

Letter

Study of the Adhesion Mechanism of *Acidithiobacillus ferrooxidans* to Pyrite in Fresh and Saline Water

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Abstract: In the present work, the streaming potential of *A. ferrooxidans* and pyrite was measured in two environments: fresh and saline water (water with 35 g/L of NaCl) at different pH values. Also, attachment kinetics of *A. ferrooxidans* to pyrite was studied in fresh and saline water at pH 4. The results show that *A. ferrooxidans* and pyrite had lower streaming potentials (comparing absolute values) in saline water than in fresh water, indicating the compression in the electrical double layer caused by Cl^- and Na^+ ions. It was also determined that the bacteria had a higher level of attachment to pyrite in fresh water than in saline water. The high ionic strength of saline water reduced the attractive force between *A. ferrooxidans* and pyrite, which in turn reduced bacterial attachment. Electrostatic interactions were determined to be mainly repulsive, since the bacteria and mineral had the same charge at pH 4. Despite this, the bacteria adhered to pyrite, indicating that hydrophobic attraction forces and Lifshitz–van der Waals interactions were stronger than electrostatic interactions, which caused the adhesion of *A. ferrooxidans* to pyrite.

Keywords: streaming potential; *Acidithiobacillus ferrooxidans*; pyrite

1. Introduction

Water is essential for human development and domestic and industrial activities. Although 70% of the Earth's surface is covered with water, historically, it has been considered a scarce resource [1]. The average water runoff in Chile is 53,000 m³/person/year, which is high compared to the world average of 6600 m³/person/year [2,3]. However, arid conditions prevail in northern Chile and the average of available water decreases to 800 m³/person/year. Most of the mining in Chile is concentrated in this area, which features the driest desert in the world (Atacama Desert). Here, the main rivers and their tributaries have been declared exhausted by the Dirección General de Aguas [4–6]. In this context, new water sources are critical for the continuation of mining activity and its sustainable development.

The processing of copper sulfides is becoming more relevant with the depletion of oxidized copper ore deposits in Chile. Copper sulfides are processed by flotation to obtain copper concentrates. It is estimated that, by 2028, 91.4% of copper production in Chile will be copper concentrates [7]. Flotation accounts for more than half the water (67%) used in mining in Chile [8]. Given the water scarcity in northern Chile, some mining companies have started using seawater in flotation.

Flotation is a physical-chemical process used to separate valuable minerals from gangue minerals due to differences in hydrophobicity. Flotation is used in copper mining to separate copper sulfides and molybdenite from minerals of no value. The main metallic gangue in copper sulfide deposits is pyrite. In the flotation process, pyrite is depressed by adding lime to the pulp until pH 10 is reached [9].

The use of seawater in flotation produces two problems: excessive consumption of lime, because seawater acts as a buffer solution making it very difficult to change its natural pH (7.8–8.2), and a low recovery of molybdenite, which occurs at pH > 9.5 [10].

It has been shown that *Acidithiobacillus ferrooxidans* can depress pyrite in flotation with fresh water [11–19]. *A. ferrooxidans* has been evaluated as an option to lime as a pyrite depressant, given the problems of seawater flotation and the fact that these bacteria can depress pyrite at the natural pH of seawater (pH 8) [20].

Several authors have established that the mechanism of biodepression is related to the adhesion of bacteria to pyrite. For example, Ohmura et al. [17] showed that the higher is the level of adhesion of *A. ferrooxidans* to pyrite, the higher is the depressant effect of the bacteria on the mineral. Chandraprabha et al. [11] and Hosseini et al. [13] demonstrated that *A. ferrooxidans* has higher adhesion to pyrite than to chalcopyrite, and that the bacteria can depress pyrite more than it can depress chalcopyrite.

The adhesion of bacteria to pyrite can be seen as a process of adsorption of colloidal particles. According to the extended DLVO (Derjaguin–Landau–Verwey–Overbeek) theory, there are three types of forces among colloidal particles immersed in a solution: Lifshitz–van der Waals interactions (G^{LW}), electrostatic interactions (G^{EL}) and acid–base (or hydrophobic) interactions (G^{AB}). Lifshitz–van der Waals forces are always attractive; they depend on the nature of the particle and the solution, and the distance between particles. They do not depend on the charge of the particles, the pH level of the medium or the electrolyte concentration of the solution (ionic strength). Electrostatic interactions can be attractive or repulsive. Electrostatic interactions are influenced by the pH of the medium and the concentration of the electrolyte. Acid–base interactions can be attractive (hydrophobic) or repulsive (hydrophilic). The total force (G^{total}) between mineral particles and bacteria is the sum of all forces: Lifshitz–van der Waals (G^{LW}), electrostatic (G^{EL}) and acid–base interactions (G^{AB}) (Equation (1)):

$$G^{total} = G^{LW} + G^{EL} + G^{AB} \quad (1)$$

If the sum is negative, there is attraction among the particles, while if the sum is positive the colloidal particles will repel each other.

The bacterium *A. ferrooxidans* is surrounded by exopolysaccharides (EPS), which confers surface properties. EPS have different compositions depending on the growth substrate of the bacteria. For example, it has been determined that EPS from *A. ferrooxidans* grown in iron (II) sulfate mainly contain sugars (52.2%), while a growth substrate of sulfur results in the major component of EPS being lipids (53.8%) [21]. EPS influence bacterial adherence to minerals. It has been demonstrated that without EPS, *A. ferrooxidans* has less attachment to minerals than with EPS [22–24]. Bacterial charges are due to functional groups such as amino (NH₂), carboxyl (COOH), and hydroxyl (OH), which are constituents of lipids, sugars and proteins in their EPS [25–27].

The present study aimed to determine the behavior of surface charges of *A. ferrooxidans* and pyrite in fresh and saline water (water with NaCl at the concentration of seawater, 35 g/L) by measuring streaming potential, which in turn indicates whether electrostatic forces are responsible for bacteria attachment to pyrite.

2. Materials and Methods

2.1. Mineral Preparation

The pyrite used was handpicked and prepared by a sequential process of manual crushing and sieving until the required amount of material between 37 µm and 212 µm (mesh # 70 and # 400) was obtained. The samples were cleaned with a solution of hydrochloric acid 6 N to remove oxidized species from the pyrite surface. The samples were analyzed by X-ray diffraction with a Bruker D8 powder XRD instrument. The purity of the pyrite was determined to be above 99%.

2.2. Microorganisms Cultivation

The bacteria used were *Acidithiobacillus ferrooxidans* strain ATCC19859 cultivated in sterile medium at 30 °C. Table 1 shows the composition of the culture medium. All the reagents were from Merck, with a purity of $\geq 99\%$. The pH level of the culture medium was adjusted to 1.6 by adding sulfuric acid (H_2SO_4). The sterile medium (95 mL) was inoculated with 5 mL of an active inoculum of *A. ferrooxidans* and cultivated in a rotary shaker for 48 h. At the end of the incubation, the solution containing the cells was filtered using Whatman 42 filter paper to remove iron precipitates. The filtrate was 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 H_2SO_4 solution at pH 2. The re-suspended cells were filtered using a 0.22 μm Millipore membrane to obtain free cells. Finally, the cells retained in the membrane were re-suspended in a H_2SO_4 solution at pH 2 to obtain iron-free cells of *A. ferrooxidans*. The bacterial concentration was monitored by direct counting in an Axio. Lab.A1 Zeiss microscope using a Neubauer counter.

Table 1. Composition of the *A. ferrooxidans* culture medium.

Reagent	Chemical Formula	Concentration (g/L)	Molecular Weight (g/mol)
Ammonium sulfate	$(\text{NH}_4)_2\text{SO}_4$	0.4	132.14
Di-potassium hydrogen phosphate trihydrate	$\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$	0.056	228.23
Magnesium sulfate heptahydrate	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.4	246.48
Iron sulfate heptahydrate	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	14.85	278.02

2.3. Effect of Aqueous Medium on the Adhesion of Bacteria to Pyrite

Attachment kinetics experiments of *A. ferrooxidans* to pyrite were carried out in fresh water and saline water at pH 4. The fresh water was distilled, while distilled water with NaCl at a concentration of 35 g/L (concentration of sodium chloride in seawater) was used as the saline water. The experiments were conducted at an initial bacterial concentration of 2.52×10^8 bacteria/mL. The tests were performed in a 50 mL Erlenmeyer flask, where 1 g of pyrite was mixed with 19.5 mL of water and 0.5 mL of a solution with iron-free cells of *A. ferrooxidans*. The flask was shaken in a rotary shaker for 3 h. The number of cells in solution was determined at different times (15, 20, 30, 45, 60, 90, 120 and 180 min) by direct counting in a Neubauer camera using a microscope Axio. Lab. A1 Zeiss. The number of attached cells was calculated as the difference between the initial number of cells and the bacteria in the liquid at a certain time. The attachment kinetics experiments were carried out in duplicate. Control experiments were carried out in fresh and saline water to determine if bacteria attach to the internal walls of the flask. This experiment was conducted with only bacteria and water, no minerals were added.

2.4. Effect of pH on Streaming Potential Values

Streaming potential was measured with a particle charge detector (Mutek PCD 03). The streaming potentials of *A. ferrooxidans* and pyrite were measured in the presence of fresh and saline water at different pH levels (4, 6, 8, 10 and 12). Pyrite (0.5 g) was mixed with 10 mL of water and conditioned 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 applied with *A. ferrooxidans* by mixing 150 μL of a solution with iron-free cells with 9.85 mL of water. The concentration of cells was 2.39×10^8 bacteria/mL (0.2805 g of biomass). The pH levels in all the experiments were adjusted by adding either a solution of potassium hydroxide (KOH) or sulfuric acid (H_2SO_4). All experiments were conducted in duplicate.

3. Results and Discussion

3.1. Effect of Aqueous Medium on the Adhesion of Bacteria to Pyrite

Figure 1 shows the attachment kinetics of *A. ferrooxidans* to pyrite in fresh and saline water. The controls were experiments without minerals to determine if the bacteria adhere to the internal walls of the flask. It can be observed that in the controls the density of attached bacteria (bacteria attached per gram of pyrite) did not increase over the time, indicating that bacteria did not adhere to the internal walls of the flask. When pyrite was added, the density of attached bacteria increased. This occurred with either fresh or saline water. It can also be observed that the adhesion of the bacteria to pyrite was greater in fresh water than in saline water.

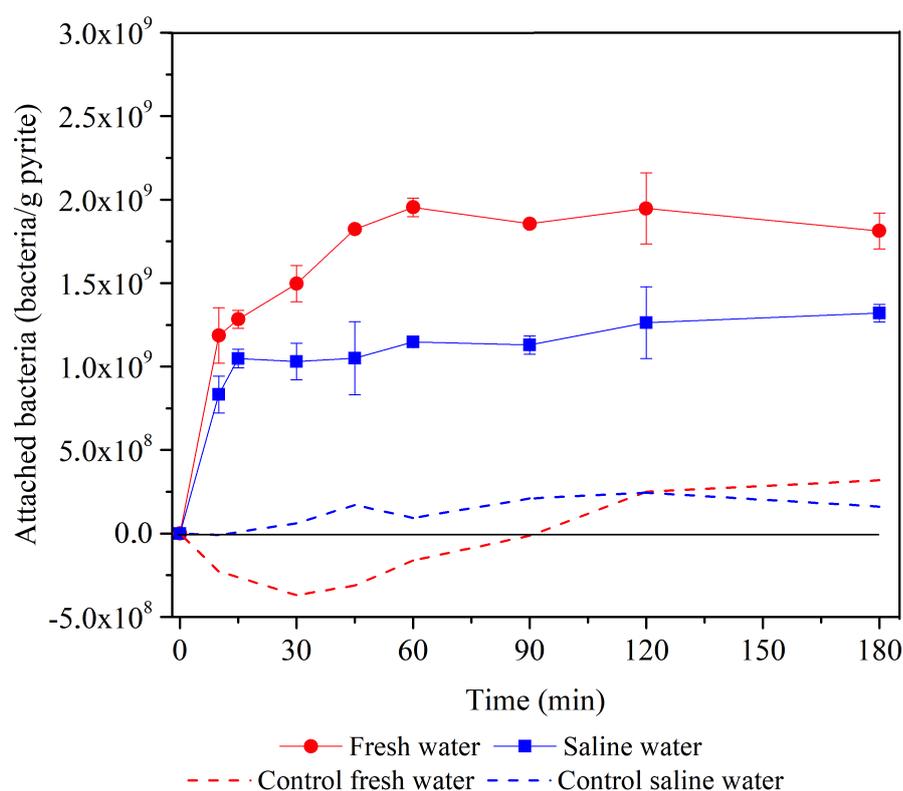


Figure 1. Attachment kinetics of *A. ferrooxidans* to pyrite in fresh and saline water (35 g/L NaCl or 0.6 M). The controls were experiments without minerals (only bacteria and water).

3.2. Effect of pH on Streaming Potential

Streaming potential was measured to evaluate the role of surface charges on the attachment of *A. ferrooxidans* to pyrite. Electrophoresis is the most common technique used to determine the charge of particle surfaces. However, it only can be used with particles smaller than 10 μm . Since the mineral sample used in this work was between 37 and 212 μm , streaming potential is a more appropriate method because it can be applied with particles as large as 300 μm [28].

Figure 2 shows the streaming potential of *A. ferrooxidans* and pyrite in fresh water at different pH levels. Both the bacteria and pyrite were negatively charged throughout the studied pH range (4–12). Other authors [11,29] have reported isoelectric points (IEP) of pyrite equal to 3.25 and 2.9. Therefore, pyrite does not have a positive charge at the studied pH range. Similarly, IEP values of 2.3 and 2.0 have been found for *A. ferrooxidans*, which is much lower than the lowest pH level used in this study [11,15,25,26,29].

Figure 3 shows the streaming potential of *A. ferrooxidans* and pyrite in saline water at different pH levels. Pyrite presented two IEPs (pH 5 and 10.2), which implies that it had a positive charge at $\text{pH} < 5$ and $\text{pH} > 10.2$. From pH 4 to 6, the streaming potential of pyrite was increasingly negative, while at pH 6 and above, it increased until pH 12 was reached. The bacteria had an IEP of 4.6, thus it was positively charged at $\text{pH} < 4.6$.

The positive charge of pyrite at low pH was due to the formation of FeOH_2^+ and FeSH_2^+ species. By raising the pH level, the pyrite streaming potential became progressively negative due to the formation of FeO^- and FeS^- on the mineral surface. In the case of bacteria, the negative charge in most of the studied pH range might be due to the presence of sugars. It was determined that 52.2% of the EPS of *A. ferrooxidans* cultured in iron (II) sulfate were sugars (monosaccharides) [21]. Monosaccharides have carboxyl groups (COOH) that can lose their protons, leaving a negative charge (COO^-).

The magnitude of streaming potential of pyrite and *A. ferrooxidans* was lower in saline water than in fresh water. This was because of the presence of sodium (Na^+) and chloride (Cl^-) ions that increased ionic strength and compressed the electrical double layer. The magnitude of the electrostatic charges depends on the ionic strength. The greater the ionic strength, the lower the magnitude of the surface charges and the lower the attractive force between pyrite and bacteria. If the ionic strength is low, the magnitude of the surface charges is high, therefore the force of attraction between the bacteria and the pyrite is high [30]. This is why the attachment density was higher in fresh water than in saline water (Figure 1).

Electrostatic interactions also depends on the pH of the medium. Figure 2 shows that at pH 4 both the bacteria and pyrite were negatively charged in fresh water, while Figure 3 shows that both the bacteria and pyrite were positively charged in saline water at the same pH level. This indicates that, although pyrite and bacteria should repel each other, this did not happen. Experiments in adhesion kinetics at pH 4 showed that the bacteria *A. ferrooxidans* adhered to pyrite in fresh water and in saline water (Figure 1). Thus, the attraction forces were higher than the repulsion forces. According to the extended DLVO theory, there are three kinds of interactions: Lifshitz–van der Waals (G^{LW}), electrostatic (G^{EL}) and acid–base (or hydrophobic) (G^{AB}). If the attraction force is not electrostatic, Lifshitz–van der Waals interactions and acid–base (or hydrophobic) interactions were possibly the motive forces that produced the attachment of *A. ferrooxidans* to pyrite. Acid–base (hydrophobic) and Lifshitz–van der Waals interactions were stronger than electrostatic repulsion interactions, making the adhesion of the bacteria to pyrite possible, even though their charges had the same sign. These results are in agreement with those of Tan and Chen [31], who also determined that surface charges are not the main motive force of *A. ferrooxidans* adhesion to pyrite. In saline water at pH 10, the bacteria and pyrite had opposite signs, so that a higher level of attachment could be reached at alkaline pH.

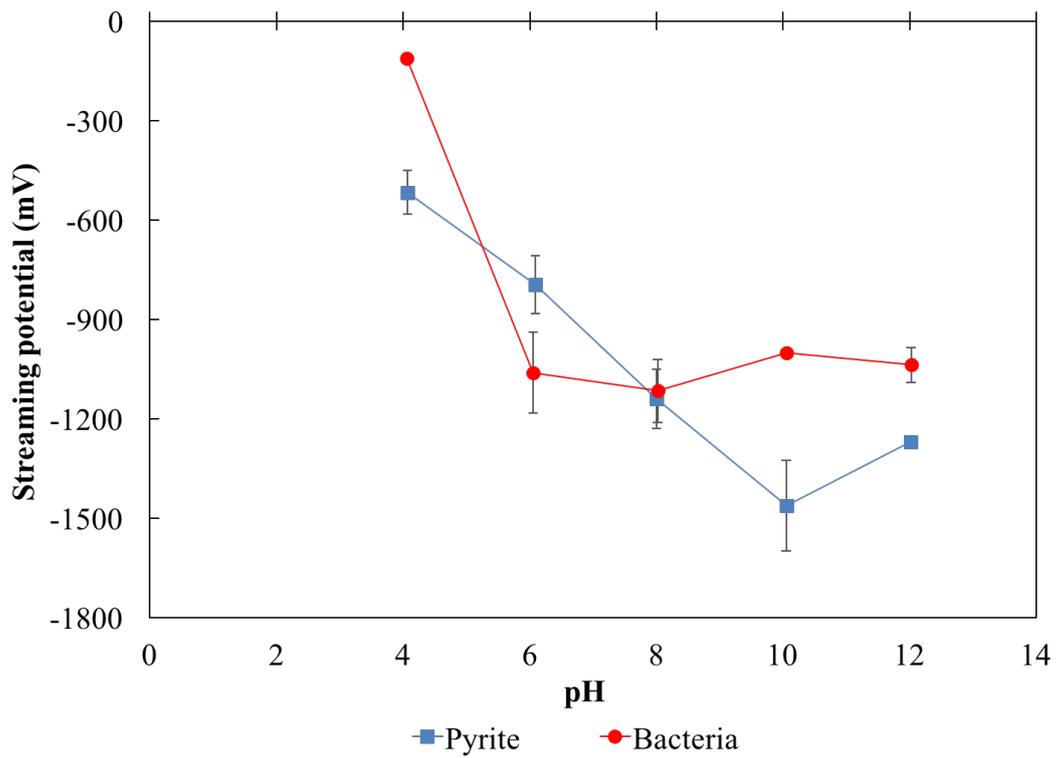


Figure 2. Streaming potential of *A. ferrooxidans* and pyrite in fresh water.

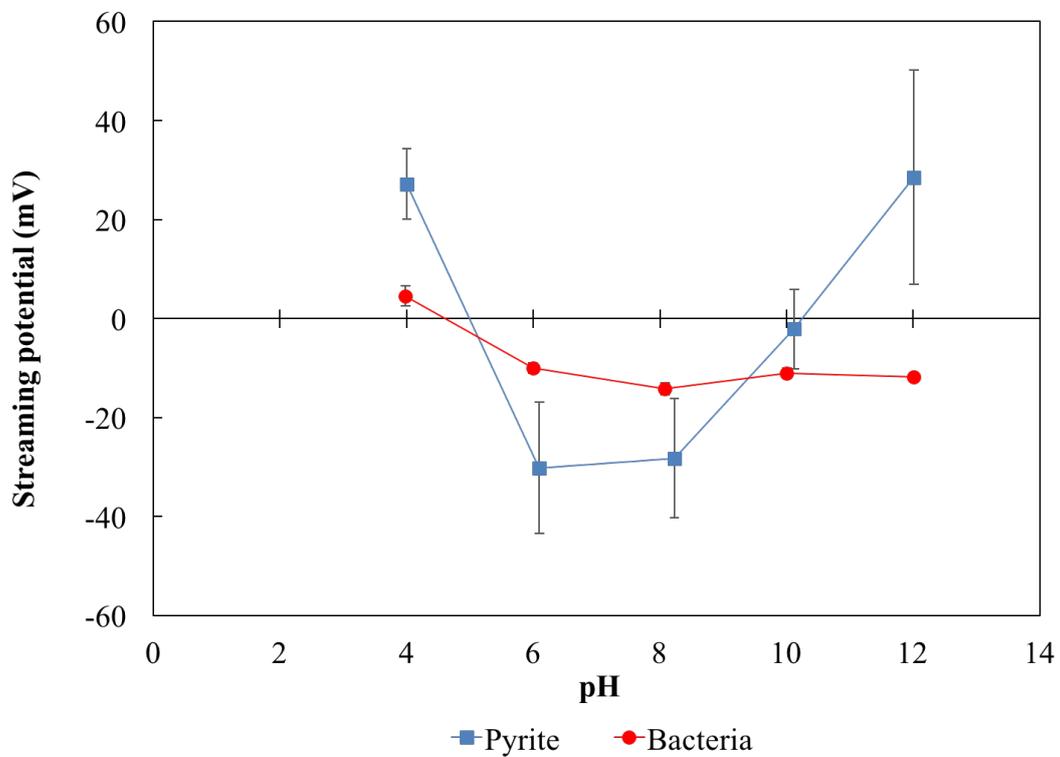


Figure 3. Streaming potential of *A. ferrooxidans* and pyrite in saline water (35 g/L NaCl or 0.6 M).

4. Conclusions

Electrostatic interactions to alter the adhesion of bacteria to pyrite can be manipulated by modifying the pH level or the ionic strength of the medium. Seawater has high ionic strength due to its high NaCl content (35 g/L). The use of raw seawater in a flotation process implies that a fixed value of ionic strength will be used. In this case, the pH level should be raised to alkaline values to increase electrostatic attraction and thus increase the level of bacterial attachment.

This work determined that the bacteria *A. ferrooxidans* and pyrite adhere even though electrostatic interactions have a repulsive effect. This indicates that hydrophobic attraction forces and Lifshitz–van der Waals interactions are stronger than electrostatic interactions, according to the extended DLVO theory.

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