Environmental Pollution 76 (1992) 169–175

Microbiological and chemical characteristics of an acidic stream draining a disused copper mine

Kenneth C. Walton

Institute of Terrestrial Ecology, University of Wales, Bangor, Gwynedd, LL57 2UW, UK

&

D. Barrie Johnson

School of Biological Sciences, University of Wales, Bangor, Gwynedd, LL57 2UW, UK

(Received 7 May 1991; revised version received 8 July 1991; accepted 22 July 1991)

Water samples draining a disused copper mine (Parys Mountain) in Anglesey, North Wales, were analysed for distribution of acidophilic bacteria (iron oxidising and heterotrophic) and for changes in physicochemical composition along the length of the drainage stream. Ten samples were taken at regular distance intervals along a 1 km stretch from the source of the acid mine drainage. The stream remained highly acidic ($\text{pH} < 2{\cdot}8$) although a slight decrease (0·6 pH unit) in acidity with distance from source was observed. Concentrations of most metals measured decreased with distance along the length of the stream, although some showed a gradual increase and others peaked at *c.* 200 m from source. Most dissolved iron was in the ferrous form in the upper reaches of the stream, but ferric iron became increasingly dominant downstream as a result of microbial oxidation. Although concentrations of nutrients such as nitrogen and phosphorus were low in the acid mine drainage, they were not limiting rates of bacterial iron oxidation, which appeared to be limited more by temperature. The iron oxidising bacteria *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* were both isolated from all sampling sites, although their relative abundances varied; *L. ferrooxidans* accounted for 57% of all iron oxidising isolates. Numbers of iron oxidising bacteria decreased with distance from drainage source, in contrast to those of acidophilic heterotrophic bacteria which increased. The diversity of heterotrophic isolates also increased with distance. The relationship between the chemistry and microbiology of the stream is discussed.

INTRODUCTION

Acid mine drainage (AMD) is formed by the activities of a number of obligately acidophilic bacteria which are capable of catalysing the oxidation of pyrite and other sulphidic minerals. Bacteria include *Thiobacillus thiooxidans*, which oxidises reduced forms of sulphur, *Thiobacillus ferrooxidans*, which oxidises reduced sulphur and ferrous iron, and *Leptospirillum ferrooxidans*, which oxidises ferrous iron (Harrison, 1984). The general form of the reaction can be given as:

$$MS_2 + 3O_2 + 2H_2O \longrightarrow M^{2+} + 2H_2SO_4,$$

where M is a metal (or more than one metal in the case of mixed sulphides). If M is, or contains, ferrous iron, this is further oxidised:

$$Fe^{2+} - e^- \longrightarrow Fe^{3+},$$

and hydrolysis of ferric iron (which may then precipitate, depending on pH) produces more acidity:

$$2Fe^{3+} + 6H_2O \longrightarrow 2Fe(OH)_3 + 6H^+.$$

AMD tends, therefore, to have high concentrations of sulphate and iron (ferrous and ferric), and a low pH

Environ. Pollut. 0269-7492/$05.00 © 1992 Elsevier Science Publishers Ltd, England. Printed in Great Britain

(often < 3). In addition, depending on the geochemistry of the environment in which the microbial oxidation is taking place, soluble concentrations of other metal ions may reach levels at which they are toxic to many aquatic organisms. Analysis of AMD from various sites has been described in the literature (e.g. Johnson *et al.*, 1979; Filipek *et al.*, 1987).

Studies on the microbiology of AMD have tended to concentrate on chemolithotrophs in general (bacteria which obtain energy from the oxidation of inorganic materials and fix carbon dioxide) and *T. ferrooxidans* in particular. Problems with the handling of acidophiles (e.g. their reluctance to grow on solid media) has meant that information on their distribution in AMD and similar environments is somewhat sparse. Also, the significance of *L. ferrooxidans* relative to *T. ferrooxidans* in AMD is unclear, although Johnson (1991) reported that up to 30% of iron oxidising plate isolates from one mine were *L. ferrooxidans.* More recently, it has been discovered that obligately acidophilic heterotrophic bacteria are also present in AMD and many other extremely acidic environments (e.g. Wichlacz & Unz, 1981). Many of these bacteria, such as *Acidiphilium cryptum*, are oligotrophic and may survive on, for example, trace amounts of organic materials leaked by chemolithotrophic bacteria such as *T. ferrooxidans.*

Parys Mountain, Anglesey, North Wales (Grid Ref. SH 44–45.90) was extensively exploited for copper ore during the 18th and 19th centuries. Ore was mined from shafts when the mine first opened, but their early collapse forced opencast working, so that part of the mine more resembled a quarry. Many new shafts were sunk during the 19th century. All have recently been filled or capped and the site now consists of these together with one large void containing, in its lower depths, a pool of acidic water (Parys Mine, *c.* 3 ha) with a smaller void to the north-east (Mona Mine, *c.* 1 ha). The entire site (approximately 200 ha) is covered by mine spoil, except where there are precipitation ponds. These are normally filled with water in the winter, but sometimes dry out during the summer. They were originally used for extracting copper from drainage water by adding scrap iron, thereby displacing copper from solution as a sludge (Rowlands, 1966). The geology and ore mineralogy of the site have been described by Pointon & Ixer (1980). Sulphide minerals are dominated by pyrite, together with chalcopyrite, sphalerite and galena, with lesser amounts of other minerals.

There are three main streams draining the Parys Mountain area, two of which have the same name, Afon Gôch ('red stream') reflecting their colour. One of these drains northwards to the sea at Amlwch, the other southwards, reaching the sea at Dulas; the latter is the subject of this article. It issues from the almost completely blocked adit of the Mona Mine (referred to below as the 'source'), runs in a straight channel for about 180 m, and then traverses several precipitation ponds. A second stream joins it before it leaves the immediate area of the mountain, 1 km from the source. Some details of the geochemistry of streams in Anglesey are contained in Cooper *et al.* (1982).

Although the chemistry and microbiology of AMD are inexorably linked, there have been very few reports in which both have been described in detail. An integrated approach to the problem, using the Afon Gôch at Parys Mountain as a model system, is presented in this paper.

MATERIALS AND METHODS

Some investigations of the physicochemical features of the Afon Gôch were made during 1988–89, but most of the data reported here are for samples collected during December 1990. The 10 sampling points (Fig. 1) were visited in sequence on the same day (14 December 1990) and samples taken from each. Those for microbiological analysis were put into sterile polypropylene bottles, those for chemical analysis into acid-washed polyethylene bottles, those for DOC analysis were filter sterilised (Millipore 0·2-μm membranes) *in situ* into chromic acid-washed glass bottles, and those samples used for ferrous iron analysis were acidified *in situ* by the addition of 6 M HCl (1 ml/20 ml AMD).

In-situ measurements

The following were recorded at each sampling site: temperature (to 0·1°C) using a digital thermometer; acidity, measured as pH, using a combination low conductivity pH electrode, reading to a voltmeter; redox potential (Eh) using a platinum electrode, reading to a voltmeter.

Chemical analysis

Samples for chemical analysis were filtered the same day through 0·45-μm Millipore membranes. The following cations were measured, using flame AAS (or AE in the case of sodium and potassium): Fe, Cu, Zn, Mn, Mg, Ca, Na, K, Cd, Pb and Al. Normal procedures were adopted, such as using La as a releaser for Ca, and using N_2O/C_2H_2 flame with an ionisation suppressant for Al. For arsenic, AAS with a graphite furnace was used. Sulphate, phosphate-P, and nitrate-N were measured by ion chromatography (Dionex 2000), and dissolved organic carbon (UV oxidation) and ammonium-N on a continuous flow autoanalyser (Skalar). Ferrous iron concentrations were determined by titrating 5 ml aliquots with 1 mM $KMnO_4$ in 1 M H_2SO_4.

Rates of ferrous iron oxidation in Parys mine AMD were determined in water samples collected from the AMD source. Samples (in duplicate) were incubated in the laboratory at 4°C, 10°C and 30°C (the approximate

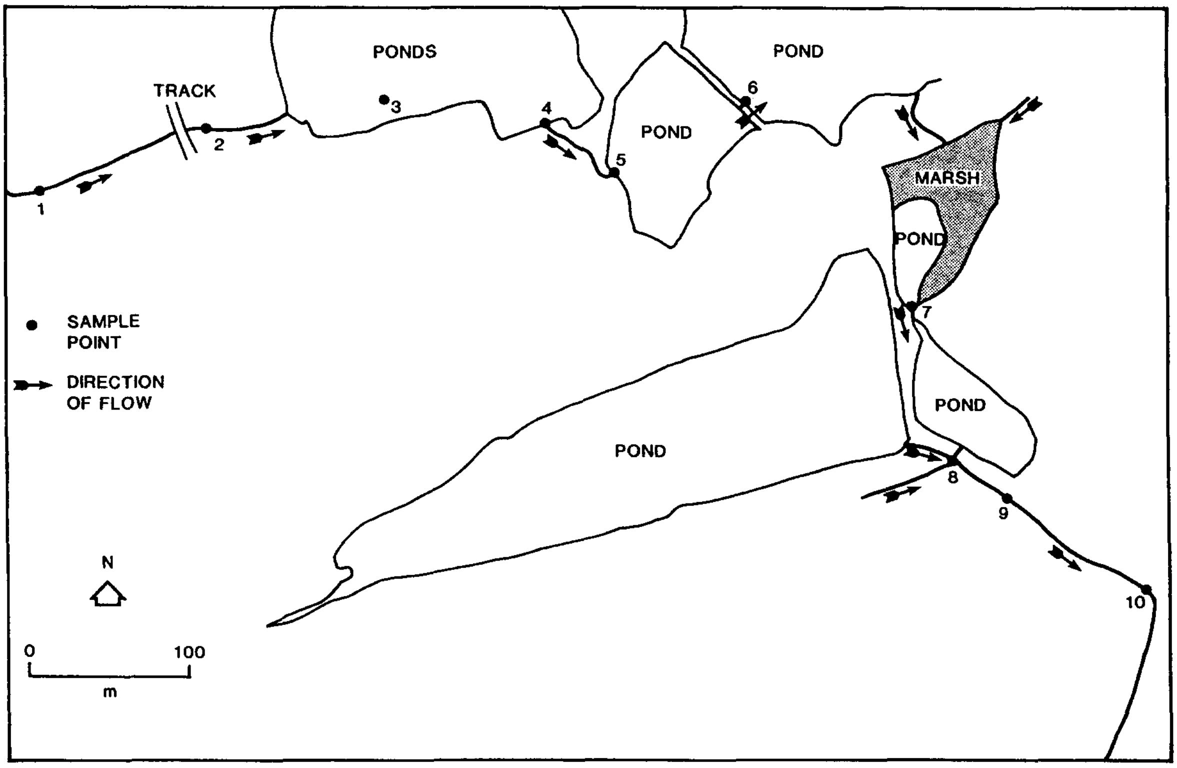

Fig. 1. Map of part of Parys Mountain, Anglesey, showing points 1–10 where water samples were taken from the Afon Gôch.

optimum temperature for most isolates of *T. ferrooxidans*) shaken at 100 rpm. In addition, separate samples at 30°C were amended with a basal salts concentrate (McCready *et al.*, 1986) which contained ammonium, phosphate, potassium and magnesium, to ascertain whether the rate of oxidation was limited by the availability of these nutrients. Subsamples were withdrawn at regular intervals and titrated with $KMnO_4$.

Microbiological analyses

Total numbers of bacteria were determined by acridine orange direct counts (AODC) using Nuclepore 0·2-μm membrane filters (Johnson & McGinness, 1991*a*). Numbers of viable acidophilic iron oxidising bacteria were evaluated by plating serial dilutions of AMD samples on overlaid FeTSB medium, which has been shown to have a plating efficiency of over 90% (Johnson & McGinness, 1991*a*). Heterotrophic acidophiles were enumerated by plating diluted AMD samples on a solid medium containing 0·02% (w/v) yeast extract, 2·5 mM ferrous sulphate, gelled with 0·7% (final concentration, w/v) agarose (Sigma, Type 1, pre-washed with distilled water). The final pH of this medium was *c.* 2·8.

Plates were incubated for 6 weeks at 30°C, at which time final counts were made, and isolates partially characterised. For heterotrophic isolates this entailed recording colony morphologies and noting basic cellular characteristics. Iron oxidising isolates were also differentiated initially on colony morphologies (Johnson, in press), and isolates (*c.* 10 from each plate) examined by phase contrast microscopy to, again, ascertain basic cellular characteristics. In particular, note was taken whether cell morphologies corresponded to those of *T. ferrooxidans* (straight rods of variable motility) or *L. ferrooxidans* (curved, highly motile rods).

RESULTS

Changes in water pH with distance from AMD source are shown in Fig. 2. Throughout the 1-km stretch monitored, the stream remained extremely acidic. Temperature profiles for different times of the year are shown in

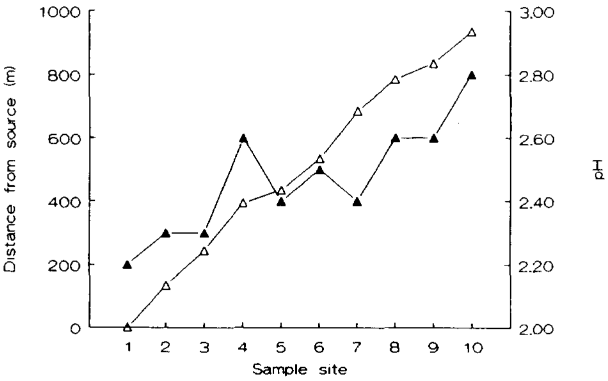

Fig. 2. Sampling distances (Δ) and pH trends (▲) in Afon Gôch water samples.

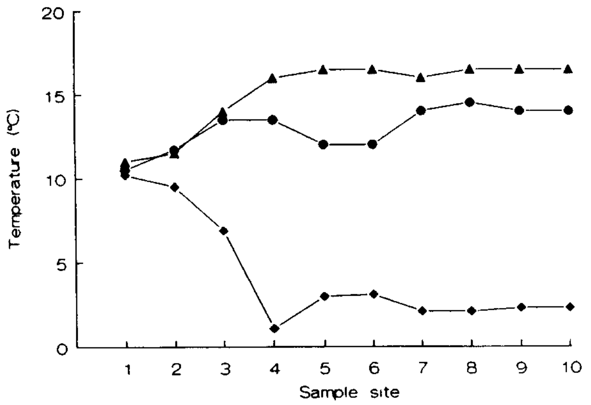

Fig. 3. Temperature profiles in the Afon Gôch: samples collected ● April 1988; ▲ September 1989; ◆ December 1990.

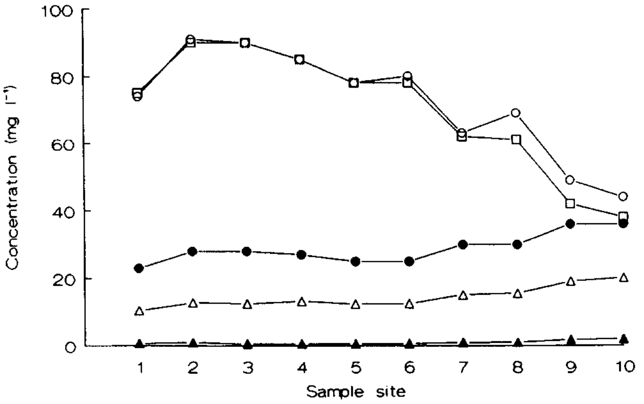

Fig. 5. Concentrations of some basic cations and aluminium in Afon Gôch water samples: Δ sodium; ▲ potassium; O magnesium; ● calcium; □ aluminium.

Fig. 3; whilst at the AMD source the temperature was almost constant (10–11°C), its gradient downstream was highly dependent on season. Redox values (Fig. 4) indicated that Eh increased downstream from AMD source, and that the environment remained oxidising throughout.

Concentrations of dissolved ions (Figs 5–7) ranged from <1 (e.g. K) to >2500 mg litre^{-1} (SO$_4$). Concentrations of most cations decreased downstream, as did that of sulphate, whilst Na, K and Ca all increased with distance from source, and Mn and Pb changed little. However, a number of ions (Mg, Fe, Mn, Cu, Al, As and SO$_4$) initially increased at sampling points 2 and 3, before declining. Concentrations of total and ferrous iron call for special comment. Both increased considerably between AMD source and points 2 and 3 (Fig. 4). Total iron then maintained a steady decline, whilst ferrous iron fell to a very low level at point 5 and beyond, presumably due to bacterial oxidation. Concentrations of inorganic nitrogen and phosphorus were low in all water samples (complete data not shown). Ammonium-N fell from 2·5 mg litre^{-1} at AMD source to 1·4 mg litre^{-1} at site 10, whilst NO$_3$-N increased from 1·1 to 1·4 mg litre^{-1} over the same distance. Soluble phosphate remained at <0·2 mg litre^{-1} throughout. Dissolved organic carbon varied from 4·4 (site 6) to 8·0 mg litre^{-1} (site 2), but showed no trends with distance from AMD source.

Plate counts of iron oxidising and heterotrophic acidophiles followed different trends (Fig. 8), and tended to be 1–2 orders of magnitude below total counts. Whilst numbers of iron oxidising bacteria increased from sampling points 1–3 and decreased thereafter, the general trend for heterotrophic bacteria was that numbers increased with distance from AMD source (where they were very low); the peak in numbers of acidophilic heterotrophs at sampling point 9 corresponded to the point just after the Afon Gôch was joined by a smaller stream.

Both *T. ferrooxidans* and *L. ferrooxidans* were isolated from all samples on solid media. However, the relative abundance of the two bacteria varied from site to site (Fig. 9). *L. ferrooxidans* was numerically dominant at AMD source, but the situation was reversed at sampling points 2 and 3. Further downstream, *L. ferrooxidans* re-emerged as the more frequent plate isolate. It was noted that *L. ferrooxidans* isolates tended to have a single, quite distinct colony morphology on the solid iron medium, but four other, readily distinguish-

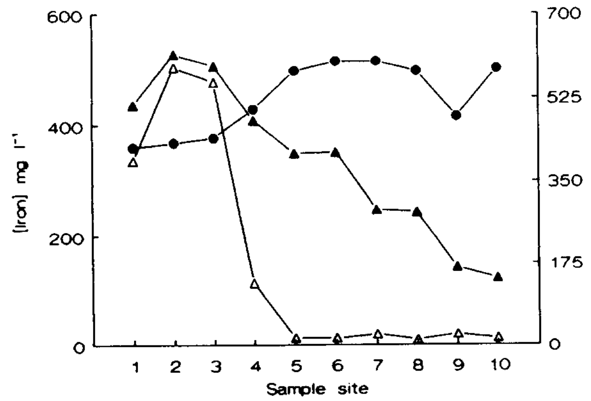

Fig. 4. Total iron, ferrous iron and Redox (Eh) values in Afon Gôch water samples: ▲ total iron; Δ ferrous iron; ● Eh.

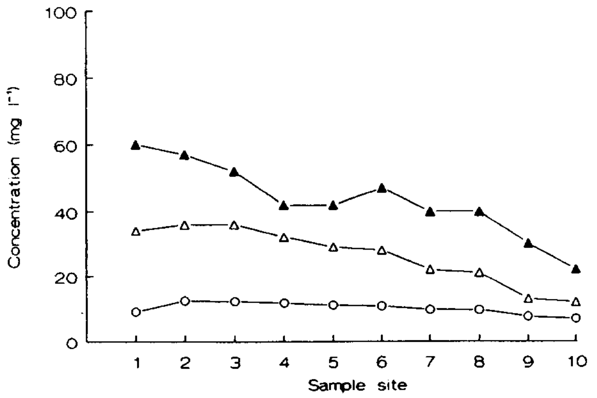

Fig. 6. Concentrations of some heavy metals in Afon Gôch water samples: Δ copper; O manganese; ▲ zinc.

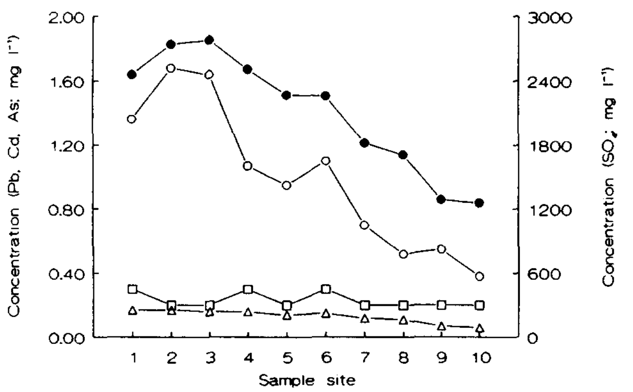

Fig. 7. Concentrations of sulphate and some toxic metals in Afon Gôch water samples: ○ arsenic; Δ cadmium; □ lead; ● sulphate.

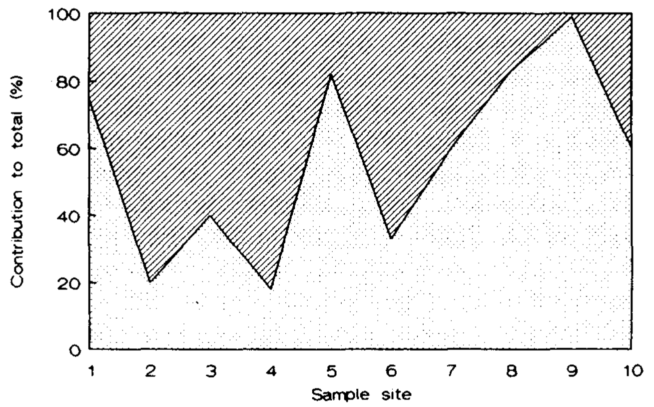

Fig. 9. Relative abundance of *Thiobacillus ferrooxidans* (▨) and *Leptospirillum ferrooxidans* (░) isolates in Afon Gôch water samples.

able (from size and morphology) colony forms were all identified as *T. ferrooxidans*, indicating possible strain diversity amongst this species of iron oxidiser at Parys mine. Rates of ferrous oxidation at 30°C corresponded to culture doubling times of 7·3 ± 0·5 h, a figure which is typical of *T. ferrooxidans* rather than *L. ferrooxidans*; microscopic examination of cultures at the end of ferrous oxidation also showed the population to be dominated by *T. ferrooxidans*-like cells. Culture doubling times at 10°C were 44 ± 2 h. There was no enhancement of ferrous oxidation by the addition of basal salts, and no detectable oxidation was observed, over 2 weeks, in cultures incubated at 4°C.

Colonies of heterotrophic bacteria formed on the overlaid iron medium as well as the yeast extract medium, indicated that the heterotrophs were very competitive scavengers (overlaid FeTSB medium contains an indigenous heterotrophic acidophile which tends to inhibit the development of heterotrophs in the inoculum; Johnson & McGinness, 1991*a*). Three distinct colony morphologies were observed for acidophilic heterotrophs. Type 1 were gelatinous, cream, entire, raised/umbonate colonies of *c.* 1 mm diameter, formed by Gram-negative motile rods, 2–3 μm long; Type 2 were light red, round, entire colonies formed by small (*c.* 1 μm) rod-cocci; Type 3 were off-white, round, flat colonies formed by motile, fine rods (2–3 μm long) which tended to aggregate. Ratios of the three types, based on plate isolation, varied from site to site (Fig. 10).

DISCUSSION

Over the length of stream sampled (almost 1 km) the Afon Gôch draining Parys mine remains a highly acidic stream, although some decrease in acidity was detected with distance from its source. This change, although small (0·6 pH unit) has a large influence on water chemistry. For example, it was noted that ochreous precipitates (presumably of ferric hydroxysulphates) were particularly abundant downstream of sampling point 3; this would account for the gradual decrease in not only iron and sulphate, but also anions such as arsenate which would be readily adsorbed onto such

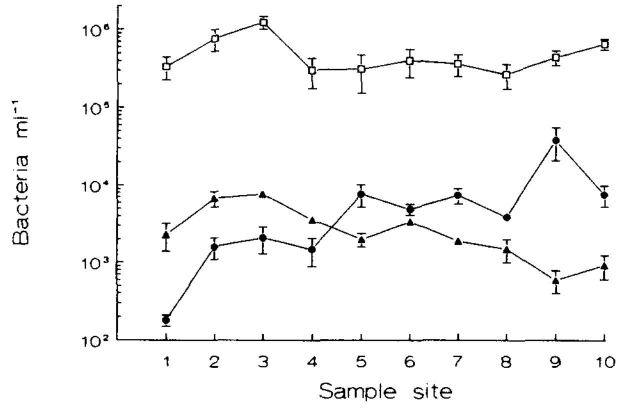

Fig. 8. Numbers of bacteria in Afon Gôch water samples: □ total counts (AODC); ▲ acidophilic iron oxidising bacteria (colony forming units ml⁻¹); ● acidophilic heterotrophic bacteria (colony forming units ml⁻¹).

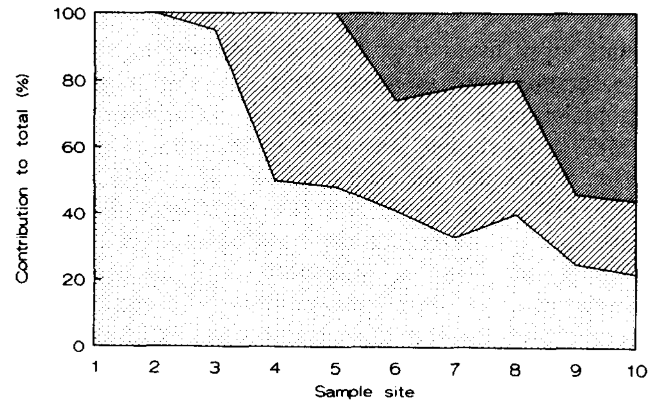

Fig. 10. Distribution of acidophilic heterotrophic isolates in Afon Gôch water samples: ░ Type 1; ▨ Type 2; ▩ Type 3.

materials. The increase in several species, such as ferrous iron and sulphate, suggests that there were further additions of these beyond the source point from lateral seepage from the surrounding, highly acidic waste ground. It is also possible that some increase in ferrous iron arose from bacterial reduction of ferric iron (Johnson & McGinness, 1991*b*), although this would not account for the observed increase in total iron. Filipek *et al.* (1987) measured a similar series of ions in AMD from West Squaw Creek in California. At only three of their sampling points were concentrations found approaching those in the Afon Gôch; in each case the pH value was $\leq 2{\cdot}45$ and the sulphate concentration >2200 mg litre^{-1}, the highest value found in the present study. Results from Foster *et al.* (1978) for the lower reaches of the Afon Gôch show an obvious continuation of the trends described here for Fe, Cu, Zn, Mn and pH. Their highest sampling point appears to have been some hundred or so metres downstream from our lowest (site 10).

Changes in AMD pH seem to have affected the distribution of heterotrophic acidophiles in the stream. There was a readily detected increase in heterotroph diversity with distance from AMD source (although the diversity was still apparently very limited 1 km from source). The most acidophilic of the heterotrophs, estimated by comparing both their relative abundances and colony sizes on the two media (the pH of the iron medium was about 0·5 unit lower than that of the yeast extract medium) was Type 1, which was the only heterotroph isolated from sites 1 and 2. On the same basis, the least acidophilic heterotroph was Type 3, which was detected only at points 6 and beyond; its numerical dominance at points 9 and 10 indicate that it was abundant in the stream that drained into the Afon Gôch just before sampling point 9. Although total numbers of acidophilic heterotrophs showed a general increase with distance from AMD source, this did not correlate with DOC levels, which remained similar throughout.

The influence of changing pH on numbers of iron oxidising bacteria was probably less significant, and it seems more likely that numbers declined as substrate (i.e. ferrous iron) concentrations fell. The reasons for the variation in *T. ferrooxidans* : *L. ferrooxidans* ratios at different sampling sites seem to be more complex. In those sites (with the exception of the AMD source) which contained appreciable concentrations of ferrous iron, *T. ferrooxidans* was more abundant. In laboratory cultures the faster growth rate of *T. ferrooxidans* (Eccleston *et al.*, 1985) means that *L. ferrooxidans* is eventually eliminated when mixed cultures of iron oxidisers are subcultured in ferrous sulphate medium. In the majority of 'lean' environments, however, *L. ferrooxidans* was the more abundant, possibly reflecting its higher affinity for ferrous iron (Norris *et al.*, 1988). The survey revealed that, far from being a less significant iron oxidising bacterium, *L. ferrooxidans* was, in fact, more abundant overall than *T. ferrooxidans*, accounting for 57% of total plate isolates. It is also clear that this fact would not have been apparent if the more conventional method of isolation from enrichment cultures rather than plate isolation had been carried out; oxidation of ferrous iron in incubated AMD samples was primarily by *T. ferrooxidans*, and resulted in an oxidised culture dominated by this iron oxidiser.

As well as being responsible for the chemical environment of AMD, the activities of chemolithotrophic bacteria are, in turn, influenced by it. *L. ferrooxidans* is more sensitive to many heavy metals, such as copper, than is *T. ferrooxidans* (Eccleston *et al.*, 1985). It may therefore appear somewhat surprising that *L. ferrooxidans* was the dominant bacterium in AMD draining a disused copper mine, very much higher in copper than 'normal' streams. However, the maximum copper concentration (36 mg litre^{-1}, or 0·57 mM) is well below that noted to inhibit *L. ferrooxidans* in laboratory cultures (*c.* 5 mM). The concentration of zinc in AMD samples was greater than that of copper, but again below levels which have been recorded as inhibiting iron oxidising bacteria; indeed, one strain of *L. ferrooxidans* has been shown to be stimulated by zinc over the range 5 μM–5 mM (Norris, 1989). Anions, other than sulphate, tend to be more acidic to acidophilic bacteria than cations (Eccleston *et al.*, 1985). Other heavy metal cations, such as cadmium and zinc, were also below toxic levels. Arsenic would occur primarily as arsenate, with some arsenite, in the Afon Gôch waters, although no attempts at speciation were made in the present study.

The rate of ferrous iron oxidation in the Afon Gôch does not appear to be limited by the availability of nutrients. Accelerated weathering of minerals under the extreme pH regime of the environment, releases appreciable quantities of some nutrients, such as magnesium. Other macronutrients, such as nitrogen and phosphate, although present in relatively low concentrations, were not apparently limiting the rate of bacterial iron oxidation. Temperature, on the other hand, greatly affected rates of iron oxidation. Laboratory data suggest that psychrophilic strains of iron oxidising bacteria (capable of functioning at 4°C, the approximate temperature of most of the water samples on the day of collection) were absent from Afon Gôch AMD. The much slower rate of ferrous iron oxidation in AMD incubated at 10°C compared with 30°C, suggests that the iron oxidising bacteria present were typical mesophiles.

The Afon Gôch draining Parys mountain, described in some detail in the present text, is a typical example of acid mine drainage associated with microbially-oxidising sulphidic mines and mine spoils. Whilst the characteristics of AMD streams vary from site to site (e.g. Johnson *et al.*, 1979; Filipek *et al.*, 1987) basic properties (low pH, high iron and sulphate) are common to all. Because of the presence, at Parys Mountain, of metal sulphides (e.g. sphalerite and chalcopyrite) other than those of iron, concentrations of heavy metals such as zinc and copper exceed those which might be expected in AMD from coal spoils and other materials in which most of the sulphide is present in pyrite and/or marcasite.

We did not examine the suspended material collected on the filters during the preparation of water samples, nor did we collect and analyse samples of sediment from the bed of the stream. It seems likely that these sediments contain large amounts of those elements for which the concentration decreases down the course of the stream. Concentrations of ions could also alter by dilution, as suggested by Foster *et al.* (1978). This would have to be by seepage from the stream banks, as there are no major inflows along the first part of the stream where considerable reductions occur. Evidence for such seepage is provided by the increase in concentration of some cations, such as Ca and Na. Apart from bacteria in suspension, the stream bed has a layer of organic material which includes bacteria, but also contains fungi and algae. This biofilm must play a significant role in the changes in water chemistry. We hope to address these topics in future research.

ACKNOWLEDGEMENTS

The authors thank Chris Freeman and Steve Hughes for their help with some analyses, and David Norris for drawing Fig. 1. Christopher Smith confirmed the main cation figures using ICP–MS.

REFERENCES

Cooper, D. C., Nutt, M. J. C. & Morgan, D. J. (1982). A reconnaissance geochemical survey of Anglesey. Report 51. Institute of Geological Sciences.

Eccleston, M., Kelly, D. P. & Wood, A. M. (1985). Autotrophic growth and iron oxidation and inhibition kinetics of *Leptospirillum ferrooxidans*. In *Planetary Ecology*, eds. D. E. Caldwell, J. A. Brierley & C. L. Brierley. Van Nostrand Reinhold, New York, pp. 263–72.

Filipek, L. H., Nordstrom, D. K. & Ficklin, W. H. (1987). Interaction of acid mine drainage with waters and sediments of West Squaw Creek in the West Shasta mining district, California. *Environ. Sci. Technol.*, **21**, 388–96.

Foster, P., Hunt, D. T. E. & Morris, A. W. (1978). Metals in an acid mine stream and estuary. *Sci. Total Environ.*, **9**, 75–86.

Harrison, A. P. Jr. (1984). The acidophilic thiobacilli and other acidophilic bacteria that share their habitat. *Ann. Rev. Microbiol.*, **38**, 265–92.

Johnson, D. B. (1991). Diversity of microbial life in highly acidic, mesophilic environments. In *Diversity of Environmental Biogeochemistry*, ed. J. Berthelin. Elsevier, Amsterdam. pp. 225–38.

Johnson, D. B. & McGinness, S. (1991*a*). A highly efficient and universal solid medium for growing mesophilic and moderately thermophilic iron oxidizing, acidophilic bacteria. *J. Microbiol. Meth.*, **13**, 113–22.

Johnson, D. B. & McGinness, S. (1991*b*). Ferric iron reduction by acidophilic heterotrophic bacteria. *Appl. Environ. Microbiol.*, **57**, 207–11.

Johnson, D. B., Kelso, W. I. & Jenkins, D. A. (1979). Bacterial streamer growth in a disused pyrite mine. *Environ. Pollut.*, **18**, 107–18.

McCready, R. G. L., Wadden, D. & Marchbank, A. (1986). Nutrient requirements for the in-place leaching of uranium by *Thiobacillus ferrooxidans. Hydrometallurgy*, **17**, 61–71.

Norris, P. R. (1989). Mineral-oxidizing bacteria: metal–organism interactions. In *Metal–microbe Interactions; Special Publications of the Society for General Microbiology*, **26**, eds. R. K. Poole & G. M. Gadd. IRL Press, Oxford, pp. 99–117.

Norris, P. R., Barr, D. W. & Hinson, D. (1988). Iron and mineral oxidation by acidophilic bacteria: affinities for iron and attachment to pyrite. In *Biohydrometallurgy, Proceedings of the International Symposium, Warwick 1987*, eds. P. R. Norris & D. P. Kelly. Science & Technology Letters, Kew, pp. 43–59.

Pointon, C. R. & Ixer, R. A. (1980). Parys Mountain mineral deposit, Anglesey, Wales: geology and ore mineralogy. *Trans. Instn. Min. Metall.* (Sect. B: Appl. Earth Sci.), **89**, 143–55.

Rowlands, J. (1966). *Copper Mountain.* Anglesey Antiquarian Society, Llangefni, U.K.

Wichlacz, P. L. & Unz, R. F. (1981). Acidophilic, heterotrophic bacteria of acidic mine waters. *Appl. Environ. Microbiol.*, **41**, 1254–61.