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Adaptation of *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* strains on sphalerite concentrate from mining waste

Adaptación de las cepas de *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* y *Leptospirillum ferrooxidans* en el concentrado de esfalerita de residuos mineros

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Keywords: *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans,* Adaptation, Biolixiviation, *Leptospirillum ferrooxidans.*

ABSTRACT

One of the main characteristics of the microorganisms used in the leaching process is their capacity to adapt to aggressive environments, characterized by a notable presence of heavy metals. In this study the adaptation of the strains *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* was evaluated on a sphalerite concentrate from mining waste. In the adaptation tests, the energy source (ferrous sulphate) was gradually replaced by percentages of mineral pulp, ending with subcultures without the addition of an external energy source. The results show that the strains A. *ferrooxidans* and A. *thiooxidans* are more resistant to high concentrations of sphalerite, compared to the strain of L. *ferrooxidans*, since, in the case of this strain, it was necessary to repeat some tests (8% of pulp), since a deficient development was evident. This was associated with factors such as the decrease of the Fe+2 energy source, the increase of the pulp density, the accumulation of toxic metals and secondary products of the dissolution of minerals and the increase of the pH.

Palabras clave: Acidithiobacillus ferrooxidans, *Acidithiobacillus thiooxidans,* Adaptación, Biolixiviación, *Leptospirillum ferrooxidans.*

RESUMEN

Una de las principales características de los microorganismos empleados en el proceso de lixiviación, es su capacidad de adaptación a ambientes agresivos, caracterizados por una notable presencia de metales pesados. En este estudio se evaluó la adaptación de las cepas *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* y *Leptospirillum ferrooxidans*, sobre un concentrado de esfalerita, proveniente de residuos mineros. En los ensayos de adaptación, se suplementó paulatinamente la fuente de energía (sulfato ferroso) cambiándola por porcentajes de pulpa mineral, finalizando con subcultivos sin adición de fuente de energía externa. Los resultados muestran que las cepas A. *ferrooxidans* y A. *thiooxidans* son más resistentes a altas concentraciones de esfalerita, frente a la cepa de L. *ferrooxidans*, puesto que, en el caso de esta cepa, fue necesario repetir algunos ensayos (8% de pulpa), ya que era evidente un desarrollo deficiente. Lo anterior se asoció con factores como la disminución de la fuente energética Fe+2, el aumento de la densidad de pulpa, la acumulación de metales tóxicos y productos secundarios de la disolución de los minerales y el aumento del pH.

Introduction

Biolixiviation consists of the application of biological agents in dissolution processes for the treatment and recovery of metals of economic interest [1] - [4].

A wide variety of microorganisms are widely used, mainly bacteria and filamentous fungi, which participate in the cycle of inorganic and organic compounds, allowing the transformation of minerals

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[5]. Research highlights autotrophic leaching, performed by acidophilous bacteria (pH<3), which obtain their source of energy from the oxidation of reduced sulfur compounds, ferrous ion and/or insoluble metallic sulfides, by means of electron transfer in environments with or without the presence of oxygen [6], [7].

Thus, today we see a greater number of works where autotrophic microorganisms are used, among the main and most widely studied are Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans and Leptospirillum ferrooxidans [6], [7]. A. ferrooxidans is a Gram-negative, bacillus-shaped, mesophilic acidophilus bacterium that lives optimally at a pH between 1 and 3, at a temperature of about 28°C. The bacterium A. ferrooxidans is a Gram-negative, bacillus-shaped, mesophilic acidophilus bacterium that lives optimally at a pH between 1 and 3, at a temperature of about 28°C. This microorganism stands out for obtaining its energy source from the oxidation of the ferrous ion (Fe+2) and reduced sulfur compounds, where the main byproducts of its energy transduction process, under aerobic conditions, are the ferric ion (Fe+3) and sulfuric acid (H2SO4), which participate in the mobilization of metals [5], [8], [9]. Works in which genome analysis is performed with bioinformatics tools indicate that this microorganism has a versatile metabolism, being able to grow in anaerobic or microaerophilic environments, using Fe+3 or elemental sulfur (S0) as alternative electron acceptors [8], [10]. On the other hand, this microorganism stands out for tolerating high concentrations of heavy metals (values higher than 300 mM of Cu, Zn and Fe) [7], [8] and is capable of fixing atmospheric CO2 and N2 as sources of carbon and nitrogen, respectively, gaining an important role in the treatment process of drains and acid soils with nutrient deficiency, where it is suggested that it is the primary producer of carbon and nitrogen [8]. A. thiooxidans, proteobacteria, acidophilus, mesophilic and bacillus-shaped, oxidizes only, reduced sulfur compounds such as hydrogen sulfide (H2S), inorganic sulfur (S0) and thiosulfate (S2O2-2), generating sulfuric acid (H2SO4) as part of its metabolic process [11]. Finally, L. ferrooxidans is characterized as an acidophilous, spiral-shaped, obligatory chemolithotrophic bacterium, using Fe+2 as an energy source. It is usually distinguished by tolerating higher concentrations of uranium, molybdenum and silver, as well as oxidizing pyrite at higher speeds than Acidithiobacillus species. However, it is usually sensitive to copper and has a reduced spectrum of substrates that it can assimilate [10], [12].

These characteristics make these microorganisms preferred for use in bioleaching for the treatment of industrial wastes [13]. In the case of mining waste and low grade minerals with zinc sulphide content, research has been carried out with important benefits in the recovery of Zn, which represents a challenge to be processed by conventional techniques, given the energy, environmental and economic consequences that these constitute [6], [14] - [16].

To name a few recent works, Ye et al. [17], investigated the recovery of the metals Zn, Pb and Fe from the tailings of a Pb-Zn mine in Shaoguan (China), by means of A. ferrooxidans in a bioreactor with pulp percentages of 10%. Extractions of 0.82% Pb, 97.38% Zn and 71.37% Fe were obtained after 50 days, for subsequent recovery by precipitation processes with sodium sulphide (Na2S), recovering more than 99% Zn. Conić et al. [18], treated a low-grade polymetallic sample from the Majdanpek mine (Serbia), in the presence of a mixed culture with A. ferrooxidans, A. thiooxidans and L. ferrooxidans in agitated tanks with 8% pulp, achieving recoveries of 89% Zn, 83% Cu and 68% Fe in 40 days. On the other hand, Ghassa et al. [19], reached optimal conditions with a pulp density of 5%, achieving a recovery of 98.5% Zn after 25 days of treatment, on a sample of sphalerite from the Anguran mine, Zanjan (Iran), where they worked with a consortium of A. ferrooxidans, A. thiooxidans and L. ferrooxidans, native strains of the place.

Exposure to mineral residues, especially at high concentrations, has a detrimental effect on the development of the micro-organism and, consequently, the dissolution of the minerals [20]-[23]. For this reason, the adaptation stage is necessary to adapt the metabolism of the bacteria to factors associated with bioleaching such as the release of

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metal ions, the production of inhibitory compounds such as jarosite, pH changes, among others [24].

The purpose of this publication was to compare the adaptive capacity of A. ferrooxidans, A. thiooxidans and L. ferrooxidans strains on a sphalerite concentrate from mining waste, by gradually increasing the percentage of pulp and decreasing its conventional energy source.

Materials and methods

Mineral: Obtaining and initial characterization

The sample was selected from the mining waste of La Gabia Gold Mine, located in the Quiebralomo zone in the municipality of Riosucio, northwest of the department of Caldas (Colombia). The polymetallic mineral was chosen for its appreciable content of sphalerite, pyrite, chalcopyrite, galena and gangue minerals.

The sample was subjected to a process of comminution and concentration by hydrophobic flotation. The comminution involved primary, secondary and grinding, performed in a Blake type jaw crusher at a speed of 250 rpm and a ball mill, reaching through material Mesh 200 Tyler. The hydrophobic flotation was performed in flotation chambers, in aqueous suspension, assisted by injection of air bubbles. A pulp percentage of 30% was used with copper sulfate as activator, Aero 404 as collector, lime as pH controller and pine oil as foaming agent, with operating conditions of 1200 rpm and conditioning and recovery time of 8 and 15 min, respectively.

For the initial characterization, polished sections of ore were made, according to ASTM D2797, previously washed and sterilized in an autoclave.

The sample was characterized by polarized flat light optical microscopy, Reflected Light mode (MOLPP/LR), X-ray diffraction (XRD) and Fourier Transform Infrared Spectrometry (FTIR). The mineralogical composition of the sample (Standard D2799) was estimated using the MOLPP technique in a Leitz Laborolux 11POL polarized flat light optical microscope, reflected light mode of operation, with magnification objectives of 10X and 4X. The XRD analysis was performed using a PANalytical Xpert Pro MPD diffractometer, set up spiner mode using a standard reflection in an operating range of 2θ: 10° - 90°, with a running speed of 0.05°/s, using copper tube. The FTIR analysis was performed on a Shimadzu Advantage 8400 equipment using KBr tablets (transmittance mode). The total number of scans was 24, with spectral resolution of 4cm-1, in a wave number range of 400 - 4000 cm-1, with Happ-Henzel apodization.

Microorganisms

The strains Acidithiobacillus ferrooxidans DSMZ 9465, Acidithiobacillus thiooxidans ATCC 15494 and Leptospirillum ferrooxidans DSMZ 2705 were used.

The microorganisms were previously activated at a pH of 1.8, adjusted with sulfuric acid, temperature of 30±1°C and stirring speed 180±10 rpm, during 21 days. The strains of A. ferrooxidans and L. ferrooxidans were cultured in medium T&K [25], with a composition of (NH4)SO4: 0.5 g/L, MgSO4.7H2O: 0.5 g/L and K2HPO4: 0.5 g/L, with addition of ferrous sulfate solution, FeSO4.7 H2O at 33.3%p/v. On the other hand, the strain of A. thiooxidans was grown in medium 9K [26], with a composition of (NH4)SO4: 3 g/L, MgSO4.7H2O: 0.5 g/L, K2HPO4: 0.5 g/L, KCl: 0.1 g/L and Ca(NO3)2: 0.1 g/L with 1%p/v sulphur addition.

Adaption Tests

The microorganisms were independently adapted to sphalerite concentrates, with progressive increases of the mineral concentrate.

These were inoculated at the orbital agitator level, using 250 mL erlenmeyer, with a working volume of 100 mL. For A. ferrooxidans and A. thiooxidans pulp percentages of 5 %p/v, 8 % w/v, 10 % w/v, 15 % w/v and 20 % w/v, with ferrous sulphate content of 15 % w/v, 12 % w/v, 10 %p/v, 5 % w/v and 0 % w/v, respectively, were used. In the case of the L. ferrooxidans strain, tests were prepared with pulp percentages of 1 % w/v, 3 % w/v, 5 % w/v, 8 % w/v and 10 % w/v, since these strains, starting the adaptation process, were affected in the tests with initial percentages of 5 % w/v.

The assays were incubated at a pH of 1.8 adjusted with sulfuric acid, temperature of 30±1°C and stirring speed 180±10 r.p.m. during 21 days. Evaporation control was done every 3 days due to volume loss; all conditions were replicated and the respective abiotic control was included.

Measurement Parameters

The kinetics of the adaptation process were monitored by pH and redox potential measurements, daily during the first 3 days and every 3 days thereafter.

The measurement of pH and redox potential was made in a multiparametric equipment HACH HQ40d multi PHC30103, with electrolyte KCl and reference electrode Ag0/AgCl respectively. The measurements were made in a horizontal laminar flow chamber, to guarantee the sterility conditions in the electrodes used and in the system.

Results and Discussion

Initial Characterization

The petrographic analysis made through the observations of polished sections of the concentrate of the initial sample, presented sphalerite as the majority mineral, accompanied by smaller quantities of pyrite, chalcopyrite, galena and minerals from the gangue (Qz) (figure 1.a). It can be observed that the grains are presented in diverse sizes, with anedral morphology strongly angular, with some grains of subedral pyrite (Figure 1, above left).

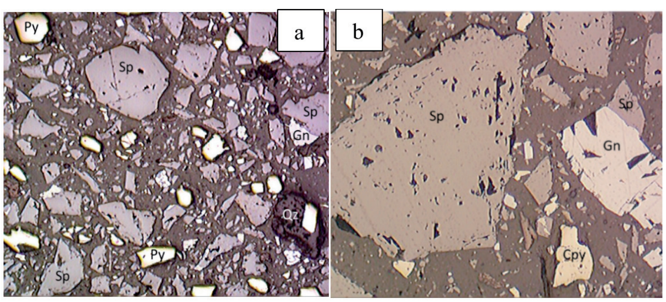


Figure 1. MOLPP images. a) 40X magnification. High concentration of sphalerite grains (Sp, blue-grey); inter-growth of sphalerite grain next to galena grain (Gn, upper and lower left). Pyrite grains (Py, brass yellow) with high reflectance. b) 100X magnification. Sphalerite grain with some porosity. Galena grain with triangular holes caused by polishing and cubic cleavage, with simple inter-growth next to sphalerite grain. Chalcopyrite grain.

Quantification of mineral phases using the point count technique, ASTM D2799, indicated an approximate content of 83.3% sphalerite, 3.6% pyrite, 3% chalcopyrite, 7.8% galena and 2.3% gangue minerals. These results were confirmed by DRX (figure 2) and FTIR (figure 3), where quartz, moscovite and illite were also identified as gangue minerals.

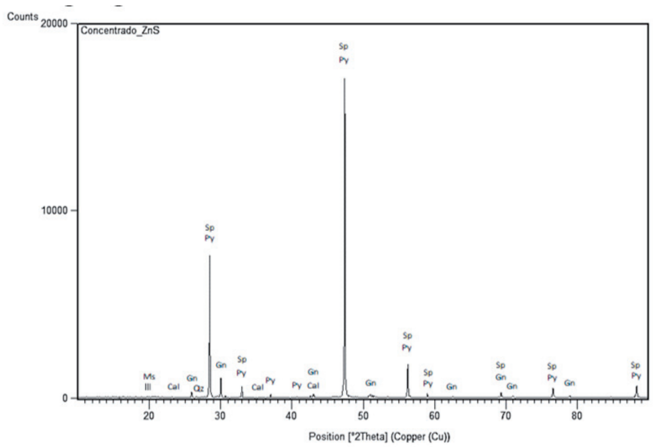


Figure 2. X-ray diffractogram of the concentrated sphalerite sample. (Sp: sphalerite, Py: pyrite, Cpy: chalcopyrite, Gn: galena, Qz: quartz. Ill: Illita, Ms: Muscovite, Calcite).

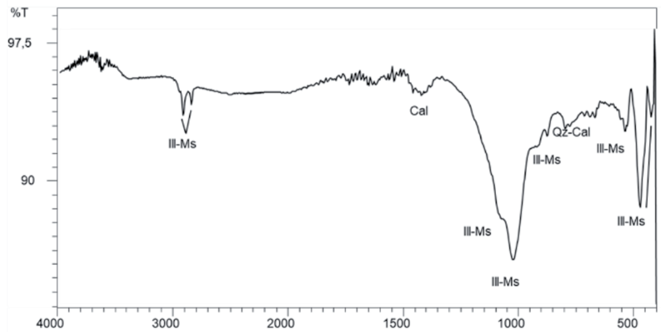


Figure 3. FTIR spectrum of the initial polymetallic sample and sphalerite concentrate. Qz: quartz. Ill: Illita, Ms: Muscovite, Lime: calcite.

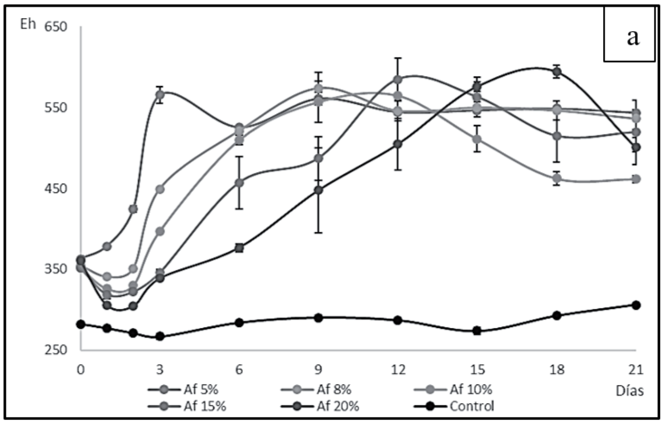
A. ferrooxidans. Redox potential (Eh) y pH

In general, the redox potential, for the different percentages of pulp (figure 4.a) presented a similar behavior, where the latency phase was clearly appreciated during the first two days, presenting a slightly different behavior as the proportion of pulp increased, where, for the higher percentages, a fall in the Eh value was observed on the first day, which was losing its importance with the decrease in the percentage, reaching an extreme in the trials with 5%, where a slight increase is observed. Similarly, the exponential growth phase was also more delayed depending on the percentage of pulp, where the greater the percentage of pulp the longer the exponential phase was and therefore more delayed to start the stationary phase. Finally, it is worth noting that in the trials with the highest percentage of pulp (10%, 15% and 20%), there was practically no stationary phase. In this sense, in the trials using 10% and 15% of pulp a decline was observed from day 12 to day 18, subsequently showing a small lag phase until the end of the experiment, while in the trials with 20% of pulp, in addition to a clear delay in reaching around 550 mV on day 18, after this there was an abrupt fall, insinuating a phase of death.

It is evident that the increase in the percentage of pulp had a negative effect on the microorganism, since, as mentioned above, the phases of adaptation and exponential growth took more days as the quantity of the mineral increased; in addition, as the percentage of pulp increased, the stationary phase lasted a shorter time, rapidly reaching the phase of decline.

The tests where the microorganisms were exposed to 5% of mineral, showed the best adaptation, evidenced by a minimum lag phase of one day and an exponential growth phase of 2 days, after which a stationary phase was observed, which was maintained until the end of the test. With a higher percentage of 8% of pulp, the behavior of the microorganism was very similar, showing short phases of adaptation and exponential growth and a stationary phase that was conserved during the trial, however, this phase only began after the sixth day. With higher percentages of pulp (10%, 15% and 20%), the latency and growth phases had a longer duration, reaching the stationary phase on day 12 and, unlike the previous ones, the phase of decline or death was appreciated.

In relation to pH (Figure 4.b), the different tests with increasing percentages of pulp presented a similar behavior, with increase in the first days and subsequent fall and stabilization until the end of the experiments. The main differences were in the sense of a greater increase in pH and a greater delay for the onset of the fall, as the percentage of pulp increased. The highest pH was reached by the tests with 20%, with values around 3.5. With the tests using 8%, 10% and 15% of pulp values between 2.5 and 2.6 of pH were observed, being that the test with 5% of pulp showed the lowest value of around 2.2. In the tests using 5% and 8% of pulp the pH started to decrease after the first day, whereas in the other tests it only started to decrease after day 3. The final pH was also dependent on the percentage of pulp, with the highest value for tests with 20% (about 2), followed by tests with 15% pulp (about 1.6), while the other tests presented similar final pH values of about 1.3.



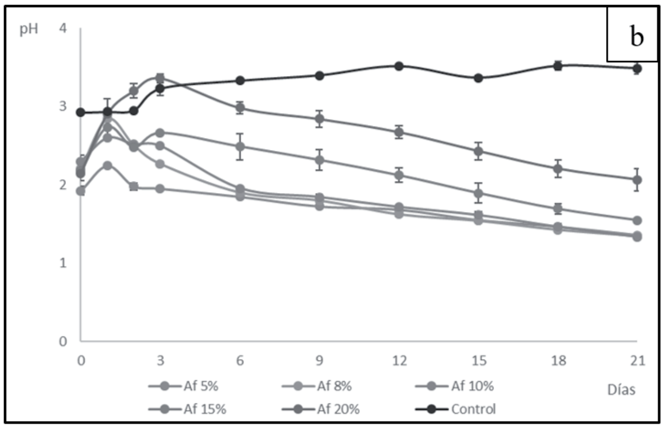


Figure 4. a) Eh curve for the A. ferrooxidans bioleaching process. b) pH curve for the A. ferrooxidans bioleaching process.

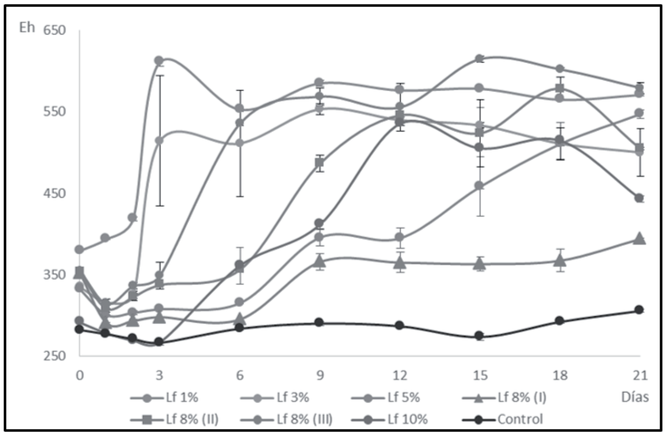
L. ferrooxidans. Behaviour of the potential redox (Eh) y del pH

In preliminary trials with L. ferrooxidans, where adaptation was attempted using initial percentages of 3% and 5% pulp, the microorganism did not proliferate, showing values of redox potential, indicator of the development of this microorganism, lower than 300 mV throughout the trial (data not shown), being that the authors in the literature report redox potentials higher than 600 mV [27], [28].

For this reason, it was decided to start adaptation trials with 1% of mineral percentage. As can be seen in figure 5.a, a similar behavior is presented for all percentages of pulp, showing an initial lag phase, followed by an exponential phase, culminating with a stationary phase in almost all the assays. However, with a percentage of pulp of 8%, it was necessary to carry out several tests, since the microorganism had difficulty adapting to this mineral content. Thus, a total of 3 tests (8% (I), 8% (II) and 8% (III)) were carried out, where the gradual differential behavior evidenced in the tests with the strains of A. ferrooxidans and A. thiooxidans was observed, as the microorganism adapted to this mineral content. The best behavior was observed by the tests using 1 and 3% of pulp, being that after these percentages of pulp the behavior was half erratic, since the tests with 5% and 8% (I-II) of pulp showed the lowest performance with a lag phase until day 6, after which Eh gradually increased, without reaching stationary phase. The trials with 8% (III) and 10% showed a lag phase until day 3, after which the trials using 8% (III) of pulp showed an exponential phase until day 15, with a subsequent slight drop until the end of the experiment. On the other hand, the test with 10% reached its maximum a little late (on day 9), after which Eh began to fall rapidly until the end of the experiment, evidencing a phase of mortality.

In relation to pH, it had a stable behavior over time in the different subcultures, with values between 1.5 and 2.5 (Figure 5.b), and without major variations. The tests in which the microorganisms did not present a stable behavior (8% (I-II)), the decrease in pH was much milder, reaching a minimum value of 2.

If these data are compared with those of the assays using *A. ferrooxidans*, it can be seen that in the latter a drop in pH is evident, which is not so in the assays with *L. ferrooxidans*, as well as a greater generalized increase in Eh values in the assays with *L. ferrooxidans*.



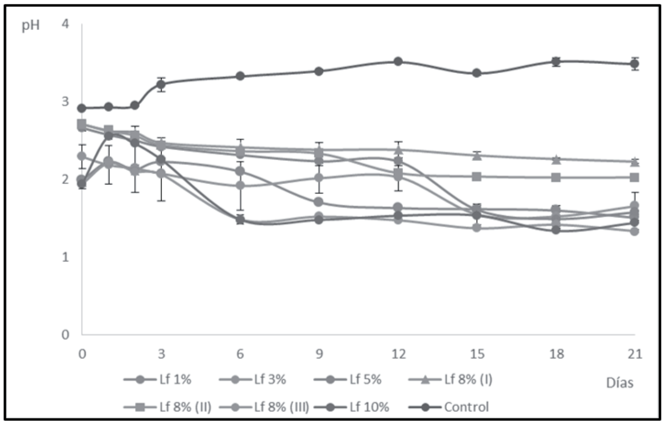


Figure 5. a) Eh curve for the bioleaching process by L. ferrooxidans. b) pH curve for the bioleaching process by L. ferrooxidans.

A. thiooxidans. Redox potential (Eh) and pH

In the trials using the A. thiooxidans strain, the redox potential had a similar behavior for the different subcultures, where a potential decrease was presented until day three, after which it gradually ascended, reaching a higher redox potential at a lower percentage of pulp (Figure 6.a). In the trials with 5% of pulp, the initial drop was practically not observed, which was evident in the other experiments.

In relation to pH, the general behavior was similar for all cases, starting with a strong increase in pH on the fi rst day (very little evident in the trials using 5% pulp, where what appears to be a lag phase was given until day three), and then starting to descend vertiginously until reaching optimal conditions (pH lower than 1) on day 6, from which it continued to descend slowly until reaching an average pH of 0.5 (Figure 6.b).

The difference between the tests is observed in the increase of the pH of the fi rst day, which is more accentuated, as the percentage of pulp increases, thus, with pulp density of 5%, the pH was 1.2, while with 20% of pulp, it was around 3.4.

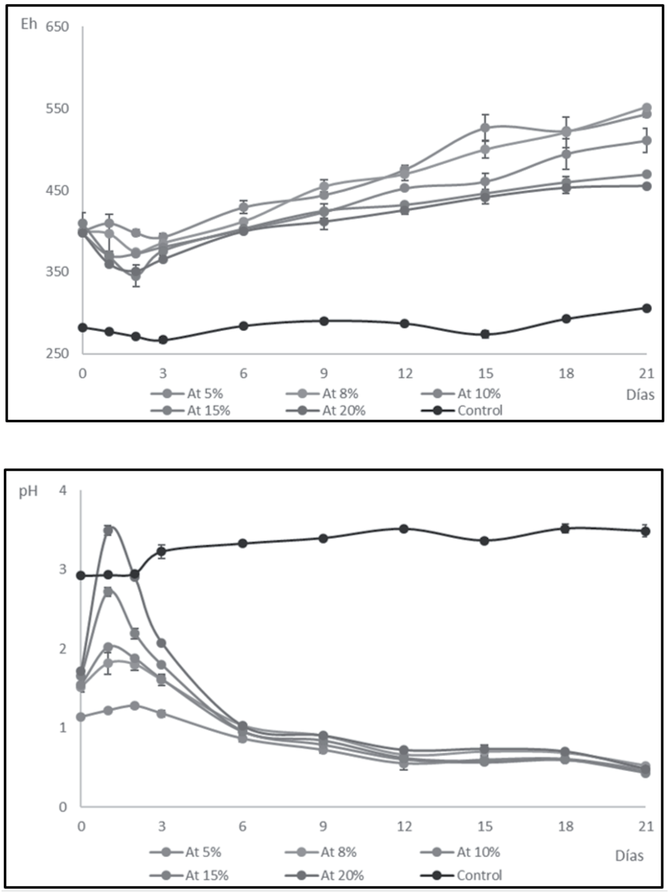


Figure 6. a) Eh curve for the bioleaching process by A. thiooxidans. b) pH curve for the bioleaching process by A. thiooxidans.

Factors involved in altering the behaviour of micro-organisms

Variables such as increased pulp density, accumulation of toxic metals and by-products of mineral dissolution and increased pH may be related to inhibition of the behaviour of A. ferrooxidans, A. thiooxidans and L. ferrooxidans during the adaptation process on Zn concentrate [6], [7], [10], [12].

With respect to the pulp density, this factor represents the surface area of mineral available to the microorganism and is inhibitory mainly at high concentrations, both mechanically and chemically. This has been explained by different authors, as the result of a greater number of collisions and frictions between mineral particles and bacterial cells, as a consequence of the increase in pulp density [22], [23], [30], [31]. This was postponed by Ban et al [22], who obtained a greater dissolution of marmatite, at low percentages of pulp, obtaining the greater dissolution speed of Zn (88.2%) with 1% of ore; this speed was decreasing as the percentage of pulp increased, obtaining around 9% of dissolution speed with 10% of ore, after 21 days of operation.

On the other hand, this increase in pulp density generates a lower availability of oxygen and carbon dioxide, necessary in the metabolism of bacteria, as has been demonstrated by different authors [23], [32]. This is the case of Mousavi et al [32], who studied the effect of pulp density on the bacterial leaching of a concentrated sample of sphalerite, using native cultures of A. ferrooxidans and S. thermosulfidooxidans. The authors found that at a pulp density of 2%p/v, dissolved O2 was approximately 2.5 ppm, but this gradually decreased as pulp density increased, reaching 1.5 ppm at a 10%p/v pulp density. The results show that leaching was affected by increased pulp density, with a recovery of 85% Zn (pulp density 2% (w/v)), over 40% Zn (pulp density 10% (w/v)) in 30 days. With this, the authors concluded that, at higher concentrations (10% of pulp), the oxygen demand by Fe+2 ions exceeded the oxygen supply rate by gas-liquid mass transfer, affecting bioleaching.

Additionally, Ye et al. [17], pointed out that a greater release of heavy metals is significantly influenced by the density of the pulp. With respect to the above, exposure to a greater amount of harmful elements makes it necessary to increase the tolerance of bacteria. To name some toxic influences of the metals associated to the sphalerite concentrate on the microbial cell, it has been detected the inhibition of transcription and translation by Pb and Cd, DNA damage by Pb, Cd, As, inhibition of enzymatic activity by Pb, As, Cd, Cu, alteration of cell membrane by Pb, Zn, Ni, Cu, and Cd, inhibition of cell division by Pb, Cd and Ni, and denaturation of proteins by Pb and Cd, among others, where the limits of toxicity by these elements over A. ferrooxidans are: 0.4 g/L Cr+3, 10 g/L Cu+2, 10 g/L Cd+2, 30 g/L Ni+2 and 30 g/L Zn+2 [30], [33].

The results obtained from the biolixiviation process, with 10% of pulp, of the different strains, indicated that surely the strains were affected by these

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elements, mainly ions of Zn+2, which was reported with values higher than 30 g/L Zn (Table I).

Table I. Content of Zn+2 in solution of the different cepas on day 21.

Subculture	*A. ferrooxidans*	*L. ferrooxidans*	*A. thiooxidans*	Control
Zn+2 content (g/L)	27,01	14,81	12,20	0,81
% of recovery	54,9%	30,1%	53,5%	1,7%

On the other hand, the inhibition of the development of the three strains was also attributed to the formation of undesirable compounds associated with the dissolution of minerals. The jarosite, precipitate of secondary formation, products of the oxidation of iron sulfides, is highlighted to a greater extent. This compound has an unfavorable effect on the adaptation of microorganisms and the dissociation of mineral species, since it inhibits the access of the microorganism to the mineral, causes the alteration of nutrients necessary for the microorganism, affects the process of diffusion of leaching agents between the surface of the mineral and causes the exhaustion of ferric ions [20].

The jarosite to an iron hydroxysulphate, which is produced by the oxidation of ferrous iron according to equation 1, where X can be occupied by up to 16 ions: K, NH4, Na, Pb, H3O, Ag, Cu, Tl, Ca, Ba, Sr, Ce, La, Nd, Bi, and Th [39]. This depends on the presence of these cations, generally obtained from the culture medium in the form of salts, or released from mineral species, associated with the process.

$$X^{+}+3Fe^{+3}+2HSO_4^{-}+6H_2O \rightarrow XFe_3(SO_4)_2OH_6+8H^{+} \quad (1)$$

Various investigations have indicated that the conditions necessary for the precipitation of these compounds depend mainly on pH, Fe+3 content and temperature. With regard to pH, this is usually between 1.8 and 2.5. As for the iron content, the lower limit of the molar concentration of Fe+3 is approximately 10-3 M (0.001 M). And the laboratory tests, in the production of synthetic jarosite, report formation temperatures between 20°C and 150°C, where the increase in temperature improves the reaction kinetics and allows the formation of higher purity jarosite [39].

Thus, taking into account the operating conditions of the adaptation process, the presence of this precipitate in the different samples was considered. However, in the case of the tests in which the A. thiooxidans strain was used, where decreases in pH of up to 0.44 were achieved, the appearance of this compound was considered to a lesser extent, compared to the A. ferrooxidans and L. ferrooxidans strains. This would indicate a lesser influence of jarosite on crops with the presence of this strain.

Regarding the increase of pH, common in the three strains, in the first days of the trials, this has been attributed by different authors, as the consequence in the variation of acid consumption, according to the dissolution reactions of the mineral and the oxidation of Fe+2 to Fe+3, the consumption of protons on the part of monosulfides as the sphalerite and the galena of the mineral sample and the presence of carbonate phases (calcite), which consume H+ ions [34]. Consequently, the increase in the percentage of pulp (appreciable Zn content) favours these reactions, causing a greater increase in pH.

Adaptative capacity of micro-organisms

Several studies have demonstrated the adaptive capacity of microorganisms, under extreme conditions, where acclimatization stages favour good microbial growth and development, through the activation of protection mechanisms against an external agent [24]. Thus, microorganisms possess mechanisms of resistance and detoxification in response to metals in the environment, which explains their relationship with the biogeochemical cycle of minerals. Many of the determinants of resistance are encoded on the chromosome or in mobile genetic elements such as plasmids and transposons. These activate resistance mechanisms against a non-metallic tension, metal or a specific metal, fulfilling the function of cell protection [33]. In this way, the microorganisms A. ferrooxidans, A. thiooxidans and L. ferrooxidans necessarily had to develop global mechanisms of resistance and detoxification for the protection of the cell, as the dissolution of the metallic ions associated to the mineral sample was carried out, taking into account the toxicity limits reported previously [33], [35].

The mechanisms of protection are still the subject of study among the main researchers [33]. In the case of A. ferrooxidans, it has been predicted that this strain has more external membrane receptors (OMRs) for the recognition of Fe+3 siderophores, as well as transporters involved in Fe+2 uptake systems, compared to other Acidithiobacillus [35]. This system could facilitate the metabolic process of iron oxidation, which takes place within the cell. This coindice with the data obtained in the redox potential profile for A. ferrooxidans, which indicates the changes of the concentration of Fe+3 and the molar relation of Fe+3 to Fe+2, and which presented a better metabolic development compared to the other two strains.

In addition, the response of the microorganism to oxidative stress (caused by the metallic ions dissolved in the medium from the mineral sample) favors the stimulation of several protection mechanisms such as the repair of oxidative damage, the omission of functions damaged by isoenzymes and the exclusion of oxidative stress agents by pumping toxins outflow [8]. This would explain the greater resistance of this microorganism over the other two, considering that in the cultures with the presence of A. ferrooxidans, they had a higher content of Zn+2, and despite this, the bacterial growth profiles (redox potential) were outstanding.

On the other hand, the pH profiles showed a remarkable decrease in pH of the A. thiooxidans strain over A. ferrooxidans, suggesting a good metabolic development of the latter. Studies identified the presence of a large number of unique genes in the A. thiooxidans strain, related to resistance to heavy metals, such as Zn and Hg [11], which would justify the existence of mechanisms to eliminate metallic toxic elements, mainly Zn, from sphalerite and the good metabolic development of A. thiooxidans.

In addition, studies show that the A. thiooxidans strain removes sulphur layers from the surface of the minerals, which passively dissolves the process. This reduces the resistance to diffusion and increases the rate of leaching, which in turn promotes the release of sulfur associated with sulfur, which is used as an energy source by this microorganism [36]. In the case of sphalerite, the solution releases elemental sulfur, which effectively, has been demonstrated in previous work, is located on the surface of the mineral, which could affect the bioleaching. However, the results of good performance.

On the other hand, with respect to the L. ferrooxidans strain, genome studies have not yet succeeded in identifying mechanisms associated with the resistance of metallic elements involved in the bioleaching of sphalerite. On the other hand, the identification of transposons related to resistance, mainly arsenic, has been detected, which suggests that these microorganisms are better able to treat minerals containing this element [37], [38].

Conclusions

The adaptation of A. ferrooxidans, A. thiooxidans and L. ferrooxidans strains with gradual increases in the percentage of pulp and a decrease in the conventional energy source obtained good results compared to trials without adaptation stages. However, a greater adaptive capacity of A. ferrooxidans and A. thiooxidans with respect to L. ferrooxidans is highlighted.

It was evidenced the effect of the increase in the percentage of pulp and the decrease of the energy source, which provoked increases in the times in the bacterial growth curves, mainly in the strain with L. ferrooxidans, probably associated to the stress conditions of the system, as the increase of the frictions between the mineral particles and the bacterial cells, the liberation of a greater concentration of metallic toxic ions and the production of compounds, product of the dissolution of the minerals.

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