The Evolution, Current Status, and Future Prospects of Using Biotechnologies in the Mineral Extraction and Metal Recovery Sectors

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Abstract: The current global demand in terms of both the amounts and range of metals for industrial and domestic use greatly exceeds that at any previous time in human history. Recycling is inadequate to meet these needs and therefore mining primary metal ores will continue to be a major industry in the foreseeable future. The question of how metal mining can develop in a manner which is less demanding of energy and less damaging of the environment in a world whose population is increasingly aware of, and concerned about, the environment, requires urgent redress. Increased application of biotechnologies in the mining sector could go some way in solving this conundrum, yet, biomining (harnessing microorganisms to enhance the recovery of base and precious metals) has remained a niche application since it was first knowingly used in the 1960s. This manuscript reviews the development and current status of biomining applications and highlights their limitations as well as their strengths. New areas of biotechnology that could be applied in the mining sector, and their potential impact in terms of both their potential environmental and economic benefits, are also discussed.

Keywords: base metals; bioleaching; bio-oxidation; biosulfidogenesis; deep *in situ* biomining; natural biomines; reductive mineral dissolution; sulfur-enhanced bioleaching

1. Introduction

Homo sapiens has had, for thousands of years, an insatiable appetite for metals. While in previous eras this was restricted to relatively few base and precious metals, in the 21st century vast amounts of a wide array of different metals are used in industrial and domestic applications, which has resulted in the mushrooming of the scale of metal mining. Estimates are that as much metal ore has been mined in the last 30 years or so as in the entire preceding time of human history. One consequence of this is that most of the accessible "rich" ore bodies have by now been exploited, and mining companies need increasingly to use lower grade primary ores and to consider other sources, such as marine deposits and those buried deep within the lithosphere. Overall, the demand for metals in general is expected to double over the next 15–20 years (e.g., Reference [1]), with the major drivers being: (i) population growth (anticipated to increase by 30% by 2050); (ii) the gradual equalising of the gaps between "poor" and "rich" nations; (iii) changing lifestyles and greater use of resources, including metals; (iv) urbanisation, and the transition towards renewable energy resources, electronic devices etc. which use a wide array of metals, including rare earth elements. Recycling, although a significant source of metals, is generally considered to be grossly inadequate to meet future demands. The large-scale mining of primary mineral ores will, therefore, be necessary to meet the requirements of a growing, more affluent and more environmentally aware global population for the foreseeable future. The question of how this can be done in a manner which is less demanding of energy and less

damaging of the environment, in a world whose population is increasingly aware of, and concerned about, the environment, requires urgent redress.

Mining in general and metal mining, in particular, does not have the best environmental credentials and suffers from the burden of historic bad practices where mine operators have often abandoned sites that have been worked out, leaving legacies of ecosystem destruction and long-term pollution hazards. Modern mines, especially opencast operations, tend to have large footprints, making them untenable to establish as new operations in populated areas. Large amounts of energy (estimated at 5–7% of global energy production) are used to haul rocks to the land surface and to grind them to fine-grain particles (comminution). Noxious chemicals are frequently used in mineral processing, such as in froth flotation to concentrate target minerals. The smelting of ores and concentrates, the most widely used end-processing stage, again consumes significant amounts of energy and, in most cases, is responsible for the large-scale production of carbon dioxide. In addition, mining produces vast amounts of waste materials, as rock dumps and fine-grain mineral tailings that are mostly deposited on the land surface and which pose a potential long-term environmental hazard.

In the light of these negative perceptions, the metal mining industry is increasingly aware of the need to incorporate new practices that would be accepted by society in general. Among these are biotechnologies that not only offer more environmentally-benign approaches for extracting and recovering metals, but are also competitive in their cost-effectiveness. One of these, generically referred to as "biomining", is well established as a technology for extracting base and precious metals from reduced (sulfidic) primary ores (reviewed in References [2–4], and elsewhere). Alternative opportunities for the applying biotechnologies in the mining and mineral processing sectors have also been reviewed [5,6].

2. History and Current Status of Biomining

The modern era of biomining began within a decade of the discovery of a bacterium that was shown to catalyse the oxidation of ferrous to ferric iron in low pH water bodies (*Thiobacillus ferrooxidans* [7], which was later renamed *Acidithiobacillus ferrooxidans* [8]), and the demonstration that this isolate and some related bacteria could accelerate the oxidative dissolution of pyrite (FeS_2), the most abundant sulfide mineral in the lithosphere. These bacteria have the ability to indirectly harness the energy "locked up" in sulfide minerals (as reduced sulfur and metals), though their actual immediate source of energy is the pre-existing proton gradient between their cytoplasms and the acidic liquors in which they bathe. In degrading sulfides they release metals that are integral to or associated with these minerals, most of which are retained in solution in low pH bioleach liquors, facilitating their downstream capture. Many commercially-important transition metals, such as copper, cobalt, nickel and zinc, occur either predominantly or in significant amounts in the lithosphere as sulfide minerals, whereas others, such as native gold and uranium (in uraninite) are associated with sulfidic minerals (pyrite, arsenopyrite, etc.) in some ore deposits. Depending on the nature of the target metal, these are either solubilised (bioleaching) or made accessible to chemical extractants (bio-oxidation) in biomining operations.

The microorganisms involved in the bioleaching/bio-oxidation of sulfidic ores, and the mechanisms by which they accelerate their oxidative dissolution, are now well known (e.g., References [9,10]. Rather than this being the remit of a unique bacterium, >30 species are now known to play direct and indirect roles in biomining, and the key to their success is the fact that they operate as microbial consortia, rather than as individuals [11]. A fundamental characteristic of all these species is that they are extremely acidophilic (i.e., they grow optimally at pH 3 or less) as biomining is carried out using low pH lixiviant solutions. Three groups of acidophilic prokaryotes (bacteria and archaea) interact to form stable and efficient microbial sulfide mineral-degrading consortia. These are (i) the "oxidant manufacturers", acidophiles that oxidise ferrous to ferric iron, which acts as the primary chemical oxidant of pyrite and other sulfidic minerals; (ii) the "acid generators", that oxidise the sulfur moieties in the minerals, generating sulfuric acid and thereby help to maintain acidity levels in the range that are suitable for biomining consortia (generally below pH 2) and the chemical attack of the minerals by soluble ferric iron; (iii) the "janitors", which degrade organic carbon compounds exuded by active prokaryotes and lysed

from dead cells, which would otherwise potentially accumulate and inhibit groups (i) and (ii). While some acidophiles perform only single roles in bioleaching consortia (e.g., *Leptospirillum* spp., which are known only to oxidise iron), others can perform multiple roles, such as iron-oxidising *Acidithiobacillus* spp. that both generate ferric iron and sulfuric acid, and the archaeon *Ferroplasma acidophilum*, which oxidises iron and degrades organic carbon [12].

Although the modern era of biomining began in the mid-1960s with the dump leaching of run-of-mine copper waste rocks in mines operated by the (then) Noranda corporation in the USA, bioleaching had been unknowingly used in mediaeval times to extract and recover metals [13]. This was an *in situ* approach, which is still being used and refined, as described below. Miners at copper mines in Spain, China, Wales, and probably elsewhere learned that, if they allowed shafts and adits to flood periodically, released the water into lagoons, and then added metallic iron to ponds, it was possible to recover metallic copper. The "transformation" of iron to copper was often perceived as a form of alchemy, but the same electrochemical reaction is still used widely to produce "cement copper" from pregnant leach solutions generated at dump biomining operations. *In situ* bioleaching was also used to extract residual uranium from worked out mines in Canada in the 1970s (e.g., Reference [14]). New engineering approaches (heap leaching of copper ores and concentrates, and tank leaching of refractory gold ores) for applying bioleaching/bio-oxidation developed in the late 20th century chiefly in Chile and South Africa, provided a major boost for biomining. The diversification of the biotechnology in the 1990s, targeting transition metals other than copper and the bioleaching of polymetallic ores further highlighted its potential, though it has remained restricted, at full scale, to processing sulfidic ores and concentrates.

3. Biomining: A Niche Technology

Despite its apparent "green credentials", biomining has remained a niche rather than mainstream technology in the metal mining sector. Current estimates are that biomining accounts for ~15% of global copper production, 5% of gold, and smaller percentages of other metals (e.g., zinc, nickel, and uranium). There are, however, a number of reasons for this, one being the relative slowness of the process compared with pyrometallurgical and alternative hydrometallurgical (e.g., pressure leaching) options. Bioprocessing in tanks generally takes several days to complete, heap leaching takes 1–3 years, while dump leaching can last up to or even well beyond five years. Other reasons include, as mentioned, the current limitation of bio-processing, at full-scale, to reduced ores only, the occasional mistrust of "bio"-based processes in some parts of the mining sector, and the need to maximise the use of existing large-scale investments, such as smelters, rather than to diversify into alternatives. There have also been a few situations where biomining has failed or has been linked with environmental problems. One example of the former was described by Brierley and Kuhn [15]. Analysis of a low-grade secondary sulfide copper ore deposit in North America had indicated that it would be suitable for bioleaching. However, laboratory tests carried out in shake flasks and columns had failed to identify that fluoride released from the relatively high content of acid-soluble minerals (credite and grearksutite) present in the ore body would inhibit the bioleaching bacteria, which resulted in the failure of the commercial-scale operation. The flooding and escape of metal-enriched waters from Europe's first large-scale heap bioleaching operation (at Talvivaara, Finland) caused major societal concern. Metal-enriched water overflowed from a gypsum waste pond due to the engineering design of the site underestimating the impact of prolonged intense rainfall, whereas the bioheap leaching operation itself was operating effectively. Protagonists of biomining have often also overplayed its green credentials. As illustrated in Figure 1, many of the energy-demanding and CO_2-generating processes involved in conventional (smelting-based) mining are also, by necessity, used in biomining operations, in particular, tank leaching of mineral concentrates. Although the main microbial players in conventional biomining are autotrophic bacteria (i.e., they capture carbon dioxide) this minor advantage in net carbon budget is more than offset by the CO_2-producing pre-processing steps required. However, as described below, some of the emerging applications of mineral bioleaching

can minimise or avoid some or all of the ore processing stages and thereby have potential major advantages in terms of carbon/energy budgets.

Figure 1. The comparison of the stages involved in conventional metal mining, and current and biomining technologies. The light purple shading indicates where these are necessary and light green shading where they are not. Abbreviations: "pyro" = pyrometallurgy; "repro" = reprocessing solid wastes; DISB = deep *in situ* biomining. * heap leaching sometimes uses crushed ores but other time mineral concentrates.

There are a number of existing situations where biomining does have significant advantages over conventional mineral extraction and processing. These include (i) extracting and recovering metals (principally copper) from low-grade/run-of-mine ores; (ii) bioleaching primary and secondary copper sulfide ores in sites where solvent extraction-electrowinning (SX-EW) plants have previously been installed for processing pregnant leach solutions (PLS) from acid-leaching of copper oxide ores; (iii) for bio-oxidising refractory gold ores. These are predominantly carried out in dumps, bioheaps, and stirred tanks, respectively, as described above. Bio-processing is also a feasible alternative in situations where producing a mineral concentrate is problematic. This was the case with the polymetallic black schist ore at Talvivaara, Finland, where the high (~10%) graphite content of the deposit precluded the production of a suitable concentrate, and the decision was made to bioleach the crushed ore in heaps [16]. Metal sulfide ores often contain significant concentrations of arsenic, which occurs in arsenopyrite (FeAsS), orpiment (As_2S_3), realgar (AsS/As_4S_4) and other minerals. Smelting mineral concentrates that contain high levels of arsenic incurs a penalty, which is often circumvented by blending these with other concentrates with lower arsenic contents, though the net amount of the metalloid released in gas plumes is ultimately unchanged. In hydrometallurgical mineral processing (which includes biomining), the arsenic released by oxidative dissolution of sulfide minerals is retained in solution (principally as arsenate, $H_2AsO_4^-$) and can be effectively removed downstream by, for example, the adsorption onto positively charge ferric iron minerals or as the mineral scorodite ($FeAsO_4·2H_2O$) (though the latter has to be crystallized to enhance its stability, which adds to costs).

One particular area where bioleaching can be highly effective, both in terms of metal recovery and environmental protection, is for processing solid mine wastes in general, and mine tailings in particular. Due to the fact that mineral separation techniques in the past were not as efficient as they are at present, historic tailings may still contain significant concentrations of valuable metals. These may be the original metal(s) that was mined or one or more other metals that were not considered at the time, on economic grounds, to warrant extraction. Since tailings deposits have already been through the cycle of extraction and haulage, grading, comminution, and froth flotation, bio-processing of these has particularly strong "green" credentials (Figure 1). An excellent example of the combined economic and environmental benefits to be gained from bioleaching tailings in Kasese, Uganda, was described by Morin and d'Hugues [17]. Tailings (*ca.* 900,000 tonnes) generated by copper mining were left untreated at the abandoned site, where high temperatures and rainfall facilitated both the accelerated oxidative dissolution of the residual sulfide minerals, erosion of the tailings deposits, and the transportation

of highly acidic, metal-rich waters they generated towards the vicinity of communities living in the vicinity of the Queen Elizabeth National Park and Lake George. Analysis of the tailings showed that they contained significant concentrations (~1.4%) of cobalt (an estimated reserve of ~11,300 tonnes), a base metal that has been increasingly in demand, with consequent hikes in its commodity value, in the past 20 years or so. The cobalt was present as "cobaltiferous pyrite" and initial laboratory and later pilot-scale tests confirmed that it could be readily solubilised by oxidative mineral dissolution (Figure 2). A full-scale commercial operation was commissioned to reprocess these tailings in stirred tanks (a rare example of this engineering approach being used to recover a metal other than gold) by the Kasese Cobalt Company. The cobalt solubilised by bioleaching was recovered by SX-EW, generating a cathode cobalt of 99.9% purity. The operation lasted from 1999 to 2013, producing (in 2012) >600 tonnes of cobalt per annum, and only ceasing operation when the tailings reserves expired. The solid waste produced from bioleaching the tailings (an oxidised rather than a reduced material) was secure and posed little or no long-term threat to the environment.
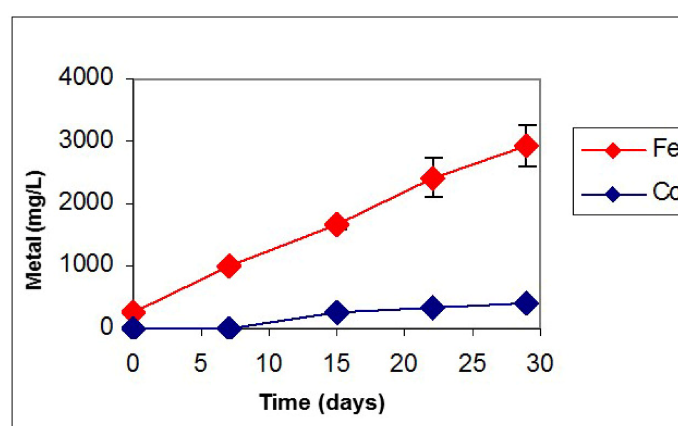

Figure 2. Laboratory-scale solubilisation of iron and cobalt from cobaltiferous pyrite present in mine tailings at Kasese, Uganda due to bioleaching by a consortium of acidophilic micro-organisms. The symbols depict the mean values of triplicate shake flasks and the error bars (where visible) standard deviations.

4. New Developments in Biomining Technologies

4.1. Sulfur-Enhanced Bioleaching (SEB)

Elemental (or zero-valent) sulfur (ZVS) is used by many species of acidophilic bacteria and archaea as an electron donor, and more rarely as an electron acceptor [18]. In aerobic environments, ZVS oxidation is coupled to the reduction of molecular oxygen, generating sulfuric acid (Reaction (1)):

$$S^0 + 1.5\,O_2 + H_2O \rightarrow HSO_4^- + H^+ \tag{1}$$
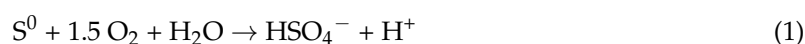
In the absence of oxygen, some acidophiles can couple the oxidation of ZVS to the reduction of ferric iron (Reaction (2)):

$$S^0 + 6\,Fe^{3+} + 4\,H_2O \rightarrow SO_4^{2-} + 6\,Fe^{2+} + 8\,H^+ \tag{2}$$
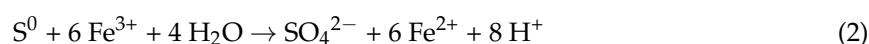
Sulfur is one of the most abundant elements in the lithosphere and ZVS is produced in vast quantities as a waste material, for example, in removing hydrogen sulfide from natural gas reserves. Since most acidophiles that oxidise ZVS are autotrophic, using this material to enhance bioleaching consumes rather than produces CO_2. In addition, the amount of microbial biomass produced by autotrophic bacteria is much less than that from the equivalent amount of ZVS oxidation by heterotrophic micro-organisms. To date, SEB has been described for two contrasting applications: (i) to
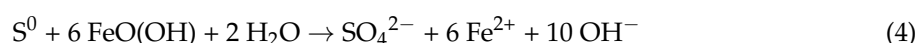
promote the reductive dissolution of oxidised metal ores, and (ii) to accelerate the oxidation and to facilitate control of redox potentials, of acid-deficient reduced ores and wastes.

4.1.1. Reductive Bio-Processing of Oxidised Mineral Ores

Sulfur-enhanced bioleaching has been most intensively investigated for extracting base metals (nickel and cobalt) from lateritic ores (e.g., [19–22]). These are widely distributed in low latitude countries and represent very large reserves of both metals (e.g., an estimated 72% of terrestrial Ni reserves, though more nickel is currently recovered from reduced ores). Laterites are layered deposits, with limonitic ore (which typically contains 1–1.5% (by weight) of Ni and 0.1–0.25% Co) sandwiched between underlying magnesium-rich saprolite and a soil/ferricrete overburden. Saprolite is mined commercially but, in many cases, limonite is stockpiled as a waste material. Nickel in limonite is associated mainly with ferric iron minerals, such as goethite (FeO(OH)). Consortia of acidophilic bacteria have been demonstrated to couple the oxidation of sulfur to the reductive dissolution of goethite, leading to the release of nickel from limonite ores [19]. The process appears to involve two processes: (i) abiotic acidic dissolution of goethite (Reaction (3)):

$$FeO(OH) + 2\,H^+ \rightarrow Fe^{3+} + OH^- + H_2O \qquad (3)$$
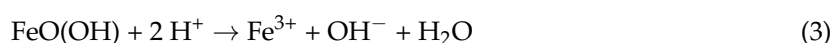
and the bacterially-catalysed reduction of the ferric iron liberated to ferrous, coupled to oxidation of ZVS (Reaction (2)). As shown in Reaction (4), the combined reactions are net alkali-generating/acid consuming:

$$S^0 + 6\,FeO(OH) + 2\,H_2O \rightarrow SO_4{}^{2-} + 6\,Fe^{2+} + 10\,OH^- \qquad (4)$$

Cobalt (together with some nickel) is chiefly deported in manganese (IV) minerals such as asbolane ($(Ni,Co)_x Mn(O,OH)_4 \cdot nH_2O$). The ferrous iron generated in Reaction (4) can accelerate the reductive dissolution of asbolane, leading to the solubilisation of both manganese (as Mn^{2+}) and cobalt (which has been shown to be closely synchronised in laboratory experiments; Figure 3).
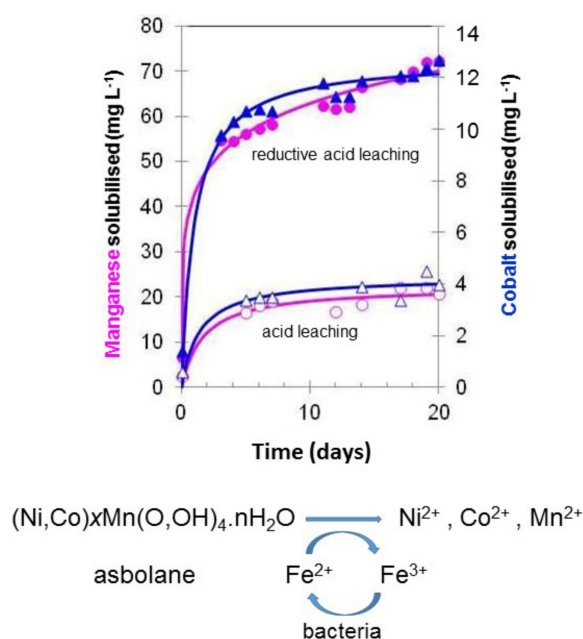

Figure 3. The correlation between cobalt and manganese solubilised from a lateritic ore by reductive mineral dissolution, and comparison of bioleaching under aerobic and anaerobic conditions [23].
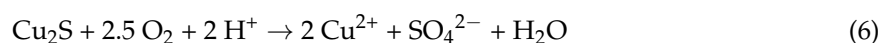
The reductive bio-processing of metal ores (which has been referred to as "biomining in reverse gear" [23]) since it operates on opposite principles to conventional biomining (though both operate at low pH) represents a radical new direction for mineral bioprocessing. Table 1 compares the major differences between microbially-catalysed oxidative and reductive mineral dissolution. The major operational cost of reductive dissolution is for sulfuric acid to maintain a pH within an optimum range for the process. As indicated in Reaction (1), some acidophilic bacteria (including those involved in reductive bioleaching) can generate acid directly from ZVS. However, this requires oxygen which conventionally precludes the reduction of iron. Alternating cycles of active aeration (to promote acid production) and zero aeration (to generate reducing conditions) have been used to address this impasse. Intriguingly, some acidophiles, such as the moderate thermophile *Acidithiobacillus caldus*, have been reported to reduce ferric iron to ferrous under aerobic conditions, with the simultaneous generation of biogenic sulfuric acid [24]. This gives the potential for combining simultaneous biogenic acid production and reductive dissolution in aerated stirred tanks and bioheaps.

Table 1. The comparison of the operational parameters and key requirements of oxidative and reductive mineral dissolution, catalysed by acidophilic micro-organisms.

Parameter	Oxidative Mineral Dissolution	Reductive Mineral Dissolution
Key reaction	iron oxidation	iron reduction
Acidity	often generated	consumed
Aeration required	yes (aerobic process)	no (anaerobic process)
Additional energy source required?	no (Fe^{2+}, reduced S in minerals)	yes (elemental sulfur)
Electron acceptor	Oxygen	Fe^{3+} (in minerals)
PLS generated	Oxidized	reduced

4.1.2. Using SEB to Enhance Oxidative Processing of Sulfidic Ores and Tailings

Pyrite is one of the major sources of acidity in oxidising sulfide ores and concentrates (Reaction (5)) while, in contrast, the oxidative dissolution of many other sulfide minerals (e.g., chalcocite) is a net consumptive of acid (Reaction (6)):

$$FeS_2 + 3.75\,O_2 + H_2O \rightarrow Fe^{3+} + 2\,SO_4{}^{2-} + H^+ \tag{5}$$

$$Cu_2S + 2.5\,O_2 + 2\,H^+ \rightarrow 2\,Cu^{2+} + SO_4{}^{2-} + H_2O \tag{6}$$

Large concentrations of soluble iron can be generated by Reaction (5) and, although ferric iron is required as the primary oxidant involved in the oxidative dissolution of sulfide minerals, excess amounts can be problematic, requiring removal and precipitation in bleed streams. SEB avoids this problem and can be used where acid-generating minerals are present in relatively small amounts in ores and tailings. SEB also provides the potential for ultra-low pH (<1.5) bioleaching, and the opportunity to change redox potential rapidly in bioleach liquors, which can be achieved by removing aeration of tanks or bioheaps (sulfur-oxidising bacteria rapidly consume the remaining dissolved oxygen before switching to ferric iron as the alternative electron acceptor). An example of applying SEB to extract copper from tailings at mine sites in Spain and Serbia has been described in Reference [25]. Bioleaching was carried out in stirred and temperature-controlled bioreactors; the most efficient extraction (84 to >90%) of copper was achieved by adding ZVS to mineral tailings and using defined microbial consortia (dominated by *Acidithiobacillus caldus* and *Sulfobacillus thermosulfidooxidans*) at 45 °C and where pH was allowed to decline to ~1.0. Switching from aerobic to anaerobic conditions generated reduced PLS and precluded the formation of ferric iron precipitates and co-precipitation of some of the soluble copper (Figure 4). The economics of bio-processing the tailings wastes was also considered in this study. The site in Spain generates ~1.5 million tonnes of tailings, containing ~0.72% copper deported mostly in chalcopyrite, per annum. This is equivalent around 10.8 kilotonnes of copper dumped in tailings ponds/annum, and the extraction of this (at 84% efficiency) was estimated to potentially provide an additional revenue stream of ~$70 (US) million/annum to the mining operation.
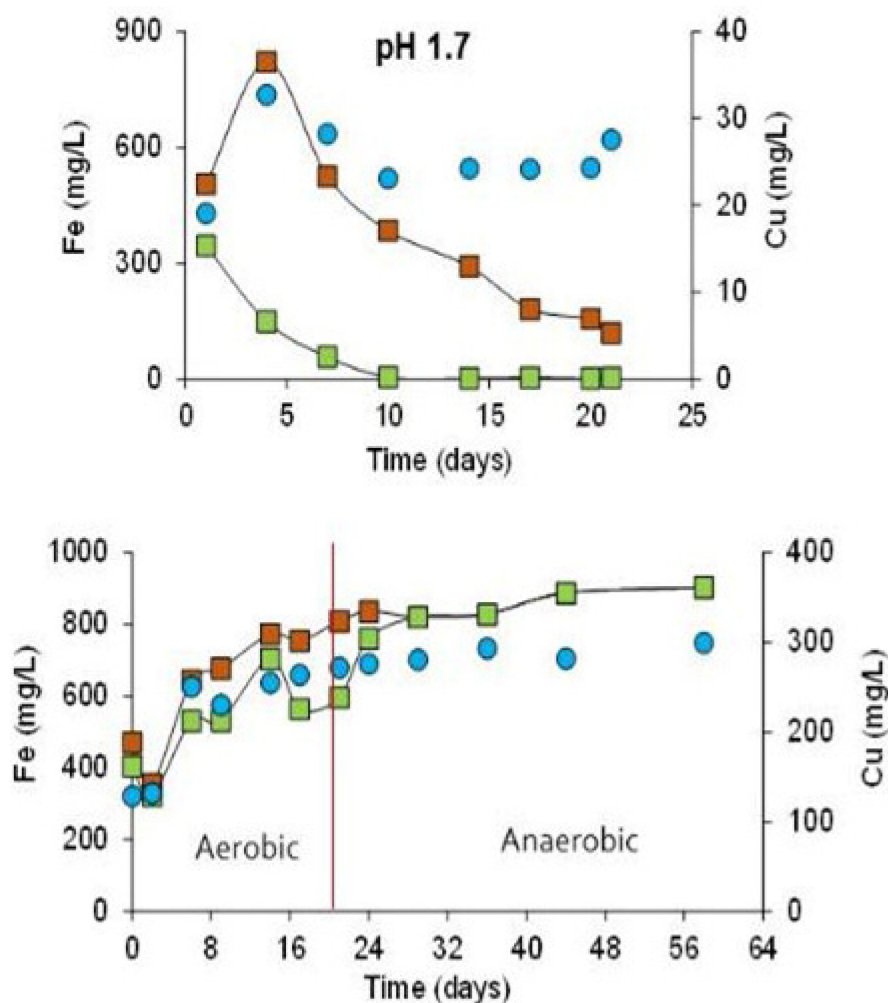
Figure 4. The comparison of aerobic bioleaching of tailings from a copper mine in Spain maintained at a pH of 1.7 by the automated addition of sulfuric acid (top) and SEB of the tailings (with no pH control) carried out under alternating aerobic and anaerobic conditions (bottom), in bioreactors maintained at 45 °C. Key: •, copper; ■ total soluble iron; ■ soluble ferrous iron.

4.2. Deep In Situ Biomining

The term "deep *in situ* biomining" (DISB) has been used to describe an emerging approach for extracting and recovering base metals buried 1–2 km in the lithosphere [26–28]. DISB combines indirect bioleaching, where the abiotic dissolution of a sulfidic ore or concentrate by an acidic, ferric iron-rich lixiviant and the biological regeneration of oxidised iron following the stripping of the target solubilised metal(s), are spatially separated (e.g., Reference [29]) with *in situ* leaching (ISL) which is, as noted, not a new concept. An ISL process for recovering uranium was first introduced in the USA in 1959 and was estimated to account for 51% of global U production in 2014 [30]. Copper was also recovered using ISL in, e.g., Nevada and Arizona in the 1970s–1990s [31]. Major differences between DISB and conventional ISL are the depths of the target ore bodies and the techniques used to expose surfaces to flowing lixiviant solutions when this is necessary. The ISL of uranium carried out in Kazakhstan, Uzbekistan, Australia, the USA and other countries mostly involves extracting the metal from porous sediments and rock strata at relatively shallow depths, whereas hydrofracturing would be required to open up flow channels in a deep-buried massive sulfide deposit. While a paradigm shift would be required in both the mining industry and society, in general, to get to the point where DISB is both technically feasible and accepted, the potential advantages of this approach are enormous.

Most high-grade base metal ores located close to the land surface have been worked out, but can still be found deep within the lithosphere (e.g., Reference [32]). The green credentials of DISB are also strong: it avoids haulage and comminution, and therefore incurs greatly reduced energy costs and carbon-footprints, material of no value (~99% of a typical ore body) would remain buried, leading to a vast reduction in waste material generated and dumped on the land surface, in contrast with current mining practices. The land surface area ("footprint") required by a DISB operation would also be a fraction of that required by a conventional opencast metal mine.

Conceptual aspects (Figure 5) and laboratory- and pilot-scale application of DISB have been the subject of a large-scale recent European Union-sponsored project, "BIOMOre" [33]. The test ore selected in this study was *kupferschiefer*, a copper-rich deposit that occurs widely in central and north-eastern Europe. The saline and calcareous nature of *kupferschiefer* meant that leaching the ore with water and then with sulfuric acid was required before leaching with a biologically-generated ferric iron lixiviant could be carried out. The proof of concept of indirect leaching was achieved in laboratory tests, with a 50–87% extraction of copper achieved, which is currently being mimicked in a pilot-scale underground test [28]. Modelling toolboxes generated to simulate DISB of *kupferschiefer* were also closely aligned to experimental results.
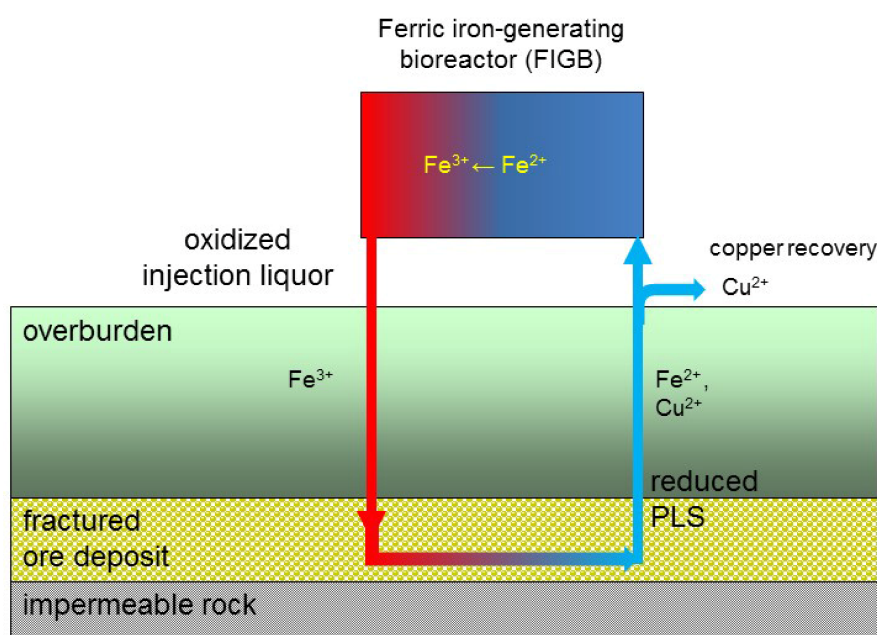

Figure 5. Conceptual configuration of a deep *in situ* biomining operation to extract copper from an ore body located 1–2 km below the land surface. An oxidised, extremely acidic lixiviant generated in a ferric iron-generating bioreactor located at the surface of the mine is injected to the fractured ore body at a depth where it reacts chemically with metal-containing sulfide minerals, solubilising copper and becoming increasingly reduced. The PLS is pumped back to the surface where copper is stripped and the oxidised lixiviant regenerated.

4.3. Exploiting "Natural Biomines"

The term "solution mining" has been used to include both abiotic (e.g., acid leaching of Cu oxide ores and *in situ* leaching of U) and biologically-catalysed metal ore processing, as both involve the extraction of metals into acidic liquors and the downstream recovery of metals using SX-EW, ion-exchange or alternative methods. However, the microbially-catalysed dissolution of metal sulfides also occurs, advertently, both at operating mines and, more significantly, at abandoned mine sites.

Natural biomines (as differentiated from engineered heap and dump biomines) are relic features (e.g., underground mines, mine wastes such as rock dumps and tailings) that generate run-off waters

which contain concentrations of base metals that are high enough to make their recovery economically viable, and which require zero or minimal engineering to achieve this. Sites of this kind are widespread throughout Europe and the USA, as well as other parts of the world that have historic mining operations. Although they can give the impression of being devoid of life, being often sparsely colonised, if at all, with vegetation, they are actually hot-spots of microbiological activity, particularly chemo-lithotrophic ("rock eating") bacteria and archaea that thrive by oxidising sulfide minerals made accessible by mining operations. Recovering metals from waters draining natural biomines has particularly strong green credentials, as not only are metal resources recovered without recourse to the energy-demanding steps shown in Figure 1, but environmental pollution is also simultaneously abated.

One example of a natural biomine is the abandoned Mynydd Parys copper mine site in Anglesey, north Wales (Figure 6a,b). This was operated initially as a deep mine and later as an opencast operation and was, in the late 18th century, the largest copper-producing mine in the world. Extraction of ore ceased in the 19th century, though cement copper was recovered at the site (via ISL) well into the 20th century. Water percolating through the surface mine wastes is impounded in an underground reservoir, which overflows into a drainage stream (the *Afon Goch*) which flows, untreated, into the Irish Sea [34], discharging ~315,000 m^3/year of extremely acidic, metal-rich water (Table 2a) directly into these marine waters. The two commercially valuable transition metals (copper and zinc) present in the mine water were recovered sequentially by sulfide precipitation (Carmen Falagan and D. Barrie Johnson, unpublished data) using biogenic hydrogen sulfide generated in a low pH sulfidogenic bioreactor [35,36] (Figure 7). Hydrogen sulfide was added directly to the extremely acidic mine water to selectively precipitate CuS, which was then removed and the mine water pH increased to 4.0 and ZnS precipitated. Table 2b compares the consumable costs (glycerol, the substrate used for low pH biosulfidogenesis, and sodium hydroxide to increase water pH for ZnS precipitation) and the value of the metals recovered in this scenario. Both sulfide precipitates produced were essentially free of iron (the major metal in the mine water), which has a much smaller solubility product of its sulfide phase and FeS does not form in acidic (pH < 6) solutions. The selective sulfide precipitation is particularly suitable for recovering metals from mine drainage waters as these tend to be present in relatively low concentrations (compared to typical PLS) and techniques such as SX-EW and ion exchange are therefore ineffective.

Table 2. (**a**) Concentrations of the major transition metals present in the *Afon Goch* (in mg/L) and those remaining after the selective precipitation of copper and zinc (as sulfide phases); (**b**) comparison of reagent costs and metal values in the proposed recovery of copper and zinc from the mine water using biosulfidogenesis.

(a)			
Parameter	**Afon Goch**	**Post Cu Removal**	**Post Zn Removal**
pH	2.37	2.38	4.05
Total Fe	512	510	506
Cu	42	0	0
Zn	58	53	0
Mn	14	14	14
(b)			
Metal	**Consumable Costs/Year $**	**Metal Value/Year $**	
Cu	10,500	88,000	
Zn	34,500	56,500	
Total	45,000	144,500	

Figure 6. Examples of "natural biomines": (**a**) the abandoned opencast operation and (**b**) the *Afon Goch* draining the former Mynydd Parys mine in north-west Wales; (**c**) the *Rio Tintillo* in south-west Spain, the catchment of waters percolating through a vast area of abandoned mine waste (shown in the back of the image).
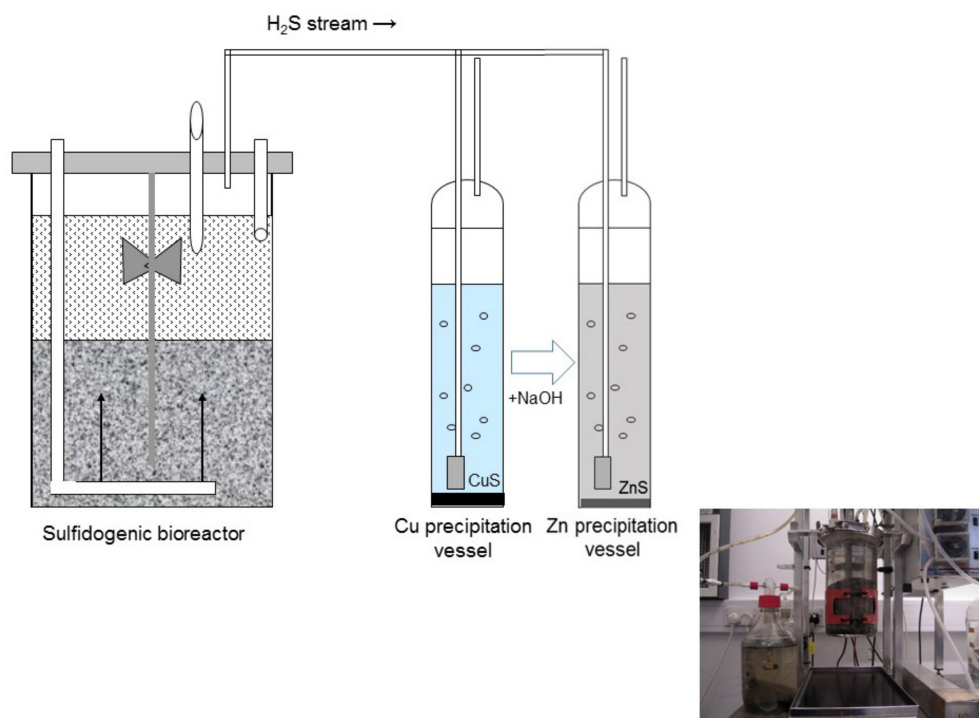

Figure 7. The schematic representation of the protocol used to remove copper and zinc selectively from the *Afon Goch* mine drainage stream, and (**inset**) an image of a low pH sulfidogenic bioreactor (**centre**) being used to precipitate copper in an off-line vessel (**left**).

Biosulfidogenesis can be used either directly (as in the Mynydd Parys mine) or to concentrate metals that are present in even smaller concentrations, prior to remobilisation and recovery [36].

A second example of a water body draining a natural biomine is the *Rio Tintillo* in south-west Spain (Figure 6c). This collects water from a vast area of biologically-active rock waste accumulated over centuries around the Rio Tinto mine and contains significant concentrations of cobalt, as well as (like at Mynydd Parys) copper and zinc [37]. Although cobalt concentrations are the lowest of these three metals, its far greater value as a commodity (2018 prices) means that it is the most valuable resource in the river water (Table 3). Since the unit cost of recovering divalent cationic transition metals by sulfide precipitation is the same (as all of these require the same stoichiometric amounts of hydrogen sulfide to form solid sulfide phases), the potential profit margin from recovering these metals is directly related to their relative market values.

Table 3. The comparison of the values (in $ US, May 2018 commodity prices) of the major transition metals present in the *Rio Tintillo*, Spain.

	Iron	Zinc	Copper	Cobalt
Concentration (mg/L)	~1750	~550	~180	~45
Price ($/kg)	0.07	3.07	6.78	88.9
Value ($/m^3)	0.12	1.69	1.23	3.99

5. Retrospective Critique and Potential Future Developments in Mining Biotechnologies

Although biomining is now well established as a proven biotechnology, it has not, as many had thought during its development, revolutionised the mining industry but rather has remained as a more specialised, and often peripheral, application. For the past 30 or so years, biomining has mostly focused on fine-tuning sulfide mineral bioleaching than more blue sky thinking and finding radically new directions and opportunities for using biological processing in the mining sector. Even so, areas of (mostly) laboratory-based research, such as reductive mineral bio-processing, have suggested how bio-processing could be used outside of conventional bioleaching and bio-oxidation of sulfide ores and concentrates. By combining low carbon/energy solutions for metal extraction with environmental protection (e.g., considering the natural biomines as resources rather than wastes) in approaches that are economically sound, biotechnology has the potential to bring about major changes in the direction of how the 21st-century global community wins and recycles metals.

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