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Biodepression of pyrite using *Acidithiobacillus ferrooxidans* in seawater

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ABSTRACT

In Cu-Mo deposits it is usual to find considerable amounts of pyrite (FeS_2). When processing this kind of ore by flotation, pyrite is rejected using lime to increase the pH to alkaline conditions (pH ~ 10–12). In the case of using seawater without pre-processing (raw seawater), the lime consumption increases dramatically and the recovery of molybdenite drops due to the precipitation of secondary ions (e.g. magnesium, sulfate, calcium and bicarbonate) on its surface. In order to avoid these negative effects, alternative ways of depressing pyrite should be considered.

The current work introduces the use of *Acidithiobacillus ferrooxidans*, bacteria commonly used in bioleaching, as an alternative to depress pyrite in seawater flotation. This work presents the results of biodepression, at microflotation scale, in three systems: fresh water, saline water (35 g/l of NaCl, which corresponds to the salt concentration in seawater) and seawater. It was determined that when pyrite is bio-conditioned with *A. ferrooxidans* before flotation, recovery of pyrite at pH 8 drops from 99% to 24% and 34% in fresh water and saline water, respectively. A similar behavior is observed when running the experiments in seawater, where recoveries drop from 97% to 36% in the presence of *A. ferrooxidans* in flotation at natural pH (7.8–8.2). Results show that it is possible to bio-depress pyrite with *A. ferrooxidans* in seawater flotation at natural pH.

1. Introduction

Water is a scarce but essential resource for human development. Although 70% of the earth's surface is covered with water, only 3% corresponds to fresh water and an important part of this water is frozen, so that only 1% of fresh surface water can be used for human activities. In Chile, most of the mining activity is concentrated in the north of the country, located in the Atacama Desert, which is considered the world's driest desert. According to a study of CEPAL (2012), most Chilean mining locations will see less water availability than before, caused by an increase in water evaporation (due to the increase in temperatures) and a decrease in rainfall. In this context, the use of seawater appears as an alternative resource for the mining activity.

Seawater represents an alternative to fresh water in mineral processing; however, when used without desalination in copper sulfide flotation, it generates some difficulties. Copper sulfide ores are usually associated to pyrite and molybdenite. In order to selectively separate copper sulfides from pyrite it is necessary to float in an alkaline medium, at a pH 10–12 or higher (Napier-Munn and Wills, 2006). Natural pH of seawater is between 7.8 and 8.2, depending on the salinity, carbonate/bicarbonate and borate/boric-acid concentration, which are responsible of a buffer effect in seawater (Pytkowicz and Atlas, 1975). In order to increase pH up to 10–12, lime is used, with a consumption that may be as high as 10 kg/ton of processed ore when seawater is used, compared to a lime consumption of ca. 1 kg/ton in fresh water (Castro, 2012). On the other hand, molybdenum recovery in seawater is strongly affected at high pH (Castro, 2012). The depression of molybdenite at high pH may be due to the precipitation of secondary ions. Maximum sodium chloride (NaCl) concentration in seawater is around 0.6 M which is equivalent to 35 g/l, with an important amount of secondary ions such as sulfate (2.7 g/l), magnesium ions (1.29 g/l), calcium ions (0.41 g/l), bicarbonate ions (0.145 g/l), borate ions (0.027 g/l), etc. These ions may influence the surface chemistry of some minerals by forming colloidal precipitates at pH > 10 (Castro, 2012). Calcium and magnesium ions may influence molybdenite flotation due to adsorption in the edges of its crystalline structure, rendering it hydrophilic or being able to attract negatively charged gangue minerals that make it hydrophilic as well (Zanin et al., 2009; Ramos et al., 2013; Hirajima et al., 2016; Laskowski et al., 2014).

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1.1. Bioflotation

Bioflotation is the technique of floating minerals with the aid of microorganisms. In this technique, the microorganisms must be capable of modifying the surface properties of the minerals prior to separation. The advantage of bioflotation is that bacteria can have the same functions as conventional reagents, but being biodegradables. There are no known records of bioflotation being used at industrial scale, however there are several studies about this topic at laboratory scale where the microorganisms used are the same that are used in bioleaching (e. g. *Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans, Acidithiobacillus thiooxidans*).

A. ferrooxidans is a bacterium widely used in bioleaching processes due to its capacity to oxidize minerals. It is a chemiolitotroph, autotrophic microorganism, since gets energy from the oxidation of inorganic compounds (Fe (II) and reduced sulfur compounds) and fixes carbon from CO_2 by Calvin-Benson cycle.*A. ferrooxidans* is also aerobic, acidophilic (pH < 4), mesophilic (optimum growth temperature around 30 °C) and Gram-negative (Rawlings, 2005).

It has been demonstrated that *A. ferrooxidans* can selectively depress pyrite in flotation with fresh water. It is believed that depression of pyrite is produced due to the attachment of the microorganism to the surface of the mineral, which changes their surface properties making them more hydrophilic. *A. ferrooxidans* shows a greater affinity for pyrite over other minerals (e.g. chalcopyrite, molybdenite, millerite, galena, arsenopyrite, chalcocite), meaning that it is able to selectively depress pyrite (Nagaoka et al., 1999; Chandraprabha et al., 2004, 2005; Hosseini et al., 2005; Misra et al., 1996; Mehrabani et al., 2011; Ohmura et al., 1993). Ohmura et al. (1993) in studies of bioflotation demonstrated that *A. ferrooxidans* with their oxidative capacity inhibited by sodium cyanide treatment, were able to adhere and depress pyrite. This suggests that biodepression may be possible even at conditions where *A. ferrooxidans* is inhibited, e.g., at high concentration of NaCl and natural pH, which would be the case if seawater was used.

In most ecosystems, bacteria are more likely to adhere to solid surfaces than remaining suspended in solution, as planktonic bacteria. The reason for this is because on solid surfaces microorganisms have greater access to nutrients and are more protected from hostile environments (Schaechter, 2009). The attachment of microorganisms to pyrite is mediated by the extracellular polymeric substances (EPS). EPS are metabolic products of the bacteria that surround the cell and have an influence on its surface properties (surface charge and hydrophobicity). It has been demonstrated that bacteria without EPS present less attachment to minerals that bacteria with EPS (Chandraprabha and Natarajan, 2013; Yu et al., 2011; Harneit et al., 2006; Zhu et al., 2012). The composition and quantity of EPS depends on the growth substrate, but generally EPS are composed of uronic acids, lipids, sugars and Fe (III) ions. Sugars are the main component of bacteria's EPS cultivated in iron (II) sulfate, corresponding to 52.2% wt/wt of the total EPS of *A. ferrooxidans* (Gehrke et al., 1998). EPS can be removed from *A. ferrooxidans* cells at high pH. It has been reported that bacteria subjected to pH 6.5 and 8.0 for 1 h release lipopolysaccharides which does not occur at pH 1.5 (Arredondo et al., 1994).

In this work the effect of *A. ferrooxidans* as pyrite flotation depressant is evaluated. As a comparison, results of biodepression in fresh water, and saline water (35 g/l NaCl, which corresponds to the concentration of sodium choride in seawater) are also presented. In addition, the effect of the EPS in pyrite flotation in seawater is evaluated. Using *A. ferrooxidans* instead of lime to depress pyrite in seawater at natural pH (7.8–8.2) would solve the problems associated with seawater flotation of sulfide copper ores, namely, the use of large quantities of lime would be avoided and molybdenite flotation would not be affected.

2. Experimental

2.1. Mineral preparation

The pyrite used in this work corresponds to hand picked mineral samples that where manually crushed. Molybdenite and chalcopyrite correspond to concentrates. The samples were dry screened between mesh # 70 and # 400 and cleaned with 6 N hydrochloric acid solution to remove the oxidized species from their surfaces. The minerals were cleaned batchwise and stored in a desiccator. The particle size distribution of the samples were determined with a laser diffraction sensor HELOS KR SympaTEC. It was determined that the P_{80} was 242 μm, 99 μm and 85 μm for pyrite, chalcopyrite and molybdenite, respectively. The surface area of pyrite was calculated assuming that the particles are cube-shaped. It was determined that the surface area was 0.0103 m^2/g. The purity of pyrite was ascertained by X-ray diffraction and was determined to be higher than 99%.

2.2. Microorganisms

The bacteria used in this work correspond to *Acidithiobacillus ferrooxidans* strain ATCC19859. The bacteria were grown at 30°C in sterile basal medium containing 0.4 g/l of ammonium sulfate ($(NH_4)_2SO_4$), 0.056 g/l of di-potassium hydrogen phosphate trihydrate ($K_2HPO_4 \times 3H_2O$) and 0.4 g/l of magnesium sulfate heptahydrate ($MgSO_4 \times 7H_2O$) at pH 1.6. Iron sulfate heptahydrate ($FeSO_4 \times 7H_2O$) was used as substrate. The sterile medium was inoculated with an active inoculum of *A. ferrooxidans* and a 33% (wt/v) solution of iron sulfate heptahydrate obtaining a concentration of 0.05 M of $FeSO_4 \times 7H_2O$. All experiments were carried out with fresh cells, harvested on the third day of incubation. At the end of the incubation, the solution containing the cells was filtered using Whatman 42 filter paper to remove precipitated solids. The filtrate was then centrifuged at 12,000 rpm for 20 min in a Sorvall RC-5B refrigerated Superspeed Centrifuge, at 5°C. The pellet obtained was re-suspended in a sulfuric acid (H_2SO_4) solution at pH 2. Re-suspended cells were filtered using a 0.22 μm Millipore membrane in order to obtain metabolite free centrifuged iron-free cells. Finally, cells retained in the membrane were re-suspended in pH 2 H_2SO_4 solution again. Bacterial concentration was monitored by direct counting in an Axio. Lab. A1 Zeiss microscope using a Neubauer counter.

2.3. Bioflotation

Bioflotation experiments were carried out in a 100 ml Hallimond tube at various pH's (4, 6, 8, 10 and 12) with fresh water, saline water and seawater. Fresh water corresponds to distilled water, saline water is a solution of distilled water with a concentration equal to 35 g/l of NaCl, which is the concentration of sodium chloride in seawater; and seawater corresponds to real seawater extracted from the central coast of Chile. Experiments in saline water were performed in order to determine whether NaCl is detrimental to biodepression, since it is known that *A. ferrooxidans* is inhibited at concentrations greater than 6 g/l of NaCl (Lawson et al., 1995). Bioflotation experiments were carried out in two ways: bio-conditioning at pH 3 or bio-conditioning at variable pH (4, 6, 8, 10 and 12). Prior to flotation, 1 g of pyrite was contacted with 19.5 ml of water and 0.5 ml of a solution with metabolite free centrifuged iron-free cells of *A. ferrooxidans* with a concentration equal to 1.2×10^{10} bacteria/ml and bio-conditioned for 15 min. The bio-conditioning was carried out for 15 min because it has been determined that this is the equilibrium time for attachment of *A. ferrooxidans* to pyrite (Chandraprabha et al., 2004). After bio-conditioning, 150 μl of 0.1% (wt/v) solution of collector sodium isopropyl xanthate (6.3×10^{-3} M) was added, corresponding to a collector dosage of 150 g/ton. The mineral was conditioned with the collector for 5 min at the desired pH. Subsequently, flotation was conducted by blowing nitrogen

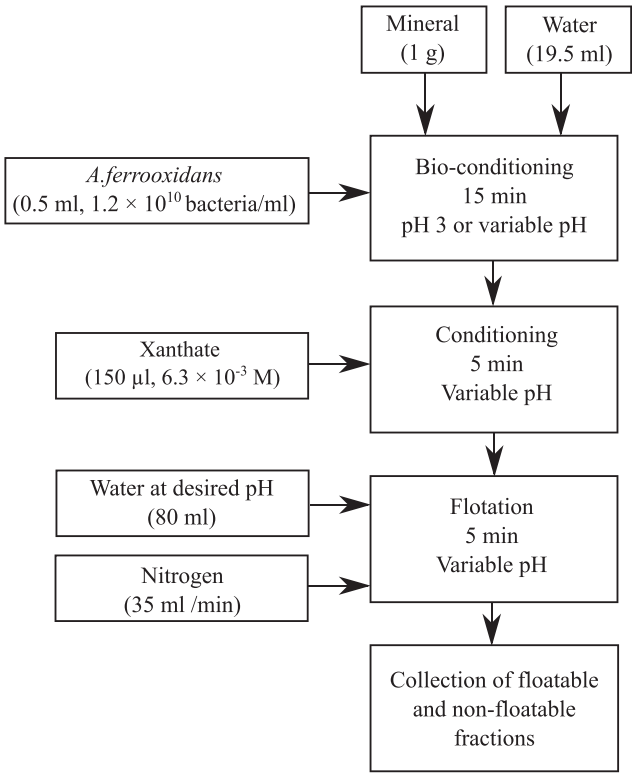

Fig. 1. Flowsheet of experimental bioflotation procedures.

at a flow rate of 35 ml/min for 5 min. Finally, floatable and non-floatable fractions were separately collected, filtered, dried and weighed. A flowsheet of the bioflotation experimental procedure is shown in Fig. 1. Flotations without microorganisms were performed in the Hallimond tube in the same way that bioflotations but without bio-conditioning. Bioflotation experiments of molybdenite and chalcopyrite in seawater at pH 8 were conducted following the same procedure that was described for pyrite. In all experiments the pH was adjusted by adding either a solution of potassium hydroxide (KOH) or sulfuric acid (H_2SO_4). All experiments were conducted in duplicate.

2.4. Attachment of A. ferrooxidans to pyrite in seawater

An experiment of attachment of *A. ferrooxidans* to pyrite was carried out in seawater at pH 8. This test was performed in a 50 ml Erlenmeyer flask, where 1 g of pyrite was contacted with 19.5 ml of seawater and 0.5 ml of a solution with metabolite free centrifuged iron-free cells of *A.ferrooxidans*. The resulting slurry was agitated on a rotary shaker. The number of cells per gram of mineral was measured at different times by direct counting in a Neubauer camera using a microscope Axio. Lab. A1 Zeiss. The concentration of cells adhered to the mineral was calculated as the difference between the cells in the liquid at a certain time and the initial cell concentration. This experiment was carried out in duplicate.

2.5. Determination of EPS released in seawater

In order to determine if bacteria were releasing EPS in seawater, the sugars in solution were quantified, because it is known that 51.3% of EPS correspond to sugars (hexoses) (Gehrke et al., 1998). A solution with metabolite free centrifuged iron-free cells of *A. ferrooxidans* (0.5 ml) and 19.5 ml of seawater were added to a glass beaker achieving a concentration approximately equal to the concentration in bioflotation experiments. The solution was magnetically agitated for 15 min at pH 8. Then, it was filtered with a 0.22 µm Millipore membrane to retain the bacteria and collect the EPS that were released into the solution. Sugars in solution were determined with the colorimetric method of Dubois et al. (1956) at a wavelength 490 nm in a Agilent 8453 UV–visible spectrophotometer. This method can be used to measure the kind of sugars hexoses in solution. The calibration curve was performed with glucose solutions between 0 and 35 µg/l. The absorbance of pure seawater filtered through a 0.22 µm Millipore membrane was measured as a control. The quantity of sugars released from the bacteria was calculated as the difference between the amount of sugars in seawater and the amount of sugars in the seawater contacted with bacteria. The experiment was conducted in duplicate.

2.6. Flotation with EPS in seawater

In order to evaluate the effect of EPS in flotations, flotation experiments were performed in a Hallimond tube conditioning the mineral with a solution containing EPS from *A. ferrooxidans* instead of using bacteria. The solution with EPS was obtained as was described in the previous section. Pyrite was conditioned with this solution for 15 min at pH 8 in seawater. After that, it was conditioned with the collector and the flotation was carried out, as it was described before. The experiment was conducted in duplicate.

3. Results and discussion

Fig. 2 shows the results obtained in flotation tests carried out with fresh water. When flotations were performed without cells, only conditioning with collector (squares), recoveries were near to 100% between pH 4 and 10 and dropped to 50% at pH 12. Bio-conditioning with *A. ferrooxidans* at pH 3 (circles), decreased recoveries at all pH's to values betwen 36% and 20%. This results are in agreement with the results obtained by Chandraprabha et al. (2004) in fresh water, where they determined that the recovery of pyrite decreased in all the studied range of pH (4–9) when the mineral was conditioned with *A. ferrooxidans*. It can be seen that when bio-conditioning is carried out at different pH (triangles), depression of pyrite is not so good at pH 6 and 8, reaching recoveries of 84% and 50%, respectively. Therefore, *A. ferrooxidans* has a better depresant effect on pyrite in fresh water at pH 6 and 8 when bio-conditioning is at pH 3.

Fig. 3 presents the results of flotations performed in saline water. When flotation tests were carried out only with collector (squares), recoveries were near to 100% until pH 10, decreasing to 74% at pH 12. The results of flotations performed when the bio-conditioning is conducted at pH 3 (circles) were similar to those carried out at different bio-conditioning pH (triangles). This indicates that bio-conditioning pH does not affect the performance of *A. ferrooxidans* in flotation with

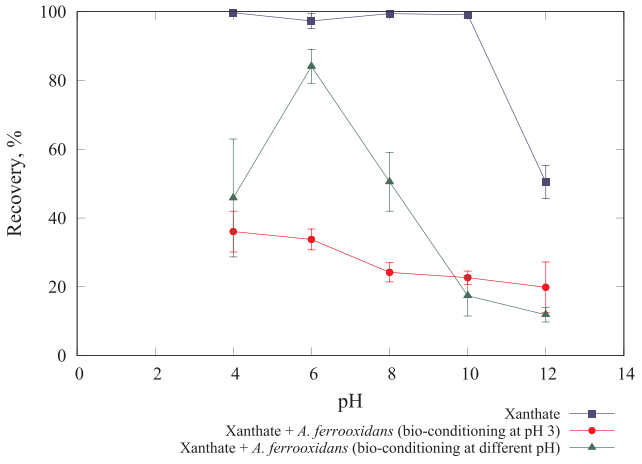

Fig. 2. Effect of *A. ferrooxidans* on pyrite recovery in fresh water. The concentration of *A. ferrooxidans* and xanthate in the conditioning was 3×10^8 bacteria/ml and 4.74×10^{-5} M, respectively.

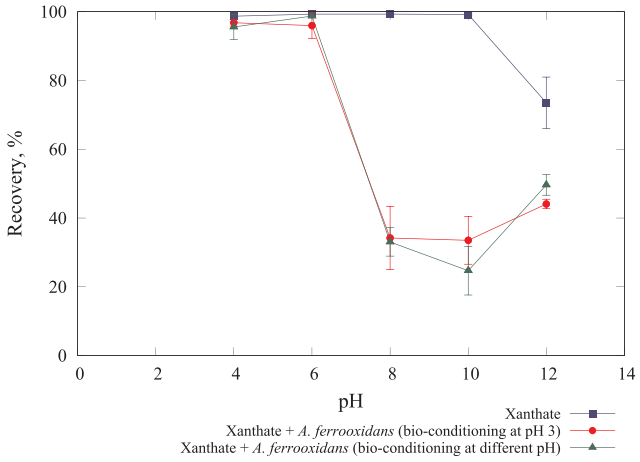

Fig. 3. Effect of *A. ferrooxidans* on pyrite recovery in saline water, 35 g/l NaCl. The concentration of *A. ferrooxidans* and xanthate in the conditioning was 3×10^8 bacteria/ml and 4.74×10^{-5} M, respectively.

water with NaCl at 35 g/l. It can be seen that between pH 4 and 6, *A. ferrooxidans* does not have any effect in pyrite recovery, but at pH 8 recovery drops sharply to 34% and is maintained low until pH 10. At pH 12 recovery slightly increases but is still lower than recovery without bacteria.

In the case of seawater flotation (Fig. 4), when tests were carried out only with collector (squares), recoveries were near to 100% between pH 4 and 8 and decreased to 29% and 33% at pH 10 and 12, respectively. The results of flotations performed bio-conditioning at pH 3 (circles) were similar to those carried out at different bio-conditioning pH (triangles). This indicates that bio-conditioning pH does not affect the performance of *A. ferrooxidans* in flotation with seawater. It can be seen that at pH 4 *A. ferrooxidans* does not have any effect on pyrite recovery, but at pH 6 and 8 the presence of microorganisms produces a significant decrease in pyrite floatability, reaching values equal to 70% and 36%, respectively.

Differences between bioflotations in saline water (35 g/l NaCl) and seawater show that not only Na^+ and Cl^- ions affect cell surface properties, but other ions present in seawater (e.g. calcium, borate, magnesium, sulfate and bicarbonate) are likely affecting the surface chemistry of both bacteria and pyrite.

Table 1 presents the recoveries of molybdenite, chalcopyrite and pyrite with and without *A. ferrooxidans*. The tests were performed at natural pH 8 (bio-conditioning with *A. ferrooxidans*, conditioning with

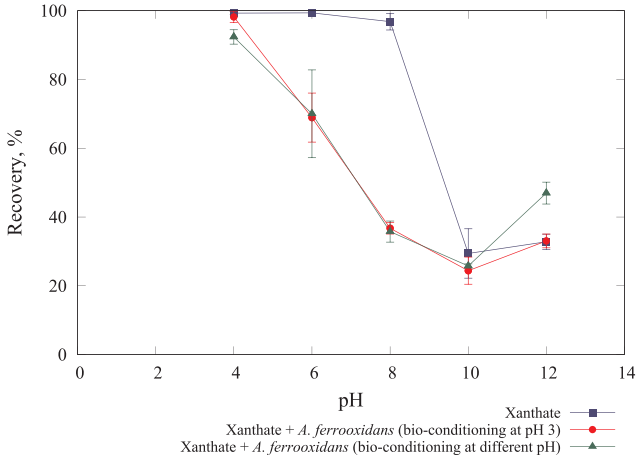

Fig. 4. Effect of *A. ferrooxidans* on pyrite recovery in seawater. The concentration of *A. ferrooxidans* and xanthate in the conditioning was 3×10^8 bacteria/ml and 4.74×10^{-5} M, respectively.

Table 1
Flotation recovery of molybdenite, chalcopyrite and pyrite with and without bio-conditioning with *A.ferrooxidans*. The bioflotations were performed in seawater at natural pH 8. The concentration of *A. ferrooxidans* and xanthate in the conditioning was 3×10^8 bacteria/ml and 4.74×10^{-5} M, respectively. The results are from single mineral flotation.

	Recovery	
	Without bacteria	With bacteria
Pyrite	97% ± 2.4%	36% ± 3.1%
Chalcopyrite	97% ± 1.1%	94% ± 0.7%
Molybdenite	97% ± 0.03%	96% ± 0.5%

collector and flotation). It is observed that *A. ferrooxidans* produced a decrease from 97% to 36% in pyrite recovery, while the recovery of molybdenite and chalcopyrite was always over 90%.

An experiment of bio-flotation was carried out in order to know if the sequence in which collector and bacteria are added influence the depressant effect. It was determined that when pyrite is conditioned with collector before than bacteria, the recovery only drop until 64% ± 18%. This means that the depressant effect of bacteria is more pronounced when pyrite is conditioned first with bacteria where the recovery was dropped until 36% ± 3.1%.

In the case of the attachment experiment, the initial concentration of bacteria in the solution was 3.5×10^8 bacteria/ml. After 15 min, it was determined that 2.5×10^8 bacteria/ml remain in solution, which means that ca. 28% of initial bacteria were attached to pyrite. The adsorption density of *A. ferrooxidans* in 15 min was 1.99×10^9 bacteria/g pyrite. The coverage pyrite by bacteria was calculated using the surface area of the bacteria and pyrite particles. For the calculation it was assumed a cell size equal to $1.3 \times 0.5\ \mu m$ ($6.5 \times 10^{-13}\ m^2$) (Rao and Subramanian, 2007). The surface area of pyrite was calculated assuming that the particles were cube-shaped ($0.0103\ m^2/g$). It was determined that attached bacteria cover 12.59% of pyrite surface.

Fig. 5 shows SEM images of non-floatable fraction (tailings) of pyrite obtained by performing bioflotations in seawater at pH 8. The low presence of *A. ferrooxidans* observed over pyrite does not agree with the calculated coverage surface area, which is explained by the possibility of bacteria being removed from the surface during the sample preparation. Therefore, only those bacteria that were more strongly attached remained on the surface. Nevertheless, the images show that *A.ferrooxidans* are able to attach to pyrite despite adverse conditions for growth and development in seawater (salinity and pH).

In order to know whether EPS of *A. ferrooxidans* were being released from bacteria in seawater at pH 8, the sugars in solution were measured. It was found that 3×10^8 bacteria/ml release $1.18\ \mu g/ml$ of sugars (hexoses), which is equivalent to $2.29\ \mu g/ml$ of EPS. To evaluate the effect of EPS on pyrite flotation, a test was carried out conditioning with EPS instead of bacteria. The recovery of pyrite when conditioning with EPS was 96% ± 0.5%. Therefore, EPS have a negligible effect on the depression of pyrite compared with the result obtained when bio-conditioning with cells was performed (36% ± 3.1%), which indicates that bacteria are required for the depression to take place. Ohmura et al. (1993) determined that the metabolic products, which include EPS, have a depressant effect over pyrite. Sugars are the main components of EPS and they are polar molecules that can react with minerals by acid-base or hydrogen bond interactions and can change the surface properties of the minerals (Laskowski et al., 2007). However, in this work it was determined that EPS are not causing the depression.

4. Conclusions

By bio-conditioning pyrite with *Acidithiobacillus ferrooxidans* for 15 min, it is possible to decrease flotation recovery in fresh water at pH > 4, in saline water (35 g/l of NaCl) at pH > 8 and in seawater at pH

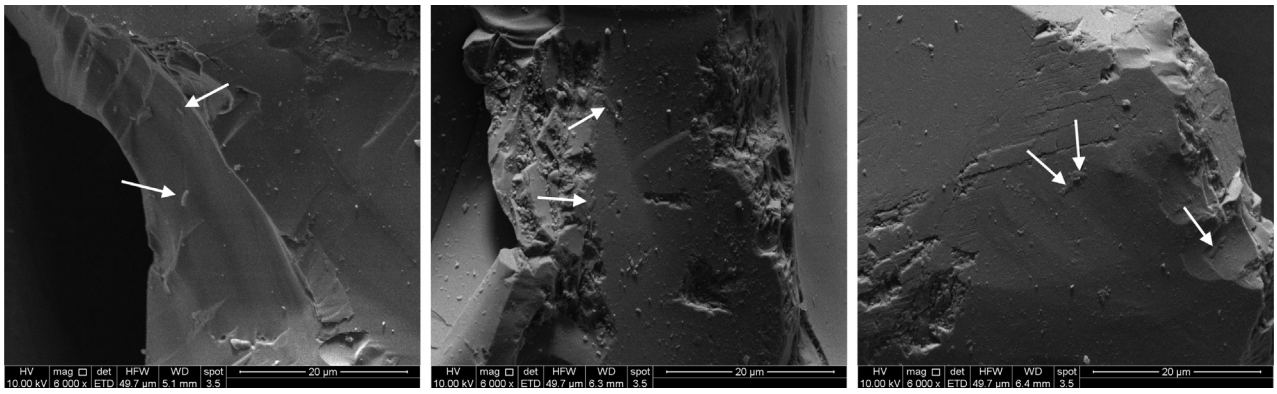

Fig. 5. SEM images obtained in the non-floatable fraction of bioflotation experiment in seawater at pH 8 with bio-conditioning with *A. ferrooxidans*. Arrows indicate the attached bacteria.

> 6. Although, concentrations higher than 6 g/l of NaCl are toxic to *A. ferrooxidans*, it was shown that bacteria are able to depress pyrite in seawater, which has a concentration equal to 35 g/l of NaCl. Therefore, *A. ferrooxidans* can serve as a depressant of pyrite even at conditions where the bacteria might be inhibited. While pyrite can be successfully depressed in seawater at pH 8 (which is the natural pH of seawater), it does not have a depressant effect on molybdenite or chalcopyrite. The results show that there is no difference between conditioning pyrite with *A. ferrooxidans* at pH 3 or 8 in seawater, therefore, it would not be necessary to acidify the pulp to carry out the bio-conditioning. On the other hand, the order in which bacteria and collector are added influence the efficiency of biodepression, getting a better performance when *A. ferrooxidans* is added first. These results indicate that *A. ferrooxidans* could be used as a pyrite depressant in seawater flotation at natural pH without affecting the flotation of molybdenite and/or chalcopyrite.

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