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Bioleaching of marmatite flotation concentrate by *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* ①

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Abstract: Bioleaching of marmatite flotation concentrate by *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* was investigated at 35 ℃, the initial pH value of 2.0 on an orbital shaker with 160 min^{-1} over a period of 10 days. Experimental results indicate that the adapted strains increase markedly the dissolution rate and the leaching ratio of marmatite. Pulp density also affects the bioleaching of marmatite. Massive elemental sulfur and jarosite form during the leaching process in the systems inoculating the adapted strains in pure and mixed cultures; and acid product is enhanced, which decreases the pH below to 2.0 in latter leaching period. Marmatite preferentially dissolves during the bacterial leaching of complex sulfides. Compared with the pure cultures of original and adapted strains, the adapted strains of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* in mixed cultures are more efficient in the oxidation of marmatite.

Key words: marmatite; bioleaching; *Acidithiobacillus ferrooxidans*; *Leptospirillum ferrooxidans*
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1 INTRODUCTION

The bacterial dissolution of metal sulfide was called as bioleaching. The biooxidation of metal sulfides to form soluble metal sulfates, elemental sulfur and sulfuric acid, was affected by some special bacteria. Some of mesoacidophilic chemolithetrophic bacteria, such as *Acidithiobacillus ferrooxidans* (*A.f.*), can oxidize sulfur compound to sulfate and ferrous to ferric ions; on the other hand, some of bacteria, such as *Leptospirillum ferrooxidans* (*L.f.*), can oxidize only ferrous ions[1, 2]. It was reported that a considerable accumulation of elemental sulfur occurred in the case of pyrite bioleaching by Leptospirillum bacteria, while only slight amounts of elemental sulfur were detectable in the case of bioleaching by *Thiobacillus ferrooxidans* [3]. The association of Leptospirillum with sulfur-oxidizing bacteria could provide a more rapid and complete oxidation of pyrite than the pure culture of the same strains[4, 5].

Bioleaching of zinc sulfide was investigated by some researchers[6-8] due to its lower redox potential and rapid oxidation rate, but its commercial acceptance still remains restricted due to poor leaching kinetics compared with other alternative techniques. Moreover, these results were obtained from the bioleaching of zinc sulfides (contained iron< 10%) in pure culture. The leaching effects of marmatite flotation concentrate by *A.f.* and *L.f.* in pure and mixed cultures were studied in this work. The mineral composition and the surface erosion of the residue samples were analyzed by X-ray diffraction (XRD) and scanning electron microscope (SEM), respectively. The cell density, pH and E_h in the liquid phase were measured as the important factors affecting the leaching process.

2 EXPERIMENTAL

2.1 Mineral

The marmatite flotation concentrate used in the experiments was provided by a Lead-zinc Mine in Yunnan Province of China. The concentrate sample contained 40.61% Zn, 15.81% Fe and 31.66% S. The main minerals in the sample were marmatite and some pyrite. Before preleaching, the sample was ground for 15 min. Its particle size was < 35.5 μm (over 90%).

2.2 Microorganisms

The mesoacidophilic strains used in this work were *A.f.*, provided by Institute of Microbiology, Chinese Academy of Science, and *L.f.* obtained from the German Collection of Microorganisms and Cell Cultures. The original strains were cultured in Leathen medium[9] (with 10% Fe^{2+}) and were adapted to the medium containing the marmatite flotation concentrate (50 g/L) as the sole energy

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source. The culture used in these experiments was adapted to the marmatite sample through continuously subcultured over a year.

2.3 Experimental methods

In 250 mL Erlenmeyer flasks, the amount of marmatite sample required by experiments was added into 90 mL Leathen medium (without iron) and preleached statically under the pH value of around 2.0 at the room temperature for 3 d. These flasks were inoculated with the inoculum of a five-day-old culture: the original and adapted strains of 10 mL of *A.f.*, the original and adapted strains of 10 mL of *L.f.*, and the adapted strains of 5 mL of *A.f.* plus 5 mL of *L.f.*, respectively, in a batch experiment. The experiments were carried out on an orbital shaker with 160 min^{-1}. The initial bacterial densities were about 2.0×10^7 cell·mL^{-1} in the all inoculated systems. In each set of experiment, uninoculated controls were also run, in which 1 mL formaldehyde (AR) was added as bactericide.

2.4 Analysis methods

The concentration of Zn^{2+} in the leaching solution was measured by EDTA titration. The pH value and redox potential E_h value in the leaching solution were measured with a pH meter and a Pt electrode in reference to a saturated calomel electrode, respectively. The leached residues of marmatite were periodically collected from flasks and filtered, air dried and then analyzed by XRD and SEM. The samples were scattered and mounted on sheet glass, then coated with gold before examination with a scanning electrode microscopy operating at 10 kV or 15 kV.

3 RESULTS AND DISCUSSION

3.1 Leached Zn in process

Fig. 1 shows the leached Zn of marmatite flotation concentrate by the original and adapted strains of *A.f.* and *L.f.* in pure and mixed cultures, and the sterile control at 35 ℃ and the pH of about 2.0 over a period of 10 d. It is found that the adapted strains of *A.f.* and *L.f.* increases markedly the dissolution rate and the leaching rate of marmatite. Moreover, the association of *A.f.* with *L.f.* can provide a more rapid and complete oxidation of marmatite than the pure cultures of the same strains. The concentrations of Zn^{2+} in the leaching systems inoculating microorganisms are higher than that in the uninoculated system after leaching for 10 d, especially in the systems inoculating adapted strains. It means that the bacterial strains play a key role in the dissolution of marmatite during the leaching process. It is also found from Fig. 1 that the concentration of Zn^{2+} in the presence of the original or adapted strains of *L.f.* is slightly higher compared correspondingly with that inoculating the original or adapts strains of *A.f.*. The leaching rate of marmatite by the adapted strains of *A.f.* and *L.f.* is rather low in the initial leaching period, which increases after an induction period about 1 – 2 d. To explain the greater efficiency of mixed inocula, it was considered that the different species had different affinities for the energetic substrates provided by sulfide minerals (sulfide, sulfur and ferrous iron)[5]. However, the interactions between *A.f.* and *L.f.* were not shown during the leaching experiment and still need to examine further.

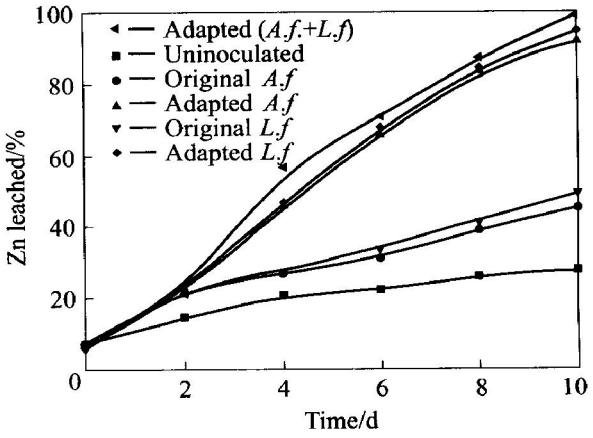

Fig. 1 Leaching curves of marmatite flotation concentrate under sterile controls, original strains and adapted strains of *A.f.* and *L.f.* in pure and mixed cultures

3.2 Evolutions of pH, E_h and cell density during leaching process

During the leaching process, the pH value was measured and then adjusted to around 2.0 (the initial pH value) by adding 3.0 mol·L^{-1} H_2SO_4 solution at the same time intervals in all experiments. The changes of pH during the leaching process in different systems are shown in Fig. 2(a). It is found that the bacteria oxidation of marmatite flotation concentrate is an acid-consuming reaction. The values of pH in the leaching solution changes in terms of the same trend in all systems inoculating adapted strains. Moreover, the changes of pH in the presence of adapted strains of *A.f.* and *L.f.* in pure and mixed cultures are more markedly than that in the presence of original strains, and so need to add more 3.0 mol·L^{-1} H_2SO_4 solution to maintain the pH around 2.0 in the previous leaching period. While the change of pH in the solution decreases with time and the addition of 3.0 mol·L^{-1} H_2SO_4 solution decreases in the latter leaching period. The pH values are near and even less than 2.0 at the end of leaching experiment in the systems inoculating adapted strains. It is the reason that

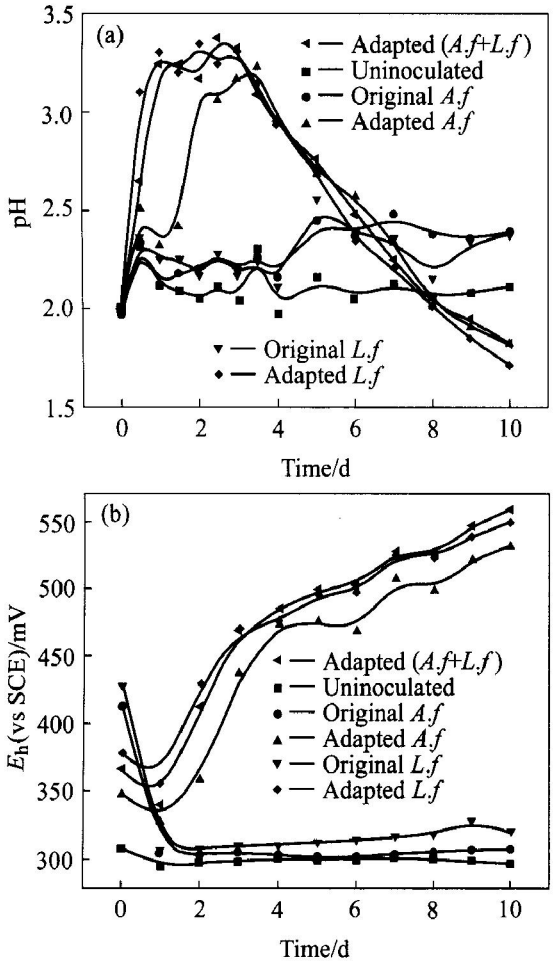

Fig. 2 Evolutions of pH (a), E_h(b) in solution during leaching process under sterile control, original and adapted strains of *A.f.* and *L.f.* in pure and mixed cultures

a mass of jarosite and H^+ form by the reaction (1) due to the higher concentration of Fe^{3+} as follows:

$$M^+ + 3Fe^{3+} + 2SO_4^{2-} + 6H_2O \rightarrow MFe_3(SO_4)_2(OH)_6 + 6H^+ \quad (1)$$

where $M^+ = K^+$, NH_4^+ and H_3O^+. The changes of pH values are smaller in the systems inoculating the original strains, but its changes increase in the latter leaching period due to the adaptation of original stains. While the pH value of the uninoculated system almost remains constant during the experiment, except for the first day of the experiments when it increases due to the dissolution of alkaline ingredients in ore sample. In the latter leaching period, the increase of pH might result from the contamination of microorganisms.

Except for the sterile controls, the redox potential E_h values of the leaching solution are higher at first due to the higher concentration of Fe^{3+} added by the inoculum and decreases due to the depletion of Fe^{3+} (Fig. 2(b)). The values of E_h increase with time during the leaching process in the system inoculating adapted strains of *A.f.* and *L.f.* in pure and mixed cultures after an induction period around 1 – 2 d. It suggests that the adapted strains oxidize ferrous ions to ferric ions and maintain the higher concentration of Fe^{3+} in the solution during the leaching process. The difference of E_h between pure and mixed cultures is very small, especially in the presence of *L.f.* strains. Except for the higher initial values, the E_h values are small in the system inoculating original strains, in which the E_h value in the presence of *L.f.* is higher slightly than the one in the presence of *A.f.* strains. It suggests that *L.f.* has a higher affinity to ferrous iron as compared with *A.f.*, as reported in the literature[10]. The uninoculated tests show the higher initial potential values due to the presence of a little amount of Fe^{3+} in the solution, while the potential remains very low and almost constant, about 300 mV(vs SCE), throughout the sterile experiment, suggesting that few change take place in the ore.

For some of sulfide minerals. such as zinc sulfide and pyrite, the dissolution results mainly from the indirect mechanism, ie the chemical oxidation of Fe^{3+}, during the leaching process[11, 12]. As the E_h value in leaching systems depends mainly on the ratio of $\rho(Fe^{3+})/\rho(Fe^{2+})$, the fact that there is higher E_h value in the mixed cultures can make marmatite oxidized easier by *A.f.* and *L.f.* in the mixed cultures. It is consistent with the actual dissolution rate of marmatite in the mixed cultures.

The cell density also increases in terms of the same trend in the systems inoculating adapted strains during the leaching process. It reaches the maximal value, about 4.0×10^9 cell·mL^{-1}, about at the 5th day after inoculating microorganisms, and almost remains the value along the leaching experiment. But in the system inoculating original strains, the cell density keeps lower during the leaching process, only increases a little in the latter leaching period. It is obvious that the activity of original strains is lower compared with that of the adapted strains.

3.3 Effects of pulp density on marmatite leaching

Under the experimental conditions mentioned above, the leaching effect of marmatite flotation concentrate by the adapted strains of *A.f.* and *L.f.* in a pure culture was examined when the pulp densities were 5%, 10%, 15% and 20%, respectively. The experimental results(Fig. 3) show that pulp density can affect the leaching rate of marmatite. The changing trend of the leaching rate is similar with the increase of pulp density under inoculating the different

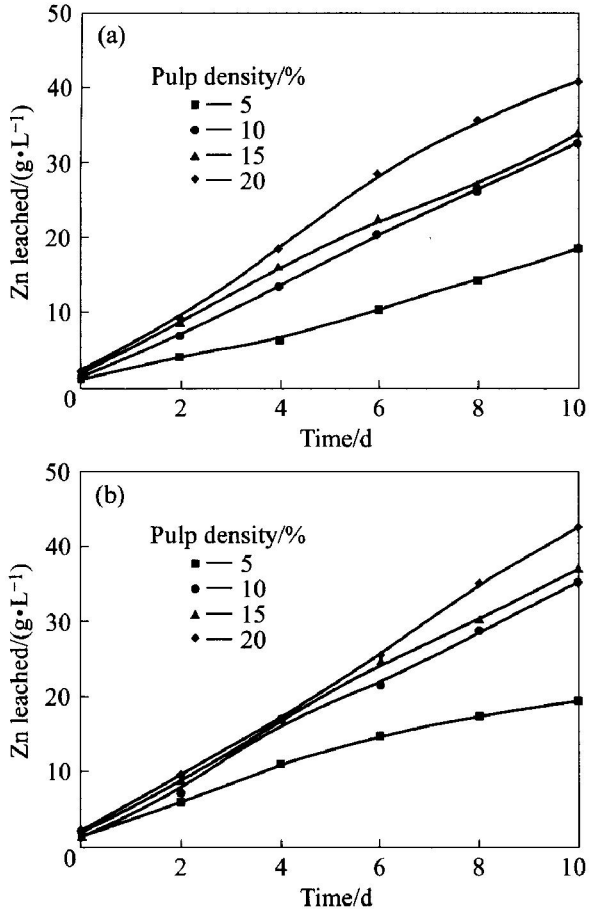

Fig. 3 Leaching curves in different pulp densities (a) —*A.f.*; (b) —*L.f.*

strains. The leaching rate reaches the highest when the pulp density is 5%, in which the concentration of Zn^{2+} in the solution is around 20 g·L^{-1} after 10 d of bioleaching. The leaching rate decreases with the increase of pulp density. When the pulp density is 20%, the concentration of Zn^{2+} is around 40 g·L^{-1} after leaching for 10 d. While the pulp density is 10%, the concentration of Zn^{2+} is close to that of the pulp density of 15%, in which the concentration of Zn^{2+} is around 32~36 g·L^{-1}. The results indicate that the leaching rate of Zn from marmatite flotation concentrate decreases, but the concentration of Zn^{2+} in the leaching solution increases in a unit time with the increase of pulp density.

The reason that pulp density affected the bioleaching of marmatite flotation concentrate is the increase of sharing force with the increase of pulp density, which leads to the decrease of biological activity of the bacterial strains. On the other hand, the alkaline ingredients in marmatite flotation concentrate increase with the increase of pulp density, which leads the changing amplitude of pH value to increase, thus affects the growth and activity of leaching strains further.

Under different pulp densities, the changing amplitude of redox potential E_h decreases with the increase of pulp density, in which the E_h keeps at lower value during the leaching process when the pulp density is 20%. The E_h value is related closely to the proportion of $\rho(Fe^{3+})/\rho(Fe^{2+})$, and chemical oxidation of ferric ions plays a key role in the dissolution of marmatite. The leaching rate is higher when the E_h value is higher. It is shown that the dissolution of marmatite during the leaching process is affected by the indirect action, ie the chemical oxidation of Fe^{3+}. It is also noted that the cell densities are not markedly different among the different pulp densities. To a certain extent, the increase of nutrition substrate, ie pulp density is favorable to the growth of bacterial strains.

3.4 SEM images of residues under differently leaching conditions

SEM has been used to investigate the attachment of bacteria to mineral surface[13, 14]. In this work the formation of corrosion pit was observed in mineral surface, which correlated with bacterial activity[15]. Typical SEM images of the leached residues in the systems inoculating adapted strains are shown in Fig. 4. There are no much changes on marmatite surfaces leached by mixed cultures and pure strains of *L.f.* after leaching for 5 d, while the corrosion pits on the mineral surface are observed clearly in the presence of *A.f.* strains. A product layer on the surface of mineral substrate is found in the presence of pure strains of both *A.f.* and *L.f.* in latter leaching period as shown in Fig. 4(a′) and 4(b′), but it isn't observed in the mixed cultures. The corresponding EDX analyses show that the product layer also contains the leaching products, such as S^0 and jarosite, in addition of extracellular polymeric substance released by inoculated microorganisms. It was reported that leaching bacteria attached to the particle of mineral or sulfur by means of extracellular polymeric substance[16]. It was also reported that the extracellular polymeric substance, such as lipopolysaccharides, had come into the substrate surface inhibited the attached of cells to minerals and negatively affected the bioleaching[17]. At the same time, the reaction products, such as elemental sulfur and the precipitate of iron compound, which covered on the surface of mineral substrate, also negatively affected the dissolution of marmatite. It might be one of the reasons that the dissolution rate of marmtite in the mixed cultures, in which no product layer formed on the substrate surface, was higher compared with any one inoculating the adapted strains in pure cultures.

3.5 XRD analyses of residues under differently leaching conditions

The composition of leached residue in the systems inoculating adapted strains was analyzed by

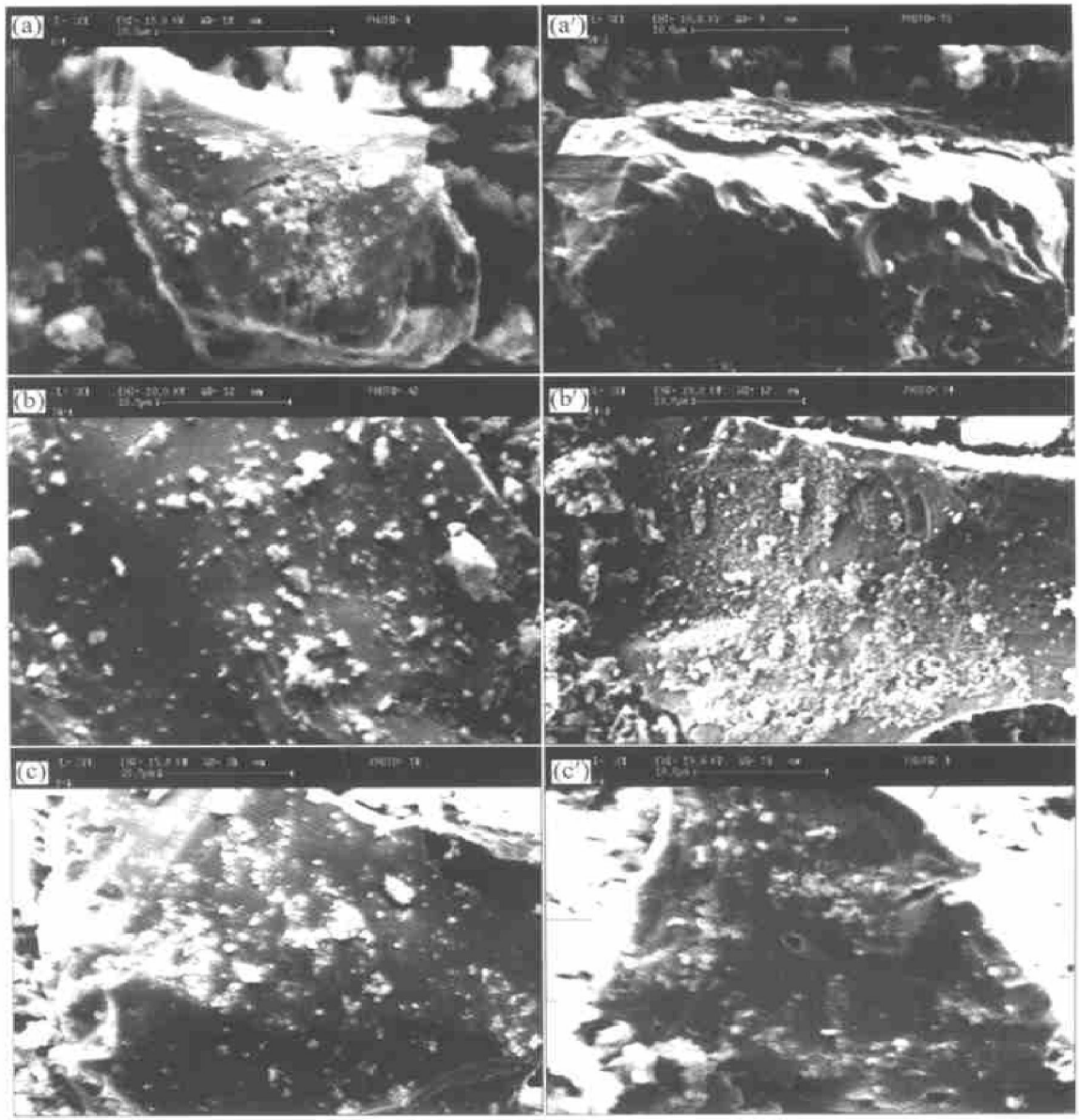

Fig. 4 Typical SEM images of leach residues of marmatite flotation concentrate in systems inoculating adapted strains

(a) —After 5 d interaction with *A.f.*; (a′) —After 10 d interaction with *A.f.*;
(b) —After 5 d interaction with *L.f.*; (b′) —After 10 d interaction with *L.f.*;
(c) —After 5 d interaction with *A.f.* + *L.f.*; (c′) —After 10 d interaction with *A.f.* + *L.f.*

X-ray diffraction. XRD patterns of the leached residue at the dif ferent leaching periods in pure and mixed cultures are shown in Fig. 5. It is found that the mineral compositions of leached residue are almost similar, but the contents of the various components in the leached residue are different. Compared with the systems inoculating the mixed cultures and in the presence of *L.f.*, the contents of elemental sulfur are lower in the case of inoculating the adapted strains of *A.f.* after leaching for 10 d due to the reason that *A.f.* are able to oxidize elemental sulfur into sulfur compounds, but *L.f.* are only able to oxidize ferrous ions. The fact that the content of pyrite, as the impurityin the mineral sample, increases with the leaching time, indicating that its dissolution rate in the leaching process is lower than marmatite. Marmatite is essentially depleted from the residues after 10 d. XRD analysis also shows that a mass of jarosite form during the bioleaching process in pure and mixed cultures, especially in the presence of Leptospirillum bacteria.

The results of energy dispersive X-ray (EDX) analysis of the leached residues of marmatite inoculating the adapted strains in mixed cultures during the different leaching periods is shown in Fig. 6, in which the contents of Zn, S and Fe contain in mineral sample and residues after leaching for 5 or 10 d are listed in Table 1. Combined with XRD analysis, it suggests that the leached residues are composed mainly of the

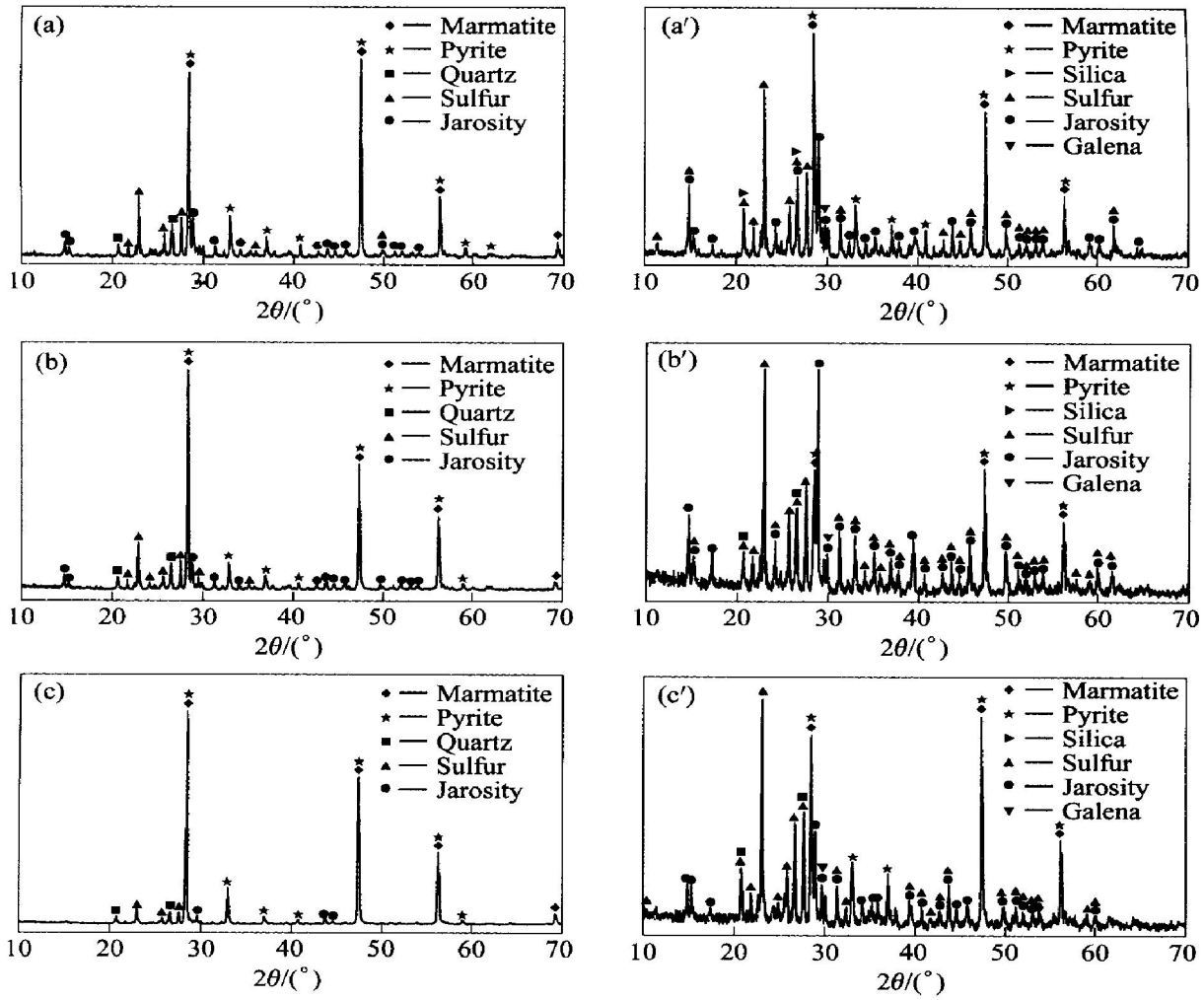

Fig. 5 XRD patterns of leached residue of marmatite flotation concentrate in systems inoculating adapted strains

(a) —After 5 d interaction with *A.f.*; (a′) —After 10 d interaction with *A.f.*;
(b) —After 5 d interaction with *L.f.*; (b′) —After 10 d interaction with *L.f.*;
(c) —After 5 d interaction with *A.f.* + *L.f.*; (c′) —After 10 d interaction with *A.f.* + *L.f.*

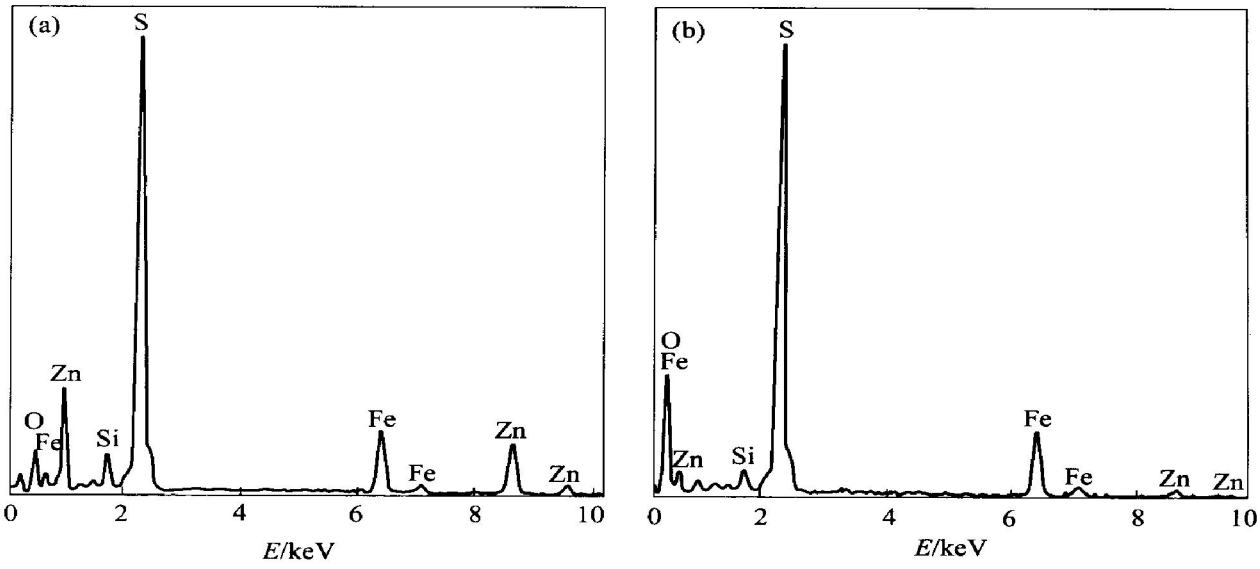

Fig. 6 EDX patterns of leached residue of marmatite inoculating adapted strains in mixed cultures after different leaching periods

(a) —5 d; (b) —10 d

reaction products, which include elemental sulfur and the precipitate of iron compound, and mineral impurities undissolved. The content of Zn decreases with the time, and only a little of marmatite undissolved contain in the residue after leaching for 10 d. According to its lower redox potential value in the sulfide series[18], zinc sulfide preferentially dissolves as anode in oxidation reactions during the bacterial leaching of complex sulfides.

Table 1 Contents of three main elements in concentrate and residues (mass fraction, %)

Contents	Zn	S	Fe
Concentrate	42.93	24.90	14.89
Residue(5 d)	6.54	41.75	15.96
Residue(10 d)	3.62	33.67	26.74

4 CONCLUSIONS

The experimental results show that the adapted strains of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* increase markedly the dissolution rate and the leaching rate of marmatite. The association of the different adapted strains provides a more rapid and complete oxidation of marmatite than the pure cultures of the same strains. The pulp density also affect the bioleaching of marmatite by adapted strains of *A.f.* and *L.f.*. The increase of pulp density decreases the leaching rate of Zn from marmatite, but the increase of total concentration of Zn^{2+} in the leaching solution in a unit time.

Massive elemental sulfur and jarosite form during the leaching process in the systems inoculating the adapted strains in pure and mixed cultures; the acid product is enhanced in the latter leaching period, which decreases the pH value to below 2.0. In the systems inoculating the adapted strains of *A.f.* and *L.f.* in mixed cultures, no product layer forms and more elemental sulfur contained in the leached residue. The content of Zn contained in minerals decreases with the increase of leaching time, and only a little marmatite undissolved contains in the residue after leaching for 10 d. Marmatite preferentially dissolves during the bacterial leaching of complex sulfides.

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