Chapter

Analysis of the Oxidation: Reduction Potential and Bacterial Population of *Acidithiobacillus ferrooxidans* during the Bioleaching Study of Sulfide Ores

Vladimir Arias-Arce, Daniel Lovera-Dávila, José J. Guerrero-Rojas, Fanny Blas-Rodriguez and Ismael Molina-Pereyra

Abstract

The analysis of the variables, bacterial population, and oxidation-reduction potential (ORP) during the bioleaching of sulfide ores by a bacterial strain of *Acidithiobacillus ferrooxidans*, isolated from acid mine effluent, aims at the solubilization of copper and the liberation of the gold present in an ore containing more than 80% sulfides. It was studied at different pulp densities (1, 2, and 6% - W/V) and with a 9 k medium at different ferrous sulfate concentrations (0, 3, 6, 9, 12, and 15 g/L), keeping temperature and pH constant. The tests were carried out in three consecutive stages, starting with inoculum, whose cell content was $7.05x10^7$ Cell/mL, then the strain with the highest population obtained in the previous stage was used, observing the variation in the periods of adaptation and growth. During the bioleaching of sulfide ores, in the first stage, the maximum bacterial population achieved was $4.75x10^7$ Cell/mL in 24 days with 6 g/L ferrous sulfate, in the second stage, the maximum population was $6.30x10^7$ Cell/mL without the addition of ferrous sulfate, and in the third stage, the bacterial population became $4.51x10^7$ Cell/mL. The exponential characteristic growth of the population started at approximately 13, 8, and 3 days, respectively in each stage.

Keywords: bacterial population, bioleaching, sulfide minerals, *Acidithiobacillus ferrooxidans*, redox potential

1. Introduction

The redox potential of a mineral solution or ore pulp is a measure of electron activity that can be influenced by the presence of pyrite in its natural status, considered electrochemically passive, a favorable condition for the galvanic effect with other sulfide compounds to be enhanced and the formation of elemental sulfur to be

achieved [1]. Therefore, several works are carried out to identify the variables and parameters that allow the liberation of metallic and nonmetallic ions that are present in a mineral.

Specifically, the identification of the oxidation-reduction potential (ORP), which is a critical factor in the development of the inoculum and in the evolution of the oxidation of inorganic compounds, is determined with a platinum reference electrode and a hydrogen electrode connected to a potentiometer. It is quantified in volts, which represent the energy released by all components of the system in a fraction of time when a number of electrons move from one phase to another: namely, between the bioleaching substrate and the platinum electrode.

The biological oxidation of sulfide to elemental sulfur, sulfate anion, and other sulfur compounds and the reduction of oxygen in water are the main redox changes that occur in this process. The measured redox value of the medium in which a process takes place will be the result of the set of chemical reactions occurring in it.

Likewise, the thermodynamic relationship of the oxidation-reduction potential (ORP) represented by the potential (Eh) of the solution is known as the Nernst equation, nevertheless, in practice, the ORP value is determined mostly by ionic compounds with high current exchange density, in other words, the ability they have to exchange electrons on the surface of the platinum electrode; In this sense, several researchers have found that there are compounds that have a great aptitude to exchange their valence electrons on the platinum surface, such is the case of hydrogen sulfide, for which there is a linear relationship between the ORP measurement and the logarithm of the concentration of hydrogen sulfide in natural environments [2]. The process of dissolution of chalcopyrite with sulfur-oxidizing microorganisms and iron oxidizers depends mainly on the redox potential. Chalcopyrite was preferentially oxidized to polysulfide when the redox potential is approximately less than 350 mV with reference to Ag/AgCl electrode and at higher potential approximately between 350 and 480 mV, chalcopyrite was mainly transformed to Cu_2S, intermediate species, resulting in high dissolution rate and when the redox potential is higher than that of 480 mV, chalcopyrite was mostly oxidized to polysulfides, causing passivation of chalcopyrite [3].

The usefulness of ORP data may be questionable because the measuring probe is directly in contact with the sinus of the extracellular environment, which is totally different from the intracellular environment. A disadvantage of ORP is its strong dependence on pH. In this regard, decreases in potential of up to 33 mV have been reported with a one-unit increase in pH [4]. However, real-time potential monitoring offers many advantages [5].

It is known that in culture media, microorganisms show very different sensitivities to the oxidation-reduction potential. Therefore, it is believed that the redox potential is a very particular and important factor in each of the environments where the substrate probably determines the presence of a variety of microorganisms and their metabolic evolution [6].

By means of bioaugmentation processes under conventional bioleaching conditions, the growth of diverse bacteria was achieved, which contributed to improve copper dissolution, achieving the extraction of 90.2% after 24 days. In the final stage, the formation of a passivating layer of jarosite decreases the copper release rate; resulting that, the increase of iron-oxidizing cells negatively influencing the leaching of chalcopyrite [7].

It should be noted that sulfide minerals often coexist with several mineral species, which act synergistically during the leaching process, allowing the dissolution of certain elements. In the case of leaching of chalcopyrite and silver-bearing

bornite [8], by thermophilic culture (50°C), 94.6% copper was extracted, quite superior to the results of separate leaching of chalcopyrite and bornite, silver was released to the solution, forming Ag_2S on the surface of chalcopyrite. In recent studies of bioprocesses, bioleaching is considered a process with multiple advantages such as low cost, environmental friendliness, simplicity of requirements, and suitability for the treatment of low-grade ores. In the analysis of commercial processes, the evolution of the problem is identified as the lack of definition of the parameters due to the synergic effect of microorganisms, the role of extracellular polymeric substances, the passivation phenomenon, the galvanic interaction between minerals, the mode of application, and the environment [9].

2. Background of bioprocesses

In recent years, the development of microbiological processes for the extraction of metals from ore bodies has generated much interest and the approach to biotechnological processes such as biooxidation, which are already applied in many parts of the world. These are the fundamental reasons for research and for providing incentives for new discoveries and are also likely to become the cause of the development of the mining and metallurgical sector [10, 11]. Bioleaching is a clean technology for processing complex and low-grade ores because of reduced energy and water consumption and low CO_2 and SO_2, emissions compared to pyrometallurgical processes [12].

In the bioleaching of copper sulfide ores using *At. ferrooxidans* and subsequent characterization of the residues by scanning electron microscopy (SEM) and X-ray diffraction (XRD), it has been identified that dissolution occurs in the following preferential order: bornite, pyritic chalcopyrite, covellite, and porphyry chalcopyrite; the latter as a surface layer can hinder the dissolution of other compounds and thus, the extraction of copper [13, 14]. Polysulfides, elemental sulfur, and insoluble sulfates are the main constituents that determine the redox potential [15].

It can be asserted that electron donor sources are abundant and diverse in nature and can be of anthropogenic, geological, biological, and inorganic materials. An important source of inorganic compounds is volcanic activity as reduced sulfur compounds and others. All compounds derived from the mining and agricultural industries, products from the burning of hydrocarbons, and other industrial activities release reduced sulfur compounds into the environment, which donate or receive electrons and thus energy through chemolithoautotrophic sulfoxidizing bacteria [16].

During the last three decades, the application of bioleaching for the treatment of sulfide ores has reached its industrialization, the sequential use of biooxidation — bioleaching—electrowinning, for the extraction of copper, uranium, gold, and zinc, providing satisfaction in the mining sector. In addition, in recent years, its application is being sought for the extraction of copper from refractory ores [17]. In the dissolution of chalcopyrite promoted with ferric ions, Hiroyoshi et al. [18] presented a two-stage dissolution model: first, the reduction of chalcopyrite to Cu_2S by ferrous ions in the presence of cupric ions. Second, the oxidation of Cu_2S to cupric ions and elemental sulfur. Reactions achieved at solution potentials below the predicted critical potential as a function of ferrous, ferric, and cupric ion concentrations.

In studies by Nazari et al. [19], it was observed that a ferric precipitate in the form of jarosite was produced at 50 g/L ferrous sulfate at an initial pH of 2.2 and a temperature of 32°C. The effects of ferric iron precipitation on other ions are important for *At. ferrooxidans* bacteria in the aqueous phase, that is, sulfate, phosphate, magnesium,

and potassium ions. The results showed relatively similar patterns for potassium and ferric ions, and this could be explained by the coprecipitation of these ions as constituents of jarosite, increased at higher pH.

The copper extraction yield from thermophilic bioleaching of chalcopyrite depends on temperature, pH, and oxidation-reduction potential (ORP), as well as the activity of the thermophile used [20]. The copper extraction yields obtained with thermophilic microorganisms at different pH and temperature conditions, and with different initial amounts of Fe^{3+}, generate high biomass concentrations at an ORP close to a critical value (450 mV, with reference to the Ag^0/AgCl electrode) and high copper extraction, attenuating at higher ORP values and causing Fe3+ precipitation as jarosite [21]. However, the dissolution of chalcopyrite might not be hindered even though large amounts of jarosite are produced and the free jarosite would be easily detached from the ore surface [22, 23].

Through tests with different electrochemical circuits [24] for the dissolution of chalcopyrite, it was identified that the increase in potential caused the formation of a CuS layer, hindering the dissolution speed of the electrode. The formation of CuS is concomitant with the formation of $Fe_2(SO_4)_3$ and the latter can act as a precursor to jarosite nucleation at potentials around 750 mV (referring to the $Hg°/Hg_2SO_4$ electrode). Concluding with the modeling of the experimental results. Also, Zhao et al. [20], through thermodynamic calculations and electrochemical measurements, established the conditions to determine the optimum potential in the leaching of chalcopyrite, having as main variables the temperature and the concentrations of Cu^{2+} and Fe^{2+}, managing to establish a model to predict the potential range, becoming inhibited due to the formation of jarosite, requiring the periodic addition of Cu^{2+} and Fe^{2+} ions to improve the bioleaching of chalcopyrite. In this line, Yang et al. [23], during the electrochemical oxidation of a chalcopyrite electrode at potentials between 550 and 630 Mv (having as reference an Ag/AgCl electrode), finds an anodic dissolution region with S_2^{2-} and covellite species and two very close passive regions coated with a thin sulfur-rich layer, which could be responsible for the passivation, concluding that chalcopyrite can passivate in the potential range of 748–828 mV with respect to the standard hydrogen electrospray (SHE). On the other hand, at an applied potential of 415 to 750 mV, a thin film of copper and iron sulfide was produced, exhibiting passivation properties and, at 1070 mV, the film formed dissolved and the rate of chalcopyrite dissolution was enhanced; when the potential continued to increase, CuS was formed and hindered chalcopyrite dissolution; finally, at 1400 mV, jarosite was formed, which hindered chalcopyrite dissolution [24]. Subsequently, to investigate the roles of dissolved oxygen (O_2), Fe^{3+} and Fe^{2+} and their interactions during the leaching of chalcopyrite in a basic culture medium at atmospheric pressure and 45°C, it was shown that Fe^{3+} significantly promoted the dissolution of chalcopyrite in the initial stage, then in the final stage it caused the passivation by the formation of jarosite due to the oxidation of Fe^{3+}, it was also tested adding oxygen, which caused an appropriate potential range between 380 and 480 mV (with respect to the Ag/AgCl electrode), eliminating the passivation species caused by polysulfides and favoring the formation of jarosite [25].

The hindrance or delay in the dissolution of minerals by bacterial action is due to the formation of a surface layer, a phenomenon known as passivation, which is the subject of debate. In leaching tests, in the presence and absence of mixed culture, it was found that the presence of jarosite and elemental sulfur in an abiotic experiment does not hinder copper dissolution and the dissolution curves do not represent signs of postdissolution but indicated hindered dissolution. In bioleaching and abiotic tests

with chalcopyrite samples, it was identified that the common phases on the surface of the leached samples during different periods of time were elemental sulfur and iron oxyhydroxides, which were identified by XPS spectrometer as jarosite, being the cause of the difficulty in dissolution [26]. The kinetics of chalcopyrite dissolution is fast when the solution potential is lower than 648 mV (SHE), and it cannot be effectively leached when the solution potential is higher than 698 mV due to the production of polysulfides, elemental sulfur, and jarosite on the surface, reaching surface passivation; it is not possible to oxidize with Fe^{3+}, but it can be oxidized by stronger oxidants [8].

3. Experimental procedure

In leaching with *At. ferrooxidans*, the conditions at which various works have been carried out by several researchers, including Wang et al. [17], identify that at the temperature of 30 ± 1°C and pH of 2.0 achieve concentrations of $2.24x10^7$ Cell/mL and recovery of 45% copper after 75 days of leaching. Liu et al. [22], during the bioleaching of chalcopyrite at different times, identifies the presence of various species such as bornite, chalcocite, covellite, and their respective redox potentials and, finally, after 30 days of processing, identifies as iron species approximately 26% chalcopyrite, 10.2% bornite, and 74% jarosite.

Seeking to contribute to the knowledge of the mechanisms of bioleaching of sulfur minerals, in this study, the experimental design, implementation, and execution of the tests were carried out in the Laboratory of Biometallurgy of the School of Metallurgical Engineering of the Universidad Nacional Mayor de San Marcos (UNMSM) with the participation of teachers and students of the faculties of Chemistry and Chemical Engineering and Biological Sciences. Potential (Ev) measurements and bacterial population determinations were carried out at different concentrations of 9 k substrate and mineral substrate, maintaining constant pH, temperature, and agitation speed. The test medium consisted of sulfide mineral, 100 mL of 9 k solution, 10 ml of inoculum, pH of 1.8, average ambient temperature of 22°C, and constant agitation of 150 rpm. The analyses to determine the extraction of Cu and other elements were carried out by Atomic Absorption and Induced Plasma Spectrometry, in the laboratory of the School of Metallurgical Engineering and by third-party service, respectively. The bacterial population count was carried out at the Environmental Microbiology and Biotechnology laboratory of the School of Biological Sciences, UNMSM.

3.1 Mineral substrate as metabolic medium

An important factor of the ore under investigation is its nature; with the presence of diverse sulfides, being of interest the copper sulfides. The sulfide ore was milled at 94% -200 Mesh to facilitate its oxidation and the supply of nutrients required by the inoculated microorganism.

The mineralogical composition of the sulfide ore that forms part of the substrate in the bioleaching was identified, containing mainly: iron, sulfur, copper, gold, and silver. As well as high content of gangue with sulfide compounds that will increase the pH in the leach liquor, with the consequent inhibition and suppression of microbial activity [27]. The degree of leaching to be achieved will depend on the type of surface of the mineral substrate, as a decrease in particle size means an increase in specific surface area so that dissolution or oxidation yields can be obtained without any

alteration of the total particle mass. A particle size of approximately 42 μm is considered optimal [28]. Additionally, the provision of a 9 k culture broth modified in its ferrous sulfate content favors the reactivity of the medium [29].

3.2 Isolation in solid and liquid media 9 k

First, colony morphology, cell morphology, ferrous ion oxidation, and tetrathionate oxidation were considered in the presumptive identification of the isolated bacteria. The colonies are then poured into solid medium (Petri plates) and allowed to gel by cooling. In total, 0.1 ml of bacterial strain from liquid culture was added. It is placed in an incubator at 28°C for periods of 5 to 10 days. The evaluation of growth was by direct observation from the fifth day based on the methodology of Hallberg et al. [30]. Reddish brown colonies were obtained due to the formation of ferric iron. The identified colony is reseeded in fresh liquid medium, thus achieving the enrichment of the strain and obtaining the intrinsic characteristics of *At. ferrooxidans*. Iron is used in both isolates because it is an essential micronutrient for bacteria and because of its oxidative and reductive properties, it acts as an electron transporter and as a cofactor for many enzymes. Subsequently, the pure strain was sent to the UNMSM Molecular Biology laboratory for identification and final characterization using the polymerase chain reaction (PCR) technique.

The Wizard Genomic DNA Purification Kit (Promega) protocol was used for chromosomal DNA extraction. We then proceeded to design the universal primers that amplify the 16S ribosomal RNA coding gene. DNA sequencing used the dye termination method and the Applied Biosystems 3730 system from Macrogen USA was used. And finally, molecular identification was carried out by comparing the 16S rRNA gene sequences (16S rDNA) of the native strains with those available in the databases using the program BlastN version 2.0. The 16S rRNA sequences were obtained from GenBank/EMBL/DDBJ databases, according to the percentage of similarity, the RecB1a isolate was identified as *At. ferrooxidans* in 98% [31].

3.3 Bacterial adaptation in the presence of metallic sulfides

Adaptation tests are carried out by several researchers seeking to obtain a bacterium or bacterial consortium capable of growing in media consisting of mineral sulfides and sulfides refractory to conventional mineral extraction processes. We cite some studies carried out on the adaptation in minerals with the presence of arsenic and silver sulfides, being these compounds inhibitors to bacterial development. The tests consisted of adapting the bacterial strain to different media with different amounts of ferrous sulfate and pyritic sulfide mineral [32].

Once the 9 k medium was identified, modifying its iron content and at a favorable pH, adaptation was sought in the presence of minerals containing about 70% sulfides of various mineral species such as chalcopyrite, pyrrhotite, arsenopyrite, and others. The evolution or progress of the adaptation phase is determined by the variation of the redox potential [33], determining that above 500 mV a marked bacterial growth occurs, then inhibition, and finally the decrease of the population; possibly, by the saturation of the medium and/or by nutrient depletion.

The objective of this adaptation stage in the application of a bioprocess such as bioleaching is to obtain a bacterial microorganisms capable of growing in sulfur media, without the addition of iron sulfate; in other words, to make the microorganisms feed themselves with the iron contained in the material subjected to the bioleaching process,

this would help us to later carry out oxidation tests on refractory minerals containing high contents of pyrite, arsenopyrite and also, with arsenic and silver contents.

The isolated and identified bacterium (*At. ferrooxidans*) is subjected to media with mineral of approximately 80% sulfides, 10% arsenic, and 20% silicates, taking different amounts of mineral (1, 2, 3, and 4 g) and at different particle sizes. The basic conditions of the adaptation were: room temperature at an average of 20°C, pH 1.8, and shaker agitation at 150 rpm [34].

3.3.1 Adaptation to different quantities of ore

Four assays were carried out, with 1, 2, 3, and 4 grams of mineral pulverized at −200 mesh. In 250 mL Erlenmeyer flasks, 100 mL of 9 k medium and 10 mL of solution with *At. ferrooxidans* (pure culture) were added. The population and redox potential increased over time. The increase in potential occurs after a short latency period of approximately 10 to 12 hours. See **Figure 1**.

3.3.2 Adaptation to different ore particle sizes

Tests were also carried out with 100 ml of 9 k medium modified in its ferrous sulfate content and 10 mL of bacterial strain. Tests were carried out with the ore pulverized at −200 mesh and fractioned in three sizes: −200 + 325, −325 + 400, and − 400 mesh, taking 5 grams of each of the ore fractions. The ferrous sulfate content was limited to 11 g/L. The test with the mineral whose fraction corresponds to sizes smaller than 37 microns (−400 M) was of particular note. See **Figure 2**.

3.3.3 Prolonged adaptation

The test is carried out with the mineral pulverized at 80% - 400 mesh in 9 k liquid medium with 5.5 g/L of ferrous sulfate and 7.5 grams of the mineral. The adaptation

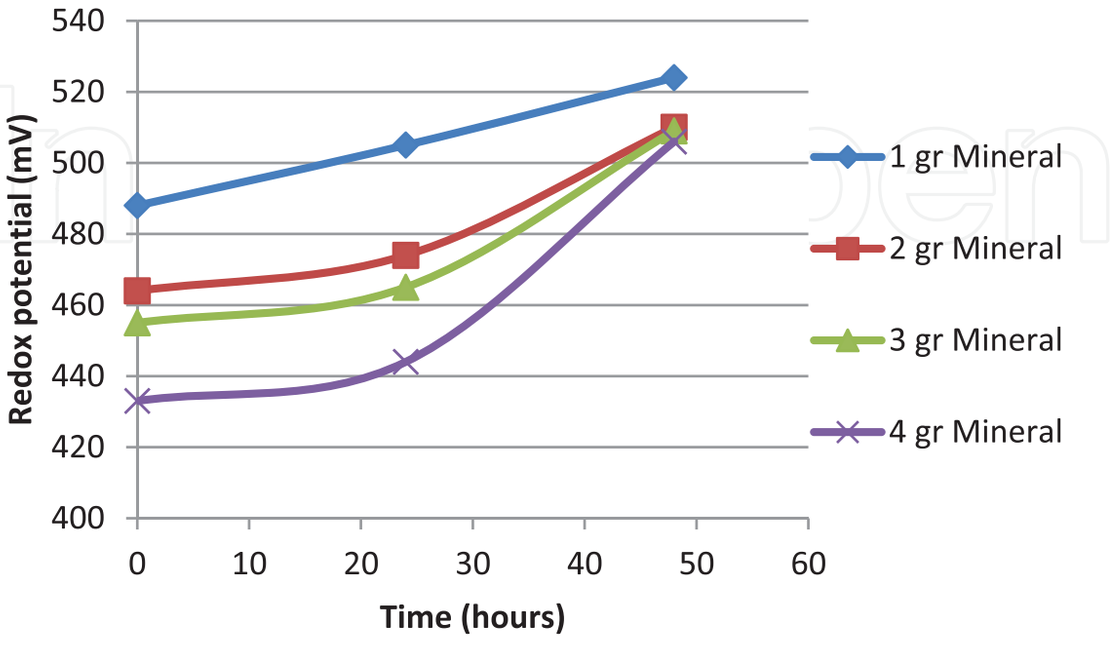

Figure 1.
Increase of the redox potential as a function of time. A greater increase in potential is achieved in the test with less mineral, consequently, less friction and less dissolution of its components, and less damage to the bacteria.

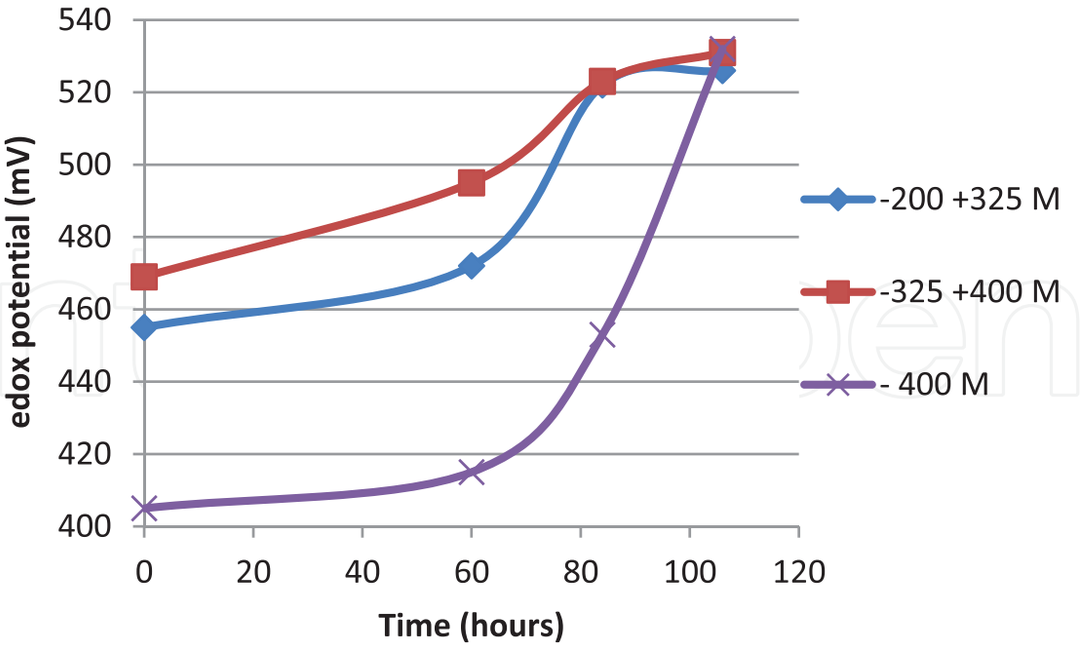

Figure 2.
Oxidation potential changes as a function of time with particle size fractions. A latency period of approximately 40 hours is observed in the smallest size fraction, followed by an exponential increase in redox potential.

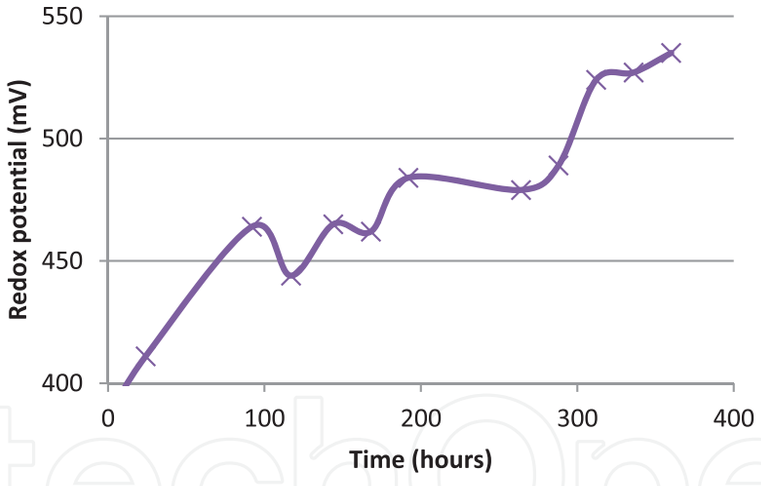

Figure 3.
Redox potential vs. adaptation time. The increase of the redox potential over a prolonged period represents the adaptation of the bacteria, the growth of its population, and consequently, the oxidation of the mineral.

is carried out for 380 hours, observing the increase of the potential with periods of inhibition. See **Figure 3**.

4. Bioleaching tests

The first tests performed corresponded to the chemical analysis of identification by elements, the results of which were subjected to theoretical analysis based on bibliographic information in order to define the operating parameters, as was done for the bioleaching tests of copper sulfide ores [28].

It is known that in bioleaching tests over a prolonged period of 60 days, the bacterial population is maintained around 550–590 mV [35]. Also, the evolution of the

bacterial population shows increases in a certain period of time, for example, between 6 and 21 days of processing, the average bacterial population was $1.70x10^8$ Cell/mL and $8.00x10^7$ Cell/mL in the bioleaching of ore whose granulometries corresponded to −200 and − 325 Tyler mesh, respectively [33].

Bioleaching is performed in three consecutive stages with 1.0, 6.0, and 2.0% (W/V) of sulfide ore, the second and third stages using the best inoculum from the previous stage and under the conditions detailed in **Figure 4**.

A 9 k solution with different concentrations of ferrous sulfate is provided as nutrient substrate. During the tests, measurements of oxidation-reduction potential (ORP) and pH are taken; in addition, periodic sampling is carried out to determine the metals present, such as copper, iron, arsenic, and zinc.

4.1 First stage of bioleaching

The bioleaching solution had as main nutrient substrate medium 9 k at different concentrations of $FeSO_4.7H_2O$ between 0.0 to 15.0 g/L. For the assays, 500 mL Erlenmeyer flasks were used, where 3 g of mineral (1% W/V), 30 mL of bacterial strain of $7.05x10^7$ Cell/mL, and 300 mL of 9 k solution containing: 3.0, 6.0, and 9.0 g/L of $FeSO_4.7H_2O$ were added. The pH was regulated to 1.8 by adding sulfuric acid solution. The process was carried out in an agitation platform (Orbit Shaker) at 150 rpm. According to research, the dose of ferrous ions for the bioleaching of sulfides such as pyrite and chalcopyrite differs depending on the characteristics of the mineral that contains them [36].

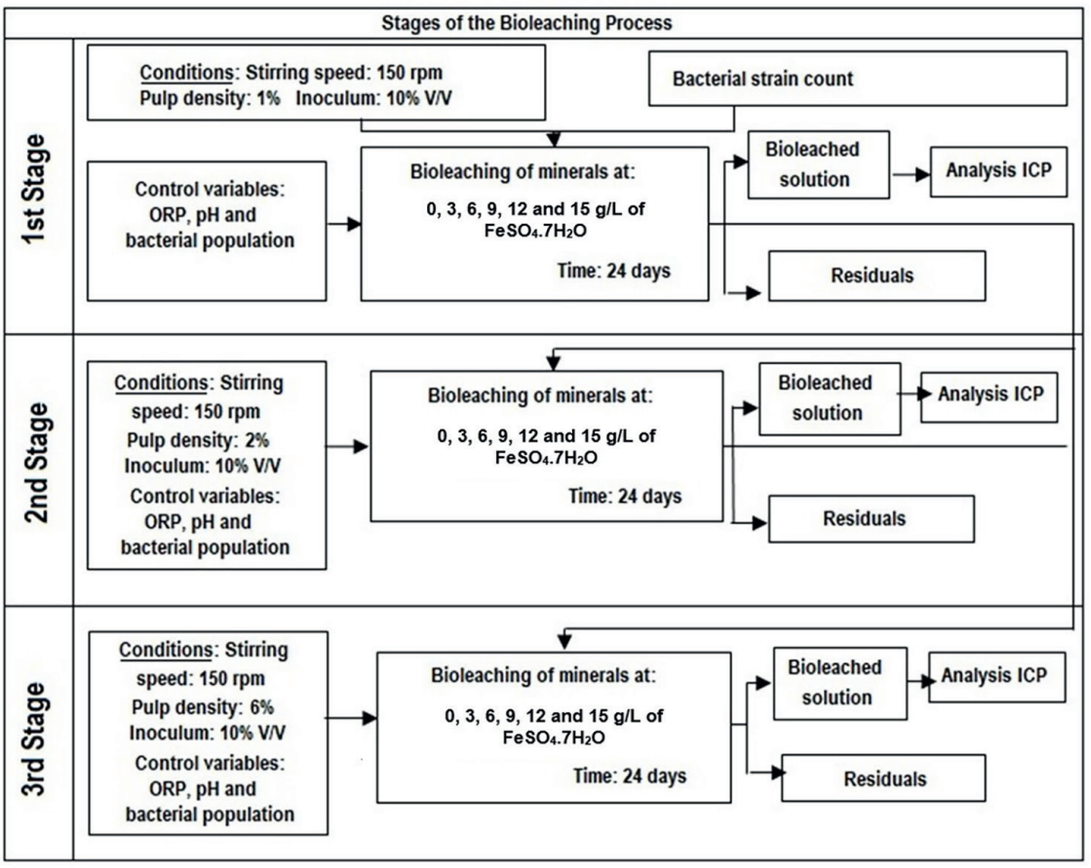

Figure 4.
Stage bioleaching design with 1, 2, and 6% solids.

The maximum copper recovery obtained in this stage was 72.64%, with 6.0 g/L of $FeSO_4.7H_2O$ and the minimum recovery was 38.96% with 15.0 g/L of $FeSO_4.7H_2O$. Approximately 20 days after the start of the process, it is observed that the copper dissolution is drastically reduced. See **Figure 5**.

4.1.1 Effect on bacterial population growth

Bacterial growth was identified as a function of the ferrous sulfate content provided, with an accelerated increase observed between approximately the 12th and 24th day, followed by a break, indicating the end of the bacterial population growth stage. The maximum population density reached was at 24 days with $4.75x10^7$ Cell/mL with 6.0 g/L of $FeSO_4.7H_2O$ reaching 67% of the inoculum, as shown in **Figure 6**.

The methodology used in bioleaching processes takes into account the adaptation stage, which is progressive for *A. ferrooxidans* bacteria in the presence of nutrient ions (Arias et al., 2015), where the reproduction of microorganisms is achieved; in parallel, the metal compounds in solution are increased [33, 37].

4.1.2 pH variation

The tests are started at pH 1.8 and at different concentrations of $FeSO_4.7H_2O$. During the first stage period, which lasts 19 days, the variation is observed and regulated. The pH varies from a minimum of 1.5 to a maximum of 2.2, on average 1.9. Finally, the trend is downward, probably due to the appearance of H^+ and the formation of sulfuric acid, which can be seen in **Figure 7**.

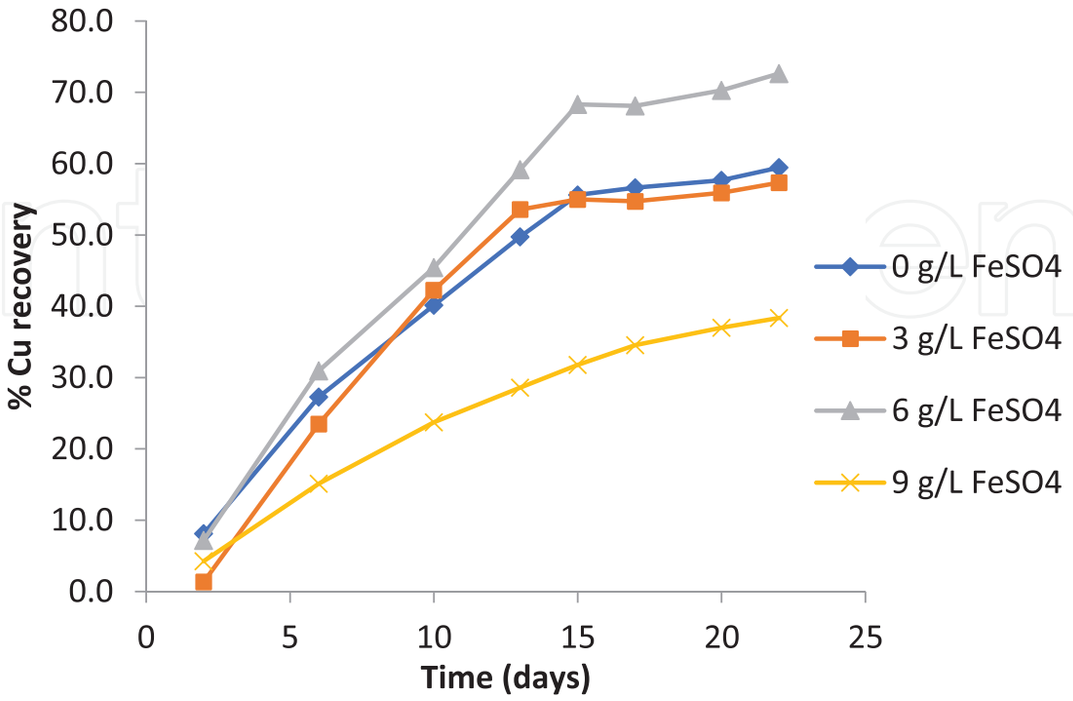

Figure 5.
Bioleaching in pulp containing 1% solids.

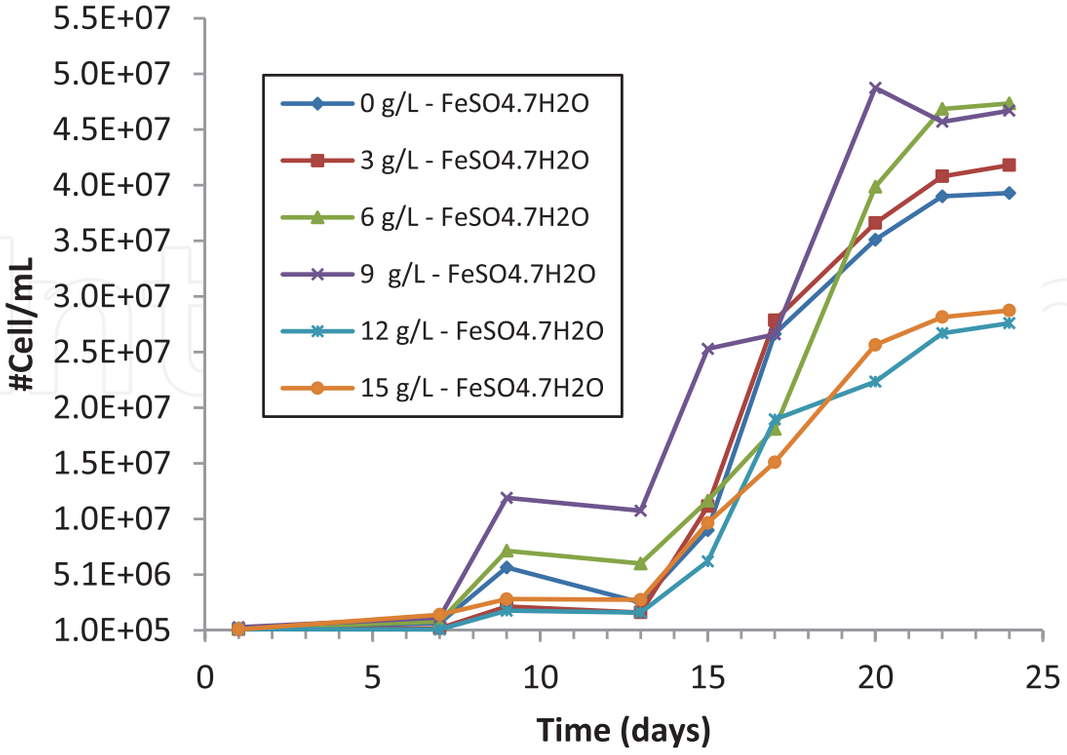

Figure 6.
Variation of the bacterial population during the first stage of bioleaching.

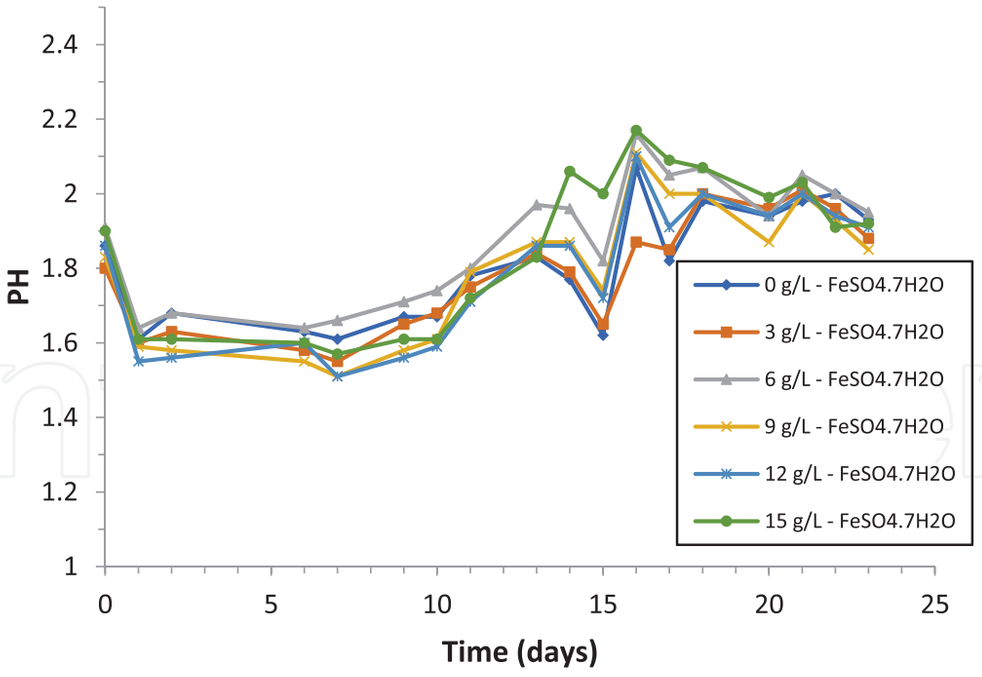

Figure 7.
pH variation during the first stage of bioleaching.

4.1.3 Measurement of oxidation: reduction potential

Figure 8 shows the ORP values for each test performed; in the first 6 days, the test containing no ferrous sulfate increases from 360 mV to 585 mV on approximately

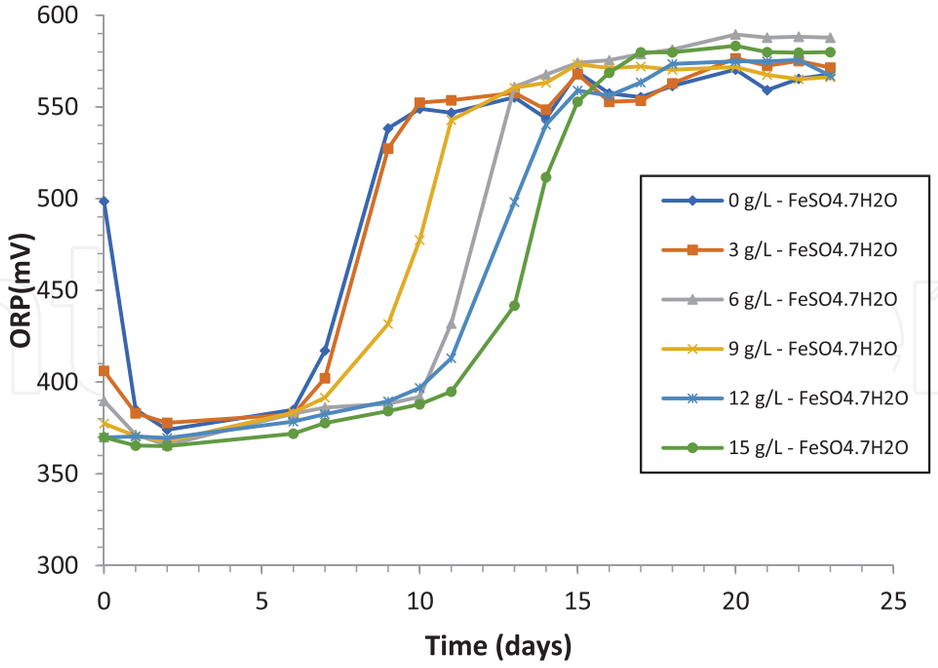

Figure 8.
Measurement of oxidation-reduction potential (ORP). First stage of the bioleaching process.

the tenth day. On the other hand, the sample containing 15.0 g/L of $FeSO_4.7H_2O$ achieves its increase after 10 days from the start of the test to approximately 560 mV. Subsequently, all samples are maintained at around 575 mV.

4.2 Second stage of bioleaching

Tests were performed in 500 mL Erlemeyer flasks, adding 18.0 g of mineral (6% W/V), 30 mL of bacterial strain (10% V/V), and 300 mL of 9 k Medium with 0.0, 2.0, 4.0, and 6.0 g/L of $FeSO_4.7H_2O$. The pH is regulated to 1.9 with sulfuric acid solution, the process was continued on a stirring platform at 150 rpm. Bacterial strain from the first stage is used. After 22 days of leaching, 89.38% copper extraction is achieved in a medium without the addition of ferrous sulfate. See **Figure 9**.

4.2.1 Effect on bacterial growth

Observed from the inoculation with the highest population strain obtained in the previous stage, whose concentration was $4.75x10^7$ Cell/mL.

It is observed that the adaptive and exponential phases show the same trend in all tests. The exponential growth phase occurs approximately between the 8th and 12th day, achieving a maximum bacterial population of $6.30x10^7$ Cell/mL in the test without the addition of $FeSO_4.7H_2O$, higher than the concentration of the initial inoculum compared to the concentration of the first stage. See **Figure 10**.

4.2.2 pH variation

The pH of the solution increases during the first 7 days, possibly due to the increase in pulp density, being controlled with sulfuric acid solution until it recovers

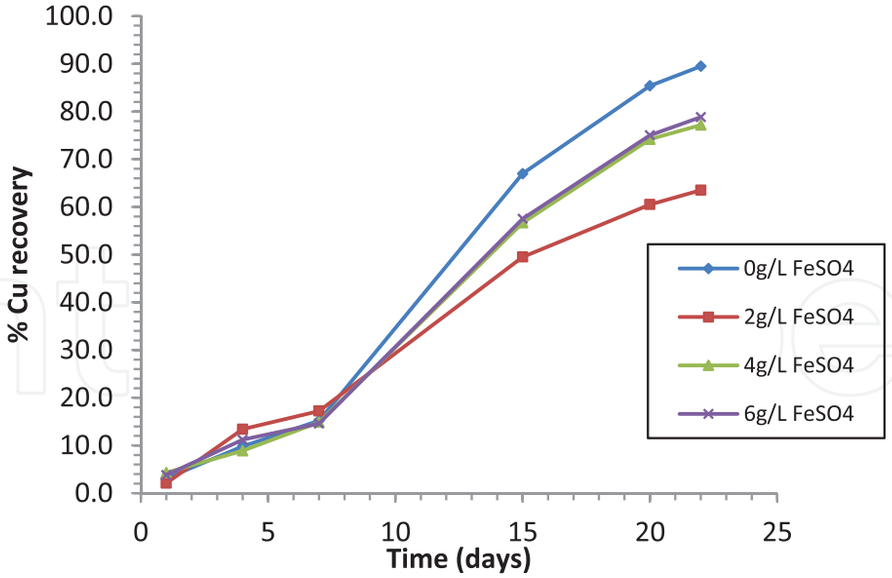

Figure 9.
Bioleaching in pulp containing 6% solids.

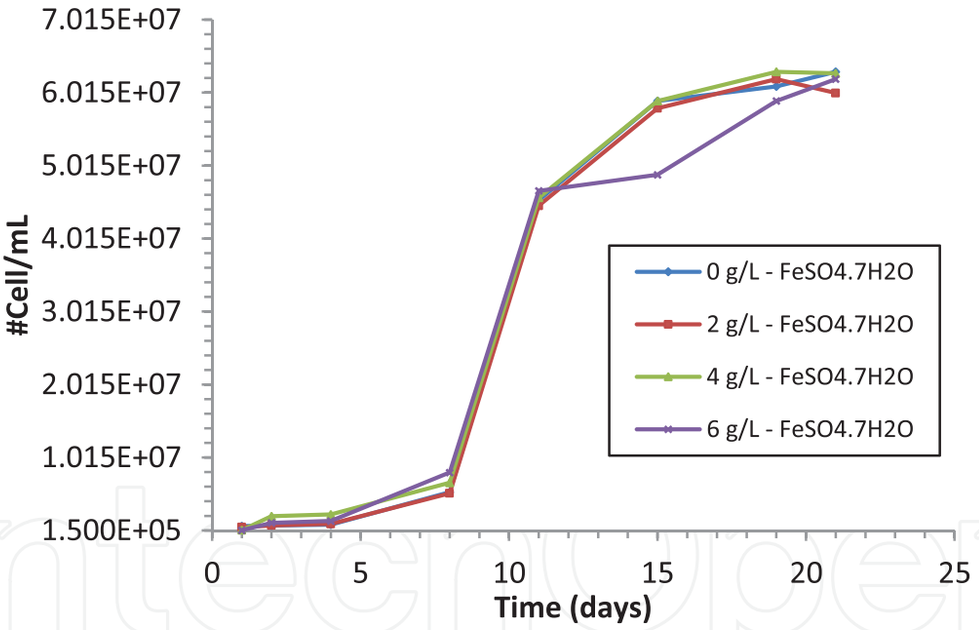

Figure 10.
Variation of the bacterial population during the second stage of sulfide ore bioleaching.

its initial value. As time goes by, the decrease is observed, being necessary for its recovery to the initial value of 1.8. See **Figure 11.**

4.2.3 Measurement of oxidation: Reduction potential

Figure 12 shows the behavior of the oxidation-reduction potential of all the tests. Achieving a maximum of 613.2 mV with the lowest amount of $FeSO_4.7H_2O$. It is also observed that they reach 600 mV approximately on the 8th day of leaching.

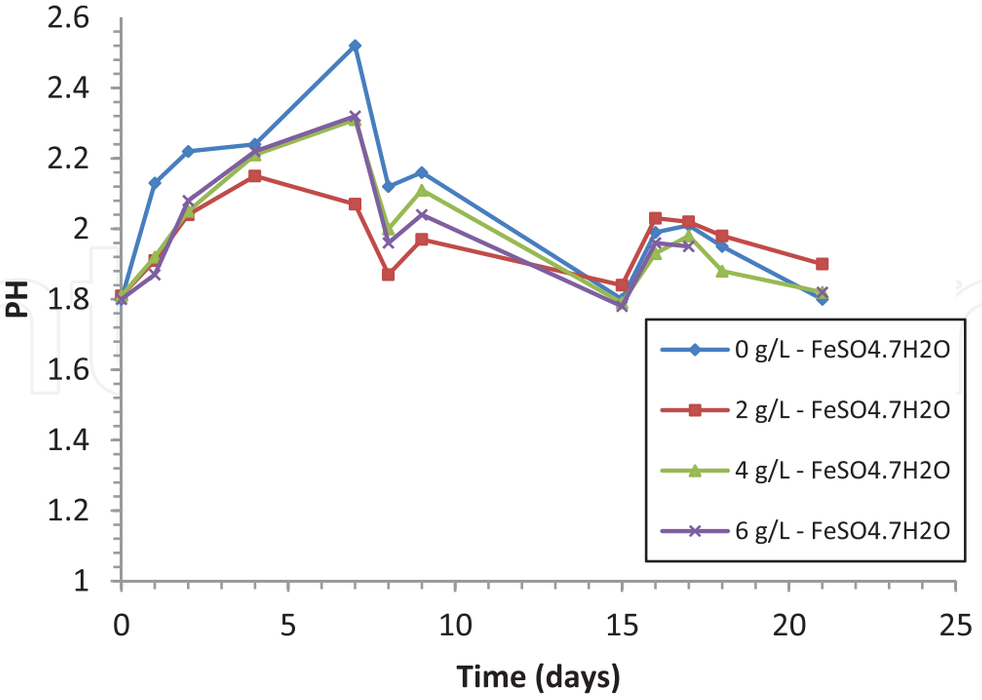

Figure 11.
pH variation during the second stage of bioleaching.

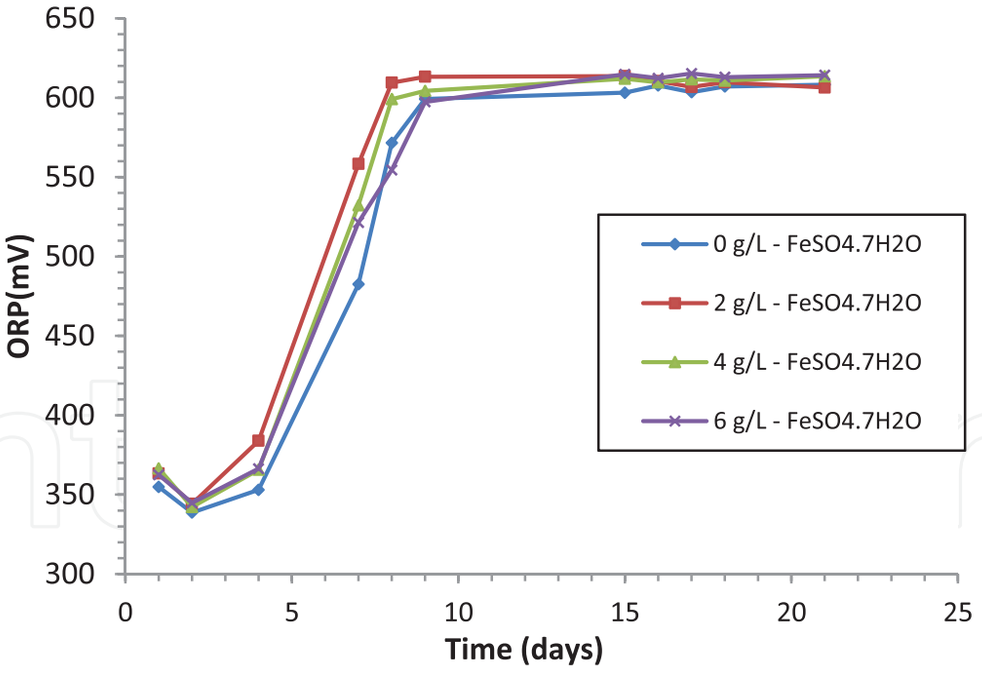

Figure 12.
Measurement of oxidation-reduction potential (ORP). Second stage of the bioleaching process.

4.3 Third stage of bioleaching

The use of Medium 9 k solution as leaching substrate is continued, varying the concentrations of $FeSO_4$ $7H_2O$. For the assays we continue using 500 mL Erlenmeyer flasks, add 6.0 g of sulfide mineral (2% W/V), 30 mL of bacterial strain (10% V/V), and 300 mL of 9 k substrate containing 0.0, 3.0, 9.0 and 15.0 g/L of $FeSO_4.7H_2O$.

The pH is regulated with sulfuric acid solution. The process was continued on a stirring platform at 150 rpm. The inoculum is obtained from the previous stage. Controls of pH, oxidation-reduction potential, and bacterial population are carried out.

After 25 days of leaching, an extraction of 85.6% copper was achieved in the test without the addition of ferrous sulfate. See **Figure 13**.

4.3.1 Effect on bacterial growth

In the tests, they were inoculated with the strain with the highest bacterial population resulting in the effluents of the second stage, whose bacterial population was $6.30x10^7$ Cell/mL. Exponential growth is achieved approximately after the second day of experimentation. In contrast to the first stage, the reduction was achieved in 8 days and compared to the second stage, in 5 days. Exponential growth ends after approximately 10 days. Higher growth ($4.51x10^7$ Cell/mL) is achieved in the test lacking the ferrous salt. It is concluded that bacterial adaptation and growth with the provision of mineral as a nutrient-supplying medium is a chemolithotrophic characteristic of the bacterium *Acidithiobacillus ferrooxidans*. See **Figure 14.**

4.3.2 pH variation

Increases and decreases in pH were observed during the first three days. The solution with 15 g/L of $FeSO_4.7H_2O$ reaches a pH of 2.08 and is corrected with sulfuric acid solution, then an increase in acidity is observed, reaching a pH of 1.3, possibly

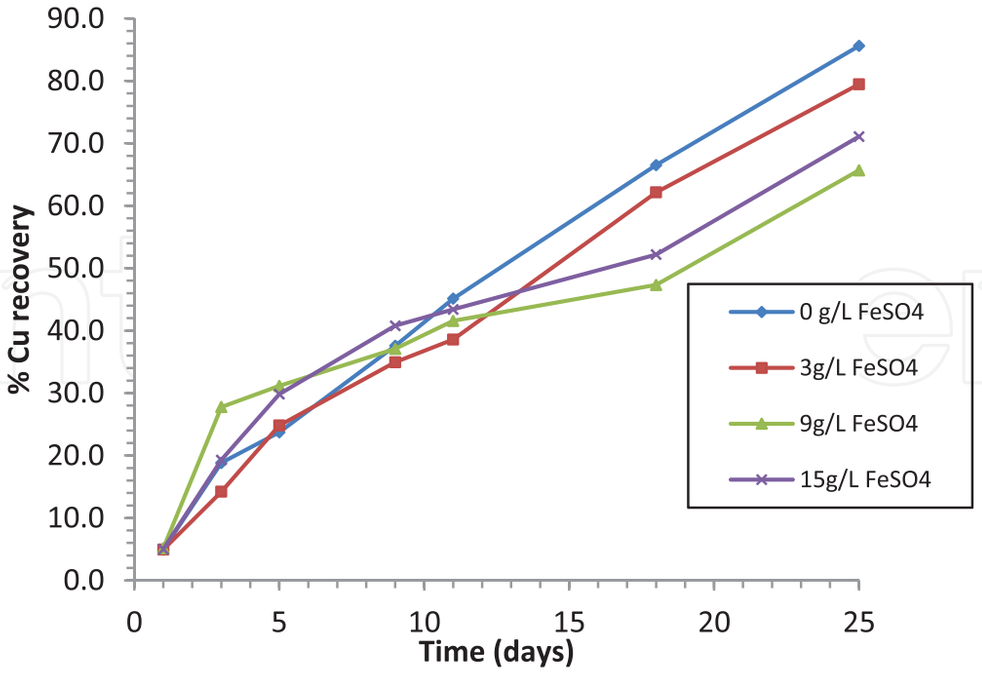

Figure 13.
Bioleaching in pulp containing 2% solids.

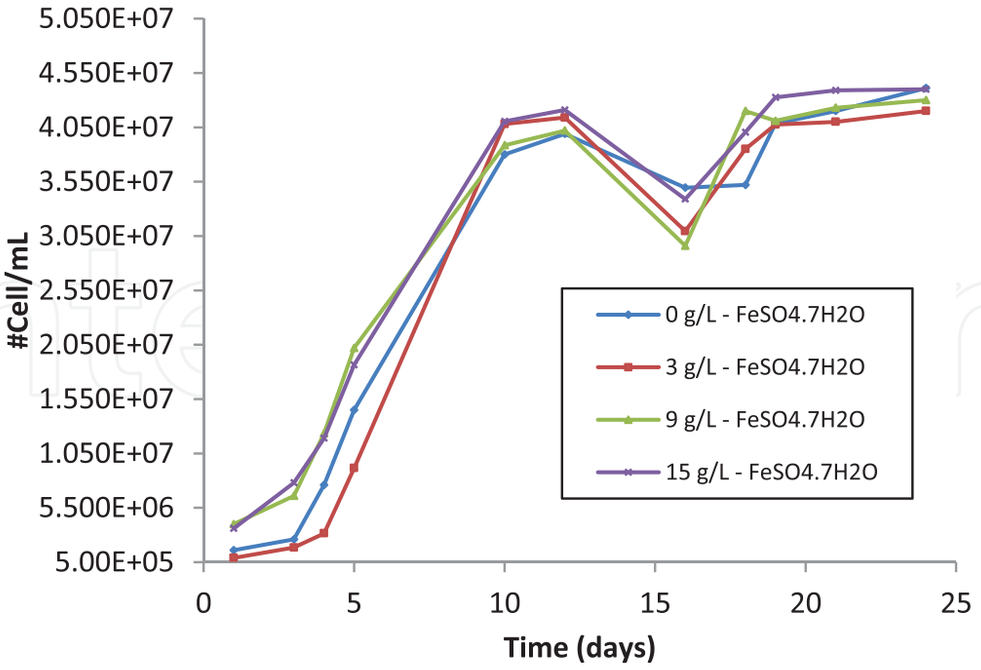

Figure 14.
Growth of the bacterial population. Third stage of experimental processing.

caused by the solubilization of the acid components of the mineral. In the following 17 days approximately, the variation is lower and is controlled with sulfuric acid solution, seeking to maintain around 1.8, then a marked decrease is observed (**Figure 15**).

4.3.3 Measurement of oxidation: Reduction potential

As can be seen in **Figure 16**, on the third day values close to the maximum are obtained, remaining almost constant during the rest of the test period. In contrast to the first stage where growth occurs between days 7 to 15 approximately, and in the second stage growth occurs between days 4 to 8 approximately. In this stage, the average maximum values oscillate around 585 mV for each of the tests.

5. Results

The level of adaptation to the new conditions is proportional to the amount of reseeding carried out and to the conditions of the substrates to which they are subjected with the possibility of making modifications. In the adaptation stage, the highest population growth of the bacteria isolated from the recovered mining unit is determined by the iron sulfate content in the substrate and the strict control of pH. These values were 22.2 g/L and 1.8, respectively.

Jarosite formation can occur at different potentials. The study by Ghahremaninezhad et al. [24], in several electrochemical circuits, identifies the formation of CuS at potentials around 750 mV and at 1400 mV the formation of jarosite, consequently, the hindering of the process. Yang et al. [23], in dissolution of a chalcopyrite electrode at potentials between 748 and 828 mV identified electrode passivation. In the present study, copper dissolution occurs throughout the test period and at the potentials revealed at each of the stages.

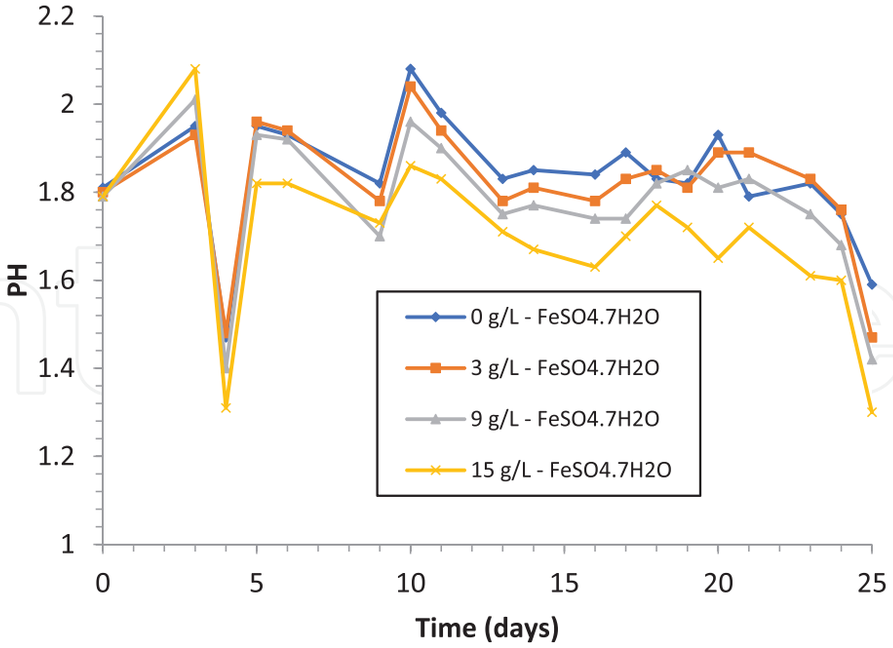

Figure 15.
pH variation during the third stage of bioleaching.

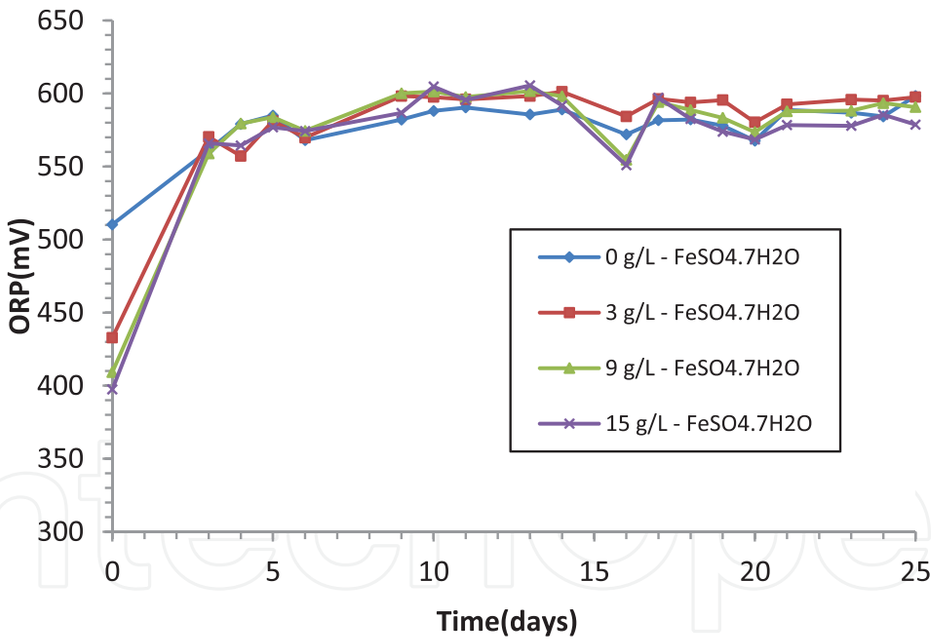

Figure 16.
Measurement of oxidation-reduction potential (ORP). Third stage of the bioleaching process.

In the first stage of bioleaching, population growth is achieved approximately in the period from the 12th to the 24th day, followed by a break and with a tendency to remain constant during the duration of the stage. The highest bacterial population was $4.75x10^7$ Cell/mL after 24 days with 6.0 g/L of $FeSO_4.7H_2O$ substrate and reaching only 67% of the initial inoculum. While the oxidation-reduction potential shows a varied behavior during the growth period. In the first 6 days, the sample without the ferrous salt increases from 360 mV to 585 mV on about the 10th day. The sample of 15 g/L of $FeSO_4.7H_2O$ has a delayed increase but reaches a maximum of 560 mV. The remaining samples, on average, reach 575 mV.

In the second stage, the inoculated strain had a concentration of $4.75x10^7$ Cell/mL, the adaptation and growth phases were observed to have the same growth trend in all tests; the exponential phase began on the eighth day, reaching a maximum of $6.30x10^7$ Cell/mL with 0.0 g/L of $FeSO_4.7H_2O$. The bacterial concentration is 42% higher in relation to the inoculum. The maximum value of oxidation-reduction potential is 613.2 mV. In the test, with 2 g/L of $FeSO_4$ $7H_2O$, it is observed that it exceeds 600 mV.

In the third stage, the beginning of exponential bacterial growth occurs on the third day after the start of bioleaching, 9 days shorter than in the first stage and 5 days shorter than in the second stage. The exponential growth ends after approximately 10 days. At this stage, it is observed that, in the absence of the ferrous salt, the concentration of $4.51x10^7$ Cell/mL is achieved with a certain similarity to other concentrations of the iron salt. Meanwhile, ORP values have a remarkable evolution during the first 3 days and then tend to remain constant throughout the test period with an average value of 585 mV, 15 mV lower than in the second stage and 10 mV higher compared to the first stage.

Compared to the ORP increase, in the first stage, it occurs between days 6 to 10 approximately, in the second stage it occurs between days 4 to 8 and in the third stage it occurs in the first 3 days; with redox potential increases between 420 and 560 mV approximately. Contrasted with the results of the instrumental analysis carried out to determine the presence of chalcopyrite, disulfides, and polysulfides on the surface of the mineral causing the passivation and hindering the dissolution of the mineral, several redox potential ranges are identified. Thus, chalcopyrite is predominantly oxidized to polysulfide when the redox potential is below 350 mV and a low dissolution rate occurred when the redox potential is in the range of 350–480 mV, chalcopyrite was mainly transformed into Cu_2S intermediate species instead of polysulfide, increasing the dissolution rate, and when the redox potential is above 480 mV, chalcopyrite was directly oxidized to polysulfide, which causes passivation of chalcopyrite [3]. Also mentioned is the dissolution of iron from the chalcopyrite surface in the 475 to 700 mV potential range, leaving a slowly dissolving S_2^{2-} and S_n^{2-}, layer above 700 mV [38].

The measurement of the potential (Ev) is the dissolution of the electron giver and electron acceptor at varying substrate concentrations at pH 1.8 and at 22°C, showing increasingly positive values due to the increasing tendency to accept electrons with the consequent formation of sulfates. In this regard, Vilcáez et al. [21] mention that optimal temperatures for thermophile growth did not always mean high copper extraction yields, suggesting that with a high pH (pH 2.0), the bioleaching of chalcopyrite is more efficient, concluding that the bioleaching of chalcopyrite is controlled by ORP rather than by pH or temperature.

6. Conclusions and recommendations

The acid drainage of the mine workings studied (Huancavelica – Peru) is acidic, with a pH in the range of 3.0 to 4.5 pH, with a significant amount of metals in solution and abundant microorganisms such as the bacterium At. ferroooxidans.

Bacterial species are satisfactorily adapted to different media containing varying amounts of iron as sulfides and oxides, coming from highly mineralized quarries (presence of iron, copper, lead, zinc, sulfur, silica, gold, silver, and others). However, the qualitative and quantitative determination of the bacterial strain is still under investigation and will depend on the constitution of the mineral substrate provided.

The redox potential as a determinant of the growth and metabolism of the culture indicates its capacity to accept or donate electrons, that is, the oxidizing or reducing characteristics of the components of the medium or substrate, determined in part by the oxygen concentration. These oxidizing characteristics are those required by bacteria of the genus *thiobacillus*, favoring their growth and the development of an oxidative metabolism.

Also, the redox potential indicates the metabolic activities of living microorganisms and can be used to specify the environment in which microorganisms are able to generate energy and synthesize their enzymes or generate new cells without resorting to molecular oxygen.

Undoubtedly, the mineralogical composition of the mineral, as well as the structure of the species, together with the temperature, pH, and physical conditions of the mineral, will determine the bacterial growth, the redox potential, and the degree of dissolution and extraction of the elements of interest during the leaching process with sulfur and iron oxidizing microorganisms.

In the bioprocesses applied to sulfur minerals, the simple and compound ions, together with the bacterial consortium, transfer the electrons coming from the oxidation of inorganic matter to the available electron acceptors of a more oxidizing nature, allowing to obtain the greatest margin of energy gain for the oxidation of the mineral substrate present, from which the carbon and energy necessary for its evolution are provided, being a mechanism typical of chemo lithotrophic organisms.

The oxide reduction potential offers many advantages in real-time monitoring. The variation in the dissolution of mineral sulfides can be attributed mainly to two factors, 1. the type of measuring electrode and 2. the composition of the mineral substrate, the dissolution of some of its components will determine the change in pH and consequently the increase or decrease of the potential.

The different oxidation statuses of sulfur (−2, 2, 2, 4, and 6) provide a redox potential and a great variety of enzymes that can oxidize different inorganic sulfur compounds; for this reason, it is advisable to identify them, as well as the metabolic routes, allowing to optimize the conditions of the sulfur oxidation reactions and to improve the bacterial catalytic activity.

During bioleaching, after a period of time, the oxidation rate and/or the dissolution of the valuable elements present in the ore show a decrease or even interruption caused by the passivation of the ore surface, as well as by the saturation of the medium with ionic compounds. Therefore, it is recommended to purify or change the enriched solution.

Acknowledgements

The authors would like to thank the Vice-Rectorate for Research of the Universidad Nacional Mayor de San Marcos (UNMSM); which, through the Superior Research Council, financed the execution of projects C17162131, C18160202, C19161651 and C23160791, of the Research Group Clean Technologies for Environmental Coexistence (TELICMA); likewise, the students and teachers of the Faculties of Biological and Chemical Sciences and Chemical Engineering, who participate in the achievement of the objectives of the research group.

Author details

Vladimir Arias-Arce[1*], Daniel Lovera-Dávila[1], José J. Guerrero-Rojas[2], Fanny Blas-Rodriguez[3] and Ismael Molina-Pereyra[4]

1 Metallurgical Engineering Deparment, FIGMMG-UNMSM, Lima, Perú

2 Universidad Privada Norbert Wiener, Lima, Perú

3 Chemical Engineering Deparment, FQ&IQ - UNMSM, Lima, Perú

4 Unidad de Posgrado, FIGMMG-UNMSM, Lima, Perú

*Address all correspondence to: variasa@unmsm.edu.pe

References

[1] Majima H. How oxidation affects selective flotation of complex sulphide ores. Canadian Metallurgical Quarterly. 1969;**8**(3):269-273. DOI: 10.1179/cmq.1969.8.3.269

[2] Fagundo Castillo JR, González Hernández P, Suárez Muñoz M, Melián Rodríguez C. Relaciones entre potenciales redox y concentraciones de sulfuros en aguas termales de Cuba. Contribución a la educación y protección ambiental. 2005;**6**:31-44. Available from: https://www.researchgate.net/profile/Patricia-Gonzalez-Hernandez/publication/

[3] Wang J, Gan X, Zhao H, Hu M, Li K, Qin W, et al. Dissolution and passivation mechanisms of chalcopyrite during bioleaching: DFT calculation. XPS and electrochemistry analysis. Minerals Engineering. 2016;**98**:264-278. DOI: 10.1016/j.mineng.2016.09.008

[4] Alvarez M. Microbial Treatment of Heavy Metal Leachates. Spain: Gráficas Terrasa. Department of Biotechnology, Lund University; 2009. Available from: https://lup.lub.lu.se/search/publication/24654

[5] Arias-Arce VA, Lovera Dávila DF, Paucarima AF, Rojas TL. Correlación del potencial óxido reducción y la población bacteriana durante el estudio de biolixiviación de sulfuros de cobre. Revista del Instituto de investigación de la Facultad de minas, metalurgia y ciencias geográficas. 2021;**24**(47):19-28. DOI: 10.15381/iigeo.v24i47.20639

[6] Kaksonen AH, Plumb JJ, Franzmann PD, Puhakka JA. Simple organic electron donors support diverse sulfate-reducing communities in fluidized-bed reactors treating acidic metal- and sulfate-containing wastewater. FEMS Microbiology Ecology. 2004;**47**(3):279-289. DOI: 10.1016/S0168-6496(03)00284-8

[7] Zhang L, Wu J, Wang Y, Wan L, Mao F, Zhang W, et al. Influence of bioaugmentation with Ferroplasma thermophilum on chalcopyrite bioleaching and microbial community structure. Hydrometallurgy. 2014;**146**:15-23. DOI: 10.1016/j.hydromet.2014.02.013

[8] Yang C, Jiao F, Qin W. Co-bioleaching of chalcopyrite and silver-bearing Bornite in a mixed moderately thermophilic culture. Minerals. 2017;**8**(1):4. DOI: 10.3390/min8010004

[9] Li J, Yang H, Tong L, Sand W. Some aspects of industrial heap bioleaching technology: From basics to practice. Mineral Processing and Extractive Metallurgy Review. 2022;**43**(4):510-528. DOI: 10.1080/08827508.2021.1893720

[10] Sand W, Gerke T, Hallmann R, Schippers A. Sulfur chemistry, biofilm, and the (in)direct attack mechanism a critical evaluation of bacterial leaching. Applied Microbiology and Biotechnology. 1995;**43**(6):961-966. DOI: 10.1007/BF00166909

[11] Rohwerder T, Sand W. Mechanisms and biochemical fundamentals of bacterial metal sulfide oxidation. Microbial Processing of Metal Sulfides. 2007:35-58. DOI: 10.1007/1-4020-5589-7

[12] Anjum NA, Ahmad I, Mohmood I, Pacheco M, Duarte AC, Pereira E, et al. Modulation of glutathione and its related enzymes in plants' responses to toxic metals and metalloids—A review. Environmental and Experimental

Botany. 2012;**75**:307-324. DOI: 10.1016/j.envexpbot.2011.07.002

[13] Fu KB, Lin H, Mo XL, Wang H, Wen HW, Wen ZL. Comparative study on the passivation layers of copper sulphide minerals during bioleaching. International Journal of Minerals, Metallurgy, and Materials. 2012;**19**:886-892. DOI: 10.1007/s12613-012-0643-x

[14] Crundwell FK. The semiconductor mechanism of dissolution and the pseudo-passivation of chalcopyrite. Canadian Metallurgical Quarterly. 2015;**54**(3):279-288. DOI: 10.1179/1879139515Y.0000000007

[15] Zhao H, Zhang Y, Zhang X, Qian L, Sun M, Yang Y, et al. The dissolution and passivation mechanism of chalcopyrite in bioleaching: An overview. Minerals Engineering. 2019;**136**:140-154. DOI: 10.1016/j.mineng.2019.03.014

[16] Espinoza J, Revah S, Le Borgne S. Rutas metabólicas de oxidación del azufre en bacterias quimiolitoautótrofas, relevancia ambiental y biotecnología. Mensaje Bioquímico. 2010;**XXXIV**:101-120. Available from: http://bq.unam.mx/mensajebioquimico

[17] Wang J, Zhu S, Zhang YS, Zhao HB, Hu MH, Yang CR, et al. Bioleaching of low-grade copper sulfide ores by Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans. Journal of Central South University. 2014;**21**(2):728-734. DOI: 10.1007/s11771-014-1995-3

[18] Hiroyoshi N, Miki H, Hirajima T, Tsunekawa M. A model for ferrous-promoted chalcopyrite leaching. Hydrometallurgy. 2000;**57**(1):31-38. DOI: 10.1016/S0304-386X(00)00089-X

[19] Nazari B, Jorjani E, Hani H, Manafi Z, Riahi A. Formation of jarosite and its effect on important ions for Acidithiobacillus ferrooxidans bacteria. Transactions of Nonferrous Metals Society of China. 2014;**24**(4):1152-1160. DOI: 10.1016/S1003-6326(14)63174-5

[20] Zhao H, Wang J, Yang C, Hu M, Gan X, Tao L, et al. Effect of redox potential on bioleaching of chalcopyrite by moderately thermophilic bacteria: An emphasis on solution compositions. Hydrometallurgy. 2015;**151**:141-150. DOI: 10.1016/j.hydromet.2014.11.009

[21] Vilcáez J, Suto K, Inoue C. Bioleaching of chalcopyrite with thermophiles: Temperature–pH–ORP dependence. International Journal of Mineral Processing. 2008;**88**(1-2):37-44. DOI: 10.1016/j.minpro.2008.06.002

[22] Liu HC, Xia JL, Nie ZY. Relatedness of Cu and Fe speciation to chalcopyrite bioleaching by Acidithiobacillus ferrooxidans. Hydrometallurgy. 2015;**156**:40-46. DOI: 10.1016/j.hydromet.2015.05.013

[23] Yang Y, Harmer S, Chen M. Synchrotron-based XPS and NEXAFS study of surface chemical species during electrochemical oxidation of chalcopyrite. Hydrometallurgy. 2015;**156**:89-98. DOI: 10.1016/j.hydromet.2015.05.011

[24] Ghahremaninezhad A, Asselin E, Dixon DG. Electrochemical evaluation of the surface of chalcopyrite during dissolution in sulfuric acid solution. Electrochimica Acta. 2010;**55**(18):5041-5056. DOI: 10.1016/j.electacta.2010.03.052

[25] Zhao H, Wang J, Tao L, Cao P, Yang C, Qin W, et al. Roles of oxidants and reductants in bioleaching system of chalcopyrite at normal atmospheric pressure and 45 C. International Journal

of Mineral Processing. 2017;**162**:81-91. DOI: 10.1016/j.minpro.2017.04.002

[26] Khoshkhoo M, Dopson M, Shchukarev A, Sandström Å. Chalcopyrite leaching and bioleaching: An X-ray photoelectron spectroscopic (XPS) investigation on the nature of hindered dissolution. Hydrometallurgy. 2014;**149**:220-227. DOI: 10.1016/j.hydromet.2014.08.012

[27] Johnson DB. The biogeochemistry of biomining. In: Barton L, Mandl M, Loy A, editors. Geomicrobiology: Molecular and Environmental Perspective. 2010. pp. 401-426. DOI: 10.1007/978-90-481-9204-5_19

[28] Arias V, Lovera D, Quiñones J, Flores A, Gil J, Ramírez L, et al. Biolixiviación de cobre a partir de minerales sulfurados con altos tenores de pirita y arsenopirita. Revista Del Instituto de Investigación de La Facultad de Ingeniería Geológica, Minera, Metalurgica y Geográfica. 2015;**18**(36):157-164. DOI: 10.15381/iigeo.v18i36.12164

[29] Acevedo F, Gentina J. Biolixiviacion de minerales de cobre. Fundamentos y Perspectivas de las Tecnologías Biomineras. Archivos de Ingeniería Bioquímica. 2005:45-61. Available from: https://docplayer.es/10211791-Fundamentos-y-perspectivas-de-las-tecnologias-biomineras.html

[30] Hallberg K, Johnson B. Novel Acidophiles isolated from moderately acidic mine drainage waters. Hidrometallurgy. 2003;**71**(1):139-148. DOI: 10.1016/S0304-386X(03)00150-6

[31] Arias V, Rodríguez C, Ramírez P, Nonones E, Salazar D, Gil J, et al. Aislamiento de bacterias acidófilas a partir del drenaje ácido proveniente de las inmediaciones a las unidades mineras de Julcani y Recuperada, Huancavelica. Revista del Instituto de investigación de la Facultad de minas, metalurgia y ciencias geográficas. 2012;**15**(30):59-66. DOI: 10.15381/iigeo.v15i30.3450

[32] Akcil A, Ciftci H, Deveci H. Role and contribution of pure and mixed cultures of mesophiles in bioleaching of a pyritic chalcopyrite concentrate. Minerals Engineering. 2007;**20**:310-318. 2. DOI: 10.1016/j.mineng.2006.10.016

[33] Ospina JD, Mejía Restrepo E, Osorno Bedoya L, Márquez MA, Morales AL. Biooxidación de concentrados de arsenopirita por Acidithiobacillus ferrooxidans en erlenmeyer agitados. Revista Colombiana de Biotecnología. 2012;**XIV**(1):135-145. Available from: https://revistas.unal.edu.co/index.php/biotecnologia/article/view/31851#textoCompletoHTML

[34] Arias V, Anaya F, Quiñones J, Salazar, D, Gil J, Jamanca G. Adaptación del Thiobacillus Ferrooxidans a sustratos conformados con especies de minerales piríticos. Revista del Instituto de investigación de la Facultad de minas, metalurgia y ciencias geográficas. 2013;**16**(31):40-46. DOI: 10.15381/iigeo.v16i31.8339

[35] Rivera R, Camejo P, Moya F, López J, Munguía M. Estudio de biolixiviación de un mineral de sulfuros de un mineral de sulfuros de cobre de baja ley con bacterias Tio- y Ferro-oxidantes en condiciones termófilas. Revista de La Facultad de Ingeniería. 2011;**26**:65-73. Available from: http://www.revistaingenieria.uda.cl/Publicaciones/260009.pdf

[36] Pradhan N, Nathsarma KC, Srinivasa Rao K, Sukla LB, Mishra BK. Heap bioleaching of chalcopyrite: A review. In Minerals Engineering. 2008;**21**(5):355-365. DOI: 10.1016/j.mineng.2007.10.018

[37] Rawlings DE. Characteristics and adaptability of iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. In Microbial Cell Factories. 2005;**4**:1-15. DOI: 10.1186/1475-2859-4-13

[38] Yang C, Qin W, Zhao H, Wang J, Wang X. Mixed potential plays a key role in leaching of chalcopyrite: Experimental and theoretical analysis. Industrial & Engineering Chemistry Research. 2018;**57**(5):1733-1744. DOI: 10.1021/acs.iecr.7b02051