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Effects of microorganisms on surface properties of chalcopyrite and bioleaching

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Abstract: The alteration of surface properties of chalcopyrite after biological conditioning with *Acidithiobacillus ferrooxidans* and *Acidithiobacillus caldus* was evaluated by Zeta-potential, adsorption studies, FT-IR spectra and contact angle measurement. The Zeta-potential studies show that the iso-electric point(IEP) of chalcopyrite after bacterial treatment moves towards the IEP of pure cells, indicating the adsorption of cells on chalcopyrite surface. The FT-IR spectra of chalcopyrite treated with bacterial cells show the presence of the cell functional groups signifying cells adsorption. Due to the formation of elemental sulfur and intermediate copper sulphides on chalcopyrite surface, the contact angle and surface hydrophobicity of chalcopyrite increase at the initial bioleaching stage. Chalcopyrite bioleaching by *Acidithiobacillus ferrooxidans* has higher copper extraction, which agrees with the fact that *Acidithiobacillus ferrooxidans* adsorbed on chalcopyrite surface is much more than *Acidithiobacillus caldus.* The results support the direct mechanism of sulfide oxidations in bioleaching chalcopyrite.

Key words: chalcopyrite; microorganism; bioleaching; adsorption; surface properties

1 Introduction

Bacterial adhesion to mineral surfaces plays an important role not only in bacterial survival in natural ecosystems, but also in mining industry applications. The bacteria can utilize various minerals, and bacterial adsorption to minerals is an initial step in bacterial leaching for metal recovery[1].

Chalcopyrite, $CuFeS_2$, is the most important copper-bearing mineral in the world and unlike many other ores it is known to be recalcitrant to hydrometallurgical processing[2]. Researchers have strived for decades to accelerate the speed of chalcopyrite in the biological leaching. The selection of suitable microorganisms for leaching tests is one of the important factors[3]. Bacterial adhesion is dependent not only on the biochemical properties of the organism but also on the interfacial properties of the various interfaces existing in a bioleaching system[4]. It has been reported that the microorganism-mineral interaction results in changes in the surface properties of the microorganisms and mineral surface. The surface charge on cells grown in media with soluble iron (Fe^{2+}) was different from that grown on a solid substrate (sulfur, pyrite). The altered cell surface charge was attributed to higher protein content in the latter[5]. The results of surface studies always are related to the bioflotation and bioflocculation of sulphide mineral. EDWARDS and RUTENBERG[6] concluded that small local surface alterations due to the bacterial metabolism could strongly affect the local adhesion parameters and result in bacterial adhesion on mineral surfaces. Sulphides can be separated from quartz through selective flocculation and dispersion after bio-treatment[7−9]. Bacterial adhesion changes the surface properties of pyrite in crushed fine coal from hydrophobic to hydrophilic[10]. The surface change makes it possible to remove pyrite from coal in flotation systems[11]. It has also been shown that conditioning minerals with *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*), could induce either hydrophilicity or hydrophobicity, making the minerals either floatable or nonfloatable[12−13].

In this work, the alteration of surface properties of chalcopyrite after biological conditioning with *Acidithiobacillus ferrooxidans* and *Acidithiobacillus*

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caldus was studied, and the changes of surface properties caused by bacterial adsorption were discussed with reference to bioleaching behavior of chalcopyrite.

2 Materials and methods

2.1 Mineral

The sample of chalcopyrite used in this study is from Yushui Mine in Guangdong Province, China. Chemical analyses showed that the ore contained 31.36% Cu, 30.50% Fe, and 34.38% S. The fine copper ores with particle sizes of 0.045−0.074 mm were used for bioleaching experiments. The size less than 5 μm was used for zeta-potential, adsorption and FTIR studies. Pure solid chalcopyrite crystals were cut into certain thin sections, and then polished to the exposed faces. The mineral pieces washed with acetone were used to measure contact angle.

2.2 Microorganisms and culture media

A. ferrooxidans type strain (ATCC23270) and *A. caldus* (DQ256484) used in the experiments were conserved by the Key Lab of Biometallurgy in Central South University.

A. ferrooxidans and *A. caldus* were grown in 9K medium at an initial pH of 2.0, optimum temperature 30 ℃ and 45 ℃, respectively. The 9K medium compositions are $(NH_4)_4SO_4$ 3g/L, KCl 0.1 g/L, K_2HPO_4 0.5 g/L, $MgSO_4·7H_2O$ 0.5 g/L, $Ca(NO_3)_2$ 0.01 g/L, $FeSO_4·7H_2O$ 44.7 g/L or S 10 g/L.

2.3 Zeta-potential measurements

Zeta-potential measurements were made with the DELSA440SⅡ Type electrokinetic instrument. The chalcopyrite particles had a concentration of 1 g/L with the ionic strength(I) of 10^{-2} mol/L maintained with NaCl. Chalcopyrite was interacted with cells by magnetic blender for 90 min and a cell concentration of 1×10^8 cells/mL was used. The bacteria soliquid was put into NaCl solution with ionic strength of 10^{-3} mol/L and mingled slightly. The cell density was about 2×10^8 cells/mL. Measurements were performed as a function of pH adjusted with HCl and NaOH.

2.4 Adsorption measurements

Tests were performed with 1 g mineral in 100 mL NaCl solution with the ionic strength of 10^{-2} mol/L and pH 2.0, containing initial cell concentration of 1×10^8 cells/mL. Chalcopyrite was interacted with cells for 90 min by conditioning the suspension using a magnetic stirrer. After conditioning, the suspension was filtered to separate the solids and the bacterial cells remaining in the liquid phase were estimated by microscope.

2.5 FTIR measurements

FTIR analyses were performed with Nicolet FTIR-740 instrument. The spectrum of *A. ferrooxidans* and *A. caldus* cells alone and chalcopyrite interacted with cells were recorded. The cell mass was collected, washed, dried in a vacuum drying incubator and used for measurements. Chalcopyrite was interacted with cells for 90 min using intensive stirring at pH=2. After conditioning, the suspension was filtered and mineral samples were washed with deionised water to remove the loosely holding cells and air-dried.

2.6 Contact angle measurements

The contact angle of the distilled water on the chalcopyrite surface was measured with the JJC-I wetting angle measurement instrument produced by the Changchun Optics Instrument Factory, China. All the measurements were operated at the room temperature (25 ℃).

2.7 Bioleaching experiments

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 mineral concentration was 1%(w/v). The leaching solutions were periodically analyzed for copper concentration and the cell density everyday.

3 Results and discussion

3.1 Zeta-potential

The surface of a bacterial cell is charged due to the presence of functional groups such as carboxyl(—COOH), amino (—NH_2) and hydroxyl (—OH), originating from the cell wall components of lipopolysaccharides, lipoprotein and bacterial surface proteins[14]. The Zeta-potentials of the different types of *A. ferrooxidans* and *A. caldus* as a function of pH are shown in Fig.1.

A. ferrooxidans grown in ferrous exhibited an isoelectric point (IEP) at pH 2.0. However, *A. ferrooxidans* grown on elemental sulfur and chalcopyrite showed IEP around 3.3−3.5. The IEP is generally higher and typical for *A. ferrooxidans* grown on solid substrate cells with higher amount of EPS and proteins. *A. caldus* grown on sulfur and chalcopyrite have the similar IEP at pH=3.2−3.5 and beyond this pH the magnitude of negative potential increased with rise in pH.

The Zeta-potentials of chalcopyrite before and after interaction with bacterial cells are presented in Figs.2−3.

The IEP of chalcopyrite displayed at pH 5.1, after which interaction with cells moved towards the IEP of cells. In strong acidic pH medium, the Zeta-potential of chalcopyrite decreased and became negative. Beyond pH 5.5, the Zeta-potentials of chalcopyrite interacted with

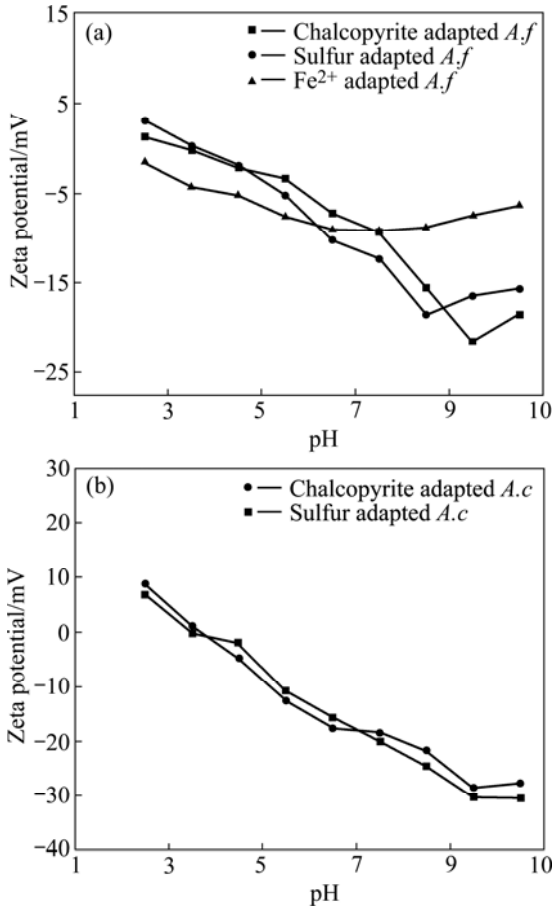


Fig.1 Zeta-potential of *A.ferrooxidans* (*A.f*) (a) and *A caldus* (*A.c.*) (b) at *I*=0.001 mol/L

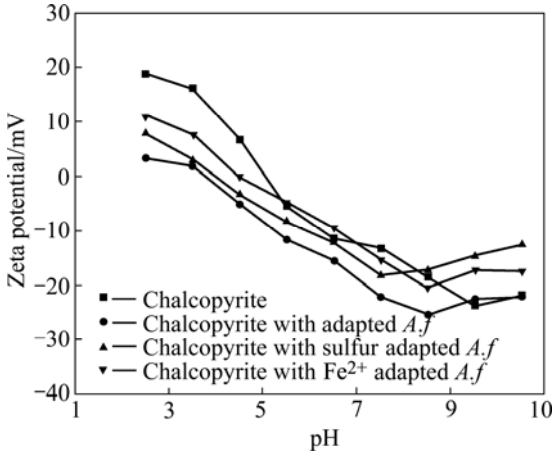


Fig.2 Zeta-potential of chalcopyrite before and after conditioning with *A. ferrooxidans* at *I*=0.01 mol/L

unadapted cells are similar to pure chalcopyrite potentials. There is hardly any influence of cells on chalcopyrite potentials in the basic pH region. The effects of adapted cells on the Zeta-potential of chalcopyrite were more significant than that of unadapted cells. The observed difference in the electrokinetic behavior of chalcopyrite and cells consequent to their mutual interaction confirms that the

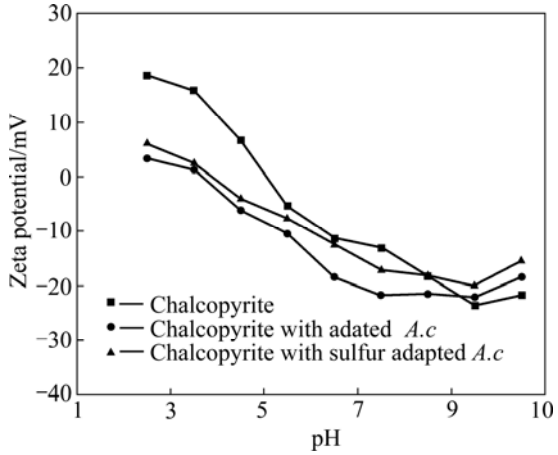


Fig.3 Zeta-potential of chalcopyrite before and after conditioning with *A. caldus* at *I*=0.01 mol/L

cells grown on different energy resources exhibit different abilities and affinities towards chalcopyrite.

The change of Zeta-potential of chalcopyrite after interaction with microorganisms may be related to the reactions for the oxidation of chalcopyrite as follows:

$$CuFeS_2+2H^++(1/2)O_2 \xrightarrow{\text{Bacteria}} CuS+Fe^{2+}+H_2O+S^0 \quad (1)$$

$$Fe^{2+}+H^++(1/4)O_2 \xrightarrow{\text{Bacteria}} Fe^{3+}+(1/2)H_2O \quad (2)$$

$$CuS+Fe^{3+}+4H_2O \rightarrow Fe^{2+}+Cu^{2+}+8H^++SO_4^{2-} \quad (3)$$

$$2S^0+2H_2O+3O_2 \xrightarrow{\text{Bacteria}} H_2SO_4 \quad (4)$$

Unequal dissolution of mineral surface ion by direct action of *A. ferrooxidans* and *A. caldus* on chalcopyrite resulted in the decrease of Zeta-potential. The direct biooxidation mechanism at the initial stage was considered as a main action in chalcopyrite bioleaching.

3.2 Adsorption researches

The adsorption curves of *A. ferrooxidans* and *A. caldus* on chalcopyrite are presented in Fig.4.

The kinetics of adsorption of *A. ferrooxidans* and *A. caldus* grown on different energy were similar to chalcopyrite respectively. And the adsorption equilibrium was attained in about 30 min for *A. ferrooxidans*, 60 min for *A. caldus*. The adsorption of *A. ferrooxidans* was faster than *A. caldus* cells onto chalcopyrite, while the *A. ferrooxidans* adhered to chalcopyrite was more than *A. caldus* under the same equilibrium concentration (1×10^8 cells/mL). After 60 min, *A. ferrooxidans* and *A. caldus* cells adhered to chalcopyrite were about 88%, 84% of the initial cells, respectively. It also could be seen that, compared with unadapted bacterial cells, the adsorption of adapted bacterial cells was faster and higher. This explains the observed difference in the electrokinetic behaviour of chalcopyrite consequent to

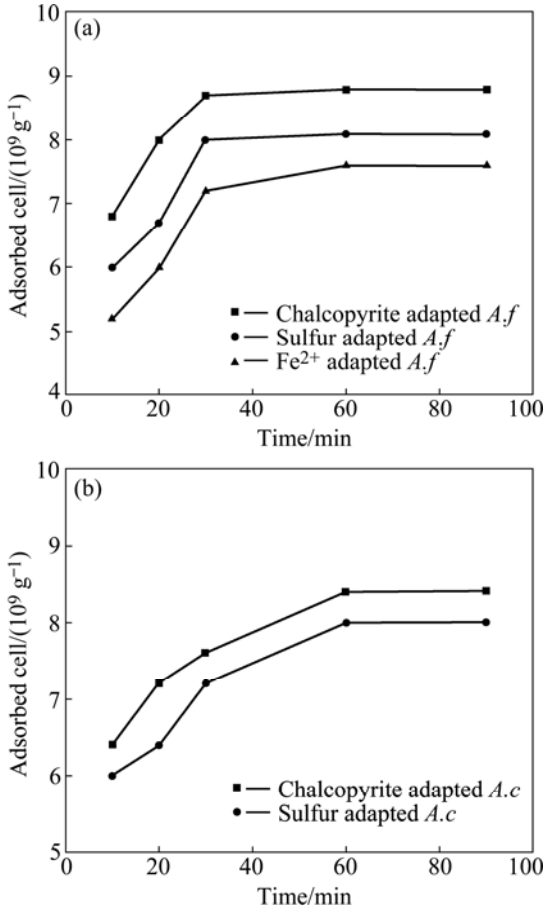


Fig.4 Adsorption curves of *A. ferrooxidans* (a) and *A. caldus* (b) on chalcopyrite

the interaction with the bacterial cells.

3.3 FTIR studies

The FTIR spectra of *A. ferrooxidans* and *A. caldus* (Fig.5) show several peaks for typical organic compounds such as carbohydrates CH_3, CH_2, CH, nitrogenous substances. The broad and strong band near 3 300 cm^{-1} represents asymmetric stretching of —NH_2 group. The band near 2 930 cm^{-1} characterizes —CH stretching mode of CH_2 group. The intense band near 1 650 cm^{-1} indicates the presence of an amide group (amide I band). The band at 1 540 cm^{-1} is attributed to NH bending of the secondary amide group —CONH (amide II band). The bands near 1 450 cm^{-1} and 1 230 cm^{-1} are attributed to —CH_3 bending and —CH_3 wagging respectively. The band at 1 050 cm^{-1} is attributed to —CH_3 rocking and —CH_2 wagging modes. Comparing the spectrum of *A. ferrooxidans* with *A. caldus*, it was illustrated that the two type bacterium had similar surface chemical compositions.

Spectra of chalcopyrite interacted with *A. ferrooxidans* and *A. caldus* are shown in Fig.6. The changes on chalcopyrite interacted with *A. ferrooxidans* were more observable than those on chalcopyrite interacted with *A.caldus*. From the adsorption, *A. ferrooxidans* had stronger ability to adhere to chalcopyrite surface than *A. caldus*. Compared with the pure chalcopyrite spectra, some peaks become less intensive and some new small characteristic peaks from bacterial spectra appeared. This proved that the cells adsorbed on chalcopyrite surface.

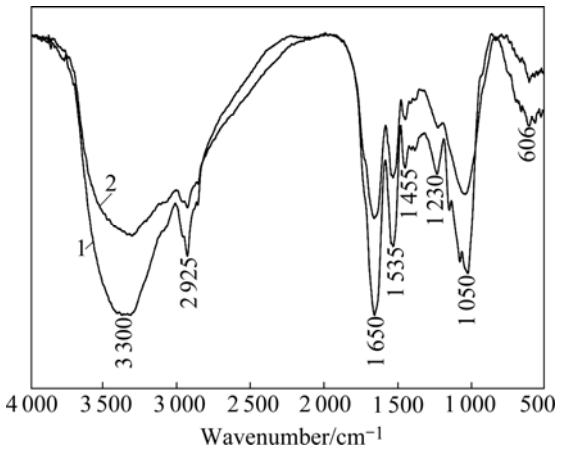


Fig.5 FTIR spectra of chalcopyrite-adapted *A. ferrooxidans* and *A. caldus*: 1—Chalcopyrite adapted *A. f*; 2—Chalcopyrite adapted *A. c*

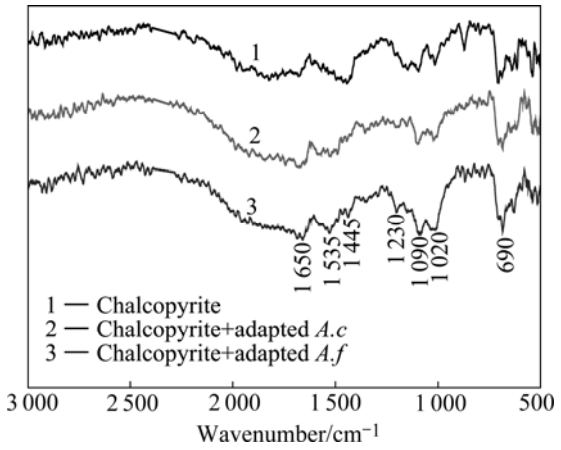


Fig.6 FTIR spectra of chalcopyrite interacted with adapted *A. ferrooxidans* and *A. caldus*

3.4 Contact angle measurements

Fig.7 shows the change of chalcopyrite contact angle interacted with different cells. The contact angle and surface hydrophobicity of chalcopyrite surface increased at initial bioleaching stage. This may be caused by the formation of elemental sulfur and intermediate copper sulphides on chalcopyrite surface (Reaction (1)). With the interaction time increasing, the contact angle of chalcopyrite surface decreased, which inferred the oxidation of elemental sulfur by the sulfur-oxidizing microorganisms (Reaction (4)). Previously published work also reported the initial chalcopyrite oxidation led to the formation of a passive layer of growing thickness, possibly elemental sulfur and bornite, which was further

oxidized to nonstoichiometric copper sulphides[15−18] (Reactions (1), (3) and (4)).

The change of chalcopyrite contact angle implies that direct action at the initial stage is more important when bioleaching chalcopyrite with *A. ferrooxidans* and *A. caldus*.

3.5 Chalcopyrite bioleaching

Comparisons of chalcopyrite leaching by *A. ferrooxidans* and *A. caldus* grown on different energy sources are shown in Figs.8−9.

The growth rate of *A. ferrooxidans* and *A.caldus* cells was increased during the leaching time, and both bacterium adapted by chalcopyrite grew faster than

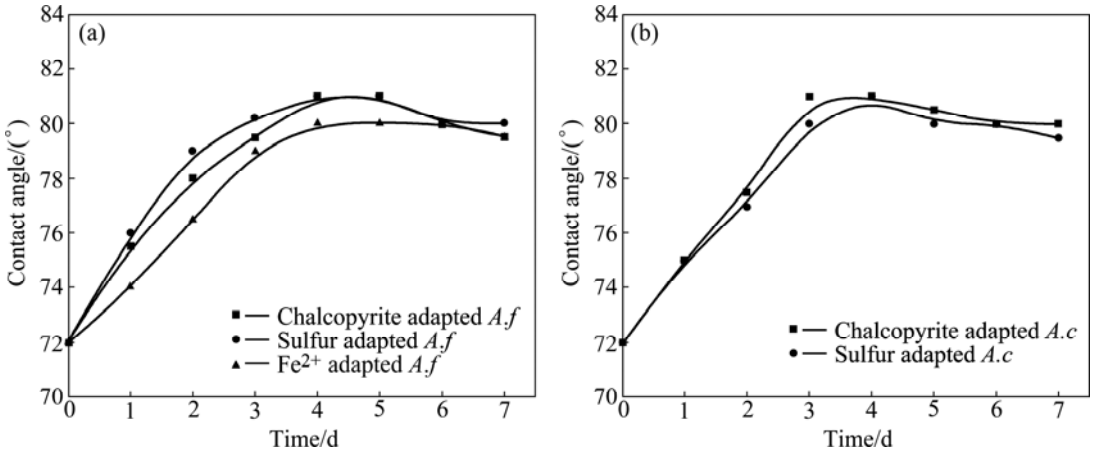


Fig.7 Changes of contact angle of chalcopyrite leached by *A. ferrooxidans* (a) and *A. caldus* (b) under different bioleaching conditions

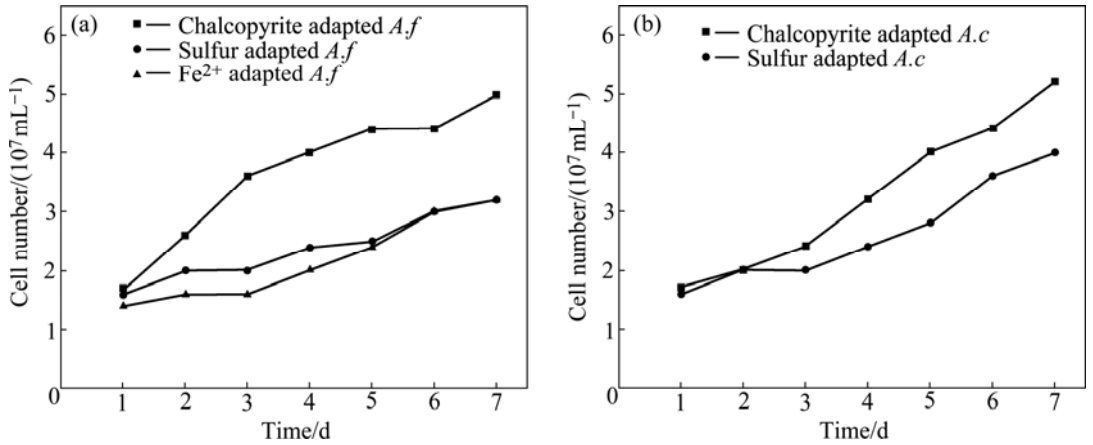


Fig.8 Growth curves of *A. ferrooxidans* (a) and *A. caldus* (b) in different bioleaching time

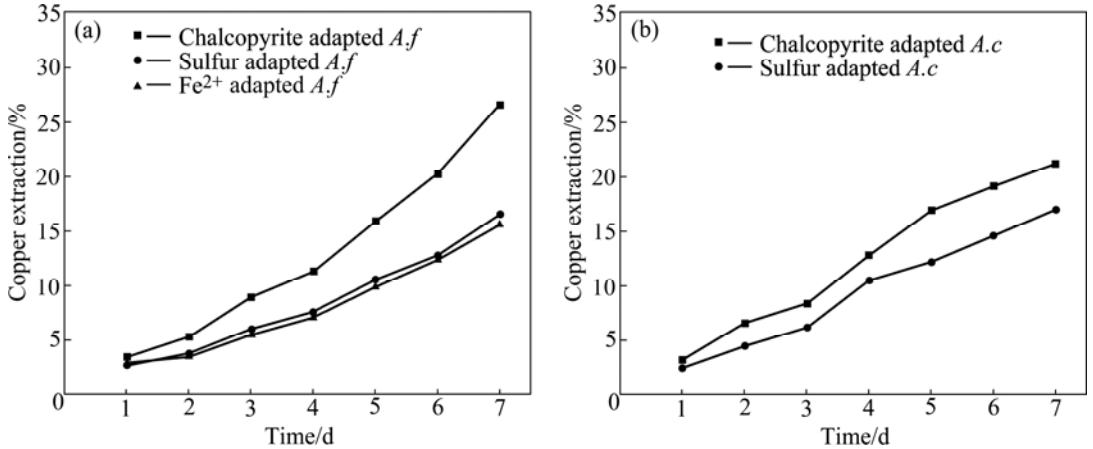
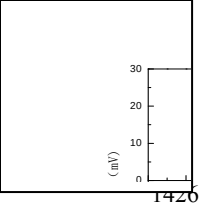


Fig.9 Copper extraction of chalcopyrite leached by *A. ferrooxidans* (a) and *A. caldus* (b)



unadapted bacterium. After 7 d, the copper extraction by *A. ferrooxidans* and *A. caldus* on chalcopyrite reached 26.6% and 21.1%, respectively. The reason that *A. ferrooxidans* could oxidize iron and sulfur and the *A. caldus* just had the ability to oxidize sulfur could explain this phenomenon. However, at the initial bioleaching stage, the increasing trend of chalcopyrite leaching rate by *A. ferrooxidan* was nearly the same as that by *A. caldus*. This indicates that bacteria with the ability to oxidize sulfur play a dominant action. Linking to the results of surface properties, our studies support that the direct action is dominant at the initial stage of bioleaching.

4 Conclusions

1) The IEP of cells grown on solid substrate is generally higher for EPS and proteins with higher amount. Conditioning with bacterial cells, the IEP of chalcopyrite moves towards the IEP of pure cells indicating the adsorption of cells on chalcopyrite surface.

2) The changes on chalcopyrite FT-IR spectra conditioning with *A. ferrooxidans* are more observable than those with *A. caldus*. However, the higher amount of adsorbed *A. ferrooxidans* cells on chalcopyrite surface than *A. caldus* cells can explain this phenomenon.

3) Due to the formation of elemental sulfur and intermediate copper sulphides on chalcopyrite surface, the contact angle and surface hydrophobicity of chalcopyrite surface increase at the initial bioleaching stage.

4) The mesophilic *A. ferrooxidans* has the ability to oxidize ferrous ion and elemental sulfur has stronger leaching ability for chalcopyrite than moderately thermophilic *A. caldus* only oxidizing sulfur.

5) The direct action is dominant at the initial stage of bioleaching chalcopyrite. As the oxidation of sulfur by bacteria, both direct and indirect actions enhanced the metal dissolution in bioleaching process.

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