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Elective culture of bacteria used in bioleaching on pyrrhotite①

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Abstract: Elective culture of bacteria on pyrrhotite was researched, and the selected bacteria were tested on bioleaching of marmatite and zinc sulfide ore. The results show that the microorganism cultured on pyrrhotite with various S/Fe ratios is a mixed culture of *thiobacillus ferrooxidans* and *thiobacillus thiooxidans*, of which the integral activity and the oxidation capability of Fe^{2+} and S are enhanced. With the high Fe and low S content of pyrrhotite, the oxidation capacity of ferrous ion is improved; on the contrary, the oxidation capacity of sulfur is advanced. The bioleaching capacity of bacteria cultured on marmatite is better than that of the bacteria cultivated by conventional methods.

Key words: pyrrhotite; elective culture; bioleaching; marmatite
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1 INTRODUCTION

Biohydrometallurgy is characterized by low cost, short flowsheet and low contamination. With the escalating depletion of mineral source and the strengthened mind of environmental conservation, biohydrometallurgy presents more advantages over the conventional metallurgy methods in treating low-grade and complicated ore.

Bioleaching has been widely used in the commercial extraction of uranium, copper, gold from ore, and it is being exploited in the extraction of other cheap metals and rare noble metals such as zinc, cobalt, nickel, molybdenum, gallium, germanium etc. In some cases it has been in the phase of pilot scale experiment[1].

However, the slow leaching rate is the bottleneck of bioleaching, which limits bioleaching to only treating the ore abandoned by traditional processing methods. Hence, to enhance the leaching rate, many studies have been done at the aspects of microbiology, electrochemistry, metallurgy etc. One of the solutions proposed by researchers is to cultivate effective microorganism used in bioleaching[2].

There have been more than 20 strains of microorganism used on bioleaching of sulfide ore in which the most familiar and widely used are *thiobacillus ferrooxidans*, *thiobacillus thiooxidans* and *thermoacido philic achaebacterial*. In natural environment, the microorganism with the capacity to dissolve mineral is a mixed culture of different bacterial strains. Cultivated with the conventional methods of domestication, mutation, crossbreed, cell syncretizing and gene engineering, some capabilities of the microorganism such as the activity, the tolerance of heavy metal ions and the adaptability to sulfide ore were improved in some extent. However, the microorganism derived from the above methods usually is a single strain, and the improved capability shown in bench scale test may degenerate in industrial cases, so its effect on bioleaching is limited.

The bacteria inhabiting sulfide deposit can use sulfide mineral as energy source by dissolving mineral and oxidizing ferrous ion and elemental sulfur. According to literature reports, the role of bacterial in bioleaching includes the direct attack on minerals, the oxidation of Fe^{2+} and S. Therefore, we suppose to cultivate bacteria with an appropriate mineral as selective culture, which can enrich strains of microorganism and improve their integral capability on bioleaching of the similar mineral.

Pyrite(FeS_2) and pyrrhotite($Fe_{1-x}S$) are the familiar components of sulfide deposit, of which Fe and S are the essential element to cultivate bacteria, so both are the perfect substrate for selective culture of bacteria used in bioleaching. Considering that pyrrhotite is easier to be dissolved than pyrite, and has various ratios of Fe/S, experiments were carried out to explore the elective culture of bacteria with pyrrhotite as the substrate.

2 MATERIALS AND METHODS

2.1 Pyrrhotite

The chemical components of pyrrhotite are not fixed. The iron atom of the crystal is imperfect. In addition to Fe and S, a small quantity of Ni, Co, Mn and Cu can re-

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place Fe, so the structural formula could be illustrated as $Fe_{1-x}S$ (where $x = 0 - 0.223$). While $x = 0$, the iron atom is perfect in the crystal, for the only example of troilite. The reason for the imperfect crystal of pyrrhotite is commonly considered to be that part of Fe^{2+} is oxidized to form $Fe^{2+}_{1-x}Fe^{3+}_{2/3x}S$, where $2/3x$ turns into Fe^{3+} and $1/3x$ is empty, so the charge is not balanceable[3, 4].

With the increasing ratio of S/Fe, the magnetism of pyrrhotite increases. While the value of S/Fe is about 0.647, a change of crystal structure from hexagonal syngony to monocline syngony with worse symmetry happens because of the vacant site of atom in pyrrhotite, following the magnetism of pyrrhotite changing from paramagnetism to ferromagnetism. Therefore pyrrhotite can be separated into various parts with corresponding S/Fe ratios by magnetic separation.

Table 1 shows the results of magnetic separation of a pyrrhotite collected from Dachang Mine, Guangxi province, China. The sample was ground to powder with a particle size of less than 180 μm. Sample 1# − 4# was the concentration with the magnetic intensity of 0.05, 0.1, 0.2 and 0.5 T respectively, and sample 5# is the tailing with the magnetic intensity of 0.5 T. It can be seen from Table 1 that the five samples obtained from magnetic separation have remarkably various S/Fe ratios. Because of containing impurities and different particle sizes, the S/Fe ratio was not so consistent with the proper magnetism of pyrrhotite theoretically.

Table 1 Samples with various S/Fe ratios obtained from magnetic separation of pyrrhotite

Sample No.	Mass fraction/%					
	S	Fe	Si	Zn	Mg	Ca
1	30.64	67.61	1.75	−	−	−
2	32.34	62.01	5.65	−	−	−
3	28.98	58.92	6.76	5.34	−	−
4	28.59	45.75	11.58	14.09	−	−
5	30.07	35.91	22.31	7.50	3.94	0.27

2.2 Elective culture of bacteria on pyrrhotite

The original microorganism was collected from a zinc sulfide mine. The microorganism was cultivated in 250 mL shake flask containing 150 mL iron-free 9K nutrient medium with 15 g sample 1# − 5# of pyrrhotite as substrate respectively. The flasks were placed on an orbital shaker (170 r/min) and incubated at 30 ℃. Periodically, the pH was measured and when pH > 1.80, it was adjusted to 1.80 with 5 mol/L sulfuric acid. The number of bacterial cells in solution was determined by counting with hemacytometer under 1000X biomicroscope. Repeatedly, when the bacteria reached an exponential growth phase, one-fourth of the culture volume was transferred to the next incubation. After three times of transfer, the suspended solution of the culture was filtrated with millipore filter, and the bacteria enriched on the membrane were washed with sulfuric acid solution (pH = 1.80) to reduce iron contamination. The concentration of bacterial cells was diluted with iron-free 9 K nutrient solution to the density of 10^8 cell/mL, which was ready for the inoculum of the next experiments.

2.3 Bacterial leaching experiments

Bacterial leaching experiments were performed in 250 mL shake flasks containing 150 mL iron-free 9 K medium, and a sulfide ore and marmatite were used for bioleaching, of which the pulp density was 10% and 5% respectively. Because there were some acid-consuming minerals in the samples, the pH value of solutions must be adjusted to a steady level of about 2.0 before inoculating 3 mL bacterial solution. The flasks were placed on a orbital shaker (170 r/min) and incubated at 30 ℃.

At intervals, the water lost by evaporation were compensated by the addition of deionized water, and if the pH > 2.0, it was adjusted to 2.0 by sulfuric acid. Periodically, 5 mL supernatant liquid sample was drawn from the flasks to determine the content of zinc and iron. The 5 mL samples were replaced with an equal volume of iron-free 9 K nutrient medium.

2.4 Analytical methods

The chemical components of pyrrhotite samples were determined by electron probe. The total content of zinc and iron was determined by Atomic Absorption Spectrophotometry. The ferrous ion content was determined by titration with potassium dichromate ($K_2Cr_2O_7$, 0.002 mol/L), with barium diphenylamine sulphonate as indicator (0.2% water solution, mass fraction), and the content of ferric ion was calculated by differencia.

3 RESULTS AND DISCUSSION

3.1 Mechanism of elective culture on pyrrhotite

Elective culture of bacteria on pyrrhotite was also a course of bacterial leaching of pyrrhotite, in which pyrrhotite was a sole substrate. Usually, bioleaching of pyrrhotite bearing nickel or gold to extract nickel or gold was reported[5, 6]; but bioleaching of pure pyrrhotite mineral is relatively unavailable in the literature. The course of bioleaching of pyrrhotite can be illustrated as follows[6].

1) Acid leaching

$$Fe_{1-x}S + 2H^+ \rightarrow (1-3x)Fe^{2+} + 2xFe^{3+} + H_2S \quad (1)$$

2) Bacterial catalyze

$$2Fe_{1-x}S + O_2 + 4H^+ \xrightarrow{Bact} (2-6x)Fe^{2+} + 4xFe^{3+} + 2S + 2H_2O \quad (2)$$

$$S + 1.5O_2 + HO_2 \xrightarrow{Bact} SO_4^{2-} + 2H^+ \quad (3)$$

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{Bact} 4Fe^{3+} + 2H_2O \quad (4)$$

3) Oxidizing action of Fe^{3+}

$$Fe_{1-x}S + (8-2x)Fe^{3+} + 4H_2O \rightarrow (9-3x)Fe^{2+} + SO_4^{2-} + 8H^+ \quad (5)$$

$$Fe_{1-x}S + (2-2x)Fe^{3+} \rightarrow (3-3x)Fe^{2+} + S \quad (6)$$

4) Hydrolyzation of Fe^{3+}

$$Fe^{3+} + 2H_2O \rightarrow FeOOH + 3H^+ \quad (7)$$

Bioleaching of pyrrhotite includes two mechanisms: the direct one(Eqn. (2)) and the indirect one(Eqns. (3)−(6)). The mechanisms of the bioleaching of metal sulfides are usually both of these two ones or either of them[7]. In the direct mechanism, it is assumed that the bacteria attack and oxidize the minerals directly by an enzyme with oxygen to sulfate and metal cations. The indirect mechanism is that the oxidizing behavior of ferric ion dissolves sulfide minerals, and the products of the reaction, ferrous ions and elemental sulfur, are biologically oxidized to ferric ions and sulfate. Thus, if the bacteria are cultivated purposefully to accelerate the chemical oxidation of sulfide minerals by ferric ion or the biooxidation of sulfides and elemental sulfur to sulfate, the rate of bioleaching will be increased. A mixed culture can be obtained from the cultivation of bacteria on pyrrhotite with various S/Fe ratios. Accordingly the mixed culture has a certain strengthened capability of oxidizing ferrous ion or sulfides and elemental sulfur, and the adaptability to minerals was also improved, which could accelerate the bioleaching rate of some sulfides.

Bioleaching of pyrrhotite was an acid-consuming course(as shown in Fig. 1), and the acid consumption of the five samples of pyrrhotite was in the order: 3# > 2# > 1# > 4# > 5#. With increasing time, the pH had a decreasing tendency. Except sample 3#, the bacterial numbers of other samples were above 10^6 cell/mL in the third day. The low growing rate of bacteria in sample 3# was because of the high pH value. Usually the best range of pH for bacterial growth is below 3.0. After the sixth day, the pyrrhotite was dissolved and a lot of iron was released, which made the solutions take a brown yellow color. From the depth of color it was determined semi-quantitatively that the concentration of iron was in the order: 1# > 2# > 3# > 4# > 5#. Here the numbers of bacteria in the samples are all above 10^7 cell/mL. In sample 1#, the high concentration of iron ion leads to a precipitation, so the bacteria should be transferred to the next incubation with fresh pyrrhotite in time.

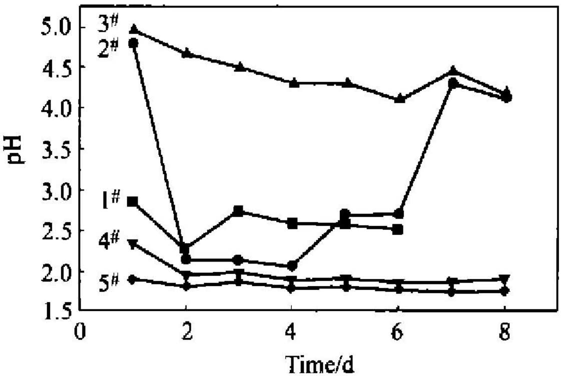

Fig. 1 Variation of pH in cultivation of bacteria on pyrrhotite with various S/Fe ratios

3.2 Characteristic of selected bacteria

By the microscopic examination and observation of solid culture, the bacteria obtained from pyrrhotite cultivation were characterized. The main strains were *thiobacillus ferrooxidans*, *thiobacillus thiooxidans*, *ferrobacillus ferrooxidans* and *leptospirillum ferrooxidans*. In sample 1#, *thiobacillus ferrooxidans* was dominant; *thiobacillus thiooxidans* was in the majority in sample 3# and 4#; in sample 2# and 5#, the two strains of *thiobacillus ferrooxidans* and *thiobacillus thiooxidans* were equiponderant approximately. It can be seen here that the bacteria obtained from pyrrhotite cultivation was a mixed culture, in which some strains were dominant in numbers and in the oxidizing capacity corresponding to the variation of S/Fe ratio.

Fig. 2 shows the oxidation of ferrous ion by bacteria in 9 K medium(the original ferrous concentration was 9 g/L), and the inoculation was 3 mL bacterial medium. It can be seen the most fast oxidizing ratio belongs to bacteria 1#, and the ferrous ion was completely oxidized to ferric ion after 40 h. The times for oxidizing the ferrous ion for bacteria 5# and 2# were 80 h and 100 h

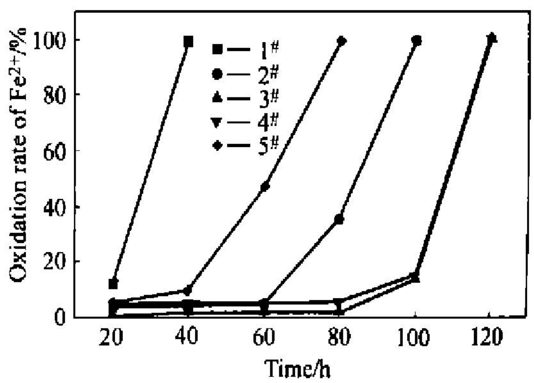

Fig. 2 Ferrous ion oxidation of five bacterial cultures

respectively, for both bacteria 3# and 4# were 120 h. The results were consistent with the bacterial characterization above, that is, the bacterial sample in which *thiobacillus ferrooxidans* was dominant has the higher capacity of ferrous oxidation. Correspondingly, if the *thiobacillus thiooxidans* dominate, a higher capacity of elemental sulfur oxidation was presented. Contrasting the bacterial culture with the S/Fe ratio in Table 1, it can be concluded that ferrous oxidizing bacteria was dominant in the pyrrhotite culture with low S and high Fe oxidizing ratio. On the contrary, the sulfur oxidizing bacteria was dominant in the culture with high S and low Fe oxidizing ratio.

It was interesting that the increase of ferrous oxidation in Fig. 2 was the step change, and the oxidizing ratio from zero point to 100% was only 20－40 h. It accounted for that though the ferrous oxidizing bacteria was not dominant, and its activity could be still enhanced after residence time.

3.3 Bioleaching of marmatite

Bioleaching of marmatite can be presented as follows.

1) Biochemical reactions

Direct mechanism:

$$ZnS + 2O_2 \xrightarrow{Bact} Zn^{2+} + SO_4^{2-} \tag{8}$$

$$2FeS + 3O_2 + 2H_2O \xrightarrow{Bact} 2Fe^{2+} + 2SO_4^{2-} + 4H^+ + 4e \tag{9}$$

Biooxidation:

$$2S^0 + 3O_2 + 2H_2O \xrightarrow{Bact} 4H^+ + 2SO_4^{2-} \tag{10}$$

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{Bact} 4Fe^{3+} + 2H_2O \tag{11}$$

2) Chemical reactions

$$ZnS + 2Fe^{3+} \rightarrow Zn^{2+} + 2Fe^{2+} + S^0 \tag{12}$$

$$FeS + 3Fe^{3+} + 4H_2O \rightarrow 9Fe^{2+} + SO_4^{2-} + 8H^+ \tag{13}$$

The results of bioleaching of marmatite are shown in Fig. 3. It can be seen that bacteria 1# had the fastest leaching rate before the 20th day. After the 20th day, the leaching rate of zinc by bacteria 3# and 4# exceeded that by bacteria 1#. The zinc extraction of bacteria 3# and 4# reached 90% and 85.9% respectively in 24 d, and that of bacteria 1# reached 80.3%. The zinc extraction of bacteria 2# and 5# was 77.0% and 78.3% respectively. Thus, the bacteria with the high capacity of ferrous oxidation or sulfur oxidation had better effect on bioleaching of marmatite.

These results were consistent with the literature report in which the indirect mechanism was dominant in bacterial leaching of sphalerite[8, 9]. The bacteria 1# had a high capacity of oxidizing ferrous ion to ferric ion, thus the generation of ferric ion accelerated sphalerite dissolving, and the products of dissolving reaction were ferrous

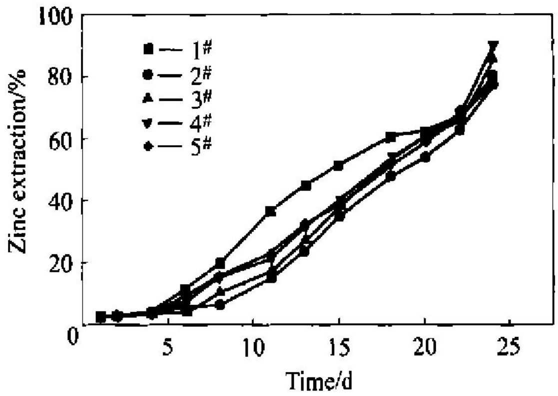

Fig. 3 Effect of five bacteria samples obtained from pyrrhotite culture on bioleaching of marmatite concentrate

ion and elemental sulfur. With the increase of elemental sulfur, the transfer of reactant and reaction product was baffled to some extent if the elemental sulfur was not eliminated in time, which decreased the leaching rate. In the cases of bacteria 3# and 4#, the bacteria had a high capacity of sulfur oxidation. With the leaching time increasing, the capacity of ferrous ion oxidation was enhanced, so in the later stage both of these strengthened capability accelerated the zinc leaching rate. Compared with a mixed culture of *thiobacillus ferrooxidans* and *thiobacillus thiooxidans* obtained from conventional induction mutation, all the five bacteria samples had more positive effect on bioleaching of marmatite. Though the bacteria also had the high capacity of ferrous ion oxidation and sulfur oxidation respectively, the zinc extraction of the mixed culture was 62% after 24th day (as shown in Fig. 4).

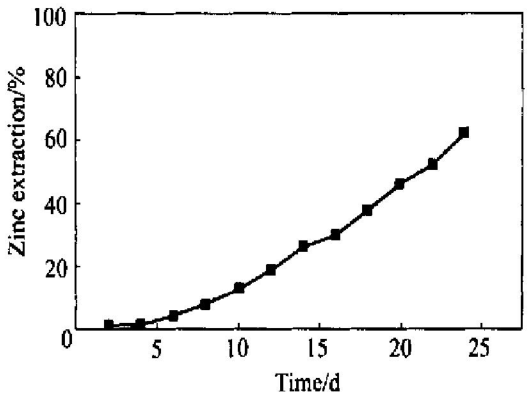

Fig. 4 Bioleaching of marmatite with a mixed culture obtained from conventional induction mutation

The results of bioleaching of zinc sulfide ore were quite different from those of marmatite (as shown in Fig. 5). The bacteria 2# and 5#, which show the lowest zinc extraction in bioleaching of marmatite, had the highest zinc extraction of 99.8% and 96.1% respectively in 9 d; while the zinc extraction of bacteria 1#, 3# and 4# was 71.0%, 75.9% and 83.4% respectively. The curves didn't pass through the original point because portions of zinc released during pH adjustment before inoculation.

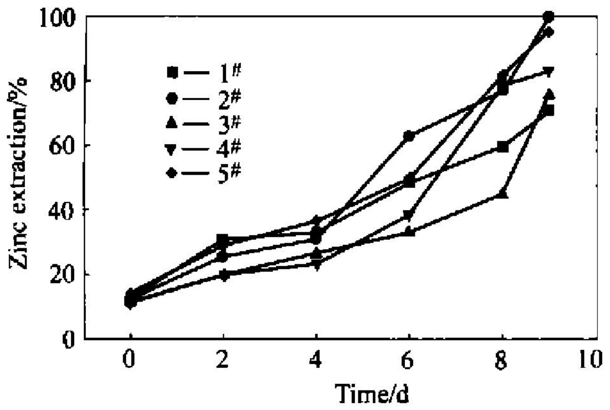

Fig. 5 Effect of five bacteria samples obtained from pyrrhotite culture on bioleaching of zinc sulfide ore

It can be seen that a mixed culture of *thiobacillus ferrooxidans* and *thiobacillus thiooxidans* being equiponderant approximately had better effect on bioleaching of zinc sulfide ore. The iron in the ore was more likely to be released because portion of the pyrrhotite and pyrite was dissolved by chemical or biochemical process. The iron concentration reached about 5.0 g/L after the 9th day in bioleaching of zinc sulfide ore, and in bioleaching of marmatite that was about 1.8 g/L after 24th day. With the galvanic reaction and the high concentration of iron in leach solution, the leaching rate of sulfide ore was faster than that of concentrate. The sulfur product didn't become the baffle of leaching because of low sulfur content in zinc sulfide ores.

4 CONCLUSIONS

Pyrrhotite is a familiar associate in many metal sulfide ore. Bacterial leaching of pyrrhotite is a typical illustration for bioleaching of any other metal sulfides. On the basis of the above results, the following conclusions can be made:

1) The bacteria culture on pyrrhotite was a mixed culture of *thiobacillus ferrooxidans*, *thiobacillus thiooxidans* and portion of *ferrobacillus ferrooxidans*, *leptospirillum ferrooxidans* etc. The leaching capacity of the culture was improved remarkably.

2) The capability of bacteria cultured on pyrrhotite varied with the S/Fe ratio. With the low S and high Fe content, the ferrous oxidizing bacteria was dominant; on the contrary, the sulfur oxidizing bacteria was dominant.

3) Bacteria with high capacity of ferrous oxidization or sulfur oxidization adapt to bioleaching of marmatite. A mixed culture of equivalent *thiobacillus ferrooxidans* and *thiobacillus thiooxidans* adapt to bioleaching of zinc sulfide ore. The role of bacteria in bioleaching of marmatite was primarily to oxidize the chemical reaction products of ferrous ion and elemental sulfur, thus the dominant mechanism is the indirect one.

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