



Bioleaching and electrochemical property of marmatite by *Sulfobacillus thermosulfidooxidans*

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Abstract: Bioleaching and electrochemical experiments were conducted to evaluate marmatite dissolution in the presence of pure *S. thermosulfidooxidans*. The effects of particle size, pH controlling and external addition of Fe^{3+} ions on the zinc extraction were investigated. The results show that in the bioleaching process the best particle size range is 0.043–0.074 mm and adjusting pH regularly to the initial value has a profound effect on obtaining high leaching rate. External addition of Fe^{3+} ions could accelerate the bioleaching, while the concentration of additional Fe^{3+} over 2.5 g/L weakens the positive effect, and even hinders the dissolution of marmatite. SEM and XRD analyses of the leaching residues reveal that a product layer composed of elemental sulfur and jarosite is formed on the mineral surface, which results in a low leaching speed at later phase. The results of electrochemical measurements illustrate that additional Fe^{3+} ions could increase the corrosion current density, which is favorable to zinc extraction. The EIS spectra show that rate-limiting step does not change when Fe^{3+} ions are added.

Key words: marmatite; *S. thermosulfidooxidans*; bioleaching; jarosite; electrochemical property

1 Introduction

Nowadays, it is widely accepted that certain microorganisms play a major role in the leaching of base and precious metals from various mineral resources [1–4]. The bioleaching of zinc sulphide using acidophilic autotrophic microorganisms has been studied extensively by some researchers in shake flasks and in columns [2,5–9], though the leaching effects reported are different in each study due to different leaching conditions and distinct properties of the mineral specimens used in the various experiments. However, there is neither total agreement on the mechanism of zinc sulphide bioleaching nor a unanimously accepted theory that explains the bioleaching process.

Marmatite is an important resource of zinc ore in China, which is difficult to be processed effectively by traditional technologies due to its high content of iron. But it is more suitable for bioleaching and the rate of oxidative dissolution is higher compared with sphalerite [10]. As moderately thermophilic microorganisms have received increasing attention since their

optimal growth temperature presents a kinetic advantage over mesophilic microorganisms. Recently, WANG et al [11] and SHI and FANG [12] investigated the bioleaching of marmatite using a moderately thermoacidophilic iron-oxidizing bacterial strain (MLY). SHI and FANG [12] demonstrated the technological feasibility of a microbiological process using MLY for extracting zinc from marmatite concentrate. And under the leaching conditions of the initial pH value 1.5, temperature 50 °C, pulp density 5%, particle size less than 35.5 μm (over 90%), over 95% of zinc dissolution was obtained [12]. WANG et al [11] discovered that, comparing with *A. ferrooxidans*, the supplementary addition of Fe^{2+} as a source of Fe^{3+} to be generated by bacteria was not necessary because the higher temperature improved the dissolution of marmatite in sulphuric acid, and thus acidic leaching happened before ferric ion leaching. *Sulfobacillus thermosulfidooxidans* is a moderately thermoacidophilic bacterium. Its optimum growth temperature is 50–53 °C and the effective growth temperature can be up to 58 °C. *Sulfobacillus thermosulfidooxidans* can take ferrous iron and sulphide ores as energy substances to grow autotrophically. Also,

it can utilize yeast extraction to grow heterotrophically. It can be able to oxidize ferrous iron and elemental sulfur in the presence of yeast extract. Some investigations of sulphide mineral leaching were carried out by using *Sulfobacillus thermosulfidooxidans*. XIA et al [13] investigated the surface sulfur speciation of chalcopyrite leached by *Sulfobacillus thermosulfidooxidans*. *Sulfobacillus thermosulfidooxidans* was used to study the effect of temperature on nickel sulfide bioleaching by CRUZ et al [14], compared with mesophile (*Acidithiobacillus ferrooxidans*) strains, the dissolution was higher, and meanwhile the external ferrous iron adversely affected the nickel leaching at higher temperatures.

In order to discover the effect of *Sulfobacillus thermosulfidooxidans* on the dissolution of natural marmatite and further understand the mechanisms of marmatite bioleaching, the effects of some factors, such as particle size, pH controlling and the external addition of different concentrations of Fe^{3+} ions, on the marmatite dissolution by bioleaching experiments were studied. The mineral composition and the morphological features of the residue samples were analyzed by XRD and SEM. The mechanism of marmatite bioleaching was also studied by Tafel and electrochemical impedance spectroscopy.

2 Experimental

2.1 Marmatite preparation and mineral characters

The bioleaching tests were performed using pure marmatite (approximately above 96%). The natural marmatite ores were obtained from a lead–zinc mine in Dachang, Guangxi Province, China. The ores were splintered into small fragments with a geological hammer and selected carefully by hand. After hand-selected, the obtained samples were dry-ground by a porcelain ball milling and then sieved into different fractions.

The samples of marmatite used in the bioleaching tests were from Dachang Mine in Guangxi Province of China. The samples were splintered into small fragments with a geological hammer and dry-ground in a porcelain ball milling. Then, the products were sieved into different fractions for bioleaching experiments. The chemical composition of different fractions is listed in Table 1. X-ray diffraction (XRD) analysis in Fig. 1 shows that marmatite is the major mineral component and there is also a little amount of quartz in it.

2.2 Microorganisms and culture media

A moderately thermoacidophilic bacterium of *Sulfobacillus thermosulfidooxidans*, which was grown in medium using rotary shakers at 160 r/min with the initial

Table 1 Chemical composition of different fractions

Particle size/mm	w(S)/%	w(Fe)/%	w(Zn)/%
0.074–0.150	32.41	13.02	51.56
0.043–0.074	31.91	13.20	51.00
<0.043	31.88	13.10	51.52

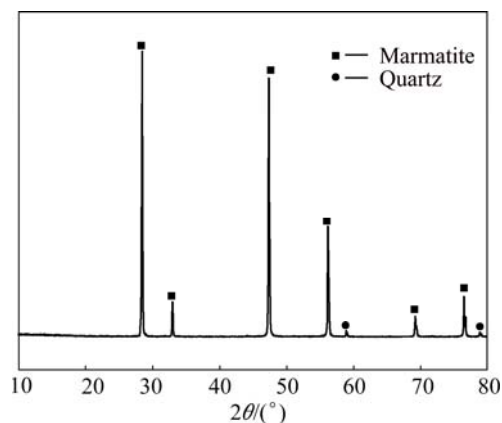


Fig. 1 XRD pattern of mineral sample

pH of 1.6 and temperature of 53 °C and used in all experiments, was obtained from the Key Laboratory of Biometallurgy in Central South University, China. A sterilized iron-free 9K medium was used as a substrate for bacterial growth. The iron-free 9K medium consisted of $(\text{NH}_4)_2\text{SO}_4$ 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 and $\text{Ca}(\text{NO}_3)_2$ 0.01 g/L. The bacteria were routinely sub-cultured using 20 g/L ferrous sulphate and 0.02% yeast extraction as energy source in an incubator. Then, the solution was filtered through a Whatman filter No. 1 to remove the precipitate and centrifuged at 9000 r/min for 20 min using a centrifuge, Model TGL16M. The centrifuged cells were washed by sterilized sulphuric acid at pH 1.6. Washing and centrifugation were repeated three times before the cells were free from the precipitates. After that, the cells were re-suspended in the sterilized iron-free 9K medium with a concentration of 2×10^8 cell/mL.

2.3 Bioleaching experiments

Bioleaching experiments were carried out in 250 mL Erlenmeyer flasks. The iron-free 9K salt medium (135 mL) adjusted to the required pH was transferred into each flask. The flasks were then autoclaved at 1×10^5 Pa and 121 °C for 20 min. Following autoclaving, each flask was inoculated with 15 mL bacterial suspension, producing a final volume of slurry of approximately 150 mL. In all experiments, the initial bacterial population was 2×10^7 cell/mL. The flasks were shaken in rotary shakers for 30 d with a constant speed of 160 r/min and temperature of 53 °C. Ferric irons used in the experiments were added properly by analytical reagents of ferric sulfate.

During the bioleaching process, if the pH changed, it was adjusted to $\text{pH } 1.6 \pm 0.03$ with diluted sulfuric acid or 2 mol/L sodium hydroxide. 4 mL liquid sample was removed periodically from the flasks, and then used for the analysis of dissolved zinc content by atomic absorption spectrophotometry (AAS). The removed solutions were replaced with an equal volume of sterilized iron-free nutrient medium. The redox potentials were measured using a platinum electrode with reference to a saturated calomel electrode. The bioleaching residues were examined using X-ray diffraction (XRD) and scanning electron microscopy (SEM).

2.4 Electrochemical study

Electrochemical studies were conducted using a conventional three-electrode system electrochemical cell. The cell consists of a Ag, AgCl/KCl(saturated)-reference electrode with a salt bridge (capillary), two graphite counter electrodes, and a particularly made working electrode by the method in Refs. [12–14]. 1 g base conductor material (powdered graphite) was mixed with 1 g powdered marmatite sample and 0.5 mL paraffin oil. They were compressed under pressure using a carbon paste electrode holder.

The Tafel curves were tested with a sweep rate of 1 mV/s and applied potentials of ± 250 mV vs open circuit potential (OCP). The electrochemical impedance spectroscopy studies were carried out by applying a 5 mV amplitude sine-wave signal perturbation in the frequency range of 10^{-2} – 10^{-5} Hz under the open circuit potential and the impedance diagrams were shown in the form of Nyquist plots. Before all the measurements, the pH of the electrolyte was adjusted to 1.6. There was no further adjustment when Fe^{3+} ions were added into the electrolyte.

3 Results and discussion

3.1 Bioleaching of marmatite

3.1.1 Effect of particle size

The effect of particle size on the marmatite bioleaching was studied at a rotating speed of 160 r/min. It can be seen from Fig. 2(a) that the zinc dissolution increased with decreasing particle size to a certain size (0.043–0.074 mm), below which a reverse trend was noted. A similar situation was observed by OLUBAMBI et al [15], where particles less than 75 μm did not improve the bioleaching rates, but resulted in lower recoveries. This could be explained from the view point of the surface area of the mineral, which was available for microbial attack. By decreasing the particle size, the surface area per unit mass of the mineral increased, and therefore, improved mass transfer and enhanced

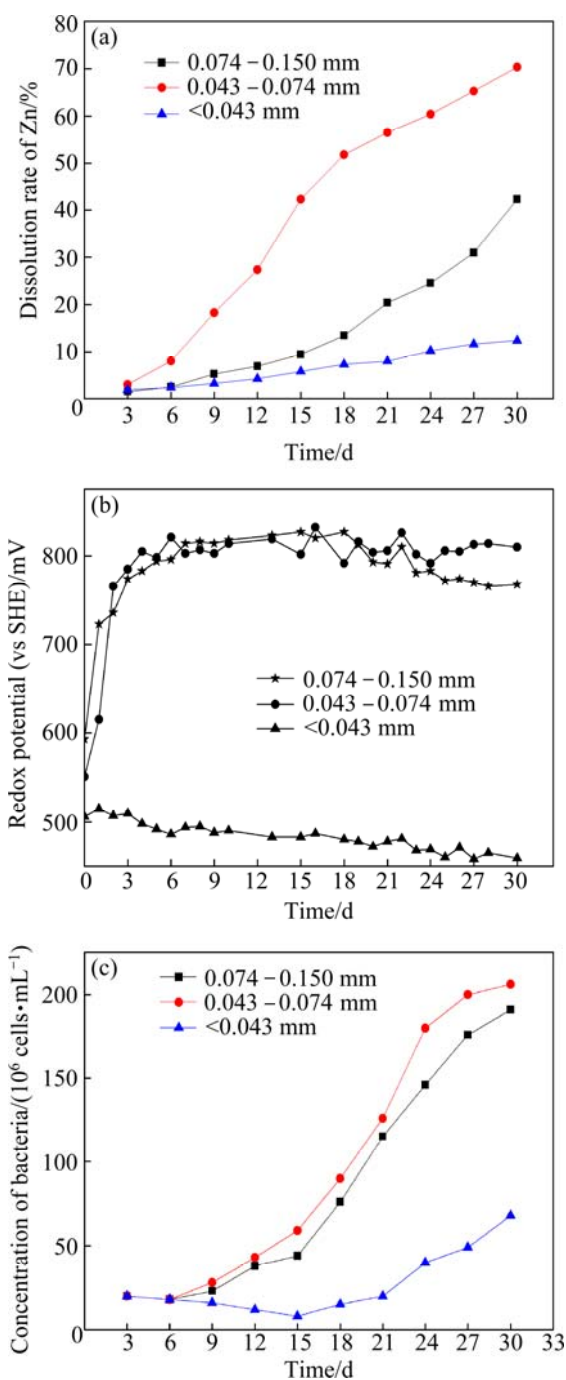


Fig. 2 Effect of particle size on zinc extraction by *S. thermosulfidooxidans* (3% (w/v) pulp density, 10% inoculums addition in volume fraction, and adjusting pH to 1.6 everyday)

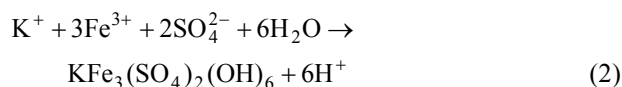
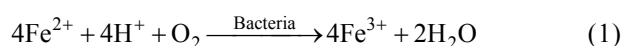
bioleaching rates are achieved [16]. Lower dissolution rate at particle size fraction of 0.074–0.150 mm could be attributed to the negative influence of larger particle size. Lower surface area at the larger particle size fraction could result in a decrease in the number of active microbial attachment sites and a decrease in cell viability [17]. Lower recovery at finer particle size fractions might be attributed to possible cell damage and deactivation [18], which could be caused by finer

particles arising from probable oxygen deficiency due to limiting air flow rate. At the same time, reduction of particle size below a critical level could increase the extent of the particle–particle collision and impose severe attrition which might disrupt the structure of the cells [15,16]. In a word, the larger particles and finer particles both had impeding effects on microbial–mineral activities and the growth of *S. thermosulfidooxidans*, which could be proved by Figs. 2(b) and (c). Therefore, the particle size fraction of 0.043–0.074 should be chosen as the optimum size for bioleaching experiments.

3.1.2 Effect of controlling pH

There usually was an optimal pH for the bacteria to keep them the best activity. But the pH changed during the leaching process due to the complex redox reactions in the system. In this work, the effect of controlling pH to the initial value of 1.6 at regular intervals on the dissolution of marmatite was examined. The experimental results are shown in Fig.3. It indicates that the zinc dissolution was higher when pH was adjusted to the initial value of 1.6 during the experiment process (Fig. 3(a)). This suggested that adjusting pH could promote the marmatite bioleaching by *S. thermosulfidooxidans*. The low zinc extraction could

be credited to the higher pH values (Fig. 3(c)) without controlling pH, which might make it difficult for the thriving of *S. thermosulfidooxidans* and the generation of ferric ions according to the Reaction (1). At the same time, higher pH values during the leaching process without controlling pH might accelerate the formation of jarosite (Reaction (2)). It can be seen from Figs. 3(b) and (d) redox potential and concentration of iron ions were both higher under the condition of controlling pH. The results were consistent with the tendency of the dissolution rate change of marmatite.



3.1.3 Effect of external addition of Fe^{3+} ions

The effects of external addition of Fe^{3+} ions on the marmatite bioleaching are shown in Fig. 4. It can be seen that the addition of Fe^{3+} ions accelerated the marmatite dissolution at the beginning of the leaching experiments, since the marmatite oxidation occurred by a chemical mechanism according to Reaction (3) and the rate of bacterial oxidation of ferrous ion was low in the initial

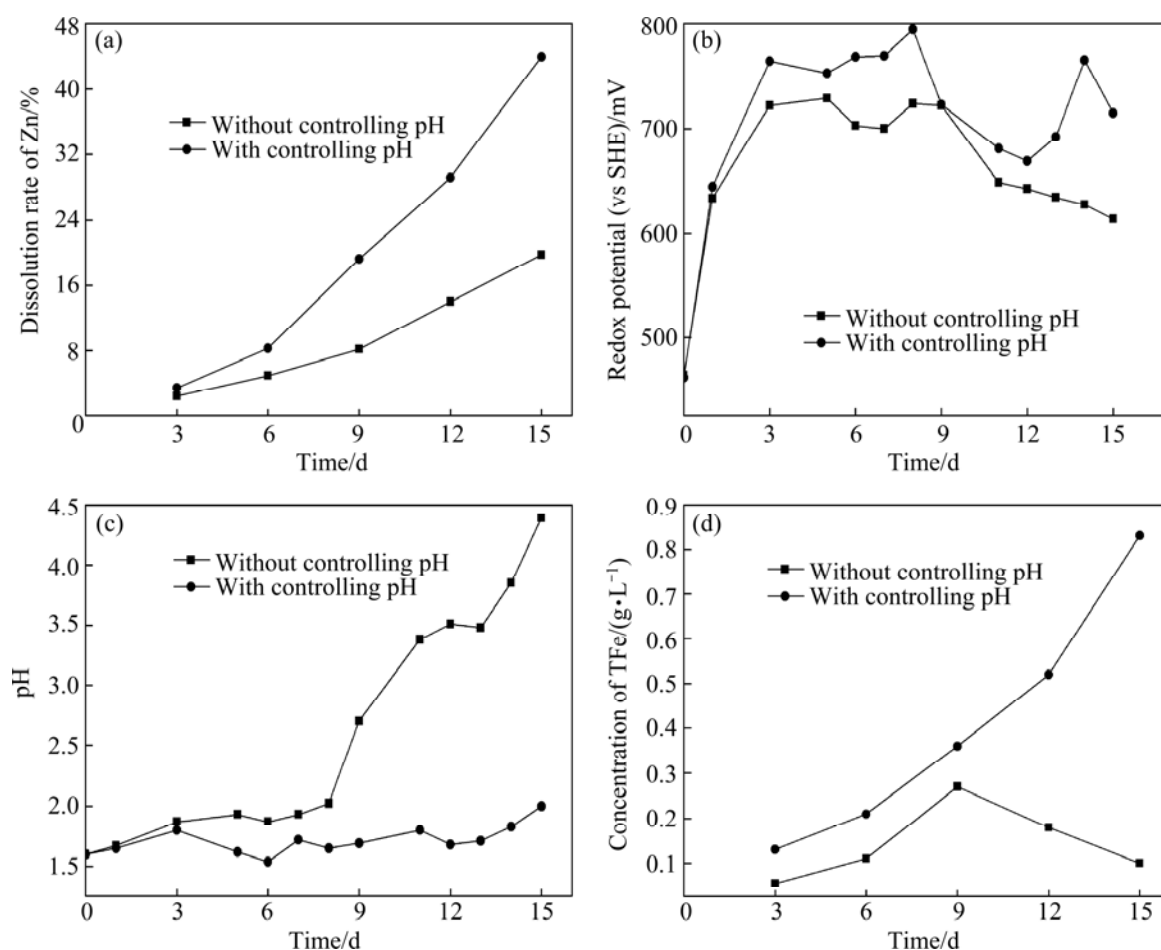


Fig. 3 Effect of controlling pH on zinc extraction by *S. thermosulfidooxidans* (3% (w/v) pulp density, 10% inoculums addition in volume fraction, and rotating speed 160 r/min): (a) Dissolution of Zn; (b) Redox potential; (c) pH; (d) Concentration of TFe

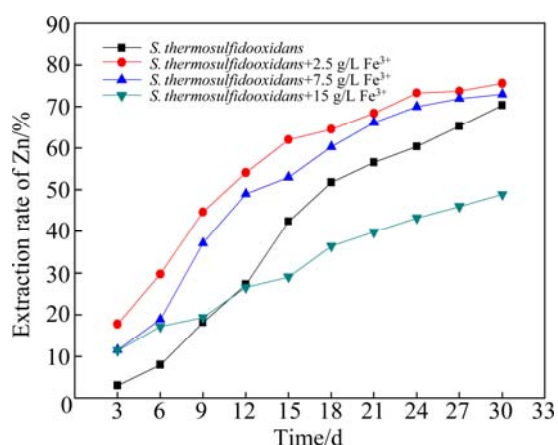
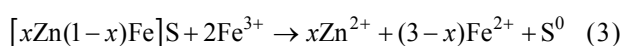


Fig. 4 Effect of external addition of Fe^{3+} ions on zinc extraction by *S. thermosulfidooxidans* (3% (w/v) pulp density, rotational speed 160 r/min, temperature 53 °C, 10% inoculums addition in volume fraction, and adjusting pH to 1.6 everyday)

stage of experiments [7,8]. In the first 3 d, the zinc extraction rate obtained in the experiments with 2.5 g/L Fe^{3+} was 17.8%, which was almost five times that achieved in the experiments without Fe^{3+} (3.0% zinc dissolution). When the supplementary addition of Fe^{3+} was up to 7.5 g/L, the promoting effect on zinc dissolution became weaker, but the leaching rate was still higher than that without additional Fe^{3+} ions. Whereas the inhibited effect was observed when additional concentration of Fe^{3+} was up to 15 g/L. This was because high Fe^{3+} concentrations might affect the activity of bacteria [19]. Another reason for the low zinc dissolution with high Fe^{3+} concentration might be attributed to the formation of precipitation (such as jarosite) according to Reaction (2), which occurred much more easily and earlier under the condition of high Fe^{3+} concentrations. The results indicated that the chemical oxidation of Fe^{3+} ions played an important role in the zinc extraction during the leaching process, especially in the initial stage when the cell density and bacterial oxidation ability of ferrous ions were low. While it was also suggested that too high concentration of additional Fe^{3+} ions in the leaching system could inhibit the zinc extraction.



3.1.4 SEM and XRD analyses of leaching residues

The SEM analysis results of bioleaching residues in different leaching solutions are shown in Fig. 5. It can be seen that the surfaces of the residue particles were wrapped by a reaction product layer, which inhibited the flow of microorganisms, nutrients and reaction products from mineral surface [4,10]. The layer on the residue obtained from the bioleaching with 2.5 g/L external additional Fe^{3+} ions was less porous than that without additional Fe^{3+} ions.

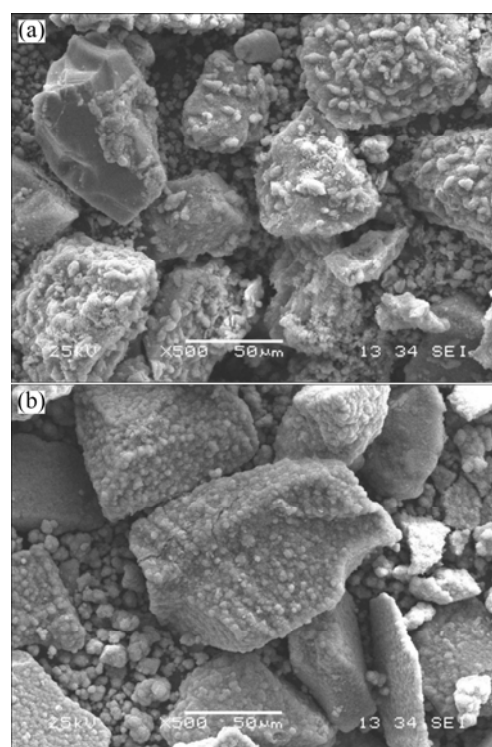


Fig. 5 SEM images of leaching residues: (a) Residue after 30 d of bioleaching; (b) Residue after 30 d of bioleaching with 2.5 g/L external additional Fe^{3+} ions

The components of leaching residues were analyzed further by X-ray diffraction (Fig. 6). In Fig. 6, the X-ray diffraction patterns of residues leached by original *S. thermosulfidooxidans* after 30 d show that jarosite and sulfur formed during the leaching process. The lower leaching rate might have a relation with the passivation layer formed on the particle surface, which could hinder the bioleaching of marmatite. It can also be seen that with additional 2.5 g/L Fe^{3+} ions the intensity of peaks corresponding to jarosite was stronger than that without additional Fe^{3+} ions. This suggested that there were more jarosites produced on the surface of particle and then inhibited the dissolution of marmatite.

3.2 Electrochemical analysis

The Tafel polarization curves of the marmatite-carbon paste electrode with various concentrations of Fe^{3+} ions are described in Fig. 7. From the Tafel polarization curves, the electrochemical corrosion kinetic parameters can be obtained, which are listed in Table 2. It can be seen that the corrosion potential (ϕ_{corr}) had a significant increase with the addition of Fe^{3+} ions. The result was consistent with the tendency of open circuit potential (OCP) change. The open circuit potentials were 347, 567 and 612 mV for the Fe^{3+} concentrations of 0, 1.25 and 2.5 g/L, respectively. The J_{corr} in the electrolyte with Fe^{3+} ions was much higher than that without

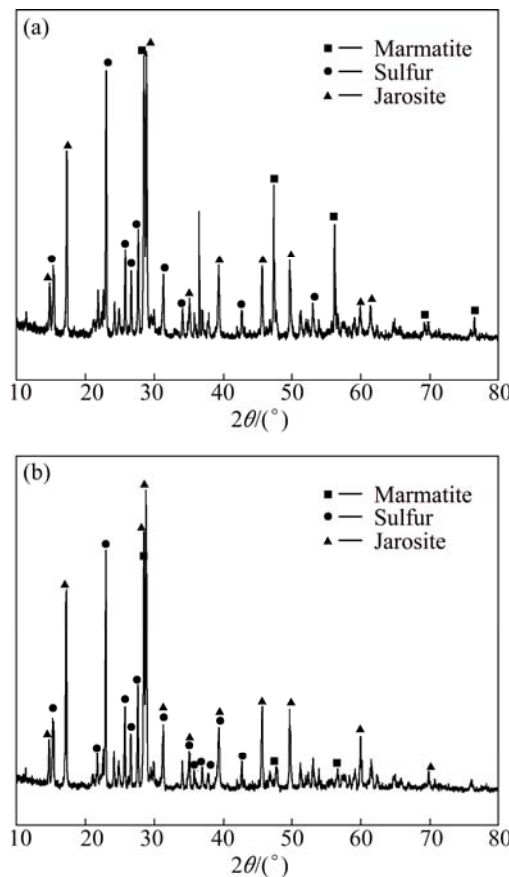


Fig. 6 XRD patterns of leaching residues: (a) Residue after 30 d of bioleaching; (b) Residue after 30 d of bioleaching with 2.5 g/L external additional Fe^{3+} ions

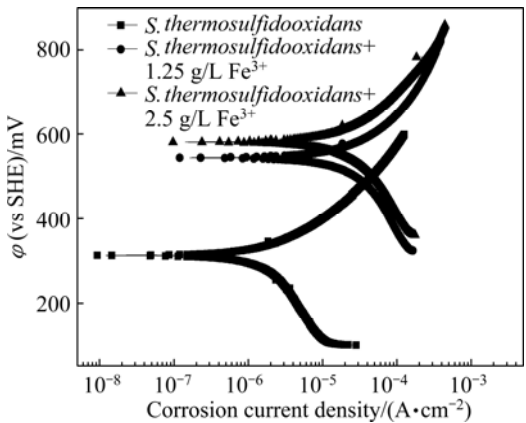


Fig. 7 Effects of Fe^{3+} ions on Tafel curves of marmatite-carbon paste electrode in the presence of pure original strain of *S. thermosulfidooxidans* at 53 °C and scan rate of 1 mV/s with sweep range of ± 250 mV (vs OCP)

additional Fe^{3+} ions. The higher corrosion current density suggested that the dissolution rate of marmatite was higher during the experiments. In other words, the external addition of Fe^{3+} ions facilitated the extraction of marmatite [20,21]. Under the condition of 2.5 g/L additional Fe^{3+} ions the corrosion current density was $41.00 \mu\text{A}/\text{cm}^2$ and declined slightly when compared with that under the condition of 1.25 g/L additional Fe^{3+} ions. This is because the higher the concentration of additional Fe^{3+} ions was, the earlier the passivation phenomenon appeared during the leaching process (Fig. 7).

Figure 8 illustrates the electrochemical impedance

Table 2 Tafel parameters of marmatite-carbon paste electrode with different concentrations of Fe^{3+} ions in acid solution with *S. thermosulfidooxidans*

Condition	$B_a/(\text{mV} \cdot \text{decade}^{-1})$	$B_c/(\text{mV} \cdot \text{decade}^{-1})$	$\phi_{\text{corr}}(\text{vs SHE})/\text{mV}$	$J_{\text{corr}}/(\mu\text{A} \cdot \text{cm}^{-2})$
<i>S. thermosulfidooxidans</i>	180.0	992.5	311.7	4.290
<i>S. thermosulfidooxidans</i> + 1.25g/L Fe^{3+}	342.6	878.4	543.7	76.16
<i>S. thermosulfidooxidans</i> + 2.5g/L Fe^{3+}	252.6	382.7	580.4	41.00

B_a : Anodic Tafel slope; B_c : Cathodic Tafel slope; ϕ_{corr} : Corrosion potential; J_{corr} : Corrosion current density

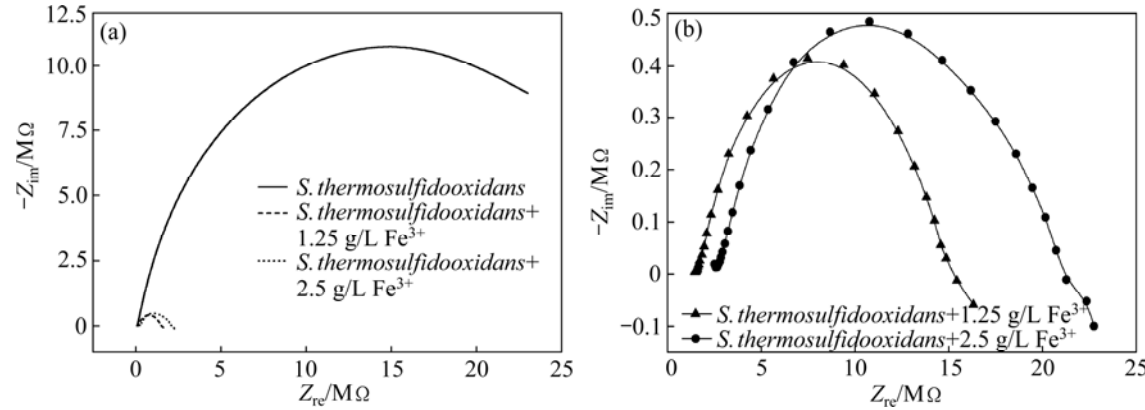


Fig. 8 Electrochemical impedance spectroscopy (EIS) of marmatite-carbon paste electrode in the presence of *S. thermosulfidooxidans* at 53 °C with different concentrations of additional Fe^{3+} ions under open circuit potential

spectroscopy (EIS) of the marmatite–carbon paste electrode with different concentrations of additional Fe^{3+} ions under the open circuit potential. From Fig. 8, it can be seen a large capacitance loop in the tested frequency scope when the EIS measurement was carried out in the electrolyte without additional Fe^{3+} ions in the presence of *S. thermosulfidooxidans*, which suggested that the oxidation reaction of the marmatite was controlled by the charge transfer [21–24]. Compared with Fig. 8(a), Fig. 8(b) shows an obvious change in the EIS spectra in the system with additional Fe^{3+} ions. The loops were dramatically depressed with the addition of Fe^{3+} ions, which implied the improving function of Fe^{3+} ions on the dissolution of marmatite. But the shapes of electrochemical impedance spectroscopy were similar. These results illustrated that the rate-limiting step was not changed.

4 Conclusions

1) Controlling pH had a positive effect on marmatite leaching by *S. thermosulfidooxidans* and the best particle size for bioleaching experiments was 0.043–0.074 mm. External addition of Fe^{3+} could promote zinc extraction, while the positive effect might decline when the concentration of additional Fe^{3+} ions was too high. Fe^{3+} ions played an important role in the dissolution of marmatite, especially in the initial stage when the cell density and bacterial oxidation ability of ferrous irons were low.

2) The SEM and XRD analyses showed that there was a layer wrapping on the surface of residues, which was composed of sulfur and jarosite formed in the process of bioleaching by *S. thermosulfidooxidans*. With additional 2.5 g/L Fe^{3+} ions more jarosites were produced than that without additional Fe^{3+} ions.

3) The electrochemical behaviors of the marmatite–carbon paste electrode showed that external addition of Fe^{3+} ions increased the corrosion current density, which meant that the dissolution of marmatite became rapid, accordingly promoted the dissolution of marmatite. The EIS spectra indicated that the rate limiting step was not changed when Fe^{3+} ions were added. The dissolution of marmatite was still controlled by the charge transfer process.

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铁闪锌矿的 *Sulfobacillus thermosulfidooxidans* 菌 浸出及电化学性能

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摘 要: 采用纯种 *Sulfobacillus thermosulfidooxidans* 菌进行铁闪锌矿的生物浸出及电化学实验, 研究颗粒大小、pH 值控制 and 外加 Fe³⁺ 离子对锌浸出的影响。结果表明: 在生物浸出过程中铁闪锌矿生物浸出的最佳粒度范围为 0.043~0.074 mm; 定期调整 pH 值至初始值对获得较高的浸出率有重要影响; 外加 Fe³⁺ 离子能加速铁闪锌矿的生物浸出, 但当外加 Fe³⁺ 离子浓度超过 2.5 g/L 时, 促进作用变弱, 甚至阻碍铁闪锌矿的溶解。SEM 和 XRD 分析浸渣发现, 在矿物表面形成一层由单质硫和黄钾铁矾组成的产物层, 并导致后期的浸出速度低。电化学测试实验结果表明, 外加 Fe³⁺ 离子可以增加腐蚀电流密度, 有利于锌的提取。交流阻抗谱表明, 添加 Fe³⁺ 离子后没有改变反应过程的控制步骤。

关键词: 铁闪锌矿; *S. thermosulfidooxidans* 菌; 生物浸出; 黄钾铁矾; 电化学性能

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