CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF MINERAL SPOILS AND DRAINAGE WATERS AT ABANDONED COAL AND METAL MINES

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Abstract. Mining is a long-established human activity. Abandoned mines, tailings, and mine spoils may have considerable impact on neighboring environments long after the sites are abandoned. Of greatest concern are derelict mines and wastes that generate acidic discharge waters that are enriched with iron and other metals and metalloids. The chemistry and microbiology of these sites are intricately intertwined. Whilst some indigenous microorganisms are responsible for accelerating sulfide mineral oxidation, thereby generating acidity and mobilizing metals, others catalyze reductive processes that essentially reverse these reactions and thereby ameliorate polluted mine waters. This article reviews current knowledge on the chemistry and microbiology of abandoned coal and metal mines, mine spoils and tailings.

Keywords: acid pollution, acidophiles, biodiversity, metal pollution, mine wastes

1. Introduction

Homo sapiens have, for thousands of years, exploited mineral resources in order to obtain metals considered to be of use and value. In contrast, extraction and burning of coal is a much more recent phenomenon in western civilization, though it has a much longer history in the far east. Mineral mining necessarily impacts the local (and sometimes the wider) environment, and the legacy of mining may persist for many years after the site is abandoned. In scale and global impact, however, the mining of coal and metals remained relatively minor until the advent of the Industrial Revolution and subsequent technological advances. These, coupled with the growth and affluence of human populations have placed on-going demands for many natural resources, including metals and coal. Worries about global warming and acid rain has led to questions about the use of coal as a fuel for energy generation, and concerns about dwindling resource pools has prompted schemes for recycling metals. However, mining of both coal and metal ores is still a major human activity. Mines themselves have limited lives; ultimately the ore or coal seam expires, or it becomes no longer cost-effective to continue mining. The impact that on-going operations have on the local environment may also be a contributory factor leading to mine closure, though the perception (and legislation) of environmental impact varies from country to country.

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Mining of metals and coals inevitably produces large amounts of waste materials, or 'spoils'. Frequently, these are gathered into large heaps or mounds on the land surface. The composition of these spoil heaps is highly variable, though they usually contain a mixture of chemically-inert and reactive components. Safety and aesthetic concerns about coal and metal spoils have led to improvements in engineering aspects, such as landscaping, sealing and surface treatment of these heaps, at least in more affluent countries. A second vestigial problem associated with metal mining is the production of more reactive mineral wastes, generally referred to as 'tailings'. These tend to be fine-grain deposits, typically rich in sulfide minerals, causing them, on both counts, to be of greater environmental concern than spoil heaps. There are alternative strategies for disposing of mineral tailings, all of which entail the prevention of access to oxygen and/or water. These include burial in clay-sealed chambers and storage as sediments in lakes and pools ('tailings dams'). The surrounding walls of tailings dams are necessarily built up as the volume of tailings disposed within them increases. Catastrophic failure of retaining walls has occasionally led to serious pollution of surrounding areas, most notoriously in recent years at the Aznacollar-Los Frailes mine complex in southern Spain, which led to the release of an estimated 2 million cubic meters of sulfidic tailings and 4 million cubic meters of acid water (Grimalt and Macpherson, 1999).

A third endemic problem with abandoned and derelict mines is the generation of acidic discharge waters that contain elevated concentrations of metals and metalloids. During the life of a mine, water that enters working areas is removed by pumping. This includes rainwater reaching the underground aquifers, and subsurface water that enters via faults and connecting adits that may extend beyond the immediate catchment area. Water treatment facilities are usually installed to treat pumped water that becomes contaminated within the mine, in order to meet discharge consent levels. However, when mines are closed, the expensive water pumping operations are generally halted and the level of the groundwater table rebounds to its 'natural' level. The time scale for this varies from mine to mine, and may be several years. For surface mines, this is dictated by the physical nature of the material used to backfill the void, while for deep mines factors such as nature and extent of interconnecting channels, fracturing of overlying strata and the extent of tunnel collapse are important factors. Release of contaminant water will occur when the rising water comes to a surface discharge point, and this may be a catastrophic event if the increasing hydraulic pressure within flooded shafts causes the failure to a retention plug, as was the case at the former Wheal Jane tin mine in Cornwall, England. Polluted waters may also be produced by the interaction of surface waters and rocks exposed during surface mining or in spoil heaps, though this tends to be less important (in scale) than that resulting from discharges from the abandoned mines themselves (Clarke, 1995). Figure 1 summarizes the origin of contaminated discharge waters associated with coal and metal mining.

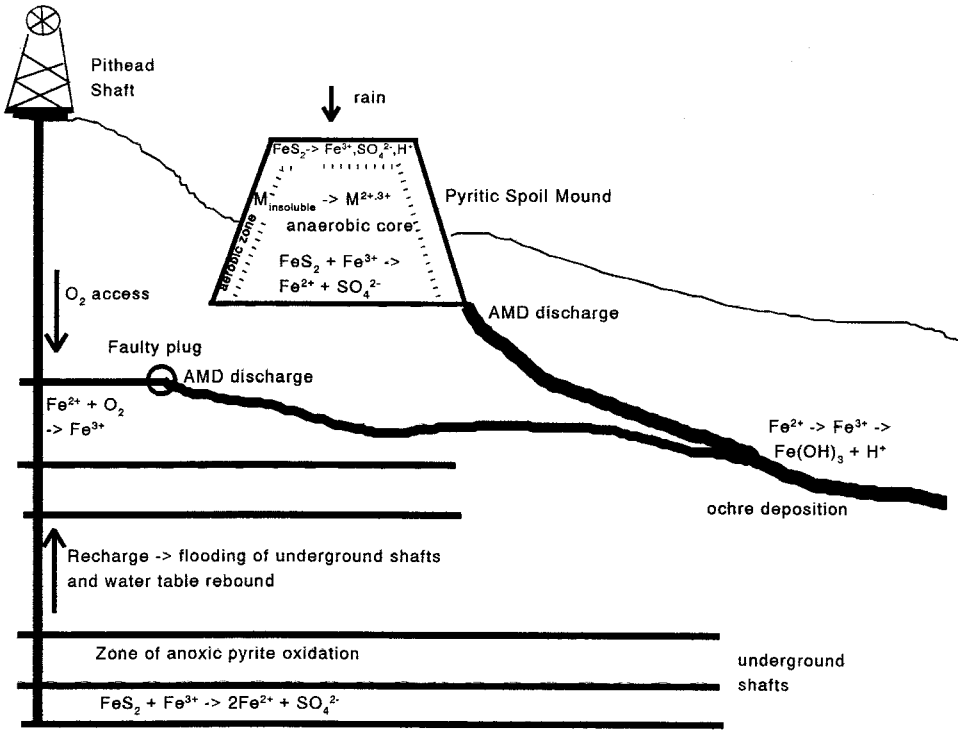

Figure 1. Schematic illustrating the genesis of acidic, metal-rich discharge at an abadoned mine site.

2. Chemistry of Mine Spoils and Discharge Waters

2.1. Abandoned Mines, Spoil Heaps and Mineral Tailings

Abandoned mines vary from site to site. They may comprise of ruined buildings, spoil heaps, unstable workings and shafts, and be used as sites for dumping of off-site spoil materials and fly-tipping. Others remain in relatively pristine states, and may be of interest to industrial archaeologists, biologists and geologists. Other abandoned mines have been subjected to minor or major reclamation schemes, designed to reduce deleterious impact on the local environment, to enhance the surrounding area aesthetically, and possibly to allow the derelict site to be re-developed. In some cases it is economically feasible to re-process spoil heaps that were produced at a time when mineral processing was less efficient, in order to recover more of their metal content.

Mynydd Parys (Anglesey, U.K.) is an example of an abandoned mine site that remains much as it was at the time operations ceased (Jenkins *et al.*, 2000). The site has a long history (of about 3500 yr) and was the world's most important copper mine in the late 18th century. Both deep mining and small-scale production of copper by precipitation had finished by the early 20th century. The 200 ha site

remains mostly unvegetated due to the toxicity of the weathering surface materials. The dominant minerals in the ore are chalcopyrite ($CuFeS_2$), galena (PbS) and sphalerite (ZnS); pyrite (FeS_2) is also abundant. In contrast, the former lead/zinc-producing Parc mine. situated some 60 km from Mynydd Parys within the Snowdonia National Park, is a more recently worked site (operations ceased in 1963) which had caused considerable pollution problems to nearby farmland due to the erosion of an estimated 13 000 tonnes of fine-grain waste materials. The site was regraded, the fine materials and slimes buried under coarser materials, and the whole site capped with a layer of quarry waste, which was sown with metal-tolerant cultivars of grasses. Figures 2a and b contrast the landscapes of these two abandoned mines.

2.2. Acid genesis

Sulfide minerals are the prime reactive components of many abandoned metal mines and spoils. This is also true of surface and deep coal mines, and of coal spoil dumps, since coals may contain up to 10% (and occasionally even more) sulfur, principally as pyrite and/or other iron sulfides. Whilst sulfidic minerals are stable in dry and anoxic environments, exposure to both oxygen and water will cause them to oxidize spontaneously, though this process is accelerated (by a factor of up to 10^6) in the presence of certain chemolithotrophic (literally 'rock eating') microorganisms (Section 4). The process of oxidative dissolution of sulfide minerals has been the subject of much debate (e.g. Schippers and Sand, 1999; Fowler *et al.*, 2001) Sulfides may be divided into those minerals which are acid-soluble (and therefore solubilized by microorganisms that oxidize sulfur to produce sulfuric acid) and those which are acid-insoluble, but which can be oxidized by ferric iron in acidic liquors (and are solubilized by iron-oxidizing bacteria). Sphalerite is an example of an acid-soluble sulfide, and is attacked by protons as in Equation (1):

$$ZnS + 2H^+ \rightarrow Zn^{2+} + H_2S\ . \tag{1}$$

The hydrogen sulfide so-formed is oxidized by sulfur-oxidizing microorganisms, thereby regenerating the protons (Equation (2)):

$$H_2S + 2O_2 \rightarrow 2H^+ + SO_4^{2-}\ . \tag{2}$$

In the case of acid-insoluble sulfide minerals, such as pyrite and chalcopyrite, the primary attack on the mineral by ferric iron produces ferrous iron, thiosulfate and protons (Equation (3):

$$FeS_2 + 6Fe^{3+} + 3H_2O \rightarrow 7Fe^{2+} + S_2O_3^{2-} + 6H^+\ . \tag{3}$$

The primary role of bacteria and archaea in this process is the oxidation of ferrous iron which regenerates the chemical oxidant, ferric iron. Thiosulfate is unstable in acidic liquors, particularly in the presence of ferric iron, and is oxidized to

Figure 2. Comparison of two abandoned metal mine sites in north Wales: (a) Mynydd Parys, a former copper mine, which is largely in the same state as when mining ceased over 100 yr ago; (b) Parc mine, a former lead-zinc mine, a site that has undergone extensive reclamation.

tetrathionate which, in turn, decomposes to form a variety of other polythionates, sulfur and sulfate (Schippers and Sand, 1999). The production of polythionates (also referred to as 'thiosalts' or 'reduced inorganic sulfur compounds' (RISCs)) as intermediates during the oxidation of these minerals supports the growth of non iron-oxidizing sulfur bacteria.

2.3. Assessment of mine spoil for potential acid production

Abandoned mines and mine spoils contain varying amounts of minerals that have the potential to generate acidity (mostly metal sulfides) and those which have potential for neutralizing acidity (mostly carbonates). Several methodologies have been devised to assess whether mine waste will produce effluents that are net acidic (described in Ferguson and Erickson, 1988). These include 'acid-base' accounting, in which the total pyritic-S and the total neutralization potential of representative samples are determined separately, and the balance between the two evaluated. This is one of the simpler and more rapid prediction techniques used, but it suffers from a number of drawbacks, including (i) it does not differentiate between various morphological forms of pyrite, which have quite different reactivities (fine-grain ($<1\mu$m) framboidal pyrite is more reactive than euhedral pyrite); (ii) it does not consider the kinetics of acid-generating and acid-neutralizing processes. The latter is important in view of the greater solubilities of calcareous materials. As a consequence, mine spoils may produce net-neutral effluents initially, but these become increasingly acidic with time. One alternative prediction technique which does take kinetic aspects into consideration is the use of 'humidity cell chambers', in which samples of 1 kg or so are placed in enclosed chambers, through which air of fixed or variable humidity is continually recirculated in order to accelerate oxidation/dissolution processes. Sample incubation generally lasts for up to one year, and aqueous extracts are analyzed on a regular basis (White and Jeffers, 1994).

2.4. Spoil heaps

Mineral spoil heaps tend to be very heterogeneous, and can have vastly differing chemical characteristics over relatively short distances. Both coal and mineral spoil heaps that contain significant amounts of sulfidic minerals may develop relatively high temperatures as a result exothermic oxidation reactions. In extreme cases, this may lead to spontaneous combustion of coal spoil heaps, which may continue smoldering for many years.

Schippers *et al*. (1995) examined the chemistry and microbiology of two uranium mine waste heaps in Ronneburg, Germany. Temperatures within the heaps varied from 7 to 45 °C, and pH between 4 and 7. Redox potentials were also noted to vary (by up to 700 mV), and oxygen concentrations declined dramatically with depth (and those of carbon dioxide increased) and were only about 3% in some

samples taken from 1 m below the heap surface. Spoil heaps generally have relatively thin aerobic surface layers overlying anaerobic (or microaerobic) cores, the latter accounting for the bulk of the spoil material.

Pichtel and Dick (1991) prepared synthetic coal spoil material by mixing crushed bituminous coal, rock (shale, sandstone and mudstone), both collected from the floor of a working opencast mine, with museum-grade euhedral pyrite. The resulting synthetic material, which contained 26 g of pyritic sulfur per kilogram of 'spoil', was incubated in 1% (w/v) aqueous suspensions and incubated, shaken at room temperature, for 28 days. The pH of the slurries fell from initial means of pH 6 to 3.8 during incubation, with corresponding increases in soluble iron and sulfate, presumably due to microbially-catalyzed oxidation of pyrite. These researchers also detected RISCs (thiosulfate, trithionate and tetrathionate) in the spoil slurries throughout the 28 day incubation period, with trithionate (present at up to 1.07 mmol kg^{-1} spoil, equivalent to 103 mg trithionate-S kg^{-1}) being the most abundant reduced sulfur anion present.

2.5. Mine tailings

There have been relatively few reported studies on the chemistry and microbiology of mine tailings. Wielinga *et al*. (1999) examined the geochemistry and microbiology of mine tailings in two sites within a flood plain near Butte, Montana. There were sharp gradations in dissolved oxygen, pH and concentrations of sulfate, iron and copper at the water table (about 1.5 m from the surface of the tailings). Most samples taken above the water table were covered in orange-brown ferric oxyhydroxides, and pore waters were also more acidic and contained more dissolved oxygen, sulfate, iron and copper than those from greater depths. Fortin and Beveridge (1997) examined mine trailings containing pyrite, chalcopyrite, sphalerite and galena in Ontario. The pH of pore waters increase from $\sim$2 at the tailings surface to over 7 at depths below 30 cm, and there were corresponding decreases (with depth of sampling) in concentrations of soluble sulfate, iron, copper and zinc.

2.6. Mine drainage waters

Waters draining mines, mine spoils and tailings dams have highly variable chemistries. They may be alkaline, neutral, or moderately to extremely acidic. Some mine waters are highly saline, while others have low solute potential. Frequently, discharge waters contain elevated concentrations of dissolved iron (ferruginous mine waters). Since the form of iron at the point of discharge is generally (though not always) as iron(II) (ferrous iron), which has only a very faint color, the waters may initially appear untainted, though oxidation of ferrous iron and the resulting hydrolysis of the ferric iron results in characteristic ochre-like precipitates and coatings in impacted streams and rivers. Mine water discharges which are of greatest environmental concern are those which are acidic, and contain significant concentrations of dissolved iron, sulfate and frequently a variety of heavy metals

and metalloids. Water of this type is generally referred to as 'acid mine drainage' (AMD) or 'acid rock drainage' (ARD). There have been a number of reviews and conference proceedings which have addressed this form of environmental pollution, and fuller accounts may be found elsewhere (Nordstrom, 2000).

2.6.1. *Acidity and Alkalinity*

Mine waters vary greatly in their pH values. Acidity arises principally from the oxidative dissolution of pyrite and other sulfidic minerals, while calcareous and other basic minerals in rock strata and spoils heaps may be present in sufficient amounts to neutralize this acidity, at least in the short term. Moderately acidic mine waters (with pH > 4.5) are classified as containing alkalinity, principally in the form of bicarbonate ions (Clarke, 1995). Acidity in mine waters has two major forms: (i) proton acidity (i.e. as measured by pH) and (ii) mineral acidity, which is due to the presence of dissolved metals, mostly iron, aluminum and manganese ('mineral acidity'). Hydrolysis of these metals (preceded by oxidation, in the case of iron) generates free protons, e.g.:

$$Al^{3+} + 3H_2O \rightarrow Al(OH)_3 + 3H^+ . \quad (4)$$

In some cases, mine waters will contain both alkalinity and mineral acidity, and whether these waters are net acid or alkaline is determined by calculating total acidity, and comparing with the total (measured) alkalinity. Total acidity of a mine water samples may be calculated from its pH and the sum of the 'acidic' metals (milli-equivalents of iron, aluminium and manganese) present (Equation (5); Hedin *et al*., 1994).

$$\text{Total acidity} = 50\left(\frac{2Fe^{2+}}{56} + \frac{3Fe^{3+}}{56} + \frac{3Al^{3+}}{27} + \frac{2Mn^{2+}}{55} + 1000(10^{-pH}\right) . \quad (5)$$

Equation (5) does not, however, take into account two other possible contributions to total acidity in mine water: organic acidity, due to organic acids (though these are likely to be of very minor importance in many mine waters) and RISCs, formed as intermediates of sulfide oxidation (Section 3.1.2). Oxidation of RISCs, mostly due to microbial activity, produces sulfuric acid, as illustrated in Equation (6) for trithionate:

$$S_3O_6^{2-} + 2O_2 + 2H_2O \rightarrow 3SO_4^{2-} + 4H^+ . \quad (6)$$

2.6.2. *Major and Trace Element Components*

Whilst the chemistry of mine waters is subject to considerable variation, many (most notably AMD and other ferruginous discharges) contain concentrations of sulfate and soluble iron that well exceed those of unpolluted waters (Table I), as a result of the dissolution of iron sulfides. In addition, contamination of waters

TABLE I

Physico-chemical data of discharge waters draining coal and metal mines and mine spoils in the U.K. and Norway. Data from Banks *et al*. (1997) and Johnson *et al*. (2001 and unpublished). All concentrations are mg L^{-1}

	pH	[Fe_{total}]	[Fe(II)]	[Al]	[Mn]	[Cu]	[Zn]	[SO_4]
Coal mines								
Bullhouse (U.K.)	5.9	61	45	1.2	15	<1	<1	–
Ynysarwed (U.K.)	6.2	160	140	20	–	–	–	460
Coal spoil heaps								
Oatlands (U.K.)	5.5	287	–	0.97	5.2	<0.007	0.05	146
Sverdrupbyen (Norway)	2.7	179	–	27.5	3.2	0.168	1.3	1077
Metal mines								
Mynydd Parys (U.K.)	2.5	650	650	70	10	60	40	3100
Roeros (Norway)	3.7	6.7	–	4.3	0.25	11	3.76	–
Wheal Jane (U.K.)	3.6	130	130	50	20	2	130	350
Metal mine Spoil heap								
Cwm Rheidol (U.K.)	2.6–2.7	–	–	104–128	–	1.2–9.35	577–978	250

– = Not determined.

draining metalliferous mines by a variety of trace elements is widely recognized. However, waters draining some coal fields may have relatively low contents of metals other than iron, whilst elsewhere they also contain elevated concentrations of aluminum, arsenic, and a variety of heavy metals.

The most important trace elements that occur in mine waters, and that have a high probability of being regulated, are arsenic, barium, cadmium, copper, manganese, molybdenum, nickel, lead, selenium and zinc (Sullivan and Yelton, 1988). The reason for enhanced concentrations of trace elements in mine waters is threefold. Firstly, many heavy metals and metalloids occur as sulfide minerals (such as asenic in arsenopyrite, FeAsS) and are themselves subject to microbially-accelerated oxidative dissolution (Section 3.1.2). Secondly, acidic ferric iron-rich liquors formed by pyrite dissolution are highly aggressive, and can degrade aluminosilicates and other minerals, releasing their metal components. Thirdly, many metals (with the

exception of those, such as molybdenum, which occur as oxyanions) are far more soluble in acidic than in circum-neutral pH waters. The presence of some trace elements, such as cadmium and arsenic, can pose the greatest toxicity hazard in mine waters, even if present only in small concentrations.

2.6.3. *Salinity*

Mine waters often have high solute potentials due to the presence of large concentrations of sulfate and dissolved cations. An additional problem in some areas is caused by the salinity of discharged waters. This is particularly the case where water is pumped from deep mines, though saline waters may also be pumped from surface mines where associated rocks and sediments have high soluble ion contents (Clarke, 1995). About 40% of water discharged from Polish coal mines in the early 1990's was classed as saline; this was pumped directly into two major rivers, causing problems to industries downstream as well as having a severe biological impact (noted in Clarke, 1995). Mining of coals and metals in arid and semi-arid areas, such as in the Hunter Valley and other parts of Australia, has also resulted in accentuated salinity problems in local rivers.

2.7. WATER CHEMISTRY OF UNDERGROUND STREAMS AND POOLS

Most published data on the chemistry of mine waters refer to samples in drainage streams and impacted rivers located outside of mines. Frequently, access to the inside of derelict mines is not possible on safely grounds or because adits and shafts have collapsed. However, there have been occasional reports describing the chemistry and microbiology of streams and pools located within abandoned mines. These are interesting in that they often have more extreme physico-chemical characteristics, and may be colonized by unusual and diverse microorganisms (Section 4). Physico-chemical data from six underground pools located in two mines in North Wales (Cae Coch and Mynydd Parys) and one in California (Iron Mountain) are shown in Table II. Mine waters within the latter mine are the most acidic to have been recorded, with pH values as low as –3.6 (Nordstrom *et al*., 2000). Evaporation of water from the acidic. metal- and sulfate-rich pools, thereby concentrating protons and other ions, is one of the major reasons why pools within Iron Mountain have such pronounced extreme physico-chemical characteristics.

3. Microbiology of Abandoned Mines and Discharge Waters

Since, as noted earlier, waters that drain mines have very variable chemical characteristics, it follows that indigenous macro- and microflora will also differ from site to site. The following account describes the biotic life that is known to occur in acidic and ferruginous mine wastes and discharge waters. While macrobiotic life is not excluded from such environments, e.g. as evidenced by successful colonization

TABLE II

Physico-chemical data of subterranean pools in three abandoned pyritic mines. Data from: (a) McGinness and Johnson (1993); (b) D. B. Johnson (unpublished); (c) Jenkins *et al*. (2000); (d) Nordstrom *et al*. (2000). All concentrations quoted are g L^{-1}

	pH	Temp. (°C)	[Fe_{total}]	[Fe(II)]	[SO_4]	[Al]	[Cu]	[Zn]
Cae Coch 1[a]	2.4	9	2.26	0.8	6.59	0.10	–	–
Cae Coch 2[b]	2.2	9	8.10	5.90	74.5	–	–	–
Mynydd Parys 1[c]	2.1	9	2.07	–	–	0.33	0.145	0.068
Mynydd Parys 2[c]	2.9	9	0.012	–	–	0.019	0.010	0.023
Iron Mountain 1[d]	1.5	41	2.67	2.47	14	–	0.293	0.058
Iron Mountain 2[d]	–2.5	42	124	34.5	760	–	4.76	23.5

– = Not determined.

of acidic mines lakes in Germany and elsewhere by *Juncus bulbosus, Typha latifolia, Phragmites australis* and other macrophytes (Pietch, 1998), the most diverse, and in many ways most significant, life-forms in these environments are microbial.

3.1. MICROBIAL DIVERSITY IN ACIDIC, METAL-RICH ENVIRONMENTS

It is now recognized that a considerable diversity of microbial life may be found in acidic, metal-rich effluents such as mine spoils, tailings and AMD. Many of these are obligately acidophilic microorganisms and grow poorly, if at all, above pH 4. Although composed of distinct microorganisms. microbial community structure and interactions in these environments are as complex and diverse as those found in more 'normal' environments (Johnson, 1998a).

3.2. ACIDOPHILIC MICROORGANISMS

Some prokaryotic and eukaryotic microorganisms are obligately acidophilic. Whilst the majority of known acidophiles are mesophilic (temperature range 20–40 °C), some are thermotolerant, (growth range 40–60 °C) and others (exclusively archaea) are thermophilic, growing at > 60 °C. Fuller accounts of the biodiversity, physiologies and phylogenies of acidophilic microorganisms may be found elsewhere (e.g. Hallberg and Johnson, 2001a).

3.2.1. *Mineral-Oxidizing Prokaryotes*

Probably the most significant (and certainly the best studied) of acidophiles are those bacteria and archaea that, by virtue of their abilities to oxidize ferrous iron and/or reduced forms of sulfur, accelerate the oxidative dissolution of sulfidic minerals (Table III). These microorganisms are used to process metal ores and concentrates in a biotechnology often referred to as 'biomining' (Rawlings, 1997)

TABLE III
Sulfide mineral-oxidizing bacteria and archaea

	Iron-oxidizers	Iron/sulfur-oxidizers	Sulfur-oxidizers
Mesophiles	*Leptospirillum ferrooxidans* *Ferroplasma* spp. *'Ferrimicrobium acidophilum'*	*Acidithiobacillus ferrooxidans* *Thiobacillus prosperus* *'Sulfobacillus montserratensis'*	*Acidithiobacillus thiooxidans* *Thiomonas cuprina*
Moderate Thermophiles	*Acidimicrobium ferrooxidans* *Leptospirillum thermoferrooxidans*	*Sulfobacillus thermosulfidooxidans* *Sulfobacillus acidophilus*	*Acidithiobacilus caldus*
Thermophiles		*Acidianus* spp. *Sulfolobus metallicus* *Sulfurococcus yellowstonensis*	*Metallosphaera* spp.

and in the depyritization of coal (Bos *et al*., 1992). The best known of all mineral-degrading prokaryotes is the mesophile *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*; Kelly and Wood, 2000) which was the first pyrite-oxidizing bacterium to be discovered. Due to its widespread distribution in natural and man-made iron-rich acidic environments, and the relative ease with which it can be cultured in the laboratory, it was for many years assumed that *At. ferrooxidans* is universally the most significant sulfide mineral-oxidizing bacterium. However, it is now known that a second iron-oxidizing bacterium, *Leptospirillum ferrooxidans* is more numerous and more active than *At. ferrooxidans* in many situations. This is probably due to *L. ferrooxidans* having a higher substrate affinity, and greater tolerance to ferric iron (which both bacteria generate) and low pH (<2) than *At. ferrooxidans*. On the other hand, most strains of *L. ferrooxidans* are less tolerant of low (<20 °C) temperatures, and have been reported to be less numerous than *At. ferrooxidans* in the few studies that have looked at the microbial ecology of cold acidic environments (Langdahl and Ingvorsen, 1997; Johnson *et al*., 2001). The significance of some of the more recently-described iron-oxidizing acidophiles (such as '*Ferrimicrobium*', and *Ferroplasma* spp.) has yet to be evaluated.

While most mesophilic mineral-oxidizing microorganisms are Gram-negative eubacteria, most of the currently known thermotolerant/moderate thermophilic iron-

and/or sulfur-oxidizers are Gram-positive bacteria, though *Acidithiobacillus caldus* is a notable exception. Many moderately thermophilic iron-oxidizing bacteria display relatively poor oxidation of pyrite when grown in pure culture, though mixed cultures with other bacteria may be very effective (Dopson and Lindstrom, 1999). Gram-positive mineral-oxidizing bacteria have much more complex physiologies than their Gram-negative mesophilic counterparts. Besides growing autotrophically on iron, sulfur or sulfide minerals, they can grow as organotrophs or mixotrophs, and are also facultative anaerobes.

The distribution of thermophilic mineral-oxidizing archaea (temperature maximum ~80 °C), such as *Metallosphaera* spp. and *Sulfolobus metallicus* in abandoned mines and spoils is not well known, though at least one isolate (*Sulfolobus* BC) was isolated from a self-heating coal spoil (Birch Coppice) in central England (Norris and Owen, 1992). Sulfide mineral oxidation is an exothermic reaction, and where this heat is conserved rather than dissipated (e.g. within the cores of spoil heaps) conditions may favor colonization by thermophiles rather than mesophilic bacteria.

3.2.2. *Iron- and Sulfate-reducing Acidophiles*

Oxidized (ferric) iron and sulfate can both act as alternative electron acceptors to oxygen as electron sinks in anoxic environments; this holds true for acidic as well as neutral pH environments. Indeed, because of the frequent elevated concentrations of iron in these environments, the solubility of ferric iron at pH < 2.3, and the high redox potential of the ferrous/ferric couple (+770 mV at pH 2), anaerobic respiration using ferric iron is both pragmatic and a thermodynamically-attractive alternative to aerobic metabolism in extremely acidic environments. A number of acidophiles have been shown to reduce ferric iron to ferrous, though whether this is an energy-transducing reaction has not been proven in all cases. A number of RISCs also react with ferric iron, reducing it to ferrous, which may lead to erroneous conclusions being drawn about the abilities of sulfur-oxidizing bacteria (such as *At. thiooxidans*) to reduce iron directly. However, it has been shown conclusively that some iron-oxidizing acidophiles, including *At. ferrooxidans*, can also, under anoxic conditions, couple the oxidation of sulfur or RISCs to the reduction of ferric iron. Iron-oxidizers which also use organic electron donors (*Sulfobacillus* spp., *Acidimicrobium ferrooxidans* and *'Ferrimicrobium acidophilum'*) can also grow heterotrophically in the absence of oxygen by ferric iron respiration (Bridge and Johnson, 1998).

All known species of the primarily heterotrophic genus *Acidiphilium* are also facultative anaerobes, coupling the oxidation of organic substrates to the reduction of ferric iron. In some *Acidiphilium* spp. this ability is constitutive, whilst in others it is inducible (Johnson and Bridge, 2002). In contrast to many other acidophiles, ferric reduction in at least some *Acidiphilium* spp. occurs even in the presence of oxygen (up to about 60% of maximum ambient dissolved oxygen); indeed, some *Acidiphilium* spp. reduce iron very slowly in the complete absence of

oxygen, though they do so at relatively rapid rates under microaerobic conditions (Johnson and Bridge, 2002). Moderately thermophilic iron oxidizers/reducers and *Acidiphilium* spp. have been shown to accelerate the reductive dissolution of many ferric iron-containing minerals, including those such as ferrihydrite, jarosites and goethite that are common in mine spoils and drainage waters (Bridge and Johnson, 1998, 2000). Solublization of ochreous sediments in AMD-impacted streams has also been demonstrated with *Acidiphilium* SJH (Johnson and Bridge, 1997).

The situation regarding the existence of acidophilic sulfate-reducing bacteria (SRB) is currently less clear. Although biological reduction of sulfate to sulfide has been observed in metal-rich acidic environments by a number of researchers and neutrophilic SRB have been found in high numbers in minerals tailings (Fortin and Beveridge, 1997), there are very few reports of the isolation and characterization of truly acidophilic SRB. Sen (2001), however, described SRB which grow at pH values as low as 3 (most SRB are inactive at pH < 5.5); most of these 'acid tolerant' SRB appear to be *Desulfosporosinus*-like Gram-positive eubacteria.

The occurrence of bacteria and archaea which can either oxidize or reduce iron (or sulfur) in juxtaposed environments can lead to the rapid re-cycling of these elements in acidic mine environments, with the reduced species (ferrous iron, sulfide etc.) acting as electron donors and the oxidized species (ferric iron and sulfate) as electron acceptors. A key limiting factor in this cycling process is the nature and amount of soluble organic carbon present, as this will strongly influence the extent of the anaerobic reductive processes.

3.2.3. *Other Acidophilic Microorganisms*

Other acidophilic microorganisms that have no direct impact on the geochemical cycling of iron or sulfur, but which may nevertheless be of considerable ecological significance, occur in mine spoils, tailings and AMD. These include other heterotrophic prokaryotes, as well as acidophilic eukaryotes (Rawlings and Johnson, 2002). The photosynthetic eukaryote *Euglena mutabilis* has been often found in AMD streams at coal and metal mines, where it is a major primary producer, contributing significantly to the carbon budgets of these ecosystems. Obligately acidophilic protozoa have also been isolated from AMD and 'acid streamers', and studied under laboratory conditions. Phagotrophic flagellates, ciliates and amoebae have been described that, by grazing mineral-oxidizing bacteria, can control numbers and activities of the latter *in vitro* (Johnson, 1998b).

3.3. MICROBIOLOGY OF MINE SPOILS AND TAILINGS: IN SITU STUDIES

In one of the earliest reports of the distribution of microorganisms in mine wastes, Belly and Brock (1974) enumerated chemolithotrophic and heterotrophic bacteria in pyritic coal spoil samples with pH from 1.1 to 2.4. Iron-oxidizing bacteria were present at between 10^4 to 4.6×10^7 g^{-1} spoil, though relatively few heterotrophic acidophiles were isolated. The first moderately thermophilic, acidophilic archaeon,

Thermoplasma acidophilum, had earlier been isolated by Tom Brock's research group (Darland *et al*., 1970) from a self-heating coal refuse pile. Goebel and Stackebrandt (1994) isolated cultivatable acidophiles from a chalcocite overburden heap in Australia, and identified isolates by 16S r RNA gene sequencing. *At. ferrooxidans, L. ferrooxidans, At. thiooxidans, At. caldus* and heterotrophic *Acidiphilium* spp. were found to be present in spoil samples. One of the few studies that has looked at vertical heterogeneity in a spoil heap was carried out by Schippers *et al*. (1995). These researchers examined two uranium waste mine heaps in Germany at depths of up to 5 m, analyzing 162 ore samples. A variety of iron- and sulfur-oxidizing and heterotrophic bacteria were recovered, though there was a notable cut off of aerobic microorganisms below 1.5–2 m depth, corresponding to a sharp decrease in oxygen concentrations. Interestingly, these authors reported large numbers of moderately acidophilic sulfur-oxidizing bacteria in one of the heaps, where the pH was generally close to neutral.

The microbial status of mine tailings has also been described on several occasions. Miller *et al*. (1987) detected *Bacillus, Arthrobacter* and fungi in uranium mill tailings in Colorado, while Southam and Beveridge (1992) reported up to 7×10^8 '*At. ferrooxidans*' (though these may have included other acidophilic iron-oxidizers, as the method used for enumeration was not species-specific) per gram of mine tailings which contained pyrite, chalcopyrite and sphalerite (pH range 1–7). Berthelot *et al*. (1997) isolated and characterized a range of *Acidiphilium*-like heterotrophic bacteria from tailings effluents from two uranium mines in Ontario. *At. ferrooxidans* was also isolated, but not *L. ferrooxidans*, though the methods used were inappropriate for the latter iron-oxidizer. More recently, Wielinga *et al*. (1999) examined the microbiology of long-deposited mine tailings in Montana. Iron- and sulfur-oxidizing bacteria were detected (at up to about 3.5×10^3 g^{-1} dry tailings) at depths of up to 175 cm (25 cm below the water table). Iron-reducing bacteria and SRB were also present, sometimes in higher numbers in the aerobic zone than beneath the water table. As with Schippers *et al*. (1995) these authors also detected significant numbers (up to 10^6 g^{-1}) of neutrophilic sulfur-oxidizing bacteria in their samples.

3.4. Microbiology of acid mine drainage: in situ studies

Walton and Johnson (1992) enumerated acidophilic microorganisms over a 1 km stretch of an acidic stream draining the Mynydd Parys copper mine in North Wales. Although measured pH increased only from pH 2.2 at the point of discharge to pH 2.8 at the last sampling point, redox potentials increased by almost 200 mV, as a result of ferrous iron oxidation. Both *At. ferrooxidans* and *L. ferrooxidans* were isolated, with the latter tending to be more numerous, particularly in downstream samples where ferrous iron concentrations were <10 mg L^{-1}. Total numbers of iron-oxidizing isolates varied from $1–8 \times 10^3$ mL^{-1} AMD and were more numerous than heterotrophic isolates in upstream samples, though the reverse was true in

downstream samples. Biodiversity of heterotrophic acidophiles also increased with distance from the discharge point, as evidenced by the range of isolates obtained on solid media. Seasonal variations in the microbiological status of a stream draining another abandoned (pyrite) mine in North Wales (Cae Coch) were reported by McGinness and Johnson (1993). Whilst there were some variations in numbers of both iron-oxidizing chemolithotrophs and acidophilic heterotrophs counted over a two year period, these did not appear to show distinct seasonal trends, probably due to the fact that most of these bacteria had originated from inside the mine itself, where conditions (temperature etc.) were stable year-round. *At. ferrooxidans* was the dominant iron-oxidizer in Cae Coch AMD, which was considered due to its ferrous iron content (often >500 mg L^{-1}). *L. ferrooxidans* was present but accounted for only 9–50% of iron-oxidizing isolates. More recently, Johnson *et al.* (2001) described the microbiology of three AMD streams at an abandoned copper mine site in Roeros, central Norway. Isolates were subjected to phylogenetic analysis to confirm identities that had been implied from physiological analyses. Numbers of iron-oxidizing acidophiles ranged from 1.4×10^3 to 5.6×10^4 mL^{-1} AMD, and again the dominant organism was a strain of *At. ferrooxidans*, though this was probably due in this case to the very low ambient temperatures at the site. These AMDs were found to contain large numbers (up to 2.1×10^5 mL^{-1}) and considerable diversity of acidophilic heterotrophic bacteria. Six were present at $>10^3$ mL^{-1} AMD, of which only two had 16S rDNA sequences which allowed them to be positively identified as species of bacteria previously isolated from acidic, metal-rich waters. Whilst three of the others were considered to be novel species of known acidophilic genera (*Acidiphilium, Acidocella* and *Acidisphaera*) the fourth was most closely related (96% gene sequence identity) to *Frataura aurantia*, a γ*-Proteobacterium* not known to occur in AMD.

3.5. Recent advances: Novel microorganisms in extremely and moderately acidic mine waters

One of the most revealing studies on mine microbiology in recent years has been carried out at Iron Mountain, California (Edwards *et al.*, 1999; Bond *et al.*, 2000). As noted in Section 3.2.5, some zones within this pyritic mine are extremely acidic, and also vary in dissolved ion contents and in temperature. A range of iron-oxidizing bacteria and the archaeon '*Ferroplasma acidarmanus*' were isolated from sites within the mine and also identified using genetic probes (FISH analysis). Microbial populations in AMD streams were found to vary in diversity and numbers both spatially and temporally. Bacteria were most numerous in January, and archaea in the summer months. This was primarily as a result of rainfall patterns, lower rainfall from July-September resulting in AMD with greater ionic strength which favored '*Fp. acidarmanus*'. *L. ferrooxidans* was abundant in the more extremely low pH environments and in slime growths. In contrast, although *At. ferrooxidans* was present, it was not a significant member of the microbial

community at acid-generating sites (considered to be those in contact with the pyrite ore body). Gram-positive *Sulfobacillus* spp. were detected in warmer zones (~43 °C) within the mine.

At the other end of the AMD pH spectrum, ferrous iron oxidation in streams which are 'moderate' in proton or mineral acidity is often considered to be primarily or exclusively abiotic. One such example is discharge from a coal mining area at Ynysarwed, south Wales. The pH of the water at point of discharge was ca. 6.2 (Table I) and, following chemical oxidation and hydrolysis of the ferric iron produced, this fell to ca. pH 4. However, in sterilized samples the remaining ferrous iron (ca. 50% of that in freshly-discharged water) was stable and remained in solution, while in native waters the iron was fully oxidized (Dennison *et al*., 2001). Examination of the oxidized AMD revealed that no acidophilic iron-oxidizers were present (plating on pH 2.5 solid media, used routinely for *At. ferrooxidans* and *L. ferrooxidans*), though ferric iron-encrusted colonies were obtained on a similar medium poised at pH 4.5. Phylogenetic analyses of the isolates obtained showed that they are novel species of *Thiomonas*, a genus which includes neutrophilic and moderately acidophilic sulfur-oxidizing bacteria, though no classified species are known to oxidize ferrous iron. Mine water effluents at Roeros, Norway (Johnson *et al*., 2001) and at the former Wheal Jane tin mine in Cornwall, England (Hallberg and Johnson, 2001b) have been found to contain both moderately acidophilic iron-oxidizers and *At. ferrooxidans*. The Wheal Jane isolates were found to be phylogenetically distinct from both the more extremely acidophilic iron-oxidizers and the Ynysarwed isolates. It is intriguing to speculate that previously undiscovered iron-oxidizing bacteria that are active pH 3 to 5 have a major role in dictating the chemistry of many mine waters.

4. Overview: Microorganisms as Causes and Cures of Mine Water Pollution

There is an intricate relationship between the chemistry and microbiology of mine spoils and drainage waters. More often than not, microorganisms are deemed to be the 'villains' of the piece, and it is certainly the case that indigenous mineral-oxidizing bacteria and archaea are responsible for generating many of the environmental problems associated with the mining of coal and metals. On the other hand, it is clear that there are other acidophilic (and neutrophilic) microorganisms which catalyze reactions that can contribute to the remediation of polluted mines and AMD. The most significant microbes here are those which catalyze the reduction of ferric iron and/or sulfate, thereby reversing, in essence, the reactions involved in the oxidation of sulfide minerals. Frequently their activities generate net alkalinity, and a further bonus, in the case of SRB, is that they very effectively remove many of the most toxic metals found in mine effluents, by causing them to precipitate as metal sulfides. These activities are harnessed, though in an uncontrolled way, in the 'passive' treatment of AMD. Current trends include the development and applic-

ation of more proactive microbiological systems for remediating acidic metal-rich wastewaters (reviewed in Johnson, 2000). Greater knowledge of the microbiology of these ecosystems will doubtless lead to more focused and efficient technologies which utilize natural processes to counteract the negative environmental impacts of mining.

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