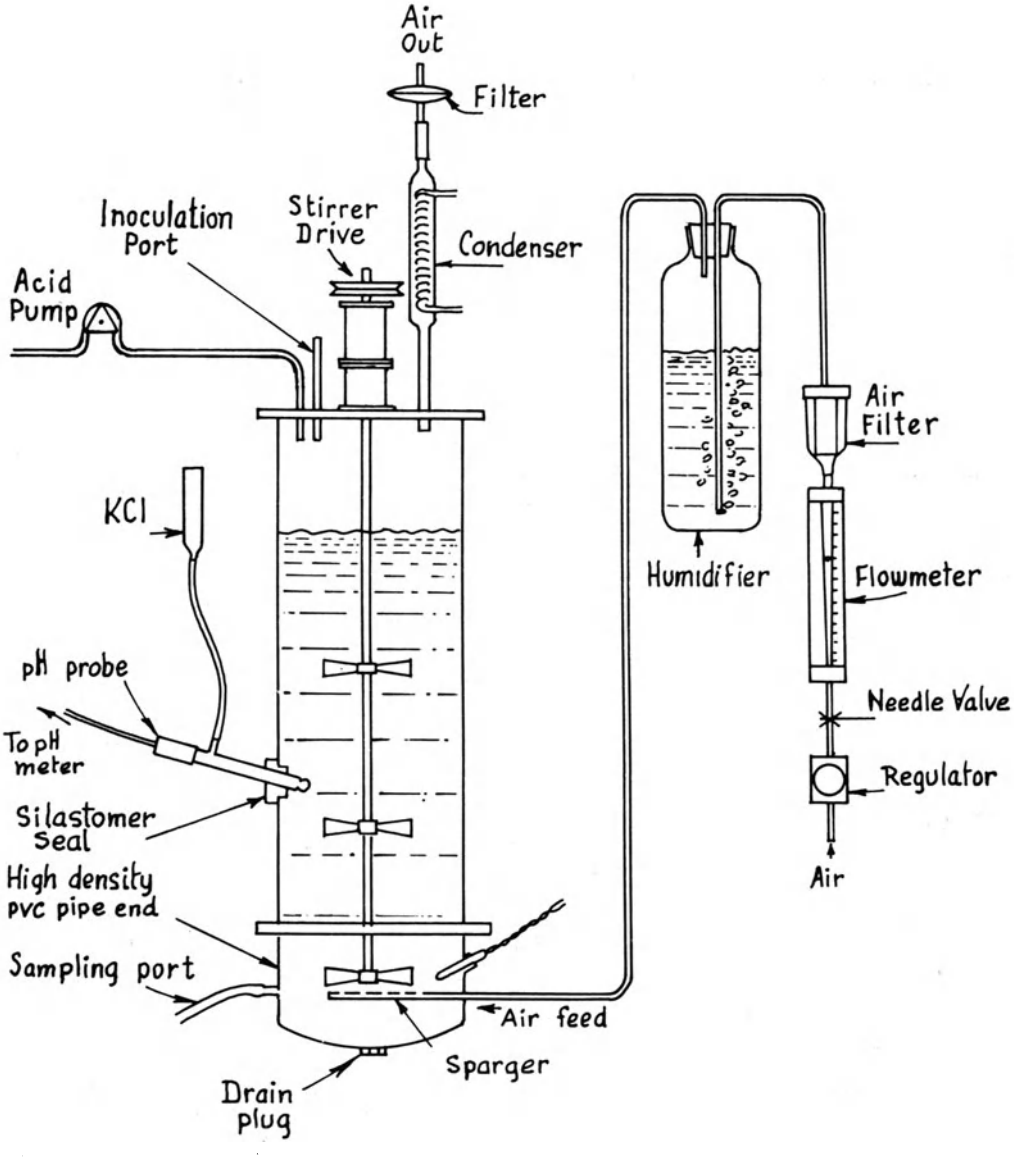


Figure 1: General layout of individual reactor system.

In the majority of reactor runs, particle-free samples of leachate are required for monitoring of solubilised materials rather than ore-leachate suspensions. The leachate samples are secured via the sintered-glass-tipped sampling tube (porosity No. 0) by gravity flow. Ore particles are restrained but suspended microorganisms pass out with the liquid sample. If suspension samples are required, an open sampling tube is employed. The horizontal teflon gas feed tube discharges directly under the lower impellor blades and may be adjusted by forward or backward movement. It is difficult to achieve this adjustment with gas inlet tubes which enter through a top-plate port.

To avoid "whip", the impellor shaft is relatively large in diameter and is further stabilised by two external bearings. In earlier models with an internal bearing, abrasion by mineral particles was extremely rapid. Since the reactors are normally used in pairs, some cost savings have been made by the use of a single drive motor (1/4 h.p.) for the two impellor systems via carefully matched pulleys. To provide good ore particle lift as well as adequate gas dispersion, two sets of impellor blades are accommodated on each shaft, with pitches to achieve these ends without undue shear. To reduce electrolytic corrosion between the impellor blades and the shaft, as well as to enable easy adjustment and removal, the blades are locked into place by means of a conical teflon washer assembly.

The gas-feed system consists of a "Manostat" pressure regulator followed by a "Flostat" flow controller and a "GAP" needle-valve flow meter. The gas flow is sterilised by an in-line filter (Whatman, Gamma-12) and is fed into the reactor through the gas-inlet tube via a sterile water humidifier. Contamination through the gas exit port is eliminated by attachment of a cooling condenser and an exit filter.

Adjustment of pH is effected by addition of acid or alkali (3M H_2SO_4 or 3M NaOH) through a top-plate port by a small peristaltic pump, activated by a pH controller and fed from a calibrated reservoir.

Temperature is maintained by means of an external infra-red lamp which is controlled by a resistance thermometer sensor coupled to an "Ether Mini" temperature controller. When the operating temperature desired is lower than ambient temperature, the reactor contents are cooled down by a "cold finger" inserted through the top-plate and connected to a refrigerated water unit.

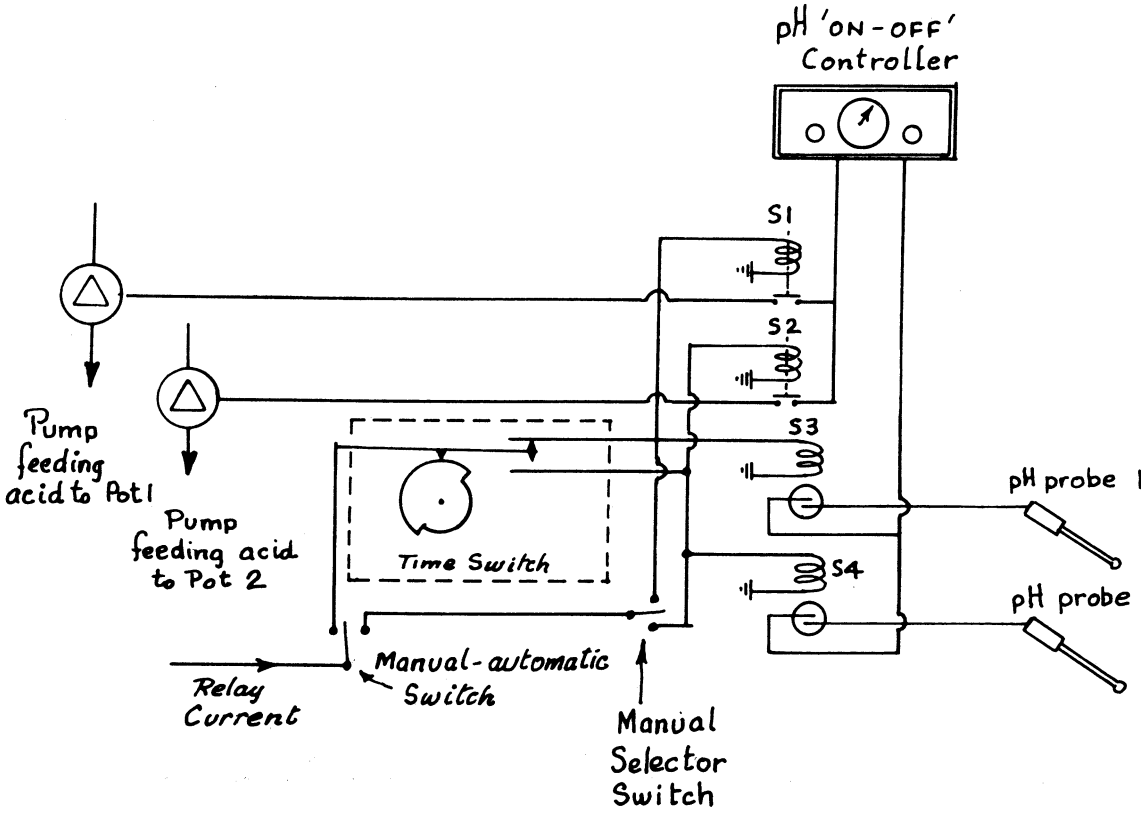
The pH in each of a reactor pair is monitored and adjusted every 15 minutes by using a "pH-alternator" device coupled to a single pH meter-controller ("Dynaco 21A"). Each reactor is fitted with a combination pH probe (Activion) which is inserted into the reactor through a hole cut in the upper glass section and held in place by a self-sealing silicone rubber sleeve. The reference solution vessel is attached to the probe by a silicone tube and raised above the operating liquid level in the reactor to provide positive pressure by the electrolyte. This pH monitoring and control system is economical in the saving of one pH-meter-controller per pair of reactors but also eliminates the discrepancies which can occur between a reactor pair due to set point drift differences associated with two independent controllers (Fig. 2).

The reactor assembly, together with ore charge (usually 320 g dry wt.) is sterilised by passing in through the gasification system from the flow meter assembly onwards, a slow stream of C.I.G. Ltd. "Sterigas 27" (12% w/w ethylene oxide in dichlorodifluoromethane) for about 4 h. The system is then closed off and left full of the sterilising gas overnight. The suspending medium (usually 8 litres) is separately cold-filter-sterilised (Babij *et al.*, 1971) and pumped into the reactor with aseptic transfer precautions. The impellor is started and the contents of the reactor aerated with sterile air until the exit gases are free of halogen. Sterility of the stirring assembly is maintained by the recirculation of sterile water through "Crane" seals. Freedom from fortuitous contamination has been maintained for reactor runs in excess of 40 days.

The currently operating system consists of four reactor pairs one of which is shown in Fig. 3. Each reactor has its own gasification and temperature control system, but the pH control systems are arranged in pairs as earlier indicated. The sterile water recirculation system for the maintenance of sterility in the stirring assemblies is common to the bank of eight reactors. A wide range of operating conditions can be set up, with respect to temperature, pH, aeration rate, composition of suspending medium, etc. Micro-aerophilic conditions can be established by the use of gasification mixtures containing nitrogen and

and carbon dioxide. The pH control system is readily disconnected if pH monitoring only is required.

Continuous monitoring and recording of process variables is carried out with a data logging system, based on a Rockwell micro-computer.



S1 & S2 Power Relay Switches

S3 & S4 Reed Relay Switch.

Figure 2: pH control circuitry

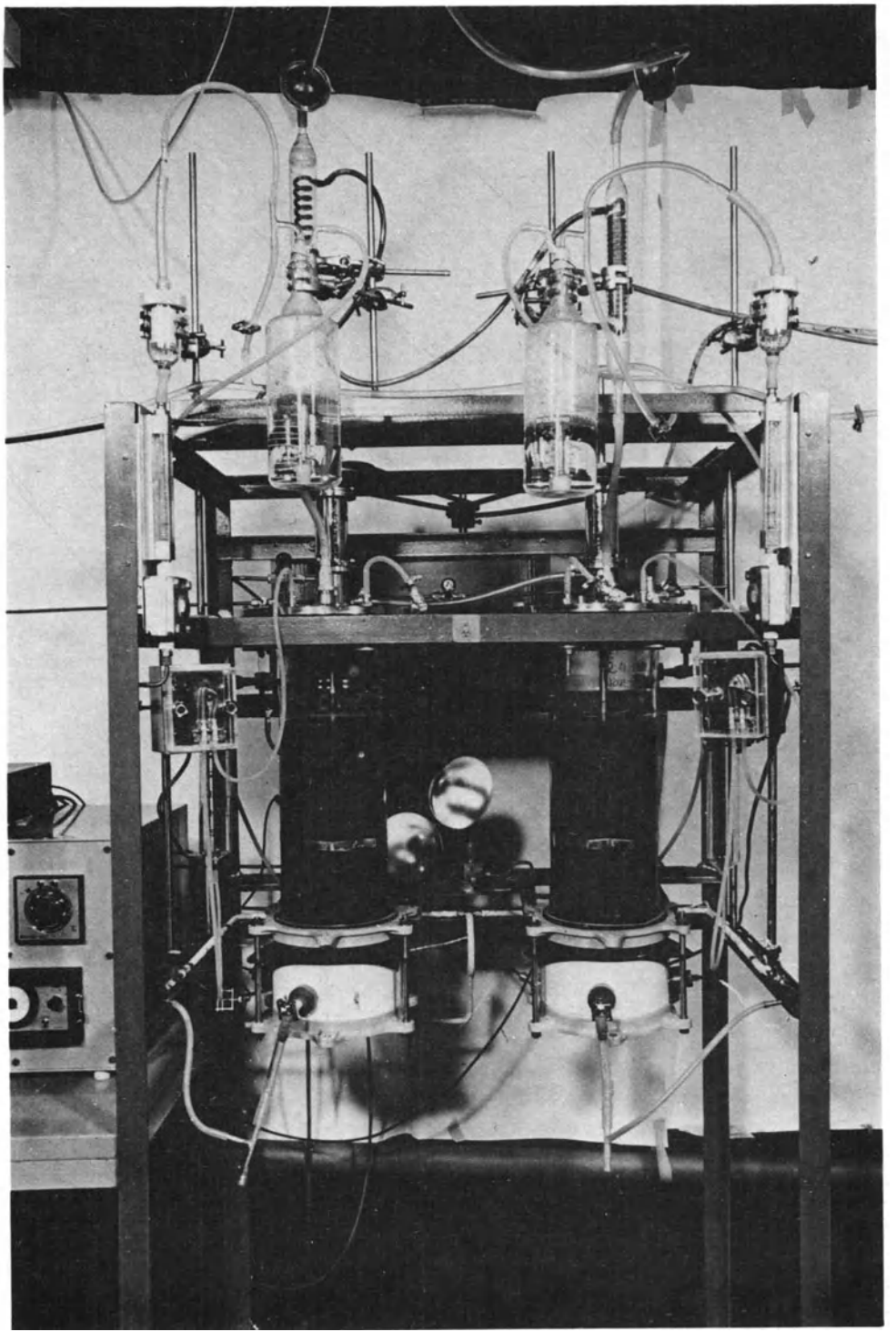


Figure 3: General view of a reactor pair

The microprocessor data logger will record sixteen channels of information, in the form c signals from zero to five volts, and record this data on both a thermal printer and on cassette tape which can later be fed into a larger computer for analysis. Data recorded the logger has a potential accuracy of 0.01% using the thirteen bit A/D converter and all timing of the recorded events is also of high accuracy since a crystal-controlled clock i used. In the event of power failure a battery backup power supply will take over and maintain logging capability for over 20 h. To date, the data logging system has been use to monitor only temperature, pH and acid or alkali additions, but it is amenable to the continuous recording of a wide range of variables.

INOCULATION OF THE REACTOR SYSTEM

In some experiments, initiation of microbial leaching will be dependent upon the indigenc microflora carried on the surface of the ore charge, and in such circumstances, the unsterilised material is added to the pre-sterilised reactor and suspending medium throug the appropriate port in the top-plate. In other circumstances, information on the behaviour of the material in respect of chemical leaching at different temperature and pH leve is desired and the first phase of the reactor run is devoted to this end, with reactor, medium and charge all sterile. At the end of this phase, the addition of a massive micro bial inoculum will rapidly initiate microbial leaching, if the material is amenable to it

The microbial inoculum is built up separately in shake flasks on an appropriate medium which usually includes the material under test to ensure acclimatisation of the microbial population. The initial inoculum is frequently a pure culture strain from a collection o mineral-degrading microorganisms, derived origianlly either from national collections or isolated by the investigators from field leaching situations.

When the shake flask cultures have grown sufficiently, they are harvested by filtration a centrifugation, washed and resuspended. A cell count is made and a measured volume of suspension added, with aseptic transfer precautions, to the reactor contents through a to plate port. A cell density in the suspending medium of 10^7 - 10^9 cells ml^{-1} is usually employed. On occasion, mixed cultures harvested directly from mine drainage waters and harvested by centrifugation, are used as an inoculum. Mixtures of two or more pure cultures, built up via shake flask cultivation, can also be employed. Routine checking of t viability of inocula is essential.

BACK-UP ANALYSES AND EXAMINATIONS

A considerable amount of back-up analysis and examination is desirable if maximum information is to be derived from each reactor run. For each particular material under examination, certain analyses will be essential; other analyses may yield information useful t later stages of investigation. Table 1 lists typical ranges of analyses and examinations of initial material, or suspending medium during reactor runs and of leach residues.

EXAMPLE OF USE OF REACTOR SYSTEM

The system has been applied in investigations of the practicability of recovery of copper zinc, cobalt and uranium from low-grade materials, to the beneficiation of gold-bearing, tin-containing and siliceous materials, and for assessing the environmental hazard presen ed by overburden heaps and tailings dams. Some results from the examination of overburde material will serve as an example of the use of the reactor system.

Table 2 summarises the chemical composition of composite samples taken from sample holes White's Overburden Heap in the Rum Jungle Mine area in Northern Territory, Australia. Th material was leached chemically under sterile conditions at pH 2.5 and pH 3.5, and in eac case subjected to further bacterial leaching with mixed microbial populations harvested from the highly polluted water of White's Opencut in the same area. Metal release and th acid consumption necessary to maintain the chosen pH of operation are shown in Fig. 4. When bacterial leaching under aerobic conditions had terminated at the 33rd day, microaerophilic conditions were established by gassing out with a 2% CO_2 - 98% N_2 mixture (Fig. 5).

TABLE 1

TYPICAL ANALYSES AND EXAMINATIONS

Initial Analyses and Examinations
Moisture Content
Acid Consumption
Particle Size Distribution*
Surface Area*
Emission Spectrographic Analysis
Principal Metals
Sulfur (Total, Sulfate, Sulfide, Elemental)
Total Carbon*
Electron Micrograph Scans*
Monitoring Analyses on Suspending Medium during Reactor Run
pH
Acid or alkali Consumption
Viable Organisms in Suspension
Other Analyses in particular cases
Principal Metals
Analyses on Washed Leach Residue
Emission Spectrographic Analysis
Principal Metals
Sulfur (Total, Sulfate, Sulfide, Elemental)
Total Carbon*
Electron Microscopy Scans*

* When required

TABLE 2

CHEMICAL COMPOSITION OF OVERBURDEN MATERIAL

Metal	$\mu g\ g^{-1}$ of ore
Ca	8500
Cu	520
Fe	46000
Mg	28800
Mn	580
Pb	160
Zn	180

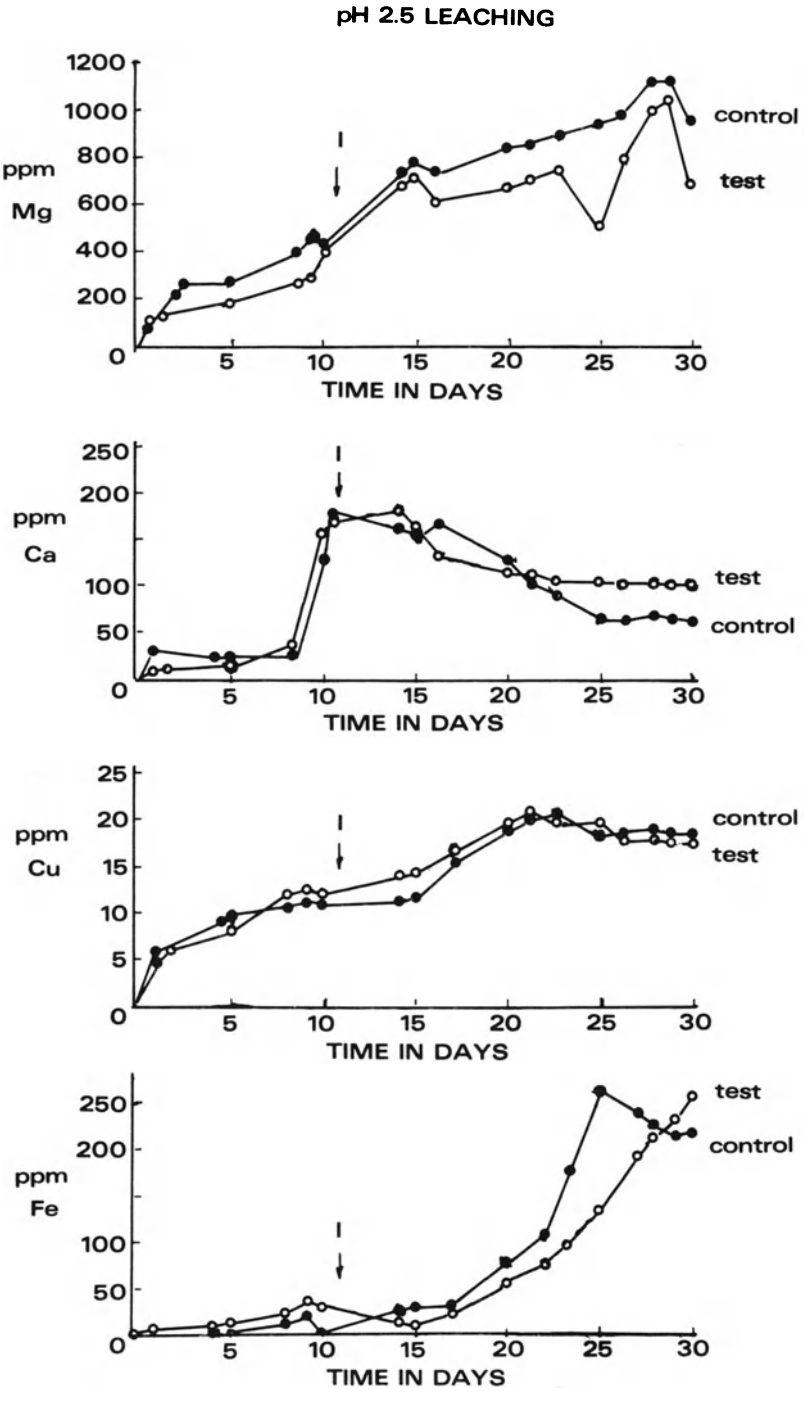


Figure 4: Leaching behaviour at pH 2.5 of a composite sample from White's Overburden Heap, Rum Jungle Mine, N.T.

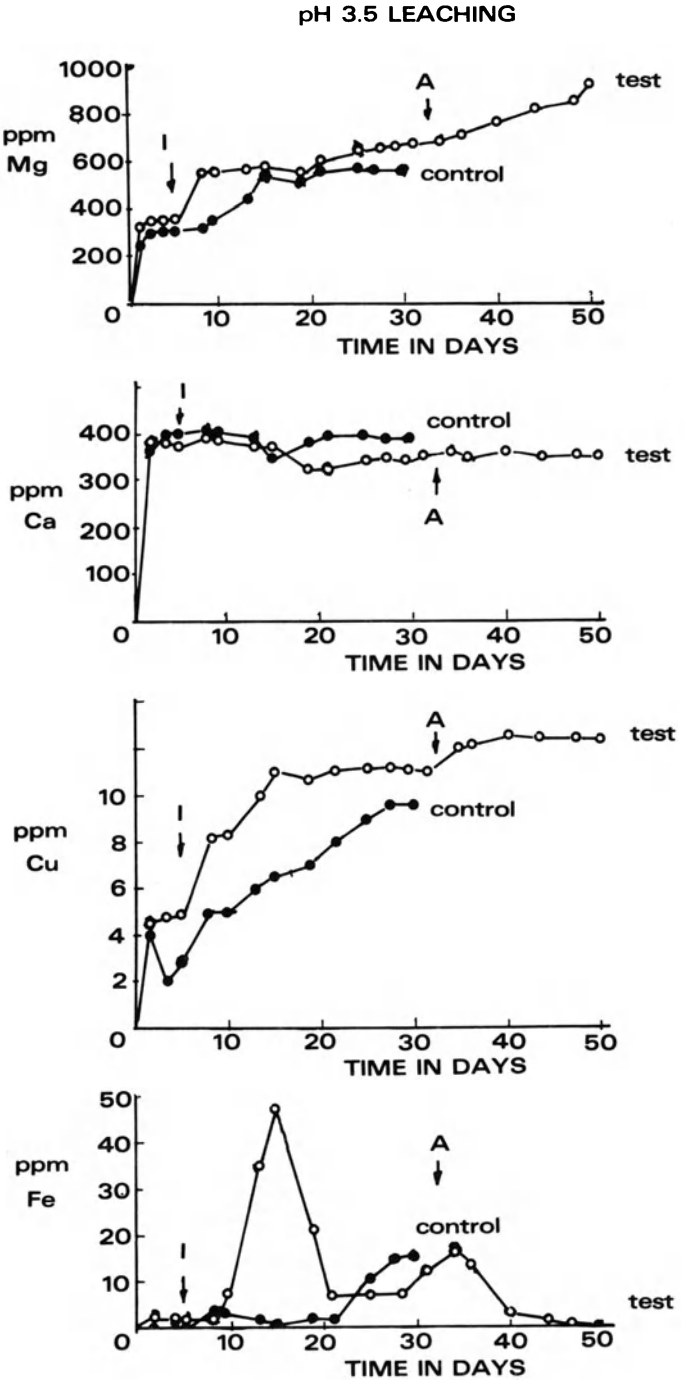


Figure 5: Leaching behaviour at pH 3.5 of a composite sample from White's Overburden Heap, Rum Jungle Mine, N.T.

DISCUSSION

Studies of Rum Jungle overburden leaching under controlled reactor conditions have demonstrated that highly acid conditions (pH 2.5) tended to mask bacterial activity, while milder leaching conditions (pH 3.5) have shown significantly different metal release patterns between the control and the inoculated test sample. Furthermore, controlled aeration and the subsequent introduction of a microaerophilic environment have revealed important changes in metal release, such as an increase in dissolved copper and magnesium and the simultaneous removal of soluble iron.

CONCLUSION

The reactor system described provides a highly versatile facility for the study of minera biodegradation under a diversity of physicochemical conditions and with a wide range of microorganism types. The reactor design allows for easy operation and facilitates mainte ance and cleaning operations. The pairing of the individual reactors and the clustering four pairs into a bank of eight, allows considerable economies to be made in respect of t impellor drive systems, the pH control equipment and the maintenance of impellor seal sterility.

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A MULTI-COMPANY SPONSORED RESEARCH PROGRAM TO STUDY THE BIOLOGICALLY ASSISTED FERRIC IRON LEACHING OF URANIUM MINERALS

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INTRODUCTION

Presently, chemical leaching of uranium, particularly from an acidic gangue, involves acidic ferric iron solutions. The 6-valent uranium is soluble at the acidic pH values involved, whereas solubilization of 4-valent uranium involved oxidation to the 6-valent species with ferric iron as the primary oxidant. Under most commercial vat leaching conditions, the pH is modestly below the level at which microbiological leaching can occur. In heap or "in situ" leaching, microbiological leaching is responsible for a substantial proportion (in mine drainage situations for all) of the uranium solubilized. The bacterium, *Thiobacillus ferrooxidans*, oxidizes the sulfides of most metals to the corresponding sulfates, thus generating acidic solutions. Where iron sulfides are involved the iron moiety is solubilized and concomitantly oxidized to the ferric form. The acidic ferric solutions, thus generated, solubilize both the 4- and 6-valent forms of uranium. The pH values over which this microbiological action can normally proceed are in the approximate range of 1.8 to 2.6; the upper limit being the less absolute. As the 4-valent uranium is oxidized to the 6-valent form, the iron which is reduced to the ferrous form is quickly re-oxidized to the ferric state by *Thiobacillus ferrooxidans*, thus enabling leaching to continue. Microbiological oxidation of ferrous to ferric iron is in no way dependent on the original source of the ferrous iron. Thus microbiological oxidation can interact with chemical leaching processes, where pH and other conditions permit.

Traditionally, microbiological leaching of uranium has been associated with pyrite-bearing ore bodies having an acidic gangue. The acidic ferric mine drainage which develops spontaneously contains solubilized uranium at levels amenable to conventional reclamation techniques.

B.C. Research has been engaged previously in studies involving enhancement of the solubilization of uranium in the drainage emanating from mined-out stopes of an operating mine and during 1978, B.C. Research conducted an evaluation of the leaching process at Agnew Lake Mines, Canada's only underground uranium leaching operation. The initial evaluation of the leaching process showed that the leach solutions sprinkled onto the surface of 100 to 200 feet deep stopes, filled with in-place broken ore, did not efficiently wet the ore when the stopes were inclined.

A series of tests subsequently performed at B.C. Research in columns holding 1-2 Mg of R.O.M. ore, were designed to assess the feasibility of floodleaching the ore in the underground stopes, a process that would ensure effective wetting of all broken ore particles. The detailed results of this testwork so far are confidential, but a number of observations made during the test program have led to a new research program to study the biologically assisted ferric iron leaching of uranium ores.

A number of the columns, which contained 1-1.5 Mg of -20 mm (-8") run of mine ore, were leached at varying pH and Eh values. When samples of pregnant solution taken from columns leached for 3-4 months at a pH of 2.0 - 2.2, were transferred into sterile 9K solution (Silverman and Lundgren, 1959) containing 9 g l^{-1} ferrous iron, active bacterial oxidation of the ferrous iron started within 48 h. However, if similar tests were performed on pregnant solutions from columns leached for 3-4 months at a pH of 1.5 or 1.6, no bacterial ferrous iron oxidation could be achieved over a 10-15 day period. Neither could bacterial activity be promoted when pH solutions were neutralized to pH 2.0 and 9K salts and ferrous iron were added. These results indicate that there is only limited bacterial activity at pH 1.5.

Razzell reported the pH limits for growth of *T. ferrooxidans* was between 2 and 4 (Razzell, 1962). Razzal and Trussell (1963) as well as Duncan *et al.* (1966) and Malouf and Prater (1961) reported the optimum pH to be 2.5, while Tomizuka *et al.* (1976) report the optimum

pH for bacterial oxidation of ferrous iron to be between 2.0 and 2.5. Manchee (1977) recognized that different conditions are required for the chemical oxidation of uranium than for the bacterial oxidation of ferrous iron. The former favours a lower pH than the latte The recently developed Bacfox process (Livesey-Goldblatt *et al.*, 1977) purposely separates the two stages by using an individual leaching circuit for the bacterial oxidation of the ferrous iron and a lower pH circuit for the oxidation of the uranium ores.

Obviously, there will be some advantage if the bacteria can be adapted to rapidly oxidize ferrous iron and metal sulfides at pH values of 1.5 or lower. Golomzik and Ivanov (1965) have shown that *T. ferrooxidans* can be made to oxidize ferrous iron and pH values as low a 1.0 if the pH of the bacterial culture is lowered in small increments. Unfortunately, little data were given on the effect of such low pH values on the rate of ferrous iron oxidation.

Very little is known about the effect of dissolved uranium values upon the activity of *T. ferrooxidans*. Studies at B.C. Research (unpublished results) showed that as little as 50 mg l^{-1} uranium inhibits the bacteria but that serial transfer techniques can be used to readily increase their tolerance to 500 mg l^{-1} dissolved uranium. Tuovinen and Kelly (197 report that growth inhibition is noted at 0.7 g l^{-1} uranyl sulfate. However, we have isolated active bacteria from samples of underground mine water at Elliot Lake which contain several grams per liter uranium, indicating that in nature the bacteria have been capable of adapting to high dissolved uranium values.

It thus became apparent to us that significant improvements in the uranium leaching proces can potentially be achieved if strains of *T. ferrooxidans* could be adapted to lower pH environments than those in which it normally operates. Similarly, if such strains can be adapted to high dissolved uranium concentrations, leaching may be expected to continue longer and at faster rates when high grade uranium ores are leached and, most importantly, when test cycles are practised, a leaching method which produces very high dissolved metal concentrations in the non-dynamic water content of a leach dump.

RATIONALE FOR THE RESEARCH PROGRAM

The chemistry and biology of the microbiological leaching of copper from its ores are relatively well understood. Considerable progress has been made in utilizing this basic information in maximizing the rate and extent of copper release from dumps of low-grade ore and from *in situ* leaching of mines. However, comparable studies to produce the same pragmatic information for uranium have been virtually non-existent. A thorough understanding of the actual operating variables which control performance of commercial dump, heap and *in situ* leaching of both low and high grade uranium ores undoubtedly will have a significantly beneficial effect on many existing and future mining operations.

This research program will elucidate those factors which affect microbiological leaching o uranium. The program is designed to produce results translatable into a set of guidelines for the leaching of various types of ores potentially amenable to the leaching process. Also, a technique will be developed for the rapid assessment of the amenability of ore samples to the leaching process.

At the same time, the knowledge gained should be of assistance in understanding the produc tion of acidic drainages from uranium tailings and waste materials and hopefully improve o ability to control the quality of such drainage.

OBJECTIVES OF RESEARCH PROGRAM

The initial studies are non-ore specific and are aimed at obtaining a better understanding of the biological uranium leaching process and at improving the state of the art. Consequently, the following research tasks are included in the initial phase of the research program.

- A systematic examination of the literature on bacterially assisted leaching of uranium.
- An evaluation of existing uranium mining operations utilizing or potentially capable of utilizing the ferric iron leaching process.

- An evaluation of the effect and interrelationship of Eh and pH upon the uranium leaching process.

- Isolation and characterization from natural sources of strains of *T. ferrooxidans* capable of oxidizing ferrous iron at low pH values.

- Isolation and characterization from natural sources of strains of *T. ferrooxidans* adapted to high dissolved uranium concentrations.

In a subsequent phase, to be started upon completion of these tasks, the B.C. Research-developed laboratory procedures for determining whether ores, notably copper sulfides, are amenable to microbiological leaching, will be adapted to evaluating uranium ores. It is anticipated that specially isolated strains of bacteria will be used in the adapted procedure. Results of this rapid assessment procedure normally are available in 5-6 days and provide the following information:

- Whether the mineral contained in the ore will leach microbiologically and if so, to what extent.

- How much of the contained mineral is acid soluble and at what acid consumption.

- What the acid demand or production will be during the overall leaching process.

It is anticipated that this procedure, when adapted to uranium ores, will have two extremely valuable functions. It will serve as an initial screening test to indicate whether bacterially-assisted leaching can play an important role in the retrieval of uranium from high and low-grade ores associated with a specific ore body. If positive results are obtained, then further confirmatory evidence is normally sought on larger-scale tests involving small or large columns and, in some instances, test heaps or dumps, where various physical constraints can be examined.

A second valuable function will be to determine whether acid mine drainage will develop, with its resultant environmental problems, if a specific ore body or tailing is exposed to the atmosphere. This can be of consequence regardless of method of uranium retrieval.

When this rapid assessment procedure has been successfully adapted to uranium ores, we hope to use the technique to determine the microbiological leachability of the various known uranium minerals.

A number of research tasks are scheduled for commencement at a later stage in the research program:

- Study of the effect of thorium upon the leaching bacteria.

- Study of the effect of initial acid strength upon the length and extent of the chemical leaching phase and subsequent lag phase.

- Study of the use of thermophilic strains of iron and sulfur oxidizing bacteria in the uranium leaching process.

- Study of the possible use of bacteria in alkaline leaching processes.

It should be emphasized that all our studies will be conducted with maximum concern for the practical value of the results obtained, and we anticipate that the 10 mining companies which are sponsoring this research program and who cooperated in establishing the initial objectives of the program will also have a significant input into the subsequent modifications of the program, the type of tests that will be performed, as well as to the size of the experiments. To ensure the close contact with the sponsors that such a research program demands, regular meetings are held with representatives of the sponsoring companies, at which time the progress of the research studies is assessed and additions or alterations to it are made in accordance with the requirements of the sponsors.

CURRENT TEST PROGRAM

At present, we have several column tests in progress. One set of tests utilizes 16 small P.V.C. columns, each containing approximately 10 lbs of -1 inch uranium ore. The 16 columns are divided into 4 sets of 4 columns. All 4 columns in each set are leached at a similar Eh but the Eh between each set of columns is varied at <350, 400, 500 and >600 mV Within each set, 4 different pH values of 1.2, 1.6, 2.0 and 2.5 are maintained. Ten litre batches of leach solutions with the required characteristics are made up and slowly percolated through the ore column at a rate of 10 1 $h^{-1}m^{-3}$. The pregnant solution is collected in a reservoir, the contents of which is returned to the feed reservoir after its pH and have been adjusted as required. By regularly following the acid consumption and uranium extraction, we hope to obtain a better insight into the effects of Eh and pH upon the uranium leaching process. Another set of tests utilizes 4 columns containing 45.4 kg of -1" ore each. Each column is operated at a different temperature, i.e. 5°, 10°, 20° and 35°. Leach solutions for each column initially were identical in make up, bacteria content and pH and were distributed from the barren reservoir over the broken ore at a rate of 10 1 $h^{-1}m^{-2}$. The pregnant solutions produced are returned to the barren reservoir after pH adjustment. Thus, we should be able to measure the difference in initial chemical and subsequent biological leaching rates as a result of the difference in temperature.

To slowly adapt selected strains of *T. ferrooxidans* to low pH values and high dissolved uranium concentrations, we utilize a series of small fermenters of 1.5 1 reactive capacity. The contents of the fermenters are turbine agitated and air is injected under the turbine to promote efficient air sparging. Each fermenter is equipped with an automatic pH control which utilizes 6 N H_2SO_4 to offset the acid consumption of the bacterial ferrous iron oxidation that takes place in the fermenter.

In a typical test, two fermenters are operated as a set. One fermenter is fed with a 9K media solution containing 18 g l^{-1} ferrous iron, while to the feed for the other fermenter 0.34 g l^{-1} dissolved uranium is added. Both fermenters are started off at pH 2.0 and the rate of ferrous iron oxidation is monitored. When the rate reaches a maximum value, e.g. 900 mg $l^{-1}h^{-1}$, the pH of the feed solution is lowered in incremental steps. Following each incremental decrease in pH, the rate of ferrous iron oxidation is allowed to stabilize at a maximum value before the pH is lowered again. It is anticipated that, once the bacteria have adapted to a sufficiently low pH value, the dissolved uranium content in one of the fermentors will be slowly increased. The effect of these increased concentrations and the capabilities of the bacteria to learn to adapt to them will be monitored through the ferro iron oxidation rates.

We sincerely hope to be able to report to you in the very near future the results of these tests and the progress of the research program.

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BACTERIAL LEACHING OF A CARBONATE BEARING URANIUM ORE

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INTRODUCTION

According to the "Club of Rome" the continuously increasing world-wide demand for raw materials will cause an exhaustion of non-reclaimable resources within the next few decades (Meadows 1972). On the other hand, the resources are not regarded as a static magnitude but as a dynamic one which increases in accordance with scientific and technological progress (Rechtziegler, 1973; Heilbroner, 1973). Hence, new deposits may be discovered by the improvement of conventional methods and by the development of new geochemical and geophysical prospecting methods. But discovering a new ore body does not mean that mining will be feasible because several factors may influence the metal winning process, e.g. low amount of total ore, low concentration of valuable metal, large dissemination of ore.

In the case of the uranium deposit at Forstau, Austria, additional problems of environmental protection have to be taken into consideration. The Forstau Valley is a resort area; therefore, the environmental impact as a result of mining has to be reduced to a minimum. Furthermore, the local facilities for the installation of a dressing plant are rather insufficient and the industrial production of sulfuric acid necessary for acid leaching is not practical in situ. Because of these difficulties, the application of conventional processing methods for the extraction of uranium from Forstau ore does not seem to be feasible.

Within the last few decades microbiological leaching methods for the recovery of valuable metals from low-grade ores have been developed. These biohydrometallurgical processes are already used commercially for the extraction of copper and uranium (Trussel *et al.*, 1964; Harrison *et al.*, 1966; Beck, 1967; Malouf, 1972: Guay *et al.*, 1976; Murr *et al.*, 1978). The solubilization of metals depends on the activity of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. Both species are chemolithotrophic bacteria which derive all of their energy from the oxidation of inorganic compounds. *T.ferrooxidans* oxidizes either reduced sulfur or iron compounds, whereas *T.thiooxidans* oxidizes elemental sulfur (Tuovinen and Kelly, 1972; Starkey, 1925). The biochemical reactions are not yet completely resolved, but two principal mechanisms for metal recovery are accepted: the direct bacterial oxidation of ferrous and/or sulfide ions and the indirect oxidation by bacterially regenerated ferric ions or a combination of both (Silverman and Ehrlich, 1964).

The efficiency of microbiological leaching depends mainly on the chemical and mineralogical characteristics of the individual ore, i.e. the leaching conditions have to be established for each type of ore.

The solubilization of uranium is an indirect bacterially catalyzed process. In the present paper investigations on the applicability of bacterial leaching of sedimentary uranium ore from Forstau (Austria) are described.

MATERIALS AND METHODS

Materials

The sedimentary uranium ore at Forstau is extracted by underground mining in the size of < 100 mm. The uranium content is between 0.03 and 0.15% U. The ore sample used for laboratory test contained 0.12% U, 1.45% FeO, 0.77% Fe_2O_3, and 4.76% CO_2. The sulfide content is < 0.1%. The main opague minerals are pyrite, chalcopyrite, and pitchblende.

Bacteria

T.ferrooxidans and *T.thiooxidans* were isolated from local underground mine water and ore

samples. For leaching experiments, however, we used pure cultures of *T.ferrooxidans* and *T.thiooxidans* originally isolated from acid mine water in the Harz Mountains and maintained as stock-cultures in our collection which distinguished themselves from Forstau strains by quicker growth and which had already been adjusted step by step to high concentrations of uranium (2.5 g l^{-1}).

T.thiooxidans was cultivated in the medium described by Starkey (1925) using elemental sulfur as the energy source, *T.ferrooxidans* was grown in 9 K-medium (Silverman and Lundgren, 1959). The bacteria were incubated on a rotary shaker at 150 rpm and 30°.

Leaching Experiments

The leachability of the ore was tested using air-lift percolators and Erlenmeyer shake-flasks. Percolator columns (4.5 cm by 30 cm) contained 200 g of unsterilized ore crushed to a particle size of about 10 mm. 150 ml culture medium inoculated with an active culture of either *T.ferrooxidans* or *T.thiooxidans* or with a mixed culture of both strains was recycled over the ore.

500-ml Erlenmeyer flasks containing 90 ml culture medium, 10 ml bacterial inoculum, and a 5% pulp density of ore were used for the extraction of uranium from fine-grained ore (initial particle size < 0.6 mm). In the sterile control flasks 10 ml of a methanol-formol solution (10 : 1) were added instead of the inoculum. The flasks were incubated on a rotary shaker at 150 rpm. All leaching experiments were operated at 30°. Periodically, water loss due to evaporation was replaced with diluted H_2SO_4 (pH 2.5) before samples were collected for measurement of pH, Fe(II and Fe(III), uranium, and bacterial activity.

Tank-leaching experiments were performed at different pulp densities with 6 l of leach suspension in a thermostated fermentor (Biostat S, B. Braun, Melsungen). The tank was aerated with air (1.3 l min^{-1}) and CO_2 (0.1 l min^{-1}) and agitated at 500 rpm.

The pH was automatically controlled and readjusted with 1 N sulfuric acid and 1 N sodium hydroxide if necessary.

Analytical Methods

The uranium content was analyzed by x-ray fluorescence analysis. After centrifugation of the leach suspension (20 min at 10,000 rpm), 5 to 10 ml of the supernatant were evaporated to dryness in platinum crucibles and, after melting the residue with 4 g sodium tetraborat uranium was assayed by a Philips PW 1450 x-ray-spectrometer. Bacterial activity was estimated after inoculation into fresh culture medium.

RESULTS AND DISCUSSION

Percolator Leaching

Leaching in percolator columns may be regarded as a simplified model test for dump and heap leaching and the results obtained at a laboratory scale will give a preliminary answer to the question if bacterial leaching is feasible.

As shown in Table 1 the recovery of uranium from Forstau ore is correlated to a substantial consumption of sulfuric acid. Because of the high carbonate content of the ore the pH values in the leach suspensions continuously increased. Sulfuric acid was added to adjust the pH to about 3 to provide better conditions for the growth of the acidophilic thiobacilli. In the presence of *T.thiooxidans* after 120 days of leaching the pH remained at 2.5. About 1 g H_2SO_4 had been added and 5.4% U was recovered from the ore. In the case of leaching with *T.ferrooxidans* 3.5 g H_2SO_4 was consumed to maintain the pH between 3.5 and 2.5. Uranium was not detected until the pH in the leach suspension decreased to about 3. After 120 days 27% U was extracted from the Forstau ore. Using mixed cultures of both strains the acid consumption decreased compared to the leaching with *T.ferrooxidans* and the uranium extraction increased in comparison to leaching with *T.thiooxidans*.

As indicated by percolator leach studies, uranium is recovered from the Forstau ore by the activity of *T.ferrooxidans*. However, *T.ferrooxidans* is active only after carbonate neutralization. The amount of acid added to dissolve the carbonate decreased in the presence of *T.thiooxidans*.

TABLE 1

PERCOLATOR LEACHING OF FORSTAU ORE; ACID CONSUMPTION AND URANIUM RECOVERY AFTER 120 DAYS

Bacteria	H_2SO_4 consumed (mg)	Uranium extracted (%)
T.thiooxidans	980	5.4
T.ferrooxidans	3528	26.9
T.thiooxidans and T.ferrooxidans	1323	11.5

Leaching in Shake-Flasks

In fairly recent studies on leaching by the submerged fermentation method (Bruynesteyn and Duncan, 1971, Torma *et al.*, 1970, McElroy and Bruynesteyn, 1978) much higher conversion rates and yields of metal amounting to almost 100% were achieved compared with conventional percolator leaching.

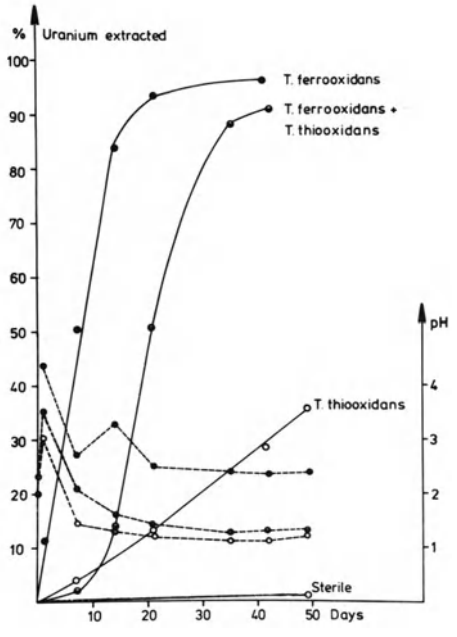

Figure 1: Uranium extraction at 30° by *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans* and mixed cultures of both strains using a 5% pulp density suspension of uranium ore (initial particle size < 0.6 mm); pH (---), uranium yield (——).

Because of the low uranium recovery by percolator leaching of the Forstau ore, the leachability in shake-flasks was investigated. The results are shown in Fig. 1. The pH values decreased immediately in samples inoculated with *T.thiooxidans*. Only in the case of *T.ferrooxidans* was sulfuric acid added to lower the pH at the beginning. With *T.ferrooxidans* 92% U was extracted after 20 days. At the same time only 11% U was recovered from the ore by use of *T.thiooxidans*. Among other factors, the velocity of uranium extraction depends on the concentration of the bacteria. In mixed culture flasks the concentration of the individual species was only half as high as in the pure cultures probably causing the lag-phase in the uranium extraction curve. After 40 days, 90% U was extracted by mixed cultures. Under sterile conditions nearly no uranium was recovered.

Tank Leaching

Shake-flask experiments indicated that almost 100% U was recovered from the Forstau ore if the pH value in the leach suspension was maintained at a suitable range for active growth of *T.ferrooxidans*. For sufficient neutralization of the carbonate about 60 - 80 kg H_2SO_4 10^{-6}g uranium ore are needed. For saving expenses, acid production with *T.thiooxidans* was taken into consideration. For feasibility studies *T.thiooxidans* was cultivated in a fermentor. During growth of *T.thiooxidans* the pH decreased continuously. After 4 days, fine-divided ore (< 0.6 mm) was added to a 10% pulp density (Fig. 2). After a short increase the pH decreased again because of continuous bacterial acid production. After carbonate neutralization at day 7 the pH was adjusted to 2.0 with 1 N NaOH and *T.ferrooxidans* was added. Furthermore, the pH was maintained automatically at about 2.0. Within the next few days the concentration of ferrous iron dissolved from the ore decreased, but no increase of uranium was observed. After supplement of *T. ferrooxidans* and $FeSO_4$ (10%) the uranium concentration increased immediately and about 90% U was extracted from the ore within less than 3 days. The maximum uranium extraction rate was 1.5 mg U l^{-1} h^{-1}. The whole process including acid production, carbonate neutralization, and uranium recovery may be accelerated after optimizing several leaching condition, e.g. better acid production with *T.thiooxidans*, a sufficiently concentrated inoculum of active *T.ferrooxidans*, and no lack of energy sources.

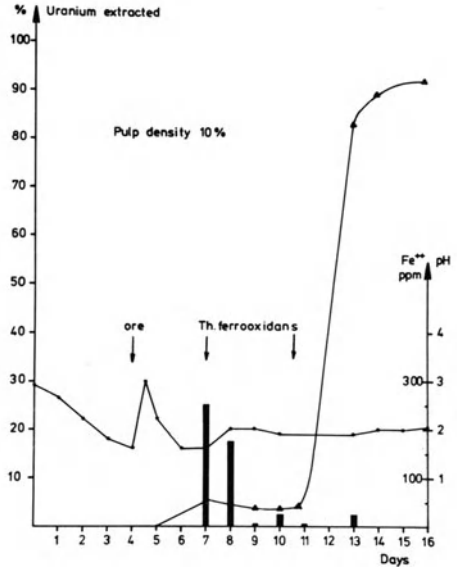

Figure 2: Tank leaching of the Forstau ore pH (o ——— o), Fe^{2+} content (■), uranium yield (□ ——— □). Further details are given in the text.

The efficiency of bacterial carbonate neutralization depends on the pulp density. At 15% pulp density the bacterial acid production was not strong enough to lower the pH, which remained at about 3.5 for 6 days until sulfuric acid was added. After inoculation with *T.ferrooxidans* at pH 2.0, 97% U was extracted within 4 days at a maximum uranium extraction rate of 3.5 mg U^{-1} h^{-1}. To maintain the pH at 2.0, continuously 1 N NaOH was added automatically indicating that *T.thiooxidans* was still active.

At 20% pulp density only 0.3% U was extracted from the Forstau ore. When inoculated into fresh culture medium *T.ferrooxidans* was inhibited once the carbonate had been neutralized probably because of increased concentrations of other easily soluble compounds of the ore (e.g. K^+, Na^+, Mg^{++}).

Leaching in a Counter-Current Process

To avoid the accumulation of toxic compounds in the course of uranium recovery, a counter-current oxidation process has been developed (Flöter *et al.*, 1979). At the beginning, fresh ore is treated with strong sulfuric acid. Later on, a pregnant solution from a previous bacterial leaching process acidified with sulfuric acid is used for the neutralization of fresh uranium ore. After sufficient carbonate neutralization the liquid phase is separated and conveyed to uranium winning. In the second leaching stage, the oxidation stage, at the beginning of the process the neutralized ore is inoculated with *T.ferrooxidans* for bacterial uranium leaching. Later, the "oldest" oxidation vat will inoculate the "younger" ones. Several oxidation vats derived step by step from the neutralization stage can be connected in parallel. Later on the "oldest" vat is separated for washing. The leached ore is discarded and the washwater is supplemented to the bacterial leach suspension after an equal volume has been separated to replace the uranium bearing solution of the "youngest" oxidation vat. This effluent, supplemented with H_2SO_4, is pumped to the neutralization vat, in order to displace the neutralization liquid containing other soluble compounds possibly disturbing the bacterial leach process. The effluent of the first leaching stage (neutralization) is conveyed to the ion exchanger for separating uranium.

The efficiency of the counter-current vat leaching process was successfully proved on a laboratory scale by leaching of 5 kg of ore with a particle size between 0.125 and 4.00 mm. After 18 days, about 80% U was extracted from the ore. According to these results a flow-sheet for a technical leaching of the carbonate bearing uranium ore is presented (Fig. 3).

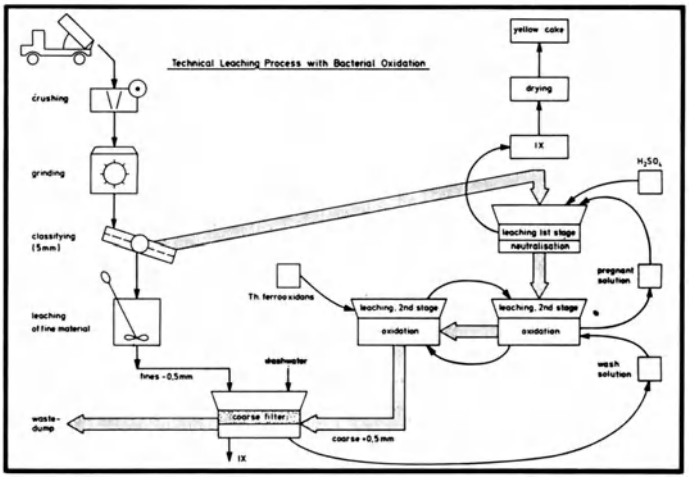

Figure 3: Flowsheet for a technical leaching process with bacterial oxidation.

As indicated in our investigations, bacterial leaching of the Forstau ore is feasible. But the results are obtained on a laboratory scale and, therefore, an extrapolation to industrial leach systems should be considered very carefully. Further studies are necessary, especially with regard to the economics of the system.

ACKNOWLEDGEMENT

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INFLUENCE OF ATTRITION GRINDING ON THE RECOVERY OF COPPER FROM A HIGH-GRADE CHALCOPYRITE CONCENTRATE BY THE BSE-PROCESS

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INTRODUCTION

In previous studies (Torma and Subramanian, 1974; Torma and Guay, 1976) we have demonstrated that a decrease in the particle size of the solid metal sulfide results in an increase in the rate/or extent of the metal extraction by the chemolithotrophic *Thiobacillus ferrooxidans*. The use of fractions of a lead-zinc sulfide concentrate (Torma and Subramanian, 1974) smaller than 5 μm in diameter resulted in zinc extractions as high as 98.1% in five days of leaching. Only limited information is available from the literature regarding the bacterial leaching of solid particles where the size of the ore would approach the bacterial dimensions (which are about 0.5 μm wide and 1.5 to 2.0 μm long). Conventional milling techniques are not practical for producing these very small particle sizes. However, the attrition grinding techniques have shown to be quite effective in producing mineral particles below the one μm range (Sadler *et al.*, 1975). The applicability of attrition grinding in hydrometallurgical operations is a subject of intensive research (Gerlach *et al.*, 1973; Beckstead *et al.*, 1976).

The present study investigated the influence of ultra-fine particles on bacterial activity by using different size fractions of an attrition ground chalcopyrite concentrate and a pure strain of *T.ferrooxidans*.

MATERIALS AND METHODS

Bacteria

Our strain of *T.ferrooxidans* was originally isolated by Torma (1971) from acid mine waters in northern Quebec. It was adapted to a modified nutrient medium of Silverman and Lundgren (1959) in which the attrition ground chalcopyrite concentrate replaced ferrous sulfate as the energy source. For subculturing and experiments an aliquot of a late log-phase was transferred into a new medium.

Substrate

The chalcopyrite flotation concentrate contained 23.4% copper, 31.6% iron, and 30.3% sulfur as major constituents and it was composed of chalcopyrite, pyrite, pyrrhotite, blend and quartz. This material was attrition ground as described by Sadler *et al.*, (1975) to yield two different size fractions whose main particle sizes were determined using a Coulter Counter to be 0.8 and 0.4 μm, respectively.

Culture Technique

All experiments were carried out in 250 ml Erlenmeyer flasks (Torma and Subramanian, 1974) containing 5 ml of an active culture of *T.ferrooxidans*, 70 ml of iron-free 9K medium (Silverman and Lundgren, 1959) and the desired quantities of substrate. In the sterile control flasks 5 ml of a 2% solution of thymol in methanol was added instead of the inoculum. The flasks were aerated with air containing 0.2% carbon dioxide and incubated on a gyrotory shaker.

Solvent Extraction and Electrowinning

Studies were done with a commercially available organic extractant (15% LIX65N in kerosene) and an electrolysis cell equipped with copper cathodes and lead anodes. These methods are described in detail by Torma *et al.* (1979).

Kinetic Determinations

The analysis of the solutions and the determination of the rate and yield of copper extraction were carried out according to Sakaguchi *et al.* (1976).

The order or reaction (n) was determined by the differential method of van't Hoff (Laidler, 1965), which relates the rate of reaction (V) to the initial concentration of substrate (C):

$$V = kC^n \qquad (1)$$

where k is the overall reaction rate constant (h^{-1}) and C represents the theoretical amount of copper (mg l^{-1}) available in the initial chalcopyrite concentration.

RESULTS AND DISCUSSION

The results obtained with the two different size fractions (0.4 and 0.8 µm) are represente in terms of final concentrations (g l^{-1}) and yield (%) of copper extraction as a functior of pulp density in Figs. 1A and 1B, respectively.

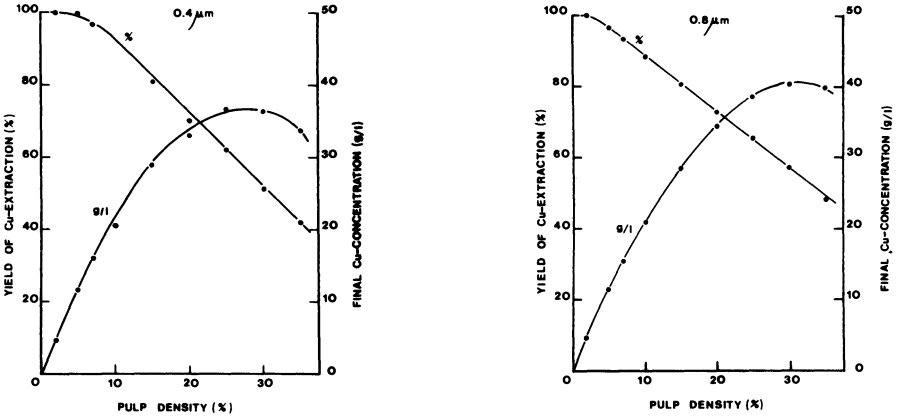

Figure 1: Influence of initial pulp density of attrition ground chalcopyrite concentrate on the final copper concentration and yield of extraction by *Thiobacillus ferrooxidans* at pH 2.3 and 35° after 7 days of leaching. 1A corresponds to the size fraction 0.4 µm and 1B to the size fraction 0.8µm, respectively. Each point on the curves is the mean of two individual measurements.

The pulp density is defined as the substrate concentration (g) x 100/ volume of the nutrient medium (ml). The copper extractions in the sterile controls were approximately 8 to 10 x less than they were in the corresponding inoculated ones. The extraction and concentration curves in Figs. 1A and 1B are very similar. At low pulp densities, the copper extraction was almost complete while it decreased linearly at pulp densities higher than 5%. At higher pulp densities, after the ferric ion concentration reached the saturation level it was partially hydrolyzed and precipitated as a basic ferric sulfate (jarosite type). The copper extraction can be expressed by the following equation:

$$2\ CuFeS_2 + 8.5\ O_2 + H_2SO_4 \xrightarrow{T.ferrooxidans} 2CuSO_4 + Fe_2(SO_4)_3 + H_2O \qquad (2)$$

and the jarosite formation is given by

$$3Fe_2(SO_4)_3 + 12\ H_2O \rightleftarrows 2\{H[Fe(SO_4)_2 . 2Fe(OH)_3]\} + 5H_2SO_4 \quad (3)$$
jarosite

This jarosite precipitate interfered with the bacterial activity by covering the surface of the unreacted chalcopyrite (Torma *et al.*, 1979). It is also possible that the high cupric ion concentration of 45-40 g l^{-1} acted increasingly as an inhibitor and contributed to the reduction of the bacterial metabolic activity. Similar observations have been reported by others (Bruynesteyn and Duncan, 1971).

The influence of pulp density on the rate of copper extraction using the two different size fractions, is shown in Fig. 2. As expected, higher rates were obtained with the smaller size fractions. The highest rate of copper extraction achieved in this series of experiments was 1,650 mg $l^{-1}h^{-1}$, which was obtained with a 25% pulp density solution of the 0.4 µm size fraction.

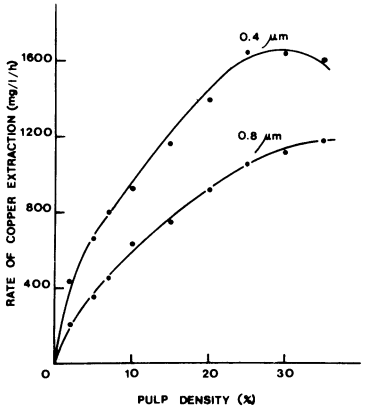

Figure 2: Influence of pulp density on the rate of copper extraction from attrition ground chalcopyrite concentrate by *Thiobacillus ferrooxidans* at pH 2.3 and 35°.

The overall reaction rate constant (k) and the order of reaction (n) for this copper extraction process have been calculated from the van't Hoff presentation (Laidler, 1965) with respect to the substrate concentration, using the two different size fractions of chalcopyrite as shown in Fig. 3. The k and n values derived were 5.63 h^{-1} and 0.52 for the size fraction 0.4 µm, and 1.06 h^{-1} and 0.66 for the size fraction 0.8 µm. Consequently, the rate of copper extraction can be approximated by the following equations as shown for:

a) the size fraction 0.4 µm:

$$V = 5.63\ C^{0.52} \simeq 5.63\ \sqrt{C} \quad (4)$$

b) for size fraction 0.8 µm:

$$V = 1.06\ C^{0.66} \simeq \sqrt[3]{C^2} \quad (5)$$

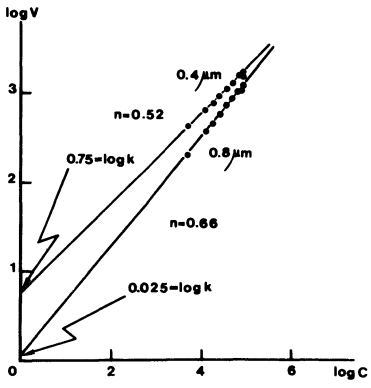

Figure 3: Determination of the overall copper extraction rate constant and the order of reaction by the van't Hoff approach for the leaching of attrition ground chalcopyrite by *Thiobacillus ferrooxidans*.

In a previous study (Torma *et al.*, 1979) using a chalcopyrite concentrate (particle size less than 37 µm) we have found that the microbiological copper extraction followed a first order reaction. In general it is recognized that the order of a metabolic reaction may change (Trumbore, 1966). For example, if the substrate level is high, the rate of the biological reaction is independent from the concentration of substrate. This situation exists mostly when the substrate is soluble. If the substrate level falls - either the substrate is used up or is inadequately exposed, as is the case for most of the solid substrates - the rate more closely approximates a first order reaction. This trend is clearly supported by the experimental evidence of this investigation and of our previous study (Torma *et al.*, 1979).

The fact that an almost complete extraction of copper can be achieved from a high-grade concentrate in a single bacterial leaching step (as shown in Figs. 1A and 1B for low pulp density suspensions) is of economical importance. Previous studies (Bruynesteyn and Duncan, 1971; McElroy and Bruynesteyn, 1978; Torma *et al.*, 1979) suggested using higher pulp density solutions to include a regrinding step after about 60% of copper extraction. The leaching is completed in the next leaching step. However, if attrition ground substrates in low-pulp density solutions would be subjected to bacterial leaching, no regrinding or releaching would be required. The dissolved copper could be purified and concentrated using a solvent extraction technique as described previously (Torma *et al.*, 1979). About 4.5 l of bacterial leach solution was collected from the leaching experiments and subjected to solvent extraction. A 99.5% pure copper was obtained by electroplating from the pregnant electrolyte solution. The overall current efficiency of the electrowinning process varied between 91.5 and 93.2%. These findings are in good agreement with those of other investigators (McElroy and Bruynesteyn, 1978; Torma *et al.*, 1979).

The findings of this investigation can be used to propose a microbiological leaching process for the extraction of copper from a high-grade chalcopyrite flotation concentrate. The schematic outline of such a process if shown in Fig. 4. This process can be carried out as a cyclic batch process or as a continuous leaching process - involving attrition grinding of the chalcopyrite concentrate, leaching of this substrate with inoculated nutrient solution containing less than 5% pulp, solid-liquid separation, solvent extraction and electroplating of the copper. In order to avoid adverse effect on the bacterial activity, the dissolved and entrained organics should be removed from the recycling liquor prior to being returned to leaching (Torma and Itzkovitch, 1976).

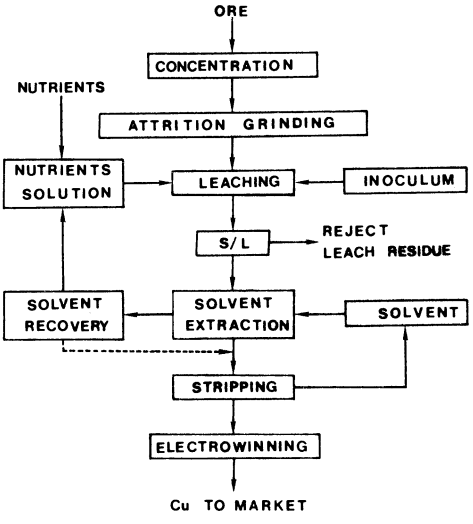

Figure 4: Schematic representation of a generalized bacterial leaching process for the extraction of copper from attrition ground flotation concentrates.

CONCLUSION

The present study has demonstrated the influence of attrition grinding on the microbiological leaching of a chalcopyrite concentrate. An important finding of this study is that copper extractions as high as 100% can be achieved at low pulp densities (less than 5%) using the particle size fractions 0.8 and 0.4 μm. The dissolved copper can be recovered by solvent extraction coupled with electroplating. A further finding of this study is that copper extraction rates above 1,600 mg $l^{-1}h^{-1}$ were realized using the 0.4 μm size fraction at 25% pulp density. Such high rates have not been reported yet in the literature. The order of reaction increased from 0.52 to 0.66 as the particle size of the chalcopyrite concentrate varied from 0.4 to 0.8 μm, respectively.

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LABORATORY STUDIES ON MINERAL LEACHING

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INTRODUCTION

There is growing interest in the bacterial leaching of low-grade ore bodies in the Australasian region. Operational aspects of local heap leaching have been described by Andersen and Allman (1968). The experimental approach to this process frequently employs the use of percolation leaching columns (Madsen *et al.*, 1975; Bruynesteyn *et al.*, 1977; Le Roux and Mehta, 1978; Murr and Brierley, 1978). The results to be presented arise from laboratory leaching studies on copper porphyry ores from Bougainville Island, Papua-New Guinea. The detailed mineralogy of these rock types have been described by Lawrence and Savage (1975). Espie (1971) has described the Bougainville Copper Project mining operation.

MATERIALS AND METHODS

Three shipments of ore from the Bougainville Copper Limited mine were used in these studies. The rock types were: Quartz feldspar porphyry (1975/76); Biotite granodiorite, Biotite diorite, Biurogranodiorite and Panguna andesite (1974). The ores were crushed and divided into various particle sizes by sieving. Percolation columns were loaded with the ore fractions in the manner detailed in Table 1.

TABLE 1

COLUMN DESCRIPTION

No.	Length x dia. (m)	Rock Shipment Date	ORE BED Particle Size (mm)	(inches)	Weight (kg)	Void (%)	BACTERIA INOCULATION	TEMP. (°C)
1.	3x0.15	1975	19-38	3/4-1½	70	52	+	Ambient
2.	"	"	5-10	3/16-3/8	70	39	+	"
3.	"	1974	"	3/16-3/8	67	32	-	"
4.	1.5x0.15	1976	"	3/16-3/8	32	46	-	30-33
5.	"	"	13-38	½-1½	28	54	-	"
6.	"	"	51-76	2-3	23	64	-	"
7.	"	"	<45μ-51	< 2	26	51	+	25-28
8.	"	"	< 44	< 2	26	50	+	30-33

The percolation columns were constructed from standard PVC piping and fittings to the dimensions indicated. Columns 1 and 2 were each fitted with 36 evenly-spaced viewing and sampling ports (12 cm^2 cross-sectional area) which could be sealed and enabled various events to be monitored throughout 80% of the ore charge depth.

The ore charges were irrigated with dilute sulfuric acid (pH 2.0) at a rate of 170 l m^{-2} day^{-1}, the feeds being maintained either by gravity from 200 litre, black, high-density polyethylene reservoirs or by Sigmamotor^R peristaltic pumps. The feed lines were glass or

silicone tubing, soft PVC tubing being avoided because it appeared to stimulate the growth of fungal contaminants. Irrigants entered the columns by a capillary drip feed (ca. 0.04 ml s^{-1}) and fell 40-70 cm before impinging upon a perforated perspex distributor disc (113 cm^2 with 20 x 1 cm holes). In some experiments, irrigation was once-through: in other experiments, the liquors were recirculated, being in these cases collected in a 20 litre, glass collection vessel whose contents were vigorously aerated (ca. 1 l min^{-1}) befc being pumped back to the top of the column. Periods of interrupted irrigation ("rest" periods) were introduced by stopping the feed to the column and allowing drainage with the base stopcock left open. Perspex lids were used to limit evaporation from the tops of the columns.

Percolated liquors were collected in 20 litre PVC drums and emptied every 3-5 days, after samples had been taken for analysis.

Acidity of leachates was measured with a Philips^R digital pH meter (PW908). Residual sulfuric acid in percolates was estimated from pH assuming a neutral salt concentration equivalent to 400 mg^{-1} sulfate ion. Copper and iron were determined by atomic absorption spectrometry (Techtron AA-5^R). Solid samples were dissolved by acid digestion; powders (< 200 mesh) were boiled with concentrated HCl (10:1, v/w) for twenty minutes followed by three successive additions of concentrated nitric acid (1/4 volume) over a further hour. Residues were filtered off and approximately normal solutions made up for spectrometry. Soluble silicon was estimated by standard colorimetric analysis after reaction with molybdate in the presence of sodium sulfite.

Qualitative elemental scans were done on a JSM-U3 scanning electron microscope with EDAX^R. In situ surface copper, chalcopyrite and pyrite were determined quantitatively with an ARL model EMX electron probe. Mineralogical examinations of surfaces and sections were done b the School of Applied Geology, University of New South Wales. Emission spectroscopy was performed by the School of Analytical Chemistry, University of New South Wales.

Microbiological methods are described in detail by Khalid and Ralph (1976). Column inoculations were made with *Thiobacillus ferrooxidans* isolated from Bougainville concentrates (10^{12} cells/addition). Temperatures of columns 4-8 were maintained in air-conditioned hot rooms to within ± 3°.

RESULTS AND DISCUSSION

The elements in Bougainville ores (average values) are listed in Table 2. Silicon, Al, Fe, Ca, Na, Mg, Ti and Cu were the main elements, with As, Ba, Mn and P of minor importanc The remaining fifteen elements were present in only trace amounts. Composition was typica of an average igeous rock type which was neither acidic nor basic in nature.

Field samples of dump leach liquors (Table 3) had high levels of sulfate and copper (e.g., 2.7 g l^{-1} and 0.5 g l^{-1}, respectively, at pH < 4). Magnesium, Ca, Si and Mn were also being solubilized (0.1 - 0.3 g l^{-1}) while effluents were enriched with small but significant levels of Co, Cr, B, Mo and Zn. However, dissolved iron (< 5 mg l^{-1}) was atypically low considering the 2 - 7% range (w/w) present in the parent rock.

EDAX^R analysis of polished surfaces showed the presence of abundant Al, Si, S, Cl, K, Ca, Ti, Mn, Fe and Cu. Most of the remaining 27 elements found by emission spectroscopy (except Sb, Pb, Mo, Co and Cd) were detected, albeit tentatively, by EDAX^R. The presence of major sulfides ($CuFeS_2$ > FeS) was confirmed by EDAX^R and microprobe analysis.

Electron probe determinations on gangue in fresh rock samples showed copper to be less tha the limits of detection (i.e., < 0.04% estimated in matrix areas). In weathered samples (e.g. Biotite diorite) non-sulfide copper was present in as much as 0.5% content in 45% of the estimated matrix area. Mineralogically, the high copper/low sulfide regions were identified as copper oxides (cuprite and tenorite). The presence of coliform banding signified that some of the copper oxides were formed secondarily on the amorphous geothite (FeO.OH) as the colloid was laid down.

Finely divided magnetite was a major mineral inclusion in fresh low grade ore but diminished in weathered samples. Haematite was found more frequently in weathered rock while rutile and ilmenite were variable. Rutile reached 1 - 2% of the total samples for Biotite granodiorite but was not observed in the fresh material.

TABLE 2

ELEMENTS IN BOUGAINVILLE ORE

Element	% (av)	Range
Ag	0.00018	< 0.001 -
Al	8.47	2.0 - 8.47
As	0.001	0.0001 - 0.001
Au	0.00008	-
B	0.005	-
Ba	0.029	0.016 - 0.053
Be	< 0.0002	-
Bi	0.001	< 0.001 - 0.001
Ca	2.66	0.2 - 6.3
Cd	< 0.001	< 0.01 - < 0.001
Ce	0.003	0.003 - 0.0009
Co	0.001	-
Cr	0.019	0.001 - 0.019
Cu	0.64	0.24 - 0.91
Fe	5.6	2.11 - 7.11
K	1.56	-
La	0.0006	0.0003 - 0.0006
Mg	2.20	0.5 - 2.14
Mn	0.04	0.00 - 0.05
Mo	0.005	< 0.0005 - 0.005
Na	1.34	0.5 - < 2.0
Nd	0.0008	0.0002 - 0.0007
Ni	< 0.001	< 0.001 - 0.003
O	38.76	-
P	0.05	0.02 - < 0.2
Pb	0.002	0.0001 - 0.006
Rb	0.005	0.002 - 0.005
S	1.00	0.005 - 1.34
Sb	0.005	-
Se	< 0.001	-
Si	27.32	-
Sn	< 0.002	-
Sr	0.09	0.028 - 0.092
Ti	0.47	0.05 - 0.47
Te	< 0.001	-
V	0.029	0.01 - 0.02
W	< 0.05	-
Y	0.003	0.0004 - 0.003
Zn	0.01	-
Zr	0.01	0.007 - 0.01

TABLE 3

ELEMENTS IN BOUGAINVILLE WASTE DUMP LEACH LIQUOR

Element	Inflow (natural stream) mg l^{-1} (I)	Outflow mg l^{-1} (O)	$\frac{O}{I}$
Ag	< 0.00005	< 0.0049	-
Al	0.05	4.9	98
As	< 0.05	< 4.9	-
B	0.005	0.98	196
Ba	< 0.05	< 4.9	-
Be	< 0.00009	< 0.01	-
Bi	< 0.00047	< 0.05	-
C	3	2	1
C (inorg)	< 1	< 1	-
Ca	6	200	33
Cd	< 0.0046	< 0.49	-
Co	< 0.0009	2.45	> 2700
Cr	< 0.0001	0.04	> 400
Cu	0.0	487.0	> 487
Fe	< 0.1	0.3	> 3
K	2	26	13
Mg	2	310	155
Mo	0.0005	0.08	160
Mn	0.05	49.0	980
N	0.3	0.3	1
Na	0.09	1.47	16
Ni	0.02	0.1	5
P	< 0.09	< 9.8	-
PO_4^{3-}	< 0.1	< 0.1	-
Pb	< 0.0005	< 0.05	-
Sb	< 0.005	< 0.49	-
Si	2.89	71.1	25
SO_4^{2-}	37.0	2721.0	73
Sn	< 0.0009	< 0.01	-
Ti	0.002	< 0.01	-
V	< 0.0002	< 0.02	-
W	< 0.02	< 2.45	-
Zn	< 0.02	4.9	> 245
Zr	< 0.005	< 0.49	-

The composition of these copper porphyry ores suggested they should be amenable to bacterial leaching. Only minor amounts of known microbial inhibitors (e.g., Ag, Mo) were present, and acid-consuming components did not predominate. However, frequent deposits of iron oxides and hydroxides and unusually low levels of leachate iron indicated that sulfidic reaction sites might eventually become inaccessible, due to iron precipitation.

Leaching Column Performance

Table 4 gives the performance data for columns 1 - 7; leaching times ranged from 1 - 3 yr for copper recoveries of 4 - 44%. Variations reflect differences in the ore, where higher grade, smaller particle size and increased porosity resulted in greater recoveries. Overall recoveries of copper were highest for columns 1 and 2, containing the brecciated, high grade, quartz-feldspar-porphyry, and least for the low grade mixture (column 3) while the compact-structured high grade ore (columns 4 - 7) gave intermediate recoveries.

TABLE 4

LEACHING COLUMN PERFORMANCE

Column No.	Period of Leaching (days)	Flow Rate 1 day^{-1}	Cu Recovery (%)	Fe Recovery (%)	Ratio Cu / Fe
1	508	2.5 ± 0.9	27	6	1.7
2	512	2.4 ± 1.0	44	8	2.3
3	1158	1.5 ± 1.0	28	0	-
4	386	3.1 ± 0.9	15	3	1.8
5	387	2.9 ± 0.8	6	2	1.2
6	386	2.9 ± 0.8	4	3	0.6
7	449	2.7 ± 1.6	12	5	0.9

Although there was more iron than copper in the ores (3 - 150X), recovery of iron in solution was low. Paucity of soluble iron resulted from acutely pH-sensitive precipitation reactions initiated by gangue acid consumption. There was a continual rise in pH as a leachant progressed down the ore column, analogous to the substitution pattern found in linear ion-exchange systems. In the extreme case (column 3), the partially weathered low grade ore, no iron showed up in effluents after three years irrigation with 1,200 l of pH 2 H_2SO_4. The familiar rust-brown iron precipitates were noted throughout all ore charges.

The recovery ratio, Cu/Fe, by weight, decreased with increasing particle size, (c.f., column 1 with 2; 4 with 5 and 6). This was due to crushed ore fragmenting preferentially along copper rich boundaries whereas iron inclusions (e.g., magnetite) tended to be evenly disseminated.

Fig. 1 compares the percentage copper recovery of columns 2, 3 and 4 (shipments 1975 and 1976, respectively), each of which contained an approximately common particle size. Recovery clearly depended upon grade and porosity.

Delays before copper release were due to differences in the amount of acid required to saturate surface gangue material (irrigation of low grade ore with water alone led to an effluent pH > 8). Porosity and degree of weathering govern *initial* acid usage. In this non-productive stage of leaching, the gangue took up 0.13, 1.47 and 1.94 g H_2SO_4 kg^{-1} ore for columns 4, 2 and 3, respectively.

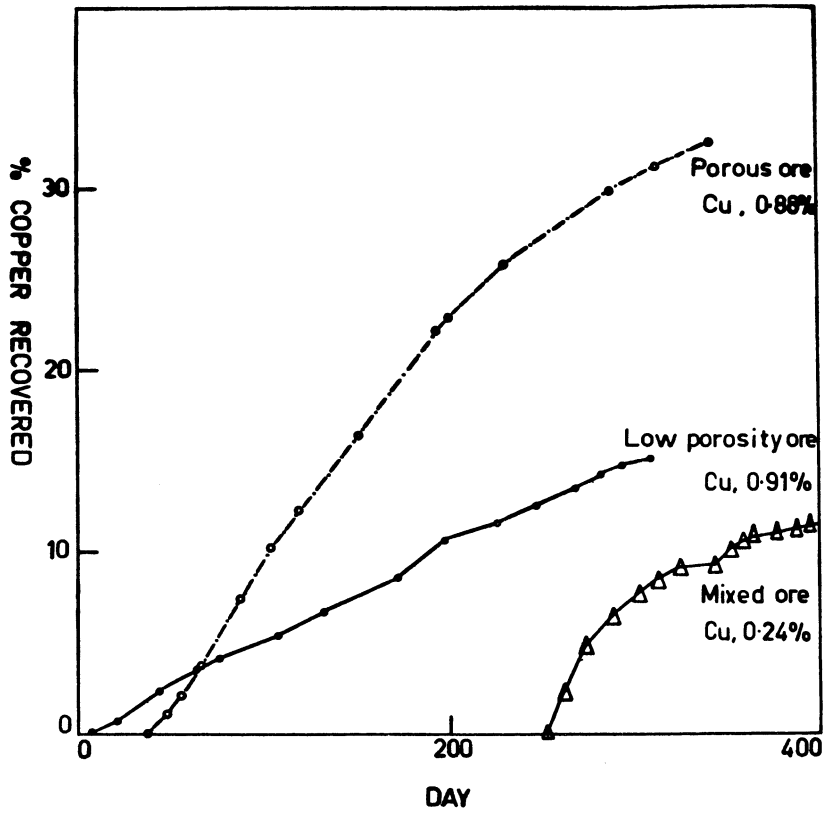

Figure 1: Recovery of copper from column leaching of Bougainville ore. Porous ore in Column 2. Low porosity ore in Column 4. Mixed ore in Column 3.

Copper Release Rates and Acid Consumption

Table 5 gives the copper leach and acid use rates for different ore loadings (rock types and sizes), calculated over comparable periods, for the same weight of ore.

The percentage acid used depended upon flow rate and surface area (particle size). Comparison of columns 1 and 2 or 4, 5 and 6 shows that while acid use was quantitative for th smaller particle sizes, at the chosen flow rate, the larger particle columns could be operated more economically at slower flow rates. However, there was only a slight difference in acid usages between the larger particle sizes (e.g. range ½" - 1'")(cf. 2" - 3", columns 5 and 6).

While there were significant improvements in leach rate for smaller particle sizes, there was surprisingly little extra acid used for the greater surface area exposed. In terms of acid use, copper yield was approximately 1.7 times greater for a reduction of 5 - 10 times in particle size and up to 3 times greater for a fifty-fold reduction. A comparison was made, prior to initiation of irrigation, of the average particle weight in each column. These were 32.8 ± 14.8, 3.8 ± 3.3, 5.2 ± 2.0, 52.2 ± 28 and 260.9 ± 62.9 (grams, average) for columns 1, 2, 4, 5 and 6, respectively. As acid use did not depend significantly on surface area (i.e. particle size), in the size range tested, there is an advantage in reducing the particle size by a factor of at least 5, provided an appropriate flow rate is used.

TABLE 5

COPPER RELEASE RATES AND ACID CONSUMPTION

Column	Period[a] (day)	Acid use[b] (%)	Cu release g Mg^{-1}	Acid use g Mg^{-1} day^{-1}	Cu/H_2SO_4 yield g kg^{-1}
1	102-200	84	6.9	31	224
2	102-200	100	11.4	29	389
3	707-796	99	0.7	22	34
4	84-150	67	4.1	48	87
5	84-150	40	1.5	31	48
6	84-150	42	1.2	40	29
7	102-164	67	3.5	69	51
8	120-193	49	1.6	52	32

[a] "Steady release phase" (i.e. where copper recovery was linear with time)

[b] $\frac{\text{Acid consumed}}{\text{Input acid}} \times 100$

Effect of "Rest" Periods

The effect of interrupted irrigation or "rest" periods was tested on the cumulative copper release from three leaching columns, each containing a different particle size.

While the "rest" periods caused immediate variations in copper concentration (tenor) upon re-irrigation, there were no obvious net gains or losses over an extended collection time. In other words, the longer term cumulative rate of copper recovery was approximately the same before as after the "rest period. Table 6 sets out the changes (ratios) in copper and iron production (flow rate x metal concentration) and bacterial numbers, before and after "rests". There was a tendency for more copper to be produced after a "rest". However, while there was always an increased tenor with the smaller particle size, the ratios for the larger particles were variable. Inconsistencies were not understood but could be the result of drying effects, which were more pronounced for the bigger particle sizes. It should be noted that no attempt was made to standardise the rate of drying (i.e. humidity control).

Soluble iron levels were higher for all particle sizes after the shortest (9 day) "rest", but were always less for the 26 and 42 day periods. Decrease in iron solubility after the longer "rests" indicates that drying of iron precipitates is partly irreversible. Re-establishment of pre-rest Fe/Cu release ratios took between 40 and 130 days after a 42 day "rest".

Bacterial numbers increased after shorter "rest" periods for smaller particle sizes. The changes in bacterial counts could not be accounted for in terms of a concentration effect by the drying process. Evidently, compared with usual irrigation rates, a more favourable moisture level for growth was encountered during shorter "rests"

This important interaction of microbial and physico-chemical phenomena, due to "rest" periods, requires further study. For example, with the present ore it seems feasible to concentrate copper release and stimulate bacterial growth with a short "rest" period. Furthermore, some attention should be given to the possibility of limiting deleterious levels of soluble iron by forming dry, yet porous, reversible precipitates of iron.

TABLE 6

EFFECT OF "REST" PERIOD ON COLUMN LEACHING

Column	Average ore particle size (mm)	Average ore particle size (inches)	"Rest" Period (days)	Production Ratio[a] Cu	Production Ratio[a] Fe	Bacterial Ratio[b]
4	5-10	3/16-3/8	9	2.1	1.2	5.8
5	13-38	½-1½		2.6	2.9	4.9
6	51-76	2-3		0.8	1.6	2.5
4	"	"	26	1.3	0.6	1.5
5	"	"		0.9	0.4	1.0
6	"	"		2.0	0.5	0.4
4	"	"	42	3.7	0.5	0.002
5	"	"		2.4	0.6	1.5
6	"	"		1.6	0.6	0.002

[a] Ratio, $\frac{\text{Flow rate x metal ion concentration AFTER "rest"}}{\text{" " " " " BEFORE "}}$

[b] Ratio, $\frac{\text{Viable } \textit{Thiobacillus ferrooxidans} \text{ AFTER "rest"}}{\text{" " " BEFORE "}}$

Effect of Recirculated Leach Liquors

Release of copper and iron was erratic during recirculation but steady in the once-through phases. Soluble iron built up faster than copper during recirculation, whereas release rates of copper and iron were similar for once-through modes. The rate of acid use was greatest for the high flow rate once-through pass, but there was little difference in the remaining irrigation modes.

Table 7 sets out data calculated from the "steady release phase" for the three irrigation modes. Most copper was recovered with the rapid once-through passage of liquors, but acid usage was comparatively high. The recirculated system proved best in terms of copper recovered per acid use.

Bacterial numbers of *Thiobacillus ferrooxidans*, increased remarkably during recirculation from $0.9 \pm 1.1 \times 10^5$ cells ml^{-1} at the standard irrigation rate (once-through) to $1.5 \pm 0.5 \times 10^6$ cells ml^{-1}. Sulfur-oxidizing bacteria decreased 200-fold during the recycle. The advantage of recycling leach liquors may accrue from the sparing effect of bacterial oxidation on acid consumption.

Effect of Increased Acidity

Table 8 (Column 2) shows that by day 350 copper production was declining. Investigation o ore surfaces showed an uneven coating of iron precipitates. The surfaces were mainly loos and friable and when scratched, revealed inclusions of chalcopyrite. The inflow pH was dropped to pH 1.5 (by fourfold increase of H_2SO_4 concentration) at day 348, in an attempt to restore a steady production rate, by removing the occluding precipitates. Fig. 2 shows the effect of changing inflow acidity from pH 2.0 to pH 1.5 on the effluent iron, iron-oxidizing bacteria and copper production.

TABLE 7

EFFECT OF RECIRCULATION (COLUMN 8)
COPPER RELEASE RATES AND ACID CONSUMPTION

Irrigation mode	Cu release g Mg^{-1} day^{-1}	Acid use g Mg^{-1} day^{-1}	Cu/H_2SO_4 yield g kg^{-1}
Recirculated	2.2	37	58
Once-through (fast)	7.3	196	37
Once-through (slow)	1.6	52	32

TABLE 8

AVERAGE RATES OF COPPER RELEASE[a]
FROM COLUMN 2

Period (days)	pH		Average copper release rate g Mg^{-1} day^{-1}
	IN	OUT	
0 - 40	2.0	6.1 - 5.4	0.6
102 - 200	2.0	4.6 - 4.1	11.4
260 - 348	2.0	4.1 - 3.7	4.8
348 - 382	1.5	3.1 - 2.4	10.1
400 - 480	1.5	2.4 - 2.0	6.0
480 - 512	1.5	2.0 - 2.0	3.1
669 - 777	1.5	2.0 - 1.8	1.1

[a] Calculated from accumulated copper recovery where net release was linear with time.

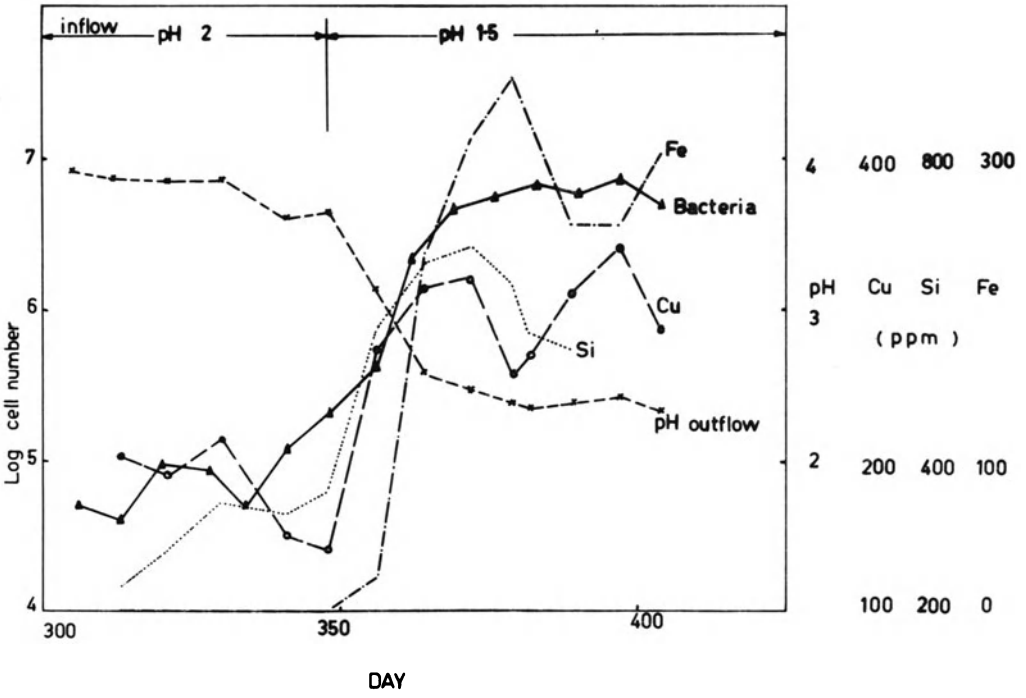

Figure 2: The effect of increased irrigation acidity on column leaching of Bougainville ore (Column 2).

Higher acidity resulted in the release of built-up surface-bound iron precipitates as soluble ferric salts. The numbers of *Thiobacillus ferrooxidans* in the effluent increased up to one hundred-fold. Copper production increased temporarily but decreased after sixteen days. Table 9 sets out comparative data for pH 2.0 and pH 1.5 H_2SO_4 irrigation.

TABLE 9

EFFECT OF INCREASED ACIDITY[a] IN COLUMN 2

Component	pH 2.0	pH 1.5
	Average release rate g day^{-1}	
Cu	0.19	0.29
Fe	0.0	0.57
Si	0.42	1.50
Total Cu release g kg^{-1} H_2SO_4	210	26

[a]Compared over a 62 day period with equivalent flow rates.

Continuous application at pH 1.5 would not be justified on an industrial scale because of acid wastage (ca. 8 times H_2SO_4 usage for the same copper recovered). However, a brief irrigation at higher acidity would partially remove rate limiting iron precipitates and inaccessible associated copper oxides.

Increase in soluble iron after 380 days (Fig. 2) was due to the greater solubility of fresh iron inclusions (e.g. magnetite) in freshly exposed surfaces rather than to continued dissolution of accumulated precipitates. Effluent ferric ion and bacterial numbers remained high after 150 days at pH 1.5.

Following the release of absorbed goethite plus copper oxides the average rate of copper production fell (Table 8) even though continued iron precipitation was no longer evidnet. The diminishing return of copper with high acid indicated an inhibition of bacterial sulfide oxidation at lower pH.

CONCLUSION

One of the more interesting results of these experiments was the decline in copper productivity at pH 1.5. Irrigation at this acidity led to an increase in iron and iron-oxidizing bacteria in the column effluent. This indicates that biodegradation of Bougainville chalcopyrite does not proceed in the main by an indirect ferric-leach mechanism. Previous workers have also shown that ferric leaching of chalcopyrite is only moderately successful, even under vigorous (non-biological) conditions (Sullivan, 1933; Woodcock, 1967).

Although more acidic irrigation (pH 1.5) removed most of the iron precipitates from the ore surfaces, and even though abundant viable leaching bacteria were present, the copper release rate did not increase to former levels. It may be inferred from this that higher acidity inhibits bacterial oxidation of chalcopyrite. The effect of a return to pH 2 irrigation is now under investigation.

In long-term leaching operations, the formation of iron precipitates has to be reduced or prevented to ensure access of leaching bacteria to chalcopyrite surfaces. Standard once-through (trickle) irrigation with dilute sulfuric acid inevitably leads to areas of undersupply of acid. Subsequent rises in pH, due to gangue acid consumption, triggers precipitation of ferric oxides and hydroxides. Furthermore, these precipitates have an affinity for divalent copper ion. It is suggested that modified acid irrigation would avoid rate-limiting iron precipitate formation. Rapid recycle irrigation has been tested on a small-scale and significantly reduced iron precipitation, in effect, by oversupplying pH 2 H_2SO_4. This technique also ensures maintenance of pH optima for bacterial oxidation and circumvents deleterious co-precipitation of soluble copper. In a large-scale recycle system, introduction of an appropriate "rest" phase would be essential to maintain aerobic conditions and to control soluble iron levels.

It has not been possible here to induce accelerated endogenous microbial copper leach rates. Inorganic phosphorus and nitrogen additions and heavy inoculation with leaching organisms (raised on Bougainville sulfides) did not improve copper release in well-established leaching columns. However, the influence of co-existing heterotrophic microbial growth on the leaching of Bougainville ore has shown some promise and is under investigation.

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AN EFFICIENT METHOD FOR THE PRODUCTION OF LEACHING MEDIUM

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The initial stages of leaching in Hungary may be traced back over a period of 500 yr (Podányi, 1977a; 1977b), whereas biohydrometallurgic research has a background of only one decade. The introduction of large scale in situ leaching experiments intensified with bacteria, to be realized in 1979-1980 may be considered as an important stage of development (Lakatos, 1976a; 1976b; Várhegyi *et al.*, 1973).

In the course of many decades of mining, copper ore resources in the Mátra region (Northern Hungary) have been considerably exhausted, as a result of which the metal content of the ores (mainly enargite, partly tennantite and lusonite) is so low that traditional mining techniques are no longer economical. Several years of researches have been devoted, therefore, to the development of a leaching (biohydrometallurgical) method, the results of which we wish to present in this communication.

The most feasible leaching medium is considered to be slack mine water, with some *Thiobacillus ferrooxidans* content, accumulated in old drifts and sumps. This medium, however, does not facilitate efficient leaching, owing to the high ratio of dissolved ferrous/ferric iron concentrations and the low redox potential, in conjunction with the low cell concentration of *T.ferrooxidans*. Some characteristic features of mine waters are presented in Table 1.

TABLE 1

CHARACTERISTIC PARAMETERS OF WATERS OF THE OLD MINE OF RECSK

Site of sampling	Fe^{2+} (g l^{-1})	Fe^{3+} (g l^{-1})	Fe^{2+}/Fe^{3+} ratio	pH value	Time of sampling
Pit 4. Level 1. running water	7.70	2.79	2.80	1.4	1.6.1977
Pit 4. Level II.	1.06	0.28	3.80	2.1	1.6.1977
sump	5.97	0.28	21.32	2.2	15.9.1977
Slack water of	4.47	0.17	26.30	2.3	1.6.1977
Pit 4/A and	5.97	0.11	54.27	2.1	15.9.1977
side-drifts					

In order to show that mine water of such composition is not suitable for leaching and to verify the mechanism of the leaching process, we carried out the following experiments.

The dissolution of laboratory-produced copper sulfide has been examined in media of varying ferric iron content, with occasional addition of *T.ferrooxidans* (Table 2). The copper sulfide sample was placed in the Beck-nutrient solution (Report, 1975) at pH = 1.9, with the following ferric iron concentrations: in cases 1 and 2, C g Fe^{3+} l^{-1}; for 3 and 4, 0.1 g Fe^{3+} l^{-1}; for 5 and 6, 0.5 g Fe^{3+} l^{-1}; and for 7 and 8, 2.0 g Fe^{3+} l^{-1}. The samples

with even serial numbers were inoculated with *T.ferrooxidans* biomass isolated from the afore-mentioned ore resources and activated in laboratory experiments so as to reach an initial cell number of 2 x 10^8 cells cm^{-3}.

The experimental results clearly show that, although *T.ferrooxidans* cells are capable of direct oxidation of copper sulfide (CuS), good results can be achieved only when the solution contains also dissolved iron. Copper sulfide is quickly oxidized by ferric ions and rapid re-oxidation of the ferrous ions formed is performed by *T.ferrooxidans*.

TABLE 2

CuS DISSOLUTION IN SOLUTION
OF VARIOUS Fe CONCENTRATIONS

Sample No.	Cu^{2+} ion concentration (g l^{-1}) from the outset of experiments									
	day 0	day 1	day 2	day 5	day 7	day 9	day 12	day 15	day 20	day 30
1[a]	0.114	0.178	0.241	0.356	0.407	0.418	0.432	0.438	0.444	0.444
2	0.114	0.184	0.273	0.413	0.470	0.489	0.502	0.509	0.514	0.514
3[a]	0.114	0.184	0.248	0.368	0.419	0.432	0.451	0.457	0.463	0.463
4	0.114	0.191	0.280	0.470	0.552	0.584	0.610	0.622	0.635	0.635
5[a]	0.114	0.202	0.280	0.406	0.463	0.476	0.495	0.508	0.520	0.527
6	0.114	0.210	0.292	0.476	0.577	0.628	0.711	0.793	0.899	1.022
7[a]	0.114	0.266	0.368	0.546	0.616	0.641	0.673	0.692	0.723	0.736
8	0.114	0.280	0.444	0.876	1.14	1.33	1.61	1.86	2.09	2.17

[a] sterile control

Initial ferric ion content of leaching medium

a. in samples 3 and 4 0.1 g l^{-1}

b. in samples 5 and 6 0.5 g l^{-1}

c. in samples 7 and 8 2.0 g l^{-1}

samples 1 and 2 are free of iron

Table 3 shows the change in ferric iron content of the samples in the course of experimen Rapid oxidation of CuS took place only in case of sufficient ferric iron concentration, ensured by means of *T.ferrooxidans*; no adequate oxidation rate could be attained either in case of a bacterium-free medium with high ferrous iron content or with an iron-free medium with bacterium content.

In these experiments our aim has been to obtain from mine water in a continuous system, l volume and high oxidation capacity, a solution of appropriate composition, with the follo ing characteristic features: high cell number of *T.ferrooxidans* and almost completely oxidized iron content. In preliminary examinations, batch cultivation parameters of appr imately 30 samples obtained from various resources have been determined and the four strains with highest oxidation and rapid multiplication capacities have been selected. Maximum cell numbers and highest multiplication rate coefficients of the four strains are shown in Table 4.

TABLE 3

Fe^{3+}-ION CONTENT OF SAMPLES IN THE COURSE OF CuS DISSOLUTION

Sample No.	Fe^{3+} concentration (g l^{-1}) from the outset of experiments									
	day 0	day 1	day 2	day 5	day 7	day 9	day 12	day 15	day 20	day 30
1[a]	FREE OF IRON									
2	FREE OF IRON									
3[a]	0.106	0.084	0.067	0.045	0.034	0.028	0.022	0.017	0.006	0.000
4	0.106	0.084	0.078	0.078	0.056	0.045	0.034	0.022	0.005	0.000
5[a]	0.497	0.313	0.218	0.140	0.090	0.056	0.039	0.022	0.006	0.006
6	0.497	0.347	0.352	0.413	0.447	0.470	0.481	0.487	0.492	0.492
7[a]	2.00	1.33	1.04	0.592	0.447	0.318	0.156	0.056	0.028	0.011
8	2.00	1.40	1.66	1.85	1.92	1.95	1.97	1.98	1.985	1.99

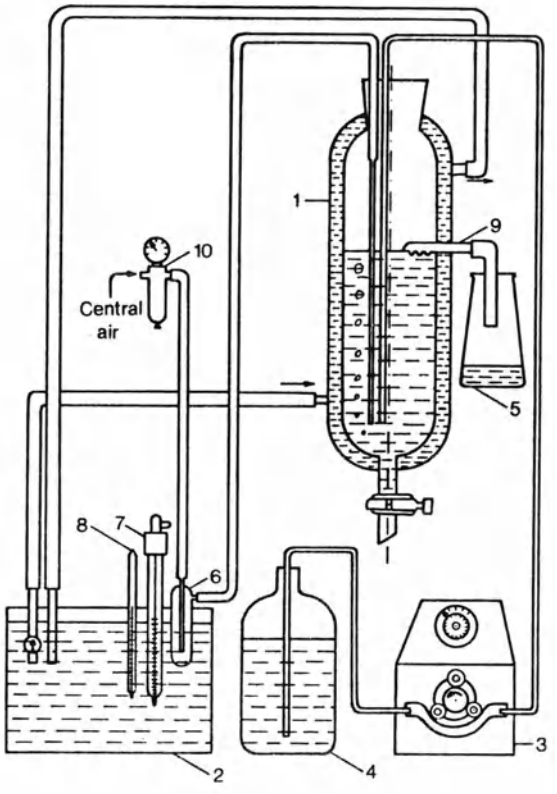
[a] sterile control

TABLE 4

COMPARISON OF KINETIC CONSTANTS OF STRAINS STUDIED

Name	k (h^{-1})	M ($\times 10^{-3}$ mm^{-3})
Parádsasvár PS-1	0.12	340
Soviet strain SU-I	0.12	290
Soviet strain SU-II	0.15	350
Strain received from ÓÁÉ FK-2	0.11	285

After selection of the most effective *T.ferrooxidans* strains by this method, we started continuous experiments in the laboratory-scale fermentor presented in Fig. 1. The useful volume of the double-wall glass fermentor could be varied between 100 and 1200 ml and optimum temperature (31^{o}) was maintained by use of a thermostat. Aeration of the solution was ensured through a stabilized valve and perforated glass pipe. The Beck-medium containing 60 g $FeSO_4.7H_2O$ l^{-1} was fed into the system by means of a peristaltic pump and the oxidized solution was allowed to leave the system through an overflow pipe.



1 Double-wall fermentor

2 Thermostat

3 Peristaltic pump

4 Nutrient solution drum

5 Product reservoir

6 Bubbler /Air saturation/

7 Contact thermometer

8 Control thermometer

9 Overflow pipe

10 Valve of stabilizer controlling air flow

Figure 1: Flow chart of continuous fermentor

Characteristic parameters of the fermentation process, measured during the continuous experiments, are depicted in Table 5. With this equipment we obtained continuously a solution in which almost all iron was in the ferric state (E>700 mV), with a high number of active cells and with a minimum amount of iron hydrate precipitation. In our experience, when the microbial culture is in the stage of logarithmic multiplication, the formation of precipitates is greatly hindered, probably due to the formation of complexing metabolic by-products. At this stage the fermenting solution has a rather characteristic smell. The main parameters of the well-controlled system are given in Table 6. Comparison with the literature data (MacDonald and Clark, 1970; Egamberdieva *et al.*, 1976; French patent 2,278,631; Soviet patent 518,435; English patent 1,409,486) shows very good results (dilution rate, D_{max} = 0.11 - 0.12, iron oxidation rate: 0.9 - 1.2 g Fe $l^{-1}h^{-1}$).

TABLE 5

EXPERIMENTAL RESULTS OF CONTINUOUS CULTIVATIONS

T.ferro-oxidans strain	D[a] (h^{-1})	Fe oxidation rate (g Fe^{2+} $l^{-1}h^{-1}$)	Fe^{2+} content (g l^{-1}) of outlet solution	Equilibrium cell number (x 10^{-3} mm^{-3})
PS-1	0.037	-	0	-
	0.062	-	0	-
	0.088	1.033	0.17	320
	0.096	0.962	1.56	264
	0.116	0.205	6.5	115
	0.125		wash-out	
SU-1	0.042	-	0	-
	0.061	-	0	-
	0.080	1.090	0.17	246
	0.094	0.933	1.5	233
			wash-out	
SU-II	0.039	-	0	-
	0.058	-	0	-
	0.084	-	0	-
	0.098	1.192	0.3	267
	0.131		wash-out	
FK-2	0.037	-	0	-
	0.061	-	0	-
	0.081	0.935	0.15	296
	0.088	0.932	1.1	260
	0.101	0.602	5.7	146
	0.110		wash-out	

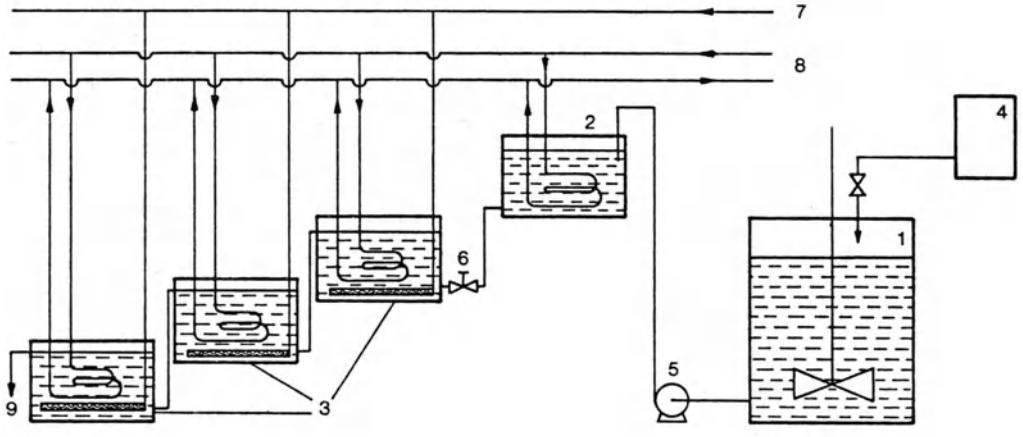
[a] Dilution rate

TABLE 6

COMPARISON OF LITERATURE DATA
AND OUR OWN RESULTS

Authors	D[a] (h^{-1})	Fe oxidation rate (g Fe $l^{-1}h^{-1}$)	Max. cell number (x 10^{-3} mm^{-3})	Nutrient solution Fe^{2+} content (g l^{-1})	Note
ALUTERV-FKI-BME lab. size/our experiment/	0.11 - 0.12	0.9 - 1.2	250	12	1 stage
Mac Donald and Clark (1970)	0.06	0.168	110 - 130	2.9	1 stage
	0.1	0.255	110 - 130	2.9	1 stage
	0.12	0.275	110 - 130	2.9	1 stage
	1.14	0.238	110 - 130	2.9	1 stage
Egamberdieva *et al.* (1976)	0.03	0.26	50	9.0	1 stage
	0.03	0.43	200	15.0	1 stage
	0.06	0.25	70	9.0	1 stage
	0.06	0.36	-	15.0	1 stage
French patent No.: 2.278.631	0.3	2.0	-	8.0	1 stage
Soviet patent No.: 518.465	-	1.0 - 1.2	10 - 30	5 - 25	3 stage

[a] Dilution rate



1 Drum for the preparation and storing of nutrient solution
2 Nutrient solution feedtank
3 Fermentors
4 Sulphuric acid feedtank
5 Feed pump
6 Feeding valve
7 Central air
8 Inlet – and outlet of warm water
9 Outlet for leaching

Figure 2: Slow-chart of pilot-plant scale system.

On the basis of calculations performed with kinetic constants (Kono and Asai, 1969) derived from the afore-mentioned results, a three-stage large scale laboratory fermentor (3 x 400 l) and a pilot-plant scale fermentor (3 x 3000 l) system have been developed and tried out, considering aspects of utilization. The experiments were completed with good results and reproduction of the data, although somewhat below the level of laboratory-scale results, may be considered satisfactory. The flow chart of the pilot-plant scale continuous fermentor operating with biocatalysis is presented in Fig. 2. The system is suitable for the production of leaching medium for the large-scale in situ leaching experiments described here. Parallel to this, laboratory-scale percolation experiments were carried out with the afore-mentioned leaching medium and enargite ore. The results of the experiments carried out over a period of several hundred days showed that with a ferric sulfate solution containing bacteria, 4% Cu/100 day can be attained while sterile control experiments resulted a dissolution rate of 0.4% Cu/100 day, which unambiguously proves the advantages of the leaching medium produced by our system.

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BACTERIAL LEACHING OF MANGANESE ORES

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The most important ores of manganese are oxides, although they may also include manganous carbonate (rhodochrosite) and silicate (rhodonite). Some of the oxide minerals of manganese are shown in Table 1. Since the manganese with oxidation states of +3 and +4 in these minerals is insoluble, its hydrometallurgical extraction requires its reduction to the divalent state. Industrially, this can generally be achieved by roasting with a suitable reductant such as gas, oil, or solid fuel, CO and H_2 being other examples of effective reducing agents (Sully, 1955). It can also be achieved by treatment with strong reductants such as SO_2 or acid ferrous sulfate which often involves a heating step (Henn *et al.*, 1968).

TABLE 1

OXIDES OF MANGANESE

birnessite	δMnO_2
hausmannite	Mn_3O_4
manganite	$Mn_2O_3.H_2O$ or - MnOOH
manganosit	MnO
pyrochroite	$Mn(OH)_2$
pyrolusite	βMnO_2
todorokite	$(Mn^{2+},Mg^{2+},Ba^{2+},Ca^{2+},K^{+},Na^{+})_2Mn_5{}^{4+}O_{12}.3H_2O$

Manganese oxides can, furthermore, be reduced in an aqueous system under mild conditions, namely at ambient temperature and near neutral, i.e., weakly acid, pH with appropriate reductants, provided a suitable catalyst is furnished. Such catalysts may be appropriate enzymes of MnO_2-reducing bacteria or the bacteria themselves. Some bacteria and fungi may also reduce manganese oxides non-enzymically. Table 2 lists microorganisms from freshwater and marine environments and from soil which have been reported to reduce higher oxides of manganese. It should be pointed out that it has so far not been clearly established whether these organisms attack only Mn(IV) oxides or also Mn(III) oxides.

Enzymically catalyzed reduction of Mn(IV) oxide can be summarized by the following reactions in which glucose is the electron donor and MnO_2 the electron acceptor (Trimble and Ehrlich, 1968),

$$\text{glucose} \xrightarrow{\text{bacteria}} n\,e^- + n\,H^+ + \text{end products}, \quad (1)$$

$$0.5n\,MnO_2 + n\,e^- + n\,H^+ \xrightarrow[\text{or uninduced cells} + Fe(CN)_6{}^{3-}]{\text{induced cells}} 0.5\,mn(OH)_2, \quad (2)$$

$$0.5n\,Mn(OH)_2 + n\,H^+ \longrightarrow 0.5n\,Mn^{2+} + n\,H_2O. \quad (3)$$

These reactions were formulated to describe the action of marine bacteria, but they shoul also apply to soil and freshwater bacteria. Compounds other than glucose, mainly other sugars, may be substituted for glucose as electron donors, but a systematic study has not been undertaken so far.

TABLE 2

MANGANESE OXIDE REDUCERS

Source	Name of organism	Reference
Fresh water and sediment:	*Bacillus cereus*	Troshanov, 1968
	B.centrosporus	Troshanov, 1968
	B.circulans	Troshanov, 1968
	B.filaris	Troshanov, 1968
	B.macerans	Troshanov, 1967
	B.mesentericus	Troshanov, 1968
	B.mycoides	Troshanov, 1968
	B.subtilis	Troshanov, 1967
	Clostridium felsineum	Troshanov, 1967
	C.polymyxa	Troshanov, 1968
	Enterobacter aerogenes	Troshanov, 1967
	Escherichia coli	Tortoriello, 1971
	Micrococcus albus	Troshanov, 1968
	M.radiatus	Troshanov, 1967
	Pseudomonas fluorescens	Tortoriello, 1971
	P.liquefaciens	Troshanov, 1967
	P.radiobacter	Troshanov, 1967
	Proteus vulgaris	Troshanov, 1967
Oceans and marine sediments:	*Bacillus* 29	Trimble and Ehrlich, 196
	Bacillus GJ33	Ehrlich *et al.*, 1973
	Coccus 32	Trimble and Ehrlich, 196
	Gram neg. rod BIII 32	Ehrlich, 1974
	Other gram neg. rods	Ehrlich, unpublished dat
Soils:	*Arthrobacter* strain B	Bromfield and David, 197
	Bacillus spp.	Perkins and Novielli, 19
	fungi	Ehrlich, unpublished dat

The enzymic steps involved in the electron transfer to nO_2 seems to vary with different bacteria. In the marine *Bacillus* 29, the pathway may be summarized by the scheme of Ghiorse and Ehrlich (1976). In this organism, as in all active marine bacteria which we have so far examined, part of the pathway is inducible. In uninduced cells, the inducible components may be replaced by a trace of ferriferrocyanide as electron shuttle. Curiously even though *Bacillus* 29 contains types -b, -c, and -a cytochromes, inhibitor studies (Table 3) with antimycin A and 2-n-nonyl-4-hydroxyquinoline-N-oxide, cyanide and azide have indicated that it employs at most cytochrome *c* in this reduction. Similar inhibitor studies (Table 3) with cultures BIII 32 and BIII41, which contain types -b, -c, and -o cytochromes (Fig. 1), indicate that they employ types -b and -c cytochromes when they redu

MnO_2. The probable electron transport pathway to MnO_2 in induced strains of cultures BIII 32 and BIII 41 are shown in Fig. 2. As in *Bacillus* 29, a branched pathway is postulated to exist in cultures BIII 32 and BIII 41 leading to oxygen via cytochrome *o* on the one hand, and via another metalloenzyme to MnO_2 on the other. However, unlike with *Bacillus* 29, MnO_2 reduction by these cultures is not stimulated by 10 mM azide. Indeed, MnO_2 reduction in culture BIII 32 is not sensitive to azide at 0.08 mM while in culture BIII 41 it is. Culture BIII 32 seems more sensitive to KCN than culture BIII 41.

Non-enzymic MnO_2 reduction may be illustrated by the following two reactions.

$$MnO_2 + HCOOH + 2H^+ = Mn^{2+} + 2H_2O + CO_2. \quad (4)$$

$$MnO_2 + HOOCCOOH + 2H^+ = Mn^{2+} + 2H_2O + 2CO_2. \quad (5)$$

Formic acid (HCOOH) is a product of glucose metabolism of enteric bacteria such as *Escherichia coli*, *Enterobacter aerogenes*, and *Aeromonas punctata*. Indeed, *E.coli* and *E.aerogenes* have been shown to reduce MnO_2 at least partly non-enzymically by the formic acid they produce (Tortoriello, 1971). Oxalic acid (HOOOCCOOH) is formed as an end-product of glucose metabolism by a number of fungi, for example *Aspergillus niger* and *Penicillium* spp. Both formic and oxalic acid are effective reductants of MnO_2 in acid solution.

TABLE 3

INHIBITION OF MnO_2 REDUCTION OF INDUCED BACTERIA

Inhibitor[a]	Bacillus 29[b]	Culture BIII 32	Culture BIII 41
atabrine	73	0; 0	27.2; 50
dicumarol	63[c]	0; 0	0
antimycin A	0[c]	100; 100	72.7; 64.3
NOQNO	0[c]	100; 100	82.4
0.1 M NaN_3	stimulated	--	--
0.08 M NaN_3	--	0	100
0.01 M NaN_3	stimulated	--	--
0.008 M NaN_3	--	0; 0	64; 55
0.1 M KCN	--	100; 100	100; 100
0.01 M KCN	100	41.7; 50	0; 0

[a] Concentration of atabrine: 1 mM for *Bacillus* 29; 0.15 mM for culture BIII 32; and 1.5 mM for culture BIII 41.

Concentration of dicumarol: 0.1 mM for *Bacillus* 29; 0.14 mM for cultures BIII 32 and BIII 41.

Concentration of antimycin A: 0.02 μmol mg^{-1} protein for *Bacillus* 29; 0.1 μM for cultures BIII 32 and BIII 41.

Concentration of NOQNO (2-n-nonyl-4-hydroxyquinoline-N-oxide): 0.045 μmol mg^{-1} protein for *Bacillus* 29; 0.54 mM for cultures BIII 32 and BIII 41.

[b] From Ghiorse and Ehrlich (1976).

[c] Tested on cell extracts.

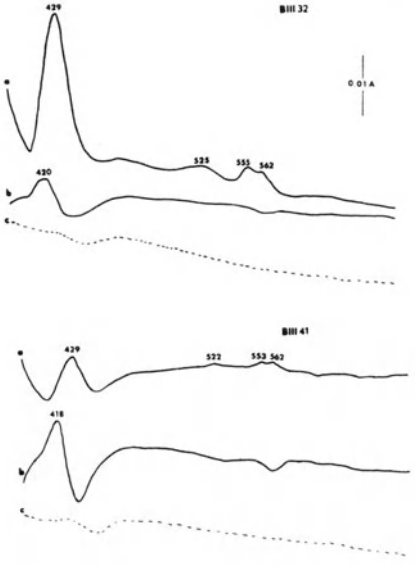

Figure 1: Difference spectra of crude extracts of MnO_2 reducers BIII 32 and BIII 41. For the method of preparation see Arcuri and Ehrlich (1979). (a) dithionite-reduced minus air oxidized spectrum; (b) dithionite-reduced plus CO minus dithionite reduced spectrum; (c) air-oxidized minus air-oxidized spectrum.

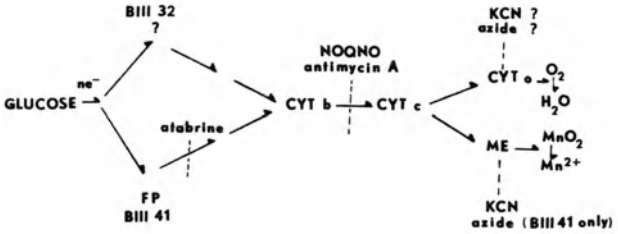

Figure 2: Probable electron transport pathways from glucose to oxygen and MnO_2 in MnO_2 reducers BIII 32 and BIII 41.

While marine bacteria from outside coastal waters have been found so far to reduce MnO_2 only enzymically, soil and freshwater bacteria have been found to reduce it enzymically and/or non-enzymically. Fungi reduce it only non-enzymically. The reason why marine bacteria seem to reduce MnO_2 mainly enzymically has probably to do with the fact that thes organisms do not produce MnO_2-reducing end-products from their energy sources. They are strongly oxidative organisms, which is not the case with many terrestrial organisms. The marine bacteria also exhibit salt requirements for MnO_2 reduction, as shown for *Bacillus* 29 by Ghiorse and Ehrlich (1974).

The only published investigations into commercial type of exploitation of microbial activity in hydrometallurgical extraction of manganese ores are those of Perkins and Novielli (1962) and by Agate and Deshpande (1977). In the first named study, the active organism was a sporeforming rod. It extracted manganese from ores whose mineralogy was unfortunately not described. The ores were low grade, containing from 2 to 30% Mn.

Extraction of manganese with help of the bacteria in a molasses medium at pH 5-6 was 72.7 and 99.9% complete in 60 days, but only 4.1 to 18.5% complete in 10 days, depending on the ore. In the study of Agate and Deshpande, ore with a manganese content of 42 to 44.6% Mn was leached in separate experiments by *Pseudomonas* sp., *Bacillus* sp. and *Arthrobacter* sp. at a pH around 6.5 in five-fold diluted nutrient broth. Using the *Arthrobacter* culture, Mn extraction was 90% complete in 90 days in small batch cultures, 71.3 - 85.2% complete in 14 days in percolation columns, and 78.4% complete in 14 days in a pilot plant test.

In a laboratory experiment, Trimble and Ehrlich (1968) leached crushed ferromanganese nodule with MnO_2-reducing bacteria resident on the nodule in seawater enriched with 0.5% glucose and 0.05% peptone. About 10% of the manganese was solubilized in 16 days related experiments using a pure culture, *Bacillus* GJ 33, Ehrlich *et al.* (1973) showed that cobalt and nickel, and to some extent copper, in ferromanganese nodule substance are solubilized as the manganese oxide is attacked by the bacteria. The iron oxide is not solubilized. Some copper in the nodule is extracted by the peptone in the medium. Compared to non-biological hydrometallurgic extraction of ferromanganese nodules (many processes have been patented), biological extraction is slow. The biological rates so far observed have not been obtained under ideal conditions, and some improvement should be possible. However, even under optimal conditions, biological rates of extraction will never equal chemical rates. In batch processes, there will be a limit to the quantity of nodule substance extractable at one time because of bacterial tolerance limits to manganese, copper, nickel and cobalt (Yang and Ehrlich, 1976; Arcuri and Ehrlich, 1977). Nevertheless, biological extraction may one day become attractive commercially in semi-continuous processes because of lesser energy and reagent requirements, which are major cost factors.

The ability of microorganisms to leach manganese oxides also has a negative aspect. Ferromanganese nodules are of commercial interest mainly for the copper, nickel and cobalt they contain and not for their manganese. Thus, in future industrial exploitation of these nodules, the manganese will be major waste product, there being much more of it than can presently be used. Huge accumulations of manganiferous wastes will pile up. Kaufman (1972) has estimated that from 1 Tg of nodules about 0.96 Tg of solid waste will accumulate of which as much as 0.23 Tg could be manganese if none of the manganese was recovered. If these wastes are not properly disposed on land, they may contribute toxic levels of manganese to soil through microbial leaching. If dumped at sea, they may interfere with life in the photic zone by scavenging trace metals required by phytoplankton, raise turbidity of the water and interfere with photosynthesis, and be attacked by bacterioplankton and raise the concentration of manganese in seawater to levels toxic to other organisms. It is clear that both positive and negative aspects of microbial leaching of manganese ores need to be studied further for their potential commercial impact.

ACKNOWLEDGEMENTS

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THE BACTERIAL LEACHING OF ANTIMONY- AND BISMUTH-BEARING ORES AND THE UTILIZATION OF SEWAGE WATERS

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INTRODUCTION

Microorganisms are widely used in many technical processes. One of the main purposes of this paper is to report some of the changes which occur in mineral ores under the influence of different types of bacteria. Studies on these changes have made it possible to develop methods for the extraction of manganese, uranium, and especially copper, from some ores and other metal-containing products (Karavaiko *et al.*, 1972; Kulebakin, 1978).

The study of the possibilities for the application of bacterial leaching to the treatment of antimony- and bismuth- bearing ores is as yet at an early stage (Batyrbekova, 1973); in the studies reported here, the prospects of bacterial leaching of these ores are examined, together with the use of microorganisms for other mineral beneficiation processes, and the optimal conditions under which they operate. The use of bacteria for the decomposition of hydrosulfide reagents in waste waters is also described.

THE LEACHING OF ANTIMONY-BEARING ORES BY *DESULFOVIBRIO DESULFURICANS*

Sulfate-reducing microorganisms occur widely in nature and according to Sokolova and Karavaiko (1964), the most typical of these types of bacteria is the species *Desulfovibrio desulfuricans*. The intact cells of sulfate-reducing bacteria (SRB) rapidly reduce sulfate, oxidizing molecular hydrogen:

$$4H_2 + SO_4^{2-} \rightarrow S^{2-} + 4\ H_2O$$

$$SO_4^{2-} + H^+ + 4\ H_2 \rightarrow HS^- + 4H_2O \quad (\Delta F = -194.2kJ)$$

To facilitate this reaction, various bacterial cultures can be used. Solozhenkin *et al.* (1979) have used a SRB culture as a sulfidizator in the concentrating of oxidised antimony and bismuth ores, where it acts as a desorbent and depressor during the flotation of mixed concentrates. It also enables the selective separation of lead from zinc and of molybdenum from chalcopyrite.

An enrichment culture of sulfate-reducing bacteria has been isolated from the Tarnyauz molybdenum-wolfram deposit. For cultivating SRB under laboratory conditions, a medium was devised by Postgate (1966). However, Lyubavina *et al.* (1976) have used an improved medium with lintary dust, a by-product of cotton seed processing, as a source of carbon; the preparation of this medium has been described in detail by Lyalikova *et al.* (1977). It has been calculated that the maximum quantity of bacteria (220 x 10^6 cells ml^{-1}) is reached by the fourth day in the enrichment culture. Over this period, 400 mg l^{-1} of hydrogen sulfide passed into solution, that is, ten times less than its maximum solubility in water (0.1M)

The present efficiency level of hydrometallurgical methods for the processing of raw antimony ores has become so high that they can successfully compete with pyrometallurgical methods. In the industry, only sulfide-alkali solvents with a high selectivity relative to the group of heavy and noble metals (which remain in leaching cakes), are applied. According to Melnikov *et al.* (1975), antimony sulfide is dissolved in alkaline sulfide solutions through the formation of sodium thioantimonates:

$$Sb_2S_3 + 3\ Na_2S = 2\ Na_3SbS_3$$

$$Sb_2S_3 + 4\ NaOH = Na_3SbS_3 + NaSbO_2 + 2\ H_2O$$

$$Sb_2O_3 + 5\ Na_2S + 3\ H_2O = Na_4Sb_2S_5 + 6\ NaOH$$

Solozhenkin (1979) investigated SRB cultures as a solvent for antimony-bearing materials. Samples of antimonite from the antimony deposits of Tajikistan and an antimony flotation concentrate containing 39.55% Sb were used. The particle size of the antimonite was 50 mesh and of the concentrate 140-65 mesh. Portions of the antimony-bearing materials were mixed with different amounts of SRB enrichment culture and sodium hydroxide solution. The pulp was heated to 32.5°; the solid/liquid ratio was 1 : 16. The concentrations of SRB culture used were calculated from the hydrogen sulfide-producing ability of the strain. The solubility of the ore in the sodium hydroxide-SRB solvent exceeded the maximum obtaine with sodium hydroxide of the same concentration (120 gm l^{-1}). The optimum time of leaching was determined to be 1.0 - 1.5 h.

A decrease in the extent of antimony extraction into solution with increasing time of contact with the SRB culture was observed, and, therefore, leaching was carried out in two stages, with the SRB culture introduced in fractions. The time of the first stage was 1 h and that of the second stage 0.5 h. The results in Table 1 show that the SRB culture effectively dissolves antimony from various antimony-bearing materials under the appropriate optimum conditions.

TABLE 1

INFLUENCE OF SULFATE-REDUCING BACTERIA (SRB) AND Na_2S ON THE LEACHING OF Sb FROM ANTIMONY-BEARING MATERIALS. NaOH 120 gl^{-1}, LEACHING TIME 1 h, TEMPERATURE 32.5°

	Antimonite	
	Stage I	Stage II
Concentration of SRB (mg l^{-1})	8.75;17.5 ;43.7 ;52.5	8.75;17.5 ;43.7 ;52.5
Sb Recovery (%)	80.53;83.15;94.90;91.82	95.6 ;93.64;96.79;98.04
	Antimony concentrate	
	Stage I	Stage II
Concentration of SRB (mg l^{-1})	25.4 ;70.9;86.7;139.1	25.4;70.9;87.7;139.1
Sb Recovery (%)	41.3 ;46.7;56.8; 77.7	92.9;92.9;94.3; 96.5
	Antimonite	
Concentration SRB (mg l^{-1})	60 ;120 ;180 ;240 ;300 ;360	
Sb Recovery (%)	51.4; 78.8; 92.2; 97.4; 98.0; 98.9	

The technology for the leaching of antimony-bearing materials, proposed on the basis of these experiments, has a number of advantages compared with the use of sodium sulfide. The presence of free alkali in solution considerably increases electrical conductivity and facilitates subsequent electrolysis. The products of sulfide oxidation are known to influence adversely the course of the electrolysis of sulfide-alkali solvents of antimony. Thermodynamic estimates have shown that the rate of oxidation of hydrogen sulfide in the solvent is lower than that of sodium sulfide. Thus, the proposed technology for the leaching of antimony using SRB cultures requires less concentration of reagent and contributes an increase in current efficiency during electrolytic recovery.

LEACHING OF COPPER AND ARSENIC BY *THIOBACILLUS FERROOXIDANS*

Copper-Bismuth Ores

According to Glembotsky *et al.* (1972), bismuth minerals accumulate in copper concentrates due to the similarity of the flotation properties of copper and bismuth sulfides. Mixed copper-bismuth concentrates can be further treated by hydrometallurgical leaching with hydrochloric acid or by pyrometallurgy. These methods of processing of concentrates have some disadvantages; one of them is the low selectivity of the processes. The use of bacterial leaching has found wide application in hydrometallurgy (Mizoguchi *et al.*, 1970; Bruynesteyn and Duncan, 1971; Stirkov *et al.* 1975).

The authors have used an enrichment culture of *Thiobacillus ferrooxidans*, isolated from the mine waters of the Kaunrad deposit. The culture is adapted to a high concentration of copper in solution. The suspending liquid for leaching experiments consisted of 9K medium containing ferric iron (5.5 gm l^{-1}) and phosphate (2 gm l^{-1}), with a concentration of the organism of 1 x 10^6 - 1 x 10^7 cells ml^{-1}. The device shown in Fig. 1 was employed.

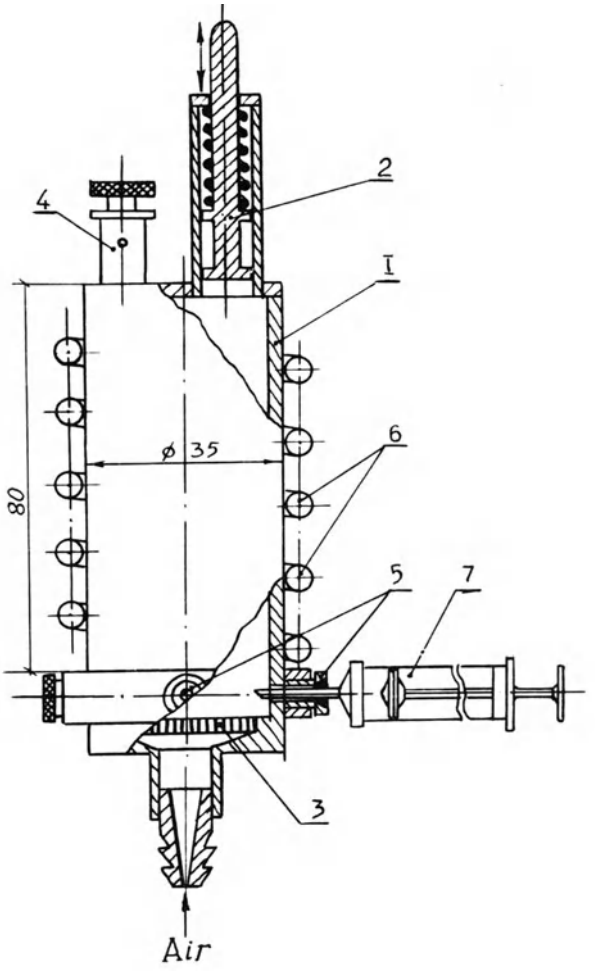

Figure 1: Device for bacterial leaching

1. Charging valve
2. Back air valve
3. Fermenteur chamber
4. Valve for probing
5. Sponge filter
6. Cooling cover
7. Syringe

The optimum conditions for copper leaching from copper-bismuth concentrates have been determined as a result of these studies. The time of leaching was 11 days; the solid/liquid ratio was 1 : 50; pH was 2.2-2.35. The extraction of copper into solution reached 97.2% (in the control test, it was 32.3%) (Table 2). Bismuth remained substantially in th leached residue which can be further processed to recover bismuth. After bacterial leaching of the copper-bismuth concentrates, the leach solutions contained 5.25-7.6 gm Cu l^{-1}; the copper could be recovered by cementation with iron shavings or by precipitation with SRB cultures. The recovery of copper by cementation was 98-99%; the precipitate containe 40-45% copper.

TABLE 2

THE RESULTS OF COPPER-BISMUTH CONCENTRATE LEACHING DEPENDING ON PULP DENSITY LEACHING TIME 11 DAYS

Product	Yield (%)	Content (%)		Recovery (%)		S:L
		Cu	Bi	Cu	Bi	
		Bacterial Leaching				
Solution	19.48	0.76	0.005	76.3	22.4	
Waste	80.52	7.3	0.53	23.7	77.6	1:25
Concentrate	100.0	24.8	0.55	100.0	100.0	
Solution	50.45	0.52		97.2		
Waste	49.55	1.52	0.53	2.8		1:50
Concentrate	100.0	26.9		100.0		
		Control Experiment				
Solution	8.75			32.3	14.12	
Waste	91.25	18.92	0.48	67.7	85,88	1:25
Concentrate	100.0	25.5	0.51	100.0	100.0	

Gold and Arsenic Ores

Ehrlich (1964) and Polkin *et al.* (1970; 1975) have shown that arsenic sulfides are readily oxidised by microorganisms. The authors have studied the role that bacteria play in the oxidative removal of arsenic from gold-bearing concentrate obtained by bulk flotation of sulfides from gold-bearing ore. The concentrate studied (100% - 74 mesh) containec 7.01% arsenic in the form of arsenopyrite. It is to be noted that reducing the particle size from 65% - 74 mesh to 100% - 74 mesh accelerates the leaching process by three times and shortens the time to six days. (Table 3). The arsenic extraction increases with time during the leaching of the concentrate in pachucas and also with increase of ferric sulfate.

Maximum extraction of arsenic into solution in these experiments has been observed to occur after 4 days with a solid/liquid ratio of 1:3. Cyanidation of the leached, gold-bearing residue enabled the extraction of 96.8% of the gold into solution. Arsenic was precipitated from the leach solution by sodium sulfide or lime. By this bacterial leaching process, mediated by *T.ferrooxidans*, arsenic can be removed from gold-bearing concentrates, and the problem of treating difficult-to-process arsenic and gold-bearing ores can be solved.

TABLE 3

THE INFLUENCE OF TIME AND DENSITY ON THE LEACHING OF ARSENIC FROM GOLD-ARSENIC CONCENTRATE

As content in waste (%)			As recovery (%)			Solid Liquid Ratio
2 days	4 days	6 days	2 days	4 days	6 days	
			Retorts mixed by vibration			
5.89	0.8	0.23	2.3	86.73	96.2	1:10
5.88	0.9	0.28	2.5	85.1	95.34	1:20
5.87	0.79	0.18	2.65	86.9	97.0	1:30
			Pachuca tanks			
0.3[a]	2.65[a]	-	12.86	98.1	-	1:30
0.39[a]	2.71[a]	-	16.71	99.9	-	1:30

[a] g l^{-1}

MICROBIAL DECOMPOSITION OF HYDROSULFIDE REAGENTS

Residual concentrations of reagents in re-cycled process waters can lead to considerable difficulties in the treatment of ores. A number of different methods for the purifying of industrial waste waters have been developed; for example, filtration, flotation, aeration, ozonization and biological purification. Ilyaletdinov *et al.* (1972) have used sulfate-reducing bacteria and microorganisms from industrial sewage for the cleaning of waste waters from copper cations and cyanide and rhodonide oxidation. Finn and Tannahill (1973) have employed *Azotobacter* for biological oxidation and *Pseudomonas fluorescens* has been used for the removal of reagents from waste water. This organism has been added to a solution of the stable radical of 2,2',6,6'-tetramethyl-imonoxyl-4-potassium xanthate and the solution analysed after various periods of time. The kinetics of decomposition of imon-oxyxanthate have been determined by measuring the intensity of the ESR-spectrum.

In the presence of bacteria, the xanthate was completely decomposed in 45 min. When bacteria were absent, only 45% of the xanthate was decomposed (Fig. 2). Analogous results have been obtained on the decomposition of considerable concentrations of butyl xanthate and sodium diethyldithiocarbamate (Fig. 3). The bacterial efficiency has been confirmed by an investigation of the purification of waste waters at the Takob lead plant. The residual concentration of xanthate in the waste waters was 0.12 mg l^{-1}; after a 5 min treatment with a bacterial suspension, the xanthate was completely destroyed (Kupeeva *et al.*, 1977).

CONCLUSIONS

The investigations described in this paper illustrate the possibilities for the improvement of metal extraction from a number of different types of ore by the use of various bacterial leaching processes.

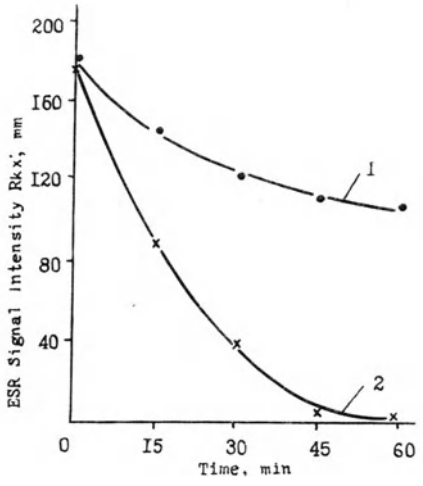

Figure 2: Influence of *Pseudomonas fluorescens* on the kinetics of destruction of the 2,2',6,6'-tetramethyl-1-iminoxyl-4 potassium xanthate (50 mg l^{-1}).

1. Initial solution
2. Solution after bacterial treatment

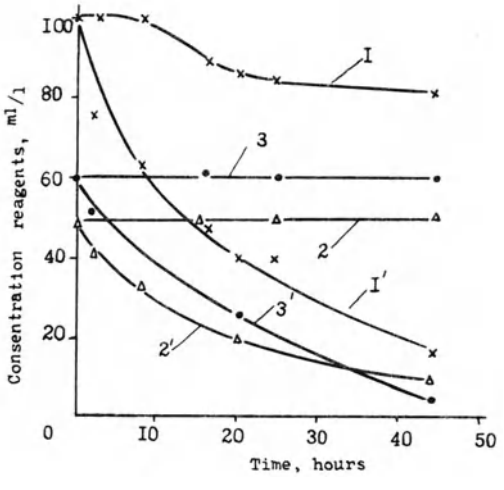

Figure 3: Influence of *Pseudomonas fluorescens* on the kinetics of destruction of the butyl xanthate (1,1´ - 100 mg l^{-1}; 2,2´ - 50 mg l^{-1}) and sodium diethyldithiocarbamate (3,3´ - 60 mg l^{-1}).

1,2,3 - Initial solutions
1´, 2´, 3´ - Solutions after bacterial treatment

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WATER MOVEMENT CAUSED BY MONSOONAL RAINFALL IN AN OVERBURDEN DUMP UNDERGOING PYRITIC OXIDATION

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INTRODUCTION

Rum Jungle, situated some 90 km south of Darwin, is reasonably well-known as a uranium mine that operated during the uranium boom of the fifties. Mining operations started in 1953 and carried through to 1971 when the mine was abandoned. Rum Jungle is also reasonably well-known as a mining operation which has had a marked impact on its environment. The major impact has been on the East Branch of the Finniss River, which flows through the mine site, and on the areas alongside the overburden dumps. This impact stemmed from the high concentration of heavy metals such as copper, manganese and zinc in the water flowing from these dumps, and other areas of the abandoned mine, into the East Finniss. The major sources of pollution (Davy, 1975a) are the overburden dumps and a dump of low grade copper ore from which extraction of the copper by a heap leaching technique was attempted.

Iron pyrites are present in the overburden dumps (ca. 3% sulfur) and the high acidity, high sulfate and heavy metal solubilisation are due to oxidation of this pyritic material. We are investigating the oxidation process within the dumps in essentially a macroscopic fashion to determine where in the dumps the oxidation occurs, the rate at which it is occurring and the parameters that limit this rate.

One parameter we are investigating is the rate at which water flows off and through the dumps. There are three main reasons for doing this. First, the overall oxidation rate within the dumps can be inferred from measurements of the quantity of the water and the sulfate concentrations in the water that runs through and off the dumps. Although the quantity and quality of run-off water from a dump, and the quality of water that appears as springs at the base of a dump can be measured, direct measurement of the quantity of water issuing from the base of the dump is not possible because most of it feeds directly into the ground water. The rate of water flowing through a dump can be inferred from the difference between rainfall and run-off and from successive measurements of the water concentration profiles within the dump. Second, water concentration profiles provide information on conditions within the dump; the extent to which the dump dries out during the dry season; the extent to which a water table forms within the dump in the wet season; and the variability of conditions in different parts of the dump. Third, water infiltration through the dump provides a significant heat transport mechanism when heat flow and heat sources within the dump are being investigated.

This paper reports measurements of soil water density within one of the overburden dumps at Rum Jungle, using neutron and gamma ray scattering techniques. Measurements have been made at various times during the wet and dry seasons to investigate changes from wet season to dry season conditions and changes within these periods.

SYSTEM STUDIED

The Overburden Dump

We decided to concentrate measurements initially on just one of the overburden dumps. White's dump was chosen, partly because it is the largest dump and the single largest source of heavy metal pollution in the mine area and partly because the springs that appear at its base are better defined and stronger flowing than those from the Intermediate dump. In addition, these springs have a simple, time-dependent decrease in flow during the dry season that indicates a simpler internal structure than Dyson's dump, which also has well defined springs (Davy, 1975b).

The top surface of the dump is about 500 m in diameter and is well graded over much of its area. Most of the top surface slopes towards the middle of the dump, and surface water

drains into a well defined, run-off channel that flows north into the East Finniss River. Flow in this channel occurs only during heavy rain and for a few hours after rain. The climate of the area has well defined dry and wet season, the dry lasting from about May to September and the wet from October to April. Rainfall in the wet totals about 1.5 m in th Rum Jungle area and falls, for the most part, in heavy storms lasting for two or three hours, with typical rainfalls ca. 10 to 50 mm and rates of 10 to 50 mm h^{-1}, although rainf and rainfall rates are frequently higher than this in particular storms. Temperature data are not available for Rum Jungle, but the annual mean temperature at Oenpelli, which is about the same distance inland as Rum Jungle, is about 28°, with the monthly mean temperature varying about the annual mean by only some 3°. The average annual pan evaporation rate at Oenpelli is about 2.5 m, with monthly rates varying from 100 mm in February to 260 mm in October. Run-off from White's dump has been measured by means of a weir built across the principal run-off channel. The fraction of the incident rain that runs off varies greatly from storm to storm in the range ca. <1% to 30%, but over the wet season, averages about 10%.

The dump is composed primarily of slates and graphitic shales, with a small portion (10 tc 15%) of dolomite which occurs mainly in the top surface of the north-west corner of the dump. Analysis of the crushed and bulked samples from an auger drill program carried out on the dump in 1969 (Cayzer, 1969) showed the average sulfur level to be just over 3% and heavy metal levels as given in Table 1. Analyses of material that resulted from the 1976 drilling program of six probe holes in the dump showed the main elements in the dump to be distributed as indicated in Table 1.

TABLE 1

ANALYSIS OF MATERIAL IN WHITE'S OVERBURDEN DUMP

Element	1969[a]	1976[b]
Cu	0.086	0.157
S	3.27	1.38
Zn	0.011	no analysis
Ni	0.026	
Co	0.013	
Mn	0.099	
Pb	0.048	

[a] Analysis of bulked auger drill samples (Cayzer 1969)

[b] Analysis of material from percussion drilling of six probe holes, 1976.

Probe System

As indicated above, six probe holes were constructed in White's dump. At the end of the 1976 dry season, the holes were drilled, using a rotary percussion drilling technique, frc the top surface of the dump some 18 m through to the underlying material on which the dump rested. Fig. 1 shows the position of the probe holes which, with the exception of hole B, were around the rim of the dump where it has its greatest height. The hole diameter of 90 mm was chosen to allow use of a 50.8 mm bore and a 3.2 mm wall thickness polyethylene liner in the probe holes. The liners were closed at the bottom with a polyethylene plug welded into the tube to stop the ingress of water and provided with a removale seal at the top to exclude rain water. After the liners had been inserted into the drill holes, sand

was poured in to fill the space between the liner and the drill hole wall. There has been no evidence of any movement of the polyethylene liners relative to the material of the dump since their insertion in September 1976.

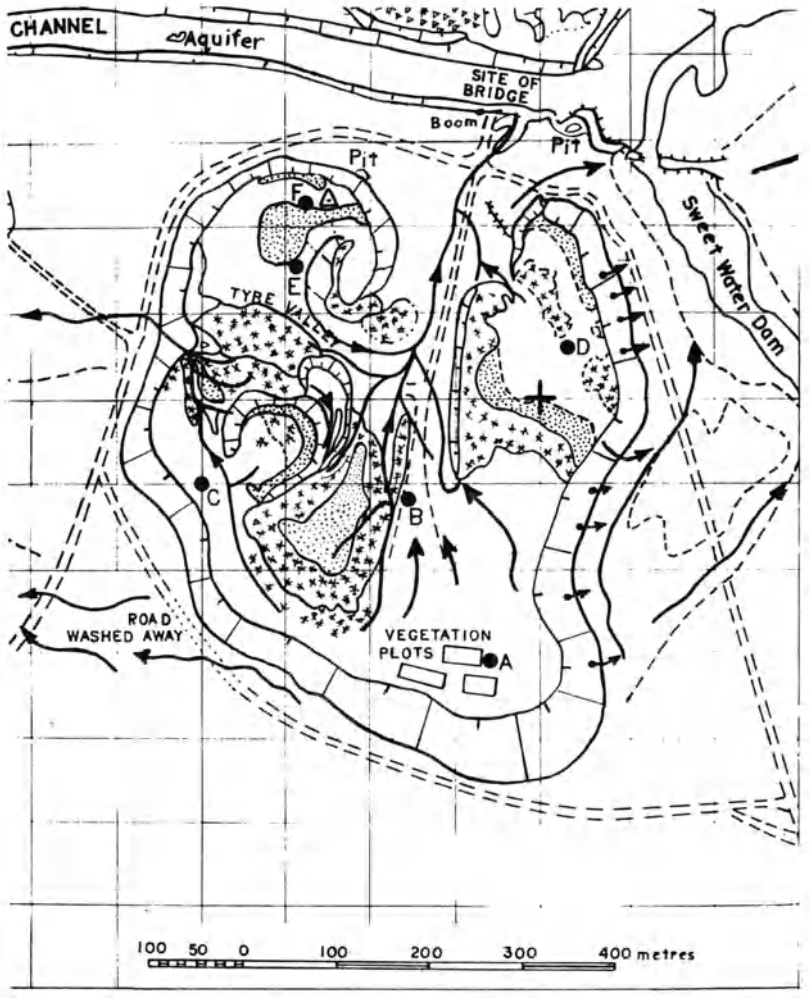


Figure 1: White's overburden heap at Rum Jungle showing the location of the probe holes.

The neutron and gamma ray probes used in the water density profile measurements are of relatively conventional design and were developed within the AAEC's Isotope Division for ground water studies. The neutron source used in the neutron probe is a 11 GBq Am-Be source and the gamma source in the gamma probe is a 0.19 GBq ^{137}Cs source. The high voltage cable connector is the same on both probes to allow use of the same set of battery operated EHT-ratemeter and scaling units in the field.

In early measurements, the probes were lowered down the probe holes by hand and the distance of the probe from the surface determined from markers on the cable. In later measurements, the cable was wound on a reel, each revolution of which was graduated in terms of distance the probe moved down the hole. This system is more convenient to operate in the field and lends itself more readily to automation. Either technique enabled the position of the probe to be reproduced to better than $\pm$10 mm.

MEASUREMENTS

Technique

Count rates from the neutron and gamma probes were determined both at spot positions down each probe hole and continuously by recording a rate-meter response as the probes were lowered down the probe holes at a rate of 0.4 m min^{-1}, by means of a stepping motor. The two measurement techniques are complementary as the motor scan gave a complete record of count rate changes down the probe holes, while the spot measurements gave results of high statistical accuracy. The quite large changes in both neutron and gamma count rates with probe movements of 0.1 m or so meant that a complete set of spot measurements would have taken an unrealistically long time.

In general, a neutron scan and a gamma scan were done on each hole during any measurement program. Prior to each scan with the neutron probe, the count rate of the probe was measured in a standard geometry rig to check for drifts in the EHT, amplifier and discrimi ation units. The rig took the form of a paraffin wax filled drum, normally used to store the neutron source when not in use. The standard geometry rig for the gamme probe took th form of a tube of concrete of sufficient size to decouple the source and detector from any external scattering bodies. As the concrete tube was too heavy to transport easily, the gamma probe was calibrated before and after each measurement program. This was adequate a the gamma probe used the same electronics as the neutron probe. Reproductibility of count rates from the neutron and gamma probes when placed in the standard geometry was ±1%.

Background count rates were measured in the probe holes with the sources removed. The neutron background was very small (typically ca. 1 count s^{-1}). The gamma background showe significant variation as might be expected in an overburden dump from a uranium mine. Generally, background levels were some 10 X less than the count rates measured with the source in position.

Neutron and gamma scans were carried out several times over the last three years and with increased frequency from November 1978 to June 1979.

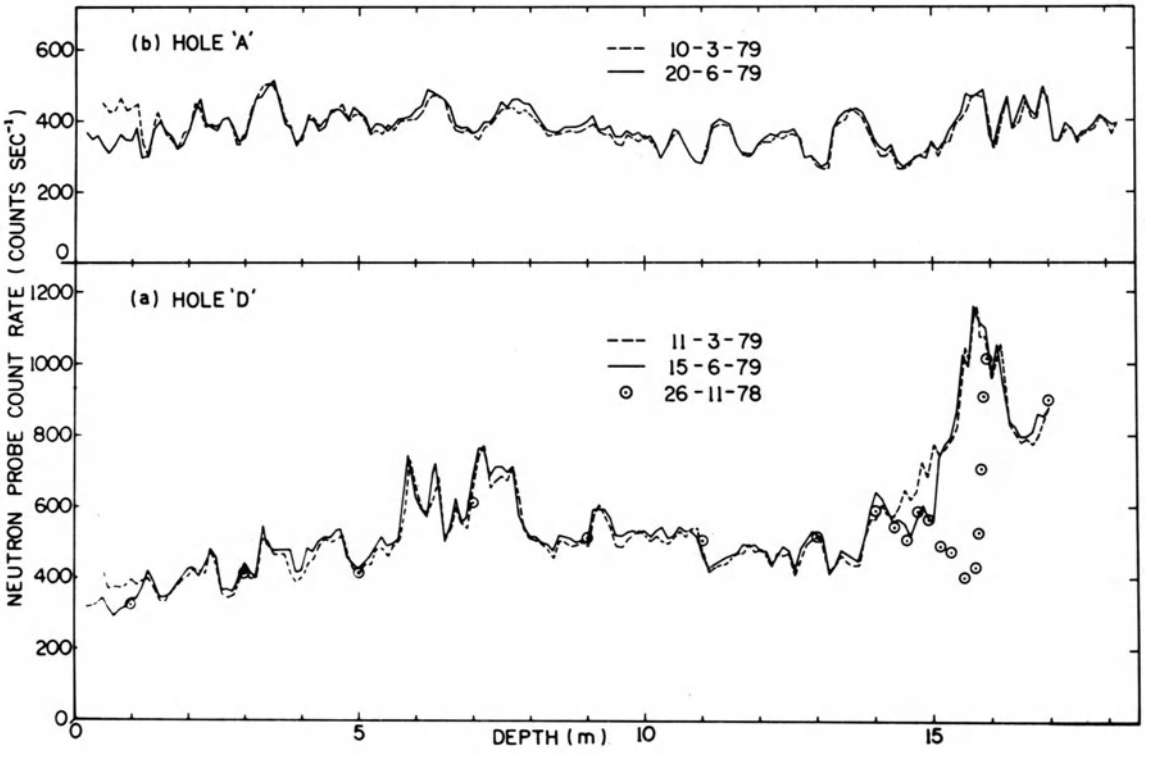
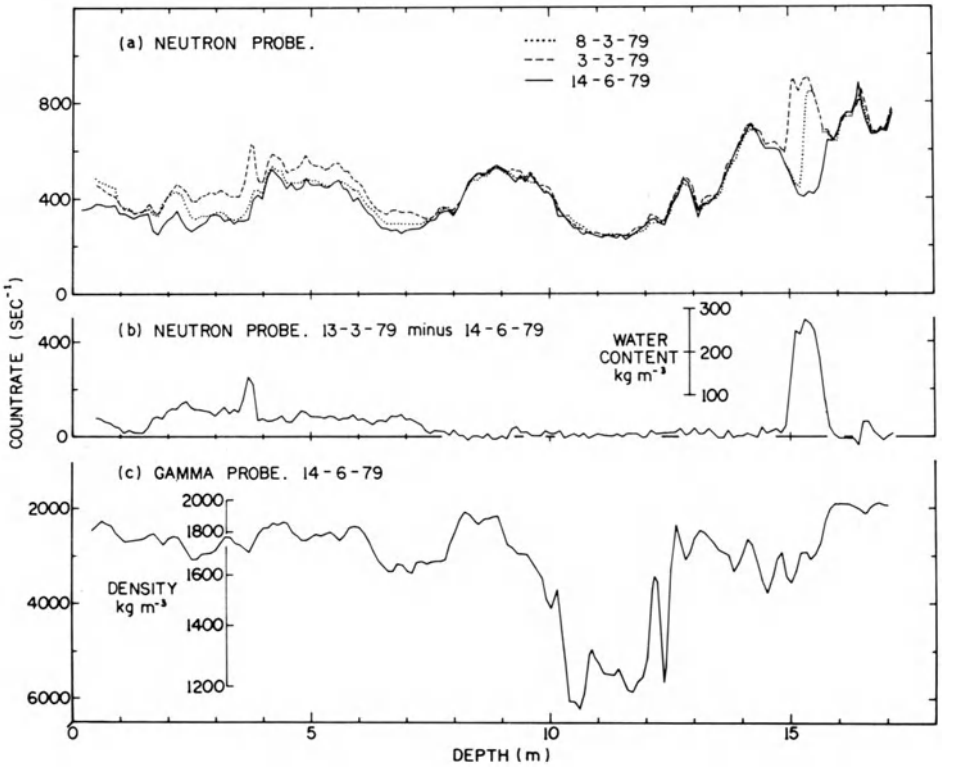


Figure 2: Neutron probe measurements for holes A and D at various periods of the wet and dry seasons.

Results

The scans show that there are quite large variations of density in the dump and hence presumably marked variations in the permeability. One result of some significance and some surprise is that the count rate from the neutron probe, and hence the soil water density, does not change much from wet season to dry season over much of the dump volume. This is particularly so in the case of hole A, where it can be seen from Fig. 2a that there is little or no change in the soil water density except in the top 1.2 m or so where there is evidence of some drying out in the change from wet season (March 1979) to dry season (June 1979) conditions. Hole A is also significant for having temperatures well above ambient (Daniel *et al.*, 1980). Hole D (Fig. 2B) also shows little change in soil water density over its 18 m length, but as in hole A, there is evidence of frying out in the top 1.2 m in the period March to June 1979. Hole F (Fig. 3A) does show evidence of changes in soil water density in the top 8 m, both from wet season to dry season conditions and during parts of the wet season, but again there is little change from 8 m to the bottom at 18 m. A scan carried out in December 1978 showed even lower count rates in the top 8 m which indicate that this region dries out even further during the dry season than implied by the measurements carried out in the middle of the dry season.



Fugure 3: Neutron and gamma probe measurements for hole F with approximate relationships shown for density and water content as a function of count rate.

The neutron scans also show evidence of fine structure in the soil water density. For example, there is a marked increase in the region of hole F in the top 2 to 4 m between measurements on 8th and 13th March, 1979. This could have been caused by the 42 mm of rain that fell between these measurements or by the 140 mm that fell in the ten days before 8th March. Subsequent measurements on 20th and 26th March showed that the soil water density in this region was decreasing. In the same hole, there was a marked change in the

region between 14.8 and 16 m in measurements made between March and June 1979. The extent of this change is more clearly seen in the difference curve of Fig. 3B. Also the gamma scan of Fig. 3C shows that the bottom of this region corresponds to a region of high density.

There is also an interesting feature in the soil water density in the region of hole D between 14.4 and 16 m. Unlike the feature at a similar depth in hole F, the levels here change by a relatively small amount from March to June, the top edge of the feature dropping from 14.4 to 15 m. However, spot measurements on 26th November, 1978, close to the er of the previous dry season, show the edge of the feature to have dropped to ca. 15.8 m. Th bottom of hole D is 7 m lower than any other hole and is close to the side of the heap where springs occur from mid-wet season to mid-dry season. This feature could well be an aquifer on the main drainage route from the heap.

An increased count rate from the neutron probe at a given position implies an increase in the soil water density at that point, although the calibration of the neutron probe varies with changes in the thermal neutron absorption cross section of the soil, with changes in the soil density and, to some extent, with changes in the scattering cross section of the soil. Similarly, a decrease in the gamma probe count rate at a given position implies an increase in the soil density, but because of the larger Z/A value for hydrogen compared tc other elements, the calibration of the gamma probe varies with changes in soil water density. Hence the best estimate of the soil density and the soil water density can be obtained by judicious use of both gamma and neutron count rates, together with a knowledge of the absorption cross section of the material in the region of the probe holes. Howevei to provide a feel for the range of soil and soil water densities, approximate calibration curves have been incorporated into Figs. 3B and 3C. The density scale of the gamma scan is based on the formula of Christensen (1973), but scaled to both the count rate observed for our probe in the polyethylene tube immersed in water and the median count rate observe in the heap, which has a mean density of 1670 kg m^{-3}. The ratio of the count rate in the water to that in the heap agrees well with the results of Christensen's calculations.

Calculations using a multigroup diffusion theory code (D.J. Wilson, personal communicatior show that, for practical values of the density ρ, the absorption cross section Σ_a and the soil water density ω, the count rate R of a neutron probe takes the form

$$R = C(\Sigma_a,\omega) + \rho \cdot M(\Sigma_a,\rho) \qquad (1)$$

The calculations are in good agreement with the experimental results of Graecan and Schral (1976) and in reasonable accord with the calculations of Ølgaard (1965). Provided the water density and absorption cross section do not vary greatly expression (1) can be simplified to

$$R = \alpha \cdot \omega + M(\Sigma_a) \qquad (2)$$

Hence at a given point, where the density and absorption cross section will remain the same, the difference in the neutron probe count rate will be proportional to the differenc in the soil water density. The water density scale of Fig. 3B was derived from an intercomparison of the calculations of Wilson (1979) and Ølgaard (1965) and the measured response of our probe in an infinite water system.

CONCLUSIONS

The count rate from neutron and gamma probes lowered down probe holes in White's overburde dump at Rum Jungle give a clear, qualitative picture of the state of the soil density and soil water density in different parts of the dump. The gamma source shows the density to be quite variable in the range 1150 to 2000 kg m^{-3}. Changes of the water density from wet season to dry season are less than expected, but there is evidence in the case of all hole that there is drying out of the top 1 to 1.5 m in the dry season. It is evident that most of the water that infiltrates through the dump does so without producing significant chang in the soil water density. In some cases, water accumulates towards the base of the heap and, in the case of hole D, there is evidence that the base is still saturated at the end the dry season.

ACKNOWLEDGEMENT

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TEMPERATURE DISTRIBUTIONS IN AN OVERBURDEN DUMP UNDERGOING PYRITIC OXIDATION

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INTRODUCTION

Rum Jungle is an abandoned uranium mine located at latitude 13°S, about 90 km south of Darwin. Mining commenced in 1953 and ore processing ceased in 1971. The abandoned mine site contains three flooded opencuts, their associated overburden heaps and the remains of the tailings dam. Copper and lead were associated with the ore, and both the ore and the overburden contained significant amounts of pyrites.

A major source of pollution is the leachate and run-off from the overburden dumps (Davey, 1975). A study of the largest overburden dump, White's dump, has been undertaken to provic an understanding of the leaching processes to evaluate the efficacy of various ways of re-habilitating the area. White's dump was completed in 1958; it contains about 4 x 10^6 m^3 c material and covers an area of 2.7 x 10^5 m^2. The top of the dump is 13 to 18 m above the original soil level. Analysis of bulked auger drill samples from White's dump in 1969 gave a sulfur content of 3.3% and a copper content of 0.09% by weight.

The climate at Rum Jungle divides into two seasons, the dry season from April to October with virtually no rain from May to September, and a wet season with, on average, 89% of the annual rainfall in the four months December to March. Most of the rain falls in localised thunderstorms, but there are some periods of continuous rain associated with barometric depressions. The mean annual rainfall is about 1.5 m.

Six probe holes were drilled into White's dump during September 1976 by rotary percussion drilling. The drill holes had a diameter of 90 mm and penetrated to the original soil level. Each hole was lined with a black polyethylene pipe (50 mm ID, 56 mm OD) which was sealed at the bottom end to exclude water. The space between the liner and hole was fillec with dry sand.

THE TEMPERATURE PROBE

Temperatures in the holes were measured using Yellow Springs Instrument Co. thermistors (YSI 44030) with a nominal resistance of 3000 Ω at 25°, and specified to conform to the standard resistance-temperature characteristic within ±0.1° over the range 0 to 80°. The thermistors were mounted in a probe assembly which was lowered by the cable through which the resistance was measured by means of a meter at the surface of the dump. In early attempts to measure the temperature distributions in the overburden dump, the thermistors were mounted in probes in which the thermistors were pressed against the polythylene liner This initial probe design resulted in a very slow response time, the resistance approachin its equilibrium value with an e-folding time of about 5 min.

A laboratory probe hole was set up in a 200 l drum using sand surrounding a short section c polyethylene tube. Heating coils around the outside of the drum produced a temperature gradient along the tube. Thermistors glued into holes in the tube allowed the temperature: to be monitored in the absence of the probe. Tests of various probe designs in this rig showed that the time constant of the probe was almost proportional to the mass of the probe Furthermore, the thermistor reading obtained with the thermistor hanging in the centre of t tube was the same as that obtained with it pressed against the wall of the liner. Anti-convection shields above and below the probe reduced the temperature fluctuations caused b convection currents. The final probe (see Fig. 1), which had a mass of about 10 g, was con-structed of tinned copper wire and fitted with two aluminium foil anticonvection shields. The e-folding time for the thermistor in this probe to approach the equilibrium temperatur was 1.2 min.

The resistance was measured in the field using a Data Precision Digital Multimeter model

245. On the 10 kΩ range used, the current used to determine resistance was between 0.22 mA and 0.273 mA, depending on the resistance being measured. The power produced in the thermistor was 0.2 to 0.07 mW, which produces a temperature rise of about 0.1 to 0.2°. Resistances were recorded at 1 min intervals, starting at 0.5 min, until the resistance changed by less than 5 Ω in 1 min. Near the surface, this sometimes took 5 min but at most positions in the holes, 1.5 min were sufficient. The final equilibrium resistance was then estimated using the known time constant of the probe, and converted to the temperature using the thermistor characteristics.

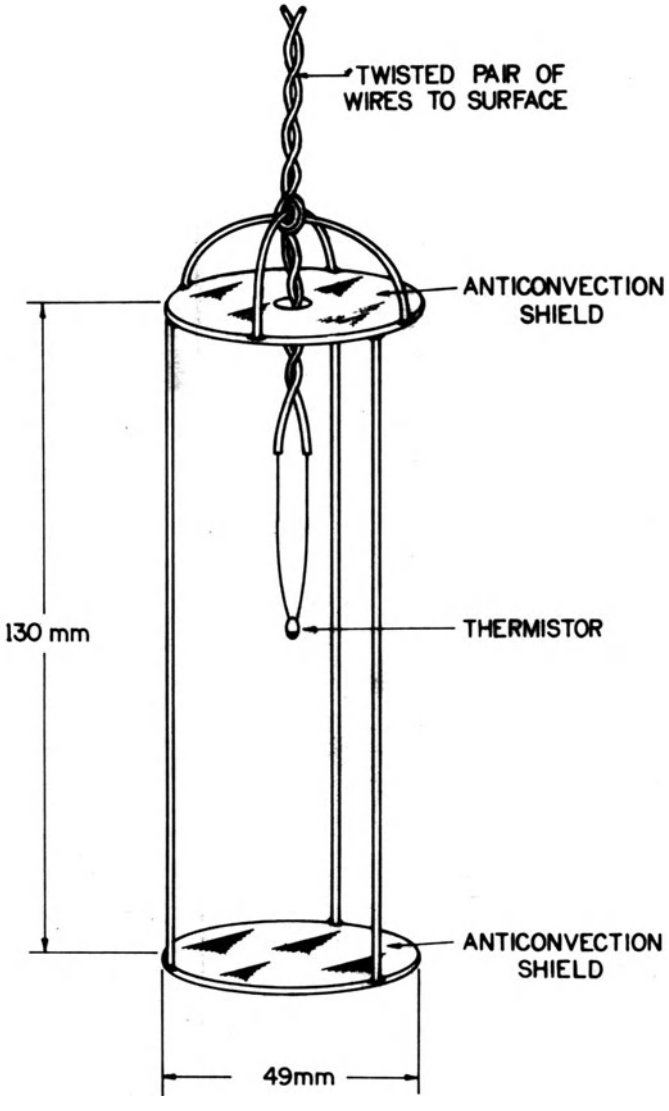

Figure 1: The temperature probe used in the polyethylene probe tubes. The probe weight is about 10 g.

TEMPERATURE DISTRIBUTIONS

The measurements with the earlier probes and temperature conversion boxes in the period September 1976 to November 1977 had suggested a possible temperature variation from wet season to dry season deep within the heap. A series of measurements, using the light weight probe and the digital multimeter to obtain more accurate data, were therefore undertaken in November-December 1978 (the beginning of the wet season), in February-March 1979 (mid to late wet season) and in June 1979 (mid-dry season). Fig. 2, which shows the temperature distributions down the holes in June 1979, indicates the wide range of temperature conditions in the heap. The temperatures in hole A, near the southern tip of the heap, reach ca. 56° at depths near 14 m. The hole C temperature distributions also show evidence of subsurface heating, but the peak temperature is only 2.4° above the near surface temperatures. Hole F shows a slight indication of a deep temperature rise. Hole D has high temperatures at depths of 3 to 4 m and some heat is conducted towards the bottom of the heap at that position.

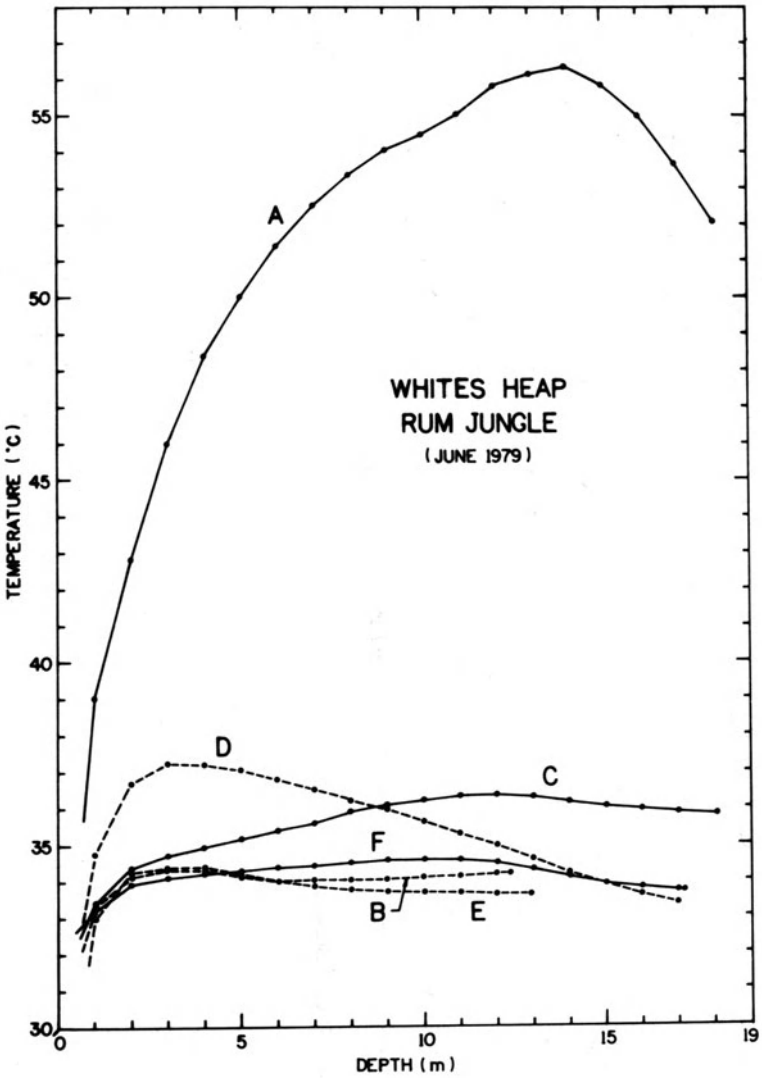

Figure 2: The temperature distributions measured in the six probe holes in White's overburden dump at Rum Jungle.

The temperature gradients observed are much greater than the normal geothermal temperature gradient of about 0.03^{o} m^{-1} in the earth's crust.

Near the surface, the temperature distributions are subject to variations in insolation, cloud cover, air temperatures and rainfall. Diurnal temperature changes do not have any significant effect at depths greater than 1 m, but weekly and monthly weather pattern changes do have an effect which can extend down to 6 m. Below 6 m the temperatures were essentially the same in the wet season and the dry season.

The maximum temperature in hole A has not changed from the range 56 to 58^{o} over the past two years. Heat production is still occurring and the reaction rate appears to be constant. Murr and Brierley (1978) also observed temperatures up to 59^{o} in a large-scale leaching test of copper-bearing waste from the Kennecott mine. Temperatures in excess of 80^{o} have been observed in dumps of low-grade copper in Bulgaria (Groudev *et al.*, 1978) and in the U.S.A. (Beck, 1967).

SOURCES OF SYSTEMATIC ERRORS IN TEMPERATURE DISTRIBUTIONS

The method used to line the probe holes and fill the outer space with dry sand raises questions about the preferential flow of rainwater alongside the tube. If water had flowed in the loose sand alongside the tube it would have caused a short-term change in temperature distribution which would subsequently have recovered as heat was exchanged with the surrounding unaffected material. There was no indication of this effect in the 1978-79 wet season suggesting that, if any flow paths did exist, they have now been blocked sufficiently by fine sediment. As a further check, the surface for a radius of 1.5 m around hole E was flooded in June 1979 and subsequent water concentration changes down the hole measured using a neutron moisture meter. These measurements showed no evidence of water trickling down the side of the probe tube.

Convection currents, likely to occur in the probe holes wherever the temperature increases with depth, will transport heat towards the surface. The air flow in the probe holes will be in the impeded laminar flow regime (Japikse, 1973) because the temperature gradients are relatively low and because the upwards flow of air near the wall is impeded by the flow of air down the centre of the probe holes. With a temperature gradient of 5^{o} m^{-1} the convection heat transfer is estimated to be about 0.4 W, based on the review by Japikse (1973) and the results of an experiment by Devyatkin (1976) on vertical air filled pipes embedded in sand. The removal of this heat from the surrounding material will cause a slight drop in the temperature at the hole. If the heat were conducted to the tube from a radial distance of 1 m, over a height of 1 m, then the temperature drop between material 1 m from the tube and the tube wall would be only 0.2 to 0.3^{o}. Hence, even for hole A, where the temperature gradients are ca. $<5^{o}$ m^{-1}, the effect of convection heat transfer can be ignored.

In parts of the holes where the temperature decreases with depth, the air column is stable and any heat transfer in the column will be by thermal conduction in air which is a factor of 40 less than the conduction in a similar cross section of soil. Perturbation of the temperature distribution by conduction of heat in the polyethylene liner can also be ignored since its thermal conductivity is about half that of soil.

CALCULATION OF HEAT SOURCES

The distribution of heat sources in the heap was determined from the temperature distributions measured at two times separated by at least one month. The calculation was carried out in terms of a one-dimensional model of the heap and using the implicit finite difference form of the heat diffusion equation. The depth of the heap was divided into 1 m intervals, corresponding to the spacing between the measurements, and a heat source to be determined was associated with each 1 m. A depth of 25 m was chosen to allow for some of the effects of heat being conducted into the ground beneath the heap with a bottom boundary condition of no heat flux at 25 m. The calculation used one-day time steps which allowed the heat transfer caused by infiltrating rain to be included.

The equations were first solved with no sources, using the first measured temperature distribution for the initial conditions, with temperatures beyond the bottom of the hole set by a linear extrapolation of the temperatures in the lowest 2 m. The top boundary condition was the mean monthly air temperature for December which varies between 25.1^{o} in

July and 29.6° in November. Source solutions were then obtained for each source using initial conditions and a top boundary conditions of zero temperature.

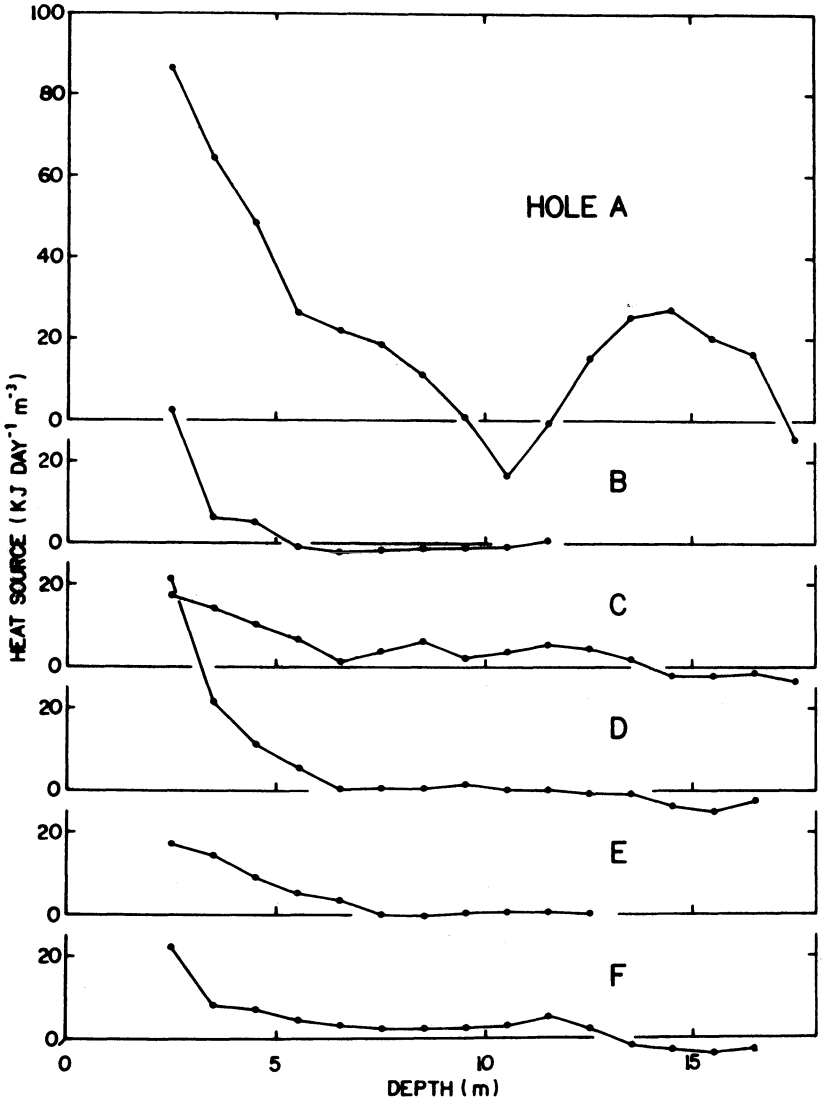

Figure 3: The heat source distribution calculated from the temperature distributions measured in March 1979 and June 1979.

If the hole was of depth N metres, there were N sources solutions and one solution based on the initial temperature distribution with no sources. The linearity of the heat conduction equation means that these solutions can be linearly combined to give the total solution. The sum of the solutions at each 1 m interval produces a set of N linear equations relating the N unknown sources to the final temperatures. The set of equations was solved to determine the heat sources which were averaged over 2 m intervals to remove a tendency for the calculated values to alternate between high and low values.

Although the Darwin air temperature assumed for the top surface is likely to be lower than the surface temperature of the dump, changing the surface temperature affects the

calculated sources at 1 m only and leaves the deeper sources unchanged.

The heat transport calculation requires knowledge of the thermal parameters of the heap material. The thermal capacity was measured for six samples in the laboratory and yielded values between 829 and 908 J kg^{-1} K^{-1} with a mean of 866 J kg^{-1} K^{-1}. The mean density of the heap has been estimated from the mass of material removed from the opencut and the volume of the dump, giving a result of 1670 kg m^{-3}. The thermal conductivity is more difficult to determine because it depends on the water content of the material and its degree of compaction. Typical thermal conductivities for soils lie in the range 0.7 to 1.2 W m^{-1} K^{-1}, while for oven-dried sand it is as low as 0.3 W m^{-1} K^{-1}. A thermal conductivity of 0.8 W m^{-1} K^{-1} was used in the source calculation. The observed temperature changes in the top 3 m of the dump caused by changing weather patterns over two weeks in the dry season were consistent with this thermal conductivity.

The sources calculated from the temperature distributions observed at the end of March 1979 and June 1979 are shown in Fig. 2; in this time interval there was no significant rain. Hole A shows a peak in the heat production at 14 to 15 m and significant heat production above 8 m. The source is negative at 10.5 m, indicating that heat is being transferred horizontally away from the immediate vicinity of hole A. Since the neutron scans (Daniel *et al.*, 1980) showed no significant change in water content at this level, the horizontal heat flow must indicate a nearby region at 10.5 m where there is a lower temperature.

All the holes show heat production near the surface. The source could not be determined in the surface layer to 1.5 m because neither the insolation or the mean surface temperature is adequately known. Holes C and F show significant subsurface heating. Holes D and E show almost no heat production below 7 m, although there is heat production at shallow depths. The neutron probe results show holes D and F penetrate to a water table which was present when the March temperature distributions were measured. Both holes show negative heat sources at the bottom which must stem from horizontal heat transfer with the ground water.

The heat released in oxidation of pyrite to ferric iron is 24 kJ g^{-1} sulfur. The total heat source for hole A between 12 and 17 m is only 88 kJ m^{-1} day^{-1} which could be produced by the oxidation per day of as little as 4 g of sulfur in the 5 m^3. The oxygen requirement would be 7.5 g O_2 day^{-1}. The oxidation of pyrites by ferric ions required 14 mol of ferric ions to each mole of pyrites, and the heat production is less than 0.2 kJ g^{-1} sulfur.

If the heat production in these six holes is representative of the heap as a whole, then the heat production below 1.5 m is 132 kJ m^{-2} day^{-1}, equivalent to a sulfur oxidation rate of 5.5 g S m^{-2} day^{-1}, and the total sulfur oxidised in the whole heap over a full year would be 530 Mg. This can be compared with the annual oxidation rate of 1600 Mg estimated from the concentration of sulfate ions in the spring water (Davy, 1975) and the amount of water infiltrating into the heap. These numbers suggest that most of the oxidation is still occurring in the surface 1.5 m layer.

CONCLUSIONS

It has proved possible to produce heat source distributions as a function of depth from the surface of White's overburden heap by analysing measurements of the temperature distributions in it. It is reasonable to associate the heat sources with the heat released in the oxidation of pyrites. The analysis has shown that significant pyritic oxidation occurs at depths of 10 m or greater in some parts of the heap. The analysis also shows that there is significant pyritic oxidation in the top 4 to 5 m in all parts of the heap. Estimates of heat input to the surface from insolation and better measurements of temperature changes near the surface will allow better estimates to be made of heat sources in the top 1 to 2 m.

Temperature measurements are comparatively straightforward and have the advantage that they are an 'integral' parameter insofar as they reflect conditions over several square metres around the probe hole. Apart from their present use in determining the distribution of pyritic oxidation within the heap, they should prove useful in monitoring in some post-rehabilitation phase the effectiveness of the rehabilitation in stopping pyritic oxidation.

ACKNOWLEDGEMENT

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ENVIRONMENTAL STUDIES OF FLOODED OPENCUTS

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INTRODUCTION

In the course of uranium mining and extraction operations at Rum Jungle in the Northern Territory of Australia over the period 1953-70, processing materials were obtained from five opencuts and a sixth was excavated to just below the level of the ore body. In the monsoonal climate of Northern Australia, with its annual average rainfall of about 150 mm during the wet season, all opencuts became water-filled on the cessation of mining operations. Those in the Rum Jungle Mine area proper (Fig. 1) have become highly acidic and metal-polluted (Intermediate, White's and Dyson's Opencuts); the water in the other three opencuts (Rum Jungle Creek South, Mt Burton and Mt Fitch Opencuts) is neutral to slightly alkaline in reaction and the heavy metal content is negligible (Davy, 1975).

Intensive studies of environmental pollution in the Rum Jungle area were initiated by the Australian Atomic Energy Commission in 1973 and have continued up to the present time. Studies on the role of microbial populations in the generation of acidic and metallic pollution in sulfidic overburden heaps, tailings dams and flooded opencuts, were commenced in late 1974 in close collaboration with the A.A.E.C. teams. The findings here reported embody a preliminary examination of the microbial status of water samples taken from the Intermediate and White's Opencuts and associated water systems, early in a dry season (June, 1978).

An examination of water quality in respect of phsico-chemical characteristics in the Intermediate, White's and Rum Jungle Creek South Opencuts was carried out by the Water Resources Branch of the Northern Territory Administration in December, 1977. The results, which were made available through the A.A.E.C., enabled the selection of a limited number of sampling sites for a preliminary microbiological examination.

Prior to the commencement of mining operations in the Rum Jungle area, the East Branch of the Finnis River passed over the White's and Intermediate ore bodies: the water flow was subsequently diverted by the construction of a river diversion channel. A portion of the old bed of the East Branch lies between White's and Intermediate Opencuts and drains into a new channel, Copper Creek, which skirts the northern embankment of the Intermediate Opencut (Fig. 1).

During the operation of the processing plant at Rum Jungle, two small leaching heaps were constructed from the overlying, low-grade copper sulfide and oxidised ores of the Intermediate ore body. They are located between the two opencuts and continue to shed acidic, copper-bearing drainage waters into the various collection ponds and thence into the old bed of the river and Copper Creek.

When all uranium ore had been recovered from White's ore body, a considerable quantity of tailings from the processing plant was returned to the opencut, about half-filling it. No tailings or other residues were deposited in the Intermediate Opencut.

METHODS

Sampling points are located by reference to survey points established by the Water Resources Branch team, using compass and range finder. The sampling sites in the Intermediate Opencut and the associated water systems are shown in Fig. 2 and in White's Opencut in Fig. 3. Temperature and dissolved oxygen concentration at various depths were measured with standard equipment. Water samples were collected with a 2-litre capacity Friedinger-type water samples, and transferred to sterile containers for subsequent chemical and microbiological examination. The pH of each sample was determined at the point of sampling.

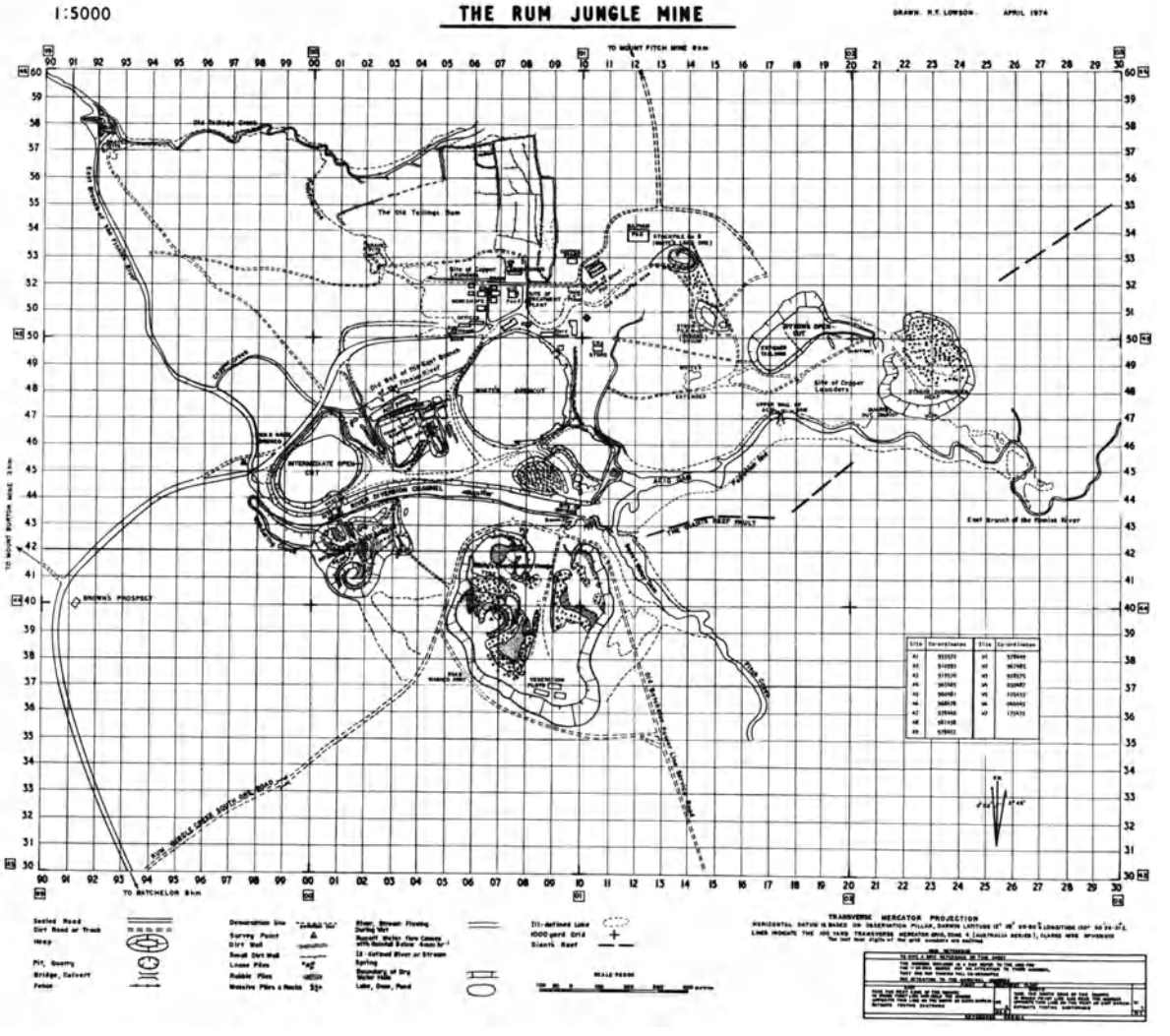


Figure 1: Map of Rum Jungle Mine area, Northern Territory, Australia. Reproduced

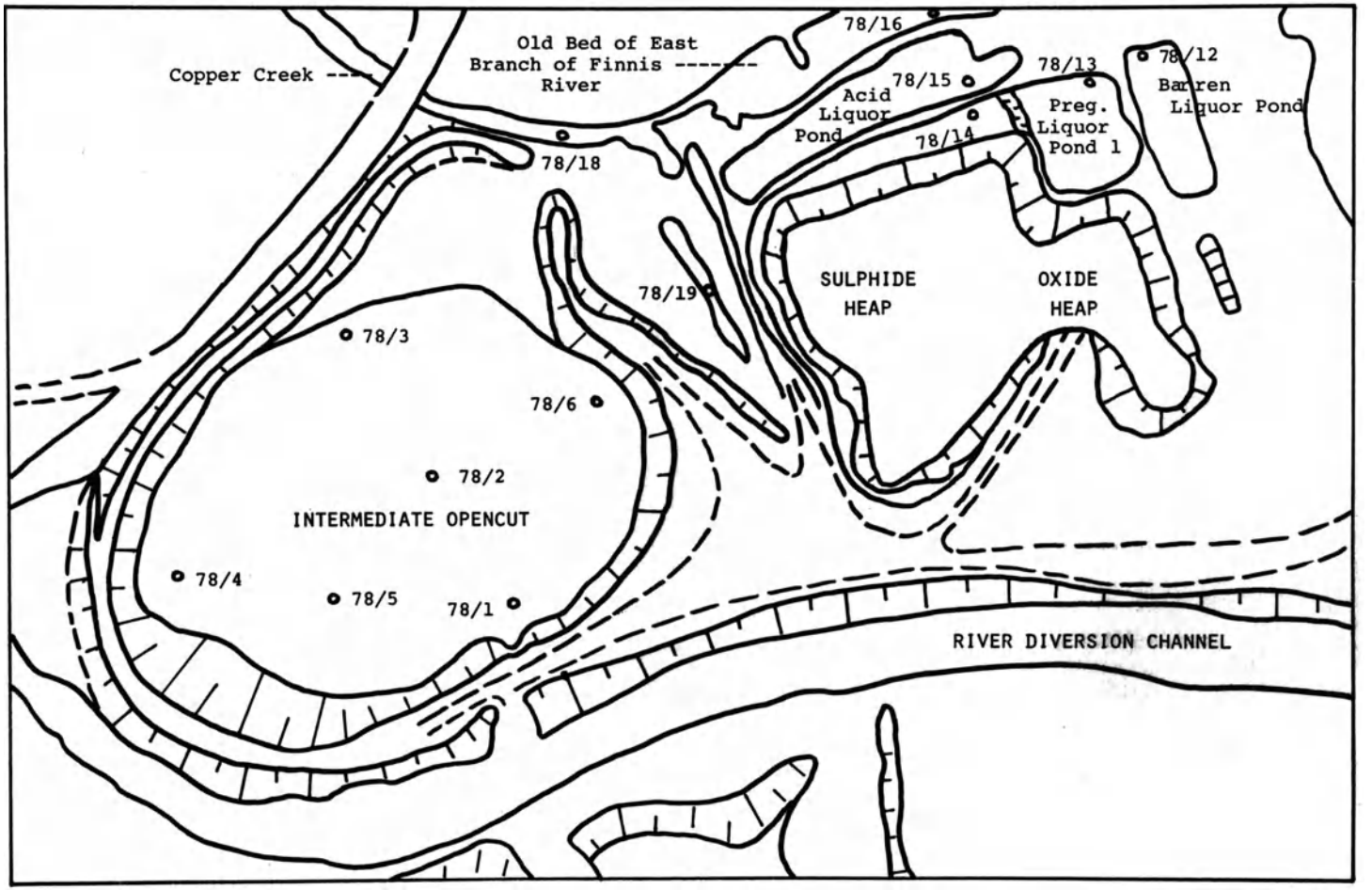
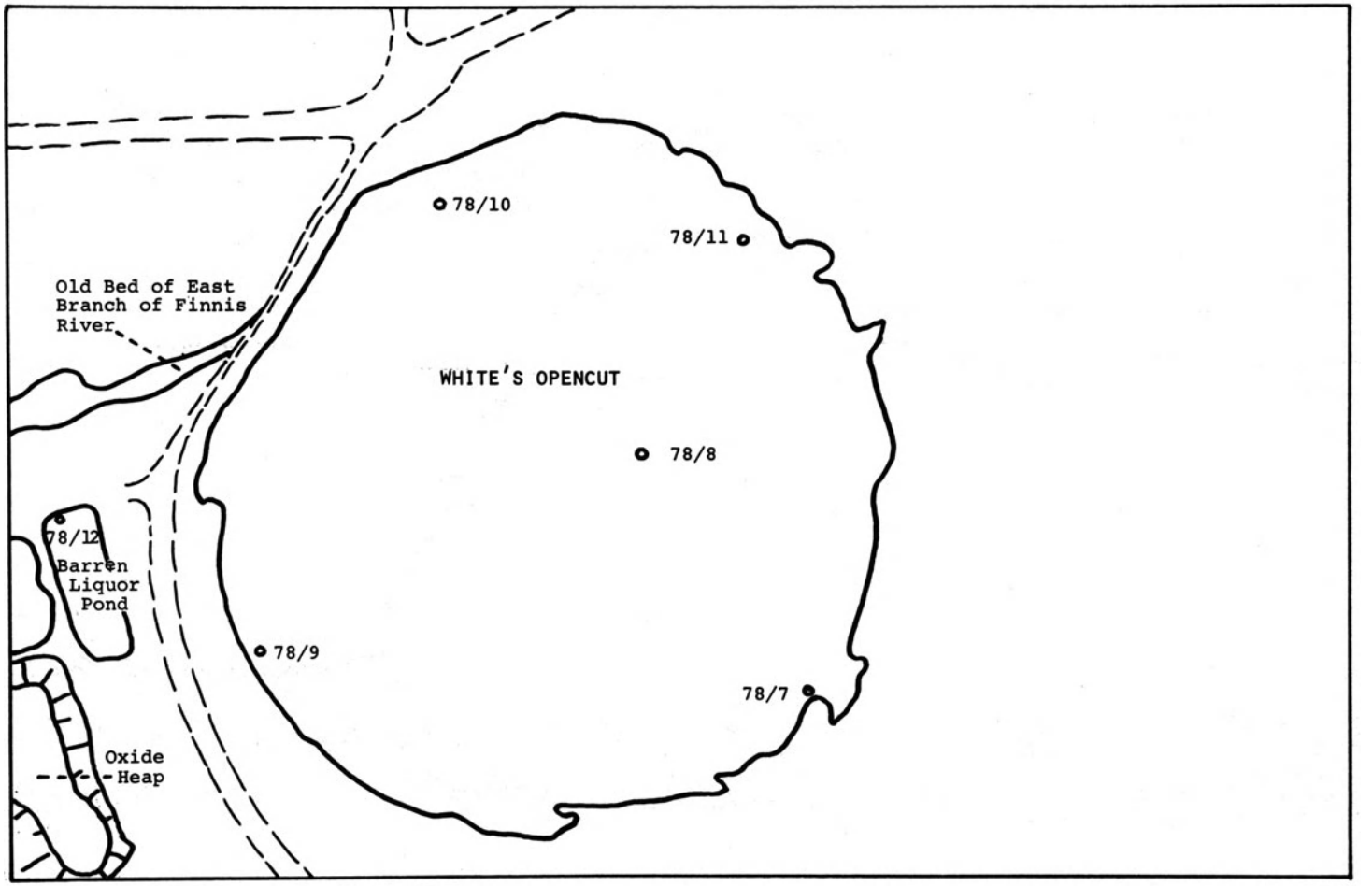


Figure 2: Sampling points in the Intermediate Opencut and associated water systems.


78/10
78/11
Old Bed of East
Branch of Finnis
River
WHITE'S OPENCUT
78/8
78/12
Barren
Liquor
Pond
78/9
78/7
Oxide
Heap

Within two hours of sampling, aliquots of each sample were membrane-filtered and both filtrate and separated microbial populations cooled to 4° for transport to Sydney. Chemical analysis and the enumeration of various microbial population types were carried out by the methods described by Khalid and Ralph (1976b) and Goodman and Ralph (1978).

RESULTS AND DISCUSSION

The physico-chemical and microbiological data respectively for the samples taken from the Intermediate Opencut are shown in Tables 1 and 2, and the similar data for the White's Opencut samples in Tables 3 and 4. The associated water systems data are set out in Tables 5 and 6.

Intermediate Opencut

The water solubles content of the samples ranged from 3780 ppm to 6760 ppm with no discernible patterns of distribution. The bottom samples did not contain a markedly higher solute concentrations than other samples taken in particular vertical profiles. The pH of samples ranged from 3.5 to 2.6, the most acidic areas being sites 78/4 and 78/6. The former site is that of the exit point of the original river bed; the latter is the closest point to the old leaching heaps. It will be noted that the soluble iron content of all samples was equal to or less than 1 ppm while the copper contents ranged between 85 and 165 ppm and zinc content between 4 and 7 ppm. A semi-quantitative examination of samples by an energy dispersive X-ray analytical system indicated high levels of magnesium, calcium and manganese, and lower levels of aluminium, silicon and chloride. The temperature profiles show a consistent slight fall from surface to bottom. Dissolved oxygen concentrations show the same pattern without sharp breakpoints.

Although the levels of soluble iron were very low, *T.ferrooxidans* populations were detected in every samples. The numbers increased markedly in the bottom samples. Sulfur-oxidizing organisms grown at pH 3.5 were detected in only two samples (78/2/12.5 and 78/2/58; middle of opencut) but those growing at pH 4.8 on thiosulfate were relatively abundant. At every site these organisms were plentiful in the bottom samples except in 78/1/1 where they occurred near the surface. Sulfur oxidizers growing at pH 6.2 on thiosulfate were not as widespread as those growing at pH 4.8 although the bottom samples from sites 78/4, 78/6 and especially 78/2 contained substantial populations.

It is interesting to note that sample 78/2/58, which is the bottom sample from the centre of the opencut, contained extremely high sulfur-oxidizing bacterial populations and also high numbers of acidophilic heterotrophs which in the main occurred only in samples at depth. The only non-acidophilic heterotrophs detected were in the bottom samples at site 78/6.

Unicellular organisms belonging to the algal genus *Chlorella* were found throughout the water of the opencut. Green colonies were isolated on acidophilic-heterotroph and thiosulfate (pH 4.8) agar plates.

All bottom samples contained viable cells of the anaerobes *Desulfovibrio* and *T.denitrificans* (except 78/3/3 in which *T.denitrificans* could not be detected). Although the dissolved oxygen concentration in the bottom samples was not less than 2.0 ppm, as measured just above the sediment, anaerobic or micro-aerophilic conditions must occur close under the sediment surface.

White's Opencut

The results show a considerable difference in both physico-chemical factors and microbial distribution between the samples from the Intermediate and White's Opencuts. In White's Opencut, the water solubles content of the samples ranged from 7160 ppm to 14,540 ppm; that is, the spread of concentrations is much more marked and all samples contained a higher level of solutes than any from the Intermediate Opencut. Further, the concentration of solutes in White's Opencut samples was highest in the bottom samples, and in all areas except 78/9, the concentration in the micro-aerophilic zone was higher than in the upper aerobic zone. This oxygen zonation is a significant feature of the waters of White's Opencut and has been previously observed by the A.A.E.C. workers and the Water Resources Branch team. The interface is relatively sharp with aerobic conditions (D.O. about 6 ppm) in the upper 4-4.5 m and dropping markedly in a metre or less to micro-aerophilic conditions (D.O. 2.0 - 0.7 ppm) below 5-5.5 m depth.

TABLE 1

INTERMEDIATE OPENCUT WATER SAMPLES -
PHYSICO-CHEMICAL DATA

Sample No.[a]	Temp. (°C)	D.O. (ppm)	pH	Total Solids (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)
78/1/1	26.8	7.4	3.1	6180	0.75	145	6
10	25.9	8.0	3.2	6760	1.0	125	4
12.5	25.0	5.2	3.2	4680	0.5	100	5
15/S	24.0	3.8	3.1	5400	nd	165	6
78/2/1	25.5	7.4	3.25	4400	0.6	120	6
10	24.0	4.3	3.2	4940	0.5	113	6
12.5	24.0	3.7	3.4	5360	0.5	120	6
58/S	23.5	3.8	3.5	6280	0.5	95	7
78/3/1	25.2	7.1	3.0	4360	0.75	125	7
2	25.2	7.4	3.0	3840	0.5	135	7
3/S	25.1	7.0	3.1	4100	0.75	125	6
78/4/1	25.3	6.0	2.7	3780	0.75	110	6
10	25.2	6.3	2.9	3940	0.5	95	6
12.5	24.0	4.5	3.0	4940	0.5	105	6
15/S	24.0	4.5	3.2	4180	nd	105	7
78/6/1	25.2	5.4	2.9	4200	0.7	125	6
12.5	24.5	5.1	2.8	5000	0.5	113	6
15	23.5	2.6	3.0	4920	0.5	113	7
28/S	23.2	2.0	2.6	5440	0.75	85	7

[a] Sample Code: Year/Location No./Depth in metres/S - Sediment

[b] nd = Not detected

TABLE 2

INTERMEDIATE OPENCUT WATER SAMPLES -
MICROBIOLOGICAL DATA

Sample No.	D.O. (ppm)	pH	Microbial Populations - Cells $ml^{-1} \times 10^{-3}$								
			A	B	C	D	E	F	G	H	J
78/1/1	7.4	3.1	0.05	nd	14.5	0.02	2.2	nd	-	-	0.72
10	8.0	3.2	0.006	nd	nd	nd	nd	nd	-	-	nd
12.5	5.2	3.2	0.008	nd	0.07	nd	nd	nd	-	-	1.18
15/S	3.8	3.1	5.35	nd	0.41	0.2	0.6	nd	+	+	1.16
78/2/1	7.4	3.25	0.02	nd	0.64	nd	nd	nd	-	-	0.86
10	4.3	3.2	0.03	nd	0.01	nd	nd	nd	-	-	0.03
12.5	3.7	3.4	0.04	nd	3.5	0.02	nd	nd	-	-	8.0
58/S	3.8	3.5	1.3	nd	500	500	500	nd	+	+	nd
78/3/1	7.1	3.0	0.002	nd	nd	nd	nd	nd	-	-	0.1
2	7.4	3.0	0.01	nd	nd	nd	nd	nd	-	-	nd
3/S	7.0	3.1	4.15	nd	1.2	0.43	0.42	nd	+	+	0.02
78/4/1	6.0	2.7	0.02	nd	nd	nd	nd	nd	-	-	nd
10	6.3	2.9	0.01	nd	nd	nd	nd	nd	-	-	nd
12.5	4.5	3.0	0.007	nd	0.57	0.04	0.21	nd	-	-	2.2
15/S	4.5	3.2	2.0	nd	10.0	5.6	11.0	nd	+	+	0.27
78/6/1	5.4	2.9	0.006	nd	nd	nd	nd	nd	-	-	nd
12.5	5.1	2.8	0.02	nd	0.01	nd	nd	nd	-	-	0.01
15	2.6	3.0	0.01	nd	nd	nd	nd	nd	-	-	nd
28/S	2.0	2.6	2.4	nd	8.5	2.0	5.5	0.05	+	+	nd

A *Thiobacillus ferrooxidans*.
B Sulfur oxidisers (growing on thiosulfate at pH 3.5).
C Sulfur oxidisers (growing on thiosulfate at pH 4.8).
D Sulfur oxidisers (growing on thiosulfate at pH 6.2).
E Acidophilic heterotrophs (Manning's glucose-salts-YE medium).
F Non-acidophilic heterotrophs (Nutrient agar).
G *Desulfovibrio* spp.
H *T.denitrificans*.
J *Chlorella* spp.

TABLE 3

WHITE'S OPENCUT WATER SAMPLES -
PHYSICO-CHEMICAL DATA

Sample No.	Temp. (°C)	D.O. (ppm)	pH	Total Solids (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)
78/7/1	23.5	6.2	2.4	9880	125	75	5
4	23.5	6.0	2.2	9640	125	85	6
5	25.5	1.3	2.1	12800	225	115	6
8/S	27.0	0.7	1.8	13420	325	80	6
78/8/1	24.0	6.3	2.5	8740	125	103	6
4.5	23.5	6.2	2.6	7520	125	100	6
6	26.5	1.5	2.4	10900	250		
40/S	25.0	1.3	2.2	14540	525	80	6
78/9/1	24.0	6.0	2.6	8100	200	120	5
4	23.2	5.8	2.5	8460	150	100	6
5	25.0	1.2	2.5	7440	100	135	6
8/S	25.2	1.1	2.3	13400	400	75	6
78/10/1	24.8	6.0	2.6	7260	125	70	6
4	23.3	5.9	2.6	7160	150	138	6
5	24.8	1.1	2.4	8940	200	80	6
12/S	25.0	2.0	2.2	14020	500	90	6
78/11/4	23.3	5.7	2.5	5800	125	80	6
5	24.8	1.2	2.4	7360	225	80	6
14/S	25.0	0.8	2.2	11700	500	90	6

TABLE 4

WHITE'S OPENCUT WATER SAMPLES -
MICROBIOLOGICAL DATA

Sample No.	D.O. (ppm)	pH	Microbial Populations - Cells $ml^{-1} \times 10^{-3}$								
			A	B	C	D	E	F	G	H	J
78/7/1	6.2	2.4	0.04	nd	0.12	nd	nd	nd	-	-	0.24
4	6.0	2.2	0.05	nd	0.16	nd	nd	nd	-	-	0.38
5	1.3	2.1	0.05	nd	1.24	nd	nd	nd	-	-	0.38
8/S	0.7	1.8	1.65	nd	79	nd	0.12	nd	+	-	8.1
78/8/1	6.3	2.5	0.05	nd	0.12	nd	0.03	nd	-	-	0.24
4.5	6.2	2.6	0.16	nd	0.02	nd	nd	nd	-	-	0.02
6	1.5	2.4	0.60	nd	0.25	nd	nd	nd	-	-	0.22
40/S	1.3	2.2	2.25	nd	25	nd	nd	0.1	+	-	0.16
78/9/1	6.0	2.6	0.16	nd	0.03	0.02	nd	nd	-	-	0.02
4	5.8	2.5	0.12	nd	0.04	nd	nd	nd	-	-	0.02
5	1.2	2.5	0.08	nd	0.12	1.0	nd	nd	-	-	nd
8/S	1.1	2.3	815	nd	500	nd	0.37	0.05	+	-	2.5
78/10/1	6.0	2.6	0.2	nd	nd	nd	nd	nd	-	-	nd
4	5.9	2.6	0.05	nd	0.07	0.04	nd	nd	-	-	0.04
5	1.1	2.4	0.08	nd	0.07	nd	nd	nd	-	-	0.08
12/5	2.0	2.2	19.5	nd	10	nd	nd	nd	-	-	1.0
78/11/4	5.7	2.5	0.07	nd	nd	nd	nd	nd	-	-	nd
5	1.2	2.4	0.22	nd	0.1	nd	nd	nd	-	-	nd
14/S	0.8	2.2	33.2	nd	2.0	nd	nd	nd	+	-	0.25

A *Thiobacillus ferrooxidans*.
B Sulfur oxidisers (growing on thiosulfate at pH 3.5).
C Sulfur oxidisers (growing on thiosulfate at pH 4.8).
D Sulfur oxidisers (growing on thiosulfate at pH 6.2).
E Acidophilic heterotrophs (Manning's glucose-salts-YE medium).
F Non-acidophilic heterotrophs (Nutrient agar).
G *Desulfovibrio* spp.
H *T. denitrificans*.
J *Chlorella* spp.

TABLE 5

ASSOCIATED WATER SYSTEMS -
PHYSICO-CHEMICAL DATA

Sample No. and Location	pH	Total Solids (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)
78/12 Barren Liquor Pond	2.8	4320	100	425	3
78/13 Pregnant Liquor Pond 1	2.1	7940	400	750	5
78/14 Pregnant Liquor Pond 2	2.6	107140	8250	25000	30
78/15 Acid Liquor	2.6	9480	300	700	5
78/16 Old Bed of East Branch of Finnis River	3.0	7640	25	205	5
78/18 Pond - Copper Creek	3.0	7520	52	171	6
78/19 Sample Ditch	2.9	22220	300	1170	13
78/17 Rum Jungle Creek S. - Composite sample	7.1	5800	nd[a]	nd[a]	nd[a]

[a] nd = not detected

TABLE 6

ASSOCIATED WATER SYSTEMS -
MICROBIOLOGICAL DATA

Sample No. and Location	pH	Microbial Populations - Cells $ml^{-1} \times 10^{-3}$								
		A	B	C	D	E	F	G	H	J
78/12 Barren Liquor Pond	2.8	0.02	nd	nd	nd	nd	nd	-	-	nd
78/13 Pregnant Liquor Pond 1	2.7	0.6	nd	0.37	nd	nd	nd	-	-	nd
78/14 Pregnant Liquor Pond 2	2.6	75	nd	3.6	nd	1.2	nd	-	-	nd
78/15 Acid Liquor Pond	2.6	0.02	nd	0.81	nd	nd	nd	-	-	nd
78/16 Old Bed of East Branch of Finnis River	3.0	0.05	nd	nd	nd	0.4	nd	-	-	0.6
78/18 Pond - Copper Creek	3.0	0.03	nd	7.5	nd	nd	nd	-	-	nd
78/19 Sample Ditch	2.9	5.5	nd	nd	nd	0.09	nd	-	-	0.04
78/17 Rum Jungle Creek S. Opencut - Composite sample	7.1	nd	nd	nd	nd	nd	nd	-	-	nd

A *Thiobacillus ferrooxidans*.

B Sulfur oxidisers (growing on thiosulfate at pH 3.5).

C Sulfur oxidisers (growing on thiosulfate at pH 4.8).

D Sulfur oxidisers (growing on thiosulfate at pH 6.2).

E Acidophilic heterotrophs (Manning's glucose-salts-YE medium).

F Non-acidophilic heterotrophs (Nutrient agar).

G *Desulfovibrio* spp.

H *T.denitrificans*.

J *Chlorella* spp.

Unlike the Intermediate Opencut samples, the samples from White's Opencut showed a high soluble iron content (100 - 252 ppm) with the highest concentrations in the bottom samples Also, except for site 78/9, the concentration of iron in the samples below the oxygen concentration interface was almost double that of samples taken immediately above the interface. The soluble copper levels (70 - 135 ppm) and zinc levels (5 - 6 ppm) were similar to those found in the Intermediate Opencut samples. EDAX examinations showed high levels of magnesium, aluminium, calcium and manganese and lower levels of sodium, silicon, silver and chloride.

The pH of the water samples was significantly lower than in the Intermediate Opencut (2.6 1.8) and decreased from surface to bottom at every sampling site. It is noteworthy also that the temperature profile, while of the same dimensions as that in the Intermediate Opencut, consistently showed higher values at depth than at the surface.

Thiobacillus ferrooxidans populations occurred in all samples with the highest levels in the bottom samples. That from sample site 78/9 contained the highest numbers of the majority of microorganism types with *T.ferrooxidans* the most abundant.

No extreme acidophilic sulfur oxidizers were isolated, but the majority of sites yielded sulfur oxidizers at pH 4.8, some in very high numbers as in samples 78/7/8 and 78/9/8. It is interesting to note that at every site except 78/10, the levels of these organisms increased by an order of magnitude from the sample directly above the interface to the sample immediately below it. Again, the bottom samples supported the highest population levels. Higher pH sulfur oxidizers were detected in only three samples. Acidophilic and non-acidophilic heterotrophs were scarce, being found in only three and two samples respectively.

Desulfovibrio spp. were detected in the bottom samples from every site except 78/10, but unlike the samples from the Intermediate Opencut those from White's Opencut did not contain *T.denitrificans*. The pH of the bottom sediments in White's Opencut is probably too low for the growth of this organism.

The occurrence of *Chlorella* in virtually all samples, including the bottom sediments, is surprising. It has been noted, however, that these organisms, from both opencuts, can develop green colonies under conditions of minimal illumination and may be capable of heterotrophic growth in the dark.

Associated Water Systems

The associated water systems comprising the old collection ponds of the leaching heaps, the old bed of the East Branch and Copper Creek contain small volumes of water as compared with the two opencuts and during the dry season may almost completely dry up. The leachin heaps are still yielding iron, copper, zinc and other metals at a low rate and this circum stance together with the evaporative effect no doubt explains the very high soluble metal content at some sampling points (e.g. 78/14, with 8 and 25 g l^{-1} of iron and copper, respectively). EDAX analysis of the sample from 78/14 showed substantial levels of magnesium, aluminium, silicon, phosphorus, calcium, manganese and nickel).

T.ferrooxidans was present in all samples and sulfur oxidizers (pH 4.8), acidophilic heterotrophs and *Chlorella* were present in a number of samples.

By comparison with the samples from the opencuts and the associated water systems, Tables 5 and 6 contain data on a composite sample from the Rum Jungle Creek South Opencut. It will be noted that although the water solubles concentration is comparable to that from the Intermediate Opencut samples, no soluble iron, copper or zinc were detected. The pH was at neutrality and no bacterial populations were isolated on any of the media employed.

CONCLUSION

It might be expected that the water quality in a flooded opencut would deteriorate and become polluted with acid and soluble metal salts if:

(i) Exposures of sulfide-bearing materials occur in the walls and/or the floor,

(ii) The opencut acts as sump for polluted water from ground drainage systems or the drainage from nearby sulfidic overburden heaps, or

(iii) Sulfidic tailings or residues from extractive processes have been dumped into the opencut and subsequently become water-covered.

The first two factors could apply in the case of the Intermediate Opencut; all three are likely contributors to the deterioration of water quality in White's Opencut. The level of the autotrophic sulfur- and iron-oxidising populations in both opencuts suggests that sulfidic material is still in process of biodegradation and that the final levels of acidic and metallic pollution have not yet been reached. Earlier data on the copper concentrations in both opencuts (Davy, 1975; Khalid and Ralph, 1976) indicate that a significant increase has occurred over the past three years.

In the Intermediate Opencut, the substantial population levels of *T.ferrooxidans* and of sulfur-oxidizing bacteria in the bottom samples suggests some remaining exposures of sulfides; the aerobic status and the pH level would favour continued biodegradation. In White's Opencut, the significant iron levels but low *T.ferrooxidans* populations in the aerobic region above the oxygen concentration interface suggests a slow penetration of ferrous iron into this zone. The high levels of *T.ferrooxidans* populations and of some sulfur-oxidizers in the deeper, micro-aerophilic regions gives rise to the speculation that these organisms could be facilitating the oxidation of residual sulfides in the tailings layer, using ferric iron as an electron acceptor (Brock and Gustafson, 1976). The higher temperature at the bottom of White's Opencut is probably maintained by metabolic heat generation and induces an upward convective flow which carries ferrous iron from the micro-aerophilic zone to the surface aerobic regions.

This preliminary examination has been fruitful in providing a general picture of the microbial status of the two most polluted opencuts and is useful as a basis for the planning of long-term, systematic investigation of the mechanisms by which deterioration of water quality in flooded opencuts occurs. It is clear that the dumping of sulfidic tailings into an opencut may be a most dubious procedure and that an oxygen-depleted water cover provides no guarantee that microbially-catalysed degradation of sulfides will not continue to occur.

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FACTORS IN METAL LEACHATE TOXICITY

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One of the by-products of oil production in the Athabasca oil sands, Canada, and indeed in most petroleum refining operations, is coke. At the Great Canadian Oil Sands plant there is a net production of about 500 Mg day^{-1} over and above that which is burned for fuel. This residual coke is currently stockpiled and could constitute an environmental hazard, depending on the susceptibility of elements in the coke to "natural" leaching. In order to assess this potential problem a number of cokes were leached with water and the toxicity of the leachates were examined (Zajic *et al.*, 1978).

In order to relate the results to species present in solution, the toxic behaviour of the individual components of the leachates must be known. Rainbow trout are usually employed as the test organism for toxicity bioassays but interest is being shown in the freshwater microcrustacean *Daphnia magna*; the use of this waterflea has certain economic advantages, such as low cost, small space requirements and short duration of tests. These are static bioassay tests. The *Daphnia* are placed in a standard test medium, in which the appropriate metal salt has been equilibrated. The pH should be as low as practicable, to minimize hydroxide precipitation; the particular medium used for these tests contained essentially the acetate anion with mineral nutrients at pH 7 (see below, Table 2).

The toxicity of a number of metals which occur in water or acid leachates of the cokes was examined; the results are presented as toxicity curves, with the median survival time plotted against total metal concentration (Figs. 1 and 2). A useful comparison for relative toxicity is the 8 h LC50; this is the concentration of toxicant which kills 50% of the *Daphnia* in 8 h (Table 1).

TABLE 1

TOXICITY OF METAL IONS TOWARDS *DAPHNIA MAGNA*

Metal	8hr-LC50	
	(mg l^{-1})	mol l^{-1}
Cu(II)	0.01	1.6×10^{-7}
Cr(VI)	0.08	1.5×10^{-6}
Cd(II)	3.0	2.7×10^{-5}
Ni(II)	25	4.3×10^{-4}
V(V)	30	5.9×10^{-4}
Zn(II)	150	2.3×10^{-3}
Mn(II)	200	3.6×10^{-3}
Co(II)	400	6.8×10^{-3}
Sr(II)	c. 2000	c. 2.3×10^{-2}
Na(I)	c. 3300	c. 1.4×10^{-1}
Mo(VI)	c. 4000	c. 4.2×10^{-2}

TABLE 2

ANALYSIS[a] OF WATER LEACHATES, TEST MEDIUM AND PONDWATER

Element[b]	Standard Test Medium	GCOS[c]	Flexi-coker[d]	Syncrude[e]	Calif.[e]	Pond-water
Ag	--	ND	NA	0.005	0.011	ND
Al	--	0.08	0.09	0.70	ND	0.16
B	--	ND	0.108	2.14	ND	NA
Ba	--	ND	ND	0.006	0.15	0.10
Be	--	ND	0.005	0.007	ND	ND
Ca	58.2	34.4	38.3	12.8	2.85	36.3
Cd	--	ND	ND	ND	0.09	0.002
Co	--	ND	1.8	0.03	0.07	ND
Cr	--	0.032	ND	ND	ND	0.008
Cu	--	ND	0.03	ND	0.022	0.01
Fe	--	ND	ND	0.008	0.024	0.654
K	11.7	1.5	ND	3.1	3.8	3.6
Mg	--	17.8	10.4	2.4	0.329	4.14
Mn	--	ND	2.75	0.032	ND	ND
Mo	--	0.25	ND	0.9	ND	NA
Na	56.1	44	ND	6	22	12
Ni	--	ND	252	ND	ND	ND
Si	--	46.3	12.4	11.0	NA	4.9
Sr	--	0.184	0.477	0.0734	0.0075	0.0637
Th	--	ND	NA	0.04	0.061	ND
Ti	--	ND	ND	ND	0.003	0.006
V	--	0.079	1.82	7.04	0.287	ND
Zr	--	ND	NA	ND	0.009	ND
Zn	--	ND	1.60	ND	ND	ND

[a] Concentration in ppm.

[b] Analysis by radio frequency plasma emission spectrometry.

[c] Duration of leach, 5 months.

[d] Duration of leach, 2 months.

[e] Duration of leach, 1 month.

NA: Not determined.

ND: Not detected

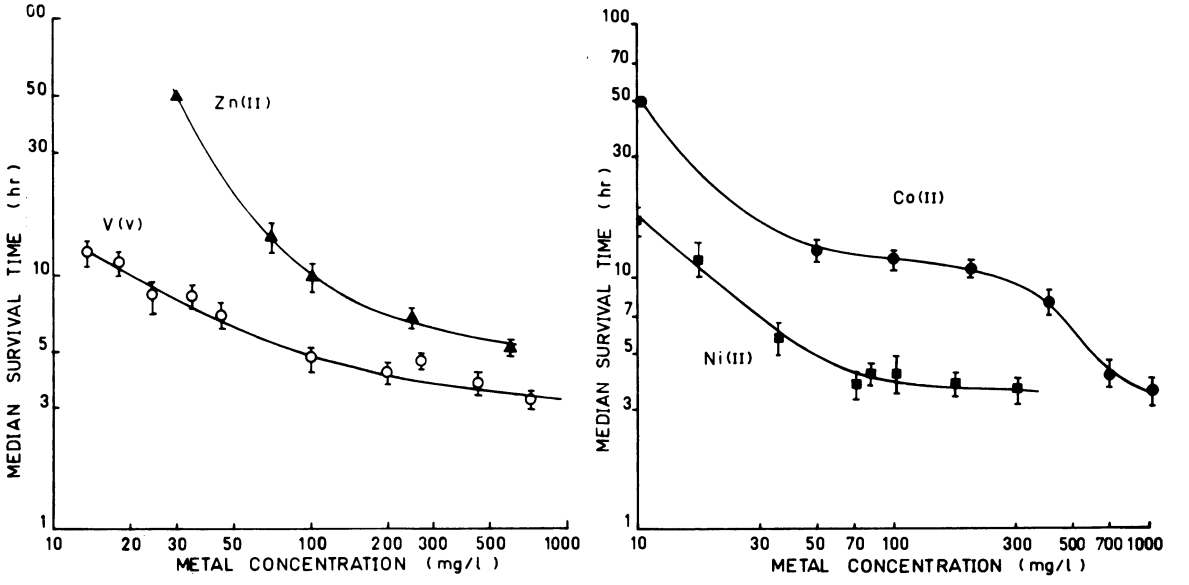


Figure 1: The toxicity of zinc(II), vanadium(V), cobalt(II) and nickel(II) towards *Daphnia magna*.

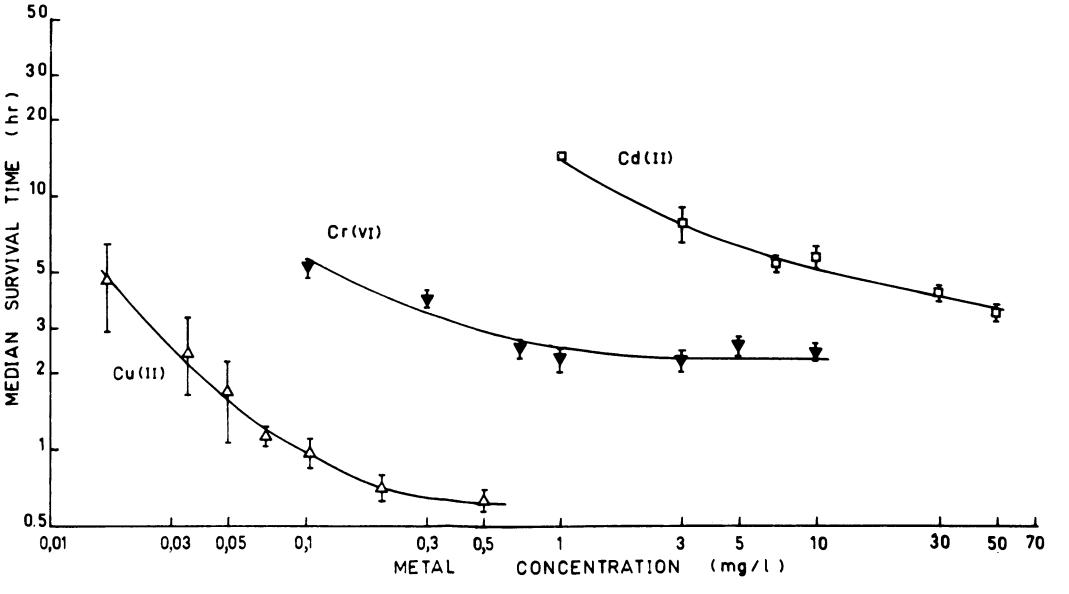


Figure 2: The toxicity of copper(II), cadmium(II) and chromium(VI) towards *Daphnia magna*.

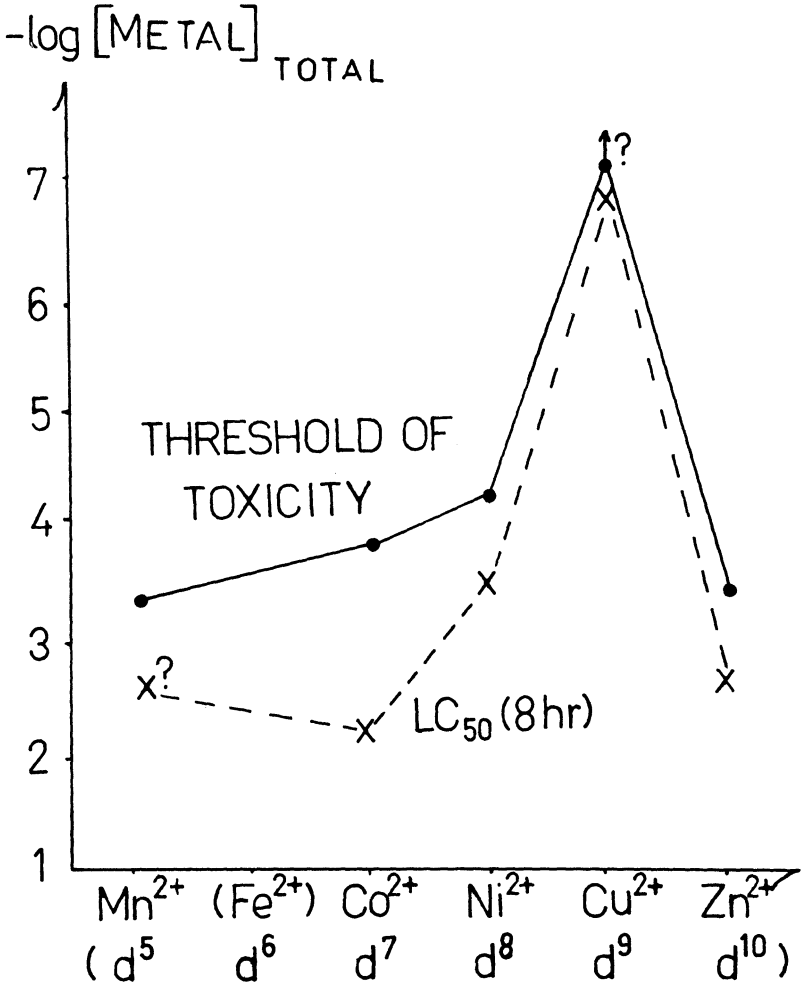


Figure 3: The toxicity of metals towards *Daphnia magna*, as a function of the electron configuration of the divalent cations.

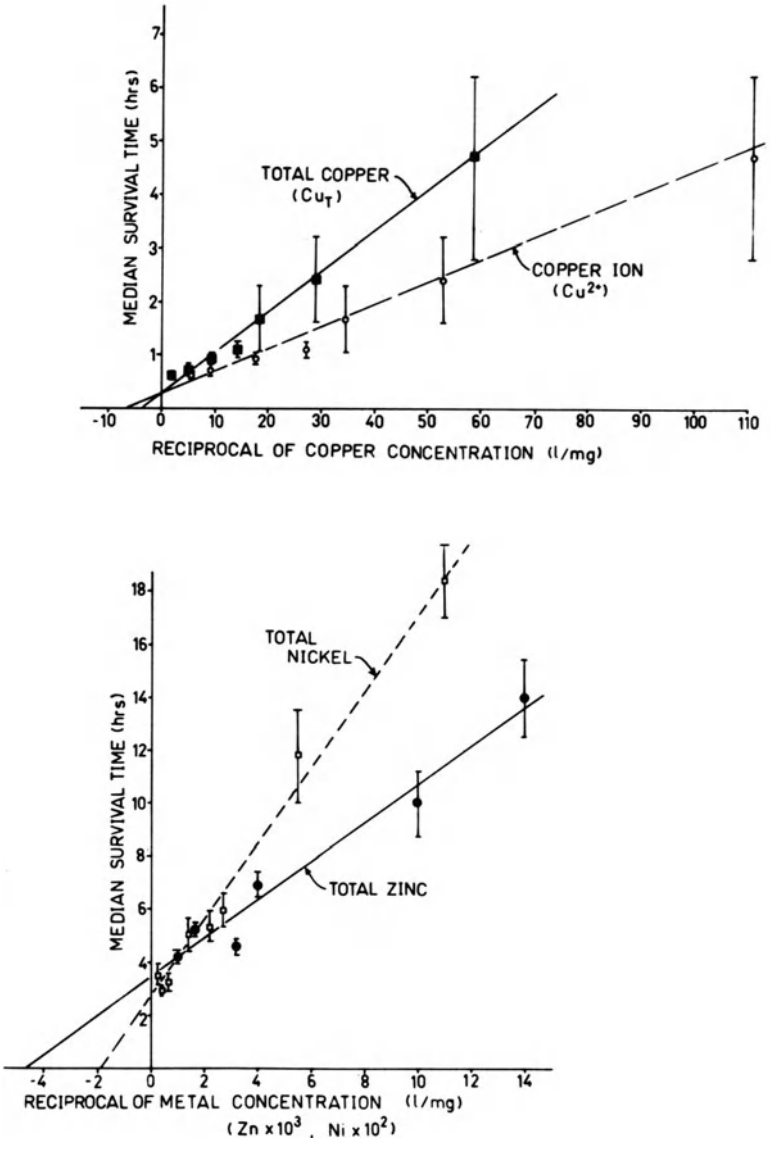
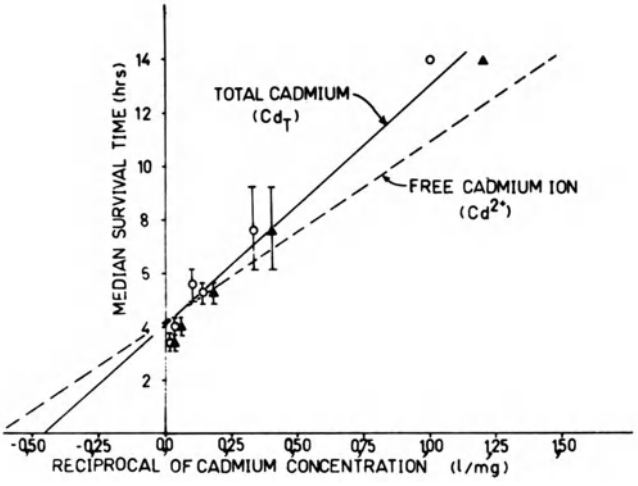


Figure 4: Reciprocal-concentration plots for the toxicity of nickel(II), copper(II) and zinc(II) towards *Daphnia magna*.



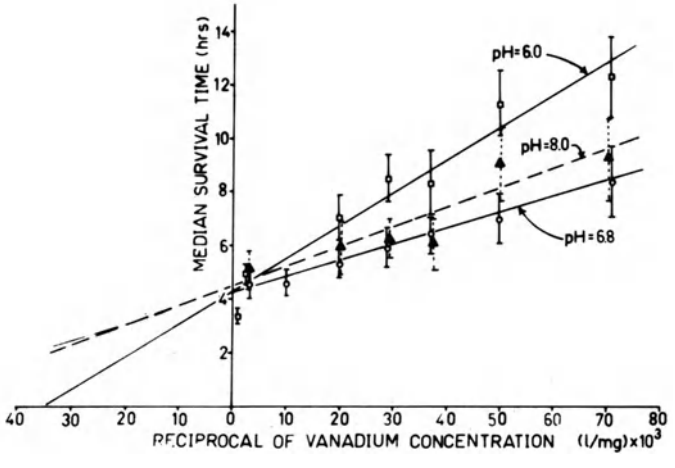


Figure 5: Reciprocal-concentration plots for the toxicity of cadmium(II) and vanadium(V) towards *Daphnia magna*.

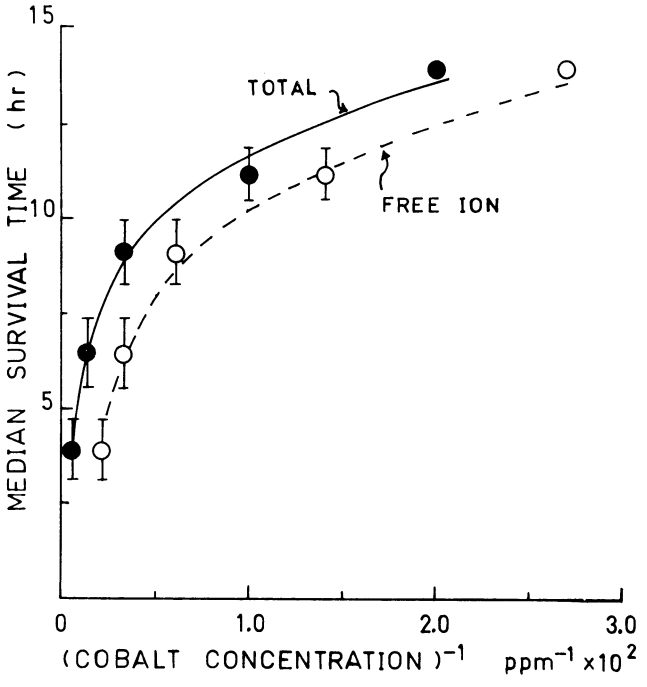


Figure 6: Reciprocal-concentration plot for the toxicity of cobalt(II) towards *Daphnia magna*.

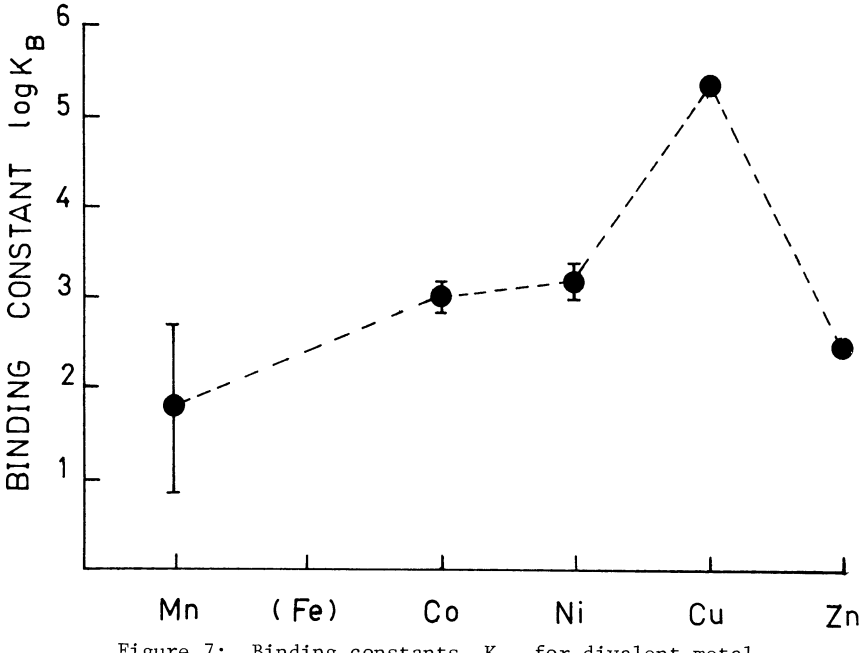


Figure 7: Binding constants, K_B, for divalent metal cations towards *Daphnia magna*.

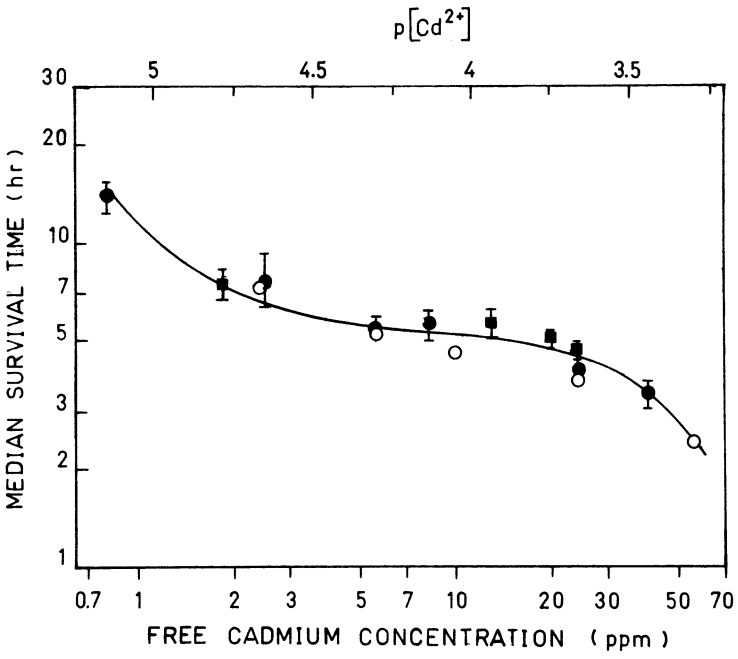


Figure 8: The toxicity of "free" cadmium ion, Cd^{2+}, towards *Daphnia magna*. Concentration of Cd^{2+} calculated for cadmium(II) in standard test medium (●), in standard test medium containing NTA (□), in pondwater (O).

The order of toxicity is interesting, particularly for the divalent cations of the first transition series. In Fig. 3, the metals show a relative toxicity Mn < Co < Ni < Cu > Zn and this relationship, with copper having the maximum toxicity, has also been observed for a number of other test species (Shaw, 1961). Moreover, the relationship follows the so-called "natural order" (Irving and Williams, 1953), as observed for the stability constants of complexes of the divalent transition metal cations. This order can be explained in terms of fundamental electronic properties of the metal ions concerned and it has been found to be largely independent of the nature of the ligands (Rossotti, 1960) The implication of these observations is that metal toxicity might be viewed empirically in terms of a simple metal-ligand interaction involving the binding of metals to various biological molecules such as enzymes (Sahe, 1954, 1961; Williams, 1977). Although certainly an oversimplification, this thermodynamic approach can provide a useful rationalization for metal toxicological behaviour.

Take, for example, perhaps the simplest model for metal toxicity towards *Daphnia*: the reversible binding of a metal at a single site of uptake, which leads directly to mortality. This can be expressed as follows:

$$\text{Metal} + \textit{Daphnia} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\text{Metal}\cdot\textit{Daphnia}] \overset{k_2}{\rightarrow} \textit{Daphnia}\ \text{mortality} \qquad (1)$$

By conventional mathematical treatment

$$\frac{V}{V_{max}} = \frac{c}{K + c}, \quad K = \frac{k_{-1} + k_2}{k_1} \qquad (2)$$

where c is the concentration of metal ion and V, the *Daphnia* mortality rate. K is a composite of the individual rate constants. Assuming that the median survival time, T, is inversely proportional to the mortality rate (T_{min} is inversely proportional to V_{max}) and that $k_2 << k_{-1}$, then

$$\frac{T_{min}}{T} = \frac{c}{c + 1/K_B} \qquad (3)$$

where K_B is the metal binding constant, k_1/k_{-1}.

Equation 3 still represents a rectangular hyperbola and most of the popular methods of presenting toxicity data can be viewed as variations on this theme. In particular, rearrangement of (3) yields T as a linear function of 1/c, i.e.

$$T = T_{min} + (T_{min}/K_B) \cdot 1/c \qquad (4)$$

This plot accommodates much of the toxicity data quite well (Figs. 4 and 5). Other results deviate quite markedly from a straight line (Fig. 6) but this is hardly surprising, in view of the assumptions inherent in this simple model.

Approximate values for the binding constants of the metals towards *Daphnia* can be calculated from the intercept ($-K_B$) on the 1/c axis. The trend of these constants is seen to follow the Irving-Williams series (Fig. 7).

Recent studies indicate that, for metals in aqueous solution, the toxic species may be the "free" (i.e. hydrated) metal ion (Sunda and Guillard, 1976; Sunda *et al.*, 1978; Andrew *et al.*, 1977.) In the presence of chelating agents, e.g. EDTA and NTA, which decrease concentration of free metal ion, metal toxicity is substantially reduced (Sprague, 1968; Biesinger *et al.*, 1974). The concentration of solution species can be readily calculated by computer programs such as REDEQL II, if the relevant stability data are known. The results of such a computation are incorporated into Fig. 8. The experimental points show the calculated concentrations of free cadmium ion in various solutions: a standard test medium; test medium with NTA; a natural pondwater (assumed non-complexing towards cadmium). For all three cases, the resultant toxicity is practically the same as that due solely to the amount of free cadmium in solution.

With a knowledge of the toxicity of the individual metals, the toxicity of water leachates from the cokes can be assessed. Since some of the metals, e.g. copper, are very toxic, even at trace levels, it is important to obtain an accurate and comprehensive analysis of the solutions; this was done using radio frequency, argon plasma emission spectrometry, and the composition of the leachates is shown in Table 2.

The water leachate from delayed coker coke (GCOS) is non-toxic as might be expected from the general absence of toxic elements. "Flexicoking" is an experimental fluid coking process developed by Exxon but not used in Canada. The coke (here derived from Athabasca bitumen) is seen to be quite susceptible to water leaching (N.B. nickel concentration). The leachate is quite toxic to *Daphnia*, in fact more so than the nickel content would indicate; the toxicity is probably associated with the copper content. Interestingly, the leachate from a conventional fluid coker coke (derived from a Californian crude) is non-toxic although it contains a comparable amount of copper as does the flexicoke leachate. The presence of trace amounts of organic material capable of binding small amounts of toxic metals might account for this non-toxic behaviour. This tendency is likely to be more pronounced for the natural environment, on account of the presence of particulate matter, humic material or other soluble complexing agents, and a comprehensive assessment of toxicity must eventually incorporate all these factors.

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BIOLOGICAL METHODS TO REMOVE SELECTED INORGANIC POLLUTANTS FROM URANIUM MINE WASTEWATER

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INTRODUCTION

In many mining operations the mines are de-watered, i.e. water is pumped from the mines to prevent flooding of operations. The water often contains low concentrations of the elements being mined as well as other associated ions. These waters must be treated to meet governmental regulatory standards before being discharged. Some of the mine waters are used in milling processes, and become even more laden with soluble inorganic ions. These brines are pumped to tailings ponds for evaporation or eventual release to surface or ground water systems. Both mine waters and tailings solutions are valuable sources of metal and other ions, and it is both economically and environmentally sound to recover these elements. In the state of New Mexico, U.S.A., industries which must remove inorganic agents from large volumes of water are the uranium and molybdenum operations. Although precipitation, ion exchange, solvent extraction and electrowinning systems are available for soluble ion removal, these systems are quite often ion specific and very expensive when treating large volumes of water. An inexpensive and ion non-specific, accumulator system is the desirable alternative. The understanding and design of a functional bio-filter system for removal of uranium and other ions associated with uranium mining operations would alleviate discharge problems in the Grants, New Mexico, U.S.A. district (the producer of 46% of the U.S. uranium). Such a system would be applicable to other uranium-producing regions. The bio-filter process may also be applicable to other industries, with wastewater effluents containing trace elements. Among the extractive industries, the molybdenum operations have considerable need for economic and environmentally safe processes for purification of mill effluents.

Because living organisms can accumulate trace elements in concentrations of geochemical significance, it is likely that these organisms can and will be economically exploited. It is foreseeable that living organisms will be used as "bio-filters" to recover metals from solutions too dilute in metal concentrations to economically use other processes for metal recovery. An example of such a process may be the removal of heavy metals from sea water. It is more likely that organisms will be used extensively in the near future to recover trace elements from industrial effluents to both remove environmentally undesirable inorganic ions from discharge waters and recover elements of economic importance. Gale and Wixson (1979) have elucidated the role of algae in removal of soluble lead in wastewater discharged from lead tailings dams in the "New Lead Belt" of Missouri, U.S.A.

Bacteria will concentrate potassium, magnesium, manganese, iron and calcium (Eisenstadt *et al.*, 1973), and the mechanism which transports these ions will also readily transport metals such as nickel and cobalt (Tuovinen and Kelly, 1973). Many bacteria also produce complexing agents which selectively extract metals from dilute solution (Pollack, *et al.*, 1970). Metal accumulation can occur on the cell walls of some bacteria, fungi, and algae; this process is believed to occur by reaction of metals with reactive groups located on the microbial cell walls. The amount of metal accumulated by this process can be substantial (Langman *et al.*, 1972; Tornabene and Edwards, 1972). The sulfate-reducing bacteria can effectively precipitate large quantities of heavy metals by the production of hydrogen sulfide (Ehrlich and Fox, 1967; Temple, 1964; Temple and Le Roux, 1964). All of these mechanisms were reviewed in an article by Kelly, Norris and Brierley (1979).

Other organisms are also know to accumulate elements. Silica is concentrated by algae; calcium carbonates and magnesium are taken up by corals and gastropods; green and brown algae and some fungi take up zinc and other heavy metals. Mercury, nickel, zinc, uranium, cesium and strontium are concentrated by mosses and higher plants (Tuovinen and Kelly, 1974).

Waste water from uranium mines in the Grants, New Mexico region must be treated to remove trace elements before discharge. Kerr McGee Corp. has a novel treatment process for

waters from two mines, sections 35 and 36. Particulate matter is allowed to precipitate in settling ponds. The water from one mine, section 35, is then passed through an ion exchange resin to remove uranium. Both mine waters have barium chloride added to precipi tate sulfate and radium. The waters are then retained in three consecutive algae ponds before discharge. Target elements, subject to removal, are uranium, selenium, radium and molybdenum.

The objectives of our experiments are: (1) to establish the concentrations of uranium, selenium, molybdenum and sulfate in the water and sediments of the settling and algae ponds of Kerr McGee Corp. sections 35 and 36 mines; (2) to identify and quantify the biological agents, particularly the algae and bacteria, in the ponds; and (3) to identi biological and chemical agents which participate in the removal of selenium, molybdenum, and uranium from the wastewaters passing through the treatment pond system.

MATERIALS AND METHODS

Water samples for chemical analyses were collected in 1.0 l, HCl-rinsed, polyethylene bottles. The samples were immediately acidified by addition of 15 ml HNO_3. The samples were filtered through 0.45 μm Millipore filters before assaying. Sediment samples were collected in polyethylene bags. The material was dried for 8 h at 105° before acid decom position (Brandvold, 1974). Total sulfur analysis on sediments was completed by an adap- tion of the Escheka fusion method (ASTM, 1969). Sulfate was gravimetrically determined (Standard Methods, 1960). Selenium was assayed fluorimetrically (Chan, 1976), molybdenum was quantitated spectrophotometrically (Meglen and Glaze, 1973), and uranium concentratio were obtained by the TOPO procedure (Johnson and Florence, 1971).

Samples for microbiological analyses were collected by the "grab" method. Sterile 250-ml polypropylene bottles were used. Samples were immediately placed in an ice-chest and refrigerated upon return to the laboratory. Analyses were performed with 24 h after sample collection.

The "total" count of microbes present was determined using the one-half strength iron pep tone agar of Ferrer *et al.*, (1963). Samples were diluted using 0.5% (w/v) peptone, and spread plates incubated at room temperature for five days. Sulfate-reducing bacteria wer enumerated using a five-tube most probable number (MPN) method with Postgate's (1966) medium E. The tubes were incubated for a period of three weeks at room temperature prior to grading. Enrichment cultures were used to demonstrate the presence of sulfur-oxidizin thiobacilli. Media numbers 125 and 290 (American Type Culture Collection, Catalog of Strains I, 13th Edition, 1978) were used respectively for sulfur-oxidizing and thiosulfat oxidizing thiobacilli.

Algal populations were identified using the keys of Prescott (1954) and Smith (1950).

Laboratory model ecosystems were set up in 2.8 l Fernbach flasks. Each contained 1 kg of bottom sediment collected from one of the ponds at the field site, plus 1.5 l of tap water. The flasks were supplemented with sulfate, molybdate, uranium and selenium to levels approximating those in the pond system, 700 ppm, 2 ppm, 5 ppm and 25 ppb, respect- ively. The compounds used were Na_2SO_4. Na_2MoO_4. $2H_2O$, UO_2SO_4. $3H_2O$ and selenium powder dissolved in tap water plus 1.5% (w/v) concentrated HNO_3. Sodium lactate, 1.4 g, was added to one duplicate set of flasks to stimulate sulfate-reducing bacterial activity. On duplicate set of flasks had no further additions, and one set was autoclaved at 124 kPa for 45 min to serve as sterile controls. The flasks were incubated at room temperature. The flasks were periodically sampled and concentrations of the respective "pollutants" determined.

RESULTS

Chemistry

Figure 1 schematically illustrates the pond treatment system. Dimensions and depths of the ponds are indicated.

In Table 1 are tabulated the average of the analytical results for uranium, molybdenum, sulfate, and selenium from four samplings. Although the samples were collected in different months (October, November, January and March), there was insignificant variatior

in the analytical data. Samples 2 and 7 were sediments and the sulfur data are recorded as ppm total sulfur. These data indicated large accumulations of all target elements and sulfur in the sediments. A reduction in uranium, selenium and molybdenum concentrations were noted as the water passed through the pond treatment system, but sulfate concentration remained unchanged. Although uranium was removed by ion exchange, this element was further reduced in concentration as the wastewater passed through the three algal ponds (note samples 6 and 8, Table 1).

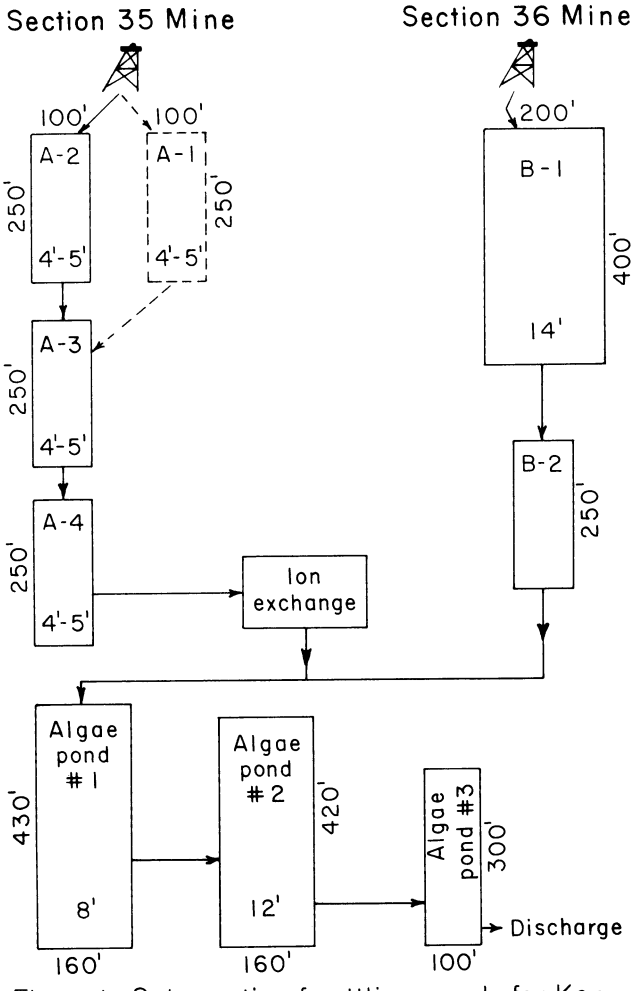

Figure 1—Schematic of settling ponds for Kerr McGee Corp. Sections 35 and 36 waste waters

TABLE 1

CHEMICAL AND BIOLOGICAL PARAMETERS OF MINE WATER TREATMENT PLANT

Sample No.	Site location	ppm				Number bacteria ml^{-1}	
		U	Se	Mo	SO_4	Total aerobic bacteria	Sulfate-reducing bacteria
1	S.35 mine water	5.8	0.03	2.3	700	2.8×10^5	3.8×10^2
2	sediment A-4	1037.0	30.00	160.00	5700[a]	1.0×10^{8}[b]	9.2×10^{5}[b]
3	effluent A-4	5.4	0.02	2.3	684	5.5×10^5	3.6×10^1
4	S.36 mine water	4.6	0.02	0.06	630	2.8×10^5	2.7×10^2
5	effluent B-2	0.8	0.004	0.05	721	1.0×10^5	2.3×10^1
6	influent algae-1	1.4	0.01	1.1	699	3.6×10^5	4.8×10^1
7	sediment algae-2	618.0	30.00	590.00	30900[a]	1.3×10^{8}[b]	2.5×10^{6}[b]
8	effluent algae-3	0.8	0.01	0.8	736	8.1×10^4	1.1×10^1

[a] ppm total sulfur

[b] Number bacteria g^{-1} dry wt.

Bacteriology

The "total" count of bacteria in suspension remained constant with a small decrease noted at the final discharge point, station 8 (Table 1). No pattern of variation was observed over the period of sampling, from October through June.

The concentration of sulfate-reducing bacteria in the water was greater in water coming directly from the mines (stations 1 and 4, Table 1). The concentration values decreased as the water flowed through the treatment system. Large populations of sulfate-reducing bacteria were found in the settling pond and algal pond sediments, having concentrations of about 10^6 cells per gram dry-weight of sediment (Table 1).

Enrichment cultures were positive for chemolithotrophic microbes able to oxidize sulfur at pH 5.0, thiosulfate at pH 6.6 and thiosulfate with production of gas (N_2). The respective enrichment cultures indicated presence of *Thiobacillus thiooxidans*, *Thiobacillus thioparus* and *Thiobacillus denitrificans*.

Algae

The predominant alga found in the initial settling ponds was *Spirogyra*. The "algal-ponds" have an abundant growth of *Chara*, growing from the ponds' bottoms, plus *Spirogyra* and some *Oscillatoria*. The *Spirogyra* are most abundant in the last algal treatment pond. Other genera of green-algae have been noted in much less abundance; they were not identified.

Laboratory Model-Ecosystem

The effect of microbial activity on concentration of soluble sulfate, uranium, molybdenum, and selenium in the model water-sediment systems was monitored with time (Table 2). The data reported were average values of duplicate flasks. Three conditions were compared: a sterile control, a system which had a viable microbial flora present, and a system to which lactate was added to increase the activity of sulfate-reducing bacteria. The sulfate level was only decreased in the system containing lactate between the 48 and 120 h sampling period. Concurrent with sulfate reduction was decrease in concentration of soluble uranium and selenium. Molybdenum levels decreased in the lactate supplemented system and the systems without added substrate. The higher level of molybdenum in the sterile control flasks was believed to have been a result of extraction of molybdenum from the sediment during autoclaving. Selenium decreased in the sterile control over 120 h, indicating non-biological processes active in removal. This experiment was repeated several times with similar results.

The variations in concentration among the different conditions were because the sediment used was not a homogenous mixture.

DISCUSSION

The mine-water treatment system was effective in lowering the concentrations of uranium selenium and molybdenum (Table 1). The uranium had the greatest apparent depletion, present in the mine's effluents at 4.6 and 5.8 ppm and reduced to 0.8 ppm in the final discharge. The mechanism(s) of depletion has not yet been defined. Settling of particulates and biological accumulation are considered to be the active processes.

Large populations of microorganisms were found in the waters pumped from the uranium mines (stations 1 and 4) and passing through the pond system. It is suspected that these microbes may play a role in removal of the target elements from the water. Of particular interest was the presence of the sulfate-reducing bacteria, believed to be *Desulfovibrio* and/or *Desulfotomaculum*. The sulfate-reducing bacteria were relatively high in numbers in water coming directly from the mines (stations 1 and 4, Table 1). Conditions for their growth must exist within the mining operations. These microbes decreased in number as the water progressed through the treatment system. This result was expected as *Desulfovibrio* and *Desulfotomaculum* cannot grow in aerobic conditions. However, very high numbers of these microbes were found in the pond sediments (Table 1). Reducing conditions, favouring the growth of the *Desulfovibrio* and *Desulfotomaculum*, were found in the sediments as indicated by an Eh of -350 mV and black coloration a few millimeters below the sediment surface.

TABLE 2

DECREASE OF SELECTED SOLUBLE POLLUTANTS IN A SEDIMENT-WATER SYSTEM

Time hours	Conditions	Concentration in solution SO_4 (ppm x 10^{-3})	U (ppm)	Mo (ppm)	Se (ppb)
0	sterile control	1.35	8.1	7.9	38.4
	no substrate	1.27	15.5	3.2	40.7
	lactate added	1.21	9.8	2.9	59.0
24	sterile control	1.18	3.1	7.5	26.5
	no substrate	1.16	13.3	3.1	31.0
	lactate added	1.29	12.1	2.8	30.0
48	sterile control	1.01	5.8	9.2	17.5
	no substrate	1.14	7.5	3.1	27.0
	lactate added	1.24	8.9	3.6	14.2
120	sterile control	1.11	7.5	8.2	10
	no substrate	1.18	8.2	2.5	14.5
	lactate added	0.69	3.8	2.4	<0.0
240	sterile control	1.03	7.5	616	14.2
	no substrate	1.19	7.6	1.9	7.7
	lactate added	0.49	2.9	1.7	<0.0

The laboratory experimentation indicated that the sulfate-reducing bacteria may have a role in removal of uranium, selenium, and possibly molybdenum from solution (Table 2). Th mechanism by which these pollutants were removed is not yet known. Sulfide production may be important; molybdenum could be precipitated as a sulfide. The reducing conditions may be responsible for converting soluble hexavalent uranium to the insoluble quadrivalent for

Jackson (1978) reported that stimulation of microbial activity, including sulfate-reductic by addition of nutrients from sewage was effective in removal of heavy metals from pollute mine and smelter waste-water.

Although a large population of sulfate-reducing bacteria was found in the pond sediments, there was no decrease of soluble sulfate in the water flowing through the system (Table 1) Reduced forms of sulfur may be oxidized by the aerobic thiobacilli in oxidizing regions, returning the sulfur to the soluble sulfate species. The system may be nutrient limited and the activity of the sulfate-reducing bacteria quite slow.

Experimentation is now in progress to determine if the algae and other bacterial populatic are effective in pollutant removal. In addition, work is continuing to define the role of the sulfate-reducing bacteria concentration of uranium, molybdenum, and selenium.

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BIOCHEMICAL BENEFICIATION OF MINING INDUSTRY EFFLUENTS

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INTRODUCTION

One consequence of the industrial development experienced over the last fifty years has been the increasing demand for water for industrial purposes. This trend has also characterized the mining industry and, particularly, the mineral beneficiation plants. An average size flotation plant treating 5 Tg of run-of-mine ore per day has a water requirement of at least 20,000 m^3. This not negligible amount of water must obviously be drawn from the natural hydrological resources of the area where the plant is located and, usually, for the most part, returns to it after the settling of the solids in appropriate tailings ponds.

Actually, the water which returns to the natural environment has usually undergone considerable alteration, with respect to its original condition, and quite often it is characterized by a notable impoverishment in oxygen and by the presence of ions and chemical compounds with a high pollution potential. These substances are often found in amounts well in excess of the limits imposed by State environment protection boards. This happens also for mineral processing plants and, in certain cases, the operation of some concentration plants nas been stopped simply on the grounds of environmental regulations.

One of the most satisfactory ways of solving this problem is the total recycling of the reject waters, and as a matter of fact, several plants where sulfide-, or uranium- bearing ores are treated successfully follow this procedure.

The recycling of water involves, in those cases, its preliminary treatment with the aim of reducing its contents in suspended solids, of neutralizing its acidity and of lowering its concentration in metal ions and residual flotation reagents (Bailey, 1970; Browne and Butler, 1970; Bell, 1974; Nagahama, 1974; Oka and Taylor, 1974; Pickett and Joe, 1974; Bell and Phinney, 1975; Müller, 1978). This procedure does not seem, however, to have found such widespread application in plants where non-metallic and/or metal oxide minerals are treated, since literature on the subject is rather limited.

THE PROBLEM OF RECYCLING REJECT WATERS FROM NON-METALLIC AND/OR METAL OXIDE MINERAL FLOTATION PLANTS.

In two previous papers published elsewhere some of the authors of the present report described the outcome of investigations aimed at finding out the most efficient water purification process for this type of plant (Carta *et al.*, 1977a,b). The problem consists fundamentally in each of the cases considered, of removing the remainder of the collectors added to the flotation circuits; for oxidized ores the most common collectors used are amine acetates of hydrochlorides, for barite flotation, alkylsulfates, and for fluorspar flotation, fatty acids.

Flotation Plants for Non-Metallic Minerals

Figs. 1, 2 and 3 refer to a plant with a capacity of 1.5 Tg per day, treating a r.o.m. assaying 2.5% galena, 41.6% fluorite and 11.5% barite. The flowsheet involves a preliminary galena flotation step followed by fatty-acid fluorite flotation and finally by alkylsulfate barite flotation. For this plant, the water flow diagram and the distribution of the carboxylate and alkylsulfate collectors, respectively, are illustrated in Figs. 2 and 3. A typical feature of this type of flowsheet consists in the considerable amount of water added to the tailings pulp before the latter is sent to the tailings pond, the objective being to dilute the pulp and consequently reduce its polluting effects when it eventually returns to the hydrologic system.

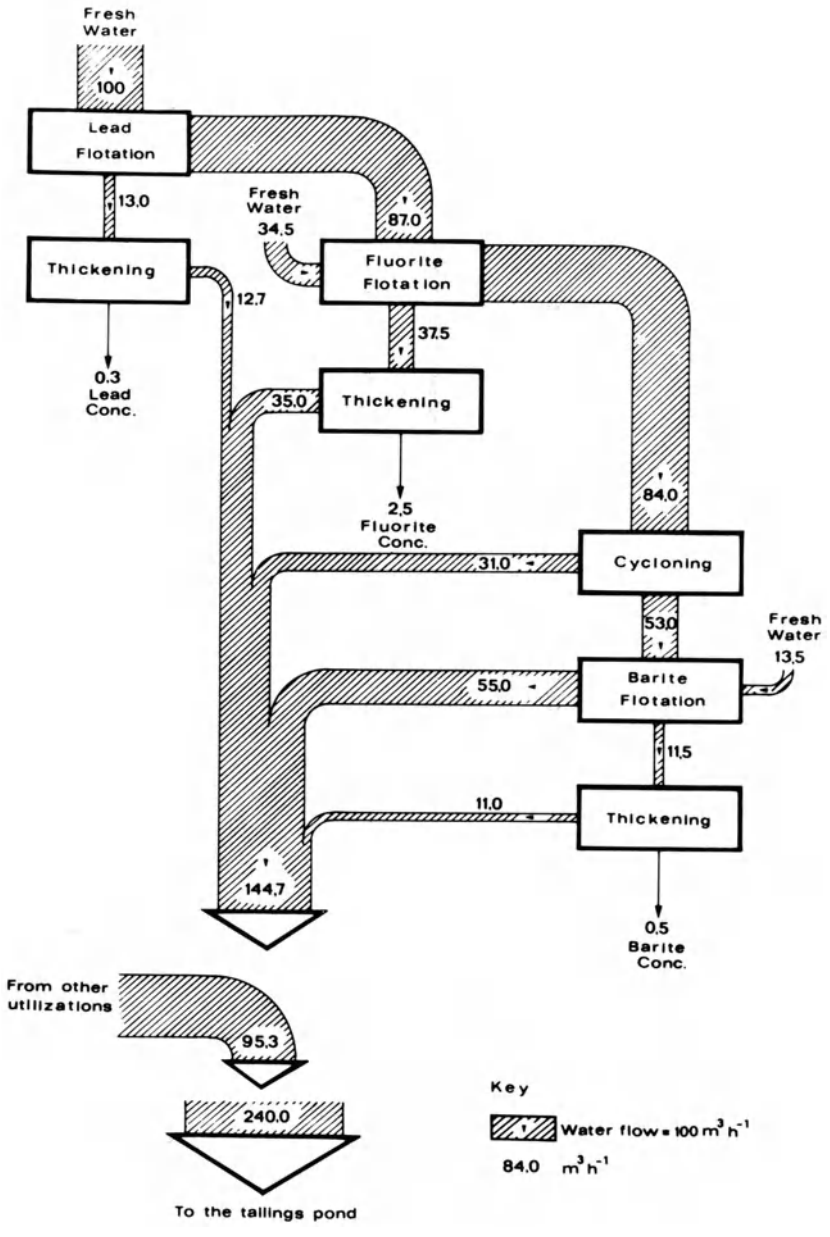


Figure 1: Water flows in a galena-fluorite-barite flotation plant.

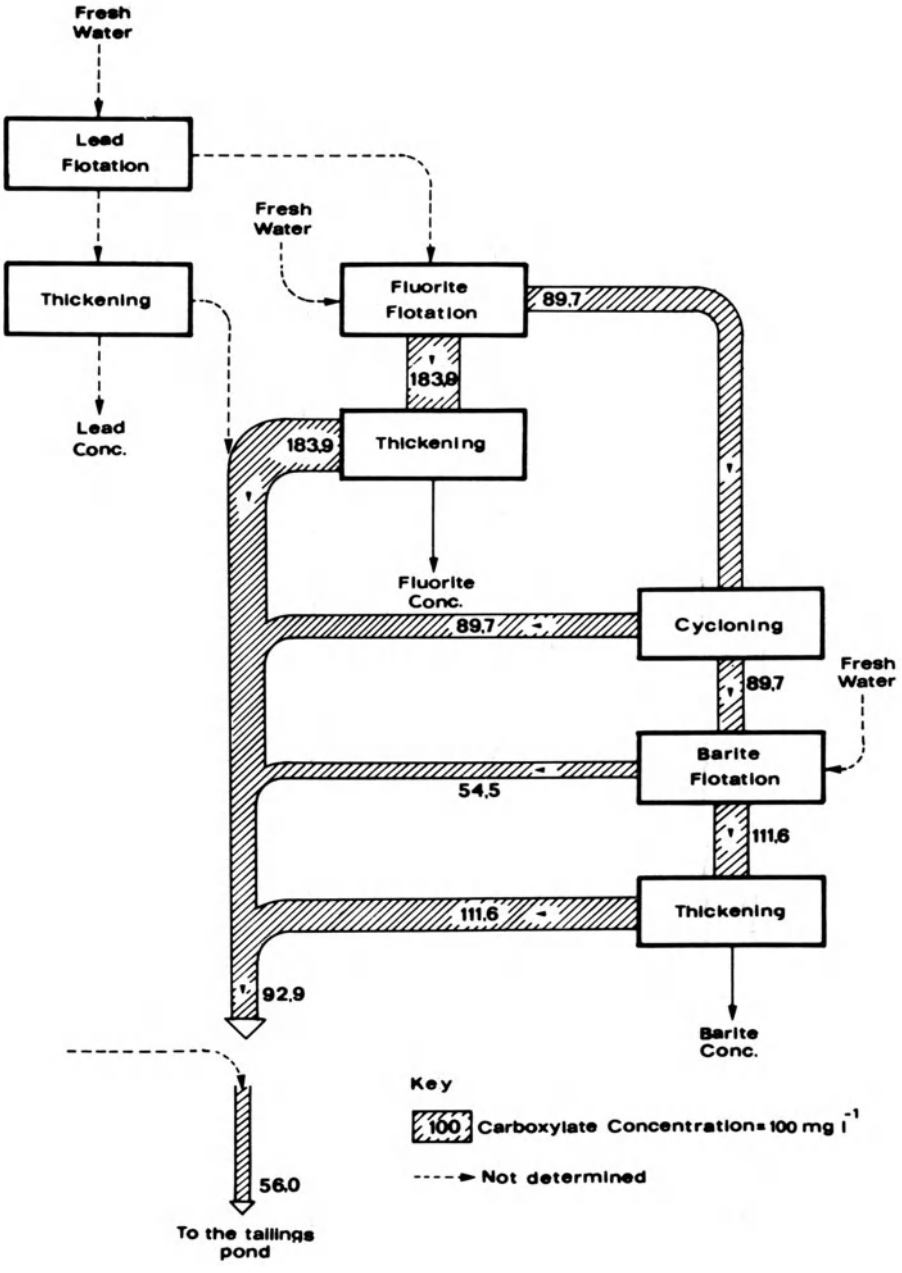


Figure 2: Galena-fluorite-barite flotation plant: carboxylate concentrations in principal water flows.

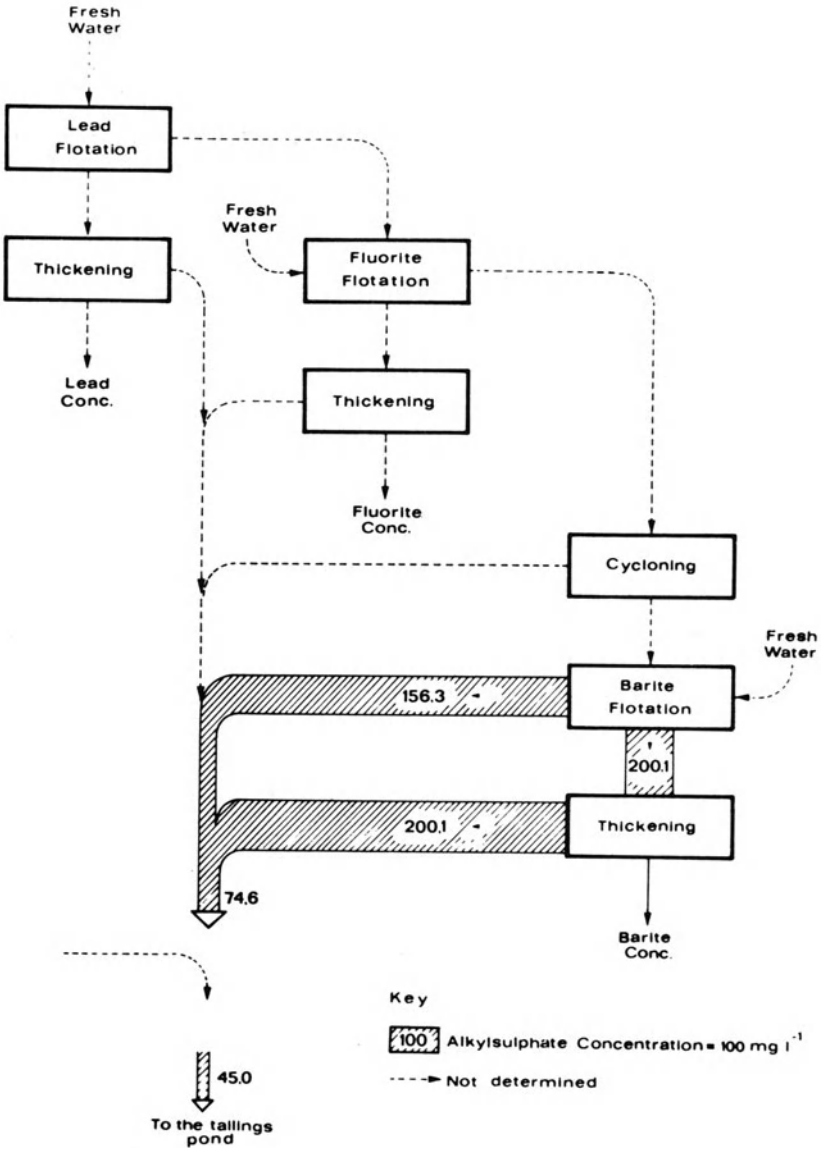


Figure 3: Galena-fluorite-barite flotation plant: alkylsulfate concentrations in principal water flows.

This plant has been thoroughly investigated by the researchers of the Mining and Mineral Dressing Institute of Cagliari University. In the first place it has been ascertained that about 45% of carboxylate and 58% of alkylsulfate added to the circuit are not adsorbed by the ore and are thus found in the reject water. The effect of the presence of these residual reagents on galena flotation has been investigated. The results of laboratory tests performed on pulps made up of feed and either fresh water or undiluted reject water or of diluted reject water have clearly shown (Table 1) that the residual reagent concentration always produced unacceptable recoveries of all the components of the feed already in the initial galena flotation stages. This means that the treatment of the reject water before recycling is absolutely necessary.

The Metal Oxides Minerals Flotation Plants

It must be pointed out in the first place that, at least as far as the Sardinian ore bodies are concerned, the lead and zinc oxide minerals are quite often accompanied by commercial grades of the respective sulfides. Since, up to the present time, no reagent has yet been discovered which acts as a collector solely for the oxides and not for the sulfides, the typical flowsheet for this type of r.o.m. must consist of a preliminary flotation stage of sulfides with xanthates followed by the lead carbonate (cerussite) flotation stage and, finally, by the zinc oxidized minerals (smithsonite, willemite, hemimorphite, etc.) flotation stage. Both the latter stages involve the use of sodium sulfide as the activating agent; after activation, cerussite can easily be floated with xanthates whereas primary amines are typical collectors of oxidized zinc minerals.

This type of flowsheet offers in principle two alternatives: (a) integral recycling to the head tanks of the water decanted in the tailings ponds, and (b) selective recycling, i.e. recycling in every stage of the water recovered from the respective tailings. The latter solution, however, seems neither technically nor economically practical at the present time. The first solution gives rise to problems quite similar to those already examined in the preceding section. Also, in the case of these circuits, high reagent concentrations are present in the final effluent (Read and Manser, 1977) even though a considerable proportion is removed during the process (by adsorption, precipitation, etc.).

As it well known, primary amines are characterized by a high adsorption potential in the electrical double layer of fine particles (Klassen and Mokrousov, 1963). Primary amine residues in the feed pulp of the first stages of the process would promote the flotation of undesirable slimes the presence of which would unacceptably spoil the quality of the product.

As for sodium sulfide residues, their presence - even in low concentrations - in the sulfides flotation section feed water would produce sensible decreases in recovery, due to the strong depressing effect of this reagent on metal sulfides when the latter are floated using xanthogenates as collectors (Fleming, 1953); this is clearly shown by Table 2. Fig. 4 shows a flow diagram of a typical plant of this type operating in Sardinia; the concentrations of amine and sodium sulfide in the main points of the circuit are indicated together with the water flows. In this case, the recycling of water necessitates its preliminary treatment in order to reduce its residual reagents content to limits which are not prejudicial to the efficiency of the process.

METHODS FOR RESIDUAL REAGENT REMOVAL

Various methods are presently employed or have been proposed for the removal of residual reagents from mineral dressing plant waste waters, the most widely used being:

Adsorption at the liquid/air interface;

Adsorption at the solid/liquid interface;

Chemical Precipitation;

Biological oxidation.

These have been thoroughly investigated on a comparative basis by the researchers of the Mining and Mineral Dressing Institute of Cagliari University, and have been the subject of a previous paper (Carta *et al.*, 1977b).

TABLE 1

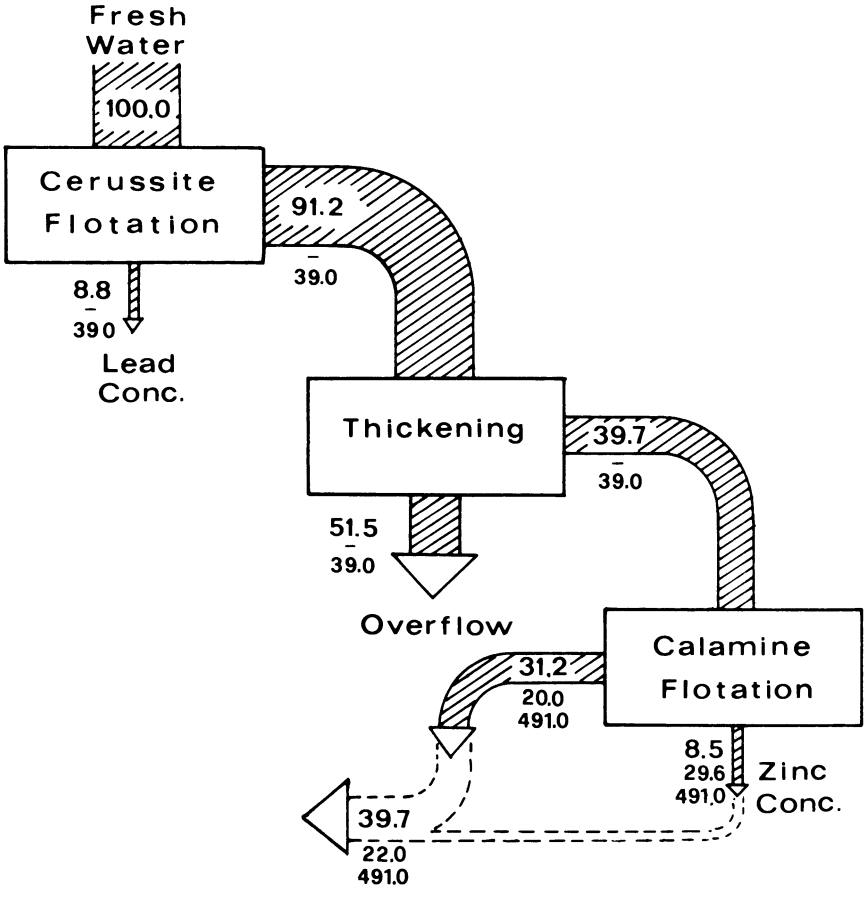
COMPARATIVE RESULTS OBTAINED IN THE GALENA RECOVERY STAGE WITH DIFFERENT KINDS OF WATER

Type of Water	Product	Contents, %			Recovery, %		
		Pb	CaF_2	$BaSO_4$	Pb	CaF_2	$BaSO_4$
Fresh Water	Rougher	51.9	14.8	5.0	86.4	1.5	2.2
	After 3 cleanings	72.4	4.0	2.6	85.5	0.3	0.6
Sample 1	Rougher	16.7	27.5	12.1	84.1	9.3	14.8
	After 3 cleanings	44.2	23.2	13.4	92.0	2.9	6.1
Sample 2	Rougher	8.0	36.3	15.6	95.4	25.9	40.4
	After 3 cleanings	23.3	33.8	24.1	91.5	8.0	20.6

TABLE 2

GALENA FLOTATION RESULTS (ROUGHING STAGE) USING PURIFIED WATER WITH VARIOUS TECHNIQUES

Type of Water	Purification Method	Contents, %			Recovery, %		
		Pb	CaF_2	$BaSO_4$	Pb	CaF_2	$BaSO_4$
1	Frothing, 20%	50.9	15.8	6.7	91.3	1.7	2.6
2	Frothing, 20%	49.2	18.9	12.9	90.5	2.1	5.2
1	Adsorption on coal (10kg Mg^{-1})	40.0	20.0	9.0	90.0	2.7	4.4
2	Adsorption on coal (10kg Mg^{-1})	38.2	25.1	13.4	91.4	3.6	7.0
1	Frothing 15% + 6 hours aeration	35.1	25.5	15.5	89.3	3.9	8.6
2	Frothing 15% + 6 hours aeration	35.1	25.5	15.5	89.3	3.9	8.6
2	Biological oxidation, 10 days	49.3	15.2	6.5	91.7	1.7	2.6
2	Frothing 20% + 14 days biological oxidation of froth	29.0	24.5	11.5	90.6	4.6	7.8



39.7 Water flow = 39.7 % of the feed

22.0 Amine Concentration = 22.0 mg l^{-1}

491.0 Na_2S Concentration = 491.0 mg l^{-1}

Figure 4: Lead and zinc oxide minerals flotation plant: flowsheet showing water flows and amine and sodium sulfide concentrations in main streams.

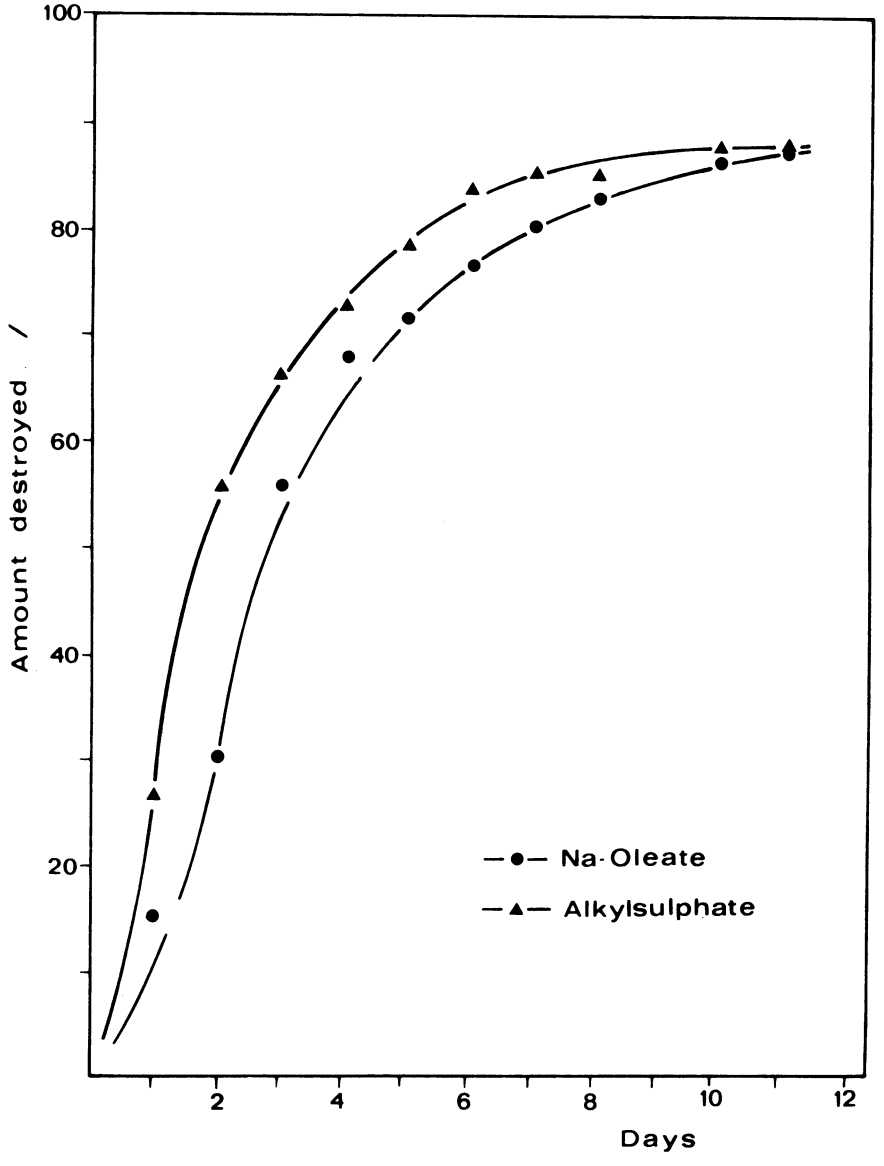


Figure 5: Biological oxidation. Percentage of reagent degraded vs. time. Inoculum: urban sewage water. Starting concentrations of both reagents: 60 mg l^{-1}.

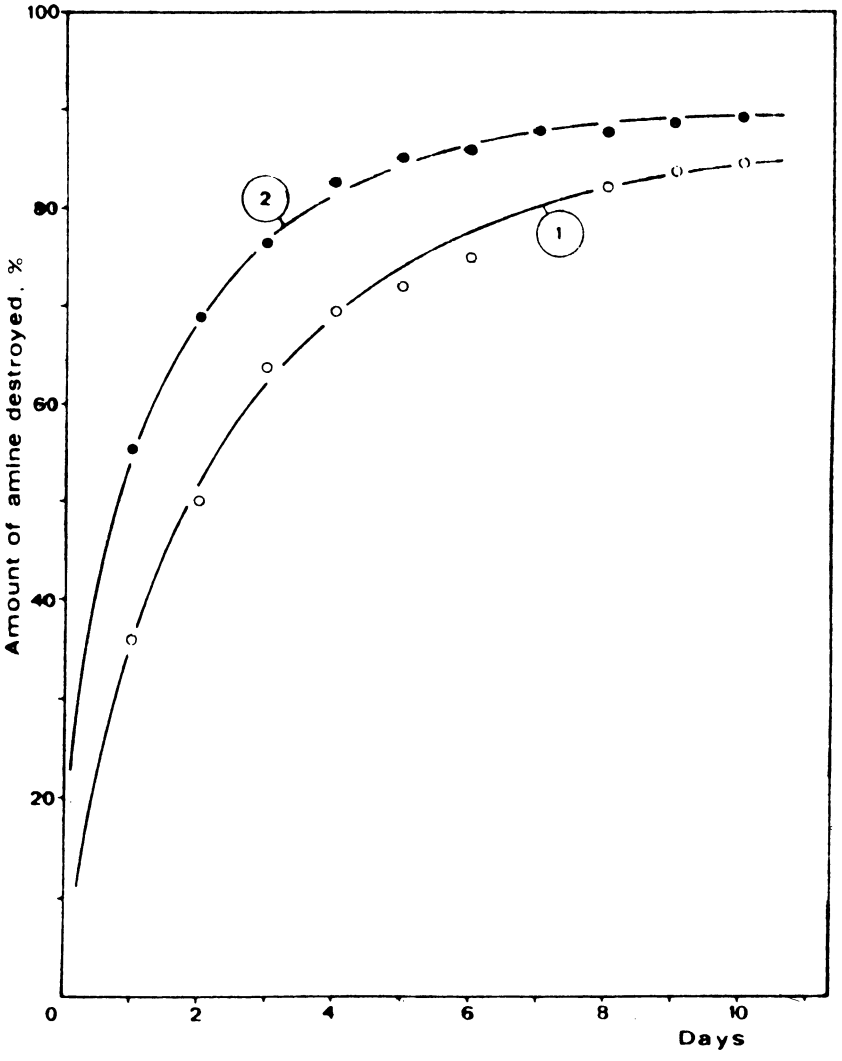


Figure 6: Biological oxidation. Percentage of reagent degraded vs. time. Inoculum: urban sewage water. Starting concentration: (1) 60 mg l^{-1}; (2) 210 mg l^{-1}.

TABLE 3

COMPARATIVE RESULTS OBTAINED IN THE ROUGHING STAGE OF CERUSSITE DURING THE FLOTATION OF THE ORE WITH VARIOUS TYPES OF WATER

Type of Water	Content, %		Recovery, %	
	Pb	Zn	Pb	Zn
Fresh water	27.9	6.7	80.1	4.9
Sample 1	7.5	10.0	77.8	27.1
Sample 2	4.9	7.7	74.9	31.3

TABLE 4

FLOTATION RESULTS FOR THE ROUGHING OF CERUSSITE USING PURIFIED WATER WITH VARIOUS TECHNIOUES

Type of Water	Purification Method	Contents, %		Recovery %	
		Pb	Zn	Pb	Zn
1	Frothing, 20%	27.4	6.9	80.3	5.2
2	Frothing, 20%	14.9	8.7	78.9	12.0
1	Adsorption on bentonite (1kg m^{-3})	28.3	6.8	79.8	5.0
2	Adsorption on bentonite (1kg m^{-3})	27.6	7.1	79.5	5.3
1	Biological oxidation 10 days	27.7	6.7	80.4	5.0
2	Biological oxidation, 10 days	27.9	6.9	80.0	5.1

Biological oxidation has been performed by simulating on a laboratory scale the conditions of the flotation pulps most frequently observed in Sardinian fluorspar plus barite and oxidized metal flotation plants. The experiments have thus been carried out with waters containing 60 mg per liter of alkylsulfates, oleate or amine, and utilizing as inoculum a certain amount of urban sewage water. The experimental procedure was identical to that which is described further on, and the results were very encouraging, as the curves of Figs. 5 and 6 show.

The biological oxidation method has thus proved to be very promising, as is also confirmed by Tables 3 and 4.

PURPOSE OF THE PRESENT RESEARCH

The critical examination of the different methods, which was the subject of exhaustive discussion in the papers already mentioned, lead to the consideration that biological oxidation is, economically speaking, the preferred method.

It was therefore decided to carry out a research program aimed at optimizing the kinetics and the technico-economical operating conditions. The process kinetics are affected by the following factors:

(a) the microbial strains utilized; it is, in fact, reasonable to assume that suitable strains can be found which metabolize every reagent or which can be adapted to them; and, also, that strains, which are specific for the different reagents, can interact with increased efficiency;

(b) the extent of the mixing of the gases necessary for microbial growth (oxygen, carbon dioxide) with the waste water;

(c) the physico-chemical factors, such as temperature, acidity, redox potential.

In this paper the results of the first phase of the program outlined above, i.e. that concerning the factors mentioned under (a) and (b), are reported. For practical reasons point (c) has been neglected for the moment; the acidity of flotation pulps depends, as is well known, on the process and, due to the considerable volumes of pulp involved, its modification would entail additional costs; as for temperature, it does not seem reasonable to consider the possibility of its regulation. The temperature of flotation pulps, thanks also to the heating to which they are subjected in the grinding mills and even in the flotation cells, never falls in Italian plants, even in winter time, below 15^{o}; the same considerations are valid for redox potentials.

During the experiments care was taken, however, to keep records of as many as possible of these parameters, which can be defined as independent. This was done so that, in the case of necessity, significant influences could be detected.

MATERIALS AND METHODS

Microbial Strains

For the tests with pure strains the following microorganisms supplied by the Microbiology Institute of the University of Cagliari were utilized: *Escherichia coli*, *Proteus rettgerii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

For the tests with the natural microbial flora samples of urban sewage water were utilized.

The Solutions of Flotation Reagents

The solutions were made up with spectrophotometric grade sodium hexadecylsulfate, or with sodium oleate produced by the reaction of analytical grade oleic acid and sodium hydroxide or with analytical grade dodecylammonium acetate, always dissolved in distilled water.

Radioactive Tracers

Sodium hexadecylsulfate, sodium oleate and dodecylammonium acetate marked with 1. ^{14}C were used.

Equipment

Most of the experiments were performed in litre pyrex graduated cylinders. Into each cylinder a 3 mm i.d. glass tube was immersed at a depth kept constant in all tests; all the glass tubes were connected to a main tube which received compressed air by a Millipore pump. The air coming out of the pump was washed twice in two bottles, one containing a KOH solution and the other distilled water. Glass wool filters were inserted in the main tube and in the rubber lines connecting the latter to the glass tubes in the cylinder.

When the microbial strains are aerobic, one of the most important factors affecting their growth is, as is well known, the availability of oxygen, which is attained by means of an adequate aeration of the culture media. This aeration can be produced either by bubbling or by simple diffusion; the latter, which is the least expensive, can be adopted provided that, for the process to be efficient, the surface of contact between the solution and the atmosphere is as large as possible. This condition can be obtained by letting the solution

percolate through a column containing a material - chemically inert toward the solution - having a very large surface on which the percolating solution spreads forming a very thin moving film. Tests were conducted with this type of device, which consisted of glass columns 50 mm i.d. and 600 mm high, full of plastics chips; by means of peristaltic pumps a total volume of 1000 ml of solution was circulated from a Florence flask through the column of chips and back to the flask. The photograph of Fig. 5 shows the above-described equipment.

For pH determinations, a Beckmann Century SS-1 pH-meter was used. The radioactivity of th solution samples was measured by means of a Geiger counter. Air flows in the graduated cylinders were measured by means of flowmeters.

The sterilization of instruments, equipment and solutions was ensured either by autoclavin or by seitz filtration.

Test Procedures

All of the solutions contained 60 mg l^{-1} of reagent dissolved in distilled water, to which 20 ml of solution of the corresponding radioactive tracer were added; the C^{14} to C ratios were between 1:30 and 1:6.

Immediately after the addition of the tracer, the solution was inoculated; according to the test, inoculum sizes ranged from 10 to 20 ml. The air flow in the cylinders was maintained between 0.5 and 0.7 ml s^{-1}. The flow of solution in the columns was maintained at constant 40 ml s^{-1}. Every day, at the same time, two 1 ml samples of solution were taken from the same point of the container by means of a sterile pipette, after having first stopped the bubbling of air or the flow of solution for half an hour. After drying under vacuum, the radioactivity of the sample was measured.

RESULTS

The results of tests using sewage water as inoculum have been described in detail in previously mentioned papers (Carta *et al.*, 1977a and 1977b); typical results are shown in Figs. 5 and 6.

The results obtained with four pure microbial strains are shown in Figs. 7 to 11. The latter shows the biodegradation produced by a mixture of *E.coli* and *Klebsiella pneumoniae*. In Fig. 10, the bubbling in cylinder is compared to the percolation in columns using a strain of *Proteus rettgerii*.

It is clear that sodium hexadecylsulfate is the most biodegradable reagent and that *Klebsiella pneumoniae* is the most active organism for that reagent. Amine, on the contrary is the reagent most refractory to biodegradation by the strains tested so far; *E.coli* looks like being the most suitable for its degradation. Sodium oleate is degraded better than amine but much worse than hexadecylsulfate; in this respect, *Klebsiella* and *Proteus* appear to be the most efficient.

The comparison between the outcomes of the tests performed in cylinders and those in percolation columns indicates that, in the long run, the amounts of oleate and hexadecyl-sulfate destroyed are the same; however, the initial destruction rates are higher in columns than in cylinders.

Finally, Fig. 11 shows, for the mixture of strains, an increase in the initial destruction rate compared to the corresponding results obtained with single strains. The amine and alkylsulfate solutions had an initial pH slightly acidic (between 5 and 6.2) whereas the oleate solutions were basic (around 9). The final pH always stabilized at about 7.

COMMENTS AND CONCLUSIONS

The results of this research confirm the efficiency of the method and indicate amine as being the most refractory to biological oxidation. The appropriate choice of the microbial strain may, however, considerably improve even the biodegradation of amines, and the cooperation of microorganisms in a mixture may produce further improvements, as Fig. 11 shows.

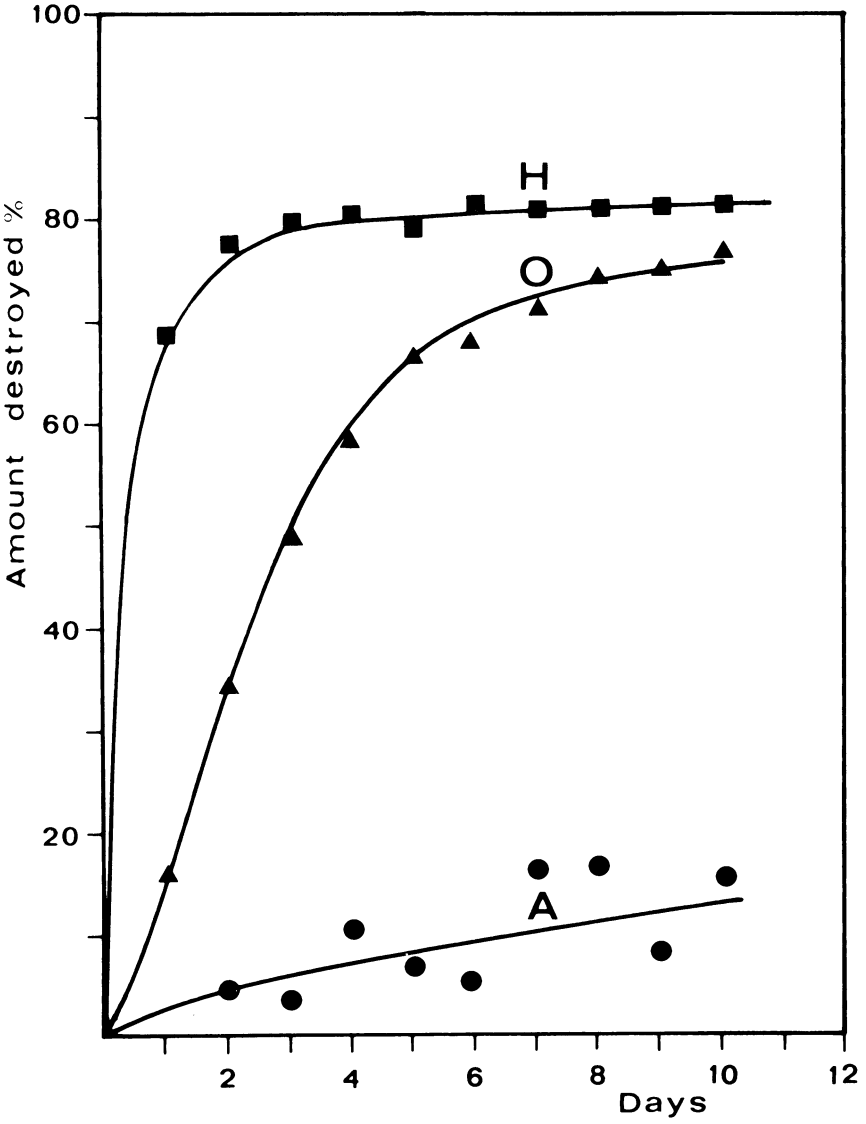


Figure 7: Biological oxidation. Percentage of reagent degraded vs. time. Inoculum: *Klebsiella pneumoniae*.

Reagents: H = sodium hexadecylsulfate;

O = sodium oleate;

A = dodecylamine acetate.

Starting concentrations: 60 mg l^{-1}

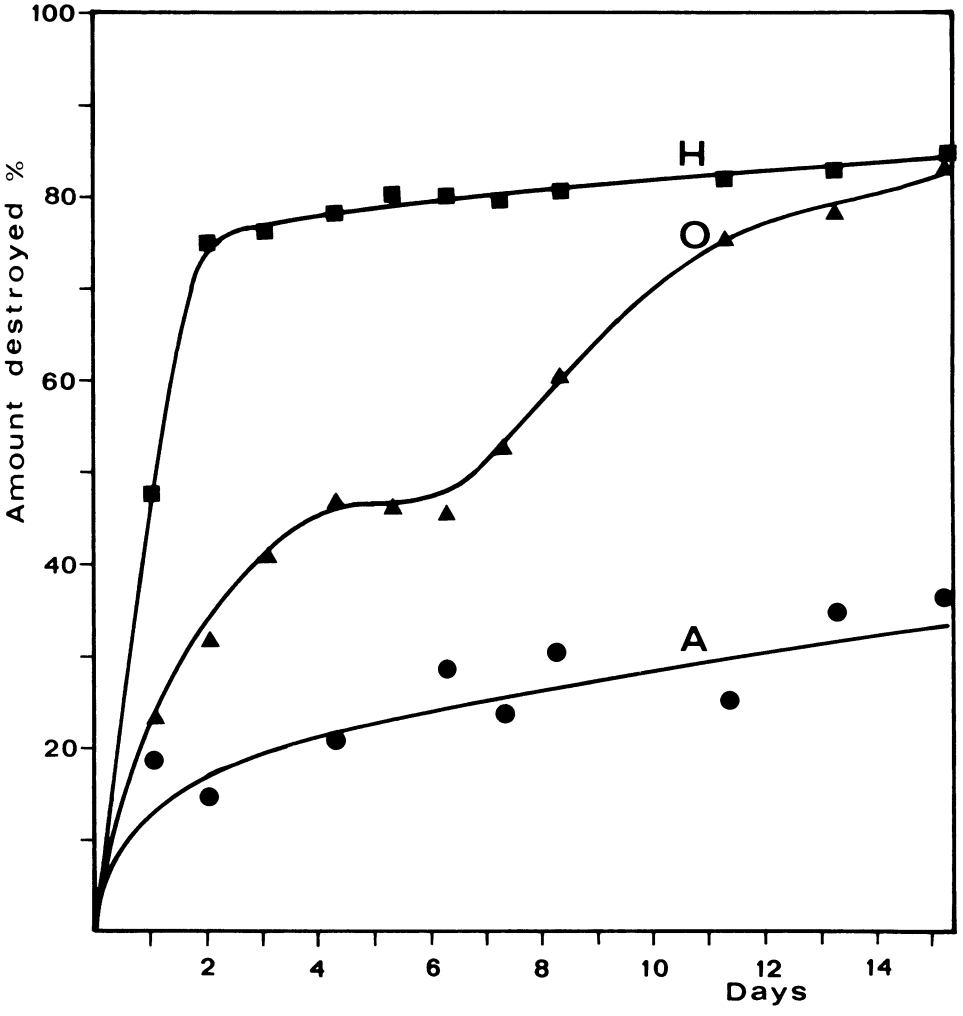


Figure 8: Biological oxidation. Percentage of reagent degraded vs. time. Inoculum: *Pseudomonas aeruginosa*. Reagents and starting concentrations as for Fig. 7.

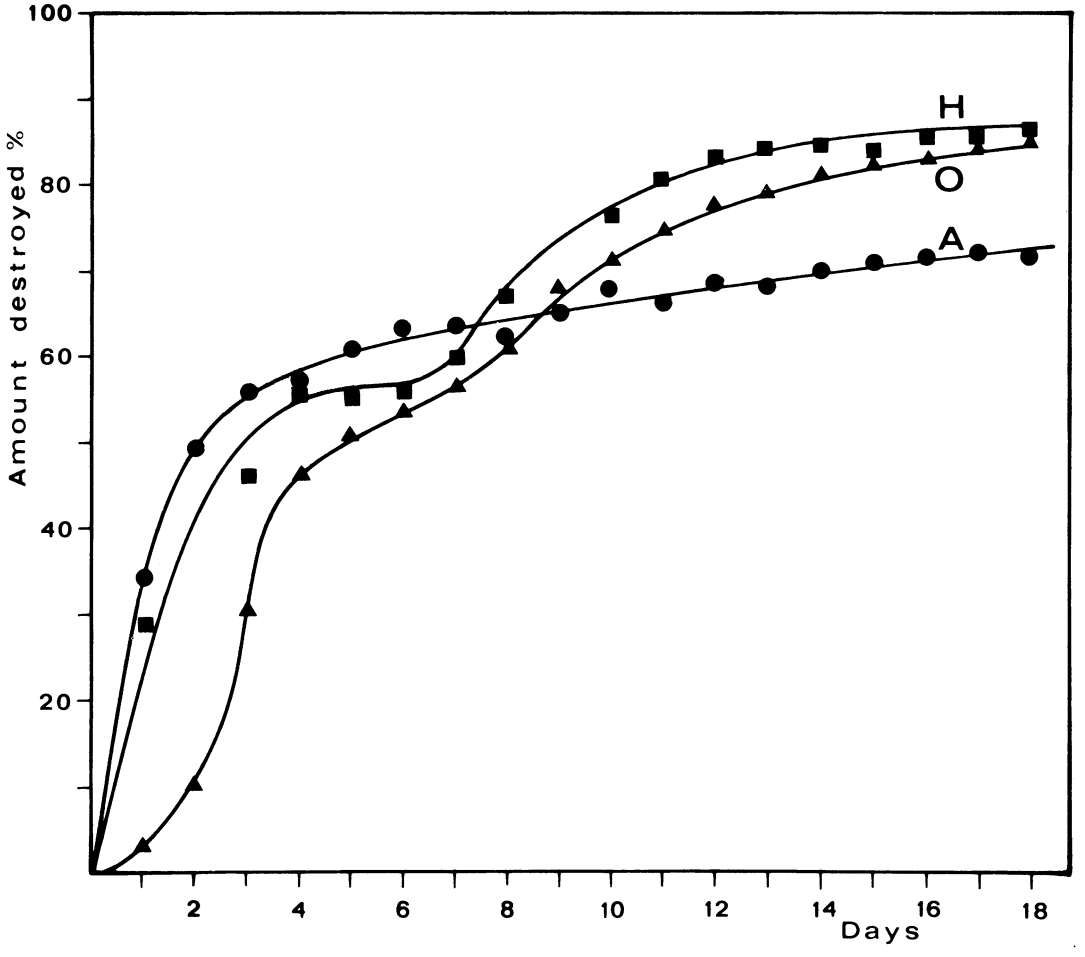


Figure 9: Biological oxidation. Percentage of reagent degraded vs. time. Inoculum: *Escherichia coli*. Reagents and starting concentrations as for Fig. 7.

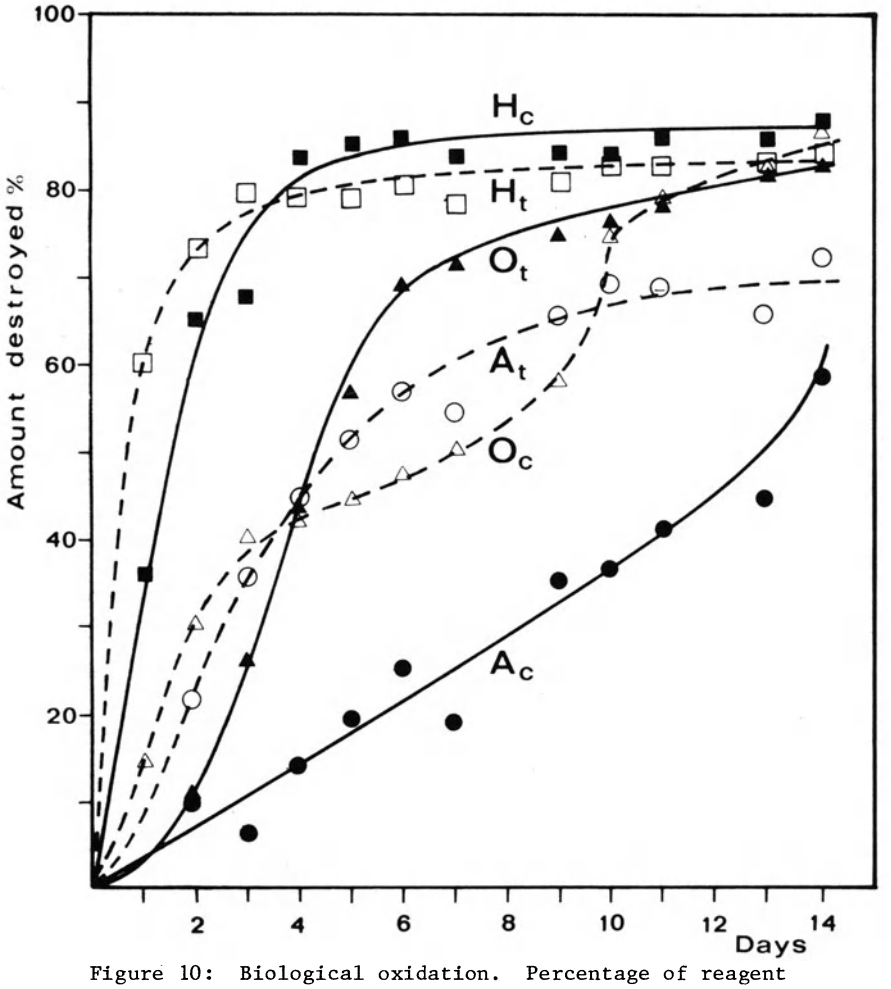


Figure 10: Biological oxidation. Percentage of reagent degraded vs. time. Inoculum: *Proteus rettgerii*. Solid lines: graduated cylinders; Dashed lines: percolation columns. Reagents and starting concentrations as for Fig. 7.

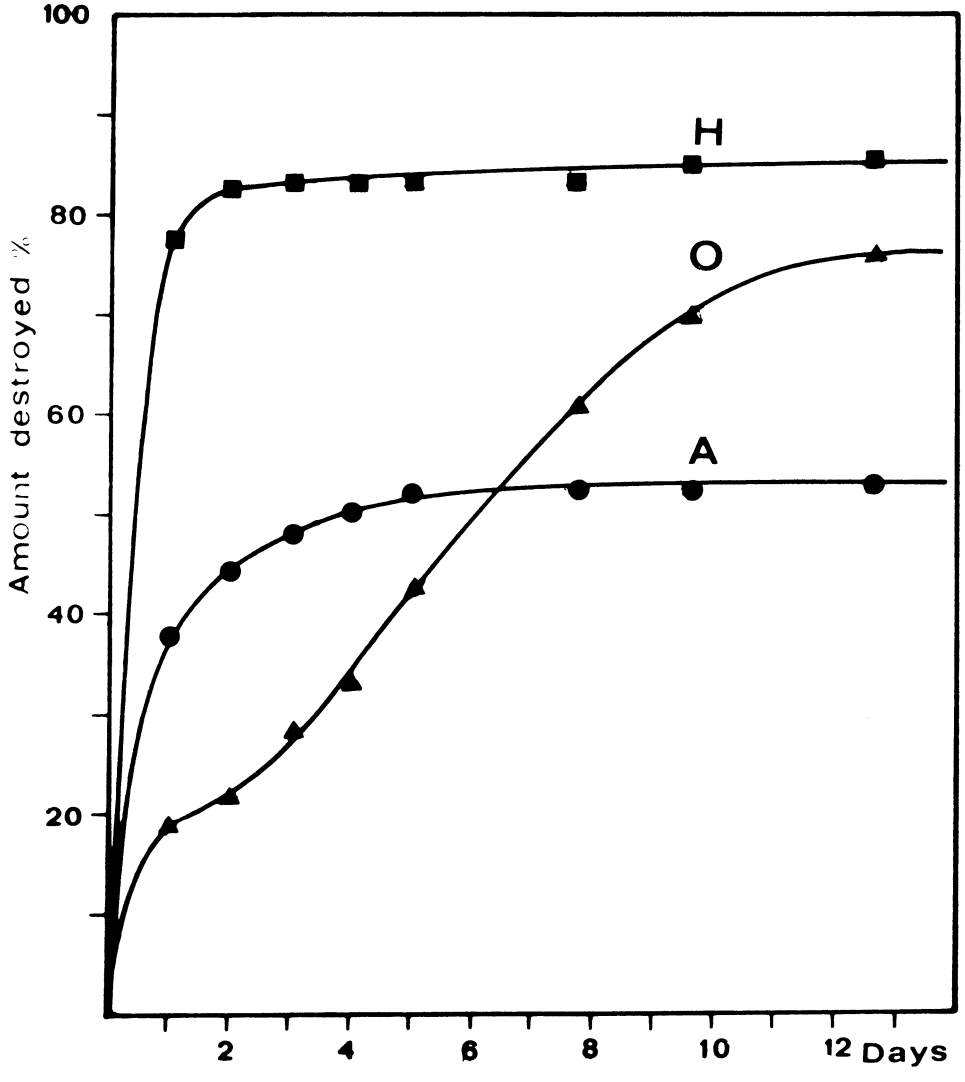


Figure 11: Biological oxidation. Percentage of reagent degraded vs. time. Inoculum: *Escherichia coli* + *Klebsiella pneumoniae*. Reagents and starting concentrations as for Fig. 7.

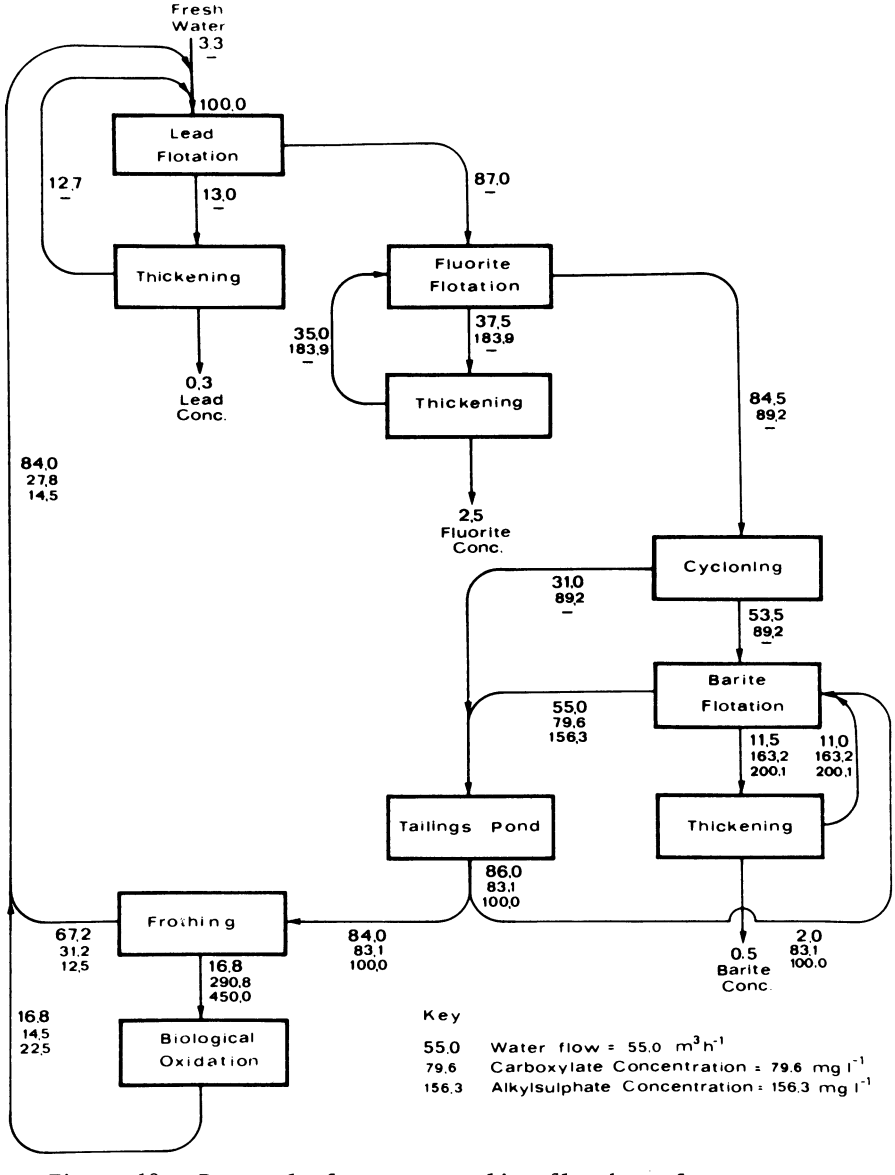


Figure 12: Proposal of water recycling flowsheet for a galena fluorite-barite selective flotation plant.

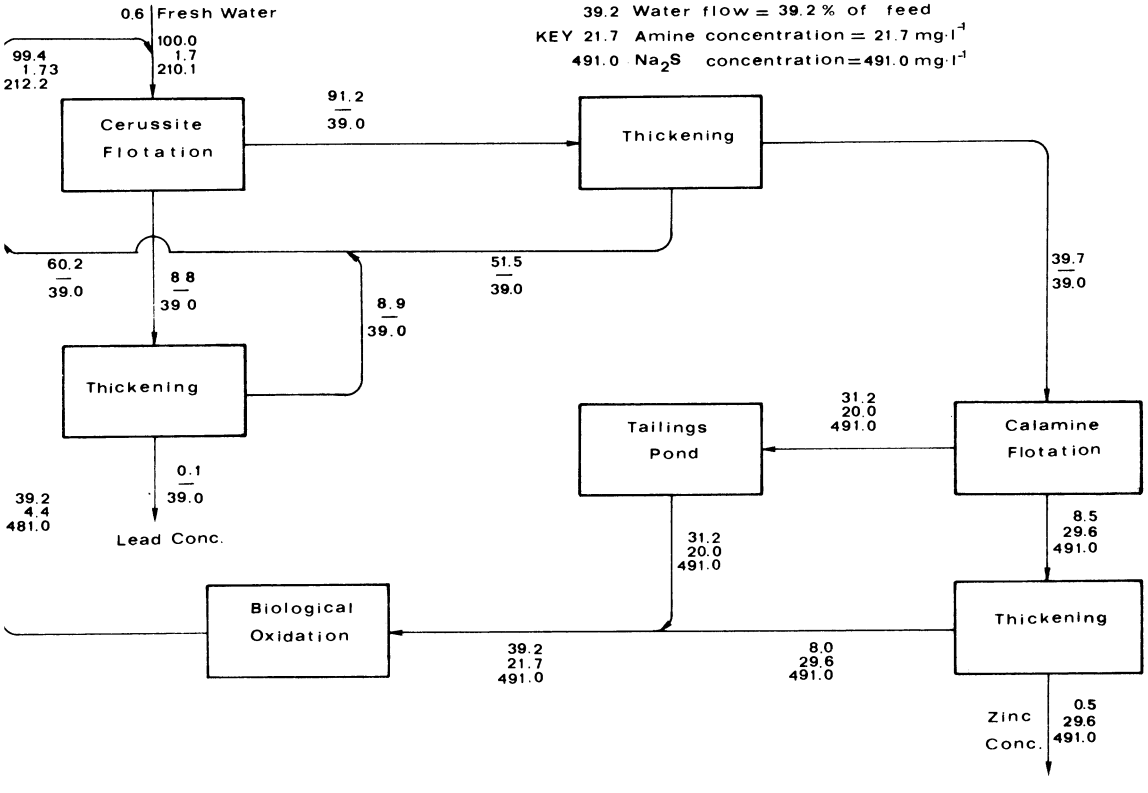


Figure 13: Proposal of water recycling flowsheet for a cerussite-calamine selective flotation plant.

Furthermore, the importance of the problem of mass transfer efficiency for the mixing of air with the solutions is once more confirmed; the better aerated the solution, the faster the biodegradation, as Fig. 10 clearly indicates. It is reasonable to belive that the faster degradation rates for all of the three reagents in the columns is directly related to the greater efficiency of air/liquid contact which occurs with the liquid film which is continually formed on the plastic chips.

Whereas the results obtained so far act as an incentive for the continuation of the research it seems appropriate, at this point, to illustrate proposed flowsheets with recycling of waste waters for the two cases which formed the object of investigation in this research program. The flowsheets for the barite + fluorspar flotation plants are shown in Fig. 12, while Fig. 13 shows those of the metal oxide minerals flotation plants. In both of these the water flows as well as the concentrations of the reagents to be removed in the various points of the circuits are indicated. In the case of Fig. 12 the carboxylate concentration (28.4 mg l^{-1}) and the alkylsulfate concentration (15.4 mg l^{-1}) and in the case of Fig. 13 the amine concentration (0.9 mg l^{-1}) appear low enough to be considered negligible insofar as their possible influence on the respective flotation performances is concerned.

ACKNOWLEDGEMENTS

The authors wish to express their greatest appreciation for the constructive suggestions and criticism granted by Prof. Antonio Spanedda, Professor Emeritus of Microbiology at Cagliari University. They would furthermore like to thank Prof. Laura Schivo, of the Microbiology Institute of Cagliari University for having kindly supplied the pure microbial strains.

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CONTROL OF ENVIRONMENTAL POLLUTION BY MINING WASTES (METALLIFEROUS MUDS, RED SEA)

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INTRODUCTION

The following paper intends to illustrate the comprehensive environmental program performed by a German mining company in order to avoid pollution of the existing ecosystem in the Red Sea. It will not present a detailed picture of the scientific results, but it intends to delineate the financial investments to be made and to show to what extent the mining industry takes care of protection of the marine environment.

Until now 18 so-called deeps have been identified in the central graben area of the Red Sea with hydrothermally influenced sedimentation; but only one of these pools seems to be of economic interest, namely, the Atlantis II deep. Initial estimates of the in situ value of sulfide ores vary between 2.5 and 3.5 billion dollars.

This mineral discovery caused the Sudan and Saudi Arabia to found a Red Sea Commission in 1974. The two countries issued decrees whereby the area beyond the depth of 1000 m is declared a common zone and is exploited jointly. A German company, Preussag, entered into an agreement with the Red Sea Commission in order to further explore the Atlantis II deposit and to investigate its economic utilization.

The amount of metals present in the Atlantis II deep are about 2.5 Tg zinc, 0.6 Tg copper and 9 Gg silver.

The metalliferous muds occur in 2100 to 2200 m water depth. They built up 10 to 20 m thick layers, deposited on volcanic rocks or older carbonatic sediments. The muds contain between 2 and 5.5% zinc, 0.3 and 0.9% copper and 60 to 100 ppm silver on a dry saltfree basis. The solid content varies between 8 and 13%.

During a pre-pilot-mining test between May and June 1979, approx. 15,000 m^3 of metalliferous mud have been mined in the Atlantis II deep, pumped to the surface, stored and processed by flotation. US drilling vessel 'Sedco 445' has been adapted by our company for the requirements of this first mining of metalliferous muds from the ocean floor. The mining equipment consisted mainly of 2000 m of drillstring, a vibrational suction head and a large pump. The mud was treated on board of the mining vessel by ultra-flotation methods. The flotation concentrate contains up to 42% zinc, 5% copper and 600 ppm silver in the dry saltfree material. The tailing material was discharged to the sea through a 6 inch pipe in 400 m waterdepth. Concentrations varied between 0.9 and 2.0% zinc, and 0.1 and 0.4% copper in the tailing material.

The environmental studies performed during March to June by a second vessel 'Valdivia' have to be divided into two categories:

A. The baseline studies to determine the background values of biology as well as physical and chemical oceanography.

B. Studies related to the disposal of 15,000 m^3 of tailing material.

This is the first time in mining history that the environment has been thoroughly investigated and data are available before anthropogenic contamination.

FIRST RESULTS OF BASELINE STUDIES

Benthos investigations

In general the observationsmade on benthos and nekton communities confirm a very low life intensity in the Red Sea. No differences in the bottom communities were encountered in the two slopes of the Red Sea. The quantity of organisms caught is strongly dependent on water depth. The poorest catches were made in about 1800-1900 m depth near the Atlantis II deep. Trap setting in the water column did not have the expected success; only a few shrimps were caught in the traps lowered. The samples collected with the pelagic trawls demonstrated a strong vertical migration of nekton organisms. Overnight all organisms concentrate in the uppermost 150 m, while during daytime they move downwards, partly up to 550 m. The distribution of lengths classes in the central area of the Red Sea shows a predominance of relatively small fishes of 20 to 50 mm length. Large numbers of molluscs were gathered in the upper part of the deep scattering layer while mesopelagic fishes and crustaceae prevailed in the collections which were made in the lower part of the scattering layer.

To determine the pathways of metal contaminants through the food chain, analysis of metals in organisms will be performed. Fifty samples from bottom communities and mesopelagic sources were stored by deep freezing and freeze drying for later analysis. Due to the lack of a rich nektonic and planktonic fauna it will be difficult to set up a useful biological monitoring system. Only shrimps seem to be suitable as they are occurring in the whole water column. Investigations on shrimps from a former environmental cruise in 1977 showed an increase of trace metals from the surface to 2000 m waterdepth, probably already reflect inga natural impact of metals from the hydrothermal source at the sea bottom.

Plankton investigations

The plankton community is characterized by a substantial decrease of invertebrate biomass and diversity from the Gulf of Aden to the centre of the Red Sea. The zooplankton density is extremely low below the 1250 m depth level; deeper than 1650 m no organisms were caught This observation demonstrates the very low population in the main deep sea graben region of the central Red Sea. Therefore a later tailing disposal of industrial size has to be restricted to the depth levels below 1250 m which corresponds to the margin of the offshore plateau.

The minimum oxygen layer occurring between 300 to 400 m is inhabited by a discrete zooplankton association. The copepods seem to be more frequent in the upper part of the minimum oxygen layer than in the lower part. The phytoplankton productivity of the euphotic zone was in each location measured at ten locations, at five depths, corresponding to light intensity levels of 1 to 100%. Final determinations will be made in home laboratories. It seems, however, from the high light penetration (down to 40 m) and low nutrient contents, that the productivity of phytoplankton is low. The short time effects of the tailing plume on the plankton community were studied by 14 zooplankton tows in the plume. A first look into zooplankton samples showed no remarkable changes in the distribution abundance of animals. No damage of specimens, caused by the tailings, could be recognized. Seven zooplankton stations were performed outside the plume for comparison studies.

Eco-toxicological experiments

Eco-toxicological experiments, performed on the research vessel and during a four weeks research campaign on a reef lagoon east of Port Sudan, concerned the following topics:

1. Sampling of fish and shellfish for later analysis of trace metals and metalloides.

2. Sampling of water by special techniques (COC-water samplers) and with special precautions (PVC-coated wire, PVC-messenger weights, etc.) for determination of trace elements.

3. Experiments on the release of metals from tailings into the water. The membrane filtered samples of up to three weeks eluation were preserved for later metal analysis.

4. Small-scale laboratory experiments, and medium-scale in situ experiments using plastic bags were performed to study the toxicity of tailings and eluates from tailings on a large number of organisms.

Effects of eluates from tailings were studied by investigation of:

1. Community structure changes of phytoplankton and zooplankton.

2. Exposure of mussels for analysis of bioaccumulation of metals.

3. Exposure of hydroids and corals to demonstrate toxic effects on benthic animals.

4. Determination of ^{14}C fixation of samples taken out of the bags to demonstrate effects on phytoplankton.

Furthermore baseline studies on metals in reef-fish were performed. Samples of dorsal muscle and liver were taken from those species which are preferably eaten by Sudanese fishermen.

Water transport and exchange investigations

Another very important sub-program of the environmental studies concerns the exchange of major water masses within the Red Sea and with the Gulf of Aden with emphasis on the renewal time of the water in the central trough. During a 12 months cycle, current data have been gathered from about 20 mooring lines on a profile from the Sudanese to the Saudi Arabian coast. Interpretation of data has started by developing computer models to predict water mass movements. At the Imperial College, London, further models are being developed to predict movement and settling behaviour of a tailing plume in time and space.

Exchange of the natural occurring brines, containing high amounts of trace metals, with the overlying seawater is studied by determination of different isotope concentrations in seawater samples, for example, ^{14}C, ^{13}C, ^{12}C, T, ^{4}He, ^{3}He, ^{39}Ar, ^{40}Ar.

RESULTS OF TAILING PLUME MONITORING

During the Pre-Pilot-Mining-Test about 15,000 m^3 of sediment brine seawater mixture have been disposed of from the mining ship into the sea. The material has been discharged through a 6 inch pipe at 400 m depth. Dumping amount and dumping time varied between 60 and 480 m^3 in 1.5. The actual or possible influence of the dumped material on the marine environment was studied by determining various chemical and physical parameters in the seawater before, during and after the operations, namely:

Light transmission at surface and at depths, amount of suspended material or seston, temperature, salinity and conductivity, oxygen, phosphate, nitrate, nitrite, silicate, pH.

Further, water sampling was made for trace element analysis, to determine release of metals from the tailing material.

Two methods were successfully employed to follow the distribution of the plume in space and time:

1. High frequency echo sounding.

2. Measuring continuously light attenuation by a STD-probe.

Best results were achieved with the 30 kHz stabilized narrow beam echo sounder. It was possible to delineate the plumes and follow them at depths for several days if the quantities discharged have been large enough. Best observation time was during night, when little influence occurred from the plankton layers. The shape of the forming and disintegrating plumes could be outlined by several profiles. The results so far obtained indicate that the dumped sediment seawater mixture disposed of in 400 m water depth flows down rather rapidly, forming jet or turbidity flows until 600 to 800 m water depth. Fall velocity and falling way seem to be dependent on the solid content of the slurry. Predominantly at 800 m the sediment slurry spreads to a plume with a lateral maximum. After a few hours the plume extends down to about 1100 m. The lateral movement of the plumes was low, between 50 and

150 m per hour, but the vertical movement relatively high (15 to 20 m h^{-1}). It can be assumed that seawater flocculates the sedimentary material thereby increasing the fall velocity of the particles. The small plumes obtained by discharge of a few tens of cubic metres could be monitored at least for a number of hours, the large scale discharges consisting of a few hundreds of cubic metres could be observed for six subsequent days, before the plume became too faint. Before and at the beginning of the tailing disposal, landscape satellite pictures were taken by the NOAA.

The second successful method to delineate the tailing plume was the measuring of the light attenuation by aid of the multisonde STD equipment. When the large scale tests started, maximum values of about 0.1 mg 1^{-1} could be measured and a number of vertical and horizonta profiles could be made through the plume for a number of days. The initial dilution within the plumes is very high and occurs immediately after discharge. The material discharged contains a solid content of about 15 to 20 gl^{-1}. Compared to values of 0.01 to 0.02 mg solids per litre in most parts of the plume a dilution of 10^6 could be determined. This high dilution rate may explain why no substantial changes in the concentrations of nutrient could be measured in the water column, neither for oxygen, dissolved phosphate, silicate nor nitrate.

Another experiment has been performed to determine the ultimate spreading area of the tailings discharged. Far away from the already-tailing-contaminated waters of the Atlantis II deep, 480 m^3 of homogenized material was discharged within 1.5 h at one point. This mud contained a tracer, consisting of 1 kg iridium in 50 kg of quartz glass granules, ground to grain sizes with the same settling characteristics as the metalliferous sediments Some sea floor samples were taken at the end of the cruise, but solids of tailings are not expected to reach the sea floor prior to a few months. A detailed inspection of the area covered by tailing material will be performed during the next environmental cruise of MESEDA III which is intended for the autumn of 1980.

Outlook

If the technical problems of large scale mining can be solved and the results of the environmental investigations point to no damage to living resources, in 1982 a pilot mining test on a 1 to 5 scale, compared to a later mining proposal, will be performed. It is intended to mine the whole deposit in a period of 15 to 20 yr. The envisaged daily production of 96 Gg of muds, corresponding to 10 Gg of solid material, will yield about 9.8 Gg of tailings, which then has to be disposed of at sea in a way that neither the deposit area would be filled up again nor the environment of the Red Sea be damaged seriously. In each case, we must be sure that mining will let it remain a Red Sea and not let it become a Dead Sea.

DESULFURIZATION OF COAL BY *THIOBACILLUS FERROOXIDANS*

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INTRODUCTION

Literature on the possibility of desulfurizing coal by microbiological leaching is limited. The earliest report was that of Zarubina *et al.* (1959) who indicated that 23 to 27% of sulfur was removed from their coal samples in 30 days. Other investigators (Silverman *et al.* 1961, 1963; Lorenz and Tarpley 1963; Clark 1966) commented on the beneficial effect of decreasing the particle size of coal samples and of adding small amounts of ferric sulfate to the leach solution. Recently, Dugan and Apel (1978) achieved an almost complete removal of pyrite from coal by using a mixed culture of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. Capes *et al.* (1973) reported that the surface properties of pyrite and coal were changed during the microbiological leaching to such an extent that the separation of pyrite from coal by the conventional flotation technique was considerably increased.

The chemolithotrophic microorganism, *T.ferrooxidans*, mainly responsible for coal desulfurization, catalyzes the oxidation of pyrite according to the following reaction (Silverman, 1967):

$$2\ FeS_2 + 7.5O_2 + H_2O \xrightarrow{\text{bacteria}} Fe_2(SO_4)_3 + H_2SO_4 \qquad (1)$$

Pyrite is insoluble in the aqueous leach medium while ferric sulfate and sulfuric acid are soluble.

The ferric sulfate produced by reaction 1 tends to hydrolyze and yield an insoluble hydroxide (reaction 2) or basic sulfates (reactions 3 and 4):

$$Fe_2(SO_4)_3 + 6H_2O \rightleftarrows 2Fe(OH)_3 + 3H_2SO_4 \qquad (2)$$

$$Fe_2(SO_4)_3 + 2H_2O \rightleftarrows 2Fe(OH)SO_4 + H_2SO_4 \qquad (3)$$

$$1.5Fe_2(SO_4)_3 + 6H_2O \rightleftarrows \underset{\text{jarosite}}{\{H[Fe(SO_4)_2 \cdot Fe(OH)_3]\}} + 2.5H_2SO_4 \qquad (4)$$

Reactions 1 through 4 result in an increase in the acidity of the leach medium or of the coal mine drainage water.

The ferric iron formed in reaction 1 is an oxidizing agent (Dutrizac and MacDonald, 1974) which can further oxidize pyrite and metal sulfides associated with coal (5 and 6):

$$FeS_2 + 4Fe^{3+} \rightarrow 5Fe^{2+} + 2S^o \qquad (5)$$

$$MS + Fe^{3+} \rightarrow M^{+2} + 2Fe^{2+} + S^o \qquad (6)$$

where M is bivalent metal. The ferrous iron and elemental sulfur are oxidized by bacteria (reactions 7 and 8; Margalith *et al.*, 1966; Trudinger, 1967; Kelly, 1968):

$$Fe^{2+} \xrightarrow{T.ferrooxidans} Fe^{3+} + e \qquad (7)$$

$$S^o + 1.5O_2 + H_2O \xrightarrow{T.ferrooxidans} H_2SO_4 \qquad (8)$$

Reaction 7 regenerates the oxidizing agent while reaction 8 contributes further to acid production. Reaction 8 can also be carried out by *T.thiooxidans* (Karavaiko and Pivovarova, 1973) which inhabits acid mine waters.

Metal sulfide can also be oxidized directly to sulfate by bacteria (reaction 9; Torma, 1971):

$$MS + 2O_2 \xrightarrow{T.ferrooxidans} M\,SO_4 \qquad (9)$$

The direct (9) and indirect (6) reactions probably take place stimultaneously in nature where an acidic environment is maintained in the presence of sulfide-bearing minerals. The metal sulfide oxidation capabilities of microorganisms have been extensively reviewed (Ehrlich and Fox, 1967; Trudinger, 1971; Tuovinen and Kelly, 1974; Torma, 1977; Brierley, 1978; Zajic, 1969; Roy and Trudinger, 1970; Karavaiko *et al.*, 1972; Brock, 1978).

The present report describes a detailed investigation of the microbiological desulfurization of two distinctly different coal samples by a pure strain of *T.ferrooxidans*.

MATERIALS AND METHODS

Bacteria

The pure culture of *T.ferrooxidans* used in this study was isolated from acid mine waters of a chalcopyrite-bearing ore deposit (Torma, 1971) and was maintained on a modified 9K-medium (Silverman and Lundgren, 1959) in which the energy source (ferrous sulfate) was replaced by pure pyrite or a pyrite-bearing coal sample. Logarithmic phase cultures were used as inocula for subcultures and experimental systems.

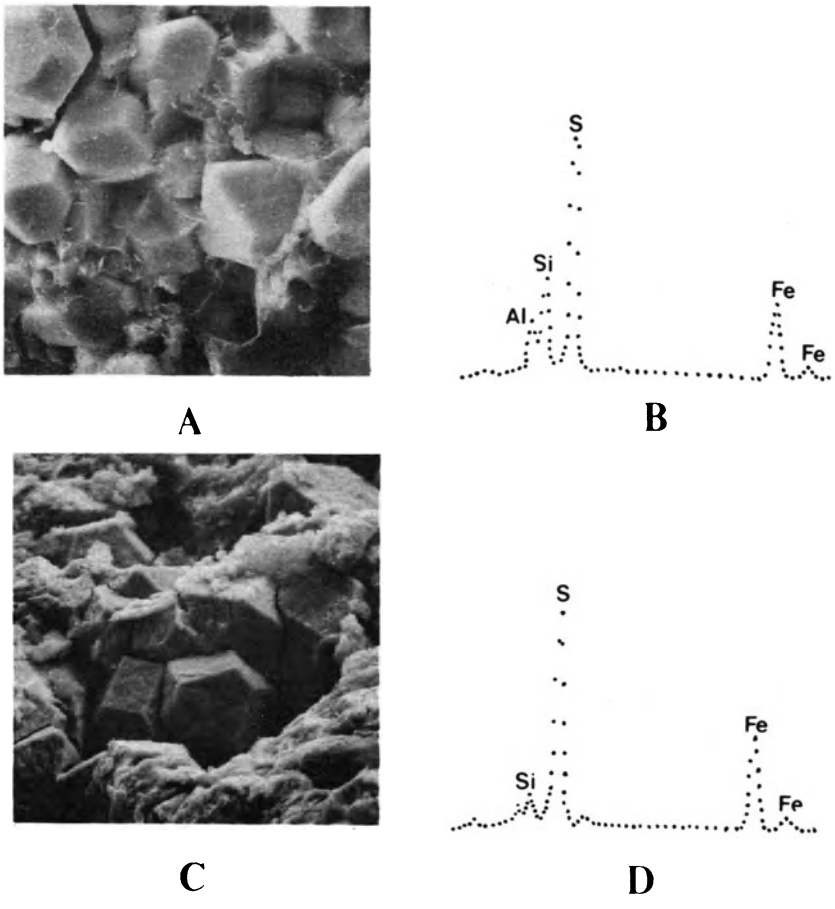
Substrates

A naturally occurring pyrite concentrate from the Sargent Welch Co., Denver, Colorado was ground to a particle size of less than 37 μm. Coal samples were obtained from the Central Ohio Coal Company and from the Hart Mine, Carthage, New Mexico. The samples were ground to less than 37 μm in size and analyzed. The coal samples differed significantly in total sulfur content, pyrite content and dissemination, and mineralogy (Table 1). The New Mexico coal contained considerable amounts of acid-soluble iron compounds. Pyrite structures in the coal samples are shown in Fig. 1.

TABLE 1

CHEMICAL AND MINERALOGICAL COMPOSITIONS OF COAL SAMPLES

Sulfur Content	Ohio Coal		New Mexico Coal	
	Chemical %	Mineralogical	Chemical %	Mineralogical
S_{total}	5.00	pyrite,	3.43	pyrite,
$S^{2-}_{inorganic}$	4.10	gypsum	1.95	gypsum
SO_4^{2-} inorganic	0.12		0.63	iron oxides
$S_{organic}$	0.78		0.85	



Fugure 1: Scanning electron micrographs of pyrite in untreated Ohio (A,B) and New Mexico (C,D) coal samples at 2,000X magnification together with their energy dispersive X-ray analyses.

Experimental procedures

Shake flask experiments were carried out as described previously (Torma, 1971) in 250 ml Erlenmeyer flasks containing 70 ml of 9K basal salts solution (Silverman and Lundgren, 1959), substrates, and 5 ml of bacterial inoculum. In the sterile controls, 5 ml of a 2% alcoholic solution of thymol replaced the inoculum. The flasks were incubated on a New Brunswick Gyrotory Shaker.

Oxygen mass transfer into the leach solutions was determined by the method of Guay *et al.* (1975). Harvesting of *T.ferrooxidans* cells free of substrate, and the manometric respirometer technique, were described by Torma and Gabra (1977). Protein was determined according to Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Shake flask experiments

The influence of pH (from 1.0-4.0) on the rates of iron solubilization from pyrite and Ohio and New Mexico coals was studied with leach suspensions containing 10% pulp density (solid to liquid ration X 100) shaken at 250 rpm at 35°. The optimum pH was 2.0 for all three

substrates. This value lies within the range, pH 1.8 to 2.5, reported as optimum for *T.ferrooxidans* grown on metal sulfides (Ehrlich and Fox, 1967; Torma, 1977).

The influence of substrate concentration (pulp density) on bacterial activity was studied using pure pyrite and the Ohio and New Mexico coals. The rates of iron dissolution (V,mg l^{-1} h^{-1}) were determined from the linear parts of curves representing iron dissolution as a function of time. The highest rates were obtained with 25% pulp density leach suspensions for all three substrates. With Ohio and New Mexico coals, more than 77% and 83% (respectively) of sulfur was removed in inoculated samples compared with about 5% and 10%, respectively, in sterile controls. Using a linear form of a modified Monod equation (equation 10; Torma, 1971),

$$V = Vm\ (PD) \ /\ (K + (PD)) \qquad (10)$$

the maximum rates of iron oxidation (Vm) and K-values (substrate concentration for Vm/2) were determined (Fig. 2).

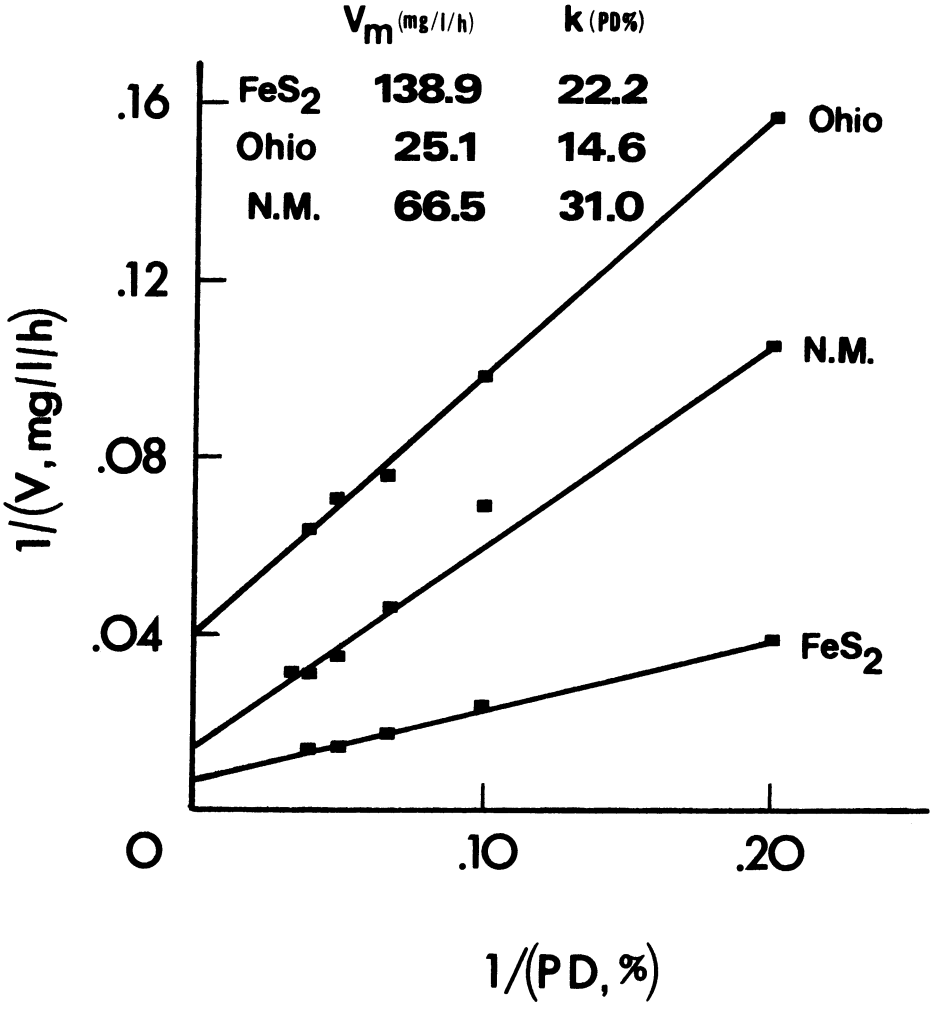


Figure 2: Effect of pulp density (PD) on the rate (V) of iron extraction by *T.ferrooxidans* from pyrite and coal: initial pH 2.0, temp. 35°.

The order of reaction (n) and the overall rate constant (k) have been determined for reaction 1 using the differential method of van't Hoff (equation 11; Laidler, 1965):

$$V = k\ C^n \tag{11}$$

where V (mg l^{-1} h^{-1}) is the rate of sulfide oxidation (in pyrite or in coal) and C(mg l^{-1}) is the initial sulfide concentration in the leach suspension. Fig. 3 shows plots of log V versus log C. Values for k are determined from the intercepts of the straight lines with the log V-axis and values for n from the slopes. The following rate equations were established:

for pure pyrite oxidation

$$V = 0.05\ C^{0.63} \simeq 0.05\ \sqrt[3]{C^2}\ ; \tag{12}$$

for oxidation of pyrite in the Ohio coal

$$V = 0.12\ C^{0.53} \simeq 0.12\ \sqrt{C}\ ; \tag{13}$$

for oxidation of pyrite in the New Mexico coal

$$V = 0.04\ C^{0.73} \simeq 0.04\ \sqrt[4]{C^3} \tag{14}$$

The differences in these rate equations reflect differences noted above in pyrite content and dissemination, as well as associated mineralogical inclusions, etc., all of which influence the raction rates. The order of reaction determined by this technique is often referred to as the order with respect to concentration of the reactant, or the true order.

The influence of particle diameter on the rate of iron extraction from pure pyrite and Ohio and New Mexico coal samples is shown in Fig. 4. For all three substrates, the rate seems to follow an exponential type of curve, which could probably be described by the following form:

$$V = k_1\ Vm\ \exp\ (k_2 d) \tag{15}$$

where k_1 and k_2 are constants, and d is the particle diameter.

Oxygen mass transfer was determined with 0.1N Na_2SO_3 solutions (in 9K medium; Silverman and Lundgren, 1959) at pH 2.0, temperatures of 25 to 45° and shaking rates of 100 to 400 rpm. Typical results are presented in Fig. 5. Slopes of the straight lines correspond to the rate of oxygen transfer (equation 16):

$$dC_\ell dt = k_{\ell a}\ (C^* - C_\ell) \tag{16}$$

where $k_{\ell a}(h^{-1})$ is the volumetric oxygen mass transfer coefficient, C_ℓ is the dissolved oxygen concentration, t is the time (h) and C* is the oxygen concentration at saturation. At 35° and a shaking rate of 250 rpm, C* was 5.74 mg l^{-1} and $k_{\ell a}$, 238.4 h^{-1}. Under the same conditions the highest rates of oxygen consumption with 25% pulp density suspensions of pure pyrite and Ohio and New Mexico coals were 146.0, 33.8 and 8.4 mg O_2 l^{-1} h^{-1}, respectively. According to the data in Fig. 5, the rate of oxygen transfer at 35° and 250 rpm was 1,368.3 mg O_2 l^{-1} h^{-1}. Clearly then oxygen was not a limiting factor in these leaching experiments.

Warburg Respirometer Studies

The influence of temperature on the oxidation of pyrite was determined by Warburg manometric technique (Torma and Gabra, 1977) using 200 mg of substrate (pure pyrite, Ohio or New Mexico coal) 2.0 ml of nutrient medium (Silverman and Lundgren, 1959) and 0.3 ml of a 10% (w/v) bacterial suspension containing 4.9 mg protein.

Arrhenius plots are shown in Fig. 6, from which activation energies of 44.3, 38.8 and 40.8 kJ mol^{-1} for pyrite, Ohio and New Mexico coal, respectively, were derived. The same order of magnitude of activation energies have been reported for heavy metal sulfide oxidation (Torma, 1977). The temperature coefficient, Q_{10}, was calculated using equation 18;

$$Q_{10} = V_2/V_1)^{10/(T_2-T_1)} \quad (18)$$

where V is the rate of oxygen uptake and T the temperature. For the interval 25 to 35° the Q_{10} - values were 2.00, 1.75 and 1.83 for pyrite, Ohio and New Mexico coal, respective

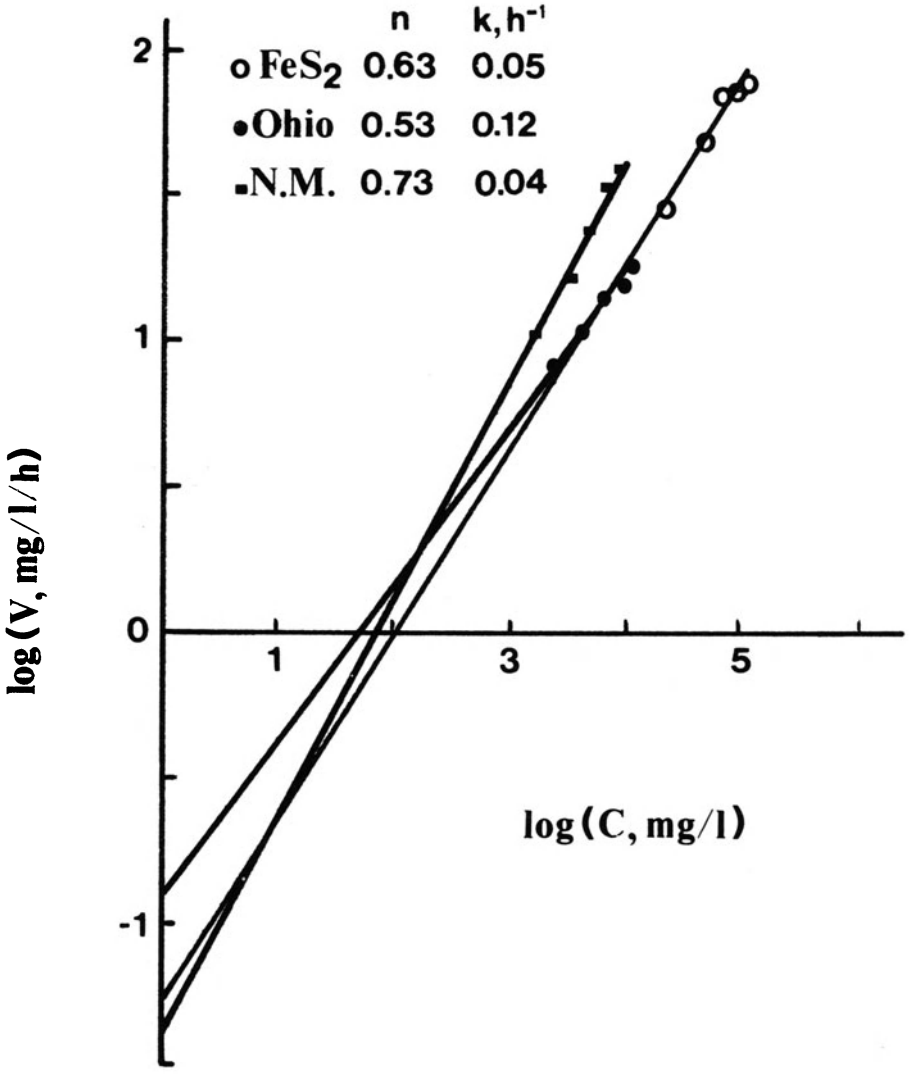


Figure 3: Rate (V) of iron extraction from pyrite and coal by *T.ferrooxidans* as a function of the initial sulfide concentration.

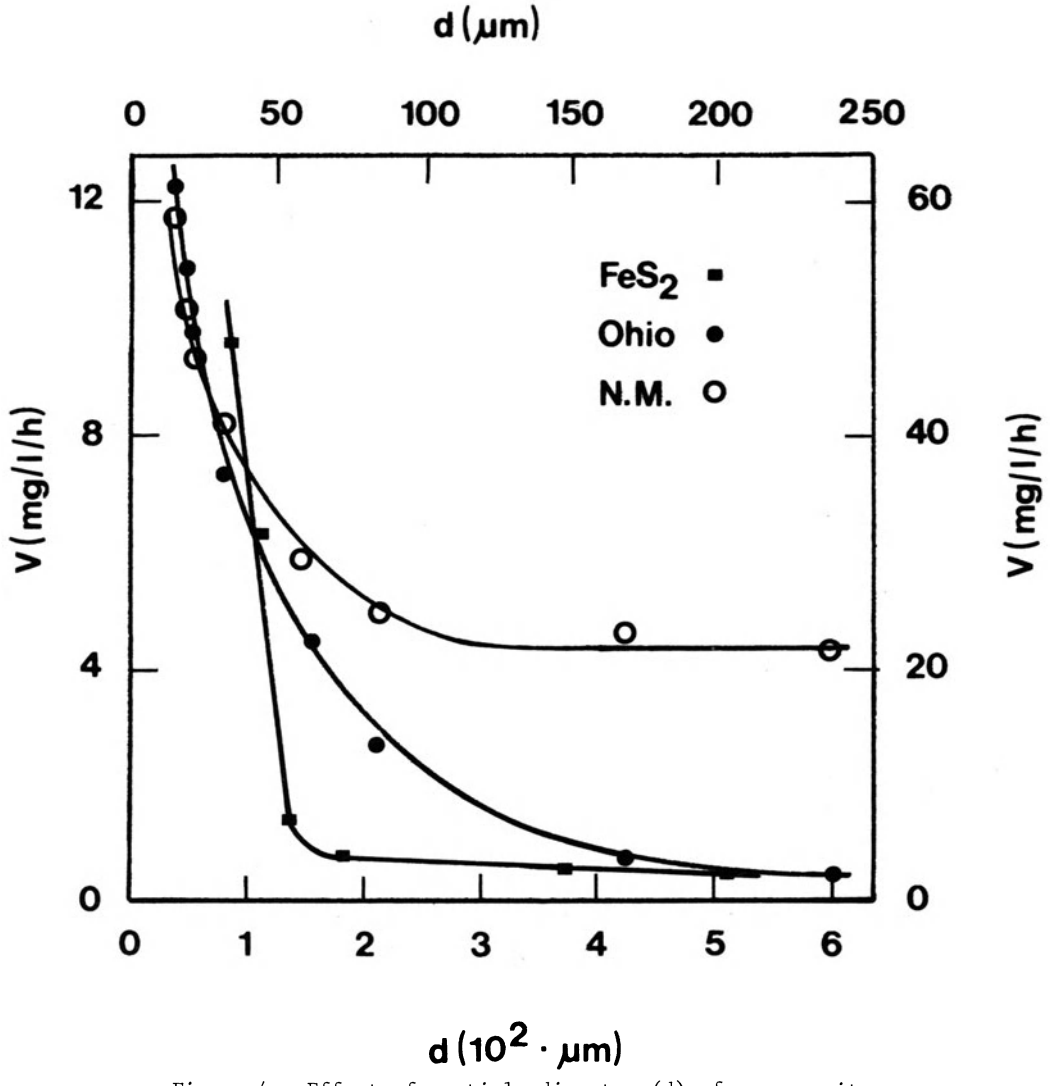


Figure 4: Effect of particle diameter (d) of pure pyrite and Ohio and New Mexico coal samples on the rate (V) of iron extraction by *T.ferrooxidans* at 10% pulp density: initial pH 2.0, temp. 35^{o}, shaking rate 250 rpm. The upper right hand side scales are applicable to pure pyrite oxidation.

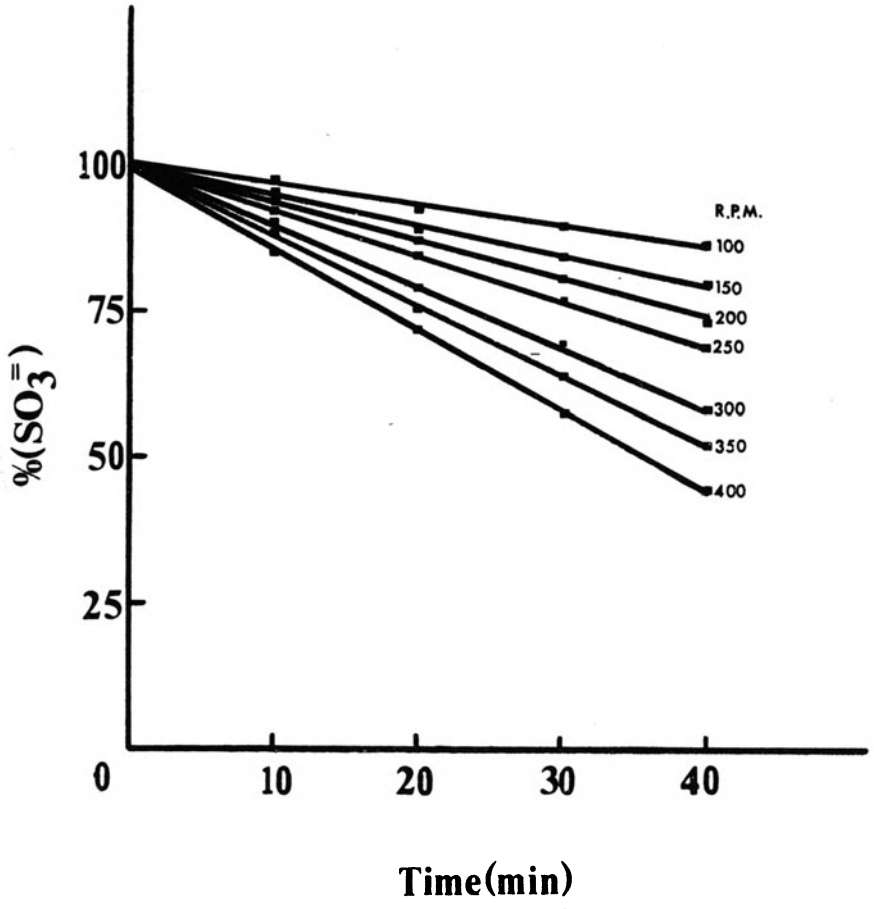


Figure 5: Sodium sulfite oxidation at 35° and pH 2.0 as a function of time and shaking rate. Each point on the figure represents the mean of triplicate measurements.

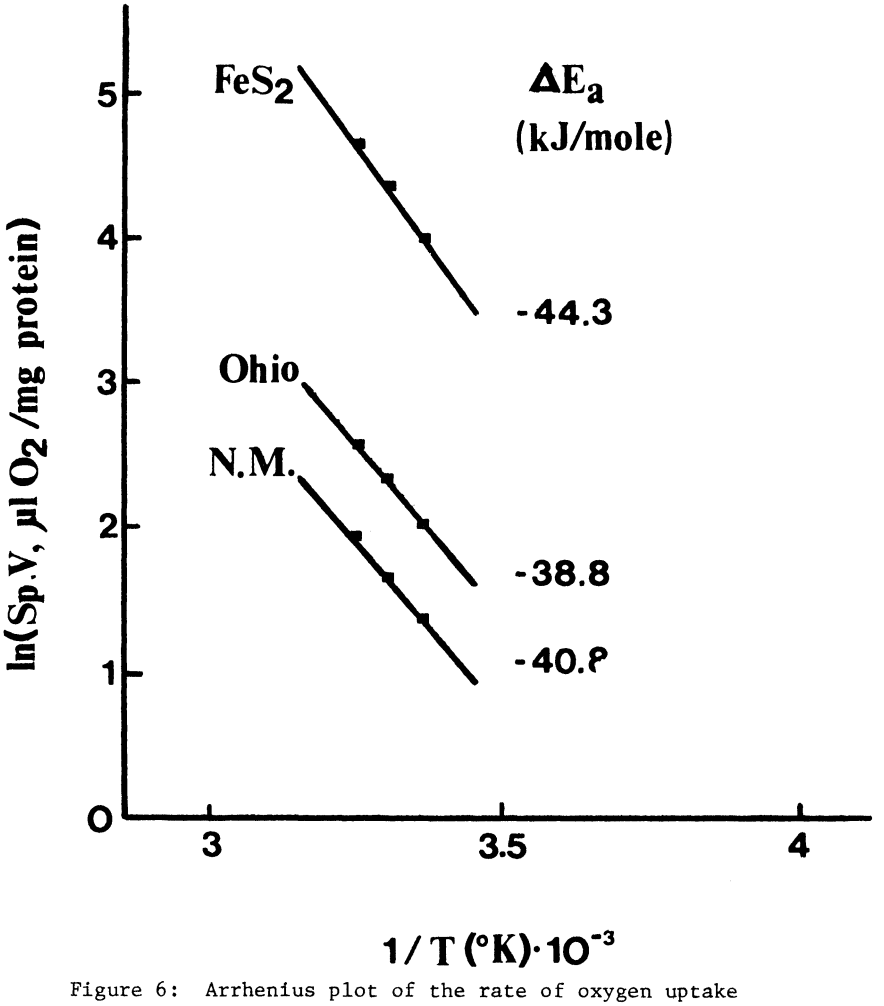


Figure 6: Arrhenius plot of the rate of oxygen uptake by *T.ferrooxidans*, with pyrite or coal as a function of temperature.

CONCLUSIONS

The present study has established many significant leads in the application of the bacteri leaching technique in the desulfurization of coal. The results indicate that *T.ferrooxida* is able to successfully remove significant concentrations of sulfur from coals of very different nature. The optimum leaching conditions have been established for a pure pyrite concentrate and coals from Ohio and New Mexico. Kinetic data on leaching and oxygen mass transfer have been determined which will provide insight into the mechanism of desulfurization and be useful parameters in the planning of commercial operations.

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MINE WASTE POLLUTION CONTROL AT CAPTAINS FLAT, NEW SOUTH WALES

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INTRODUCTION

Mine waste pollution from Captains Flat mining area in New South Wales, Australia (Fig. 1), had degraded the biology of the Molonglo River, which flows into Lake Burley Griffin in Canberra some 50 km downstream, and reduced the usefulness of its waters. In the absence of immediate remedial works, there was a risk of increased pollution from accelerating erosion of the mine waste dumps and from the collapse of unstable areas.

After discussions between the Commonwealth and New South Wales Governments, a Joint Government Technical Committee was formed in 1974 to enquire into the problem and to recommend remedial measures which would lead to permanent abatement of the pollution of the Molonglo River arising from the abandoned mining area.

THE PROBLEM

Mining commenced at Captains Flat in 1874 and continued intermittently until 1962. During this period 4 Tg of ore was milled to produce zinc, pyrite, lead, copper and gold and 2.5 Tg of mine waste was left piled in dumps covering an area of 0.15 km^2. The waste in the dumps (Fig. 2) contained significant quantities of these heavy metals (Table 1), and the pH was extremely acid - pH 2.8 for the southern dumps and 2.9 for the northern dumps (Craze, 1977a).

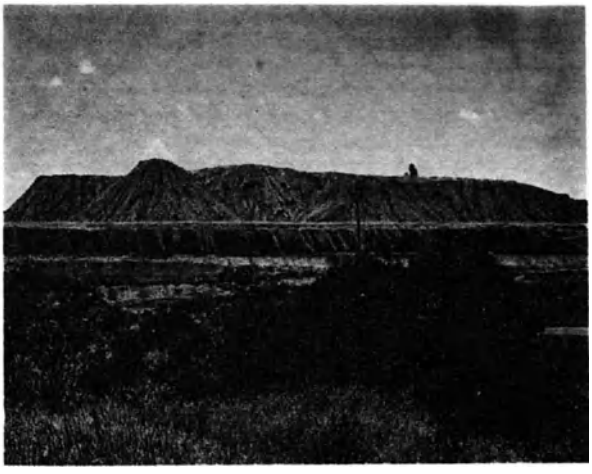

Figure 2: The northern solids dump at Captains Flat.

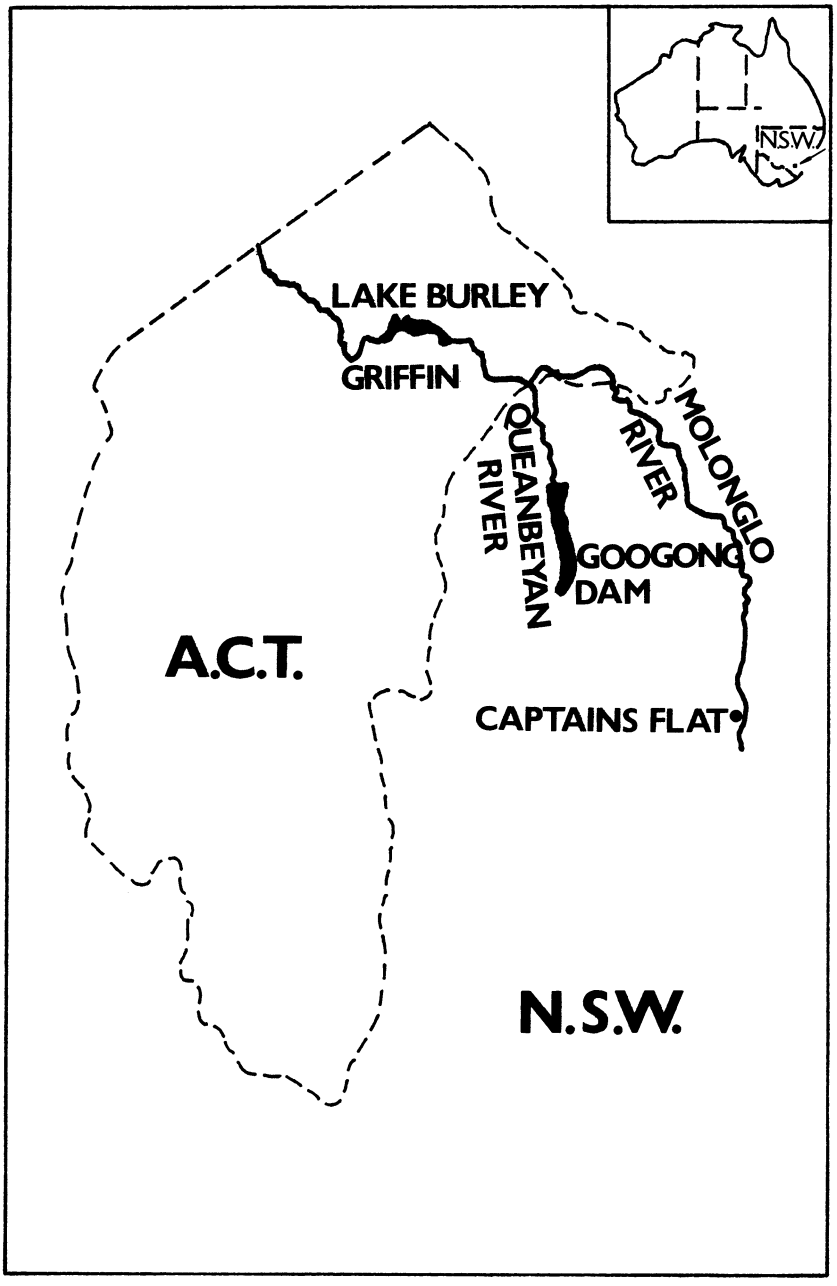

Figure 1: Captains Flat: Location map.

TABLE 1

QUANTITIES OF HEAVY METAL FOUND IN DUMPS
AT CAPTAINS FLAT

Metal	Southern Dumps (%)	Northern Dumps (%)
Total zinc	2.6	1.1
Total lead	0.8	0.5
Total copper	0.1	0.05
Total sulfur	21	17.4
Total sulfur (as FeS_2)	39.5	32.5

The southern dumps contained the finer fraction called slimes which after processing had been pumped as a slurry into earthen dam storage areas. Here the fine fraction settled out and the water either evaporated or was drained for reuse. The coarse fraction from hydrocycloning was piled high to form large dumps, referred to in Table 1 as northern dumps. These dumps besides being unsightly, were also a considerable nuisance as sources of air and water pollution (Fig. 3).

Figure 3: Drainage from the northern slimes and solids dumps passed through the pipe into the Molonglo River, causing it to be polluted and sterile for many kilometres.

They lacked stability along the outer faces and, in 1939, material from the face of one of the northern slimes dams collapsed into the Molonglo River. There was destruction of vegetation along the river valley, particularly on the flats at Foxlow, some 15 km downstream. In 1942, part of one of the southern dumps collapsed, dumping some 30 000 m^3 of tailings into the river. Major flooding of the Molonglo in 1945 carried some of these tailings onto the Foxlow-Carwoola Flats, again destroying pastures. Despite an attempt to remedy the problems by heavy liming, only sparse vegetation has returned in the intervening years (Fig. 4 and Fig. 5).

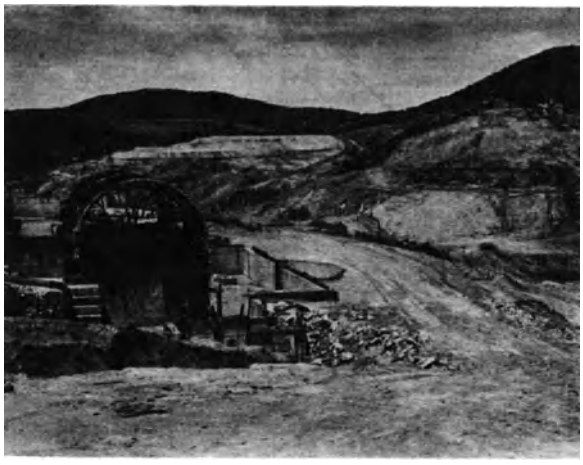

Figure 4: Abandoned treatment works at Captains Flat.

Figure 5: Foxlow-Carwoola Flats, showing the sparse vegetation that has returned after the area was covered to depths of 80 cm in places by mine waste in 1939 and 1945. The waste was washed down the Molonglo River after two slimes dams collapsed at Captains Flat, 15 km upstream.

Much of the tailings lost in these events, and through the continuing erosion of the dumps, exists today as bed load in the Molonglo and Queanbeyan Rivers down to, and including, the floor of Lake Burley Griffin at Canberra.

The extreme acidity of the tailings was a result of oxidative decomposition of pyrite, which was considerably accelerated by *Thiobacillus* bacteria. The chemical reactions resulted in the formation of soluble copper and zinc sulfate, insoluble lead sulfate and sulfuric acid. As most of these reactions require abundant supplies of oxygen and water, it was impossible for acidification to occur through the entire tailings. Generally, it was localised in the top two metres, with a zone of maximum acidity occurring in the top 30 cm. The reaction products, apart from lead sulfate, were readily leached from the dumps, and pH values as low as 1.5 were measured in the runoff.

The maximum levels of metals measured in mine drainage, waters of the Molonglo River, and river sediments at Captains Flat, are shown in Table 2. The heavy metal contamination in the river was so high that it was basically sterile for many kilometres.

TABLE 2

MAXIMUM LEVELS OF METALS MEASURED IN THE SLIMES DRAINAGE, WATERS OF THE MOLONGLO RIVER, AND RIVER SEDIMENTS AT CAPTAINS FLAT

Metal	Mine Drainage ($\mu g\ g^{-1}$)	Molonglo River ($\mu g\ g^{-1}$)	River Sediments ($\mu g\ g^{-1}$)
Zinc	6 200	130	1 800
Copper	720	1.5	1 000
Lead	70	0.7	9 000
Cadmium	8	0.03	3
Iron	5 000	130	60 000
Manganese	1 100	8	1 300
Nickel	2	0.1	40
Cobalt	21	0.1	20
Chromium	1	0.1	105
Arsenic	7	1	-

As a result of the formation of sulfates of iron, copper, lead and zinc, salinity levels were extremely high (31 to 42 mS cm^{-1}). These salt contents caused osmotic pressures of 1 to 1.5 MPa, which were too high for plants to establish in, even under saturated conditions.

The physical properties of the mine waste were also of major importance. Permeability was abnormally low due to cementation of the particles by heavy metal salts. Falling head permeameter tests carried out on cores from the dumps gave saturated hydraulic conductivities, K of 1.2 nm s^{-1} at a bulk density ot 1.31 g cm^{-3} for the southern slimes dump and K of 120 $\mu m\ s^{-1}$ at a bulk density of 1.76 g cm^{-3} for the solids dump. These rates represent rate of water movement of 4 cm and 40 cm yr^{-1} respectively. Further details of dump material are given in Craze, 1977(a).

VEGETATION STABILIZATION

Three main methods of treatment to stabilize tailings and decrease the air and water pollution have been used through the world: 1, covering with rock and slag; 2, covering

with chemical and binding agents; and 3, vegetative stabilization. Almost all published research indicates that by far the most promising stabilization method is by the use of vegetative cover. Rock and slag cover do not seal the tailings from water and air. They are also difficult to vegetate. Chemical and other binding agents deteriorate rapidly by weathering. Successful vegetation usually takes many years, and no one to date has managed to grow on tailings anything comparable to those at Captains Flat. James and Mrost (1965) used successfully a technique of spray irrigating mine dumps in South Africa to leach out the heavy metals. For this method to succeed it was important that the precipitation rate did not exceed the infiltration rate of the tailings. At Captains Flat the permeability of the tailings was so low it was practically impossible to leach them without causing increased runoff, erosion and ponding and a raising of the water table. Such water applications could have led to dump collapse.

Lime has commonly been used on acid tailings to raise the pH and lower the concentration of soluble heavy metals sufficiently for plants to grow. Lime requirement tests showed rates of 5.5 and 5 kg m^{-2} to raise pH to 7 for the southern and northern dumps, respectively. Such rates are clearly impracticable and have only a trnsitory effect (Nielson and Peterson, 1972; MacLean and Dekker, 1976). At Captains Flat, additions of lime up to 4 kg m^{-2}, for the purpose of making the tailings suitable for plant establishment, caused short term beneficial rises in pH, but also caused increased complexing of added phosphorus by the heavy metals and by the lime itself (Craze, 1977d). As much as 64% of added phosphorus was complexed in one day by tailings without lime, and with 4 kg m^{-2} of lime, 94% of phosphorus was complexed in one day. Because of the need to rapidly stabilize the reshaped dumps to prevent erosion and pollution none of these long-term direct vegetating techniques were satisfactory.

THE RECLAMATION METHOD

The results of the earlier investigations (Anon., 1974; Craze, 1977 a, b, c) made it clear that vegetative cover could not be established directly on the toxic tailings. After evaluating nine possible reclamation proposals to control the mine waste pollution, the Government Committee recommended one which would permanently abate the problem by preventint erosion and leaching of the mine waste dumps, and by reducing the flow of mine water from the main spring, which was a major contributor of pollution. After closure of the mine in 1962, the mine filled with water and overflowed into the Molonglo River through the air shaft at the Northern end of the workings and from a spring which developed close by after the air shaft was plugged.

In brief, the details of these works were as follows:

The dumps had to be reshaped, and this involved reducing the height of the main solids dump and constructing terraced slopes with grades no steeper than 1 in 3 and no flatter than 1 in 20. These slopes were chosen in order to minimize scouring, to facilitate vegetation and provide surface and internal drainage (Fig. 6).

Figure 6: Reshaping the northern solids dump.

The terraced slopes were covered firstly by about 22 cm of compacted clay to seal the tailings from air and moisture and thus prevent acidification. This was followed by 45 cm of rock-fill (shale) to prevent the capillary rise of any toxic substances that might permeate the clay from the underlying tailings and to provide a lateral drainage layer. Finally, 30 cm of soil was added as a top later to sustain a cover of vegetation (for a description of soil, see Craze, 1977a).

This reclamation method provided for lateral drainage beneath the topsoil to prevent build-up of moisture in the dumps, thereby increasing stability, and to reduce leaching. The final covering of soil was to provide a suitable medium in which an adequate cover of vegetation could be rapidly obtained. If any erosion did take place before a dense cover was established, it would be the non-toxic surface soil that reached the river. Trial embankments showed this technique to be successful.

Fertilization and sowing were carried out as soon as possible after placement of topsoil. The grass and legume mixture consisted of cereal rye *(Secale cereale)*, as a quick growing cover crop, Namoi woolly pod vetch *(Vicia dasycarpa)*, red clover *(Trifolium pratense)*, white clover *(T.repens)*, brown top bent grass *(Agrostis tenuis)*, Demeter fescue *(Festuca arundinacea)*, and couch grass *(Cynodon dactylon)*. The seed was sown through a fertilizer spreader and the batters were covered with straw mulch and bitumen. The whole of the restored area was watered by sprinkler irrigation. Fertilizer rates were 60 g m^{-2} of superphosphate, 29 g m^{-2} ammonium nitrate, 20 g m^{-2} potassium sulfate and 0.125 kg m^{-2} of lime, (Craze, 1977b). Further applications of fertilizer are being made in a 5 yr maintenance programme and the area is regularly slashed in order to build up the organic matter in the top-soil. Watering is still carried out through the dry summer and autumn months. All species are still present but some dominate certain areas more than others.

It is necessary to exercise extreme care during the entire works operations in order to avoid incorporating tailings into the covering layers. Special attention had to be paid to washing down equipment when moving off tailings surfaces. The efforts made to minimise accidential incorporation of tailings within the soil zone were largely successful. It has been necessary to ameliorate or replace only a few small areas of soil where plants would not thrive due to the presence of toxic material.

The cost of the works was estimated at $2.5 million (Australian), with provision for maintenance by the Soil Conservation Service of $200,000 over 5 yr.

TABLE 3

ANALYSIS OF WATER FROM THE MOLONGLO RIVER, AT CAPTAINS FLAT, PRE- AND POST- RECLAMATION

	Pre-reclamation[a]	Post-reclamation[a]
pH	5.3	6.6
EC ($\mu S\ cm^{-1}$)[b]	328	230
SO_4 ($\mu g\ g^{-1}$)	125	65
Zn ($\mu g\ g^{-1}$)	11	6

[a] Values are averaged over 3 and 4 yr, respectively.

[b] EC = Electrical Conductivity

An extended period of rain in September-October, 1976 yielding some 355 mm was recorded when vegetation measures were only partially complete. At the end of this period, over 107 mm fell in a single storm within 3 h. No damage of any significance occurred to any of the work and run-off was minimal due to the design of the works, the stable and loose nature of the soil and the vegetation already established.

There has been a dramatic reduction in the mineral content of the Molonglo River (see Table 3) and algae are beginning to appear in the waters immediately below the mine site. The pH of the water has risen from an average of 5.3 to 6.6. Sulfate and zinc loads have decreased by 50% and the soluble salt level (EC) has fallen 30% and is still improving. Levels in sediments do not show much of a change but as the source of pollutants has been substantially eliminated by covering the dumps, the natural occurrence of river floods will disperse in time and dilute the sediment load. The State Pollution Control Commission with the Department of Mineral Resources, continue to monitor runoff and the Molonglo River.

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MINE DUMP VEGETATION: PRACTICE AND PROBLEMS

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INTRODUCTION

Every activity of man causes some change to the environment. These changes range from barely discernable foot-tracks in recreation areas to the total reconstruction of the landscape in our cities. Of all man's activities, agriculture and grazing have had the most widespread effect yet it is mining which, although affecting only very small areas, has received the greatest public attention.

Mining causes intense devastation over a limited area and is by its very nature a terminal industry. In the past when the orebody was depleted the mine site was abandoned, leaving ruin and pollution as a monument to posterity. These effects are clear for all to see. Modern methods have reduced the problems but a certain amount of disturbance is unavoidable.

Orebodies are found in all parts of the world, in every climate, and each has its own unique chemical composition. Thus the rehabilitation of each and every mine site becomes an individual case study. However there are certain common factors which occur generally in all cases. These concern structure, pollution and appearance.

STRUCTURE

There is an obvious need for attention to structure with matters of safety. Mine dumps should be physically stable and quarries should be left free of the risk of wall collapse. These are basic engineering problems which are best covered in the original mine design when any additional cost is more likely to be minor. At this stage sites can be selected to minimise the spread of pollution and dumps designed for the control of seepage.

More often, however, structural changes are required after mining operations have ceased. Such alterations may then include reshaping the area to conform more suitably with the surroundings and to modify the surface structure as part of the vegetation process. Both these considerations applied in the case of the Captains Flat project (Craze, 1977, 1980).

A somewhat different structural problem occurs with the conical coal residue dumps at St. Etienne in central France where the steep surface was terraced using a post and mesh arrangement to hold back fine material for soil development.

Structure can also lead to control of dust as in the example of the rock meadow technique of Marshall (1970). This method has its basis in aerodynamic studies and works by placing rocks on the dump surface of such size and spacing that the wind flow pattern is lifted upwards and the air velocity at the surface is significantly reduced.

POLLUTION

Physical pollution by wind and water erosion is a problem in the immediate neighbourhood of mine dumps. Dust control, easy in temperate climates but difficult in arid areas, is most commonly dealt with by vegetation procedures. Physical methods such as that of Marshall (1970) can be also applied. A variety of surface treatments have been investigated by Dean *et al.*, (1969) and include spraying the surface with oil and polymer emulsions. These are at best only temporary and are usually applied as part of an approach to vegetation, as for example in hydromulching.

Water erosion can be overcome by increasing penetration rates, breaking up flow patterns by surface contouring or by binding the surface in some way. The use of vegetation cover goes a long way towards dealing with each of these factors. Such an approach, however, is more commonly used in revegetating the open landscape than on mine dumps (Stanley, 1978).

Chemical pollution is well known to the public from the deleterious effects around metal and coal mine dumps and causes major problems with attempts to establish vegetation. The chemical and microbiological mechanisms by which such pollution arises is covered by other papers at this conference and will not be considered here.

One mechanism which should not be overlooked is aqueous dissolution either direct or by ion exchange processes. Radium pollution from uranium waste dumps is a case in point and this will be described in detail later.

In dealing with the effects of chemical pollution one can take a number of approaches.

1. *Live with it.* The surface can be treated by, for example, the application of limestone to neutralise acidity or metal-tolerant plants may be used. The former procedure is described by Murray, *et al.* (1978) for the case of pyrite-bearing uranium waste areas and the latter by Bradshaw (1975) who has isolated a zinc-tolerant *Agrostis tenuis* for this purpose.

2. *Seal the dump.* Here a completely new surface is created by applying successive layers of clay, coarse aggregate for surface drainage and a soil layer for growth. Provision must also be made for draining the internal areas of the dump. Examples of this approach can be found in North Wales and at Captains Flat.

3. *Make use of natural processes.* In New Brunswick in Canada, Boorman and Watson (1976) found that pyritic dumps exposed to natural rainfall over many years were leached to a depth of some 20-30 cm, forming an almost impervious crust at that depth. The leached surface material, properly neutralised and fertilized, could then support good plant cover. A similar situation is described by James and Mrost (1965) for pyritic gold mine dumps in South Africa but in this case leaching was accelerated by the use of water sprays.

In the examples discussed so far the problems with the dumps have been approached with standard engineering and biological practice. With each case being different it has been recognised that investigations into hydrology, soil composition, soil microbiology and agronomy are necessary. Examples are legion and well documented, as are the methods used.

One particular example of what might be called standard technology is that prepared by Murray (1977), who has codified many of these methods. He has recognised that few mining operations have biological expertise on their staff and has translated the specialist soil science and agronomy involved into terms understandable by chemists and engineers normally present on a mine. The methods recommended by Murray are conservative and may not be ecologically perfect but they do work in the majority of cases, often very well indeed. Dumps will be made safe, pollution reduced and the result pleasing to the eye. However, some doubt may exist as to the permanence of these solutions.

SOME PARTICULAR CONSIDERATIONS

Mine dumps, however well they are constructed, are hardly objects of great beauty. Nor are the scars left on hillsides by quarrying. Where appearance is important vegetation is almost essential but the aim goes beyond simply providing cover. To be really successful the process should result in a permanent, stable vegetative cover similar to that in the surrounding area. Such an ecological climax is not easy to obtain, implying as it does a result that is maintenance-free in perpetuity.

The application of what might be called standard methods does take care of the majority of situations but there are many recalcitrant cases which require closer study. These include highly pyritic waste dumps with their consequent acid and metal pollution, the special problems of radioactive wastes, dumps of either very coarse or very fine particle size, and dumps in dry areas.

I do not propose to cover leaching as these processes are dealt with in other papers. However, I will make one point. Whether one is leaching for metal recovery or trying to prevent leaching and hence pollution one is drawing on the same body of knowledge. The processes are identical and differ only in that in one case the aim is to increase leaching rates while in the other a decrease is required.

Whatever the data that further work may produce there are several controlling factors of which use can be made. All microbiological leaching of sulfide requires the presence of oxygen and water at a favourable pH. Control of any of these can reduce the polluting effect.

In one approach the dump can be sealed off from air diffusion and a neutralising layer provided. This is the basis of the Captains Flat approach. Alternatively, by control of the water table within the dump the majority of sulfides can be flooded and rendered anaerobic. If at the same time a vegetation cover is established then the associated root and soil microflora will act as oxygen scavengers reducing the amount of oxygen diffusing to the leaching zone at the air/water interface. Thus, indirectly, vegetation can reduce pollution. This situation is inherent in the methods used in the Elliott Lake region.

One could conceivably attempt to keep the dump dry but this would mean accepting a dust problem and would not readily allow for vegetative cover. Also it has been shown that very little water is needed to initiate leaching, even high humidity being enough (B.J. Ralph, personal communication). Some leaching will always occur and inevitably a period of high rainfall will flush high concentrations of pollutants into the surrounding environment. Thus dry dumps are not really a practical proposition.

In either case control of the water table and a study of water movement within the dump is required. This is particularly the case in the special problem of the waste dumps from uranium mining. Apart from pollution from pyrite leaching radium reports in the waste material, generally as the sulfate. Some of this radium is highly mobile.

Radium sulfate is highly insoluble and the double salt with barium sulfate is amongst the most insoluble known. Use of this fact is made in uranium extraction where the effluent and residue slurry are treated with barium chloride before disposal. This, however, is not of much use as the solubility product of the double salt is of the order of 10^{-7} whereas the environmental limits for radium are of the order of 3 or 10 pg l^{-1}; i.e., radium in this form is 4 orders of magnitude more soluble than accepted limits and for these purposes must be considered a soluble salt.

Doerner and Hoskins (1925) showed that with the double sulfate part of the radium is present in the crystal structure while part is in effect adsorbed on the crystal surface. This adsorbed radium is relatively mobile and can be displaced by other ions. The conditions and rates of desorption have been well studied (Shearer and Lee, 1964; Havlik, 1968 a, b) and on the basis of this Andersen (1978) has proposed that use could be made of the ion exchange properties to remove the more mobile radium before wastes are disposed of.

The point of this example is that simple solubility considerations can be misleading and that for critical pollutants one must also consider surface chemistry and surface reaction kinetics. In any dump vegetation project one is starting essentially with an artificial, sterile sandhill with the aim of developing a stable climax vegetative cover compatible with the local environment. Crucial to this process is the formation of a suitable soil profile with its full complement of microflora and microfauna capable of maintaining normal nutrient cycles.

Initial treatment including neutralisation and fertilization can provide the basic nutrient bank and the first plantings or seeding generally proceed readily provided there is adequate rainfall or irrigation. However, to obtain a cover stable in the long term one must also consider plant selection, nutrient supply, soil composition and soil microbiology.

A simple grass seeding provides a certain degree of soil stabilization into which plants can pioneer from the surrounding area. Left to mature, a climax vegetation of sorts will become established but there is no guarantee that failure might not occur at some stage.

One must, therefore, ensure that the initial plant selection gives the balance of species that will lead to ongoing stability. Such selection will need to take account of the fact that the soil profile is in a state of development and that the dominant species at any one time will vary during this process. Plant selection must, therefore, take account of tolerance to any toxic material present, suitability for the soil texture in the particular environment, and of the stage of development of the project.

It is relatively simple to provide adequate nutrients by means of mixed fertilizers but maintenance of levels cannot be continued economically for more than a few years. The processes of litter fall and degradation will recycle most essential elements, the main exception being nitrogen which, due to denitrification processes, is a volatile component. While some nitrogen will recycle through litter fall continual replenishment is required for a stable cover. The greater part of this will come from leguminous plants but algae and soil bacteria do make a contribution. For semi-arid soils, for example, Tchan and Beadle (1955) have reported the following:

algae	0.3 $gm^{-2}yr^{-1}$
azotobacter	0.01 $gm^{-2}yr^{-1}$

More recently significant nitrogen fixation by non-leguminous associations between bacteria and plant roots has been found (L.H. Wullstein, personal communication). This points to a wider and perhaps more suitable range of plants for selection.

The value of including nitrogen-fixing plants in the vegetation can be seen at the carbonate strip mine at Nancy, France, where plantings of alders lead to rapid colonisation by surrounding species. At the Elliot Lake uranium province clover is included with the grass seed but due to the acidity of the soil it does not always take well. Where it does the general plant growth is greatly improved.

Inclusion of nitrogen fixing plants is therefore highly desirable. Further there is need of studies into the turnover and fixation of nitrogen in mine dumps as there are many cases lacking well known nitrogen-fixing plants which are doing remarkably well.

For nitrogen-fixing plants to become infected and for litter decomposition to take place there must be an adequate level of organic carbon in the soil to support the necessary microorganisms. As vegetation grows this content will build up but until it does nitrogenous fertilizer will be required.

Another effect of organic carbon in the soil is its ability by adsorption and complexing to ameliorate to some extent the effect of toxic metals in the dump material. In the work of Harris and Leigh (1976) at Broken Hill, N.S.W., pot trials on the dump material showed severe toxicity due to manganese. In dump trials where sewage effluent is used as the water supply no such toxicity effects are observed. Now this effluent is rich in nutrients and in organic carbon and probably gives rise to two effects. Firstly the soil pH is buffered to about 5.8 compared with the pH of the dump material in fresh water of 5.2. At the higher pH most manganese salts are insoluble. Secondly there is undoubtedly some complexing with the organic content. The net effect is for all practical purposes the elimination of the toxicity.

Organic carbon has a useful role, therefore, in both producing the soil conditions necessary for long term stability of vegetative cover and in reducing the toxic effects of heavy metals.

Many plants do better when infected by mycorrhizae; others do not so respond. It has been suggested by Reeves *et al.* (1979) that non-mycorrhizal plants have a competitive advantage over mycorrhizal species in the initial pioneering of sterile dumps, with the latter becoming more dominant as the soil profile matures. Little has been reported on this form of infection in severe environments. There is some doubt as to whether the Chenopodiaceae for example are mycorrhizal (Hirrel *et al.*, 1978) but there is no doubt that mycorrhizal species when infected grow much more readily. One reason is the improvement in phosphate uptake that occurs and this is likely to be very important in poor soils such as mine dumps. Another effect may well be improved moisture absorption.

In the south of France introduced species of Australian acacias and mimosa are used in the rehabilitation of degraded areas. These trees often grow far better than in their native habitat. One reason advanced for this is the very effective nodulation and mycorrhizal infection that occurs in French soils (P. Augé, personal communication).

Finally, one can consider the succession that occurs in arid areas and other places of environmental stress. On some Elliot Lake uranium dumps an initial flush of algae often develops followed by successive invasions of mosses and grasses. The same effect can be seen on the Broken Hill mine dumps and in the open range. While the crusts that form in this way stabilise the surface little has been published on the role of algae, lichens and mosses in the development of these soils. Jordan *et al.* (1978) has shown that lichens and mosses in the Canadian tundra are important sources of fixed nitrogen which suggests a role more than simple crust formation. Undoubtedly there is a valuable contribution to the level of organic carbon in the soil.

SUMMARY

Mine dumps are vegetated as part of the process of metal pollution control and aesthetic restoration of the area. For the vegetation the aim is to develop a permanent, stable cover compatible with the particular environment. One or more of the following steps are taken; engineering modification of the dump to amongst other things control water flow patterns, adjustment of soil chemical characteristics, fertilization and planting with selected species. With time, a stable soil profile develops.

Each dump is an individual system requiring some degree of special attention in one or more of the basic steps. In the past the job has been considered completed when the first plantings are firmly established. This is not necessarily so as development of a stable soil is a dynamic process requiring considerable time to reach equilibrium.

To deal with this aspect more attention needs to be paid to soil moisture movement and retention and to nutrient cycling. In the latter the nitrogen cycle is of prime importance. More attention needs to be given to plant selection with respect to metal tolerance, a balance of nitrogen fixation and the succession of dominance as the cover matures. Also the processes of maturation of the soil both in chemical composition and in microflora and microfauna content need to be better understood. The roles of mycorrhizia and other soil fungi together with algae, lichens and mosses are likely to be of greater importance than perhaps has been accepted if a stable ecological balance is to be achieved.

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