Development of an integrated microbiological approach for remediation of acid mine drainage and recovery of heavy metals

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Abstract

Various active and passive treatment technologies have been applied to treat acidic waters that drain abandoned metal mines. The most commonly used approaches are active treatment (by adding lime or other alkaline chemical) and passive remediation (utilising constructed wetlands and compost bioreactors). Both approaches result in the retention of iron and other heavy metals in the wastewaters, either in the sludge produced (lime treatment) or the wetland/compost substratum (passive treatment). An alternative approach, which facilitates the recovery and re-use of metals such as copper from mine waste streams, is to use “active biological” technology. To date, this has involved the use of off-line sulfidogenic bioreactors, wherein sulfide produced by dissimilatory sulfate-reducing bacteria (SRB) is used both to add alkalinity to the acidic waste streams, and to precipitate chalcophilic metals as highly insoluble sulfide precipitates, which may then be recovered and reprocessed. We are developing an integrated biological system for treating acidic metal mine wastewaters, in which different populations of acidophilic and acid-tolerant SRB will be used in on-line bioreactors (thereby reducing engineering costs) to selectively remove copper and zinc from mine waste streams while maintaining low pH to retain iron in solution. A second (fixed-bed) bioreactor, containing extremely acidophilic and novel strains of iron- and sulfur-oxidising moderate acidophiles, will be used to oxidise and precipitate iron, and to remove any excess sulfide produced by the SRB. With the proposed system, the essentially metal-free water would then be released, possibly following passage through a limestone drain to increase its pH. Such a biological system would be both sustainable and environmentally-benign.

1 Introduction

Acid mine drainage (AMD) is acknowledged as one of the most pernicious forms of water pollution in areas of the world that have active or historic mining operations (e.g. Banks et al. 1997; Younger et al. 2002). AMD may be generated within deep mine shafts and adits, spoil heaps and tailing deposits which contain sufficient amounts of sulfidic minerals (chiefly pyrite, FeS_2) to offset the neutralising potential of any basic minerals (e.g. carbonates) also present in these strata and wastes. Suitable conditions for the production of AMD exist at many coal and metal mines, though it is the latter that, due to the potential for water pollution by heavy metals such as copper, zinc and cadmium (in addition to elevated concentrations of iron, aluminium, manganese and sulfate that are common to both scenarios) that is often perceived to pose the more serious risk to the environment.

There are a number of alternative strategies for treating AMD, once formed; these have been described in a number of recent reviews (e.g. Younger et al. 2002; Johnson and Hallberg 2004a). These may be categorised as systems that use either biological or abiotic (chemical) means to generate alkalinity and precipitate metals, and also as systems that require continuous provision of materials for the remediation process to operate ("active" remediation technologies) or those that, once in place, require minimum management inputs ("passive" technologies). All of the established and emerging AMD remediation options have drawbacks, for example the on-going costs of both neutralising chemicals and disposal of metal-rich sludges in active abiotic systems, which have historically been the favoured option in many cases. Passive biological treatment of AMD (aerobic and anaerobic "wetland" systems) have a number of perceived advantages over chemical treatment, though their sometimes erratic performances and requirement of large land areas ("footprints") to treat high flow rate, metal-rich AMD waters (such as at the former Wheal Jane mine, Cornwall, U.K.) has tended to restrict their use to treating coal mine effluents.

Central to the operation of "active" biological systems are sulfidogenic bioreactors, wherein hydrogen sulfide (as H_2S and/or HS^-) is generated by sulfate reducing bacteria (SRB) that couple the oxidation of (mostly) small molecular weight organic compounds (such as ethanol) and/or hydrogen, to the reduction of sulfate, in reactions that are net alkali-generating (Castro et al. 2000). All of the pilot- and full-scale systems to date use "off-line" sulfidogenic bioreactors, to prevent direct contact between the AMD and the SRB, all known strains of which are highly acid-sensitive. Although using similar microbiological principles to compost bioreactors, off-line sulfidogenic bioreactors are represent a radically different engi-

neering approach for remediating AMD (Boonstra et al. 1999; Tabak et al. 2003). These engineered systems have three potential advantages over passive biological remediation: (i) their performance is more predictable and readily controlled; (ii) they allow heavy metals, such as copper and zinc, present in AMD to be selectively recovered and re-used; and (iii) concentrations of sulfate in processed waters may be significantly lower than in untreated AMD. On the negative side are the construction and operational costs of sulfidogenic bioreactor systems.

Another application of (micro)biological treatment of AMD that has been much researched (though not applied as a full-scale operation in the field) is the use of acidophilic bacteria to oxidise ferrous iron to ferric, thereby facilitating the removal of this metal as highly insoluble (at pH > 2.5) ferric iron precipitates (e.g. Long et al. 2003). Since most of these bacteria are autotrophic (i.e. they fix carbon dioxide) they do not (as do sulfidogenic systems) require on-going provision of substrate, so that they constitute another "passive" approach to AMD treatment. Whilst the oxidation of ferrous iron to ferric is rapid at and above pH 4.0, in more acidic waters its slow rate of oxidation is potentially problematic. Iron-oxidising bacteria are invariably present in mine waters (Johnson and Hallberg 2003) but their low numbers (and activities) often limit rates of iron removal. To counter this problem, immobilisation of bacteria onto solid support materials, forming biofilms, has been demonstrated to be highly effective. Most research in this area has tended to focus on a single bacterium, *Acidithiobacillus ferrooxidans.* Whilst this is the most well-known of all iron-oxidisers, it might not be those most appropriate in all scenarios. For example, *At. ferrooxidans* has a relatively low substrate (ferrous iron) affinity, so that it is not as effective at removing iron when present in small concentrations (<1 mg/l) than other bacteria, such as *Leptospirillum ferrooxidans*. Recent research has shown that, in AMD of pH 3-4, other iron-oxidising bacteria (e.g. some novel strains of *Thiomonas*) are more abundant that *At. ferrooxidans*, suggesting that these bacteria (which are moderately rather than extremely acidophilic) may be more adept at oxidising and precipitating iron in less extreme AMD waters.

This paper describes the on-going development of a novel integrated microbiological approach for remediating AMD and recovery of heavy metals, using bacteria that occur naturally in AMD-impacted and other acidic waters.

2 Materials and Methods

2.1 Mine water chemistry

The bioremediation system is targeting, initially, mine water being discharged at the abandoned Mynydd Parys copper mines located on Anglesey, north Wales. The subterranean water body held within the mine was subjected to a major partial draining exercise in 2003 (Coupland and Johnson 2004). Since then water has been draining the mine via a stream (the Afon Goch north) that flows about 3 km northwards before entering the Irish Sea at Amlwch. Water discharging into the Afon Goch north was sampled at the end of a drainage adit on a monthly basis. The pH, redox potential (Eh), conductivity (Ec) and temperature of the AMD was measured on site using a Hannah Water Test field analyser (VWR, U.K.) and dissolved oxygen (DO) was measured with a YSI95 dissolved oxygen meter (Yellow Springs Instruments, Ohio, U.S.A.). Ferrous iron was determined using the FerroZine reagent, as described by Lovley and Phillips (1987); diluted AMD samples were added to the FerroZine reagent on site, and the absorbance of the coloured complex formed was measured in the laboratory within 3 hours. Other dissolved metals (copper, zinc, aluminium, manganese, and also total iron) in filtered (<0.2 μm) acidified (with HNO_3) water samples were determined by atomic absorption spectrophotometry (AAS; Pye Unicam SP9-10). Sulfate was determined using a turbidimetric method (formation of $BaSO_4$; Hydrocheck system, WPA, Cambridge, U.K.). DOC of filtered (<0.2 μm) AMD was measured on a Protoc TOC analyser (Pollution and Process Monitoring, Kent, U.K.).

2.2 Development and operation of an acidophilic sulfidogenic bioreactor

A mixed culture of acidophilic bacteria that was capable of generating sulfide at low pH was established in a 2 l bioreactor (Electrolab, U.K.) fitted with pH, temperature and dissolved oxygen monitoring and control in a liquid medium containing 5 mM K_2SO_4, trace elements and basal salts. The medium was de-oxygenated with oxygen-free nitrogen (OFN) for 30 minutes and then sterilized (120°C, 30 minutes). Glycerol (5 mM), 5 mM $ZnSO_4$ and 0.1 mM $FeSO_4$ and a vitamin mixture (heat-sterilized, or filter-sterilized through 0.2 μm membranes, as appropriate) were added to the liquid medium after autoclaving. The bacteria used were: (i) *Desulfosporosinus* strain "M1", a spore-forming SRB that had been isolated from an enrichment culture of acidic sediment from Montserrat, and (ii) *Acidocella*

strain PFBC, an acidophilic heterotrophic bacterium that had been isolated from a supposedly pure culture of an acidophilic SRB, and which was not itself capable of dissimilatory sulfate reduction. Strains M1 and PFBC, pre-grown in pure culture, were introduced to the bioreactor, and the culture pH was set at pH 3.8 to 4.2. The control of pH in the bioreactor was maintained by automatic addition of 0.1 M NaOH or 0.1 M H_2SO_4. The amount of sulfuric acid used in pH maintenance was monitored carefully to allow accurate calculations to be made of net sulfate reduction. Concentrations of sulfate and glycerol were determined using a Dionex DX-320 ion chromatograph (Dionex, Sunnyvale, U.S.A.). Concentrations of soluble zinc were determined using AAS, and ferrous iron using the FerroZine assay.

2.3 Development and operation of the acidophilic iron oxidation bioreactors

Fixed bed bioreactors were set up using immobilised iron-oxidising bacteria, and monitored for their abilities to remove iron from synthetic AMD. Porous glass beads (8-16 mm diameter), made from recycled glass by Dennert Poraver Gmbh (Germany) provided the support matrix on which bacterial biofilms developed. The beads were acid-washed (with H_2SO_4) to remove any soda-glass present, rinsed repeatedly with distilled water and put into 10 l flat-bottomed flasks, together with 5 mM ferrous sulfate and basal salts, at pH 4 for moderate acidophiles and pH 2 for extreme acidophile, and trace elements. These were inoculated with AMD draining a deep coal mine (Ynysarwed) in south Wales (Hallberg and Johnson 2003), a moderately acidophilic iron-oxidiser (*Thiomonas* sp. Ynys4) isolated from this AMD, or the type strains of the extremely acidophilic iron-oxidisers, *At. ferrooxidans* and *L. ferrooxidans*. When the ferrous iron had oxidised (which took about 2 weeks in the first instance) the greater part of the spent medium was removed and replaced with fresh medium. This pattern was continued for at least five cycles, by which time the rate of ferrous iron oxidation had increased considerably, indicating that biofilms had established successfully on the beads. At this point the beads were put into cylindrical perspex columns (height 20 cm; diameter 9.5 cm), and covered with a layer (2 cm) of sterile gravel (5 -10 mm diameter). The columns were flooded with synthetic AMD containing 1-10 mM ferrous iron and with pH varying from 2.0 to 4.0, and were aerated at ca. 1 l air/minute. Samples were withdrawn at regular intervals to measure ferrous iron concentrations, pH and Eh.

3 Results

3.1 Mine water chemistry

Physico-chemical data of the Afon Goch north since the cessation of the dewatering of Mynydd Parys (means of 7 samples taken from July 2003 until April 2004) are shown in Table 1. The stream has remained highly acidic and rich in dissolved metals and sulfate throughout this period. At the point of discharge (the Dyffryn Adda adit) the AMD contains very little dissolved oxygen, and most of the dissolved iron is present in the reduced (ferrous) form. No flow volume data are available.

Table 1. Physico-chemical data of the AMD discharge targeted for bio remediation (Mynydd Parys outflow); n = 7 samples

Measured parameter	Mean value	Standard deviation
pH	2.74	0.23
Redox potential (Eh;mV)	+651	9.2
Conductivity (Ec; mS/cm)	1.90	0.04
Dissolved oxygen (% of saturation)	13.8	1.57
Temperature (°C)	11.2	0.3
Fe_{total} (mM)	11.1	2.0
Fe^{2+} (mM)	7.1	1.7
Cu (mM)	1.1	0.3
Zn (mM)	1.2	0.2
Mn (mM)	0.25	0.08
Sulfate (mM)	29.4	2.0
DOC* (mg/l)	5.8	2.4

3.2 Low pH sulfidogenic bioreactor

Changes in concentrations of glycerol, soluble zinc and ferrous iron in the sulfidogenic bioreactor are shown in Fig. 1. While data from a culture maintained at pH 4.2 are shown; similar trends were also found in bioreactor cultures maintained at pH 3.8 and 4.0. In each case, the oxidation of glycerol was tightly coupled to sulfate reduction (not shown), and the generation of sulfide resulted in the precipitation of ZnS,causing concentrations of soluble zinc to decline. At the pH range (3.8-4.2) at which the bioreactors were run, there was no formation causing concentrations of

soluble zinc to decline. At the pH range (3.8-4.2) at which the bioreactors were run, there was no formation of iron sulfides, which was confirmed by EDAX analysis of solid phase materials taken from the reactor (data not shown).

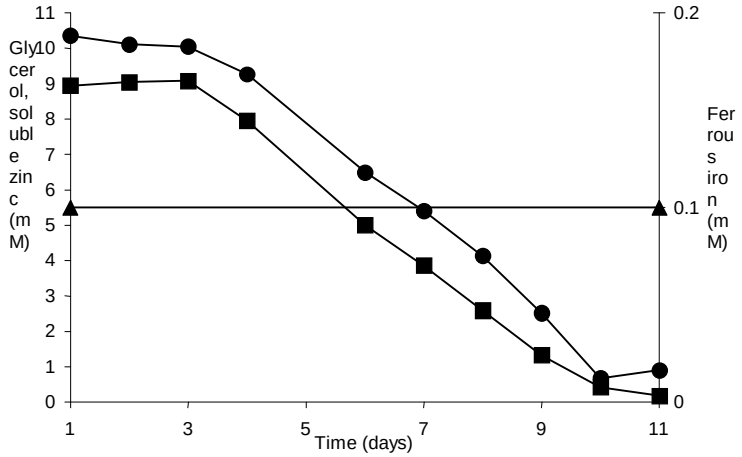

Fig. 1. Changes in solute concentrations in the sulfidogenic bioreactor operated at pH 4.2. Key: glycerol (●); soluble zinc (■); soluble ferrous iron (▲)

3.3 Ferrous iron oxidation bioreactors

All three of the pure cultures of iron-oxidising bacteria, and the undefined AMD inoculum, resulted in the successful production of fixed bed bioreactors that accelerated the oxidation of ferrous iron to ferric in synthetic AMD. The efficiencies of the bioreactors increased with time, presumably as a consequence of enhanced biofilm development on the porous glass beads. Fig. 2 illustrates iron oxidation and concomitant changes in pH and Eh for a fixed bed bioreactor inoculated with *Thiomonas* sp. Ynys4, and Fig. 3 collates data from multiple runs of the bioreactor that used AMD as inoculum. It was found that, with the latter bioreactor, a residence time of ca. 1 hour was generally sufficient for >90% oxidation of the ferrous iron in the synthetic mine water. Comparison of performance data for bioreactors inoculated with *At. ferrooxidans* and *L. ferrooxidans* showed that, whilst rates of iron oxidation were generally similar for both, residual ferrous iron concentrations tended to be smaller in the case of *L. ferrooxidans*, probably reflecting its higher affinity for ferrous iron (data not shown).

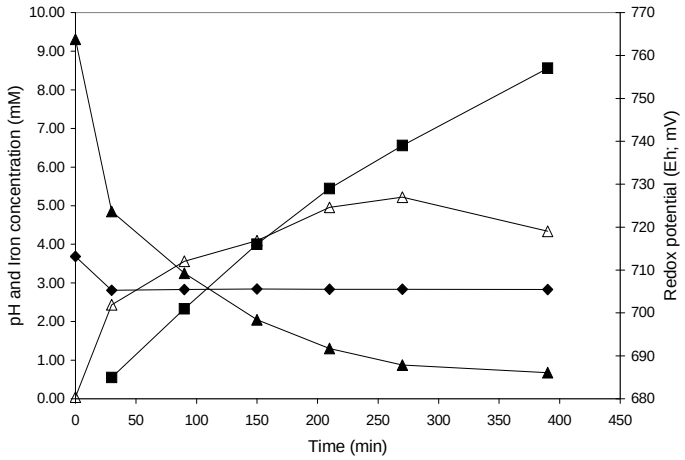

Fig. 2. Ferrous iron oxidation by a fixed-bed bioreactor inoculated with the moderate acidophile *Thiomonas* Ynys 4. Key: Fe^{2+} (▲); Fe^{3+}(soluble) (△); pH (◆); Eh (■)

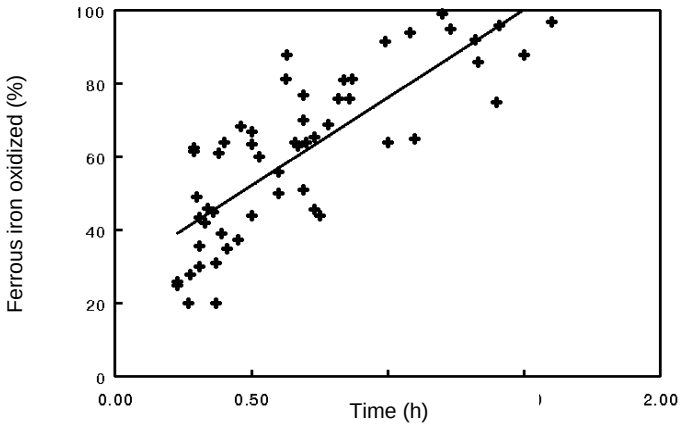

Fig. 3. Performance data of the packed-bed column bioreactor containing immobilised iron-oxidising acidophilic bacteria. Each symbol represents a result from a single experiment. A best linear fit for all data is shown (r = 0.80).

4. Proposed integrated bioremediation system

The AMD that drains the long abandoned Mynydd Parys mines contains significant concentrations of aluminium and a range of heavy metals. The solubility products of the hydroxides and (if they form) sulfides of these metals are listed in Table 2. These data suggest that: (i) zinc and copper are more effectively removed as their sulfides, and (ii) iron is optimally removed by oxidation of ferrous iron and precipitation as ferric hydroxide. Aluminium does not form a solid sulfide, and the solubility products of manganese (II) hydroxide and manganese (II) sulfide are both relatively large, and this metal is notoriously difficult to remove from AMD.

Table 2. Solubility products (log K_{sp} at 25°C) of hydroxides and sulfides of the significant soluble metals in Mynydd Parys AMD (data, except for $Al(OH)_3$, from Diaz et al. 1997)

Metal	Hydroxide	Sulfide
Al^{3+}	-24.9	-
Cu^{2+}	-19.8	-35.9
Fe^{2+}	-16.3	-18.8
Fe^{3+}	-38.6	-
Mn^{2+}	-12.7	-13.3
Zn^{2+}	-16.1	-24.5

On-going experiments have confirmed that bioreactors containing acidophilic microorganisms have the potential to remediate AMD by (i) generating alkalinity and selective removal of heavy metals such as zinc, in the case of the sulfidogenic bioreactor, and (ii) accelerating the oxidation of ferrous iron to ferric, and thereby promote the formation of insoluble ferric compounds, in the case of the iron oxidation bioreactors. The integrated modular bioreactor system that is planned, in the first instance to demonstrate amelioration of and recovery of valuable metals from AMD at Mynydd Parys, is shown in Fig. 4. Firstly, the AMD (which contains little oxygen at its point of discharge) would be fed into one (or more) sulfidogenic bioreactors. The present system (run at pH 3.8 - 4.2) would remove both soluble copper and zinc from the wastewater, but leave iron (as ferrous) in solution. Currently, we are seeking to commission a new sulfidogenic reactor that would operate at pH 2.5; solubility products (Table 2) predict that at that pH, it would be possible to segregate copper and zinc

(zinc sulfide does not form at that pH). Partly processed AMD would then flow into an iron oxidation bioreactor, which would necessarily require aeration for ferrous oxidation to proceed. The AMD entering this reactor would have a pH of ca. 4, so that moderately acidophilic iron-oxidisers such as *Thiomonas* spp. would be most appropriate. These bacteria also

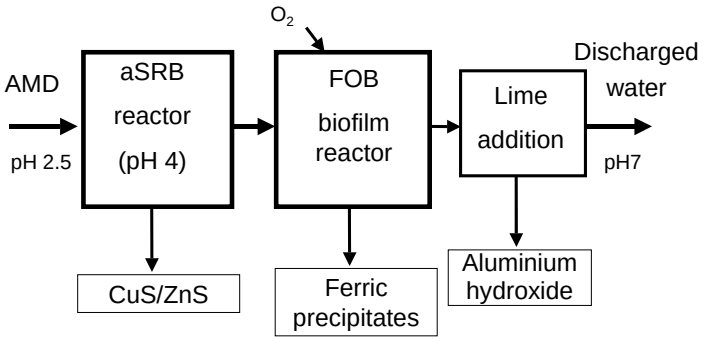

Fig. 4. Proposed layout of the integrated on-line bioreactor system for AMD remediation. aSRB = acidophilic SRB; FOB = iron-oxidising bacteria

have the ability to oxidise any hydrogen sulfide that might be excessively produced in the sulfidogenic bioreactor(s). It is important that reduced sulfur is completely oxidised before processed AMD is released into the environment, as oxidation downstream of any treatment plant can result in the severe acidification of receiving water courses, as was evident at the Wheal Jane passive treatment plant (Johnson and Hallberg 2004b). However, since hydrolysis of the ferric iron produced will generate protons, the pH of the water within the iron oxidation bioreactor would be predicted to fall, possibly to levels at which *Thiomonas* spp. are inhibited, so that inclusion of extremely acidophilic iron-oxidising bacteria (in particular of *L. ferrooxidans*, which has a high affinity for ferrous iron) would be advantageous. The acidic, metal-depleted water would require further addition of (chemical alkalinity) though, at the Mynydd Parys site, it would probably be more pragmatic to allow discharge directly into the sea.

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