Bio-mining: The Past, the Present and the Future

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Abstract

The use of microbes to extract metals from ores is simply the harnessing of a natural process for commercial purposes. Microbes have participated in the deposition and solubilization of heavy metals in the earth's crust since geologically ancient times. Most of this activity is linked to the iron and sulfur cycles. Anaerobic sulfate reducing bacteria generate sulfides that can react with a variety of metals to form insoluble metal sulfides. There are two main types of processes for commercial-scale microbially assisted metal recovery. These are irrigation-type and stirred tank-type processes. Bio-mining involves a chemical process called leaching which are actually oxidation reactions and maybe called bio-oxidation. Bio-leaching processes can be carried out at a range of temperatures and as would be expected, the iron and sulfur-oxidizing microbes present differ depending on the temperature ranges. In mineral bio-oxidation processes that operate at 40°C or less, the most important microorganisms are believed to be a consortium of gram-negative bacteria such as *Acidithiobacillus ferrooxidans*. In continuous-flow stirred tank processes, the steady-state ferric iron concentration is usually high and under such condition, *A. ferrooxidans* appears to be less important than a combination of *Leptospirillum* and *A. thiooxidans*. Microorganisms that dominate bio-leaching at 50°C include *A. caldus* and some *Leptospirillum* spp. At temperatures greater than 65°C, bio-mining microbial consortia are dominated by archaea rather than bacteria with species of *Sulfolobus* and *Metallosphaera* being most prominent.

Keywords

Archae, Bacteria, Bio-mining, Metals, Ores

1. Introduction

The use of microbes to extract metals from ores is simply the harnessing of a natural process for commercial purposes. Microbes have participated in the deposition and solubilization of heavy metals in the earth's crust since geologically ancient times. Mining activities have been carried out for thousands of years and nowadays have an enormous worldwide use to obtain important metals of industrial use [1]. Most of this activity is linked to the iron and sulfur cycles. Anaerobic sulfate-reducing bacteria generate sulfides that can react with a variety of metals to form insoluble metal sulfides. These sulfide precipitates serve as a source of mineral deposits that may become incorporated into rock formations [2]. The metal sulfides in turn can serve as electron donors for the usually aerobic sulfur-oxidizing microbes, which results in the formation of metal sulfates. Because many of these metal sulfates are soluble, this process serves as a means of leaching the metal from the mineral deposit. This ability of certain microbes to solubilize metals from ores has given rise to a growing bio-mining industry [2].

The use of microbes in ore processing has some distinct advantages over the traditional physicochemical methods. Almost without exception, microbial extraction procedures are more environmentally friendly. They do not require the high amounts of energy used during roasting or smelting and

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do not produce sulfur dioxide or other environmentally harmful gaseous emissions. Furthermore, mine tailings and wastes produced from physicochemical processes when exposed to rain and air may be biologically leached, producing unwanted acid and metal pollution. Tailings from bio-mining operations are less chemically active, and the biological activity they can support is reduced by at least the extent to which they have already been bioleached. Bio-mining also has a clear advantage in the extraction of metals from certain low-grade ores. For example, copper can be recovered from low-grade ores and dumps left behind from previous mining operations by using the biological activity that takes place during controlled irrigation of the dump [3]. Many of these metals are not economically recoverable by non-biological methods. Where ore-type and geological features permit, metal recovery using leaching solutions produced and regenerated by microbes can be carried out in situ with obvious cost advantages and minimal disturbance to the surrounding environment.

2. Characteristics of Microbes Involved in Bio-Mining

The primary bio-mining organisms have several physiological features in common. They are all chemolithoautotrophic and are able to use ferrous iron or reduced inorganic sulfur sources (or both) as electron donors. Because the by-product of sulfur-oxidation is sulfuric acid, these organisms are acidophilic and most will grow within the pH range 1.5-2.0. This extreme acidophily applies even to those bio-mining organisms that can oxidize only iron. Although bio-mining bacteria may be able to use electron acceptors other than oxygen (e.g., ferric iron), they generally grow best in highly aerated solutions. All primary bio-mining organisms fix CO_2, although there is considerable variation in the efficiency with which this is done.

The less-efficient CO_2-fixing species require either elevated levels of CO_2 or a small amount of yeast extract to grow rapidly. As may be expected, bio-mining bacteria are generally resistant to a range of metal ions, with some variation in metal tolerance between species and isolates within a species. These common properties explain why bio-mining organisms are ideally suited to growing in the inorganic environment created by the active aeration of a suspension of a suitable iron- or sulfur-containing mineral in water or during the passive aeration that takes place when a heap of the mineral is irrigated with water. Air provides the carbon source (CO_2) and the preferred electron acceptor (O_2), the mineral ore supplies the electron donor (ferrous iron and/or reduced inorganic sulfur), and water is the medium for growth. Some bio-mining organisms can also fix nitrogen from the air, although they may not be able to do so in a highly aerated environment. Trace elements are provided by the mineral and water. In commercial processes small quantities of inexpensive, fertilizer-grade, ammonium sulfate and potassium phosphate may be added to ensure that nutrient limitation does not occur.

3. Bio-mining Processes

3.1. Irrigation-Type and Stirred Tank-Type Processes

There are two main types of processes for commercial-scale microbially assisted metal recovery. These are irrigation-type and stirred tank-type processes. Irrigation processes involve the percolation of leaching solutions through crushed ore or concentrates that have been stacked in columns, heaps, or dumps. There are also several examples of the irrigation of an ore body in situ, that is, without bringing the ore to the surface. Stirred tank-type processes employ continuously operating, highly aerated stirred tank reactors [4, 5]. One feature of both types of processes is that, unlike most other commercial fermentation processes, neither is sterile, and no attempt is made to maintain the sterility of the inoculum. There is no need for sterility because the highly acidophilic chemoautolithotrophic microorganisms create an environment that is not suitable for the growth of other organisms. Second, because the aim of the process is to maximize the decomposition of the mineral, the microorganisms that do this most efficiently will out-compete those that are less efficient. There is a continual selection for those organisms that are most effective at decomposing the mineral, as the mineral provides the only energy source available.

3.1.1. Irrigation-Type Processes

The ore is crushed, agglomerated and stacked onto plastic-lined pads and irrigated with recycled leach liquor. The bacteria growing on the ore oxidize ferrous iron to ferric iron thus solubilizing the copper and the pregnant copper-containing solution is recovered from the heap. The copper is concentrated through a solvent extraction process followed by recovery though electro-winning and the spent leach liquor is recycled to the heap.

The metal recovered by far the greatest quantities using bio-leaching is copper. Recent estimates of the total quantity of copper ore treated by microbially assisted processes are difficult to obtain. However, in 1999 it was reported that the copper heap bioleaching plants built since 1991 and remaining in operation processed in excess of 30×10^6 tonnes of ore per annum [7]. All current commercial processes for copper recovery are of the dump, heap, or in situ irrigation type, although stirred tank processes for the extraction of copper

from chalcopyrite are in an advanced phase of development.

Heap leaching of copper is similar to dump leaching except that the process is designed to be more efficient. Ore is crushed, acidified with sulfuric acid, and agglomerated in rolling drums to bind fine particles to coarse particles. The agglomerated ore is stacked 2-10m high on irrigation pads lined with high-density polyethylene to avoid the loss of solution. Aeration pipes may be included during construction to permit forced aeration and speed up the bioleaching process. Although inoculation of heaps with bacteria has been considered, bio-leaching bacteria are ubiquitous and it is not clear to what extent inoculation speeds up the process. Inorganic nutrients such as $(NH_4)_2SO_4$ and KH_2PO_4 are frequently added to the raffinate prior to irrigation through drip lines placed on the surface of the heap. The increased efficiency that results from the careful construction and operation of a heap reactor results in the heap bioleaching processes being completed in a period of months rather than years.

3.1.2. Stirred Tank Processes

Finely milled flotation concentrate is added to inorganic nutrients and water in a make-up tank. The mixture is passed through a series of vigorously aerated bio-oxidation tanks. The microorganisms decompose the arsenopyrite, making the gold accessible to cyanide. Duplication (or doubling in volume) of the number of primary aeration tanks is to increase the hydraulic retention time above that of the cell doubling time to ensure that microbial cell wash-out does not occur during the mineral treatment process. The concentrate, which after pretreatment still contains the gold, is recovered in a thickening tank and sent to the gold recovery process.

The use of highly aerated, stirred tank bioreactors provides a step up in the rate and efficiency of mineral bio-oxidation processes. Because these reactors are expensive to construct and operate, their use is restricted to high-value ores and concentrates. The bioreactors are typically arranged in series and are operated in continuous-flow mode, with feed being added to the first tank and overflowing from tank to tank until bio-oxidation of the mineral concentrate is sufficiently complete [8]. The bioreactor tanks in the first stage are usually arranged in parallel to provide sufficient retention time for the microbial cell numbers to reach high steady-state levels without being washed out [9]. The feed is mineral concentrate suspended in water to which small quantities of fertilizer-grade $(NH_4)_2SO_4$ and KH_2PO_4 have been added. Mineral bio-oxidation is an exothermic process, and the bioreactors have to be cooled to remove excess heat. Large volumes of air are blown through each bioreactor, and a large agitator ensures that the solids remain in even suspension and are carried over into the next tank.

3.2. Metals Recovered in Bio-mining Processes

The metals that are currently being recovered in the greatest tonnages are copper followed by gold. In general, most copper is recovered by the irrigation of heaps and dumps, while most gold is recovered using stirred-tank technology as a pretreatment process.

3.2.1. Copper Leaching

It is difficult to obtain figures of the quantity of copper that is being recovered using bioleaching as new mines continually come on stream and while others reduce or cease operation.

Two fairly recent surveys of copper production data have been presented [4], with only a few mines that are common to both sets of data. By way of illustration, many of the largest operations are in Chile with up to 225000 tonnes of copper being produced per annum in the largest of these. Bio-leaching of copper involves the conversion of water-insoluble copper sulfides to water-soluble copper sulfates. Copper-containing minerals such as chalcocite (Cu_2S) or covellite (CuS) are crushed, acidified with sulfuric acid, and agglomerated in rotating drums to bind fine material to courser particles before stacking in heaps. The stacked heaps are irrigated with an iron-containing solution (usually recycled spent leach liquor) through a system of pipes laid on or just below the heap surface. The solution percolates through the heap and bacteria growing on the surface of the ore and in solution catalyze the release of copper. The ferric iron generated by the bacteria plays an important role in the production of copper sulfate.

$$Cu_2S + 2Fe_2(SO_4)_3 \rightarrow 2CuSO_4 + 4FeSO_4 + S$$

$$CuS + Fe_2(SO_4)_3 \rightarrow CuSO_4 + 2FeSO_4 + S$$
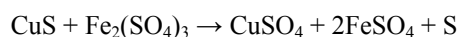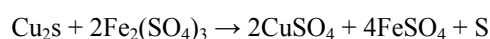
The ferrous iron and sulfur produced in the above reactions can be re-oxidized by bacteria. The pregnant leach solution containing 1.5 to 6 g/l soluble copper and up to 20 g/l iron is collected and sent to a recovery plant. The most common methods for copper recovery are by precipitation using iron filings (cementation), electro-winning or solvent extraction followed by electro-winning. The latter procedure produces the highest grade of copper. The extraction of copper from chalcopyrite using bioreactors can be faster and efficient at temperatures higher than 60°C using thermophilic archaea. These operations are easier to control compared with standard bioleaching using ore heaps [10].

3.2.2. Gold Bio-oxidation

In the 1980s, a process for the bio-oxidation of gold-bearing arsenopyrite ores in highly aerated stirred tank reactors was developed by Gencor, South Africa [6, 11]. This was at the Fairview Mine, Barberton, Mpumalanga and was designed to treat 10 tons of gold-bearing arsenopyrite concentrate per day.

Critical to the development of the process was the selection of a bacterial culture that was highly resistant to arsenic. The details of this selection process are described elsewhere. With the successful development of that process, bio-mining became a significant part of the more sophisticated mineral recovery industry. An illustration of this is that the bio-oxidation plant built at the Sansu mine in the Ashanti gold fields of Ghana [5] is currently (with the possible exception of some sewage treatment facilities) the largest fermentation plant in the world (960 tons of gold-bearing arsenopyrite concentrate is treated per day in 24 tanks of 1 million litres each).

Gold is usually extracted from ores using cyanide. Recalcitrant ores are those in which gold is encased in a matrix of arsenopyrite/pyrite, so that even after fine milling, the gold cannot be efficiently recovered. Pretreatment of the ore is required to open up the molecular structure of the ore so that cyanide can penetrate the mineral and extract the gold. Since the quantities of ore to be treated are huge and most of the gold is present in a small pyrite/arsenopyrite fraction, the ore is crushed and a gold-bearing concentrate is prepared by flotation. Prior to 1986, concentrate pretreatment processes were physicochemical. For example, the concentrate was roasted at 700°C in the presence of oxygen or digested with acid under pressure in an oxygen-enriched atmosphere (autoclaved). In contrast, bio-mining bacteria decompose ores and concentrates at atmospheric pressure and at temperatures which are close to ambient.

$$FeAsS + 13Fe^{3+} + H_2O \rightarrow 14Fe^{2+} + AsO_4^{\;3-} + SO_4^{\;2-} + 16H^+$$
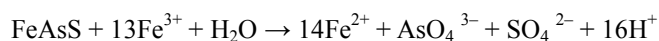
Without pretreatment, only 30-50% of the gold is recovered, depending on the concentrate, while after bio-oxidation, more than 95% of the gold is recoverable. Since gold-bearing concentrates are valuable substrates relative to copper, it is economically viable to carry out the pretreatment bio-oxidation processes in efficient, aerated, temperature- and pH-controlled tank fermenters. A typical Biox plant operates at about an 18% w/v solids concentration with a total solids retention time of about 4 days. Acidity is controlled at pH 1.6 and the temperature at 40°C. As the process is exothermic, a considerable amount of energy for cooling is required to maintain the process at this temperature [5]. An alternative to the Biox process is the Bacox process developed by BacTech (Australia) [12].

A process in which a gold-bearing concentrate is treated in a heap rather than a tank reactor has recently been reported. In this process (developed by Geobiotics, Hayward, CA, USA) the concentrate is agglomerated onto a support rock, which is then stacked on a pad, inoculated with microorganisms, and irrigated [13]. Air is blown through the stack, and after a period the partly oxidized material is removed for gold recovery. Unlike the bioleaching of copper ores, where the copper is solubilized in the biological process, the gold is not solubilized by microbial action, but a separate chemical process is required. Since gold is not leached from the ores, the term "bio-oxidation" is used to describe the treatment of gold ores as opposed to the term "bio-leaching".

3.2.3. Uranium

During 1988, approximately 300 tons of uranium with a value of over US$ 25 million was recovered from a single mine (Dennison mine, Lake Elliot district, Canada) [14]. Much like copper, uranium is recovered by the conversion of insoluble uranium oxides to soluble sulfates though the action of ferric iron and sulfuric acid produced by microbes.

$$UO_2 + Fe_2(SO_4)_3 \rightarrow UO_2SO_4 + 2FeSO_4$$

$$UO_3 + H_2SO_4 \rightarrow UO_2SO_4 + H_2O$$
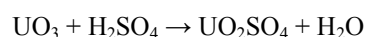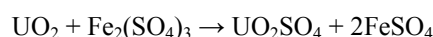
Almost all uranium recovery was carried out in situ. However, with the reduction in demand for uranium in more recent years, this mine has stopped production, and no microbially assisted uranium recovery is currently taking place.

3.2.4. Cobalt

A continuously aerated stirred-tank process is being used at Kasese (Uganda) to extract cobalt from a stockpile of 1.1 million tons of pyrite concentrate [15]. The concentrate contains 1.38% cobalt and large, 1.35 million liter tanks are used to recover about 92% of the cobalt. This process began operation in 1998 and about 240 tons of cobalt-containing pyrite is treated per day using an undefined mixture of Acidithiobacilli and Leptospirilli that grow optimally at 37°C.

3.2.5. Other Metals

Metals that are present in an insoluble reduced sulfur form and which are rendered soluble when oxidized to a sulfate may be potentially recovered by bioleaching. This includes minerals containing NiS and ZnS, etc. Although bioleaching provides the possibility of recovering metals from many low-grade deposits that would otherwise be considered waste, its application greatly depends on the value of the metal to be recovered. A major challenge is to find a suitable match between an ore body and bioleaching technology. For example, the technology for nickel recovery using the BioNic® process [5] has been thoroughly tested, but as yet no ore body of a suitable concentration and size has been identified to allow economic recovery at current nickel prices.

In 1970 it was reported that *Acidothiobacillus ferrooxidans* increased the rate of ferrous iron oxidation by half a million to a million times, compared with the abiotic chemical oxidation of ferrous iron by dissolved oxygen [16]. The ferric iron produced as a result was able to chemically oxidize sulfide

minerals. There has been a long-standing debate concerning whether the microbially assisted bio-oxidation of minerals is by a so-called direct or indirect mechanism [17]. Some of the disagreement appears to have been caused by a lack of clarity as to what is meant by direct and indirect. There is general agreement as to what is meant by the indirect mechanism, namely the chemical attack by ferric iron or protons on a mineral sulfide that results in the dissolution of the mineral and the formation of ferrous iron and various forms of sulfur. Iron-oxidizing microbes use the ferrous iron as an electron donor, re-oxidizing it to ferric iron, thereby regenerating the reactant. If the role of the microbes is nothing more than their ability to regenerate ferric iron and protons, then the efficiency of bio-oxidation should be independent of whether the microbes are in contact with the mineral or not. What is understood by the direct mechanism is not always unequivocal. In a loose sense it has meant that attachment of the microbes to the ore enhances the rate of mineral dissolution. In a more strict sense, direct attack is viewed as a process by which components within the bacterial membrane interact directly with the metal and sulfide moieties of the mineral by using an enzymatic type of mechanism [18].
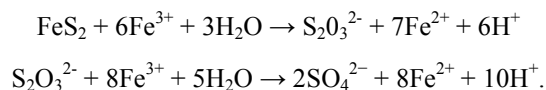
A number of scientists from a variety of disciplines have addressed this problem in recent years. It is not possible to cover fully the points made by each contributor to this debate in the space available. However, there is a growing consensus view on many aspects of the mechanism of bioleaching. An understanding of metal sulfide dissolution is complicated because different metal sulfides have different types of crystal structure, and it has been observed that the oxidation of different metal sulfides proceeds via different intermediates [19]. In addition, as is discussed below, different iron-oxidizing bacteria may use different bond-breaking strategies [20]. For example, the ability of *A. ferrooxidans* to oxidize iron is subject to ferric iron inhibition and is inhibited at high-redox potentials, whereas the ability of *L. ferrooxidans* to oxidize iron is far less inhibited by ferric iron and continues at high-redox potentials [21].

Among the most helpful contributions to an understanding of the mechanism of mineral bio-oxidation are those from the laboratories of Wolfgang Sand, Frank Crundwell, and Helmut Tributsch. Bacteria like *A. ferrooxidans* [22] or *L. ferrooxidans* [23] have a strong affinity for mineral surfaces like pyrite, to which they rapidly attach. In the case of *A. ferrooxidans* aporusticyanin appears to play an important role, especially during the early stages of attachment [24]. Sand and Tributsch and their coworkers have stressed the role played by the exopolysaccharide (EPS) layer produced by *A. ferrooxidans* [25] and *L. ferrooxidans* [26] when attached to a mineral. The EPS layer attaches the bacteria to a mineral surface and forms the matrix in which the cells divide and
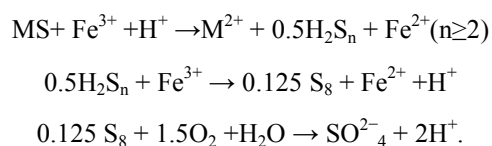
eventually form a biofilm [20]. The iron-impregnated EPS layer serves as a reaction space in which the high concentration of ferric iron mounts chemical attack on the valence bonds of the mineral [20]. In the process the ferric iron is reduced to ferrous, which is re-oxidized to ferric by the iron-oxidizing microbes:

$$14\ Fe^{2+} + 3.5O_2 + 14H^+ \rightarrow 14\ Fe^{3+} + 7H_2O.$$
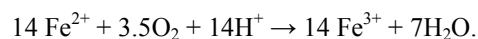
This microbially mediated re-oxidation of ferrous iron can result in a localized increase in pH within the EPS near the surface of the mineral. Researchers from the Crundwell laboratory have evidence to suggest that it is this local rise in pH that assists in mineral dissolution [27]. Sand and coworkers have observed that the oxidation of different metal sulfides proceeds via different intermediates. Therefore, the dissolution reaction is not identical for all metal sulfides. They have proposed a thiosulfate mechanism for the oxidation of acid-insoluble metal sulfides such as pyrite (FeS$_2$), molybdenite (MoS$_2$), and tungstenite (WS$_2$), and a polysulfide mechanism for acid-soluble metal sulfides such as sphalerite (ZnS), chalcopyrite (CuFeS$_2$), and galena (PbS). In the thiosulfate mechanism, solubilization is through a ferric iron attack on the acid-insoluble metal sulfides, with thiosulfate being the main intermediate and sulfate the main end-product. Using pyrite as an example, the reactions proposed by Schippers and Sand are

$$FeS_2 + 6Fe^{3+} + 3H_2O \rightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+$$
$$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \rightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+.$$

In the case of the polysulfide mechanism, solubilization of the acid-soluble metal sulfide is through a combination of ferric ions and protons, with elemental sulfur as the main intermediate. This elemental sulfur is relatively stable but may be oxidized to sulfate by sulfur-oxidizing bacteria. ZnS is an example of an acid soluble mineral:

$$MS + Fe^{3+} + H^+ \rightarrow M^{2+} + 0.5H_2S_n + Fe^{2+} (n \geq 2)$$
$$0.5H_2S_n + Fe^{3+} \rightarrow 0.125\ S_8 + Fe^{2+} + H^+$$
$$0.125\ S_8 + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+.$$

As [20] pointed out, this explains why strictly sulfur-oxidizing bacteria such as *A. thiooxidans* are able to leach some metal sulfides but not others. The role of the microorganisms in the solubilization of metal sulfides is, therefore, to provide sulfuric acid for a proton hydrolysis attack and to keep the iron in the oxidized ferric state for an oxidative attack on the mineral. The break-up of the pyrite crystal structure can be the result of one of five mechanisms:

(a) reaction of protons with a sulfide to give SH− ions;

(b) extraction of electrons from the sulfide valence band

resulting in the release of metal ions and sulfur compounds (in the case of *A. ferrooxidans*);

(c) broken chemical bonds already present in the sulfide (p-type conduction), which leads to higher interfacial dissolution;

(d) reaction with a polysulfide or metal complex-forming agent; or

(e) electrochemical dissolution resulting from multiple electron extraction and depolarization of the pyrite, which occurs at high concentrations of ferric ions (in the case of iron-oxidizing bacteria like *L. ferrooxidans*) [26].

4. Potentially Important Bacteria in Bio-mining Processes

4.1. Sulfobacillus and Relatives

Sulfobacilli are moderately thermophilic (40°C-60°C), endospore-forming, gram-positive bacteria that have been isolated from heaps of mineral waste and bio-mining operations. These bacteria are able to grow autotrophically or heterotrophically. When growing autotrophically they use ferrous iron, reduced inorganic sulfur compounds, or sulfide minerals as electron donors. However, their ability to fix CO_2 appears to be poor. To grow strongly, they require elevated levels of CO_2 in the atmosphere, small quantities of yeast extract, or close association with heterotrophic iron-oxidizing bacteria such as *Acidimicrobium ferrooxidans* of the consortium of microorganisms that are used commercially to oxidize a variety of sulfide minerals in the temperature range 45°C-55°C (possibly as high as 60°C).

4.2. Ferroplasma and Relatives

These organisms are archaea rather than bacteria, are pleomorphic in shape, and lack cell walls. *Ferroplasma acidiphilum* was isolated from a pilot plant bioreactor treating arsenopyrite/pyrite in Kazakstan [28]. It oxidizes ferrous iron but not sulfur and appears to be obligately aerobic. The archeaon is mesophilic, growing optimally at 33°C with an upper limit of 45°C. It has an optimum pH for growth of 1.7 and a lower limit of about 1.3. A closely related mixotrophic archeaon, *Ferroplasma acidarmanus*, was isolated from acid mine drainage [29]. Similar archaea have been isolated from commercial bioreactors also treating an arsenopyrite/pyrite concentrate operating at 40°C at the Fairview mine in Barberton, South Africa. These organisms appeared in large numbers when pH control of the reactor was removed and the pH was allowed to fall in an unrestrained manner to about pH 0.5.

4.3. Sulfolobus

The metabolic capability of microorganisms designated as Sulfolobus isolates is confusing because many early studies were carried out with impure cultures [30]. Many of the most important mineral bio-oxidation studies have been carried out in laboratories using a strain known as Sulfolobus strain BC, now known to be an isolate of *Sulfolobus metallicus* [31]. These obligately autotrophic archaea grow by oxidizing ferrous iron, reduced inorganic sulfur compounds, or sulfide ores. *S. metallicus* is thermophilic, with strain BC growing at an optimum of 68°C and preferably within a pH range of about 1.3-1.7. *S. metallicus* is very capable of oxidizing minerals such as arsenopyrite and chalcopyrite, especially if the air is enriched with 1% v/v CO_2 [30].

4.4. Metallosphaera

These archaea are aerobic iron- and sulfur-oxidizing chemolithotrophs that are also able to grow on complex organics such as yeast extract or casamino acids but not sugars. The species most frequently described in the context of mineral sulfide oxidation is *Metallosphaera sedula*. *M. sedula* has been reported to grow at pH 1.0-4.5 and is able to oxidize a variety of minerals at temperatures of 80°C-85°C. Metallosphaera-like organisms have been reported to be potentially the most efficient at high-temperature bioleaching of recalcitrant chalcopyrite ores [30].

4.5. Acidianus

There are several species of this group of archaea that oxidize minerals, although the industrial potential of this group is thought to be less promising than that of *Sulfolobus* and *Metallosphaera*. *Acidianus brierleyi* can grow autotrophically by oxidizing ferrous iron or sulfur, or grow heterotrophically on complex organic substrates. The optimum temperature is 70°C and the optimum pH is 1.5-2.0. *Acidianus infernus* and *A. ambivalens* are obligate chemolithotrophs that can grow either aerobically or anaerobically by the oxidation or reduction of inorganic sulfur compounds. *A. infernus* has an optimum temperature of 90°C and an optimum pH of 2.0.

5. Challenges in Applying Bio-mining Technology

While bio-mining enjoys a considerable success, technical and commercial challenges also hinder it from achieving its full potential as an applied technology. Until recently dump bioleaching has been applied much as it was 50 years ago with minimal research and development aimed at understanding the microbial populations in dumps or how to enhance the performance of dump operations. This inattention was likely the result of low copper prices and that dump bio-leaching was

an adjunct process to smelting, which was the primary source of revenues for copper operations. The material in many dumps also contained a percentage of chalcopyrite and because of the difficulty of leaching chalcopyrite, it has largely been assumed that the copper associated with chalcopyrite would not effectively leach in dumps. More recently many copper producers have realized that some chalcopyrite is indeed being leached in dumps and with this realization more substantive efforts have been made lately to understand how this occurs and how it can be enhanced.

The large underlying deposits of low-grade chalcopyrite are typically low-grade and are therefore not always amenable to conventional flotation and smelting practices. These deposits will likely have to be exploited using crushed ore heap bioleaching of the higher-grade materials with adjacent, run-of-mine dump bioleach operations to extract copper from the lower grade materials.

6. Summary and Conclusions

The use of microbes to extract metals from ores is now well established. Heap-leach and stirred tank processes that operate at ambient or temperatures of 40°C-50°C or less will continue and will be applied to new types of minerals. Exploration of the molecular biology of these organisms has begun with *A. ferrooxidans*, but studies of other, probably more important, players such as members of the leptospirilli and *A. caldus* are in their infancy. Much of the future of bio-mining is likely to be hot. Development of processes that operate at 70°C or above will make the recovery of metals from a large number of minerals economically attractive where otherwise the reaction rates are too slow. Many of these will be stirred tank-type processes, although high-temperature irrigation-type processes in which support material is coated with a mineral concentrate may also prove to be economical for copper recovery from chalcopyrite. At increased temperatures a variety of largely unstudied archaea will become important [32]. Unlike mesophiles, where iron- and sulfur-oxidizing organisms are ubiquitously associated with mineral deposits and can be readily enriched, research on thermophiles will require screening of microbes from high-temperature environments in which suitable organisms are likely to be found.

Isolation of organisms with the right combination of iron- and sulfur-oxidizing ability together with the ability to withstand abrasion (stirred tank processes) and resistance to high concentrations of metal ions is a challenge. Nevertheless, candidate organisms have already been found and are largely unexplored territory. These high-temperature organisms present a number of challenges in regard to standard microbiology techniques such as plating, incubation, and

preparation of pure cultures. Challenges such as the isolation of mutants and the selection of transformants also apply to studies on the molecular biology and genetics of these high-temperature organisms. New engineering, and especially new molecular biology tools (genomics and proteomics), make this an exciting field to investigate.

7. Future Outlook

While the demand for most metals has steadily increased in the last decade, discoveries have declined [33] and those deposits that are being discovered are declining in quality. Processing options for lower grade ore deposits and deposits of lower quality with complex polymetallic mineral assemblages are limited. Bio-mining technologies are particularly adept at technically and economically processing these types of resources. Mining companies are aware of bio-mining's unique niche and chalcopyrite heap bioleaching is already undergoing pilot- and demonstration-scale opportunities for the commercial application course, continue to be of stirred-tank bio-oxidation of sulfidic-refractory gold concentrates, because that technology has been effectively marketed for over two decades and has competed well with pressure oxidation and roasting.

Recent OMICs (genomics, proteomics, transcriptomics, metabolomics) advances have greatly helped the study of individual members of the bio-mining microbial community to understand better the mechanisms used to grow and adapt to their harsh environment [1]. This in turn would allow to genetically modify the entire extremophilic microbiome to obtain much more efficient and controlled metal concentration processes, redefining the current metal extraction sequence [34]. Because future bio-mining applications will likely be directed more on lower-grade, lower-quality, complex ores, it is important that research and development focus on the technical issues associated with these bio-mining applications. Such studies should address those issues described earlier: understanding how the different temperature groupings of microbes colonize and function within coarse ore heaps; engineering coarse ore and run-of-mine heaps to effectively exploit microbial development and activities including irrigation, aeration and heat management [35].

For bio-mining technologies to be more widely applied commercially they have to be demonstrated at scale. To achieve this there must be cooperation among the mining companies who own and exploit the deposits, and universities, government laboratories, biotechnology companies and engineering companies that develop the technologies.

Bio-mining patents are complicating the commercial development of bio-mining. Biotechnology companies have a reasonable right to be financially rewarded for their

innovations, yet the most costly and most risky part of developing bio-mining technology is demonstrating the technology at scale and the mining companies assume both the cost and the risk for this. Mining companies, too, have an obligation to protect their rights to use technology that they have developed. However, mining companies are not absolved of blame in the patent morass, because their patents effectively hinder the use of the innovations by other mining companies unless cooperative agreements are made. It is unclear how the situation can and will ultimately be resolved.

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