Developing Sustainable Biotechnologies for Mine Water Treatment in the 21ST Century

Barrie Johnson[1], Ana Laura Santos[2], Carmen Falagán[3], Rose M. Jones[4], Barry M. Grail[5], Roseanne Holanda[6] and Sabrina Hedrich[7]

College of Natural Sciences, Bangor University, LL572UW, UK
[1]d.b.johnson@bangor.ac.uk
[2]bsp41b@bangor.ac.uk
[3]c.falagan@bangor.ac.uk
[4]rosejones233@gmail.com
[5]b.m.grail@bangor.ac.uk
[6]bsp40e@bangor.ac.uk
[7]Current address: Federal Institute for Geosciences and Natural Resources (BGR), Stilleweg 2, 30655 Hannover, Germany; Sabrina.Hedrich@bgr.de

Abstract

Active chemical and passive biological treatments of acidic mine waters both have major detractions, including the need to collect and dispose of large volumes of waste materials (metal-laden sludges or spent composts) in landfill sites, and the fact that resources present in mine waters, such as potentially valuable transition metals, are not recovered and recycled. We have developed modular bioreactors that combine mine water remediation with resource recovery. Two module types have been commissioned, one designed to oxidise ferrous iron and thereby to promote the biomineralisation of pure-grade schwertmannite, and the other to generate hydrogen sulfide via the microbial reduction of either sulfate or elemental sulfur at low pH. These can be configured to treat mine waters of widely different chemical (e.g. pH and metal contents) compositions, and to promote the selective biomineralisation of different transition metals present in mine waters, thereby facilitating the production of defined mineral products that have resale value. Both the schwertmannite-generating and sulfidogenic bioreactors utilise populations of recently-described bacteria that occur naturally in acidic mine waters. More recently, we have also incorporated a third bioreactor which effectively removes soluble manganese from waters of pH 5 and above, into our design protocols. The modules have low construction costs, and the value of the metals recovered often exceeds operational costs. Even discounting the cost of an alternative mine water treatment process that does not facilitate metal recovery, the biomineralisation option would often prove to be highly competive in economic as well as environmental terms. With increasingly strict environmental regulations, diminishing availability of landfill sites, and greater pressure to recycle from waste materials, (bio)technologies that consider mine waters as potential resources as well as environmental pollutants have the potential to be the major growth areas in this sector in the 21st century.

Key words: Biomineralisation, bioreactors, bioremediation, schwertmannite, sulfate reduction

Introduction

Acidic, metal-rich waters generated in, and emanating from, active and abandoned mines and mine waste repositories (rock dumps and tailings deposits) represent a serious global threat to the environment (Blowes et al. 2014; Nordstrom et al. 2015). Various options are available to remediate acid mine drainage (AMD) water, though two approaches - active chemical treatment (aeration and addition of an alkaline material such as lime) and passive biological treatment (use of constructed wetlands and "compost bioreactors") - have been used most extensively (Johnson and Hallberg 2005). Both of these, however, have major detractions, including the need to collect and dispose of large volumes of the metal-laden sludges or spent composts in landfill sites that, in many cases, need to be designated for storage of hazardous wastes, and secondly that none of the resources present in mine waters, such as potentially valuable transition metals, are recovered and recycled.

Microorganisms play key roles in the genesis of AMD (Blowes et al. 2014), chiefly because of the abilities of some specialised species to oxidise reduced sulfur (to sulfuric acid, thereby acidifying mine waters, sometimes to extremely low pH values) and/or ferrous iron (which generates the powerful

oxidant, ferric iron, thereby accelerating the oxidative dissolution of metal-bearing sulfide minerals). However, other microbial species have the potential to facilitate the remediation of AMD by carrying out metabolic activities that either generate net alkalinity, or immobilise metals (biomineralisation), or both. An example of the latter are sulfate-reducing bacteria (SRB). The dissimilatory reduction of sulfate, coupled to the oxidation of an organic carbon substrate can, depending on pH, be either acid-generating, acid-consuming or pH neutral. In moderately to extremely acidic liquors, it is a strongly acid-consuming (alkali-generating) process as the end products are CO_2 and H_2S (as shown in equation [1], where the organic substrate shown is glycerol) rather than, as in high pH waters, HCO_3^- and HS^-:

$$4\ C_3H_8O_3 + 7\ SO_4^{2-} + 14\ H^+ \rightarrow 7\ H_2S + 12\ CO_2 + 16\ H_2O \qquad [1].$$

The hydrogen sulfide generated as a waste product by sulfate-reducing bacteria (SRB) can effectively immobilise many (chalcophilic) transition metals, forming highly insoluble sulfide minerals. Most SRB are obligate anaerobes, however, and are non-active or readily killed on exposure to oxygen. Nascent metal sulfides produced by SRB are also unstable in aerobic environments. SRB have been implicated as important in remediation systems that use constructed wetlands, though substantiated evidence in support of this is often lacking. One reason is that SRB tend, in general, to metabolise readily-degraded, small molecular weight organic substrates such as sugars, alcohols and aliphatic acids rather than more recalcitrant materials (composts, straw etc.) that are used as long-term substrates in constructed wetlands. This problem is readily circumvented when bioreactors containing SRB are used to treat acid mine drainage (AMD). Full-scale systems of this kind (sometimes using hydrogen as substrate and sulfur- rather than sulfate-reducing bacteria) have been developed and operated by the Dutch company *Paques b.v.*, and the Canadian company *BioteQ Environmental Technologies Inc.*.

Sustainable protocols for mine water remediation require capital costs of systems to be minimal, and ideally for net operating costs to be neutral. One way to achieve the latter is to recover some or all of the commercially valuable metals present in AMD in such a way that they have a saleable value, or else convert them into minerals that have useful functions. With this challenge very much in mind, modular bioreactors have been commissioned at Bangor University and tested with a variety of synthetic and actual mine waters. The objectives were to develop empirical but effective units with minimal operating costs, and which could be configured in different ways to meet the requirements of AMD with widely different chemical compositions. These may be used to promote both the selective biomineralisation of metals (and metalloids) present in waters draining metal and coal mines, and to generate alkalinity, thereby ameliorating the pH of net acid AMD waters.

Materials and Methods

The composite systems used involve two main types of bioreactors, one of which catalyses the oxidation of ferrous iron in the first stage of a process for removing iron from AMD, while the other is a low pH biosulfidogenic module that can be used to capture metals both in-line and off-line, and also to partly neutralise the acidity of mine waters. A third bioreactor, which uses undefined microbial populations to oxidise manganese (II) and thereby remove this metal as an insoluble manganese (IV) phase, has also been used occasionally.

Ferrous iron-oxidising bioreactors. These operate as continuous flow systems, where the objective is to use bacteria commonly found in acidic ferruginous mine waters to oxidise ferrous iron to ferric, a reaction that proceeds slowly below pH 3.5 in the absence of microorganisms. Two modular systems have been devised and tested with mine water from the abandoned Mynydd Parys copper mines (Anglesey, north Wales), one of which uses the autotrophic iron-oxidising acidophile "*Ferrovum (Fv.) myxofaciens*" (which is now known to have global distribution in mine waters of pH 2-4; Hedrich and Johnson 2012) and the other the heterotrophic iron-oxidising acidophile *Acidithrix (Ax.) ferrooxidans* (Jones and Johnson 2016). Both of these form the first unit in a composite system for removing iron from AMD, which also includes a second pH-controlled unit, where sodium hydroxide is added to the oxidised AMD to form the mineral, schwertmannite ($Fe_8O_8(OH)_6(SO_4).nH_2O$) and a third, fixed bed bioreactor, which is used to remove residual iron (Fig. 1). Since rates of ferrous iron oxidation far exceed those of ferric iron hydrolysis in the bioreactor unit, only small amounts of ferric iron precipitate in this unit and the accumulation of ferric minerals within it is minimal.
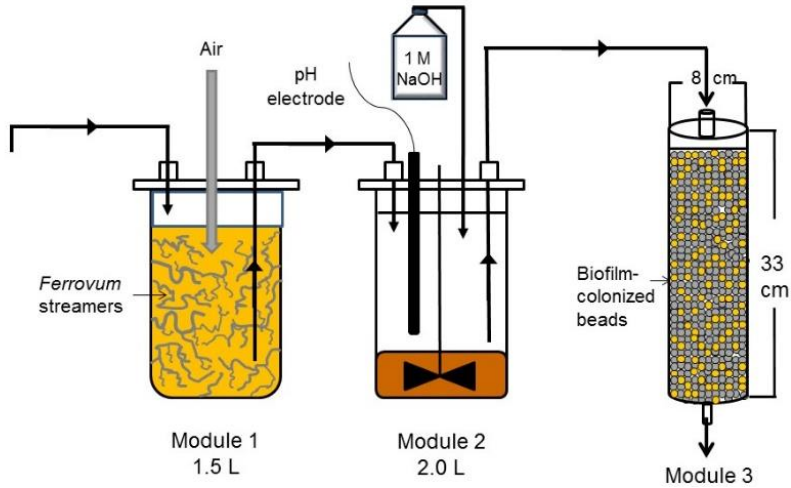
Figure 1 *Schematic of the composite modular system used to selectively remove iron from synthetic mine waters.*

Low pH sulfidogenic bioreactors. Continuous flow biofilm reactors were commissioned to produce hydrogen sulfide via the dissimilatory reduction of sulfate (Fig. 2). These contained novel species of acidophilic and acid-tolerant SRB (aSRB), and other bacteria, immobilised on 1-2 mm diameter porous glass beads. The bioreactors (FerMac 310/60; Electrolab, UK) were operated at between 20° and 45°C (30°C, typically) and at pH values between 2.5 and 5.0. The liquors used as feed solutions for the bioreactors required to be of low pH than that pre-set in the reactor vessel. Proton consumption accompanying sulfate reduction (equation [1]) caused the pH inside the reactor vessel to increase, which triggered automated inflow of the acidic feed. Flow rates (and hydraulic retention times) were therefore dictated primarily by rates of bacterial sulfate reduction. In cases where transition metals precipitated as
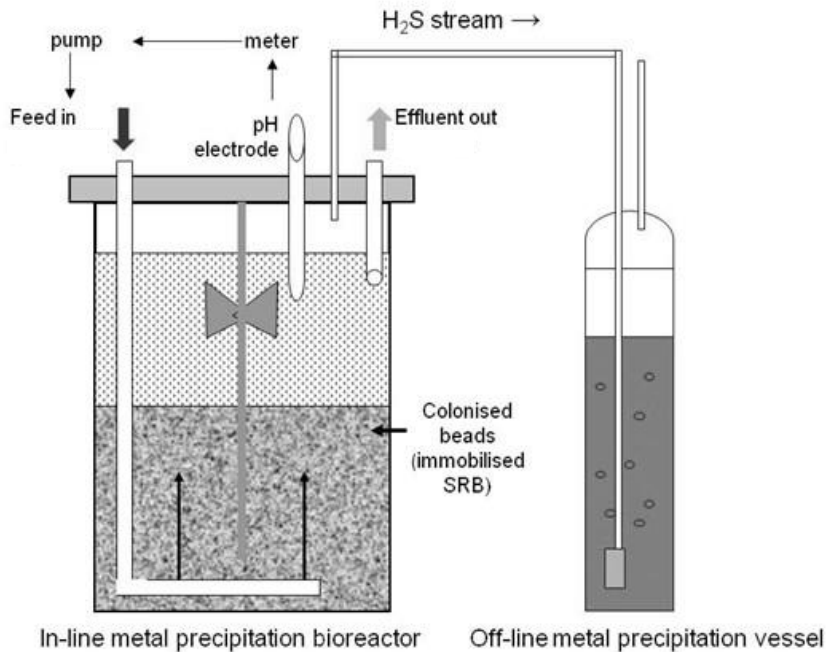

Figure 2 *Schematic of the low pH sulfidogenic bioreactor designed to both precipitate metals in-line (i.e. within the reactor vessel) and off-line (in a separate vessel, using H_2S produced in the bioreactor in excess of that required to precipitate metals in situ).*

sulfide minerals or metals (e.g. Al) hydrolysed within the reactor vessel itself, these acid-generating reactions modified the inflow of the acidic feed liquors. Full details of these low pH sulfidogenic bioreactors are given elsewhere (e.g. Ñancucheo and Johnson 2014).

Manganese bioreactors. Empirical continuous flow biofilm reactors (Mariner et al. 2008) were set up to oxidise and precipitate manganese in mine waters that had been treated previously to remove other soluble metals. These comprised Perspex columns, 30 cm by 7 cm (diameter), with working volumes of 1.08 L, that were filled with MnO_2-coated pebbles collected from a stream in the catchment of the Snowdonia mountain range (North Wales). Manganese-containing liquors were pumped into the base and out of the top of the reactors, which were aerated. No other amendment (e.g. addition of organic compounds) was required to facilitate manganese removal.

Results and Discussion

Metals and metalloids commonly found in mine waters, or of particular concern in terms of their toxicities, form sulfides and/or hydroxides that vary in their solubility products. These, and other such data, can be used to predict the most effective method for removing these elements from mine waters via (bio)mineralisation, with the general rule that, where different solid phases can be formed, that the lower solubility product is generally preferable (Table 1).

Table 1 Solubility products (log K_{sp} at 25°C) of (a) hydroxide and (b) sulfide phases of metals (and arsenic) of particular concern in mine waters. The highlighted data indicate preferable products of (bio)mineralisation (data from Monhemius 1977).

	Al	As(III)	Cd	Co	Cu	Fe(II)	Fe(III)	Mn(II)	Ni	Zn
(a)	-32.2	-	-14.3	-14.5	-19.8	-16.3	-38.6	-12.7	-15.3	-16.1
(b)	-	-16.0	-28.9	-22.1	-35.9	-18.8	-	-13.3	-21.0	-24.5

Iron. As shown in Table 1, although ferrous iron forms both sulfide and hydroxide phases, the solubility products of both of these are far greater than that of ferric hydroxide. The most effective way to remove soluble iron from mine waters is therefore to facilitate ferrous iron oxidation (using microorganisms to catalyse this reaction in acidic mine waters) and to precipitate the ferric iron formed as a hydroxy-sulfate mineral, such as schwertmannite in a separate vessel. In the system described by Hedrich and Johnson (2012), co-precipitation of copper and zinc (which were present at 45 and 70 mg/L, respectively, in the mine water tested) with schwertmannite was avoided by maintaining the pH of this unit at between 3.0 and 3.5. Schwertmannite is a useful by-product, as it can be used to adsorb toxic oxy-anions such as arsenate and antimonite, non-toxic anions, such as phosphate, in eutrophic water bodies, or used to synthesise iron pigments (Janneck et al. 2010), though relatively pure grade (not contaminated with arsenic etc.) mineral is required for schwertmannite to have commercial value. Both bacteria used to oxidise ferrous iron in AMD in bioreactors developed at Bangor University ("*Fv. myxofaciens*" and *Ax. ferrooxidans*) have the characteristic of attaching readily to surfaces and forming macroscopic streamer growths in flowing waters. This eliminates the need to fill bioreactors with inert materials that act as supports for bacterial attachment and decrease the volume available for the iron-oxidising bacteria themselves. The advantage of using *Ax. ferrooxidans* when commissioning a new bioreactor is that, as a heterotrophic species, biomass growth and colonisation is much more rapid than when (as has been common practice) autotrophic species are used. Jones and Johnson (2016) showed that *Ax. ferrooxidans* bioreactors oxidised ferrous iron effectively in extremely acidic mine waters (as low as pH 1.37) and that this acidophile was gradually displaced by other (autotrophic) iron-oxidisers when processing non-sterile AMD, with no downturn in bioreactor performance.

Chalcophilic transition metals (Cd, Co, Cu, Ni and Zn). As indicated in Table 1, these five metals can be effectively removed from AMD as sulfide phases. The data also show that there large differences in K_{sp}'s between some of these metal sulfides can be used to segregate their precipitation, e.g. by controlling solution pH. The latter is possible since pH dictates the concentration of soluble S^{2-} (the

sulfide species that reacts with the soluble metal, and which decreases in relative abundance to both HS⁻ and H_2S as solution pH falls). In practical terms, this means that biomineralisation of copper sulfide will occur at pH 2, while all of the other elements listed above (as well as ferrous iron) will remain in solution. By gradually increasing solution pH, it is possible to sequentially precipitate cadmium and then zinc, though the K_{sp}'s of nickel and cobalt sulfides are too similar to segregate them effectively by this means. Using this approach, Ñancucheo and Johnson (2012) were able to selectively recover zinc from AMD containing elevated concentration of zinc and iron, and also copper from a Cu/Fe/Zn-rich AMD.

Arsenic. This metalloid can occur both as As(III) (predominantly as uncharged arsenious acid, H_3AsO_3, in acidic mine waters) and as As(V) ($H_2AsO_4^-$ and $HAsO_4^{2-}$) all three of which are soluble. As indicated in Table 1, As(III) forms a sulfide phase and can be removed from mine waters by this means, as reported by Battaglia-Brunet et al. (2012), though given its K_{sp} it is likely to co-precipitate with other (metal) sulfides. Oxidation of As(III) to As(V) (e.g. by ferric iron) facilitates the removal of this toxic metalloid either as scorodite ($FeAsO_4$, which has a K_{sp} of -20.2) or by adsorption of the negatively-charged arsenic oxyanions onto positively-charged schwertmannite (Janneck et al. 2010). Given the concern about arsenic toxicity, when it is present in significant concentrations in mine waters it is highly desirable to remove it as the first step in a remediation/metal recovery process, as this allows downstream products to be free of this metalloid.

Manganese. Although manganese (II) does form insoluble sulfide and hydroxide phases, their relatively large K_{sp}'s (Table 1) mean that these form at relatively high pH values. Manganese (IV) is far less soluble than Mn(II) and, as is the case with iron, oxidation of the reduced cation, which can be chemically or biologically-mediated, can be used as a method of removing soluble manganese from contaminated waters, though this does not occur in extremely low pH mine waters. The empirical biofilm reactors used in the Bangor laboratories for this purpose show considerable promise for scaled-up tests, with typically 40-80% of soluble manganese removed from moderately acidic (pH 5) test solutions with hydraulic retention times of ~11 hours.

Examples of applications of the modular bioreactor units.

(i) Sequential removal of metals from a synthetic mine water, based on the composition of AMD in a copper mine in Pará state, Brazil. The chemical composition of synthetic mine water according to that draining a copper mine in northern Brazil, prior to and following processing using two of the modular bioreactors described above, is shown in Table 2, and a flow diagram showing the stages involved in the remediation protocol, in Fig. 3. In brief, copper (the major metal in the AMD) was first removed by contacting with H_2S (off-line) generated in an aSRB bioreactor. This caused the pH of the mine water to fall from 5.0 to 2.1, making it suitable as an influent liquor for the sulfidogenic bioreactor. Glycerol was added to the copper-free liquor, and pumped into the reactor, which was maintained at pH 5.0, where cobalt, nickel and zinc (but not manganese) were removed as sulfides. Finally, solutions draining the aSRB reactor were pumped through the Mn biofilm reactor, to remove this metal. This treatment also resulted in significant reductions (~40%) in concentrations of sulfate in the synthetic AMD.

Table 2 Changes in water chemistry in contaminated water at a copper mine in Brazil, following treatment for combined copper recovery and remediation. All metal and sulfate-S concentrations are mg/L.

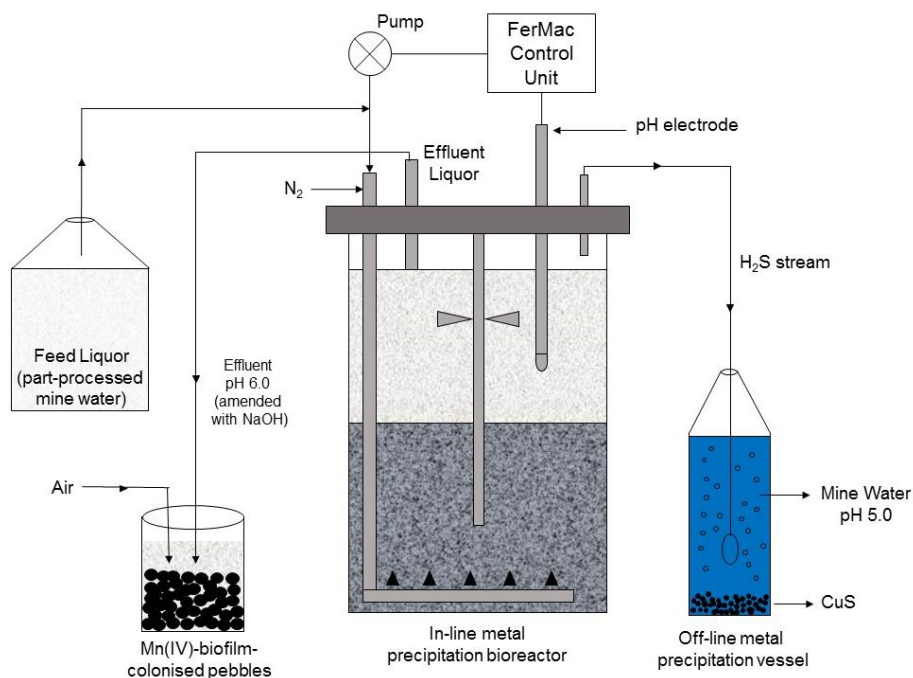|	pH	Cu	Ni	Zn	Co	Mn	SO_4^{2-}-S
Influent	5.0	476	16	1.3	2.4	10.5	660
Effluent	5.0-6.0	<0.5	<0.1	<0.1	<0.1	<0.1	393

Figure 3 *Schematic representation of the integrated system used to remediate synthetic mine water based on that at a site in Brazil, and to recover copper.*

(ii) Combined remediation of and metal recovery from an extremely acidic (pH 2.3) mine water (Hedrich and Johnson 2014). The chemical composition of AMD at the Maurliden mine (Sweden) and the protocol used to process a synthetic analogue of this water in the Bangor laboratories, are shown in Table 3 and Fig. 4. The first step in the bioremediation process was to remove soluble arsenic from the mine water by allowing it to contact schwertmannite. Next, a bioreactor was used to oxidise ferrous iron in the AMD to ferric, and to produce schwertmannite, 12% of which was used up-front to remove As. Soluble copper and cadmium were removed in the next stage by contacting with biogenic H_2S in an off-line reactor vessel, after which the part-processed mine water was dosed with glycerol and fed into an aSRB reactor. By maintaining the pH of this unit at 4.0, most of the zinc was removed (as ZnS) while co-precipitation of aluminium and manganese was avoided. No further steps were taken to remediate this synthetic mine water, though this could have been achieved by adding a second, higher pH sulfidogenic bioreactor (to remove Al as hydroxysulfate minerals) and a unit to oxidise and precipitate manganese, as described above.

Table 3 *Concentrations of the major components of Maurliden mine water (data obtained from Boliden AB, Sweden).*

Analyte	Concentration (mg/L)	Analyte	Concentration (mg/L)
Zn	464	Cd	1.02
Fe (II)	200	Co	0.4
Fe (III)	203	Ni	0.3
Al	132	Ca	271
Mn	49	Mg	123
Cu	7.72	Na	13.8
As	1.33	K	4.01

Economic considerations. One of the major advantages of the integrated bioreactor protocols developed is that, by recovering metals from AMD, a revenue is generated that can offset, at least in part, operational costs. Some products (e.g. schwertmannite) are difficult to value at present, though increasing use of this mineral in remediation protocols and as a pigment precursor could change this in the near future. Metals such as copper and zinc would be obvious saleable products. Glycerol is used as substrate for biosulfidogenesis in the Bangor reactors since it is not only an innocuous and readily

accessed material, but also (as a by-product of bio-diesel production) a relatively cheap organic compound. While relative costs of consumables and products will vary according to metal prices etc., typically $1 of glycerol consumed would produce ~$5 (copper) or $1.25 (zinc) metal value. When costs of alternative remediation treatments (e.g. high density sludge production by chemical treatment) are factored in, the biologically-based systems described in the present report become even more attractive.
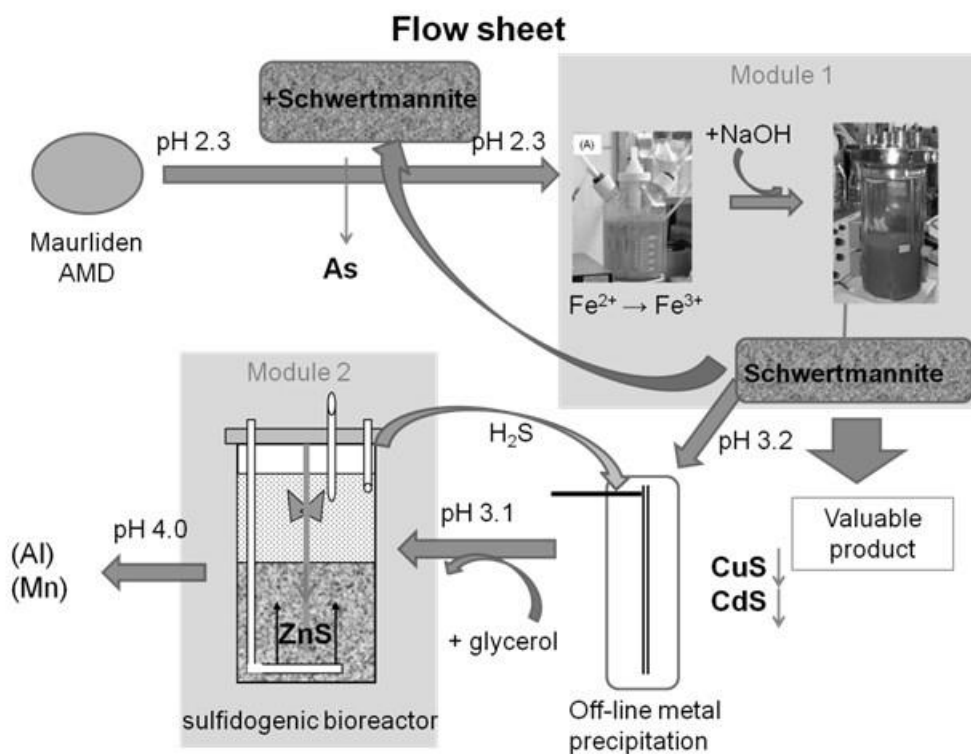

Figure 4 *Schematic representation of the integrated system used to remediate synthetic Maurliden mine water and to recover iron and zinc as mineral products.*

Conclusions

Modular bioreactor units, devised to simultaneously remediate mine waters and recover metals by catalyzing their selective biomineralisation, have been demonstrated to be both effective and economical in laboratory tests. Recovering metals from waste materials, such as AMD, should be viewed as a priority issue as sources of high-grade accessible primary resources become increasingly scarce.

Acknowledgements

References

Battaglia-Brunet F, Crouzet C, Burnol A, Coulon S, Morin D, Joulian C (2012) Precipitation of arsenic sulphide from acidic water in a fixed-film bioreactor. Water Res 46:3923-3933

Blowes DW, Ptacek CJ, Jambor JL, Weisener CG, Paktunc D, Gould WD, Johnson DB (2014) The geochemistry of acid mine drainage. In: Holland HD, Turekian KK (Eds), Treatise on Geochemistry, Second Edition, vol. 11, Oxford, Elsevier, p 131-190

Falagán C, Yusta I, Sánchez-España J, Johnson DB (2016) Biologically-induced precipitation of aluminium in synthetic acid mine water. Proceedings: Biohydrometallurgy 2016, Minerals Engineering International, U.K (in press)

Hedrich, S, Johnson, DB (2014) Remediation and selective recovery of metals from acidic mine waters using novel modular bioreactors. Environ Sci Technol 48:12206-12212

Hedrich S, Johnson DB (2012) A modular continuous flow reactor system for the selective bio-oxidation and precipitation of iron in mine-impacted waters. Bioresource Technol 106:44-49

Janneck E, Arnold I, Koch TH, Meyer J, Burghardt D, Ehinger S (2010) Microbial synthesis of schwertmannite from lignite mine water and its utilization for removal of arsenic from mine waters and for production of iron pigments. In: Proceedings IMWA Conference, Sydney, NS, Canada

Johnson DB, Hallberg KB (2005) Acid mine drainage: remediation options. Sci Tot Environ 338:3-14

Jones RM, Johnson DB (2016) Iron kinetics and evolution of microbial populations in low pH, ferrous iron-oxidizing bioreactors. Submitted to Environ Sci Technol

Mariner R, Johnson DB, Hallberg KB (2008) Characterisation of an attenuation system for the remediation of Mn(II) in contaminated waters. Hydrometallurgy 94:100-104.

Monhemius AJ (1977) Precipitation diagrams for metal hydroxides, sulphides, arsenates and phosphates. T I Min Metall 86:C202-C206

Ñancucheo I, Johnson, DB (2014) Removal of sulfate from extremely acidic mine waters using low pH sulfidogenic bioreactors. Hydrometallurgy 150:222-226

Ñancucheo I, Johnson DB (2012) Selective removal of transition metals from acidic mine waters by novel consortia of acidophilic sulfidogenic bacteria. Microb Biotechnol 5:34-44

Nordstrom DK, Blowes DW, Ptacek CJ (2015) Hydrogeochemistry and microbiology of mine drainage: an update. Appl Geochem 57:3-16