

## Study on the Mechanism of Mixed Bacterial Leaching of Pyrrhotite

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In this paper, the mechanism of mixed bacteria ( $\text{Fe}^{2+}$  grown or pyrrhotite grown) leaching of pyrrhotite was investigated through the analysis of adsorption quantity, electrokinetic potential, contact angle, shock-flask bioleaching and leaching residue of mixed bacteria cultured with different materials. The results showed that pyrrhotite-grown mixed bacteria have stronger adsorptivity and biological oxidation ability; adsorption of bacteria caused the isoelectric point of pyrrhotite to shift towards the isoelectric point of bacteria, in which the isoelectric point shifted more for minerals treated with grown mixed bacteria than did with the other bacteria. Acid dissolution was in a dominant position at the preliminary stage of pyrrhotite leaching and bioleaching was gradually taking part into work as the mixed bacteria adsorbed. Throughout the process, relative enrichment of iron element will happen in the system, which resulted in increasing of electrokinetic potential of minerals. Meanwhile, iron element easily produced hydrophilic iron precipitates on surfaces of pyrrhotite, which caused the increase of the hydrophilicity and the decrease of contact angle. Bacterial leaching was indirectly influencing the pyrrhotite leaching, since the minerals were oxidized by  $\text{Fe}^{3+}$ , which was produced by bacteria leaching in solution.

**Key words:** Pyrrhotite, Mixed bacteria, *Leptospirillum ferriphilum*,  
*Acidithiobacillus ferrooxidans*, Function mechanism.

In the wake of growing inadequacy fineness and variety, there is an increasing demand for developing new methods to explore and utilize low-grade and intractable mineral resources. Bioleaching has attracted more and more attentions due to its advantages such as low cost, less pollution, short process flow, comprehensive recovery of low-grade sulphide ore, etc<sup>1</sup>. Biohydrometallurgy has been industrially applied in dealing with sulphide ore accompanying gold,

uranium, copper, etc, but there usually are problems such as complex leaching environment, poor adaptability of strain, long leaching cycle and low leaching rate<sup>2,3</sup>. For strengthening selective leaching of sulphide ore, it is necessary to understand the mechanism of bioleaching<sup>4,5</sup>.

In general, the mechanism in microbial leaching of sulphide ore includes concurrent direct action, indirect action and indirect contact action or only includes one mechanism of action<sup>6,7</sup>. Regarding direct mechanism of action, bacteria play a role of primary oxidizing agent, which requires direct contact of bacteria with mineral's surfaces<sup>8</sup>. In indirect mechanism of action, microorganism acts indirectly and regenerates ferriions by ferrous ions in oxidizing environment<sup>9,10</sup>.

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The ratio of the two mechanisms is determined by surface types of minerals and acting environment. Pyrrhotite is a kind of familiar sulphide mineral in sulphide deposit of non-ferrous metal, which accompanies with valuable metals such as nickel, copper, lead, zinc and cobalt etc.<sup>12,13,14</sup> It is one of carriers of noble metals. Bioleaching of pyrrhotite is a complex course which mainly includes adsorption of bacteria on mineral's surfaces, various chemical reactions on surfaces of pyrrhotite and deposition of intermediate products on mineral's surfaces, etc.<sup>15</sup> Study and understanding of mechanism of bioleaching of pyrrhotite have significance to a certain extent for microbial selective leaching of low-grade sulphide ore and comprehensive recovery of valuable metals there in<sup>12,16</sup>.

There were not many reports regarding microbial leaching of pyrrhotite<sup>6,9,17</sup>. These reports concentrated on leaching of nickelic pyrrhotite<sup>18,19,20,21</sup> and intractable pyrrhotite-type gold deposit<sup>22,23</sup> and *Thiobacillus thiooxidans*, *Acidithiobacillus caldus* or *Thiobacillus ferrooxidans*<sup>6,15,17,23,24</sup>. The mechanisms of bioleaching pyrrhotite by different bacteria has been in controversy, and the investigation on the mechanism of leaching pyrrhotite using mixed bacteria cultured with different energy materials is not involved in the previous study. Therefore, studies in bioleaching of pyrrhotite are required to be carried out further. This paper focuses on qualitative changes on pyrrhotite surfaces and its effects on leaching process following actions of mixed bacteria (*Leptospirillum ferriphilum* and *Acidithiobacillus ferrooxidans*) cultured with different energies (Fe<sup>2+</sup> or pyrrhotite-grown) and then further discusses the mechanism of mixed bacteria leaching of pyrrhotite.

## MATERIALS AND METHODS

### Sample ores

Pyrrhotite used for test was taken from Jinchuan Concentrating Mill locating in Gansu Province. After rich ore blocks manually selected were subject to crushing, manual impurity removal, coarse grinding with porcelain balls and tailings discarding with dry magnetic separation, pure ore products with Fe 58.15% and S 37.85% were obtained and purity of ore products reached

94.12%. After subjecting to dry screening, part of pure minerals with granularity smaller than 0.045mm was used for shock-flask leaching and powder contact angle measurement. Other part of pure mineral underwent grinding with agates so that its granularity was reduced smaller than 5 μm and used for studies on adsorption and electrokinetic potential measurement. In addition, lump ores with good crystal were selected to undergo cutting, grinding and polishing and then used for contact angle measurement. Samples of pure mineral products were stored in dryer filling with nitrogen for future use.

### Culture and mixing of bacteria

Both *Leptospirillum ferriphilum* (*L.f.*) and *Acidithiobacillus ferrooxidans* (*A.f.*) used in the research were taken from the key laboratory of biohydrometallurgy of Education Department of Central South University. 9K culture medium was selected to culture *L.f.* and *A.f.* With 170 rpm rotary speed of constant temperature shaking incubator, initial pH value respectively was 1.6 and 2.0 and temperature respectively was 40°C and 30°C. 9K culture medium was composed of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 3g/LyKCl 0.13g/L. Fe<sup>2+</sup>-grown mixed bacteria were obtained by adding FeSO<sub>4</sub>·7H<sub>2</sub>O 44.73g/L to 9K culture medium. Mineral acclimated bacteria were obtained by repeatedly culturing strains in 9K culture medium with 1% pyrrhotite concentration and continuously increasing concentration of ore pulp to 3%. Subjected to centrifugation with centrifugal machine, cultured bacteria were used to prepare bacterial suspension with iron-free 9K culture medium and concentration of bacterial suspension can reach 1×10<sup>8</sup>/mL. Fe<sup>2+</sup>-grown mixed bacteria: mixture ratio between *L.f.* and *A.f.* for Fe<sup>2+</sup>-grown was 6:4. Pyrrhotite-grown mixed bacteria: mixture ratio between *L.f.* and *A.f.* for pyrrhotite-grown also was 6:4.

### Adsorption of mixed bacteria on mineral's surfaces

1.0 g pure mineral powder was put into NaCl solution with 100mL mixed bacteria having 1×10<sup>8</sup>/mL initial concentration and 10<sup>-3</sup>mol/L ionic strength and pH value was adjusted to 1.6. After solution was mixed with 85-2 type constant temperature magnetic stirrer for given time (temperature is controlled at 40°C), mineral particles was separated out by filtering supernatant following natural settling. Number of bacteria

remained in solution was counted through binocular microscope. Adsorption quantity of mixed bacteria on mineral's surfaces was equal to the difference between initial number of mixed bacteria in solution and number of bacteria in supernatant.

#### **Electrokinetic potential measurements**

With Coulter Delsa 440sx Zeta potentiometric analyzer produced by American Beckman Coulter, electrokinetic potentials of mixed bacteria and minerals were measured. Measurement was carried out in NaCl solution 100mL with  $10^{-3}$ mol/L ionic strength and initial concentration of mixed bacteria was  $1 \times 10^8$ /mL.

Bacterial suspension was added into the said NaCl solution and the concentration of mixed bacteria reached  $2 \times 10^8$ /mL. After 5 min mixing with magnetic stirring apparatus, electrokinetic potential measurement of bacteria was completed. 0.1g pure mineral powder ( $-5\mu\text{m}$ ) was added into the said NaCl solution to be mixed for 5min with magnetic stirring apparatus. Subsequently, electrokinetic potential measurement of mineral was completed. 1g mineral powder was put into a beaker containing NaCl solution with  $2 \times 10^8$ /mL bacteria concentration and  $10^{-3}$ mol/L ionic strength and the breaker was put on magnetic stirring apparatus to stir the solution. After 90min interaction between mineral and bacteria, mineral particles were separated out by means of centrifugation and electrokinetic potential of pyrrhotite particles subsequent to interaction with bacteria was measured. In the course of measurement, pH value of solution was adjusted with HCL and NaOH. Each sample was measured three times and average value of measurement was taken.

#### **Contact angle measurements**

##### **Contact angle measurement of lump ores**

JJC-1 wetting angle meter manufactured by Changchun Photoelectric Instrument Factory was employed to measure contact angle of distilled water on surfaces of lump ores before and after action of mixed bacteria. During measurement, distilled water was carefully dripped on solid surface to form sessile drop with 3~5 $\mu\text{L}$  volume and 1~2mm diameter. Time of measurement didn't exceed 1min and the average value of 9 readings was taken as contact angle of the sessile drop.

##### **Powder contact angle measurement**

GBX-3S surface tension tester

manufactured by French company GBX was used to measure contact angle of distilled water on pure mineral powder before and after action of mixed bacteria. 1g mineral powder was weighted and poured into a sample cell with filter paper on the bottom. After mineral powder was compacted with a compacting bar, the sample cell was installed on surface tension tester and height of mineral powder was adjusted and controlled by means of scale on sample cell so that height of mineral powder was same for each measurement. Specific operation was divided into two steps. Firstly, fixed constant of the same sample was measured out with 67mL dehydrated alcohol as reference solution; secondly, contact angle was measured out with 67mL distilled water and average value of 3 measurements was taken as contact angle of the sample powder.

#### **Bioleaching tests**

Leaching test was carried out in 250mL conical flask. 100mL 9K culture medium, with 10% (v/v) inoculum size of mixed bacteria and 2% (w/v) mass fraction of pulp and 1.8 initial pH value, was used as leaching system. Under the condition of 170rpm and 35°C temperature, the conical flask was put into air bath constant temperature shaking incubator for shaking culture of 7d.

In the course of test, pH value of leaching system was measured out 1d and 2mL leachate was taken out to analyze total iron content every two days. Leaching residues obtained by filtering was dried in the shade after completion of test and X Ray Diffraction analysis (XRD), Scanning Electron Microscope (SEM) and Energy Spectrum analysis (EDS) was performed for leaching residues.

## **RESULTS AND DISCUSSION**

### **Adsorption of mixed bacteria on mineral's surfaces**

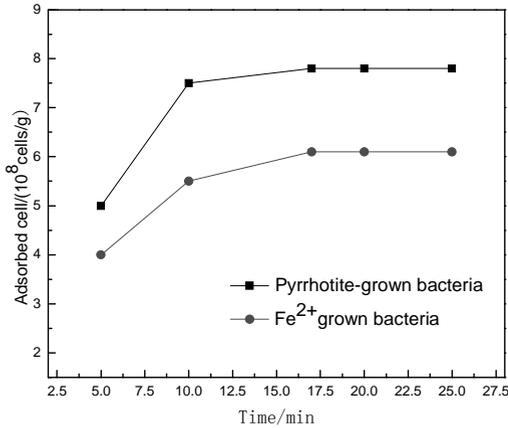
Adsorption curves of  $\text{Fe}^{2+}$ -grown mixed bacteria and pyrrhotite-grown mixed bacteria on pyrrhotite's surfaces are shown in Fig. 1. Results show that mixed bacteria cultured with different energies had similar adsorption tendency which reached saturated adsorption at 17min. Pyrrhotite-grown mixed bacteria had higher adsorption quantity than  $\text{Fe}^{2+}$ -grown mixed bacteria. After 17min, pyrrhotite-grown mixed bacteria accounts for 79% of initial concentration adsorbed and only

60% of  $\text{Fe}^{2+}$ -grown mixed bacteria adsorbed on mineral's surfaces. In terms of pyrrhotite,  $\text{Fe}^{2+}$ -grown mixed bacteria and pyrrhotite-grown mixed

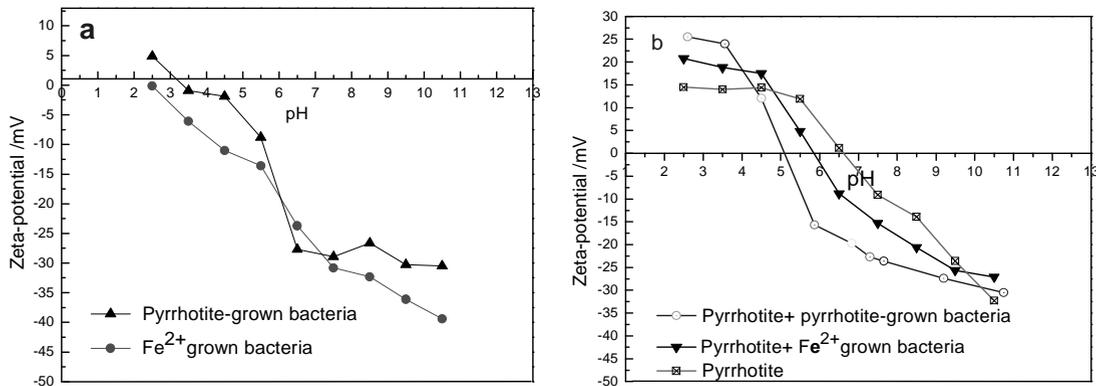
bacteria showed different adsorption capacity therefore had different dynamic bioleaching behaviours.

### Electrokinetic potential measurements

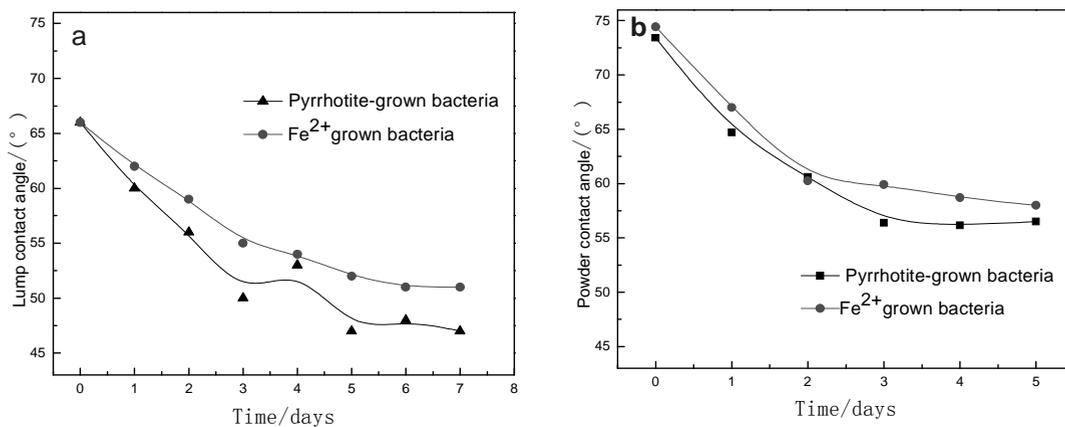
The relationship between electrokinetic potential value and pH value of  $\text{Fe}^{2+}$ -grown mixed bacteria and pyrrhotite-grown mixed bacteria is shown in Fig. 2(a). Within the range of measured pH, all electrokinetic potentials of  $\text{Fe}^{2+}$ -grown mixed bacteria were negative with an isoelectric point of about pH 2.5, while the isoelectric point of pyrrhotite-grown mixed bacteria was at pH 3.2, which indicated that the charge densities of  $\text{Fe}^{2+}$ -grown mixed bacteria and pyrrhotite-grown mixed bacteria were different. The reason might be due to the higher protein content of the pyrrhotite-grown mixed bacteria<sup>24</sup>. It can be inferred that the higher extracellular polymer and protein contents on surfaces of pyrrhotite-grown mixed bacteria



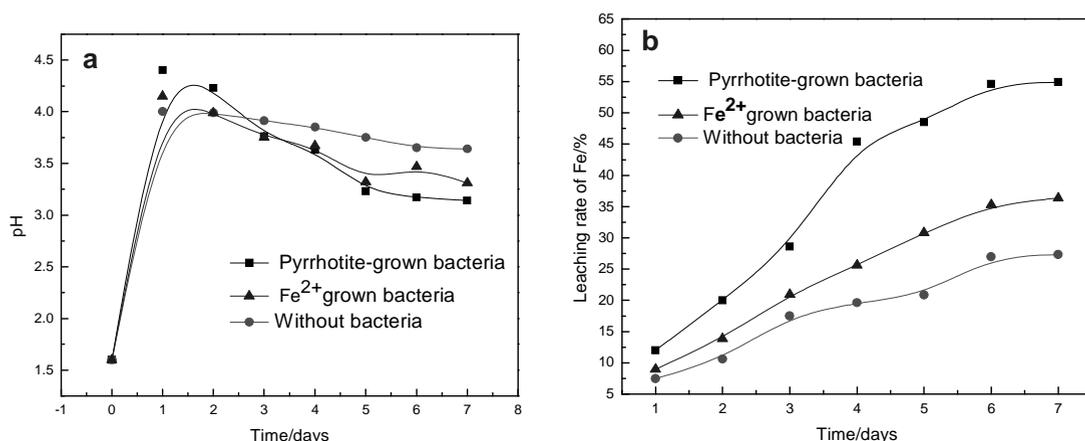
**Fig. 1.** Adsorption curves of mixed bacteria on pyrrhotite



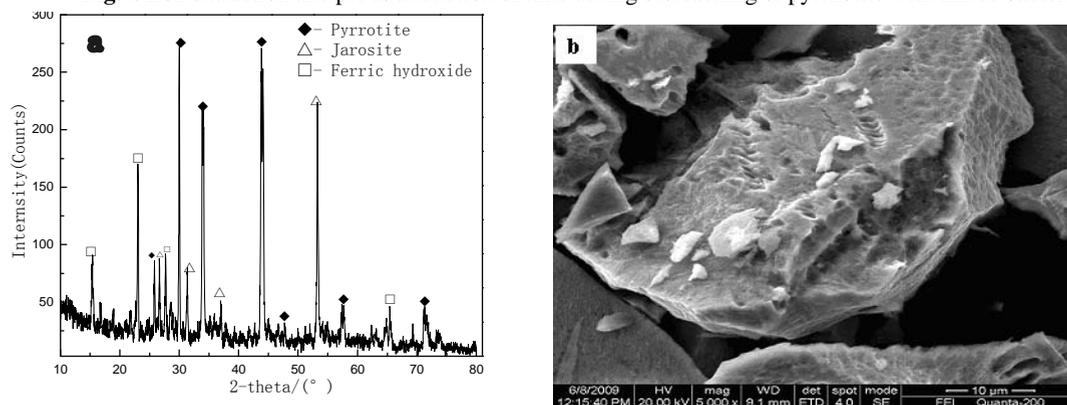
**Fig. 2.** Zeta-potential of mixed bacteria (a), pyrrhotite and pyrrhotite interaction with mixed bacteria (b)



**Fig. 3.** Changes of contact angle(a) and powder contact angle(b) of pyrrhotite leached by mixed bacteria



**Fig.4.** Iron extraction and pH as a function of time during bioleaching of pyrrhotite with mixed bacteria



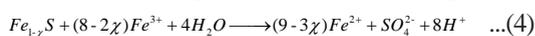
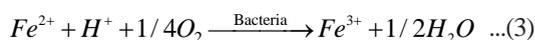
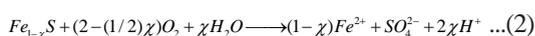
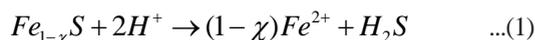
**Fig.5.** XRD pattern (a) and SEM images (b) of leached residues by pyrrhotite-grown bacteria

enable its isoelectric point higher than that of Fe<sup>2+</sup>-grown mixed bacteria.

Changes in electrokinetic potential value of pyrrhotite before and after being treated with mixed bacteria are shown in Fig. 2(b). The isoelectric point of pyrrhotite was at about pH 6.6. After treatment with bacteria, the isoelectric point of pyrrhotite shifted to close to the isoelectric point of bacteria. The greater shifts of the pyrrhotite-grown mixed bacteria indicated that the specific adsorption of bacteria occurred on mineral's surfaces and grown bacteria had stronger adsorption capacity.

It can be seen from Fig. 2(b) that, after interaction with bacteria in acid medium (pH<4.0), mineral's electrokinetic potential value increased obviously and the magnitude of increase in electrokinetic potential value caused by acclimated mixed bacteria was larger. It may be caused by

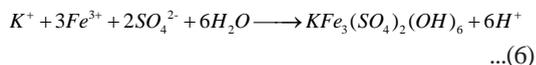
oxidization between acid solution and ferric iron. The increased concentration of ferri ions might be due to the unbalanced dissolution of metal ions from surfaces of pyrrhotite, which increased electrokinetic potential. Reaction equations triggering aforesaid changes include (1), (2), (3) and (4). Thus, we could conclude that mixed bacteria leached out pyrrhotite in an indirect manner and minerals were oxidized by Fe<sup>3+</sup>. This viewpoint will be demonstrated further in following contact angle measurement, leaching test and leaching residue analysis.



### Contact angle measurements

The relationship between changes in contact angle of the pyrrhotite lump and the pyrrhotite powder and the action time of mixed bacteria is shown in Figs. 3(a) and 3(b). According to results, change rule of lump ore was almost the same as that of powder and showed a declined tendency. Meanwhile, contact angle of powder was larger than that of lump ore, which might be resulted from that more covalent bonds or metallic bonds were uncovered and the solubility and hydrophilicity of mineral becomes stronger. According to Fig. 3, contact angle of pyrrhotite always showed a declined tendency within the time range of measurement, which might be due to that iron element relatively concentrates in system (Eqs. (1), (2), (3) and (4)) and it easily produced hydrophilic iron deposits on mineral's surfaces (Eqs. (5) and (6)). Therefore, surface hydrophilicity of mineral increased and contact angle reduced. Due to stronger effect of pyrrhotite-grown mixed bacteria on mineral, amplitude of decrease in

contact angle obtained was larger than the contact angle under effect of  $Fe^{2+}$ -grown mixed bacteria.



### Bioleaching tests

Fig. 4 shows experimental results obtained by means that pyrrhotite is leached out by  $Fe^{2+}$ -grown mixed bacteria and pyrrhotite-grown mixed bacteria. From the change curve of pH during bioleaching in Fig. 4(a), it can be seen that change tendencies of solution's pH in different leaching systems were almost the same. At early stage of bioleaching, solution of pyrrhotite gave priority to acid solution (Eq. (1)) and bacteria needed adaptation to leaching environment, which resulted into rapid increase in solution's pH at early stage of leaching. Afterwards, pH value decreased along with the progress of leaching and the action of bacteria (Eqs. (2), (3) and (4)) as well as the acid reaction (Eqs. (5) and (6)) that iron deposit occurred.

**Table 1.** Results of EDS of leached residues by pyrrhotite-grown bacteria

Sample	wt. %			at. %			Atomic ratio of Fe to S <sup>a</sup>
	Fe	S	O	Fe	S	O	
Residues with mixed bacteria	65.72	20.98	15.12	42.55	22.79	35.61	1:0.53

<sup>a</sup>Before bioleaching, the atomic ratio of Fe to S in pyrrhotite is 1/1.12

According to change curves of leaching rate of total iron of solution during leaching in Fig. 4(b), part of pyrrhotite can be solved in system without bacteria and leaching rate reaches 27.3% after 7 days. Leaching effect of pyrrhotite-grown mixed bacteria was better than that of  $Fe^{2+}$ -grown mixed bacteria. After 7 days of leaching, leaching rate of pyrrhotite-grown mixed bacteria was 54.9% and leaching rate of  $Fe^{2+}$ -grown mixed bacteria was 36.35%, which showed pyrrhotite-grown mixed bacteria had stronger adsorption capacity for and bio-oxidation effect on pyrrhotite.

### Analyses on leaching residues

X Ray Diffraction Analysis (XRD, Fig. 5(a)) Scanning Electron Microscope (SEM, Fig. 5(b)) and Energy Spectrum Analysis (Table 1) were carried out on leaching residues subjected to treatment with pyrrhotite-grown mixed bacteria in

said leaching test. X Ray Diffraction Analysis on leaching residues (Fig. 5(a)) showed that a considerable quantity of iron-containing deposits concentrated in leaching residues, including ferric hydroxide and ferrovandium (Eqs. (5) and (6)); SEM showed in Fig. 5(b) for leaching residues indicates that surfaces of pyrrhotite, after leaching, were covered by secondary products with a definite crystal structure; Analysis results of Energy Spectrum Analysis (Table 1) for leaching residues shows that, before leaching, mass fraction of iron element in pyrrhotite was 58.15% and mass fraction of sulfur element was 37.85% and atomic ratio is 1:1.14. After leaching, mass fraction of iron element in pyrrhotite was 65.72% and mass fraction of sulfur element was 20.98% and atomic ratio is 1:0.53. Hence one can see that iron element in leaching residues relatively concentrated and part of sulfur

element was oxidized into sulfate radical (Eqs. (1), (2), (3) and (4)), which was in accordance with results obtained by contact angle measurements and bioleaching tests.

Bacteria can quickly adsorb on the surfaces of pyrrhotite. Compared with Fe<sup>2+</sup>-grown mixed bacteria, pyrrhotite-grown mixed bacteria have stronger adsorption capacity and bio-oxidation for pyrrhotite. After interaction with bacteria, the isoelectric point of pyrrhotite shifts to that of the bacteria and the degree of shifts is in proportion to bacteria' adsorption capacity on mineral's surfaces.

At early stage of pyrrhotite leaching, acid solution plays a major role. Along with progress of bleaching, bacteria begin to work. Throughout the whole process, relative concentration of iron element will occur in system, which causes increase in electrokinetic potential value of mineral. Iron element easily forms hydrophilic iron deposit so as to enhance surface hydrophilicity of pyrrhotite and to reduce contact angle.

The mixture of acidophile iron oxidized bacteria and acidophile iron-sulfur oxidized bacteria leach out pyrrhotite in an indirect manner (*L.f* and *A.f*), that is minerals is oxidized and solved primarily by Fe<sup>3+</sup> in solution.

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