

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

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Abstract

In the present work electrostatic forces are evaluated as the cause of attachment of *Acidithiobacillus ferrooxidans* to pyrite. Streaming potential of *A. ferrooxidans* and pyrite was measured in two environment, fresh water and saline water (water with 35 g/L of NaCl) at different pH's values. At the same time, attachment kinetics of *A. ferrooxidans* to pyrite were carried out in fresh and saline water at pH 4. The results showed that *A. ferrooxidans* and pyrite present lower streaming potential (comparing absolute values) in saline water than in fresh water, indicating the compression in the electric double layer caused by Cl^- and Na^+ ions. Simultaneously, it was determined that the bacteria present a higher attachment to pyrite in fresh water than in saline water. This suggests that electrostatics forces do not play a leading role in the adhesion mechanism.

Keywords: Streaming potential, *Acidithiobacillus ferrooxidans*, Pyrite

1. Introduction

When particles are immersed in a fluid, they develop a surface electrical charge which is compensated by an equivalent charge distribution (of opposite sign) in the aqueous phase (Stern layer). In the transient between the Stern layer and the bulk, a diffuse layer of counterions is
5 formed. Both layers together form the so-called double electric layer. The potential between the Stern layer and the diffuse layer is known as zeta potential. At high ionic strength, the double electric layer is compressed, which means that the zeta potential approaches zero at a smaller distance from the particle surface compared to when there is a low ionic strenght (Wills

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and Finch, 2015; Hunter, 2013).

10 Zeta potential is a parameter widely used to characterize the charge of mineral and microbial surfaces (Chen et al., 2008; Devasia et al., 1993; Liu et al., 2006; Solari et al., 1992). Zeta potential can be determined by the following techniques: microelectrophoresis, streaming potential measurements and electroosmosis. Microelectrophoresis is the most common way to measure zeta potential, however it has the disadvantage that only can be used for particles
15 smaller than $10\text{ }\mu\text{m}$ (Hunter, 2013). Streaming potential could be measured for bigger particles ($>10\text{ }\mu\text{m}$) and it is related to the zeta potential (ξ) by the following equation (Fuerstenau, 1956):

$$\xi = \frac{4\pi\eta}{\epsilon} \times \frac{E\lambda}{P} \quad (1)$$

where, η is the viscosity, ϵ is the dielectric constant, λ is the specific conductivity, P is the applied pressure difference and E is the streaming potential.

20 The mineral charge depends on the adsorption density of the ions that determine the potential on its surface. In the case of many minerals, including pyrite, the ions that determine the potential are H^+ and OH^- , therefore, the charge varies with the pH. For example, at low pH, pyrite acquires a positive charge due to the formation of FeOH_2^+ and FeSH_2^+ on its surface, as the pH rises, the FeO^- and FeS^- species become predominant and the mineral becomes
25 negatively charged.

Bacterium *A. ferrooxidans* is surrounded by substances called exopolysaccharides (EPS) which give them their surface properties. EPS have a different composition depending on the growth substrate of the bacteria. For example, it has been determined that EPS from *A. ferrooxidans* grown in iron (II) sulfate contain mainly sugars (52.2%), while, if the growth sub-
30 strate is sulfur, the major component of EPS corresponds to lipids (53.8%) (Gehrke et al., 1998). EPS have an influence on the adherence of bacteria to minerals. It has been demonstrated that *A. ferrooxidans* without EPS presents less attachment to minerals than the bacteria with EPS (Yu et al., 2011; Harneit et al., 2006; Chandraprabha and Natarajan, 2013). Bacteria present charge due to functional groups such as amino (NH_2), carboxyl (COOH), and hydroxyl (OH),
35 which are constituents of lipids, sugars and proteins on their EPS (Sharma et al., 2003; Chen et al., 2008; Devasia et al., 1993).

Nowadays, with the incorporation of seawater in the flotation and leaching processes, it is important to study the properties of minerals in this type of water. On the other hand, the use of bacteria in metallurgical processes is becoming more relevant because it represents
40 a sustainable alternative to the reagents currently used (Chandraprabha et al., 2004, 2005; Hosseini et al., 2005; Mehrabani et al., 2011; Misra et al., 1996; Nagaoka et al., 1999; Ohmura et al., 1993; San Martín et al., 2018; Donati et al., 2007; Rawlings, 2005). The present study aims to determine the behavior of surface charges of *A. ferrooxidans* and pyrite in fresh water and saline water (water with NaCl at the concentration of seawater, 35 g/L) by streaming potential
45 measurements, in order to know if electrostatic forces are the responsables of the attachment of the bacteria to pyrite.

2. Experimental

2.1. Mineral

The pyrite used corresponds to hand picked mineral samples that were manually crushed.
50 The samples were dry screened between 37 μ m-212 μ m (mesh # 70 and # 400) and cleaned with 6N hydrochloric acid solution to remove the oxidized species from their surfaces. The purity of the pyrite was ascertained by X-ray diffraction and it was determined to be higher than 99%.

2.2. Microorganisms

55 The bacteria used 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 ((NH₄)₂SO₄, purity $\geq$ 99.5%, Merck), 0.056 g/L of di-potassium hydrogen phosphate trihydrate (K₂HPO₄·3H₂O, purity $\geq$ 99%, Merck) and 0.4 g/L of magnesium sulfate heptahydrate (MgSO₄·7H₂O, purity $\geq$ 99.5%, Merck) at pH 1.6. Iron sulfate heptahydrate (FeSO₄·7H₂O, pu-
60 rity $\geq$ 99.5%, Merck) 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 FeSO₄·7H₂O. 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 Cen-
65 trifuge, at 5 °C. The pellet obtained was re-suspended in a sulfuric acid (H₂SO₄) solution at

pH 2. The re-suspended cells were filtered using a $0.22\ \mu\text{m}$ 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.

70 2.3. Streaming potential

In order to evaluate the role of surface charges on the attachment of *A. ferrooxidans* to pyrite, the streaming potential was measured. Electrophoresis is the most common way to determine the charge of particle surfaces, however, it only can be used to particles smaller than $10\ \mu\text{m}$. Since, the mineral sample used in this work was between 37 and $212\ \mu\text{m}$, streaming
75 potential is a more appropriate method. 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 fresh water and saline water at different pH's (4, 6, 8, 10 and 12). Fresh water corresponds to distilled water, while, distilled water with NaCl at a concentration of $35\ \text{g/L}$ (concentration of sodium chloride in seawater), was used as saline water. Pyrite
80 ($0.5\ \text{g}$) was mixed with $10\ \text{mL}$ of water and conditioned with it for $5\ \text{min}$ 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 $150\ \mu\text{L}$ of a solution with metabolite free centrifuged iron-free cells with $9.85\ \text{mL}$ of water. The concentration of bacteria was 2.39×10^8 bacteria/mL ($0.2805\ \text{g}$ of biomass). In all
85 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 kinetics

Attachment kinetics experiments of *A. ferrooxidans* to pyrite were carried out in fresh water and saline water at pH 4. The experiments were conducted at an initial concentration of bacteria
90 equal to 2.52×10^8 bacteria/mL. These tests were performed in a $50\ \text{mL}$ Erlenmeyer flask, where $1\ \text{g}$ of pyrite was contacted with $19.5\ \text{mL}$ of water and $0.5\ \text{mL}$ of a solution with metabolite free centrifuged iron-free cells of *A. ferrooxidans*. The resulting slurry was agitated on a rotary shaker. A control experiment was carried out in fresh and in saline water to evaluate if bacteria attach to the internal walls of the flask. This experiment was carried out only with bacteria and

95 water, no mineral was added. 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 cells in the liquid at a certain time and the initial cell concentration. The attachment kinetics experiments were carried out in duplicate.

100 3. Results and Discussion

The streaming potential of *A. ferrooxidans* and pyrite in fresh water at different pH's is presented in Figure 1. At all the pH range studied (4-12), both bacteria and pyrite were negatively charged. Other authors (Chandrababha et al., 2004; Devasia and Natarajan, 2010) have reported isoelectric points (IEP) of pyrite equals to 3.25 and 2.9. Therefore, at the pH
105 range studied it is not possible to find pyrite positively charged. Alike, IEP values equals to 2.3 and 2.0 have been found for *A. ferrooxidans*, far from the lower pH analyzed in this study (Chandrababha et al., 2004; Devasia and Natarajan, 2010; Chen et al., 2008; Misra et al., 1996; Sharma et al., 2003).

Figure 2 shows the streaming potential of *A. ferrooxidans* and pyrite in saline water at
110 different pH's. Pyrite presented two IEP (pH 5 and 10.2), this implies that it was positively charged at pH<5 and at pH>10.2. From pH 4 to 6, the streaming potential of pyrite become increasingly negative but, from pH 6 up, it becomes progressively positive until it reaches pH 12. The bacteria showed an IEP equal to 4.6, then, it was positively charge at pH <4.6.

The positive charge of pyrite at low pH is due to the formation of FeOH_2^+ and FeSH_2^+
115 species. By raising the pH, pyrite streaming potential become 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 pH range studied, may be due to the presence of sugars. It has been determined that 52.2% of the EPS of *A. ferrooxidans* cultured in iron (II) sulfate correspond to sugars (monosaccharides) (Gehrke et al., 1998). The monosaccharides have carboxyl groups (COOH),
120 which can lose its proton, leaving a negative charge (COO^-). The anomalous behavior of the pyrite streaming potential in saline water (Figure 2), can be explained with the theory of the electric triple layer (Liu et al., 2006).

The magnitude of streaming potential of pyrite and *A. ferrooxidans* was lower in saline water than in fresh water. This is explained by the presence of sodium (Na^+) and chloride

125 (Cl⁻) ions which increase the ionic strength and compress the electric double layer.

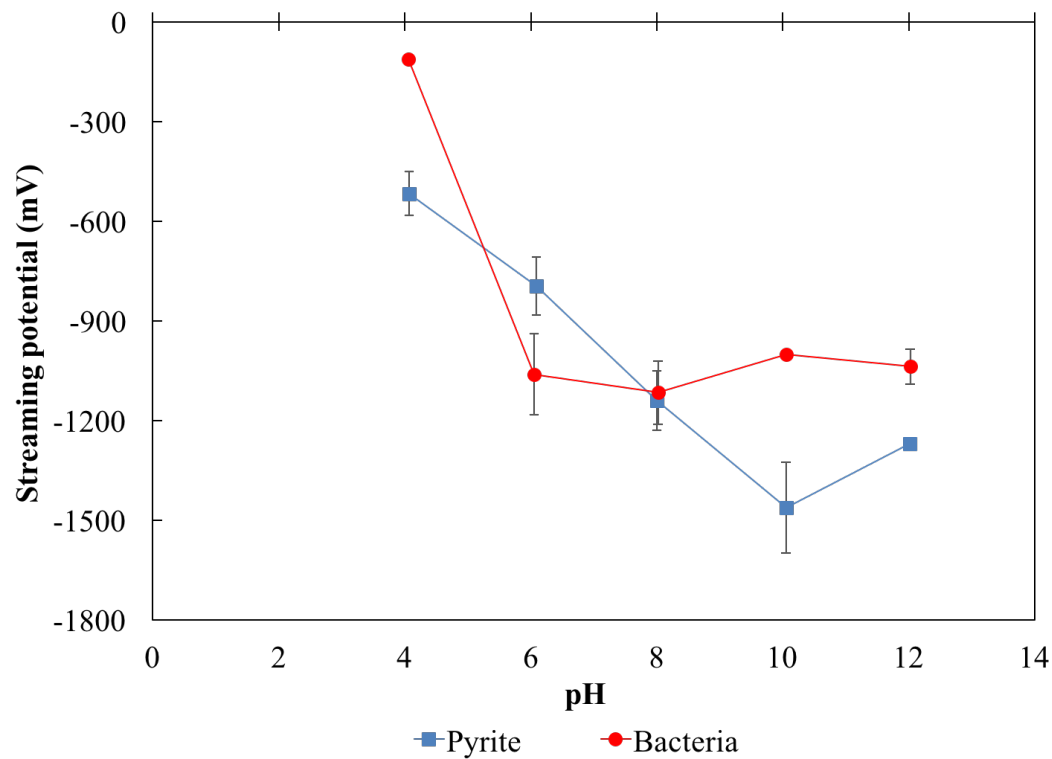


Figure 1: Streaming potential of *A. ferrooxidans* and pyrite in fresh water.

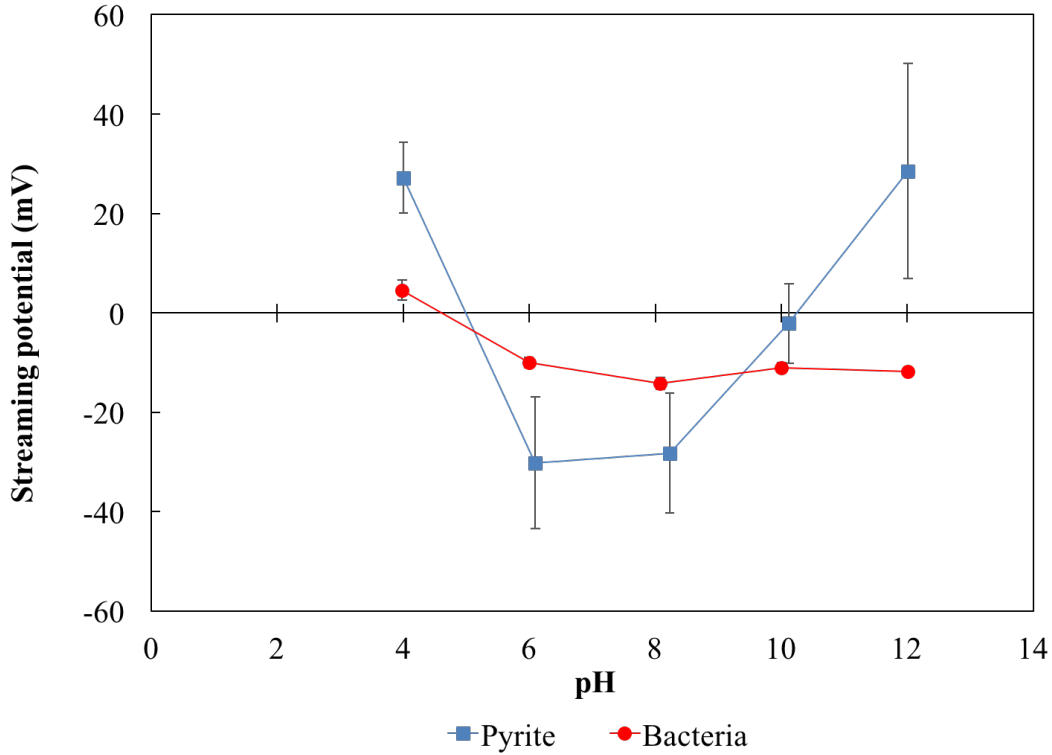


Figure 2: Streaming potential of *A. ferrooxidans* and pyrite in saline water (35 g/L NaCl or 0.6 M).

Figure 3 shows the attachment kinetics of *A. ferrooxidans* to pyrite in fresh water and in saline water. The dotted lines correspond to the controls. The controls are experiments that were carried out without mineral 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
130 per gram of pyrite) did not increase over the time, indicating that they did not adhere to the internal walls of the flask. When pyrite is added, the density of attached bacteria increases. This occurs both for fresh water and saline water. It can also be observed that the adhesion of the bacteria to pyrite is greater in fresh water than in saline water.

Due to the compression of the electrical double layer in saline water, it would be expected
135 that the attachment of *A. ferrooxidans* to pyrite would be greater in this kind of water than in fresh water. However, this does not happen but the opposite, in fresh water attachment density of the bacteria to the mineral is higher than in saline water. This suggests that the surface charges are not responsible for adhesion. These results are in agreement with the results of Tan and Chen (2012) who also determined that the adhesion mechanism is not related to surface

140 charges.

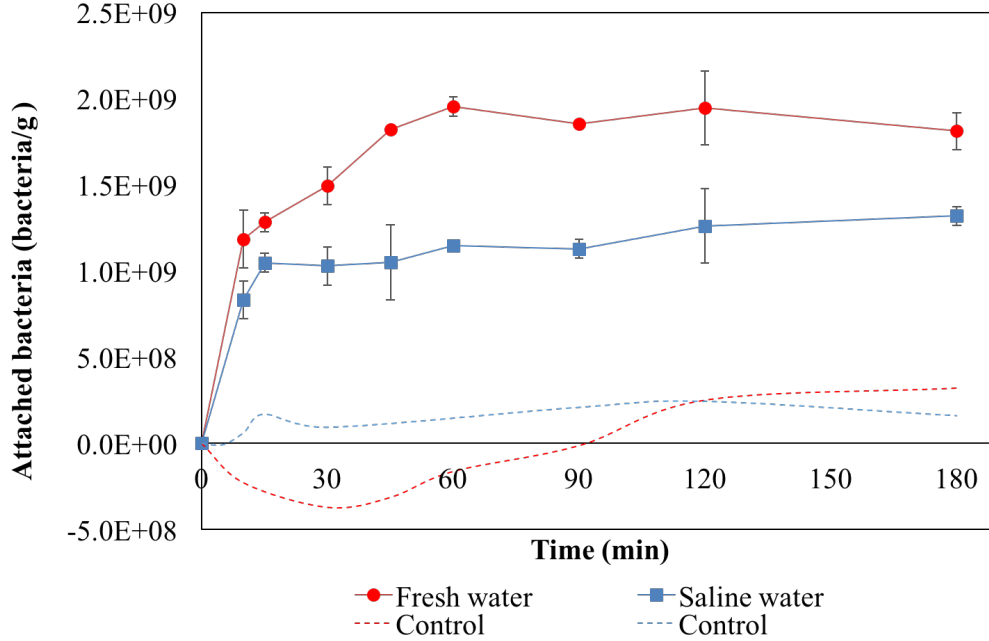


Figure 3: Attachment kinetics of *A. ferrooxidans* to pyrite in fresh and saline water (35 g/L NaCl or 0.6 M). The controls are experiments carried out without mineral (only bacteria and water).

4. Conclusions

In saline water (water with 35 g/L of NaCl), *A. ferrooxidans* and pyrite showed streaming potentials very low compared to that obtained in fresh water. This indicates the compression in double electrical layer due to the Na^+ and Cl^- ions. At the same time, a higher attachment density of *A. ferrooxidans* to pyrite was shown in fresh water than in saline water. This suggests that electrostatic forces are not the responsables of the attachment of *A. ferrooxidans* to pyrite.

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