

Attachment of *Acidithiobacillus ferrooxidans* to pyrite in fresh and saline water and its fitting to Langmuir and Freundlich isotherms

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Abstract

Attachment of bacteria *Acidithiobacillus ferrooxidans* to pyrite was investigated in two different environment: fresh water and saline water (water with 35 g/L of NaCl), in both cases at pH 4. Adsorption isotherms were fitted to the Langmuir and Freundlich models. The results showed that the bacteria adhere to pyrite to a greater extent in fresh water than in saline water. The Langmuir and Freundlich models fitted well the data obtained in fresh water, showing a coefficient of determination (R^2) approximately equal to 0.8 for both models. On the other hand, in saline water the models did not show a good coefficient of determination with a value approximately equal to 0.4 for both models.

Keywords: Langmuir, Freundlich, Isotherm, *Acidithiobacillus ferrooxidans*, Pyrite

1. Introduction

Acidithiobacillus ferrooxidans is a chemolithoautotrophic bacterium that obtains the carbon from the CO₂ of the air, to generate their cellular mass, and the energy (electrons) via the oxidation of inorganic compounds (e.g. minerals). Due to its ability to oxidize ferrous and reduced sulfur compounds, *A. ferrooxidans* has been widely used in leaching when processing copper oxides and copper sulfides (Donati et al., 2007; Rawlings, 2005). Moreover, *A. ferrooxidans* has been studied as pyrite depressant in the flotation process (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). In general, bacteria tend to adhere to solid surfaces forming biofilms rather than remain free in solution as planktonic cells because they

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find protection against hostile environments and a high concentration of nutrient (Flemming and Wingender, 2010; Schaechter, 2009; Chandraprabha and Natarajan, 2006).

In Chile most of the mines are located in the north of the country where arid conditions prevail. Due to the water scarcity in this region, mining companies have started to change the source of water that they currently use, from groundwater to seawater. The use of seawater is becoming increasingly important in mining, especially in the leaching and flotation processes.

Microorganisms, including bacterium *Acidithiobacillus ferrooxidans*, have been used in the flotation and leaching processes (Chandraprabha et al., 2004, 2005; Hosseini et al., 2005; Mehra-bani 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). In both processes, the adherence of microorganisms to minerals is an important step. For example, bacterium *A. ferrooxidans* must adhere to the mineral before leaching it in the direct bio-oxidation mechanism (Schaechter, 2009); in bioflotation it has been shown that adhesion is part of the pyrite bio-depression mechanism (Donati et al., 2007; Ohmura et al., 1993).

Langmuir isotherm is an adsorption model that has been used to determine qualitatively the degree of attachment of *A. ferrooxidans* to minerals (Xia et al., 2013). The model assumes that: (i) bacteria form a monolayer over the mineral, (ii) all sites on the surface are energetically equivalent, (iii) the attached bacteria do not interact with each other and (iv) the bacteria do not move on the surface. The Langmuir isotherm model is presented in Equation (1):

$$X_e = \frac{X_m K_L C_e}{1 + K_L C_e} \quad (1)$$

Equation (1) can be easily linearized as follows:

$$\frac{1}{X_e} = \frac{1}{X_m K_L C_e} + \frac{1}{X_m} \quad (2)$$

where, X_e is the attached bacteria per gram of mineral (bacteria/g), C_e is the equilibrium concentration of bacteria in solution (bacteria/mL), K_L is the Langmuir adsorption equilibrium constant (mL/bacteria) and X_m is the maximum adsorption capacity per unit mass of adsorbent (bacteria/g). X_m and K_L are fitted from experimental data.

Freundlich isotherm is an empirical model given by the Equation (3):

$$X_e = K_F C_e^{\frac{1}{n}} \quad (3)$$

Equation (3) can be written in the logarithmic form as follows:

$$\log X_e = \log K_F + \frac{1}{n} \log C_e \quad (4)$$

where, X_e is the attached bacteria per gram of mineral (bacteria/g), C_e is the equilibrium concentration of bacteria in solution (bacteria/mL), n indicates the adsorption effectiveness and represents the inherent properties of the adsorbent and K_F is a constant related to the adsorption capacity $((\text{bacteria/g})(\text{mL/bacteria})^{1/n})$. K_F and n are fitted from experimental data.

Because the use of bacteria and seawater in mining is gaining attention, it is important to study the adsorption behavior to pyrite in a saline environment. The present work studies the kinetics of attachment of *Acidithiobacillus ferrooxidans* to pyrite in fresh water and in water with 35 g/L NaCl (saline water), which is the concentration of sodium chloride in seawater. In addition, the attachment behavior will be modeled using Langmuir and Freundlich equations to determine which of these two models represent the real behavior.

2. Experimental

2.1. Mineral

The pyrite used corresponds to hand picked mineral samples that were manually crushed. The samples were dry screened between 37 μm -212 μm (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

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

$((\text{NH}_4)_2\text{SO}_4$, purity $\geq 99.5\%$, Merck), 0.056 g/L of di-potassium hydrogen phosphate trihydrate ($\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, purity $\geq 99\%$, Merck) and 0.4 g/L of magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, purity $\geq 99.5\%$, Merck) at pH 1.6. Iron sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, purity $\geq 99.5\%$, 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 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. 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. The 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. Attachment kinetics and isotherms

Attachment kinetics experiments of *A. ferrooxidans* to pyrite were carried out in fresh water and saline water at pH 4. Fresh water corresponds to distilled water, while, distilled water with NaCl at a concentration of 35 g/L (concentration of sodium chloride in seawater), was used as saline water. The experiments were conducted at different initial concentration of bacteria in order to construct the isotherm curves later. These tests were performed in a 50 mL Erlenmeyer flask, where 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 different cell concentrations. 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 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. The adsorption isotherms were obtained by plotting the data at equilibrium for each initial concentration of bacteria. Subsequently, the Langmuir and Freundlich adsorption isotherms

models were fitted to the experimental results.

3. Results and Discussion

90 3.1. Attachment kinetics

Figure 1 shows the concentration of bacteria *A. ferrooxidans* remaining in fresh water over time when they were contacted with pyrite. These attachment kinetics experiments were carried out at pH 4 and at different levels of initial concentration of bacteria. It is observed that the initial concentration of bacteria in solution progressively decreased and reaches a relatively
95 constant value at approximately 60 min, which indicates that the bacteria are being adsorbed on pyrite. Then, the equilibrium was reached at 60 min, time from which the adsorption no longer occurs or it occurs to a lesser extent. The trend described previously was observed independently of the initial concentration of bacteria. In the control experiment (results not shown), it was determined that *A. ferrooxidans* concentration does not decrease over time, showing that the
100 bacteria do not attach to internal walls of the flask. Figure 2 shows the concentration of bacteria in saline water as a function of time. The concentration of bacteria quickly decreased and reached a concentration relatively constant at approximately 15 min. When comparing Figure 1 with Figure 2, it is possible to observe that a lower concentration of bacteria remained in fresh water than in saline water, independently of their initial concentration. This indicates
105 that a greater number of bacteria attach to pyrite surface in fresh water than in saline water.

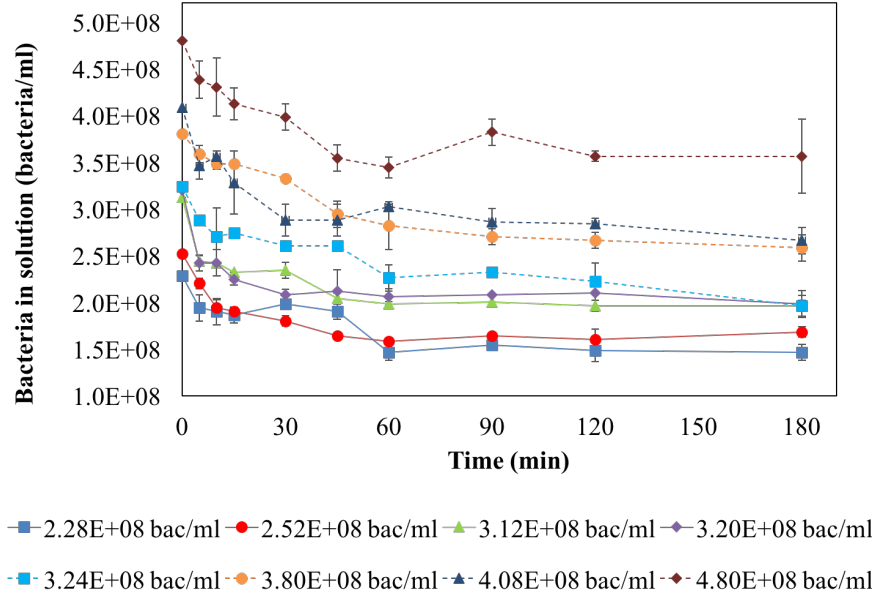


Figure 1: Concentration of bacteria *A. ferrooxidans* remaining in solution (fresh water) over time when they are contacted with pyrite. The attachment kinetics experiments were conducted in fresh water at pH 4 at different initial concentrations of bacteria.

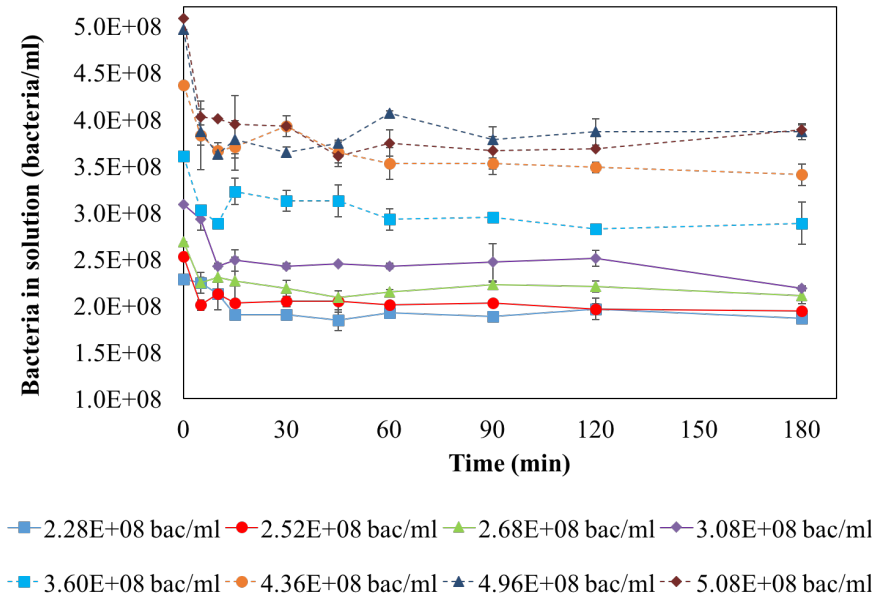


Figure 2: Concentration of bacteria *A. ferrooxidans* remaining in solution (saline water) over time when they are contacted with pyrite. The attachment kinetics experiments were conducted in saline water (35 g/L NaCl) at pH 4 at different initial concentrations of bacteria.

3.2. Adsorption isotherms

Figure 3 shows the linear fitting of isotherms obtained in fresh water and saline water to the Langmuir model, while, Figure 4 shows the linear fitting to Freundlich model. Adsorption isotherms were plotted with data of bacteria concentrations at the equilibrium from the attachment kinetics experiments. From the slope and the intersection with the Y axis, the constants of both models were obtained. In Table 1 the values of the Langmuir and Freundlich constants are presented. It is observed that the two models showed a better coefficient of determination for the experimental data obtained in fresh water than in saline water. In fresh water both models presented a $R^2 > 0.8$, while, in saline water, R^2 were approximately 0.4. Therefore, according to the Langmuir model, bacteria could attach as a monolayer to pyrite in fresh water. In saline water is not possible to assume that because the coefficient of determination (R^2) was too low. The Freundlich model is appropriate to predict the attachment behavior of bacteria to pyrite in fresh water but not in saline water. The value of X_m from the Langmuir model, indicates the maximum adsorption capacity per unit mass of adsorbent (bacteria/g). This value was equal to 5×10^9 bacteria/g in fresh water and in saline water. However, due to the low determination coefficient in saline water, the result in this case should not be considered. Figure 5 shows the adsorption isotherms and their adjustments to the Langmuir and Freundlich models. Is possible to observe that in fresh water the adsorption of *A. ferrooxidans* is higher than in saline water, showing higher attachment densities (number of attached bacteria per mass of mineral).

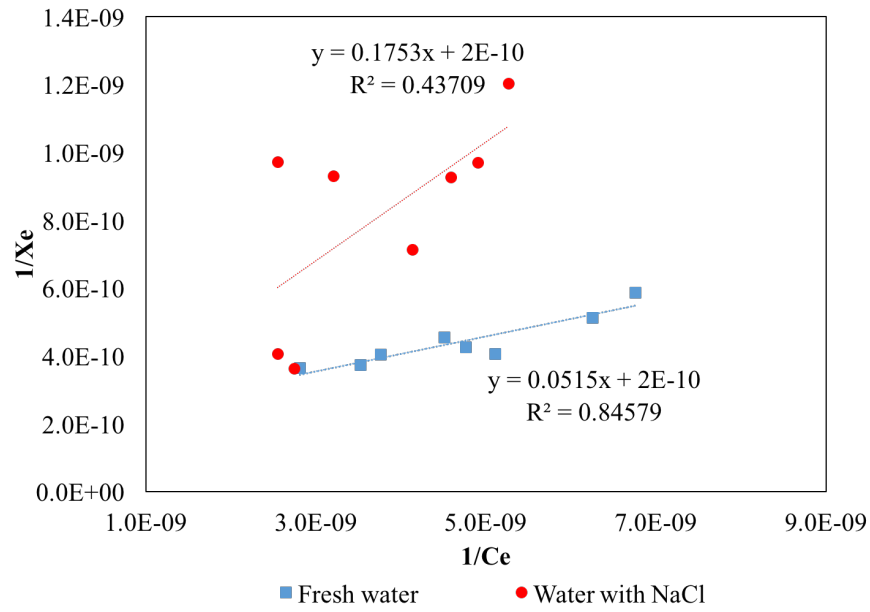


Figure 3: Linear fitting of the isotherms obtained in fresh water and saline water (35 g/L NaCl) to the Langmuir model.

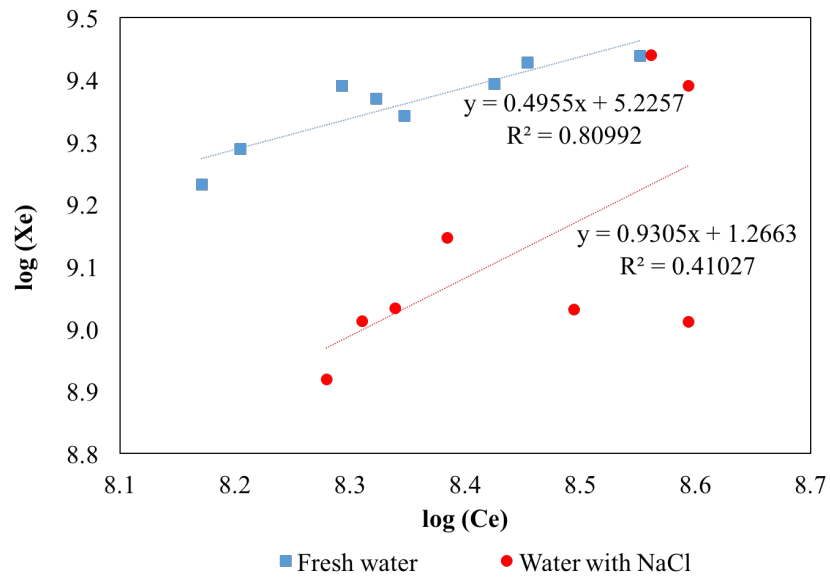


Figure 4: Linear fitting of the isotherms obtained in fresh water and saline water (35 g/L NaCl) to the Freundlich model.

Table 1: Parameters obtained from the Freundlich and Langmuir models for the adsorption isotherms in fresh water and in saline water (35 g/L NaCl).

Model	Parameter	Fresh water	Saline water
Langmuir	X_m (bacteria/g)	5×10^9	5×10^9
	K_L (mL/bacteria)	3.88×10^{-9}	1.14×10^{-9}
	R^2	0.85	0.44
Freundlich	n	2.02	1.07
	K_F (bacteria/g)(mL/bacteria) $^{1/n}$	1.68×10^5	1.85×10^1
	R^2	0.81	0.41

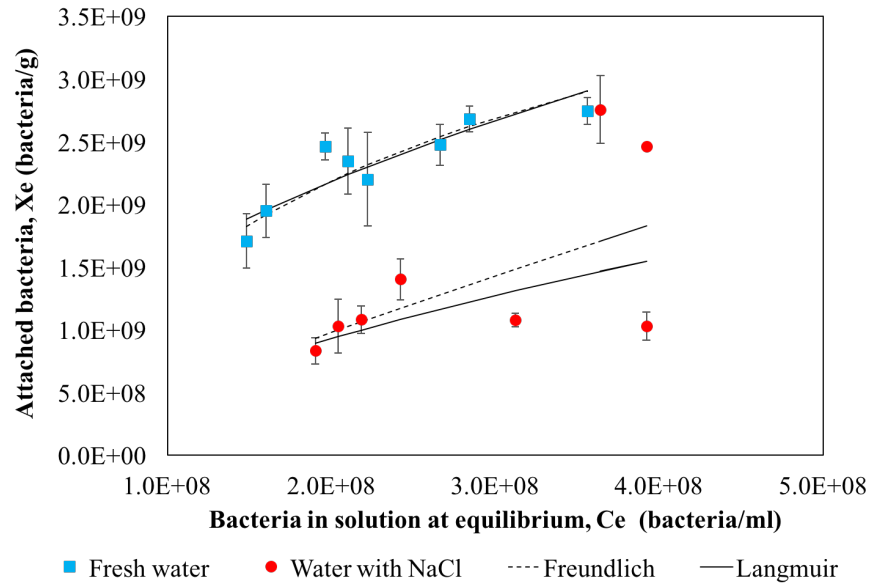


Figure 5: Adsorption isotherms in fresh water and in saline water (35 g/L NaCl) and its fitting to the Langmuir and Freundlich models.

4. Conclusions

The attachment density of bacteria *A. ferrooxidans* to pyrite was higher in fresh water than in saline water (35 g/L NaCl), showing that not only the characteristics of the adsorbent and the absorbate influence the adherence, but also the characteristics of the environment where it

is carried out the adsorption.

Freundlich and Langmuir models show to predict well the adhesion behavior of *A. ferrooxidans* to pyrite in fresh water, however, in saline water the models show a low coefficient of determination indicating that they do not predict the adhesion behavior in this type of solution.

The good fit of the experimental data to the Langmuir model suggests that the bacteria adhere as a monolayer on the surface of the pyrite when the solution correspond to fresh water.

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