Mining and Microbiology: Established, Evolving and Emerging Biotechnologies

D. Barrie Johnson
Head: *Bangor Acidophile Research Team*
College of Natural Sciences
Bangor University
United Kingdom

1. Abstract

Mining companies have become increasingly aware of the potential of microbiological approaches for recovering base and precious metals from low-grade ores, and for remediating acidic, metal-rich wastewaters. Biological systems can offer a number of environmental and (sometimes) economical advantages over conventional approaches, such as pyrometallurgy. There are two major areas in which biological systems are currently utilized in full-scale operations by the mining industries: metal extraction ("biomining") and treatment of acid mine drainage (bioremediation). Mineral processing using microorganisms has been exploited for extracting gold, copper, uranium and cobalt, and new operationss are targeting other base metals. Engineering systems ranging from crude heap leaching systems to temperature-controlled bioreactors have been used, depending on the nature of the ore and the value of the metal product. Biological treatment of acidic mine effluents mostly involves the use of constructed wetlands. More recently, compost bioreactors have been shown to be effective in both generating alkalinity and removing heavy metals (e.g. as sulfides). Although these "passive" systems generally require little maintenance once constructed, their performance may be variable and they do not allow recovery and reuse of metals from the waste streams. In contrast, "active" bioremediation systems, based on microbial sulfidogenesis, offer both system control and separation (and recovery) of metals. This paper reviews current applications and developments in the field of biohydrometallurgy, and describes how projected developments could allow future expansion of microbiological applications in the mining industries.

2. The potential impact of microbiology on mining

The mining of metal ores and coals would seem, superficially, to have little in common with the study and application of microbiology. However, microbially-based biotechnologies were developed in the latter part of the 20th century that have had major impact on the global mining industry, principally in the field of processing of copper and gold ores. More recently, new approaches to remediating polluting mine waters and recovering metals from process waters (pregnant liquors) and waste streams have been proposed and, in some cases, demonstrated at least at pilot scale.

The different areas in which microbiology can impact the mining industry include:

1. providing an alternative approach for processing ores, mineral concentrates and waste materials produced from older, less efficient mining operations. This technology ("biomining") is often considered to be more environmentally benign than conventional approaches for extracting metals, such as pyrometallurgy;
2. providing different ways to process polluting waste waters, such as acid mine drainage, using more sustainable methods (such as passive remediation systems) than chemical treatment;
3. providing new effective methods to recover and recycle metals from process waters and waste streams.

Microorganisms have also been used to extract sulfur from coals, prior to combustion, in order to minimize the production of "acid rain". Other biotechnologies are being developed for the long-term management of solid wastes from mining, such as mineral tailings and waste rocks.

3. Biomining: biotechnology based on the oxidative dissolution of sulfidic minerals by prokaryotic microorganisms

Microorganisms have had significant impact on the extraction and recovery of metals from ores and wastes long before their roles were recognised. Construction of "precipitation ponds" at the Rio Tinto mine (southern Spain) and the Parys mine (Anglesey, north Wales) to recover copper from leached rocks by cementation is documented during the 18-19th centuries. It was not until the middle of the 20th century that the first bacteria that accelerate the dissolution of metal-containing sulfide minerals at these (and other) sites were discovered. Realisation that the abilities of these microorganisms to oxidize minerals could be harnessed in more precisely engineered operations led to the emergence and establishment of biomining as a feasible technology (Rawlings and Johnson, 2007a). The advantages of bioprocessing of ores and concentrates over more conventional approaches such as pyrometallurgy include the potential for processing low-grade deposits and re-processing earlier metal-containing wastes, the production of less chemically-active tailings, lower energy inputs and other environmental benefits (zero production of noxious gases etc.).

Bioprocessing of sulfides can be sub-divided into bioleaching, which results in the solubilisation of target metals (e.g. copper from chalcopyrite and covellite) and biooxidation, whereby microbial dissolution of pyrite and arsenopyrite associated with fine-grain gold allows extraction of the precious metal by cyanidation. Besides these two metals, biomining has been harnessed to extract uranium and cobalt. Other metals, including nickel and zinc, will be bioleached from complex polymetallic ores in a heap leaching operation that is currently expanding into full-scale production, in Talvivaara, Finland.

3.1 Mineral bioprocessing: engineering options. These may be grouped conveniently into (i) irrigation-based principles (dump- and heap-leaching, and *in situ* leaching) and, (ii) stirred tank processes. The earliest engineering technology used ("dump leaching") was very basic, and involved gathering low-grade (otherwise waste) copper-containing ore of large rock/boulder size into vast mounds or dumps and irrigating these with dilute sulfuric acid to encourage the growth and activities of mineral-oxidizing acidophiles, primarily iron-oxidizing mesophiles. Copper was precipitated from the metal-rich streams draining from the dumps using by displacement with scrap iron ("copper cementation"). Later developments on the engineering and hydrometallurgical aspects of biomining have involved the use of thin layer heaps of refractory sulfidic ores (mostly copper, but also gold-bearing material) stacked onto water-proof membranes, and solubilized copper recovered using solvent extraction coupled with electrowinning (SX/EW). An innovative approach (the "Geocoat" process) involves attaching mineral concentrates to inert carrier particles (or sulfide minerals) which are then stacked into heaps and bioleached (Harvey and Bath, 2007). *In situ* bioleaching was developed to scavenge for uranium and copper in otherwise worked out mines. This involves fracturing underground workings using explosives, percolating with acidic leach liquors containing metal-mobilizing bacteria, pumping the pregnant liquor to the surface and extraction of solubilized metals. Since the 1980's, aerated stirred tanks have been used to process sulfidic ore concentrates. These tanks, which may be extremely large (up to 1,350 m^3), allow for greater control (e.g. of temperature; sulfide mineral oxidation being an exothermic process) of biooxidation of mineral ores. To date, stirred tank bioreactors used for mineral processing have tended to operate between 40°C and 50°C (i.e. where moderate thermophiles and thermotolerant acidophiles would tend to be of greatest significance), though a thermophilic stirred tank, operating at about 80°C, has been used successfully to extract copper from chalcopyrite ($CuFeS_2$), a mineral that is notoriously difficult to bioleach at low temperatures.

3.2 Microorganisms involved in the dissolution of sulfide minerals and extraction of metals. Biomining processes provide a highly specialized growth environment and, irrespective of whether tank or heap processes are used, the microorganisms that catalyze biomining processes are required to grow in an essentially inorganic, aerobic, low pH environment. The most important microorganisms are therefore autotrophic and, although the exact nature of the energy sources may vary from mineral to mineral, they grow by oxidizing reduced forms of sulfur or ferrous iron (or both). The pH within tanks and heaps might also vary, but is highly acidic and typically within the range pH 1.5 to 2.0. The characteristics of biomining microorganisms have been reviewed in detail elsewhere (e.g. Rawlings, 2005; Hallberg and Johnson, 2001) but the rather extreme conditions in stirred tanks and heaps means that the number of microorganisms that are likely to play a major role in biomining processes is limited.

3.2.1 Stirred tanks. The environment in a mineral biooxidation continuous-flow stirred tank reactor is highly homogenous as it is operated at a set pH and temperature and controlled aeration. However, conditions (such as concentrations of soluble metals and metalloids) will vary in a continuous flow series of tanks as mineral oxidation becomes increasingly extensive, and this can have a significant impact on diversity and numbers of indigenous microbial species (e.g. Okibe et al., 2003). The homogeneity within an individual tank results in a limited ecological niche that tends often to be dominated by two to four species, although smaller numbers of other microorganisms may be present (Table 1). For example, Mikkelson et al. (2006) found that the microbial populations in thermophilic (78°C) stirred tanks leaching chalcopyrite were entirely archaeal (as would be predicted from the known thermotolerance of acidophilic prokaryotes) and comprised relatively few species of the order *Sulfolobales* (Table 1). In general, the biodiversity of stirred tanks is generally limited to 2-4 different species of acidophiles, though recently work has shown that the mineral ore or concentrate being processed can have a major impact on the composition of the microbial consortia involved (Johnson et al., 2007).

3.2.2 Heap leaching operations. The engineering design of heaps used to leach ores continues to be refined. Heaps are constructed to pre-determined dimensions

Table 1. Acidophilic prokaryotes identified in stirred tank mineral bioleaching and biooxidation operations (Rawlings and Johnson, 2007b)

Mineral concentrate	T (°C)	Prokaryotes identified	Reference
Zinc/lead pyrite	35-40	*Leptospirillum ferrooxidans* [a] *Acidithiobacillus thiooxidans* [b] *Acidiphilium cryptum* [c] *Acidithiobacillus ferrooxidans* [c]	Goebel and Stackebrandt, 1994
Pyrite/arsenpyrite (gold) Biox® culture	40	*L. ferrooxidans* [a] *At. thiooxidans* [b] *At. ferrooxidans*	Dew et al., 1997
Cobaltiferous pyrite	35	*L. ferrooxidans* *At. thiooxidans* *Sulfobacillus thermosulfidooxidans*	Battaglia-Brunet et al., 2002
Polymetallic (copper, zinc and iron sulfides)	45	*Leptospirillum ferriphilum* *Acidithiobacillus caldus* *Sulfobacillus* sp. *Ferroplasma acidophilum*	Okibe et al., 2003
Pyrite, arsenical pyrite and chalcopyrite	45	*At. caldus* *Sb. thermosulfidoooxidans* *'Sulfobacillis montserratensis'*	Dopson and Lindström, 2004
Chalcopyrite	78	*(Sulfolobus shibitae* [d, e]) *(Sulfurisphaera ohwakuensis* [d, e]) *Stygiolobus azoricus* [d] *Metallosphaera* sp. [d] *Acidianus infernus* [d]	Mikkelsen et al., 2006

[a] *L. ferrooxidans* was almost certainly *L. ferriphilum* as identification methods at the time did not permit the two species to be distinguished from each other
[b] *At. thiooxidans* was almost certainly *At. caldus* for the same reason as footnote [a]
[c] These two species were found in batch tanks but not in continuous flow tanks
[d] Nearest affiliated cultivated archaea to recovered clones
[e] Clones probably represent new species within the order *Sulfolobales*

using graded ores, irrigated from above with acidic liquors and aerated from below (to provide carbon dioxide required by autotrophic mineral-oxidizing microorganisms, as well as the oxygen to promote iron- and sulfur-oxidation). However, even the most carefully engineered heap reactors are inevitably heterogeneous (both spatially and temporally), in terms of irrigation efficiency, temperature, pH, the presence of anaerobic pockets, redox potential, dissolved solutes, available nutrients etc.. This lack of homogeneity results in a large number of microenvironments compared with the relatively homogenous environment provided by a stirred tank. The variability in microenvironment would be expected to support a much greater diversity of mineral-oxidizing and other

Table 2. Acidophilic prokaryotes identified in heap reactors (Rawlings and Johnson, 2007b)

Heap type and location	Prokaryotes identified	Reference
Chalcopyrite overburden, (Australia)	*Acidithiobacillus ferrooxidans* *Acidithiobacillus thiooxidans* *Acidiphilium cryptum*	Goebel and Stackebrandt, 1994
Copper sulfide/oxide heap (south-west U.S.A.)	*Acidithiobacillus* spp. *Leptospirillum ferrooxidans* *Acidiphilium* spp. *“Ferrimicrobium acidiphilum”*	Bruhn et al., 1999
Copper sulfide/oxide heap (south-west U.S.A.)	*Sulfobacillus* spp. and other *Firmicutes* *“Ferrimicrobium acidiphilum”* *Acidisphaera sp.* *At. thiooxidans* *At. ferrooxidans*	C.G. Bryan and D.B. Johnson (unpublished)
Chalcocite heap Australia	*Leptospirillum ferriphilum* *Acidithiobacillus caldus* *“Ferroplasma cupricumulans”**	Hawkes et al., 2006
Run-of-mine copper heap, Chile	*At. ferrooxidans* *L. ferriphilum* *Ferroplasma acidiphilum* Novel *Firmicutes* Novel *Crenarchaeota*	Demergasso et al., 2005

*original proposed name (in Hawkes et al., 2006) *“Ferroplasma cyprexacervalum”*

microorganisms that colonize different zones and microsites within them. For example, temperatures will be determined by climatic conditions (particularly in the outer layers of a heap), exothermic chemical reactions and heat transfer (conduction, convection, and radiation at the heap surface). The oxidation of sulfidic minerals is an exothermic reaction, though heat generation varies between minerals, and is related to their reactivities. Mineral-oxidizing and other acidophilic prokaryotes often have widely different temperature optima and ranges, and may be conveniently grouped into mesophiles (20-40°C; predominantly bacteria) moderate thermophiles (40-60°C; bacteria and archaea) and (extreme) thermophiles (60-80°C; predominantly archaea). In a heap reactor that experiences fluctuations in temperature, these different groups would be predicted to become more or less dominant, as temperatures increase or decline, assuming that they are present in the first place. Some prokaryotes, notably *Sulfobacillus* spp. and other *Firmicutes*, are better adapted to survive adverse conditions, such as excessively high or low temperatures, or water stress (zones and microsites within heaps may experience periodic drying, in contrast to stirred tanks) due to their ability to survive as endospores. It may therefore be predicted that, unlike stirred tanks which are dominated by a indigenous prokaryotes, heap reactors contain a much greater biodiversity, and that the dominant species will vary spatially and during different stages of the life of a heap. There have been relatively few studies on the microbiology of heap bioreactors, and some of these have analyzed the liquid phases (pregnant leach solutions (PLS), raffinates etc.) rather than the ore itself. Most studies have been on chalcocite (Cu_2S) heaps, as this copper mineral is particularly amenable to bioleaching. Microbiological data from the limited analyses of heap populations that have been carried out show that a considerable diversity of acidophiles may be present in these reactors (Table 2).

3. Bioremediation of metalliferous mine waters

Acidic, sulfur-rich wastewaters are the by-products of a variety of industrial operations such as galvanic processing and the scrubbing of flue gases at power plants though the major producer of such effluents is, however, the mining industry. Waters draining active and (in particular) abandoned mines and mine wastes are often net acidic (sometimes extremely so) and typically pose an additional risk to the environment by the fact that they often contain elevated concentrations of metals (iron, aluminum and manganese, and possibly other heavy metals) and metalloids, of which arsenic is generally of greatest concern.

3.1 Active and passive remediation of mine waters. Following the axiom that “prevention is better than cure”, it is generally preferable, though not always pragmatic, to preclude the formation of AMD in the first instance. Such techniques are known collectively as “source control” measures. However, the practical difficulties entailed in inhibiting the formation of AMD at source mean the only alternative is to minimise the impact that this polluting water has on receiving streams and rivers, and on the wider environment; such an approach involves one or more “migration control” measures. Quite often, these have been divided into “active” and “passive” processes, the former generally (though not exclusively) referring to the continuous application of alkaline materials to neutralise acidic mine waters and precipitate metals, and the latter to the use of natural and constructed wetland ecosystems. Passive systems have the advantage of requiring relatively little maintenance (and recurring costs) than active systems, though they may be expensive and/or impracticable to set up in the first place. In reality, all “passive” treatment technologies require a certain amount of maintenance costs. A more useful subdivision is between those remediation technologies that rely on biological activities, and those that do not. Within these major groups, there are processes that may be described as either “active” or “passive” (Fig. 1).

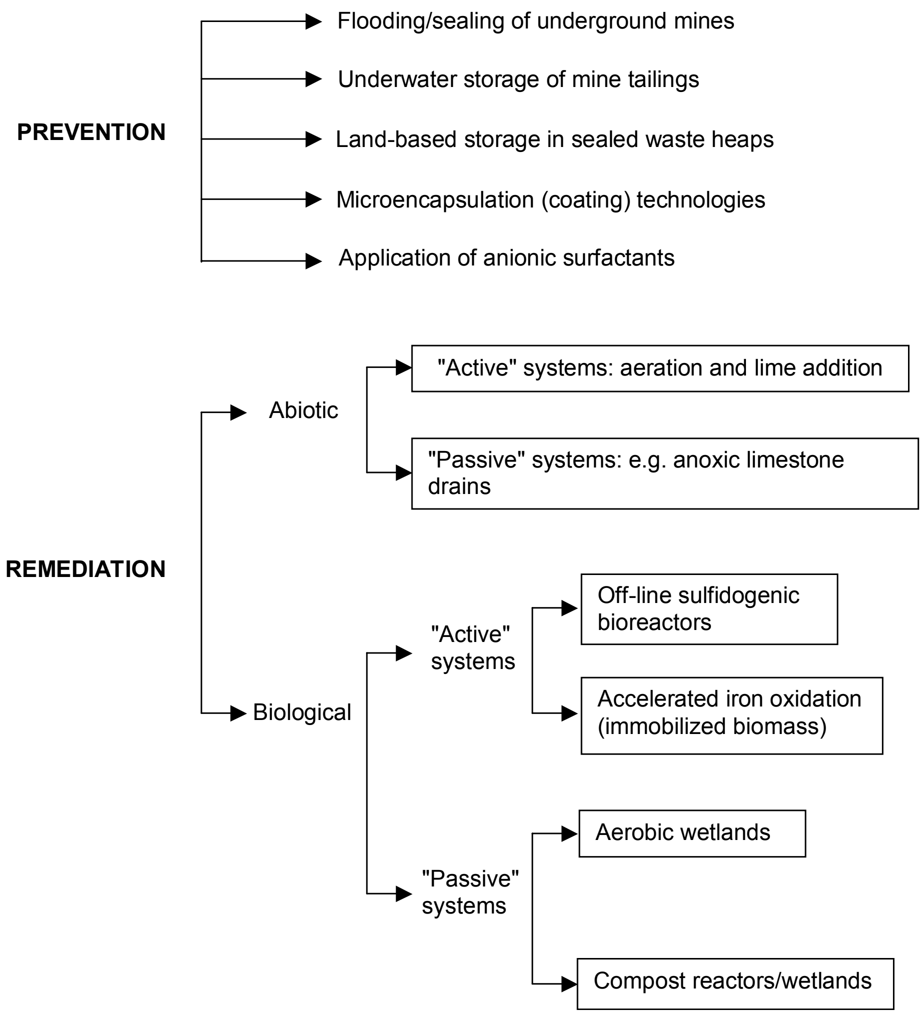

Figure 1. Options for preventing the formation of, and remediating, metalliferous drainage waters from metal and coal mines (Johnson and Hallberg, 2005)

The basis of bioremediation of AMD derives from the abilities of some microorganisms to generate alkalinity and immobilise metals, thereby essentially reversing the reactions responsible for the genesis of AMD. Microbiological processes that generate net alkalinity are mostly reductive processes, and include denitrification, methanogenesis, sulfate reduction, and iron and manganese reduction. Ammonification (the production of ammonium from nitrogen-containing organic compounds) is also an alkali-generating process. The majority of bioremediation options for AMD are passive systems, and of these only constructed wetlands and compost bioreactors have so far been used in full-scale treatment systems. The major advantages of passive bioremediation systems are their relatively low maintenance costs, once constructed, and the fact that the solid-phase products of water treatment are retained within the wetland sediments. On the downside, they are often relatively expensive to install and may require more land area than is available or suitable, their performance is less predictable than chemical treatment systems, and the long-term fate and stability (in the case of compost bioreactors) of the deposits that accumulate within them is uncertain.

3.2 Sulfidogenic bioreactors. Off-line sulfidogenic bioreactors represent a radically different approach for remediating AMD. These engineered systems have three major advantages over passive biological remediation: (i) their performances are 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; (iii) concentrations of sulfate in processed waters may be significant lowered. On the negative side, the construction and operational costs of these systems are considerable. Sulfidogenic bioreactors utilise the biogenic production of hydrogen sulphide by sulfate-reducing bacteria (SRB) to generate alkalinity and to remove metals as insoluble sulfides. SRB are, in general, heterotrophic bacteria and, unlike the iron-oxidising acidophiles described earlier, require provision of organic material as carbon and energy sources. In the first sulfidogenic bioreactor set up at the Budelco zinc refinery in the Netherlands, this was provided in the form of ethanol. However, hydrogen may substitute as electron donor for sulfate reduction:

$$SO_4^{2-} + 4H_2 + 2H^+ \rightarrow H_2S + 4H_2O.$$

4. Future potential developments and applications

The application of biological systems for ore processing and waste remediation is likely to become increasingly important in the 21st century. Driving this will be the need to process ores containing increasing small concentration of target metal(s), the potential and necessity to re-process waste spoils and tailings, economic constraints, and possible legislative changes on the environmental impact of more traditional approaches such as pyrometallurgy.

Heap leaching is likely to be a major area of expansion in biomining, particularly as the engineering options are refined and developed to allow mineral concentrates to be processed by this route. The new mining operation in Talvivaara, Finland, has demonstrated the efficacy of heap leaching complex polymetallic ores. The development of low-cost bioreactors for processing concentrates could get around the economical constraints which currently mean that , in the main, only gold concentrates are processed in stirred tanks. Patented processes such as BioCOP and BioNIC, both developed by BHP Billiton, are likely to come on stream in the next decade. Microbial systems that allow the recovery thereby the reuse of metals that are currently either dumped in oxidised sludge wastes or retained in constructed wetlands, are also likely to be further developed and utilized in future sustainable and integrated approaches to metal extraction, resource conservation and safeguarding the global environment.

5. References

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