



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

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Abstract

Objective This study aims to investigate the attachment of *Acidithiobacillus ferrooxidans* to pyrite in two different environments: fresh and saline water (water with 35 g/L of NaCl or 0.6 M). Adsorption isotherms were analyzed using the Langmuir and Freundlich models. Saline water is water with 35 g/L of NaCl (0.6 M), which is the concentration of NaCl in seawater. The use of raw seawater in mining is becoming relevant in leaching and flotation process. At the same time the use of microorganisms in both processes is gaining attention. For this reason, it is important to study the behavior of adherence of microorganisms to minerals in saline aqueous environments, similar to seawater.

Results The bacteria showed a higher level of attachment to pyrite in fresh water than in saline water. The Langmuir model fitted better the experimental data obtained in fresh water than in saline water with a coefficient of determination (R^2) of 0.85 and 0.61 for fresh and saline water, respectively.

Conclusions This suggests that the bacteria tend to adhere more as a monolayer in fresh than in saline water in the early stage of adhesion.

Keywords Langmuir · Freundlich · Isotherm · *Acidithiobacillus ferrooxidans* · Pyrite

Introduction

Acidithiobacillus ferrooxidans is a chemolithoautotrophic bacterium that obtains carbon from the CO_2 of the air, to generate cellular mass, and energy via oxidation of inorganic compounds (e.g. minerals), using oxygen as the final electron receptor (Rawlings 2005; Donati and Sand 2007). *A. ferrooxidans* has been widely used in leaching when processing copper oxides and copper sulfides because of its ability to oxidize ferrous iron and sulfur compounds. *A. ferrooxidans* has also been studied as a pyrite depressant in flotation (Ohmura et al. 1993; Misra et al. 1996; Nagaoka et al. 1999; Chandrababha et al. 2004; Hosseini et al. 2005; Mehrabani et al. 2011; San

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Martín et al. 2018). Microorganisms adherence to minerals is an important step in bioflotation and bioleaching. The bacterium *A. ferrooxidans* must adhere to the mineral before it is leached in the direct bio-oxidation mechanism or the indirect contact mechanism. In the direct mechanism, the bacteria remove the electrons directly from the mineral by an enzymatic reaction. In the indirect mechanism, bacteria oxidize ferrous iron (Fe^{2+}) in solution to ferric iron (Fe^{3+}). Ferric iron is a strong oxidant that oxidizes the mineral. The role of bacteria in this mechanism is to re-generate the Fe^{3+} ions. Bacteria can carry ferric ions in their exopolysaccharides (EPS) to the surface of the mineral, which is termed the indirect contact mechanism (Donati and Sand 2007; Schaechter 2009). Adhesion is part of the pyrite bio-depression mechanism in bioflotation. Ohmura et al. (1993) showed that the higher the depressant effect on pyrite is dependent on the level of adhesion of *A. ferrooxidans* to the mineral. Microorganisms tend to adhere to solid surfaces forming biofilms, rather than remain free in solution as planktonic cells, because they find protection against hostile environments and a high concentration of nutrients (Schaechter 2009; Flemming and Wingender 2010).

The Langmuir isotherm is an adsorption model that has been used to determine the level of attachment of *A. ferrooxidans* to sulfur, quartz, chalcopyrite, bornite and pyrite (Tan and Chen 2012; Xia et al. 2013). The model assumes that: (i) bacteria form a monolayer on 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 Eq. 1:

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

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).

The Freundlich isotherm is an empirical model without a theoretical base. The Freundlich model is given by Eq. 2:

$$X_e = K_F C_e^{1/n} \quad (2)$$

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), n is a constant and K_F is a constant related to the adsorption capacity [(bacteria/g)(mL/bacteria) $^{1/n}$].

It is important to study the adsorption behavior of *A. ferrooxidans* to pyrite in a saline environment because the use of seawater in mining is gaining attention. The consumption of raw and desalinated seawater in copper mining in Chile was 3.28 m³ /s in 2018. It is expected that by 2029 this value will increase to 10.82 m³ /s, which represents an increase of 230% (COCHILCO 2018). The salinity of seawater is 35 g/L. The main component is NaCl with a concentration between 0.5 and 0.6 M (Castro 2012). The molecular weight of NaCl is 58.44 g/mol, which means that the concentration of NaCl is between 29.22 and 35.064 g/L. It means that approximately the 100% of the salts correspond to NaCl.

The adherence of *A. ferrooxidans* to pyrite has not been studied in a saline aqueous environment. The present work studies the kinetics of *A. ferrooxidans* attachment to pyrite in fresh and saline water (water with 35 g/L of NaCl or 0.6 M). The attachment isotherms are analyzed using Langmuir and Freundlich equations.

Methods

Mineral preparation

The mineral used in this study was hand-picked and manually-crushed pyrite collected from central zone of Chile. They are samples that were selected one by one and that correspond to pure pyrite crystals that were collected in the field. The samples were crushed in a porcelain mortar. The samples were dry screened between 37 m μ -212 μ m (mesh # 70 and # 400) and cleaned with a 6 N hydrochloric acid solution to remove oxidized species from their surfaces. The purity of the pyrite was determined by X-ray diffraction to be above 99%, using a Bruker D8 powder XRD instrument.

Microorganisms cultivation

Acidithiobacillus ferrooxidans (strain ATCC19859) was grown at 30 °C in a sterile basal medium, the composition of which was: 0.4 g/L of ammonium sulfate ((NH₄)₂ SO₄, purity > 99.5%, Merck), 0.056 g/L of di-potassium hydrogen phosphate trihydrate (K₂HPO₄·3H₂O, purity > 99%, Merck), 0.4 g/L of magnesium sulfate heptahydrate (MgSO₄·7H₂O, purity > 99.5%, Merck) and 14.85 g/L of iron sulfate heptahydrate (FeSO₄·7H₂O, purity > 99.5%, Merck). The pH of the medium was adjusted to 1.6 with sulfuric acid. The sterile medium was inoculated with an active inoculum of *A. ferrooxidans*. Following 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 at 5 °C in a Sorvall RC-5B refrigerated Superspeed Centrifuge. The pellet obtained was resuspended in a sulfuric acid (H₂SO₄) solution at pH 2. The resuspended 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 resuspended in a H₂SO₄ solution at pH 2.

Attachment kinetics and isotherms

Experiments were carried out in fresh and saline water at pH 4 to study the kinetics of *A. ferrooxidans* attachment to pyrite. The pH 4 was the natural one obtained when bacteria, pyrite and fresh or saline water are in contact. The fresh water used was distilled, while, distilled water with NaCl at a concentration of 35 g/L or 0.6 M (concentration of sodium chloride in seawater), was used as the saline water. The experiments were conducted at different initial concentrations of bacteria in order to later construct the isotherm curves. The tests employed 50 mL Erlenmeyer flasks in which 1 g of pyrite was exposed to 19.5 mL of water and 0.5 mL of a solution with varying concentrations of metabolite-free centrifuged iron-free *A. ferrooxidans* cells. The resulting slurry was agitated on a rotary shaker at 180 rpm. A control experiment was carried out in fresh and in saline water to evaluate if bacteria attach to the internal walls of the flask. The control experiment was carried out with only bacteria and water, with no added mineral. The number of cells in solution was measured

at different times by direct counting in a Neubauer camera using a Zeiss Axio Lab.A1 microscope with a magnification of ×40. The number of cells attached to the mineral was calculated as the difference between the cells in the liquid at a given time and the number of cells in the initial solution. The attachment kinetics experiments were carried out in duplicate. The adsorption isotherms were obtained by plotting the data at equilibrium (120 min) for each initial concentration of bacteria. Subsequently, the Langmuir and Freundlich adsorption isotherm models were fitted to the experimental results.

Results and discussion

Attachment kinetics

Figure 1 shows the concentration of the bacteria *A. ferrooxidans* remaining in fresh water over time when they were in contact with pyrite. The attachment kinetics experiments were carried out at pH 4 with different initial bacterial concentrations. The initial concentration of bacteria in solution progressively decreased and reached a relatively constant value at approximately 60 min, which indicates that the bacteria was being adsorbed on the pyrite. Equilibrium was reached at 60 min, at which point adsorption no longer occurred or lessened. The tendency was observed independent of the initial bacterial concentration. The control experiment, with only bacteria and water and no addition of mineral (results not shown), determined that the *A. ferrooxidans* concentration does not decrease over time, showing that the bacteria do not attach to the internal walls of the flask. There were a couple of instances before reaching the 60 min point when the bacterial concentration in solution was higher than in previous instances, which may have been due to errors in counting. The direct counting method is a method that presents errors because it depends on the visual precision of the person performing the count. Before 60 min, the difference of bacteria concentration in solution between two consecutive times is very low, so it is possible to have a greater bacteria concentration in solution in previous times. However, these errors did not affect the adsorption isotherms fits, since the isotherms were constructed with the data at 120 min.

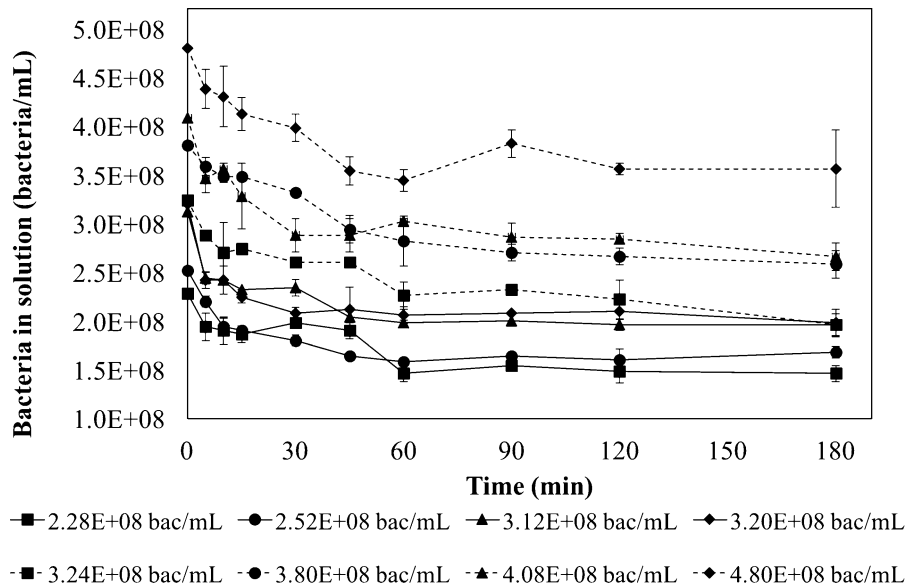


Fig. 1 Concentration of *A. ferrooxidans* remaining in solution (fresh water) over time when in contact with pyrite. The attachment kinetics experiments were conducted in fresh water at pH 4 with different initial bacterial concentrations

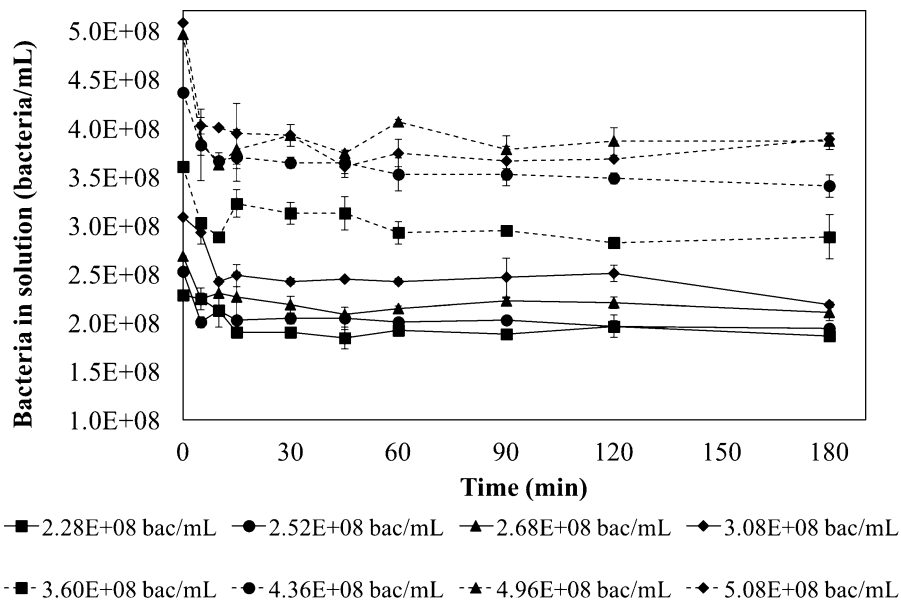


Fig. 2 Concentration of *A. ferrooxidans* remaining in solution (saline water) over time when in contact with pyrite. The attachment kinetics experiments were conducted in saline water (35 g/L NaCl or 0.6 M) at pH 4 with different initial bacterial concentrations

Figure 2 shows the concentration of bacteria in saline water as a function of time. The concentration of bacteria quickly decreased and reached a relatively constant concentration at approximately 15 min. Comparing Figs. 1 and 2 shows that a lower concentration of bacteria remained in fresh water than in

saline water, independent of the initial concentration, which indicates that more bacteria attach to pyrite surface in fresh water than in saline water. At an initial concentration of 2.28×10^8 bacteria/mL, the concentration of bacteria in solution at 60 min was 1.92×10^8 bacteria/mL in saline water and 1.5×10^8 bacteria/mL

in fresh water. Adsorption is therefore higher in fresh water than in saline water.

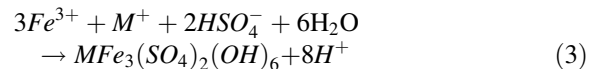
Adsorption isotherms

Langmuir isotherm

Figure 3 shows the linear fitting to the Langmuir model of isotherms obtained in fresh and saline water. The constants X_m and K_L were obtained from the slope and the intersection with the Y-axis. Table 1 shows the values of the Langmuir constants. The maximum capacity of adsorption in both fresh and saline water, X_m (Table 1), was determined to be 5×10^9 bacteria/g. This parameter is a characteristic of the adsorbent. Since the adsorbent in both cases is pyrite, the value is the same. The parameter K_L was higher in fresh water than in saline water, which indicates that bacterial adsorption to the mineral was higher in fresh water.

The coefficient of determination (R^2) was higher for fresh water (0.85) than for saline water (0.61) (Table 1). According to the Langmuir model, bacteria adhere to the mineral as a monolayer. Therefore, bacteria tend to adhere as a monolayer more in fresh water than in saline water. Other authors have shown that the Langmuir model well fits the experimental data of the adsorption isotherm of *A. ferrooxidans* in

fresh water (Tan and Chen 2012; Xia et al. 2013). The bacteria could adhere as a multilayer in saline water, which may be due to the formation of jarosite. The general formula of jarosites is $MFe_3(SO_4)_2(OH)_6$, where M may be K^+ , Na^+ , NH_4^+ , Ag^+ or H_3O^+ . Daoud and Karamanev (2006) determined that jarosite is produced at $pH > 1.6$. Formation of jarosite occurs because saline water has a high concentration of sodium ions (Na^+) and the pH is relatively high (pH 4). The reaction between the Na^+ and Fe^{3+} from pyrite generates jarosite by the reaction given by Eq. 3:



Given *A. ferrooxidans* can adhere to jarosite (Pogliani and Donati 2000), some bacteria may have attached to jarosite instead of pyrite, which would have modified adsorption behavior. Microscopic observation showed that agglomerates of bacteria were formed in the bulk solution with saline water. Therefore, bacteria may agglomerate around small particles of jarosite floating in solution. When bacteria-jarosite agglomerates reach a sufficiently large size, they precipitate on the pyrite surface, which is why adhesion is not in the form of a monolayer.

The mineral samples were analyzed by X-ray diffraction (XRD) to verify the formation of jarosite.

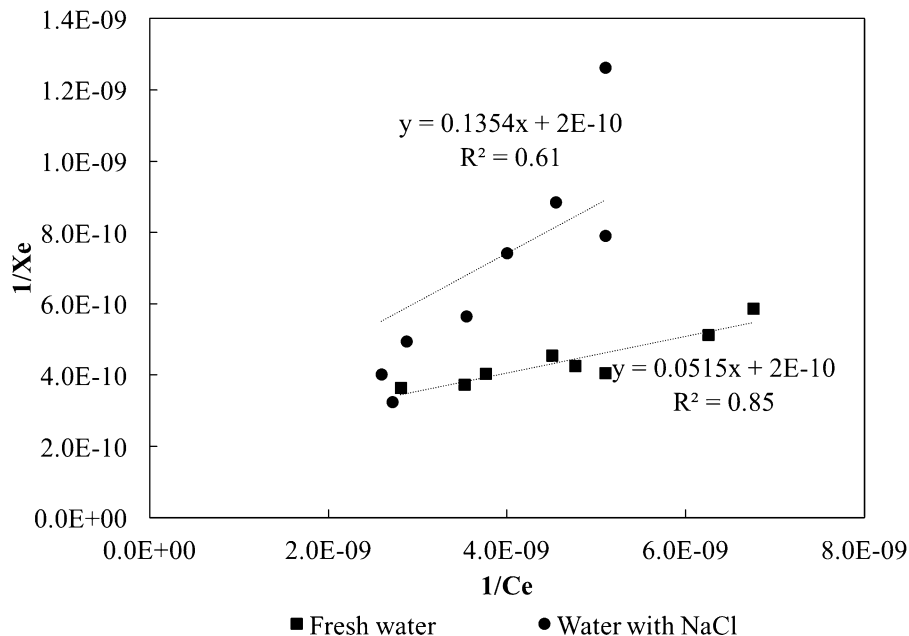
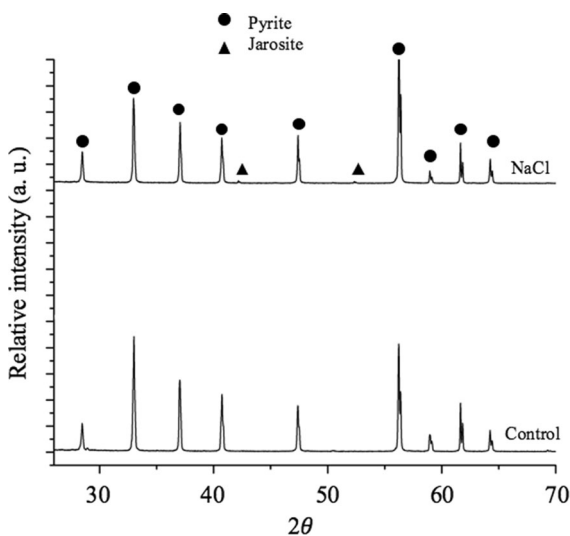


Fig. 3 Linear fitting to the Langmuir model of the isotherms obtained in fresh and saline water (35 g/L NaCl or 0.6 M)

Table 1 Parameters obtained from the Freundlich and Langmuir models for the adsorption isotherms in fresh and saline water (35 g/L NaCl or 0.6 M)

Model	Parameter	Fresh water	Saline water
Langmuir	X_m (bacteria/g)	5×10^9	5×10^9
	K_L (mL/bacteria)	3.9×10^{-9}	1.5×10^{-9}
	R^2	0.85	0.61
Freundlich	n	2.02	0.67
	K_F (bacteria/g)(mL/bacteria) ^{1/n}	1.7×10^5	4.3×10^{-4}
	R^2	0.81	0.87

A Shimadzu XRD-6000 diffractometer with Cu K α radiation was used for the analysis. The pyrite was in contact with saline water (water with 35 g/L of NaCl or 0.6 M) for 1 h, while a control sample of pyrite was in contact with distilled water for 1 h, after which both samples were filtered, dried and analyzed by XRD. Figure 4 shows the diffractogram. There was a low presence of jarosite in the pyrite in contact with NaCl, as represented by the peaks highlighted by triangles in Fig. 4. In contrast, no peaks indicating jarosite were evidenced in the pyrite in contact with distilled water. This indicates that jarosite forms in water with NaCl, but does not in fresh water.

**Fig. 4** Diffractogram of pyrite under different conditions. NaCl is pyrite in contact with saline water (water with 35 g/L of NaCl or 0.6 M) for 1 h. The control is pyrite in contact with distilled water for 1 h

Freundlich isotherm

Figure 5 shows the linear fitting to the Freundlich model of isotherms obtained in fresh and saline water. The coefficient of determination R^2 was 0.81 for fresh water and 0.87 for saline water (Table 1). The Freundlich model could be used to predict bacterial attachment to pyrite in fresh and saline water. The Freundlich equation is empirical, with no theoretical base. The constant K_F was higher in fresh water than in saline water, indicating that bacteria have a higher level of attachment to pyrite in fresh than in saline water.

Figure 6 shows the adsorption isotherms and their fit to the Langmuir and Freundlich models. It can be possible to observe that the adsorption of *A. ferrooxidans* is higher in fresh than in saline water, with higher density of attached bacteria (number of attached bacteria per mass of mineral).

Conclusions

A. ferrooxidans was more densely attached to pyrite in fresh water than in saline water (35 g/L NaCl or 0.6 M), indicating that adherence is affected by the characteristics not only of the adsorbent and the adsorbate, but also by those of the environment in which adsorption occurs.

The results show that the Langmuir model fits the experimental data obtained in fresh water better than those obtained in saline water. This indicates that in fresh water *A. ferrooxidans* has a greater tendency to form a monolayer over pyrite than in saline water. The difference in the adhesion behavior in fresh and saline

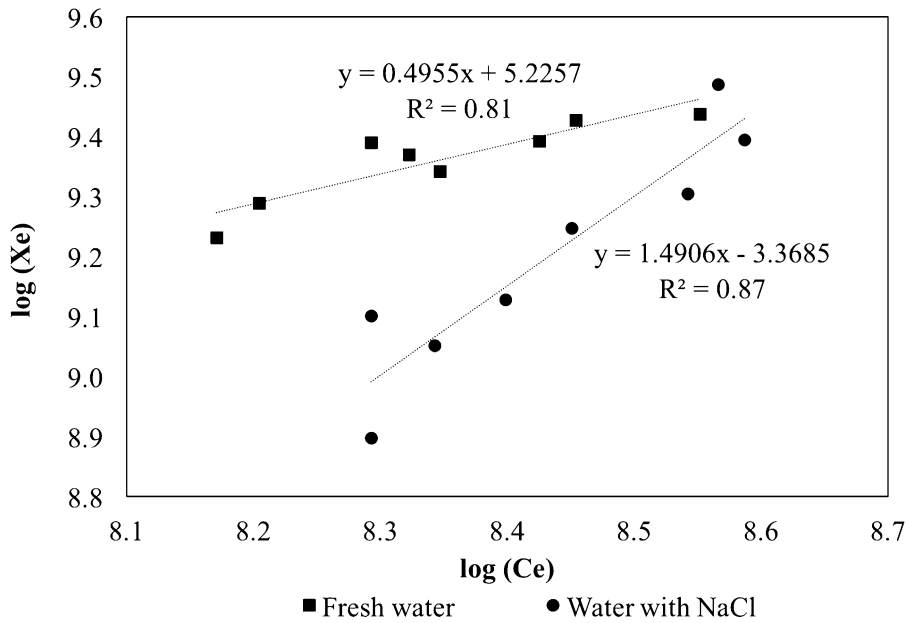


Fig. 5 Linear fitting to the Freundlich model of the isotherms obtained in fresh and saline water (35 g/L NaCl or 0.6 M)

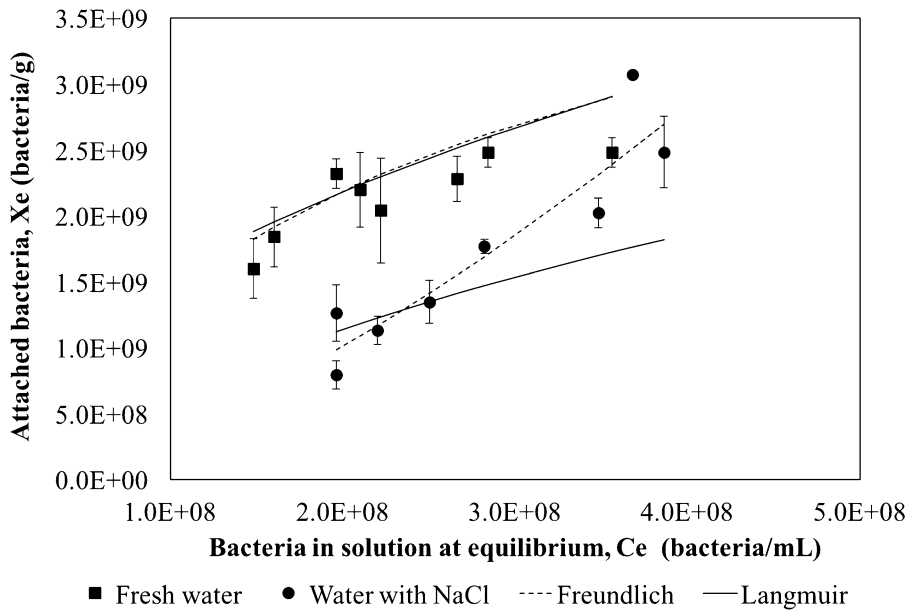


Fig. 6 Isotherms of *Acidithiobacillus ferrooxidans* adsorption to pyrite in fresh and saline water (35 g/L NaCl or 0.6 M) and the fits to the Langmuir and Freundlich models

water may be due to the formation of jarosite in saline water, which can affect adhesion.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Research involving human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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