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Abstract

It has been shown that bacterium *Acidithiobacillus ferrooxidans* can be used to depress pyrite in seawater flotation at natural pH, which opens the possibility for its use as an alternative to lime to depress pyrite in copper sulfide flotation. In order to have a better understanding of the mechanisms involved in pyrite depression with *A. ferrooxidans*, different kind of experiments were carried out, including, contact angle, attachment kinetic and streaming potential measurements. All these experiments were carried out in seawater. Biodepression of pyrite improves when increasing pH from 4 to 8, with a decrease in recovery from 92% to 36%. This increase in depressing capability is accompanied by an increase in attachment density, from 2.58×10^8 bacteria/g to 1.99×10^9 bacteria/g at pH 4 and 8, respectively. These results suggest that the mechanism of depression is related to the attachment of bacteria to the pyrite surface. The streaming potential measurements showed that both, bacteria and pyrite, were negatively charged at pH 8. On the other hand, at pH 4, pyrite was positively charged and bacteria showed negative charge. This indicates that electrostatic forces are not responsible for the attachment of bacteria to the mineral. The contact angle of pyrite conditioned with pure seawater was 16°, which increased to 54° when collector was added, indicating an increase in hydrophobicity. Nevertheless, when pyrite was previous conditioned with bacteria, the contact angle increase only to 44° when collector was added. Thus, collector has a lower influence in the hydrophobicity of pyrite when the mineral has interacted with bacteria *A. ferrooxidans*.

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1. Introduction

In order to selectively separate copper sulfides from pyrite, the flotation process is carried out in an alkaline medium. To achieve this, lime is used to increase the pH up to $10 - 12$ (Napier-Munn and Wills, 2006). However, the use of lime in seawater flotation generates some problems when processing copper sulfide ores. Seawater have a natural pH between 7.8 and 8.2 and acts as a buffer solution (Pytkowicz and Atlas, 1975). For this reason, the consumption of lime increases significantly when this type of water is used. Castro (2012) determined that, to reach pH 11, the consumption of lime in fresh water is $1\,\mathrm{kg/ton}$ of processed ore, this value increases up to $10\,\mathrm{kg/ton}$ when seawater is used. In addition, molybdenum recovery in seawater decreases significantly at pH>9.5, which does not happen in fresh water.

Bacterium *Acidithiobacillus ferrooxidans* has been successfully used to depress pyrite both in fresh water and in seawater (Chandraprabha et al., 2004, 2005; Hosseini et al., 2005; Mehrabani et al., 2011; Misra et al., 1996; Nagaoka et al., 1999; Ohmura et al., 1993a; San Martín et al., 2018). The use of *A. ferrooxidans* as pyrite depressant in flotation of copper sulfide ores with seawater, represents an advantage compared to the use of lime. This is because the use of bacteria does not imply raising the pH, which would avoid the problems that were described above. Also, bacteria are selective for pyrite, then it could be used in seawater without affecting the recovery of molybdenite and chalcopyrite (San Martín et al., 2018).

Bacteria are able to modify the surface properties of minerals by the following mechanisms: oxidation of the mineral surface by microorganisms, attachment of the microbial cells to the mineral surface and reaction of the mineral surface with the metabolic substances produced by microorganisms (Rao and Subramanian, 2007). Some studies have been carried out to clarify the mechanisms of modification when bacteria are used specifically in bioflotation. For example, Ohmura et al. (1993a) determined that, in flotation with fresh water, *A. ferrooxidans* with its oxidative capacity inhibited is able to depress pyrite to the same extent as active bacteria, concluding that biooxidation of the minerals is not the biodepression mechanism. Also, determined that metabolic substances do not have a significant effect on pyrite flotation. On the other hand, determined that the higher the attachment density of bacteria to pyrite, the greater the depression of the mineral, suggesting that the depression mechanism is the

attachment of bacteria to the mineral. In a previous work (San Martín et al., 2018), it was determined that metabolic substances released when bacteria are contacted with seawater at pH 8 for 15 min, do not affect the recovery of pyrite. Seawater has a concentration of NaCl equal to 35 g/l and bacteria *A. ferrooxidans* are inhibited with concentrations higher than 6 g/l (Lawson et al., 1995). Despite this, bacteria are able to depress pyrite in seawater showing that conditions that inhibit the oxidative capacity, do not affect the performance of *A. ferrooxidans* as depressant. Other depression mechanisms that have been proposed are that *A. ferrooxidans* can act as pyrite flocculant (Natarajan and Das, 2003; Smith and Miettinen, 2006) and that bacteria may interact with the collector preventing its action over the mineral (Chandraprabha et al., 2004).

Bacteria attach to the minerals by electrostatic forces and/or hydrophobic forces (Solari et al., 1992; Devasia et al., 1993). Both, the charge and hydrophobicity of bacteria and mineral particles are pH dependent. Therefore, pH influences the attachment kinetic of bacteria to the minerals.

In the current work an exploratory experimental campaign was carried out in order to have a better understanding of the mechanisms involved in pyrite depression with *A. ferrooxidans* in seawater flotation. The experiments include contact angle, attachment kinetic and streaming potential measurements.

2. Experimental

Mineral preparation

The pyrite used in this work corresponds to hand picked mineral samples that were manually crushed. The samples were dry screened between mesh # 70 and # 400 and cleaned with 6N hydrochloric acid solution to remove the oxidized species from its surface. The mineral was cleaned batchwise and stored in a desiccator. The particle size distribution of the samples was determined with a laser diffraction sensor HELOS KR SympaTEC. It was determined that the P_{80} was 242 μm. The purity of the pyrite was ascertained by X-ray diffraction and it was determined to be higher than 99%. One cube-shaped crystal of pyrite was not crushed, it was cut in several slides which were used to do the contact angle measurements. Prior to the measurements, the pyrite slides were prepared in order to remove the oxidized species

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and homogenizing their surface. This was done by a sequence of polishing and ultrasonic cleaning which was repeated twice. The polishing was performed using a solution with diamond nanoparticles and ultrasonic cleaning was performed for 5 min.

Microorganisms

The bacteria used in this work correspond to *Acidithiobacillus ferrooxidans* strain ATCC19859. The bacteria were grown at 30℃ in sterile basal medium containing 0.4 g/l of ammonium sulphate ($(NH_4)_2SO_4$), 0.056 g/l of di-potassium hydrogen phosphate trihydrate ($K_2HPO_4 \times 3H_2O$) and 0.4 g/l of magnesium sulphate heptahydrate ($MgSO_4 \times 7H_2O$) at pH 1.6. Iron sulphate 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 sulphate heptahydrate obtaining a concentration of 0.05 M of $FeSO_4 \times 7H_2O$. 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℃. 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.

Bioflotation

Bioflotations experiments were carried out in a 100 ml Hallimond tube with seawater extracted from the central coast of Chile. 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. Both, flotation and bio-conditioning were performed at the same pH. 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). The conditioning with collector was performed by adding 150 μl of 0.1% (wt/v) solution of sodium isopropyl xanthate, 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 at a

flow rate of 35 ml/min for 5 min. Finally, floatable and non-floatable fractions were separately collected, filtered, dried and weighed. Flotations without microorganisms were performed in the Hallimond tube in the same way that bioflotations but without bio-conditioning. 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.

Contact angle

The contact angle was measured using the drop sessile method with the measuring device OCA 50 by Dataphysics and its software SCA 20. In these experiments artificial seawater at pH $\sim$ 8, prepared following the procedure of Kester et al. (1967), was used. The pyrite slides used in the contact angle measurements were obtained and prepared as was described above. Contact angle of pyrite conditioned in different ways was compared. The contact angle of a pure pyrite slide (non-conditioned slide) was measured by putting on its surface a drop of 5 μl artificial seawater. Then, pyrite slide was immersed in artificial seawater at pH $\sim$ 8 and conditioned with it for 15 min. After this time, the slide was removed and dried by blowing air. The contact angle was measured in the same way as in the non-conditioned slide. Subsequently, the pyrite slide was immersed in artificial seawater containing bacteria (4.04×10^8 bacteria/ml) and conditioned with it for 15 min. The slide was dried by blowing air and the contact angle was measured. Pyrite slide was immersed again in seawater but this time containing bacteria and collector sodium isopropyl xanthate (4.7×10^{-4} M) and conditioned for 5 min. The slide was dried by blowing air and the contact angle was measured. The same was done for another pyrite slide but changing the sequence: conditioning first with collector followed by bacteria. These experiments were carried out in duplicate.

Attachment kinetic

Attachment kinetic experiments of *A. ferrooxidans* to pyrite were carried out in seawater at pH 8 and 4. These tests were performed in 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* with a concentration of 1.2×10^{10} bacteria/ml. The resulting slurry was agitated on a rotary shaker. The concentration of cells in solution was measured at different times by direct counting in a Neubauer camera using a microscope Axio. Lab. A1

Zeiss. The number of cells attached to the mineral was calculated as the difference between the initial cell concentration and the cells in the liquid at a certain time. These experiments were carried out in duplicate.

Streaming potential

The streaming potential was measured using the Particle Charge Detector Mutek PCD 03. Streaming potential of *A. ferrooxidans* and pyrite were measured in presence of artificial seawater at different pH's (4, 6, 8, 10 and 12). The artificial seawater was prepared following the procedure of Kester et al. (1967). Pyrite (0.5 g) was mixed with 10 ml of water and conditioned with it for 5 min at the desired pH. The resulting slurry was added to the measuring cell of the Particle Charge Detector and the streaming potential was measured. The same procedure was executed with *A. ferrooxidans* by mixing 150 μl of a solution with metabolite free centrifuged iron-free cells with 9.85 ml of water. The concentration of bacteria was 2.39×10^8 bacteria/ml (0.2805 g of biomass). 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.

Note that flotation experiments were carried out in actual seawater and measurements of contact angle and streaming potential were conducted in artificial seawater. The streaming potential also was measured in actual seawater from the central coast of Chile and the tendency and values were similar to the results obtained with artificial seawater. This indicates that the results obtained with different types of water are comparable. It was decided to use artificial seawater in the streaming potential and contact angle experiments so that the results were reproducible.

3. Results and discussion

Contact angle

The contact angle of pyrite under different conditions and the recovery associated with the respective conditioning in the flotation experiment are presented in Table 1. Pictures corresponding to each contact angle measurement are presented in Figure 1. In all the cases the conditioning was carried out in seawater at pH $\sim$ 8.

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Without conditioning, the contact angle of the pyrite was 91°. This value decreased until 16° when the mineral was conditioned with pure seawater and, in flotation experiment (with pure seawater), the recovery was equal to 18%. Seawater makes pyrite more hydrophilic, possibly due to the precipitation of calcium and magnesium ions, forming colloidal hydroxides that can coat the mineral surface. When collector was added, the contact angle increased to 54° and recovery to 97%. This agrees with the literature where it is established that the hydrophobicity of pyrite is increased by adding xanthate collector due to the formation of dixanthogen in the mineral surface (Chau et al., 2009; Hu et al., 2009; Napier-Munn and Wills, 2006). By adding bacteria after collector, the contact angle remained almost constant, increasing slightly to 59°, while the recovery dropped to 64%. The fact that the contact angle remains relatively constant and the recovery drops, indicates that the mechanism of depression is not the decrease in the hydrophobicity of the mineral. There are some works that have demostrated that *A. ferrooxidans* acts as a pyrite floculant (Natarajan and Das, 2003; Smith and Miettinen, 2006), which may be a mechanism of depression in this case. By conditioning only with bacteria, the contact angle was 42°; and by conditioning with bacteria followed by collector, the contact angle was 44° and recovery 36%. The contact angle increased with bacteria compared to the results obtained with pure seawater (16°). These results show that bacteria do not make the mineral more hydrophilic. Also, show that collector is less effective when it is added after the bacteria than when is added alone or before: the increase in the contact angle when bacteria is added before the collector was not as significantly as conditioning only with collector or with collector followed by bacteria. This suggests that bacteria could be preventing the action of collector when they are added before.

Table 1: Contact angle of pyrite in seawater at pH ~ 8 conditioned in different ways.

Condition	Contact angle (°)	Pyrite recovery
Without conditioning	91 ± 6.5	-
Seawater	16 ± 6.8	18 ± 4.7
With collector	54 ± 3.5	97 ± 2.4
With collector and bacteria	59 ± 1.4	64 ± 18.0
With bacteria	42 ± 5.4	-
With bacteria and collector	44 ± 5.8	36 ± 3.1

Previous work has shown that 15 min are not enough to reach a strong attachment of *A. ferrooxidans* to pyrite. Bacteria are weakly bound to the mineral and for this reason most of the cells are lost when the mineral is separated from the liquid to see them by SEM (San Martín et al., 2018). Then, in the experiment of contact angle it is possible that only very few cells are attached to the pyrite slide when it is dried. Despite this, it was possible to see differences in contact angle values when pyrite is exposed to different conditions.
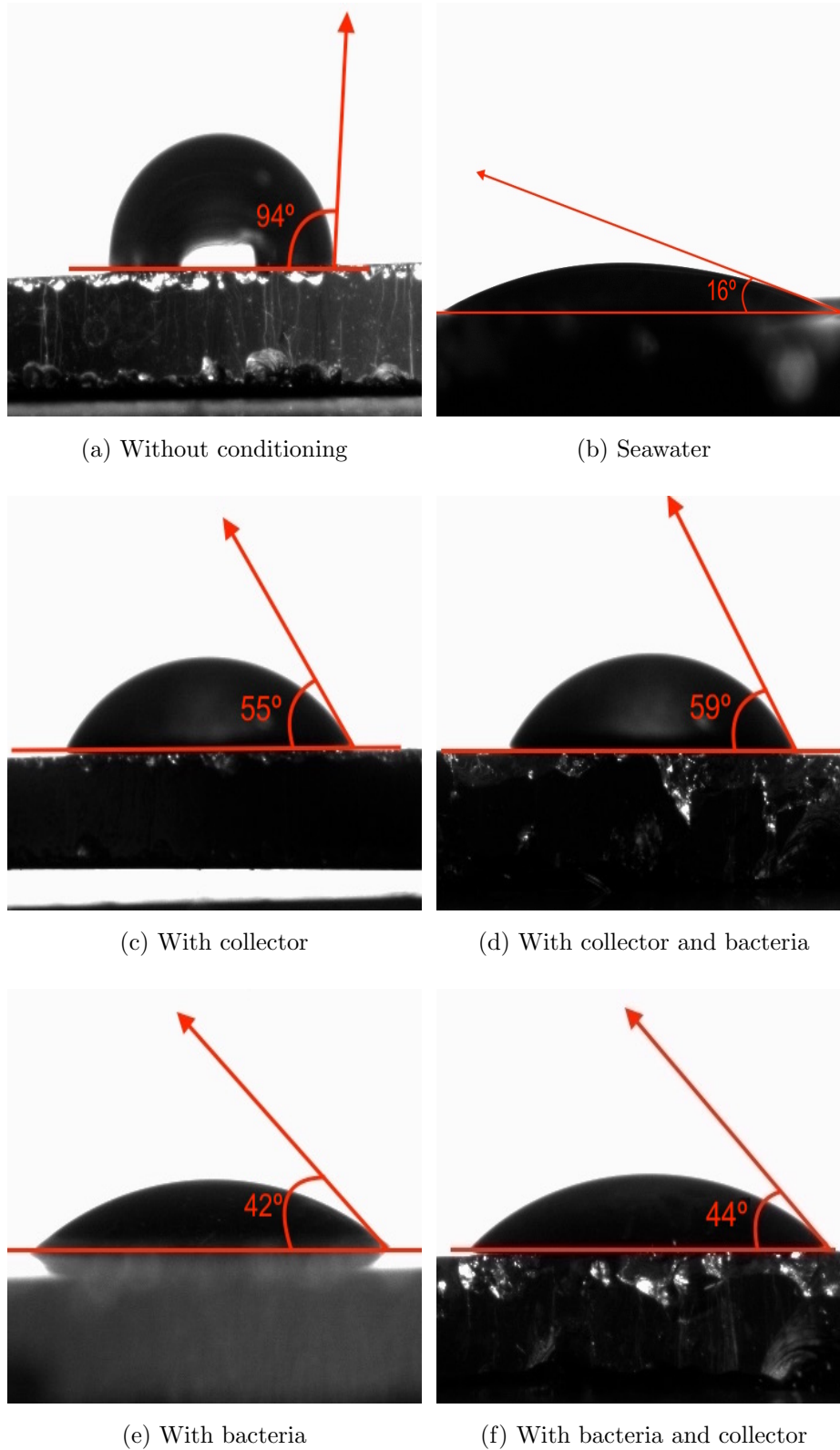
(a) Without conditioning

(b) Seawater

(c) With collector

(d) With collector and bacteria

(e) With bacteria

(f) With bacteria and collector

Figure 1: Pictures of the measurements of pyrite contact angle with the sessile drop method in seawater at pH ~ 8 conditioned in different ways. The concentration of *A. ferrooxidans* and xanthate in the conditioning was 4×10^8 bacteria/ml and 4.74×10^{-4} M, respectively.

Attachment kinetic

Figure 2 shows the attachment kinetic of *A. ferrooxidans* on pyrite in seawater at pH 4 and 8. There was a higher attachment density at pH 8 than at pH 4. Attachment kinetic curve at pH 4 shows that bacteria barely attach to the mineral obtaining an attachment density equal to 2.58×10^8 bacteria/g at 15 min. On the other hand, at pH 8 the number of attached bacteria increased with time reaching an attachment density equal to 1.99×10^9 bacteria/g at 15 min.

In bioflotation experiments with seawater it was determined that *A. ferrooxidans* is not able to depress pyrite at pH 4 obtaining a recovery equal to 92% (San Martín et al., 2018). On the other hand, at pH 8 the depressant effect is significant, decreasing the recovery until 36%. Therefore, it can be inferred that the attachment of bacteria to the mineral influences the biodepression of pyrite: the greater the attachment density, the greater the depressant effect. Several studies have demonstrated that *A. ferrooxidans* exhibits a higher attachment density to pyrite than to chalcopyrite and molybdenite. Also, have demonstrated that bacteria are not able to depress chalcopyrite or molybdenite but have a significant depressant effect on pyrite (Ohmura et al., 1993b; Harneit et al., 2006; Chandraprabha et al., 2004; Hosseini et al., 2005; Nagaoka et al., 1999; San Martín et al., 2018). This supports the hypothesis that attachment of bacteria to the mineral influence the depression of pyrite.
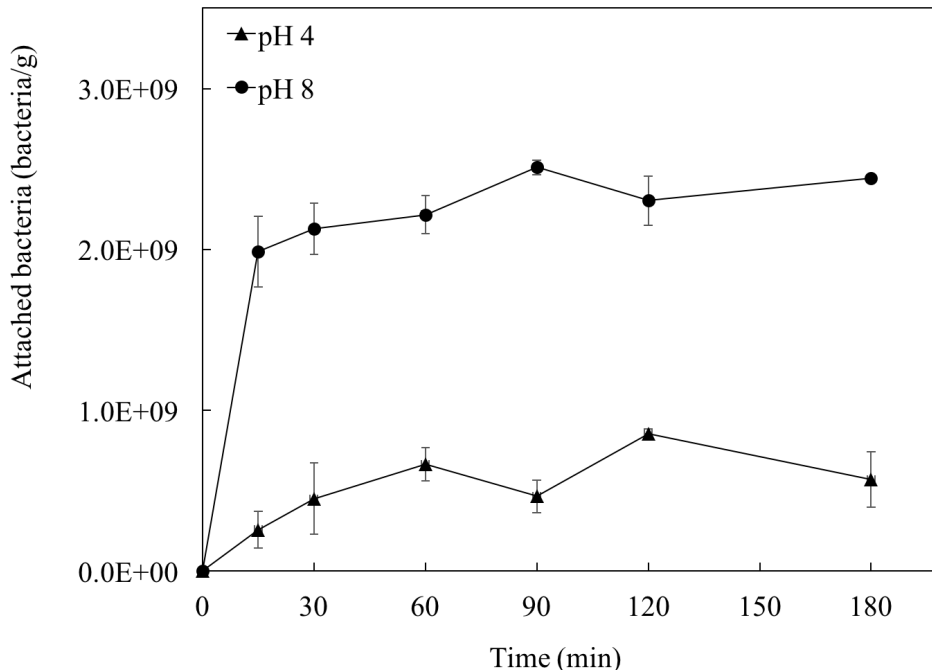
Figure 2: Attachment kinetic of *A. ferrooxidans* on pyrite in seawater at pH 4 and 8.

Streaming potential

In order to evaluate the role of surface charges on the attachment of bacteria to the mineral, the streaming potential was measured. Streaming potential is proportional to the zeta potential (Fuerstenau, 1956). Electrophoresis is the most common way to measure zeta potential, however it has the disadvantage that only can be used to particles smaller than $10\,\mu$m (Hunter, 2013). Since, the mineral sample used in this work was between 38 and $212\,\mu$m, streaming potential is a more appropriate method.

Figure 3 shows the streaming potential of pyrite and *A. ferrooxidans* in seawater at several pH's. It was determined that the isoelectric point (i.e.p.) of pyrite was close to 4: below this value the mineral is positively charged and above this value it is negatively charged. However, it is observed that pyrite potential becomes less negative at pH>10 so it will eventually be positively charged and will reach another i.e.p. Under acidic conditions, $FeSH_2^+$(sup) and $FeOH_2^+$(sup) dominate over the pyrite surface rendering the mineral positive-charged. On the other hand, under the alkali condition, mineral is negative-charged due to the presence of FeO^-(sup) and FeS^-(sup) (Liu et al., 2006). Bacteria showed two i.e.p. (pH 4 and 11),

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therefore, they are negatively charged between pH 4 and 11 and positively at pH<4 and at pH>11. The bacterial surface of *A. ferrooxidans* presents charge due to the presence of functional groups such as CH, CH_2, CH_3, NH, NH_2 (amine), NH_3, COOH (carboxyl), CONH and OH (hydroxyl)(Sharma et al., 2003; Chen et al., 2008; Devasia et al., 1993). These compounds originate from cellular components of the cell wall such as polysaccharides and proteins.

According to the results of attachment kinetic experiments in seawater, bacteria have a higher attachment density to pyrite at pH 8 than at pH 4. The streaming potential measurements showed that both, bacteria and pyrite, were negatively charged at pH 8. On the other hand, at pH 4, pyrite was positively charged and bacteria showed negative charge. Then, if electrostatic forces were responsible of adhesion of bacteria to the mineral, at pH 4 the attach- ment density should be higher than at pH 8, since equal charges repel and opposite charges attract. However, this does not happens, but the opposite. These results show that electrostatic forces do not play a major role in the attachment of bacteria to pyrite in seawater. In addition to electrostatic forces, hydrophobic forces have been pointed as responsible of attachment of bacteria to the minerals (Devasia et al., 1993; Solari et al., 1992). It is possible that this kind of forces are affecting the attachment of *A. ferrooxidans* to pyrite.
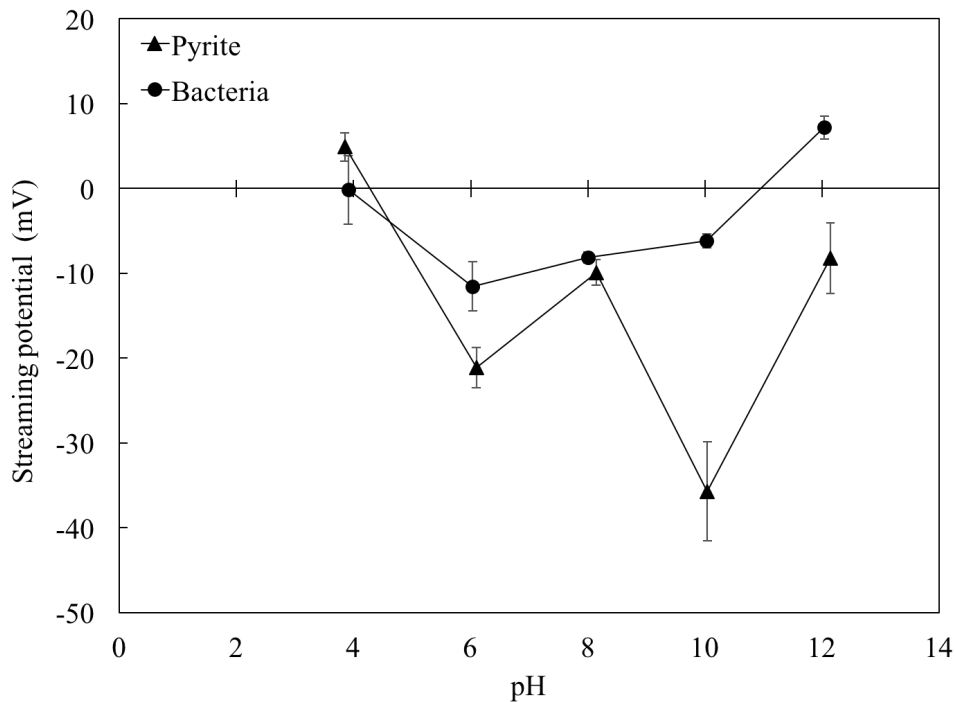

Figure 3: Streaming potential of *A. ferrooxidans* and pyrite in seawater.

4. Conclusions

Collector produces a smaller increase in the hydrophobicity (contact angle) of pyrite when the mineral is preconditioned with *A. ferrooxidans* than when it is used alone in seawater. This suggests that bacteria prevent the action of the collector, being this, one of the biodepression mechanisms.

The attachment density of *A. ferrooxidans* to pyrite in seawater at pH 8 was higher than at pH 4. At the same time, biodepression is better at pH 8 than at pH 4. Then, it can be inferred that the higher the attachment density of bacteria, the higher the depressant effect. Therefore, the results obtained in this work suggest that attachment of bacteria to pyrite is part of the biodepression mechanism.

Both pyrite and *A. ferrooxidans* showed to be negatively charged at pH 8 in seawater, while at pH 4 showed to be with opposite charge. Simultaneously, bacteria exhibited a higher attachment density at pH 8 than at pH 4. Hence, electrostatic forces are not responsible for the attachment of *A. ferrooxidans* to pyrite.

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