



# How Biomining has been Used to Recover Metals from Ores and Waste? A Review

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## Abstract

The recovery of metals present in low-grade ores and waste of electrical and electronic equipment (WEEE) is essential for the development of new technologies and the supply of existing production chains. Conventional recovery processes are energy-intensive, non-selective towards some metals, and mostly they are not eco-friendly. Thus, biomining appears as an interesting alternative, because microorganisms are used to promote the bioleaching or bio-oxidation of metals present metal-rich materials under mild conditions of pressure and temperature. Biomining can be industrially applied, but it still needs some improvement such as diminishing the time required, the robustness and reliability of biological systems, and the optimization of process parameters. In this review, we present the current frontiers in biomining scale-up and some future perspectives, a brief discussion about microorganisms involved in the mining processes to several ores, and biochemical reaction mechanisms in bioleaching and bio-oxidation processes.

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## Introduction

Due to industrial expansion and the rapid growth of the global population in the last century, the need for metals has been increased. Pyro and hydrometallurgy are considered the most traditional established physical-chemical processes for metal extraction from mineral sources [1]. However, some aspects evidence the environmental risks related to mining activity [2]. Mining waste can potentially leave negative environmental, social, and economic marks for many years, including soils and water bodies contaminated by heavy metals from acid mine drainage [3], as evidenced by the Rio Tinto in Spain, in which 4500-year-old mining pollution of water can still be observed [4].

In general, thermal-based methods are used to extract metals from raw materials, generating a considerable amount of wastes, large carbon footprints, emission of pollutants, and erosions [5]. Hydrometallurgy, for example, generates effluents composed of inorganic acids and bases, and heavy metals [6,7]. Minerals pre-treatments, such as roasting and smelting, release carbon dioxide and sulfur compounds [8,9]. Also, Anjum et al. [1] and Erüst et al. [10] emphasized the poor waste management in mining activity.

In this scenario, biomining is an alternative method to extract metals assisted by microorganisms [11,12]. It is estimated that approximately 20% of copper, 5% of gold, and lower amounts of nickel, cobalt, uranium, and zinc, are extracted by biomining processes [13]. Despite the advantages of biomining, advanced studies are required to confirm its economic viability compared to the non-biological process. Figure 1 describes the characteristics of metallurgy techniques.

The term biomining is commonly related to the extraction, processing, detoxification, and recovery of metals by microbiological activities, from ores, tailings, and non-sterile waste materials (e.g., industrial, and waste of electrical and electronic equipment). In the literature, the biomining process is referred to as biohydrometallurgy, bioleaching, and bio-oxidation [12,14]. These processes were reported in the middle of the 20<sup>th</sup> century, with the isolation of a bacterium from acid mine drainage, which plays a fundamental role in the

Fe<sup>2+</sup> and sulfur minerals oxidation [15]. However, the first use of biohydrometallurgy was only in 1950, at the Kennecott copper mine in Salt Lake City, Utah (United States of America) [16].

Biohydrometallurgy is based on two basic principles: bioleaching and bio-oxidation. While the first concept is the conversion of usually insoluble metals present in ores (or tailing) into water-soluble forms [10,11], the second relies on the decomposition of the mineral matrix by microorganisms that encapsulates the metal species, promoting its subsequent extraction [13]. The main biomining mechanism is via Fe-S oxidizing microbes, in which ferrous ions change to ferric ions, leading to sulfuroxidation, solubilizing it from minerals [17]. Otherwise, some microorganisms are capable of oxidizing metallic species on a solid mineral surface, generating water-soluble compounds. This unusual metal solubilization process is due to the production of organic acid compounds from the microbiological metabolism, which could serve as an electron acceptor [18]. Bioleaching is mainly used to recover base metals like cobalt, nickel, copper, and zinc. Usually, base metals are extracted from insoluble sulfide ores [12]. On the other hand, bio-oxidation processes aim to retrieve precious metals such as gold, silver, and uranium from oxides. In this case, bioleaching is applied only to remove interfering or toxic metals sulfides from ores containing the precious metals [13,19].

Most of the precious and base metals are occluded within sulfide minerals. In this case, the use of chemolithotrophic microorganisms to promote biomining is more appropriate since they can recover metals from low-grade ores containing mineral sulfides [21]. The most common sulfide minerals are pyrite (FeS<sub>2</sub>), chalcopyrite (CuFeS<sub>2</sub>), sphalerite (ZnS), and galena (PbS) [12,20]. Heterotrophic

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microorganisms can be also used in non-sulfide ores biomining [1]. The metabolites produced by heterotrophic microorganisms indirectly promote the dissolution of metal from ores, being used to recovery aluminum and uranium from feldspar and pegmatite minerals. Furthermore, heterotrophic microorganisms can be also used in coal ores beneficiation [19,22].

Biohydrometallurgy is quite similar to hydrometallurgy on the technical principles except for not using chemical solutions for leaching or oxidations and consumes less energy compared to the traditional processes [23]. From biohydrometallurgy consolidation, several technologies have been explored commercially and studied on pilot plants, aiming to solubilize and extract metals. Also, these processes had at its core environmental issues, due to stricter regulations in the 1990s, to the higher energy consumption associated with mining from raw materials in ores, and to economic issues, aiming to reduce the treatment costs of mining wastes [24,25].

As high-grade metal reserves were practically depleted over time, the need to explore low-grade ores, refractory ores, mine tailings, and urban wastes (e.g. waste of electrical and electronic equipment) has increased in recent years [1,13,26,27]. In this context, this review describes the current state of biomining methods for metal extraction from low-grade ores as well as recent advances in the industrial biomining process, the techniques developed, and biochemical reaction mechanisms.

## Industrial Biomining Process

Several biomining processes are industrially available and its appropriate selection depends on the ore type, geographical location, metallic content, and specific minerals present - oxides or sulfides [28]. Among the available biomining configurations, the ones that most stand out are: dump, heap, and stirred tank reactors.

Dump bioleaching is an exponent in the copper extraction industry, especially from ores that contain metal low-grade (usually 0.5% w/w).

It is characterized as being a highly economical recovering method owing to the large quantities of cupric ores that can be processed and the low production costs [14,29]. These dumps are quite deep and contain tons of run-of-mine [30]. For this reason, the methodology of dump bioleaching involves pouring acidified water on top of the dumps, causing the acidic liquid percolation in the ore and making the environment conducive to microorganisms' growth and establishment [13]. Usually, this technique is applied in the recovery of Co, Sn, Mo, Ni, and Au [31].

In situ biomining is cited as a variation from dump bioleaching, which explores underground mines for uranium extraction, for example, applying acidic solution from the surface to the underground and pumping the resulting solution back to the surface for later recovery of the metal. In situ operations requires ore already permeable [32]. For this type of methodology, recovery rates of ~75% are achieved [33].

Heap bioleaching is another biomining process widely used (see Figure 2) in the recovery of Co, Cu, Mo, Ni, U, Au, and Zn [31]. Firstly, the ores are crushed into fine particles using rotational drums with acidified water aiming to create optimal conditions for the microorganisms of interest so that bio-oxidation proceeds normally. Then, minerals are carried out in blocks that are stacked with high-density polyethylene or polyvinyl chloride, improving the material drainage and the acidic liquid percolation. Sulfuric acid or cyanide is generally used at this stage. After solvent extraction to recovery soluble metals, a weak acid effluent - raffinate - is generated, which is recycled to the next cycle as acidifying component [14,29,34]. In the heap methodology, aerators are also applied for improving the insertion of oxygen and, consequently, increasing the bioleaching rates [33,35].

The efficiency of heap leaching for copper recovering varies according to the conditions applied, but it is possible to recover about 60% of Cu in an average period of 30-48 days to achieve these results,

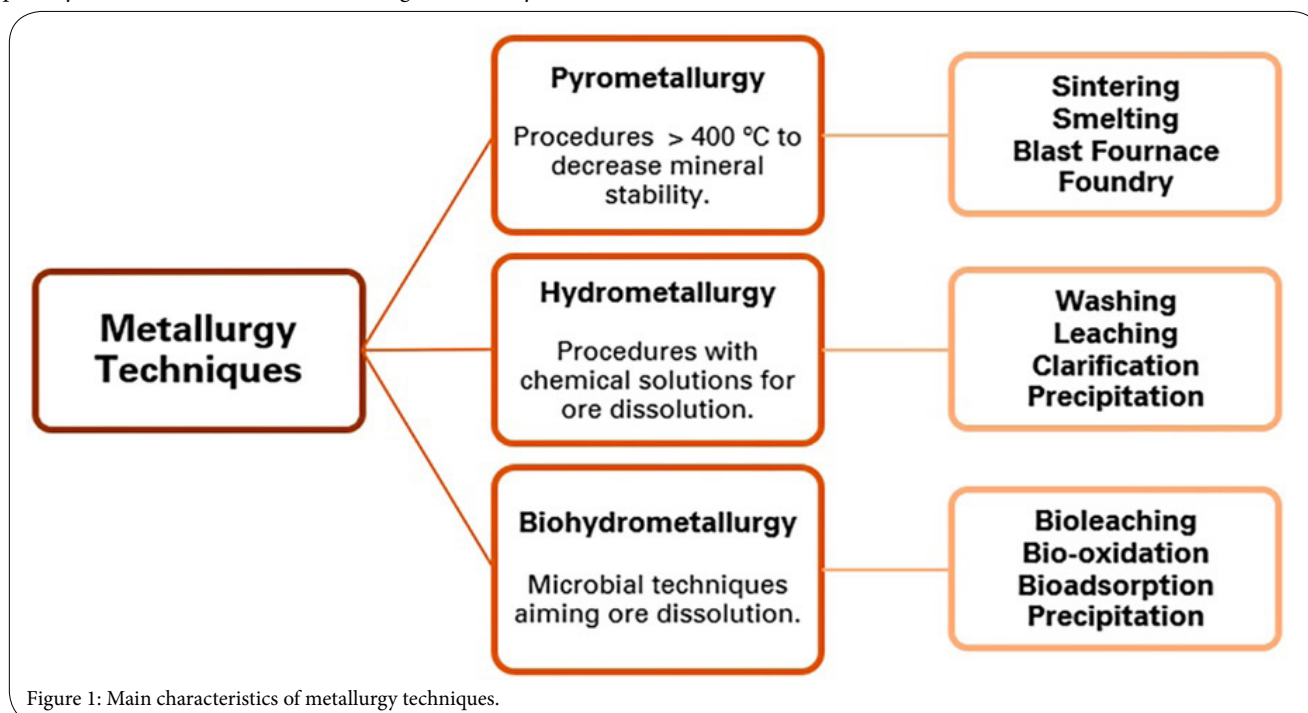


Figure 1: Main characteristics of metallurgy techniques.

with a temperature at 45°C [36]. Also, a copper recovery of 73% at 25°C can be achieved, but in a period three times longer. Above all, this method presents advantages such as a rapid start-up, consumes less water than conventional dump leaching (0.3 tons of water for 1 ton of ore) [37], commissioning of well-established operations, low capital and operating costs, in addition to toxic emissions absence. On the other hand, this process is slightly slower than the other options presented above [29].

The perspective of stirred tanks reactors (STR) for bioleaching arises because both heaps and dumps processes have limited aeration rates and less possibility of control [38]. However, due to its higher cost, the stirred tank reactor technique is more appropriate for highly concentrated ores with greater value. Also that this method presents higher recovery rates when compared to other bio-hydrometallurgical processes in the same period [18,39].

An example of STR large-scale use lies in sulfide gold mines since approximately 5% of the worldwide golden extraction occurs by using this methodology [13,14]. However, this technique has been also used in the recovery of Co, Cu, Mo, Ni, U, and Zn. The STR is equipped with agitators that affect aeration in the tank and retain the suspension of fine ores particles concentrates, also ensuring oxygen and carbon dioxide satisfactory transference to the microorganisms solution (included after prior inoculation) [12]. According to Brierley [13], tanks of over 1000 m<sup>3</sup> are used and the complete bio-oxidation takes about 3 to 5 days. It is worth mentioning that the volume processed by STR is significantly smaller in comparison to dump and

heap bioleaching, thus justifying the high process yield rates achieved [40]. In addition, depending on mineral concentration, work volume, or operation type, it can be used reactor tanks connected in parallel, increasing the operational cost [32].

## Microorganisms

Microorganisms are responsible for the recovery of metals in biomining processes. Biomining occurs owing to the energy required for microbial metabolic functions to be obtained from organic compounds, inorganic elements, or oxidation compounds, depending on microorganism strains and ores types [10]. Autotrophs take advantage of metals and sulfur as electron donors in the medium obtaining energy, while dissolved oxygen acts as an electron acceptor. Conversely, heterotrophic microbes use carbon as an energy source and generate acid by-products that help to lower the pH and solubilize metals occluded in minerals [1,19,39,41].

Most of the strains applied in sulfide metal ores biomining are acidophilic microorganisms that can oxidize sulfur - which usually generates sulfuric acid - and ferrous ion to iron-ferric when Fe is present [42]. However, the thermotolerance of leaching bacteria and fungi may vary from mesophilic (20 - 40 °C), moderately thermophilic (40 - 60 °C) to thermophilic (above 60°C). They also may differ according to their interaction with the metal, that may be by adsorption, bioaccumulation, bioprecipitation, among others [13,23,31,43].

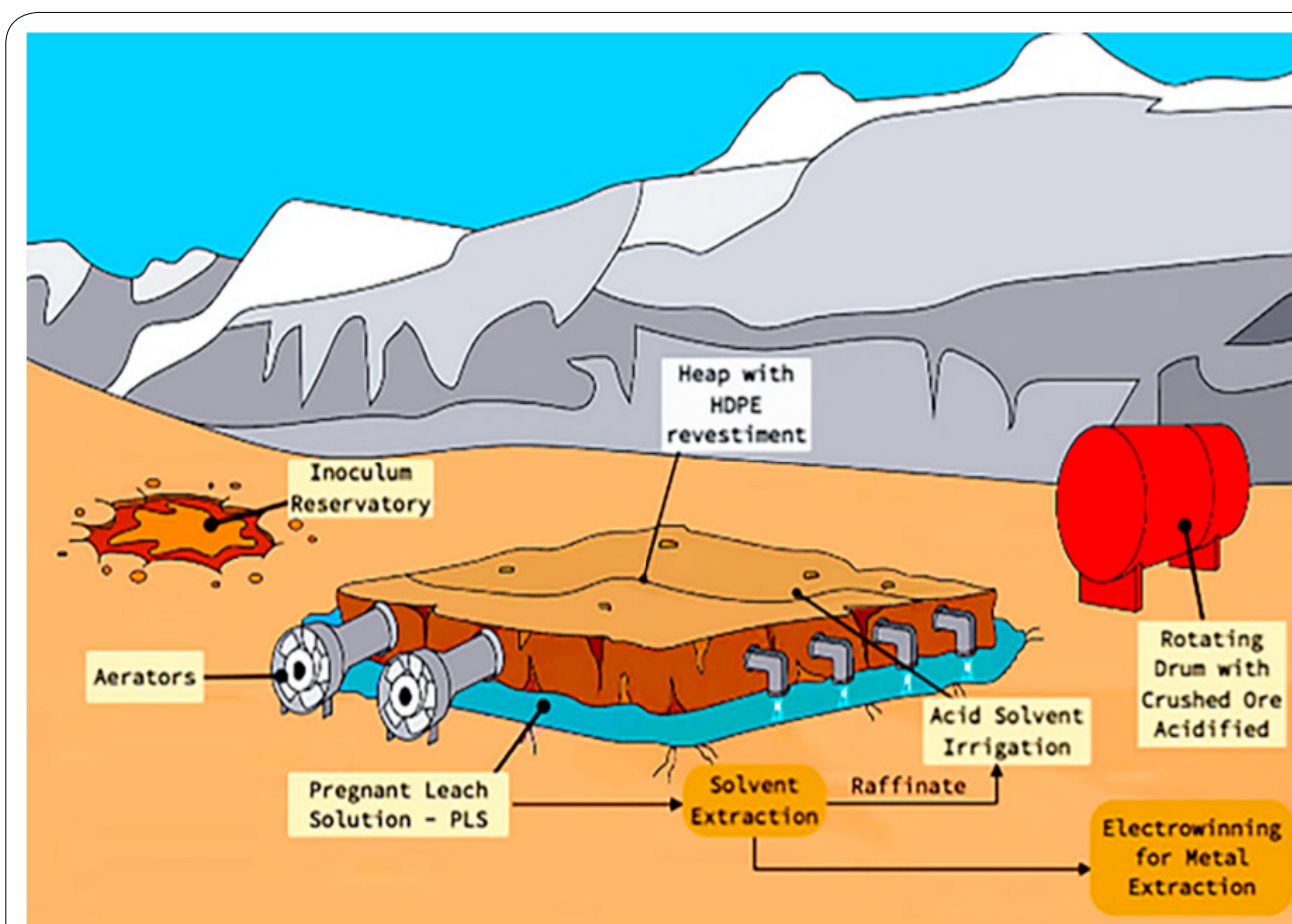


Figure 2: Heap leaching illustrative scheme. Based on Jerez [43].

Among the microorganisms used in biomining, the bacteria *Acidithiobacillus* stand out, particularly, because *Acidithiobacillus ferrooxidans* specie was the first one described. *A. ferrooxidans* was discovered in 1922 and it was isolated from AMD by Colmer and Hinkle in 1947 [44]. This specie is well-characterized and it is capable of growing in more acidic environments than most microorganisms and oxidizing iron and sulfur, justifying it as the most applied microorganism in bioleaching and bio-oxidation. *A. ferrooxidans* also can grow at higher pH values than their natural (1.5-3.0), temperatures slightly higher than their preferred mesophilic range [31,41,45].

Bacteria of, *Acidimicrobium*, *Alicyclobacillus*, *Acidiphilum*, *Leptospirillum*, and *Sulfobacillus* are quite common in the biomining process [32,46]. Particularly, some *Leptospirillum* species, e.g. *Leptospirillum ferrooxidans*, have optimum growth pH ranging between 1.5 -4.0, in moderately thermophilic conditions, and usually grow in environments with ferric and ferrous ions, but cannot oxidize sulfur and sulfur compounds such as thiosulfate. It is also worth mentioning that bacteria from Archaea species are recurrent in high-temperature bioleaching procedures, for example, *Sulfolobus* and *Metallosphaera* [10,12,45].

All those strains are generally autotrophic. Nevertheless, heterotrophic microorganisms are also present in non-sulfide ores [39]. For these microorganisms, carbon sources are necessary both for obtaining energy and for actions on the mineral surface that occludes the metals of interest. Such actions occur due to metabolites produced by carbon consumption, resulting in the release of organic acids such as citric acid, acetic acid, and sulfuric acid. Among those bacteria, several works emphasize *Bacillus* and *Pseudomonas* strains, while fungi, such as *Aspergillus* and *Penicillium* are used in biomining [1,19,47]. Table 1 [48-54] shows a list of the most recurrent microorganisms in the literature and some of their relevant characteristics for biomining.

## Bioleaching Mechanisms

Regardless of biomining type, the bioleaching pathways can occur through direct or indirect mechanisms. However, there are divergences in the literature regarding the existence of a direct contact mechanism.

In general, it is considered that there is only an indirect mechanism with contact, non-contact and cooperative sub-mechanisms [59], as illustrated in more detail in Figure 3.

In contact sub-mechanism, microbial adhesion to the mineral surface is intermediated by the extracellular polymeric substances (EPS) layer. In this layer, ferrous ions EPS restrained are oxidized into ferric ions in order to dissolve the ore surface which occludes the metal [17,60]. However, EPS adhesion does not occur on the whole microbial surface and it is attributed to metal solubilization electrochemical interactions [1,61].

At non-contact sub-mechanism, microbes reduce aqueous ferrous ions to ferric ions, and these are responsible for the chemical oxidation of the ore surface, making it an effective agent for mineral solubilization [61]. The medium must have a low pH value to make it possible to keep the iron in solution, since the ferrous ion generated at the end of the reaction can be transformed into ferric ion again, recycling it and continuing the process of indirect oxidation [12].

Cooperative sub-mechanism has been described only in sulfide minerals. There is sulfur colloids dissolution, sulfuric intermediates, and various minerals fragmented by planktonic bacterial cells, suggesting multiple bacteria patterns in the same environment. In this case, bioleaching occurs both through microorganisms' EPS - which adhere to the fragmented minerals - and through iron ions in colloid and sulfur intermediates solution [12].

Another particularity related to the dissolution of sulfide metals is that this process is performed due to the attack of protons in an acidic environment and oxidation that releases the occluded metals in ores. In this panorama, there are two possible mechanisms for dissolution reactions: thiosulfate and polysulfide pathways (see Figure 4). The pathways are determined according to minerals type, the formation of different valence bands, and through the mineral solubility in acidic environments (reactivity with H<sup>+</sup>) [32,60,62]. The thiosulfate pathway is the mechanism for minerals that are not soluble in acidic environments and end up forming valence bands only with electrons of metal atoms. The thiosulfate pathway is commonly used in the dissolution of sulfide minerals such as pyrite (FeS<sub>2</sub>),

Microorganism	Autotrophic (A)/ Facultative Autotrophic (F) Heterotrophic (H)	Temperature Range	Main Leached Metals	References
<b>Bacteria</b>				
<i>A. ferrooxidans</i>	A	Mesophilic	Al, Cu, Fe, Ni	[1,48]
<i>A. Caldus</i>	A	Moderate Thermophilic	Cu, Fe, Ni	[12,35,51]
<i>L. ferrooxidans</i>	A	Mesophilic	As, Cu, Fe	[35,50]
<i>S. thermosulfido oxidans</i>	F	Thermophilic	Cu, Fe	[55,56]
<i>Pseudomonas</i> sp.	H	Mesophilic	Al, Li, Si	[52]
<b>Archeae</b>				
<i>S. metallicus</i>	A	Thermophilic	Cu, Fe	[57,58]
<i>F. acidiphillum</i>	H	Moderate Thermophilic	Fe	[35]
<b>Fungi</b>				
<i>A. niger</i>	H	Mesophilic	Co, Cu, Ni, Mn, Zn	[1,46,53]
<i>Penicillium</i> sp.	H	Mesophilic	Au, Fe, Mn	[19]
<b>Yeast</b>				
<i>Candida</i> sp.	H	Mesophilic	Au	[19]

Table 1: Some microorganisms applicable in bioleaching and bio-oxidation processes for several metals.



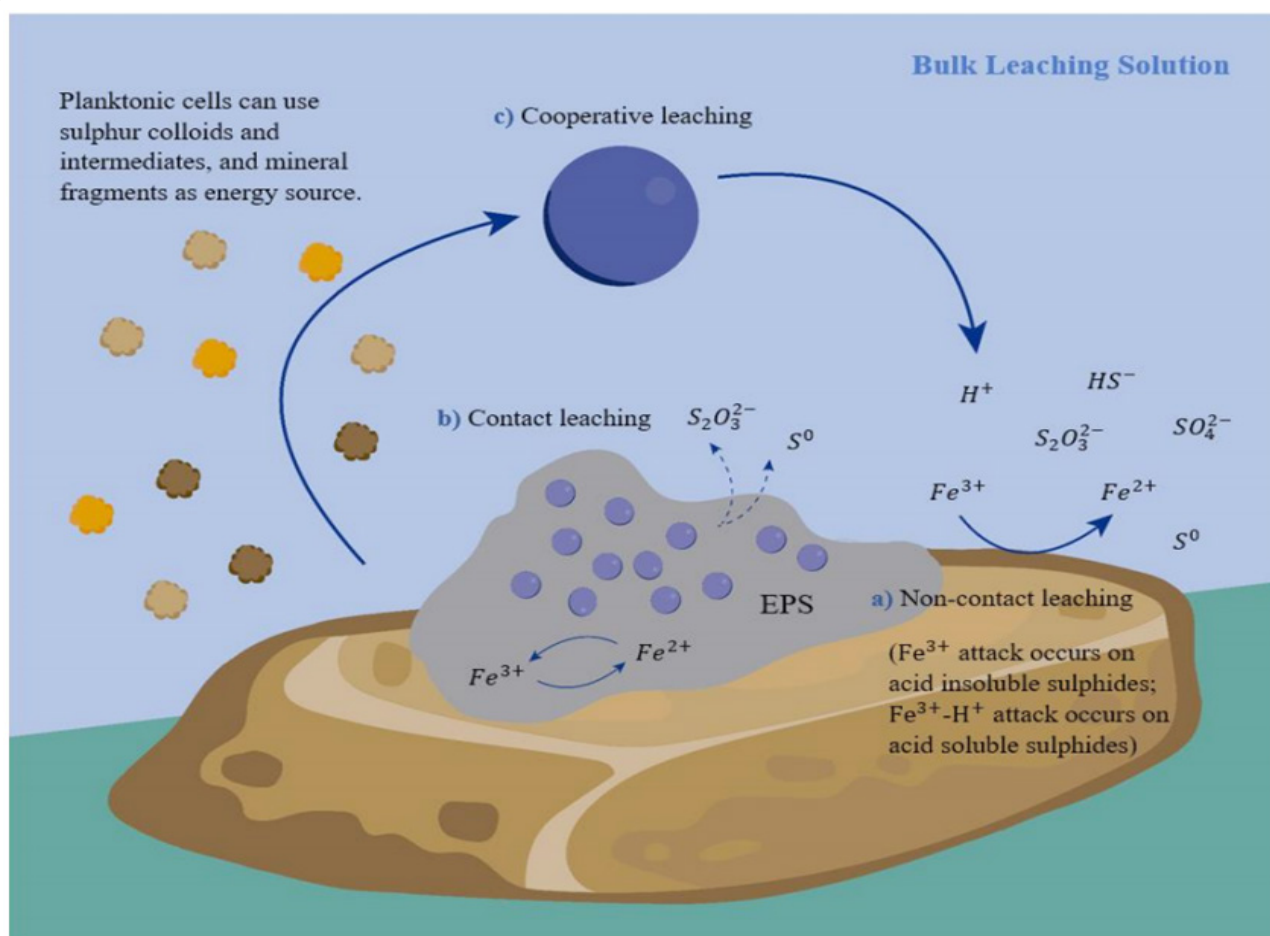


Figure 3: A comparative of leaching mechanisms. Adapted from Mahmoud et al. [12].

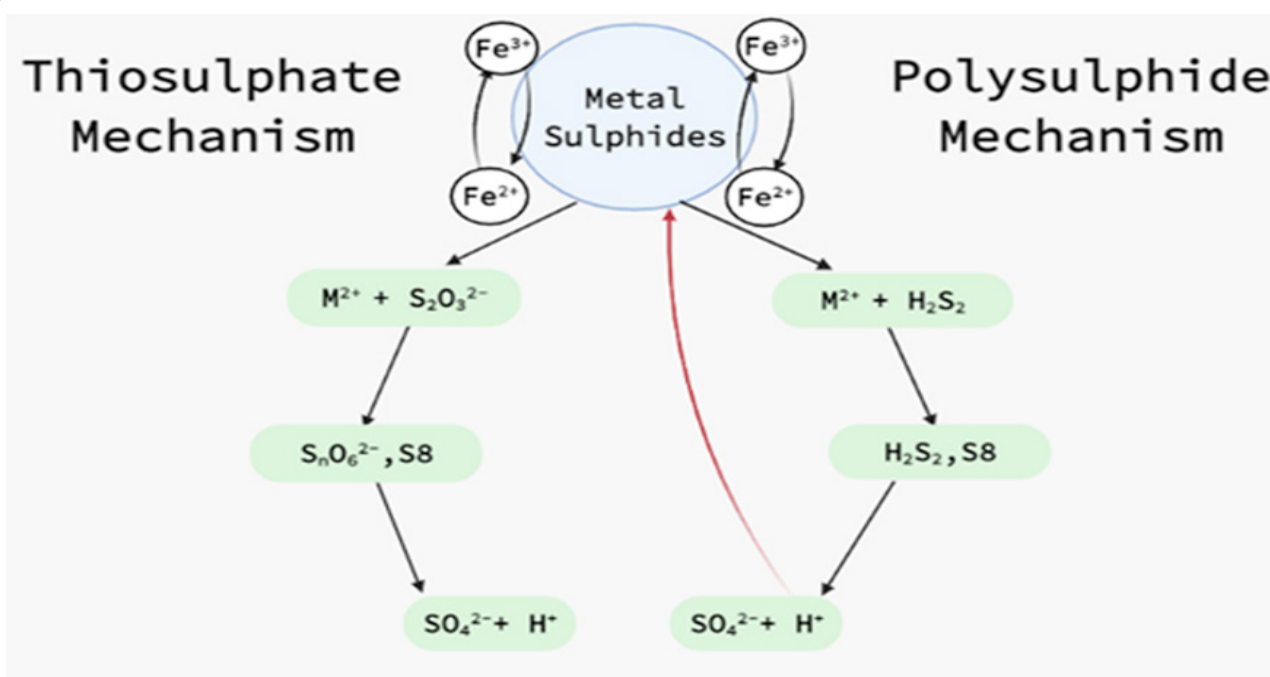


Figure 4: Thiosulphate and polysulphide mechanisms. Based on Rohwerder [62].

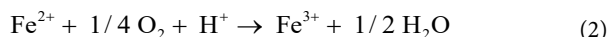
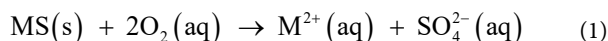
molybdenite ( $\text{MoS}_2$ ), and tungstite ( $\text{WS}_2$ ). Conversely, minerals that have their valence bands shared between metals and sulfur, and are directly soluble in acidic environments, follow the polysulfide pathway. Generally, this is what occurs with most sulfuric ores such as sphalerite ( $\text{ZnS}$ ), galena ( $\text{PbS}$ ), chalcopyrite ( $\text{CuFeS}_2$ ), and hauerite ( $\text{MnS}_2$ ) [59,61,62].

In the thiosulfate pathway, metals are extracted through the electroextraction of ferric ions present in the disulfide part where, through a sulfonic acid group, sulfur-metal bonds are broken, and ferrous and thiosulfate ions ( $\text{S}_2\text{O}_3^{2-}$ ) are formed [62]. The thiosulfate also goes through the following stages of the metabolic pathway: (i) oxidation to tetrathionate, (ii) degradation in smaller sulfur compounds, and (iii) transformation into sulfate ion [59].

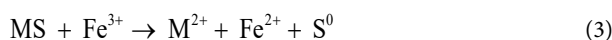
Low pH levels favor the polysulfide pathway action since, in this case, the metal is solubilized by the combination of both proton attack and oxidation of ferric ions. Therefore, elemental sulfur is released into the reaction medium, is oxidized by bacteria capable of carrying out this type of reaction and, subsequently, is transformed into sulfuric acid, decreasing the pH of the reaction medium [12,32,59].

The bioleaching mechanism is accomplished by microorganisms that obtain the required energy to grow from ferrous iron and mineral sulfides oxidation. These reactions can occur in both contact and non-contact mechanism already mentioned earlier [59].

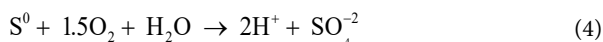
The global bioleaching reaction of a bivalent metal (M) in a mineral sulfide is given by Equation (1) and corresponds to an oxidation process [1,10,32]. Additionally, ferrous ions are oxidized to ferric species, given by Equation (2).



Ferric ions produced in the contact mechanism reactions promote the oxidation of the sulfide mineral, releasing  $\text{Fe}^{2+}$  ions to the medium, in a non-contact mechanism, given by Equation (3). Ferrous ions produced are further oxidized in the contact mechanism (Equation 2) [10,63].



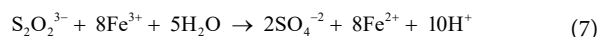
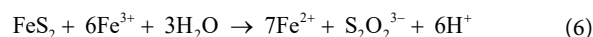
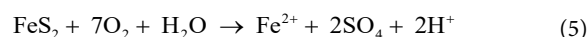
The elemental sulfur ( $\text{S}^0$ ) produced in the mineral sulfide oxidation by ferric ions are converted into sulfuric acid, given by Equation (4), responsible for the medium pH decreasing. The  $\text{H}^+$  protons released during acid generation are consumed in ferrous ions oxidation (Equation 2).



Due to the acid generation, acidophilic microorganisms are employed in biomining and also for bioremediation of removal of heavy metals, since they can grow on strongly acid conditions as well as high metal concentration [43].

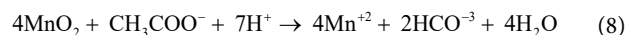
Equation (5) gives the oxidation global reaction of iron pyrite, one of the most abundant mineral sulfides. In this mechanism, iron pyrite ( $\text{FeS}_2$ ) is oxidized by ferric ions, with the formation of thiosulfate ( $\text{S}_2\text{O}_3^{2-}$ ) and ferrous ions (Equation 6). Thiosulfate is oxidized into sulfates ( $\text{SO}_4^{2-}$ ) by ferric ion (Equation 7). The ferrous ions intermediates are

further bio-oxidized to generate energy for microbial growth [31] (Equation 2).

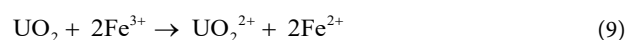


About non-sulfide minerals, bioleaching mechanisms are different (see Figure 5). These ores types are commonly bioleached by heterotrophic microorganisms which generate acidic metabolic by-products that interact with the mineral surface. Among these organic acids, gluconic, malic, succinic, citric, and oxalic stand out, which have the power to surround metals of organic compounds - organometallic complexes - by different types of chemical reactions, such as protonation and chelation [10]. Organic acids adhere to the mineral surface and extract metallic elements to be solubilized through electron transfer. Dissolution is facilitated by the generation of imbalance of anions and cations owing to the presence of these acids in the reaction medium [64]. Acid clusters can provide both protons and anions, assisted by EPS formed by bacteria and fungi, but also amino acids and proteins that aid in metallic solubilization. Also regarding acids, citrate and oxalate anions can form the most stable organometallic complexes with several metals, and oxalic acid has the ability to leach especially aluminum and iron [63,65].

About metal oxide ores, sulfide phases are inexistent because sulfur is absent. Mostly heterotrophic organisms cause metal solubilization from ores through metabolites release of organic acids. A simplified example is an action of acetate on pyrolusite ( $\text{MnO}_2$ ) as presented in Equation (8) [66]:



In some cases, the metal oxide is associated with iron ore deposits. As demonstrated in bioleaching equations, ferric iron is generated by catalysis micro-organic action and is capable of solubilizing metals. It is recurrent with uraninite ( $\text{UO}_2$ ), which ferric ion oxidizes uranium, making it soluble, and could be exemplified by Equation (9):



## Biohydrometallurgy Applications

### Sulfide and non-sulfide ores

As previously stated, biomining mechanisms and the microorganisms used in the process depends on the chemical characteristic of ores. Low-grade sulfides ores consist of the most ore group explored in the biomining process [29]. In this context, cupric extraction from sulfide ores has been widely studied. Some copper ores included chalcopyrite ( $\text{CuFeS}_2$ ), enargite ( $\text{Cu}_3\text{AsS}_4$ ), chalcocite ( $\text{Cu}_2\text{S}$ ), digenite ( $\text{Cu}_9\text{S}_5$ ), and bornite ( $\text{Cu}_5\text{FeS}_4$ ) [11].

Chalcopyrite is the most abundant copper source in the world but still requires some advances for its bioleaching. Under acid conditions, the chalcopyrite dissolution rate decreases due to the passivation phenomenon. Passivation is a surface phenomenon that may occur in the presence of iron ions or polysulfide in the bioleaching pathway [13].

Due to its chemical composition, enargite bioleaching has some environmental issues since the presence of arsenic increases the toxicity of the medium [16]. Also, some microorganisms cannot grow in the presence of arsenic, limiting the process [35].

In this context, there are some biomining industrial-scale plants for copper recovering. We highlighted two mines in Chile (Escondida and Chuquicamata), which are focused on copper extraction from a mixture composed of chalcocite, chalcopyrite, and enargite. Escondida mine, the largest copper mine in the world, started to use the bioleaching process in 2006 [29,30]. It is expected to generate 200,000 tons of copper by the year 2048, becoming the biggest biomining plant in the world [67].

Chuquicamata mine produces 20,000 tons of copper per year through thermophilic bioleaching in a continuous stirred tank reactor (CSTR) [13,68]. They use the BIOCOP™ process (BHP Billiton group), based on the use of mesophilic bacteria in tanks to extracting gold from ores and bioleaching of sulfidic minerals, such as pyrite and arsenopyrite, by the well established BIOXTM process (see Table 2). This was a successful project using CSTR to recovery copper from cupric concentrates, although it was shut down for economic issues a few years later [12,30,33,69]. Other industrial processes that contribute

to the development of BIOCOP™ process are the BIONIC™ and the BROGIM™, for nickel and cobalt extraction, respectively [20].

#### Industrial waste

Biomining is not only about recovering metals from raw ores or secondary products from mining activity, but also about recovering critical metals from several wastes containing metals in their composition and that are not usually used. These alternative wastes

Plant and Location	Year of operation start	Treatment capacity (tons/day)*
Wiluna, Australia	1993	128
Fosterville, Australia	2005	211
Jinfeng, China	2007	790
Kokpats, Uzbekistan	2008	1069
Bogoso, Ghana	2007	820
Sansu, Ghana	1994	960

Table 2: List of mining plants currently operating with BIOXTM. \*In 2013. Based on Mahmoud et al. [12].

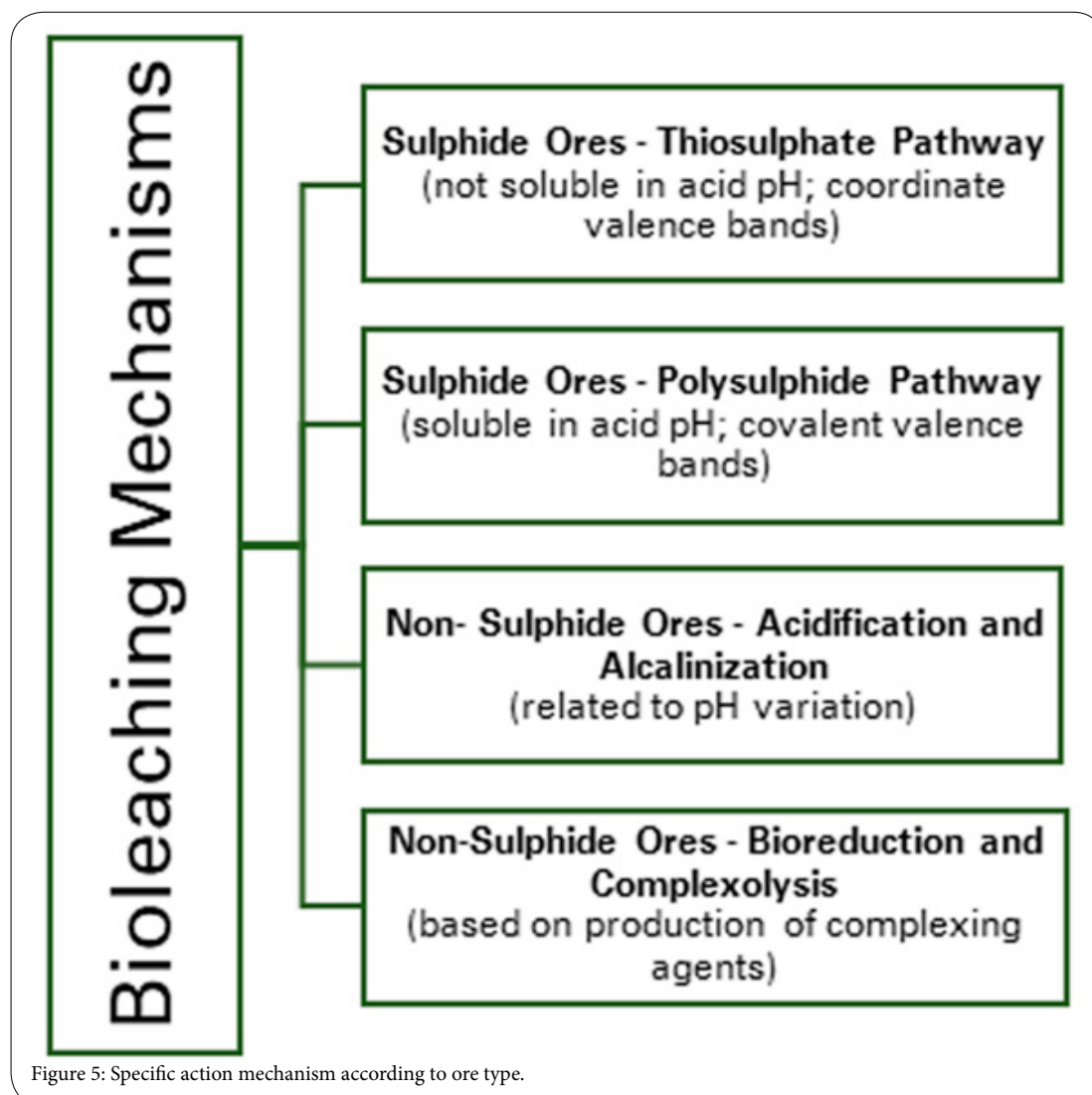


Figure 5: Specific action mechanism according to ore type.

can be from different commercial activities, e.g., contaminated sediments, industrial waste as chemical catalysts, or urban waste [19,70,71]. Table 3 shows several solid industrial wastes and the main metals leached from them [70].

Solid Mineral Waste	Bioleached Metals
Battery	Li, Cd, Co, Ni
Ashes	Zn, Al, Cd, Cu, Ni, Cr, Pb, Mn, Fe
Tannery	Cr
Sludge	Cu, Ni, Zn, Cr
Used cracking and hydroprocessing catalysts	Al, Ni, Mo, V, Sb
Steel and copper slag	Zn, Fe, Cu, Ni
Jewellery waste, automobile catalysts	Ag, Au, Pt
Wires	Cd, Cu
WEEE	As, Cu, Ni, Al, Zn

Table 3: Solid mineral wastes and major metals leached through them. Adapted from Giese, Xavier and Lins; Pryia and Hait [71,91].

Contaminating sediments originated from human activity are problematic in sanitary matters. A biomining research on dredged tailings from ships and ports was performed using a microbial consortium, with reducing agents and oxidizing agents for iron and sulfur, making it possible to recover more than 90% Cu, Cd, Hg, among other metals [72], and about 45% of heavy metals were removed with the isolated or grouping strains of oxidizing or reducing bacteria [10].

Industrial waste is considered the most harmful for aquatic and soil contamination precisely owing to its variety, which ranges from WEEE to ashes that dissipate through the air. *Aspergillus niger* was adapted to toxic metals in different concentrations and used in the bioleaching of metals from chemical catalysts. Thus, the strain was able to tolerate 100 mg/L of Ni, 200 mg/L of Mo, and 600 mg/L of Al in culture medium and also obtained relatively high leaching rates of 78.5% Ni, 82.3% Mo, and 65.2% Al [73]. Solid waste incineration usually has high metal concentration and was one of the first urban waste to arouse interest in hydrometallurgical processes. A study using a mixed culture with sulfur-oxidizing and iron-oxidizing bacteria (*Acidithiobacillus thiooxidans* TM-32 and *Acidithiobacillus ferrooxidans* ATCC23270) promoted the recovery of 67% of Cu, 78% of Zn and 100% of Cr and Cd in a culture medium containing 1% incineration ashes [74].

### Urban waste

WEEE is still incipient in the biomining aspect. However, there is an optimist projection in the use of these materials to metal recovery (see Table 4), due to the environmental damage risk linked to its incorrect disposal and low efficiency of recycling processes [25,75,76]. WEEE is often improperly deposited in open-pit landfills, remaining for long period. The natural action of oxygen and rainwater promotes the oxidation of the WEEE, leading to acid production and consequently, to heavy metals leaching that can contaminate soil and groundwater [77,78].

Printed circuit boards (PCB) are usually one of the main WEEE raw materials for metals extraction. Besides PCB have a varied composition (metal, ceramic, and polymers), about 40% (w/w) correspond to metallic. The metallic fraction is composed by 1% (w/w) of precious

metals (Au, Cu, Ag, Zn, Ni, and Sn), which represent up to 80% of the PCB market price, and 99% (w/w) of other metals (Cu, Al, Ni, Hg, Be, Pb, and Cd) [79,80]. However, this heterogeneous and complex composition of PCB and WEEE in general also represents an obstacle for metal recovery [76].

Metals	w/w proportion (%)
Cu	10-20
Pb	1-5
Ni	1-3
Hg and Pd	1-2
Ag, Au and Pt	0.3-0.4
WEEE Equipments	Amount generated in 2019 (Mt)
Small Equipments (cameras, scales, , etc.)	17.4
Large Equipments (washing machines, electric stoves, etc.)	13.1
Temperature Exchange	10.8
Screen Monitors	6.7
Lamps	0.9

Table 4: WEEE average metallic composition and the amount generated in 2019.

Adapted from Zhao et al. and Forti et al. [16, 89].

Işıldar et al. [81] listed several studies involving the biomining of critical and valuable metal recovery from WEEE using autotrophic and heterotrophic microorganisms. Usually, bioleaching of WEEE occurs between 3-7 days at a solid-liquid ratio between 1-10% (w/v), and the efficiencies achieved range between 50% and 99%.

Several works have been focused on developing bioprocess to recovery metals from PCB. Cupric extraction from crushed PCB was studied in a stirred tank reactor, using *Acidithiobacillus ferrooxidans* and *Sulfobacillus thermosulfidooxidans* in mesophilic conditions, obtaining high recovery rates (up to 94%) [82]. PCB from smartphones was used in a bioleaching process using *Chromobacterium violaceum* to extract copper and gold, obtaining 13% and 37% of recovery rates, respectively [83].

### Biomining's economic outlook

Biomining's technique has been showing as an economic alternative comparing to pyro and hydrometallurgical methods for metal recovery, most for less energy consumption in smelting or heating steps, and chemical solutions to leach or oxidize ores or another feedstock are not needed [13]. This is more evident in bioprocesses applied in both polymetallic and low-grade ores, suggesting bioleaching/bio-oxidation approach is more amenable than conventional methods to obtain metals for these sources in an abundant way [14,30]. But, there are some obstacles in the scaling-up of this technique especially in financial aspects related to capital and operation expenditures and process validation. Moreover, most mining infrastructures are not adaptable for biological approaches [29,37,84].

Other intrinsic issues end up interfering in the economic viability of the biomining process since the metal recovery is directly affected by the grade and composition of the ores [14,71]. Moreover, if the mineral present in the ores is primary or secondary also influences this aspect. For instance, secondary cupric minerals are more accessible and easier to recover than primary copper ores [30].



One of the most common ore beneficiation is crushing or grinding, used especially in heap bioleaching. This type of beneficiation improves the operation in kinetic aspects as well as increasing the metal accessibility to microorganisms. However, it can also turn the process more expensive because to make smaller particles high energy consumption is necessary [30,31,34]. Smaller particles of ore can interfere with bioleaching or bio-oxidation rates by inhibiting the microbial activity or slowing it down due to its higher toxicity [30].

About the operation mode, dump bioleaching is the cheapest technique because any pre-treatment of the ore is necessary. Otherwise, requires more time to achieve a good rate of recovery [29,30]. Heap bioleaching comes next followed by bioprocess in stirred tank reactor, because the last one demands more previous feedstock processing, like grinding, for example [33]. Other characteristics make heap leaching economic advantageous, such as low operations costs, simplicity of biomining environment, and most efficiency in front of low-grade ores. Comparing heap and tank leaching in capital and operating expenditure, the heap process has the lowest values for base metals like copper [12,29,37]. However, stirred tank process needs a shorter time to recover the metals, making it applicable and more economically feasible, especially for precious metals.

Although the biomining on WEEE through stirred tank processes has the best results in terms of sustainability there are still several economic challenges to be overcome such as operational costs, and scale-up problems that have retained the technique in bench-scale [76,85,86]. In fact, even biomining techniques demonstrating to be more economical than traditional methodologies to recovery metals, it is still challenging to understand the bioprocess dynamics for scale-up this method for industrial application [87], making BIOX™ an exception case. For instance, Potysz et al. [71] showed that copper extracted for a granulated slag is almost twice more efficient by chemical methodology than biologic (91.1% and 43.5%, respectively,) and consequently a chemical procedure value for feedstock tonne is more compensatory (US\$ 167.1 for chemical process and US\$ 79.8 for biological approach). They also exhibited the economic value balanced situation between recovery methodologies for granulated slag containing molybdenum and nickel, showing that the expectation of a quick return on invested capital in biomining slows down the progress of the scale-up [84].

## Perspectives

Biohydrometallurgy process is widely considered for metals extraction from both high-grade and low-grade mineral sulfides. Moreover, this technique can be used for the detoxification of mines and processing tailings [31]. However, Brierley [29] pointed that the specificity of the method in terms of raw materials and climate conditions requirement, as well as the paradoxical issues of industrial patents, make biohydrometallurgy less tangible for optimization and expansion. On the other hand, there are promising environmental advantages associated with biohydrometallurgy. Copper and arsenic extraction from chalcopyrite and enargite, respectively, are some of the recalcitrant compounds that can be removed from mining wastes by this technique [19,20].

Some bottlenecks in biohydrometallurgy of metals from minerals are related to non-sulfide ores and oxides bioleaching [12,14]. Ehrlich [66] pointed the prospecting of biohydrometallurgy future relies on bioprocessing of silicates and carbonates, and even on anaerobic heterotrophic microorganisms used. Microbial consortium

interaction with minerals, improvement of the microbial metabolic, and development of scale-up of bioreactors are some of the technical-commercial advances to be done [35]. Operational parameters such as temperature, pH, aeration and oxygen availability, and particle size, are directly related to these issues and must be extensively studied [13,41].

Bioleaching process shows, in general, lower kinetic constants compared to the chemical processes. Some techniques related to genetic engineering and the use of enzymatic processes [19], and specific areas, such as molecular biology and genomics within biotechnology, are increasing to solve the technical obstacles. New properties have been explored from microbial gene exchange and molecular adaptations. Considering these aspects, the omics sciences (genomics, transcriptomics, proteomics, and metabolomics) may suggest regulatory responses in the microbial consortium and molecular mechanisms in ores bio-solubilization [23,43,84,88]. DNA extraction, the analysis of microorganisms present in acid drain damage [3], and the reconstruction of *Acidithiobacillus ferrooxidans*' metabolism on a genomic scale [11] illustrate the advances in this field.

Finally, it should be noted that biomining can overcome pyrometallurgy and hydrometallurgy, especially in the metal recovering from waste. According to the Global E-Waste Monitor [89], about 53 million tons of WEEE were generated in 2019, and only 17.4 % were properly collected and recycled. This volume represents approximately US\$ 57 billion in metallic raw material. In Brazil, 2.15 million tons of WEEE are produced, which about 60% are disposed of in landfills or incinerated. Thus, WEEE biomining emerges as a promising technique for metal recovery, but, to the best of our knowledge, the development status is still in laboratory or semi-pilot stages [10,90-92]. The level of technology readiness into some WEEE biomining is higher than 4, which means that are tests being carried out on a semi-pilot scale. Some of the bottlenecks for scaling up the bioleaching of WEEE are (i) toxicity of some non-metallic fractions of WEEE to microorganisms; (ii) necessity to adjust the pH of culture medium to allow the growth of acidophilus (since some discarded WEEE materials have an alkaline nature); (iii) the decreasing into recovery rate with the increasing of pulp density; (iv) inhibition of cells growth caused by the direct contact with the metals present in WEEE [24].

## Competing Interests

The authors declare that they have no competing interests.

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