



Effects of *Leptospirillum ferriphilum* and *Acidithiobacillus caldus* on surface properties of pyrrhotite

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ARTICLE INFO

Article history:

Received 23 July 2009

Received in revised form 15 September 2009

Accepted 16 September 2009

Available online 24 September 2009

Keywords:

Pyrrhotite

Acidithiobacillus caldus

Leptospirillum ferriphilum

Surface properties

Sulfur formation

Mechanism

ABSTRACT

The variation of surface properties of pyrrhotite after biological conditioning with *Leptospirillum ferriphilum* and *Acidithiobacillus caldus* was evaluated by zeta-potential, adsorption and contact angle measurements. Previous work showed that the pyrrhotite isoelectric point (IEP) shifts towards the cell isoelectric point after interacting with bacterial cells, indicating the adsorption of cells on the pyrrhotite surface. The degree of interaction of pyrrhotite with *A. caldus* was observed to be much more pronounced than that of *L. ferriphilum*, because of the different affinity of *A. caldus* and *L. ferriphilum* to pyrrhotite. After treatment by *A. caldus*, the pyrrhotite surface formed a membrane of sulfur, which was shown by X-ray diffractograms (XRD) and the energy dispersion spectrum (EDS), which explains the increasing hydrophobicity of pyrrhotite. However, the contact angle and surface hydrophobicity of pyrrhotite treated by the *L. ferriphilum* kept decreasing during bioleaching. The results indicate that the energy source for the microorganism growth determines its function mechanism in the bioleaching system. The iron-oxidizing bacteria offer an indirect mechanism function during bioleaching of pyrrhotite and the sulfur-oxidizing bacteria offer a direct mechanism function.

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1. Introduction

Bioleaching technology plays an important role in hydrometallurgy for its simplicity, low cost and environmental acceptance (Ehrlich, 2001; Ke and Li, 2006). Bacterial adsorption to various minerals is a fundamental premise in bio-hydrometallurgy (Brierley, 1982; Preston et al., 1993). It has been reported that bacterial adhesion is not only dependent on the biochemical properties of the organism but also on the interfacial properties in a bioleaching system (Vilinska et al., 2007). The bacterial–mineral interactions change both surface properties, which are related with the bio-oxidation mechanisms (Chen et al., 2008). Currently, studies mainly focus on the bio-flotation of sulfide minerals. Attia (1990) and Ohmura et al. (1993) studied the mechanism of changes of the pyrite surface from hydrophobic to hydrophilic due to the bacteria adsorption, which makes it possible to remove pyrite from coal in flotation systems. Meanwhile Yelloji Rao et al. (1992) concluded that conditioning minerals with *Acidithiobacillus ferrooxidans* causes cell adhesion, which could induce either hydrophilicity or hydrophobicity, making the mineral separation possible.

Pyrrhotite is an important iron sulfide waste mineral in many mining environments (Jiang et al., 2006; Veglio et al., 1995), but it is usually found associated with valuable minerals and massive sulfide deposits (Belzile et al., 2004). Studies of the mechanism of bioleaching pyrrhotite (Jiang et al., 2006; Veglio et al., 2000) and leaching kinetics (Qiu et al., 2003; Schärer

et al., 1993) are at a preliminary stage. The bacteria–mineral interactions which change surface properties and the theory of different bacterial species leading to the changes of surface properties in bioleaching have to be studied further. In this work, the changes in the surface properties of pyrrhotite after contacting with *Leptospirillum ferriphilum* and *Acidithiobacillus caldus* are reported and the relative bacterial interactions are discussed with reference to the bio-oxidation mechanism of pyrrhotite.

2. Materials and methods

2.1. Mineral preparation

The sample of pyrrhotite used in this study was from Dachang in Guangxi Province, China. Chemical analyses showed the pure pyrrhotite contained 57.3% Fe, 36.8% S, and the purity reached 93.9%. The sample with particle size less than 43 µm was used for the bioleaching experiments, and the size less than 5 µm was used for adsorption and zeta-potential tests. Pure solid mineral crystals were cut in order to attain certain pieces of flat surfaces. These mineral pieces, which were used to measure contact angle, were polished successively by a polishing machine with chromic oxide as a medium.

2.2. Microorganisms and culture media

L. ferriphilum type strain (DQ343299) and *A. caldus* (DQ256484) used in the experiments were obtained from the Key Laboratory of Biometallurgy in Central South University. *L. ferriphilum* and *A. caldus*

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were grown in 9K medium using a rotary shaker at 170 rpm with the initial pH = 2.0 and 1.6 and the temperature 40 °C and 45 °C, respectively. The 9K medium compositions were $(\text{NH}_4)_2\text{SO}_4$ 3.0 g/L, KCl 0.1 g/L, K_2HPO_4 0.5 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g/L, $\text{Ca}(\text{NO}_3)_2$ 0.01 g/L. The energy source was either $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (44.2 g/L) or S (10 g/L).

2.3. Adsorption measurements

Tests were performed with 1 g mineral in a 100 ml NaCl solution with the ionic strength of 10^{-3} mol/L, containing an initial cell concentration of 1×10^8 cells/mL in order to match the conditions used for zeta-potential measurements. Cells and minerals were interacted together for 120 min by conditioning the suspension with a magnetic stirrer. After conditioning, the suspension was filtered to separate the solid and the bacterial cells which were estimated by a microscope. The amount of attached cells was calculated by subtracting the planktonic from the initial cell number.

2.4. Zeta-potential measurements

The zeta-potential of the bacterial cells was measured by a Coulter DELSA440S II Type electro-kinetic instrument in a solution of NaCl with an ionic strength of 10^{-3} mol/L, and an initial cell concentration of 2×10^8 cells/mL. The zeta-potential of pyrrhotite ($-5 \mu\text{m}$) before and after interacting with bacterial cells was also tested by the instrument. The solution was conditioned with NaCl of ionic strength 10^{-3} mol/L and initial bacteria cell concentration of 1×10^8 cells/mL. The suspension was stirred for 3 h and then put to test. All measurements were performed as a function of pH adjusted with HCl and NaOH.

2.5. Contact angle measurements

The contact angle of the pyrrhotite surface was measured with the JJC-I wetting angle measurement instrument produced by Changchun Optics Instrument Factory, China. Distilled water was dropped vertically on the solid surface with a droplet volume of 3–5 μL , and a diameter of 1–2 mm. The test time should be no more than 1 min with the room temperature at 25 °C.

2.6. XRD and EDS analysis of bioleaching residues

Bioleaching tests were carried out in 250 mL flasks containing 90 mL 9K medium and 10 mL bacterial inoculum with the cell density of 1×10^8 cells/mL. The 9K basal salts medium without iron was used in the sulfide mineral bioleaching experiments. The mineral concentration was 2% (wt./vol). The bioleaching residues were examined using X-ray diffractograms (XRD) and energy dispersive spectroscopy (EDS).

3. Results and discussions

3.1. Adsorption studies

The adsorption curve of *L. ferrophilum* and *A. caldus* on pyrrhotite is presented in Fig. 1. The adsorption kinetics of the two cells to pyrrhotite were similar and the adsorption equilibrium was attained after 15 min. However, the cell density of *L. ferrophilum* on the surface of pyrrhotite was less than that of *A. caldus*. After 15 min, about 64% of the initial cells of *L. ferrophilum* cells adhered to pyrrhotite, but nearly 80% of the initial cells of *A. caldus* cells adhered. This confirms the different selectivity of bacteria towards pyrrhotite and also explains the observed differences of the electro-kinetic behaviour of pyrrhotite after interacting with different bacterial species.

3.2. Zeta-potential tests

The zeta-potentials of *L. ferrophilum* and *A. caldus* as a function of pH are shown in Fig. 2(a). The zeta-potential of *L. ferrophilum* grown in

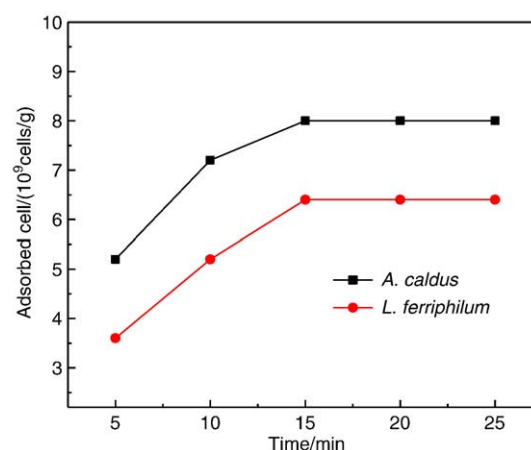


Fig. 1. Adsorption curves of *A. caldus* and *L. ferrophilum* on pyrrhotite.

ferrous ion in the whole pH range displayed a negative charge and the isoelectric point (IEP) was located about pH 2.0. However, *A. caldus* grown on elemental sulfur showed an IEP about pH 3.6. The zeta-potentials illustrate that the substrate-grown cell surfaces contain higher protein content than the ferrous ion-grown cells (Sharma and Hanumantha Rao, 2003).

The zeta-potentials of pyrrhotite before and after interaction with bacterial cells are presented in Fig. 2(b). The IEP of pyrrhotite was observed to be pH 7.0. After interaction with cells, the IEP of pyrrhotite shifted towards the IEP of the cells, which indicates specific adsorption of cells onto the minerals. It was prominent in Fig. 2(b) that in acidic medium (pH < 4.0), the change of pyrrhotite zeta-potential was different

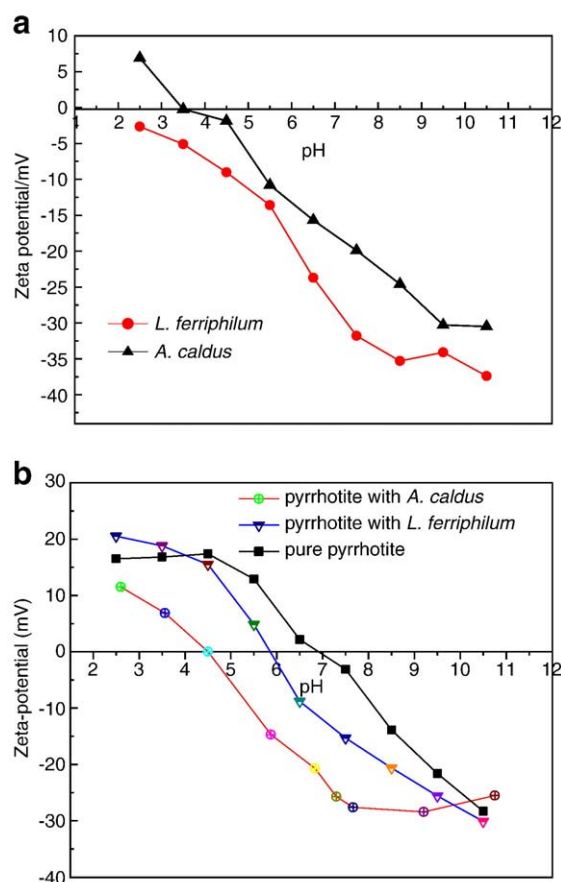


Fig. 2. Zeta-potential of different cells (a), pyrrhotite particles and pyrrhotite interaction with *A. caldus* and *L. ferrophilum* (b) with $I = 0.001$ M.

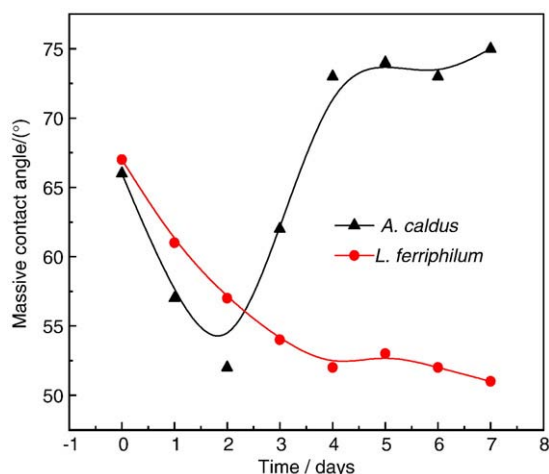
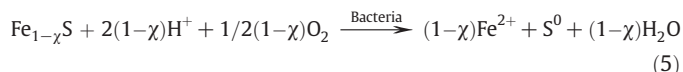
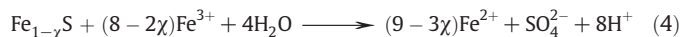
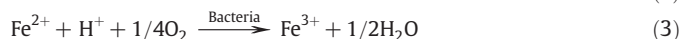
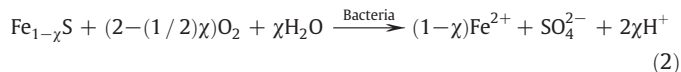
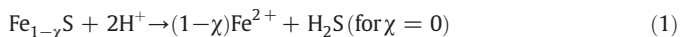


Fig. 3. Changes of contact angle of pyrrhotite leached by *A. caldus* and *L. ferriphilum*.

after interacting with different kinds of bacteria. The zeta-potential of pyrrhotite with *L. ferriphilum* increased, which could be explained by the adsorption of dissolved Fe^{3+} which oxidized the pyrrhotite. The related reaction equations, from the complete sequence below, were as follows Eq. (1), Eq. (2), Eq. (3) and Eq. (4) (Belzile et al., 2004). However, the zeta-potential of pyrrhotite which interacted with *A. caldus* decreased due to related reaction equations Eq. (1), Eq. (5), Eq. (6) and Eq. (7).



The zeta-potential tests imply that the energy for the microorganism growth determines its function mechanism in the bioleaching system. The iron-oxidizing bacteria offer an indirect mechanism function and sulfide minerals are oxidized by Fe^{3+} , whilst the sulfur-oxidizing bacteria offer a direct mechanism function. This is also further proven by the contact angle experiments and XRD and EDS results.

3.3. Contact angle measurements

Fig. 3 shows the change of the pyrrhotite contact angle after interacting with different cells. From Fig. 3, the contact angle and surface hydrophobicity of pyrrhotite treated by *A. caldus* decreased during the initial 2 days and then increased. After 4 days, the contact angle changed little. However, the contact angle and surface hydrophobicity of pyrrhotite treated by *L. ferriphilum* kept decreasing during the bioleaching process.

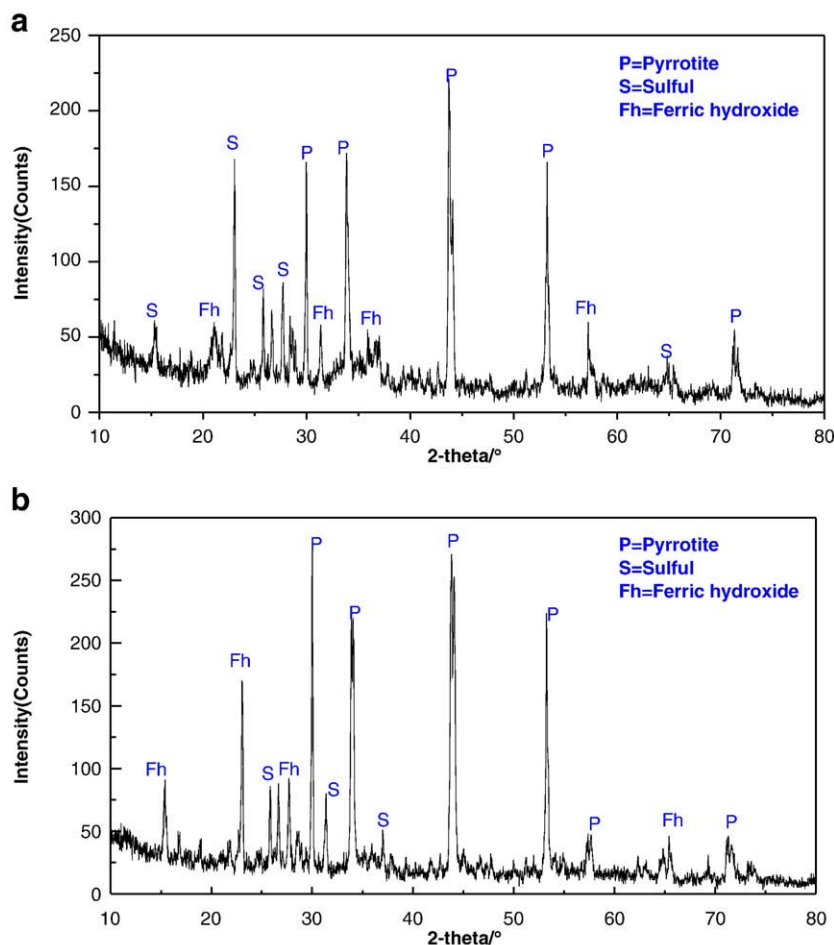


Fig. 4. XRD pattern of leached pyrrhotite residues by *A. caldus* (a) and *L. ferriphilum* (b) with mineral concentration of 2%.

Table 1The results of EDS for residues by *A. caldus* and *L. ferriphilum*.

Sample	wt.%			at.%		
	Fe	S	O	Fe	S	O
Residues with <i>A. caldus</i>	44.9	46.6	08.6	32.1	49.9	18.0
Residues with <i>L. ferriphilum</i>	62.7	22.0	15.3	40.6	23.8	35.6

During the initial 2 days, the bacteria need to adapt to the bioleaching system, and the pyrrhotite dissolution may be dominated by the acid dissolution (Eq. (1)) (Yakhontova and Nesterovich, 1982). Both of the contact angles of pyrrhotite with different bacterial cells decreased. As time goes on, due to the formation of sulfur membrane on the pyrrhotite surface (Eq. (5)), the contact angle of the pyrrhotite surface with *A. caldus* increased. When the sulfur was oxidized into sulfate with *L. ferriphilum*, and the ferric ion was relatively enriched on the surface, the contact angle decreased and the surface hydrophobicity of pyrrhotite increased (Eq. (2), Eq. (3), Eq. (4)). These phenomena were confirmed by X-ray diffraction and energy dispersion spectrum (EDS) of pyrrhotite residues by *A. caldus* and *L. ferriphilum* after 7 days.

3.4. XRD and EDS analysis of bioleaching residues

X-ray diffraction patterns (XRD) for pyrrhotite residues which had interacted with *A. caldus* and *L. ferriphilum* after 7 days show that sulfur and ferric hydroxide respectively were present as a secondary phase in Fig. 4(a) and (b). This proves that sulfur and ferric hydroxide precipitation were formed during the leaching process. From Fig. 4(a) and (b), it is also found that there are more sulfur peaks in residues with *A. caldus* than in residues leached by *L. ferriphilum*.

Table 1 shows the result of energy dispersion spectrum (EDS) for the above leached residues. Before leaching, the mass fractions of elements in pyrrhotite were Fe 57.3% and S 36.8% and the atomic ratio of Fe to S is 1/1.12. After leaching by *A. caldus*, the mass fractions of elements were Fe 44.9% and S 46.6% and the atomic ratio of Fe to S is 1/1.55 due to the formation of sulfur on the pyrrhotite surface. After leaching by *L. ferriphilum*, the mass fractions of elements were Fe 62.7% and S 22.0% and the atomic ratio of Fe to S is 1/0.59, which indicate that the ferric ion is enriched and sulfate is formed (Zhang et al., 2008). These are consistent with the zeta-potential studies, contact angle experiments and XRD results.

4. Conclusions

(1) The affinity of different bacterial cells to pyrrhotite is different. After conditioning with bacterial cells, the IEP of pyrrhotite moves to the IEP of pure cells and the range of zeta-potential is related to the affinity of bacterial cells onto pyrrhotite.

(2) During the initial stages of leaching, pyrrhotite dissolution is dominated by the acid dissolution. After 2 days conditioning with *A. caldus*, the contact angle of pyrrhotite increased due to the formation

of sulfur on the pyrrhotite surface. While using *L. ferriphilum*, the ferric ion on the surface is enriched and sulfur is oxidized into sulfate, leading to a decrease of the contact angle. These results are consistent with the XRD and EDS studies.

(3) The energy source for the microorganism growth determines its reaction mechanism in the bioleaching system. The iron-oxidizing bacteria offer an indirect reaction mechanism during bioleaching of pyrrhotite and the sulfur-oxidizing bacteria offer a direct reaction mechanism.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No 50621063), and the National Basic Research Program of China (No 2004CB619204).

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