Available online at www.sciencedirect.com
SCIENCE DIRECT®
Science Press
Trans. Nonferrous Met. Soc. China 16(2006) 937−942
Transactions of Nonferrous Metals Society of China
www.csu.edu.cn/ysxb/

Microbial leaching of marmatite by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*

WANG Jun(王 军), QIU Guan-zhou(邱冠周), QIN Wen-qing(覃文庆), ZHANG Yan-sheng(张雁生)
School of Resources Processing and Bioengineering, Central South University, Changsha 410083, China

Received 28 October 2005; accepted 17 March 2006

Abstract: The bioleaching of marmatite in shaken flasks was studied. After leaching for 29 days, the leaching ratio of zinc was 91%. Three kinds of bacteria, mixture-based bacteria, 9K-based bacteria and sulfur-based bacteria were used in marmatite leaching, of which the mixture-based bacteria have the best leaching result while the sulfur-based bacteria have the worst. By analyzing the leaching residue using SEM and EDXA, the marmatite leaching mechanism was discussed.

Key words: marmatite; bioleaching; leaching ratio; *Acidithiobacillus ferrooxidans*; *Acidithiobacillus thiooxidans*

1 Introduction

Microbial leaching of metals from sulfide minerals has been practiced over hundreds of years without realizing that microorganisms were involved. Copper, zinc, gold, etc can be recovered from sulfide ores by microbial leaching[1−5]. Zinc sulfide bioleaching was first done over 30 years ago while this process was carried out with other metal sulfides over 60 years ago[2−9]. Moderate thermophilic and extreme thermophilic microorganisms were used in the bioleaching of zinc sulfide[2,10]. Zinc can also be bioleached from industrial waste sludge using *Acidithiobacillus ferrooxidans*[4]. Bioleaching of zinc sulfide concentrates by *Acidithiobacillus ferrooxidans* was also studied[11]. However few studies on the mechanisms of zinc bioleaching were carried out[3,7,12−17].

Marmatite in Dachang, Guangxi Province, China is special for its composition of high iron and little pyrrhppite and amesonite. The main aim of this work is to examine the surface features of mineral samples, leaching residues and reaction products formed in marmatite bioleaching and to assist in understanding the mechanisms of zinc bacterial leaching. The bacterial leaching of marmatite by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* (denoted as *A.f* and *A.t* respectively) is examined in comparison with the chemical leaching by sulfuric acid. The mineral samples and leaching residues are characterized by scanning electron microscopy (SEM), energy dispersive X-ray analysis(EDXA) and X-ray diffractometry(XRD). The mechanism of marmatite bioleaching by *A.f* and *A.t* is also discussed.

2 Experimental

2.1 Materials

1) Bacteria and growth condition

The bacteria used in this experiment were isolated from the zinc waste water taken from Dachang Mine (Guangxi Province, China). Bacteria were first cultured in 9 K media. The characterization of the culture showed that it consisted mainly of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. The bacteria was cultured in media salts with three energy sources which were Fe^{2+}, S and a mixture of Fe^{2+} and S, adjusted to pH value 2.0 by sulfuric acid. The chemical composition of the three media containing the individual energy source is shown in Table 1.

2) Marmatite sample

The marmatite sample used in this experiment was obtained from No.92 ore in Dachang. The main elements of marmatite are listed in Table 2. The marmatite was ground to a particle size (over 75%) of less than 0.074 mm. XRD analysis showed that the mineral was mainly

Foundation item: Project(50321402) supported by the National Natural Science Foundation of China; Project(2004CD619201) supported by the National Basic Research Program of China
Corresponding author: WANG Jun; Tel: +86-731-8879815; E-mail: wjwq2000@126.com

Table 1 Chemical composition of three kinds of cultures(g/L)

Composition	9K medium	Mixture medium	S medium
$(NH_4)_2SO_4$	3.0	1.6	0.2
KCl	0.1	0.05	-
K_2HPO_4	0.5	0.25	-
$MgSO_4 \cdot 7H_2O$	0.5	0.5	0.5
$Ca(NO_3)_2$	0.01	0.005	-
$FeSO_4 \cdot 7H_2O$	44.7	22.3	0.01
$CaCl_2 \cdot 2H_2O$	-	0.13	0.25
Sulfur powder	-	10	20

Table 2 Major element analysis of marmatite(mass fraction, %)

Zn	Fe	S	Others
47.4	17.17	34.43	1

composed of marmatite and a small quantity of amesonite.

2.2 Chemical reagents

All the chemical reagents used were analytical grade. All solutions were made up of twice distilled water produced by a special machine.

2.3 Methods

The bacterial leaching experiments were carried out in 250 mL Erlenmeyer flasks that were shaken in an air-conditional shaker. The temperature and rotation were constantly maintained at (30±1)℃ and 160 r/min, respectively. The bacterial activity was monitored by the oxidation rate of Fe^{2+} and sulfur element. The progress of bioleaching was monitored through the measurement of the concentration of Fe^{2+}, Zn^{2+}, and pH value. The concentration of ferrous irons in the solution was determined through titration method. The concentration of Zn^{2+} in the solution was measured using an atomic adsorption spectophometer. In order to determine the mineral solubility, the specific surface area of both the original mineral sample and the leaching residuum was measured. The pH value in the leaching solution was measured with a pH-meter (PHS-3C) and kept constant (pH 2.0) throughout the leaching process using a solution of H_2SO_4 (4.0 mol/L). Solid samples were also collected, filtered, dried in air and analyzed using SEM, EDXA and XRD.

3 Results and discussion

3.1 Activity of bioleaching bacterium

The bacteria were cultured in media salts with three energy sources, Fe^{2+}, S and a mixture of Fe^{2+} and S. The oxidation rate of Fe^{2+} and the pH value of the solution are shown in Figs.1 and 2, respectively. The oxidation rate of $Fe^{2+}$and S indicated, when cultured using different energy sources, the bacteria exhibited different oxidation activity. When bacteria were cultured using Fe^{2+} as an energy source, over 95% Fe^{2+} was oxidized in less than 40 h. When bacteria were cultured by mixed culture energy, over 95% Fe^{2+} was oxidized in more than 45 h. While bacteria were cultured using S as an energy source, less than 30% Fe^{2+} was oxidized even after 80 h. The final pH values of the 9K solution were 1.42, 1.02 and 0.80, respectively after 102 h. In conclusion, bacteria cultured using Fe^{2+} exhibited the highest oxidation activity; while bacteria cultured using S showed the lowest oxidation activity.

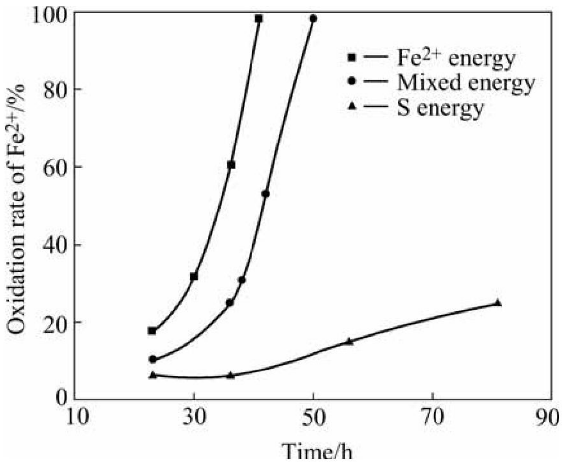

Fig.1 Oxidation rate of Fe^{2+} in 9K solution

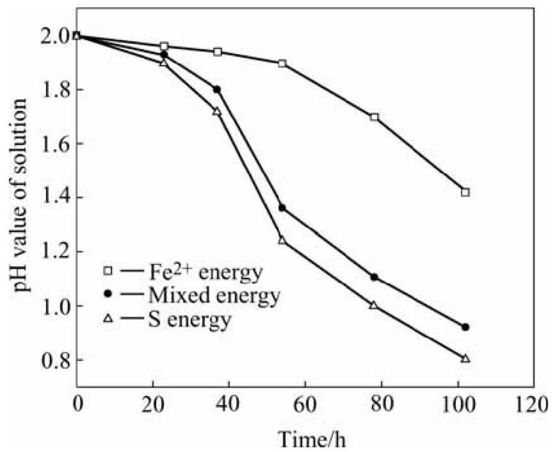

Fig.2 pH value of 9K solution

3.2 Leaching in presence and absence of bioleaching bacteria

Fig.3 shows the rate of marmatite bioleaching in the presence and absence of bioleaching bacteria. The results suggest that in the presence and absence of bioleaching bacteria, the zinc-leaching rates are 91% and 10% respectively after marmatite is leached for 29 days. The bioleaching rate is much higher than the sulfuric acid leaching rate.

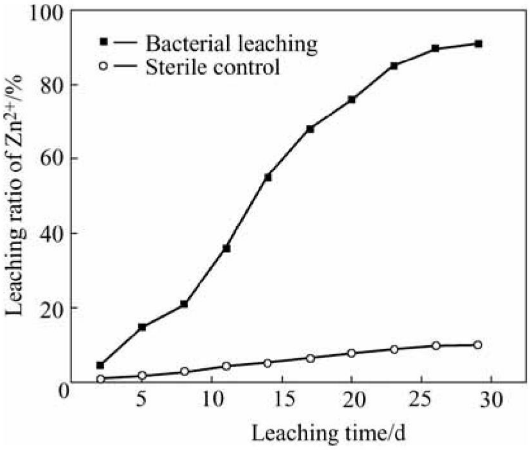

Fig.3 Bioleaching of marmatite in presence and absence of bioleaching bacteria (bacteria were cultured using Fe^{2+} energy source, 5% pulp density)

3.3 Effect of pulp density on bioleaching

Fig.4 shows the effect of pulp density on the bioleaching of marmatite (bacteria were cultured by Fe^{2+}, 5% pulp density). The results show that the zinc bioleaching ratio is 91%, 77%, and 65% when the pulp density is 5%, 10% and 15%, respectively. This demonstrates that the pulp density has great effect on the bioleaching rate of marmatite, as the pulp density increases the zinc bioleaching rate also increases.

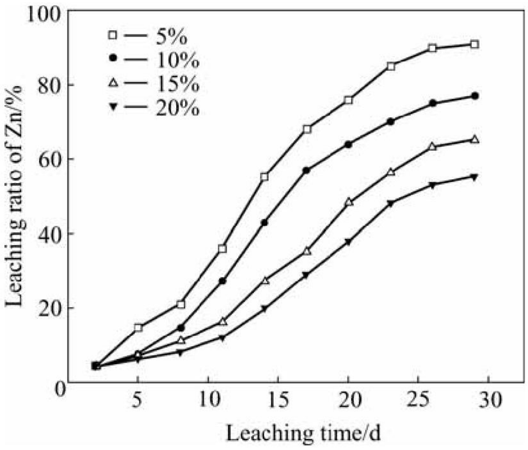

Fig.4 Effect of pulp density on leaching ratio of Zn^{2+}

3.4 Bioleaching of marmatite by 3 kinds of bacteria

The bioleaching of marmatite by three kinds of bacteria was done and the results are shown in Fig.5. It shows that the zinc bioleaching ratio is 95%, 91% and 85% after leaching for 29 days using Fe^{2+},S, and Fe^{2+} and S (mixture) as an energy source respectively. It is obvious that among the three kinds of energy sources, the mixed energy cultured bacteria have the highest zinc-leaching ratio while the S energy cultured bacteria have the lowest zinc-leaching ratio.

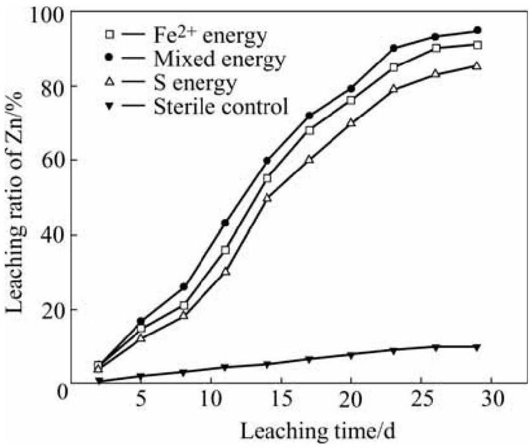

Fig.5 Bioleaching of marmatite by three kinds of bacteria

3.5 Mechanism of bacterial leaching

1) Variation of pH value in bioleaching process

Fig.6 shows the variation of pH value in the bioleaching process. The initial pH value of solution is 2.0 and the final pH value is about 3.7 when marmatite is leached using both S and Fe^{2+} (energy sources) cultured bacteria, while using mixed energy cultured bacteria the range of pH value is 2.0−2.4. When marmatite is leached by sulfuric acid, the pH value of solution is constantly about 2.0. The acid consumption is large during bacterial leaching. From these observations, we may deduce that marmatite reacts with H^+ in the presence of bacteria. Considering the oxidation of Fe^{3+} on marmatite in acid solution, the biochemical reactions of marmatite bioleaching are as follows:

$$Zn_{(1-x)}Fe_xS+2H^+\rightarrow(1-x)Zn^{2+}+xFe^{2+}+H_2S \quad (1)$$

$$Zn_{(1-x)}Fe_xS+2Fe^{3+}\rightarrow(1-x)Zn^{2+}+(2+x)Fe^{2+}+S \quad (2)$$

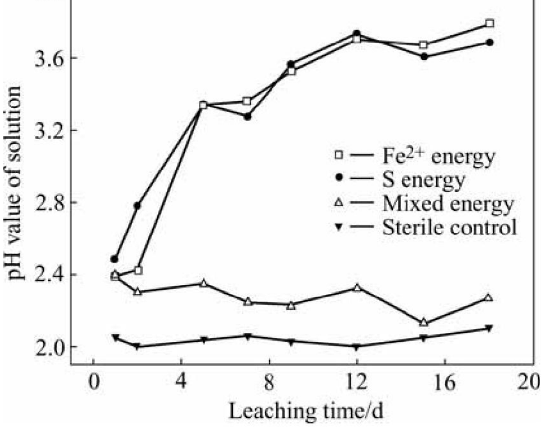

Fig.6 pH value of solution during bioleaching

As shown above, the Fe^{2+} cultured bacteria exhibit low capacity for oxidizing sulfur. Sulfur (the product of the bioleaching process) was not rapidly oxidized to sulfuric acid, and as a result the pH value of solution rises during bioleaching. Though the S cultured bacteria have the capacity of oxidizing sulfur, they have little capacity of oxidizing Fe^{2+}, there are almost no chemical leaching reactions and the marmatite is mainly leached by acid. This results in a rapid pH rise, causing a slow metal leaching rate by the two kinds of bacteria.

2) Zn, Fe bioleaching of marmatite

Fig.7 shows the Zn, Fe bioleaching ratios of marmatite. It is found that the Zn and Fe bioleaching ratios are 91% and 15%, respectively after marmatite is leached for 29 days. This demonstrates that zinc-leaching rate is much faster than iron-leaching rate.

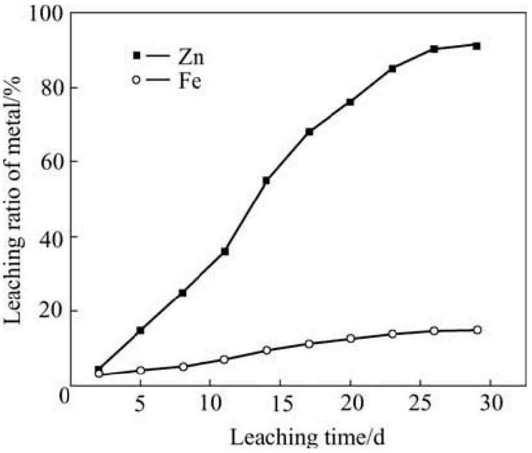

Fig.7 Zn, Fe bioleaching of marmatite (Bacteria were cultured by Fe^{2+} energy, 5% pulp density)

3) SEM analysis

SEM images of marmatite surface leached with bacteria cultured using three different energy sources are shown in Fig.8. The bioleached surface by mixed energy cultured bacteria (Fig8.(b)) is not clear; it appears to be covered with some unknown substance. However from Figs.8(a) and (c) we can clearly see the bioleached mineral surface.

It is obvious that during the bioleaching process of marmatite, elements in reactions are iron, zinc and sulfur. We can conclude that the products on the mineral surface are possibly sulfur or the hydrolysate of ferric iron. The Fe^{2+} energy and the mixed energy cultured bacteria can oxidize the sulfur and ferrous quickly in the bioleaching process. The pH value of the solution is lower than 3, at the same time, there are no solids on the mineral surface. During the sulfur energy cultured bacterial oxidization of marmatite, the pH value of the solution rises quickly, sulfur or the hydrolysate of ferric iron can be absorbed on the mineral surface.

4) XRD analysis

The XRD and EDXA patterns of marmatite and its residuum are shown in Figs.9−13. Table 3 shows the analysis of mineral sample and residuum.

Table 3 shows that after leaching for 29 days, the

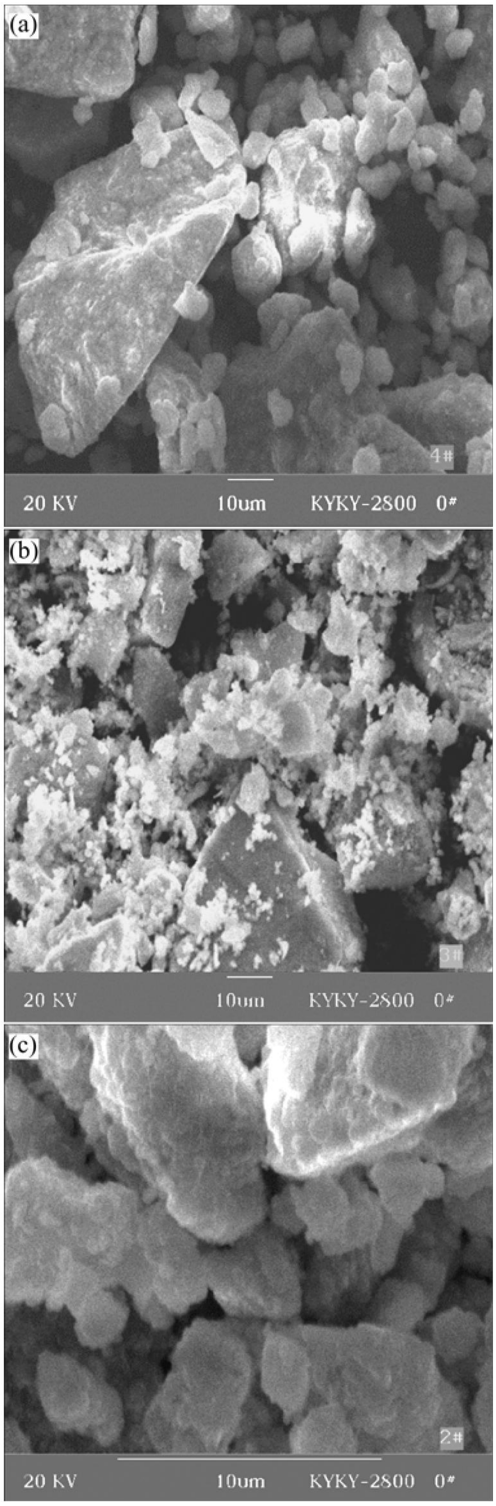

Fig.8 SEM images of marmatite surface leached with three energy cultured bacteria (30 ℃, 29 days, 160 r/min): (a) By Fe^{2+} energy cultured bacteria; (b) By mixed energy cultured bacteria; (c) By S energy cultured bacteria

total quantity of the element zinc and iron are approximately equal to the total amount of sulfur in the leached residuum. After leaching for 35 days, there is only FeS in the residuum of marmatite. It can be concluded

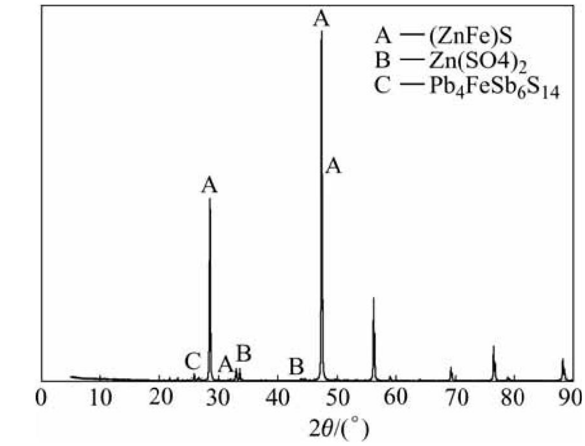

Fig.9 XRD analysis of marmatite sample leached with sulfuric acid

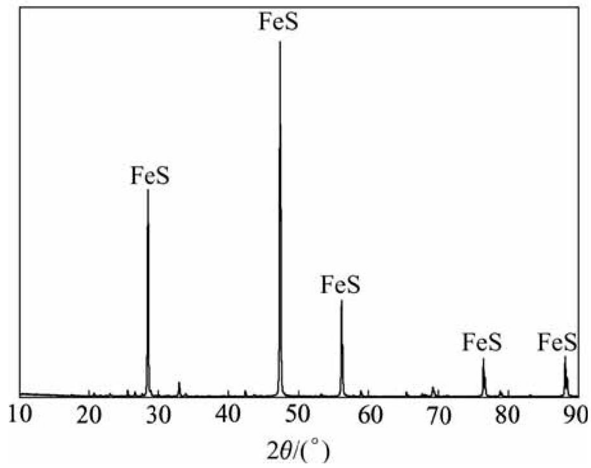

Fig.10 XRD analysis of marmatite leaching residuum bioleached with Fe^{2+} cultured bacteria for 35 days

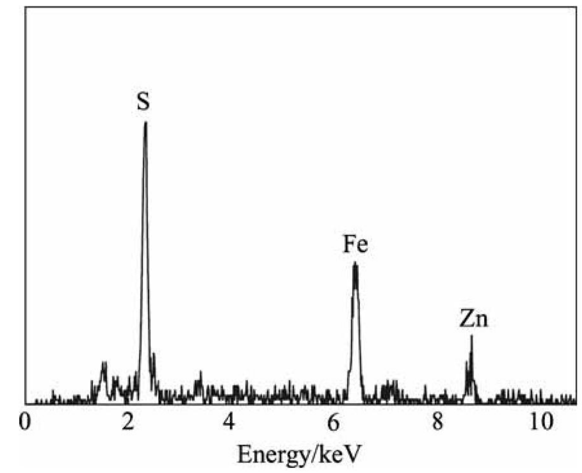

Fig.11 EDXA pattern of marmatite residuum bioleached with mixed energy cultured bacteria for 29 days

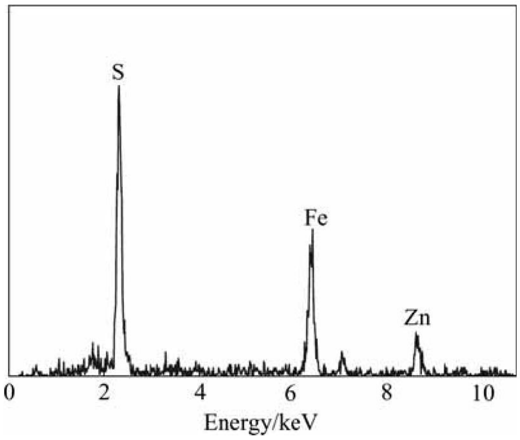

Fig.12 EDXA pattern of marmatite residuum bioleached with Fe^{2+}energy cultured bacteria for 29 days

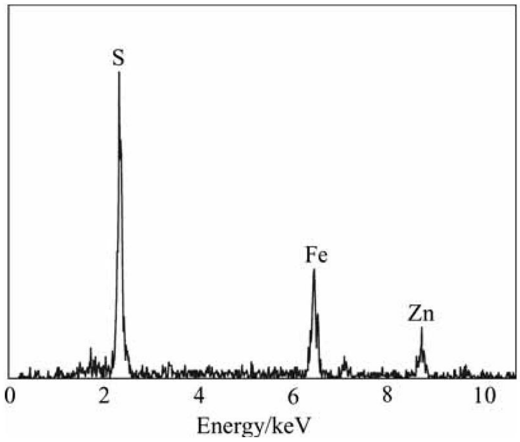

Fig.13 EDXA pattern of marmatite residuum bioleached with S energy cultured bacteria for 29 days

Table 3 Composition of marmatite sample and residuum by EDXA

Sample	Mass fraction/%			Mole fraction/%		
	Zn	Fe	S	Zn	Fe	S
Original sample	47.4	17.17	34.43	34.1	14.43	50.47
Residuum A	14.57	49.81	35.62	10.07	39.94	49.99
Residuum B	9.22	54.86	35.91	6.33	43.67	50.00
Residuum C	20.43	43.09	36.48	14.13	34.60	51.27

Residuum A is bioleached by Fe^{2+} energy cultured bacteria; B is bioleached by mixed energy cultured bacteria; C is bioleached by S energy cultured bacteria

that, during the bioleaching of marmatite, zinc is first oxidized, and its leaching rate is higher than that of iron.

4 Conclusions

1) After marmatite is bioleached for 29 days by 9K bacteria, the leaching ratio of zinc is 91% and the leaching ratio of iron is 15%. The pulp density has a great effect on the bioleaching process.

2) Among the three kinds of cultures used in marmatite leaching, mixture-based bacteria, 9K-based bacteria and sulfur-based bacteria respectively, the

mixture-based bacteria exhibit the fastest leaching rate, while the sulfur based bacteria show the lowest.

3) During the bioleaching of marmatite, zinc is first oxidized, and its leaching rate is higher than that of iron. After marmatite is leached for 35 days, only FeS is found in the residuum.

References

[1] BRIERLEY J A, BRIERLEY C L. Present and future commercial applications in biohydrometallurgy [J]. Hydrometallurgy, 2001, 59(6): 233−240.

[2] TORMA A E, GUAY R. Effects of particle size on the biodegradation of a sphalerite concentrate [J]. Nat Can, 1976, 1039(2): 133−138.

[3] AKE S, STIG P. Bioleaching of a complex sulphide ore with moderate thermophilic and extreme thermophilic microorganisms [J]. Hydrometallurgy, 1997, 46(1): 181−190.

[4] SOLISIO C, LODI A, VEGLIO F. Bioleaching of zinc and aluminum from industrial waste sludges by means of *Thiobacillus ferrooxidans* [J]. Waste Management, 2002(22): 667−675.

[5] GÓMEZ E, BALLESTER A, GONZÁLEZ F. Leaching capacity of a new extremely thermophilic microorganism sulfolobus rivotincti [J]. Hydrometallurgy, 1999, 52(5): 349−366.

[6] ROY C G, DAS R.P. Bacterial leaching–complex sulphide of copper, lead and zinc [J]. Inter J Mineral Processing, 1987, 21(1−2): 57−64.

[7] PISTORIO M, CURUTCHET G, DONATI E. Direct zinc sulfide bioleaching by *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* [J]. Biotechnology Letter, 1994, 16(4): 419−424.

[8] GROUDEV S N. Oxidation of zinc sulfides by *thiobacillus ferrooxidans* [J]. C R Acad Bulgar Sci, 1983, 36(7): 105−108.

[9] SANMUGA S, VISVANA T. Kinetic studies on the biological leaching of a zinc sulfide concentrate in two stage continuous stirred tank reactors [D]. Canada: The University of British Columbia, 1981.

[10] KONISHI Y, NISHIMURA H, ASAI S. Bioleaching of sphalerite by the *acidophilic thermophile acidianus brieleyi* [J]. Hydrometallurgy, 1998, 47(1): 339−352.

[11] KONISHI Y, KUBO H, ASAI S. Bioleaching of zinc sulfide concentrate by *Thiobacillus ferrooxidans* [J]. Biotechnology & Bioengineering, 1992, 39(1): 66−74.

[12] MERUANE G, VARGAS T. Bacterial oxidation of ferrous iron by *Acidithiobacillus ferrooxidans* in the pH range 2.5−7.0 [J]. Hydrometallurgy, 2003, 71(4): 149−158.

[13] EDWARDS K J, HU B, HAMERS R J. A new look at microbial leaching patterns on sulfide minerals [J]. FEMS Microbiology Ecology, 2001, 34(9): 197−206.

[14] TORMA A E. The role of *Thiobacillus ferrooxidans* in hydrometallurgical processes [J]. Adv Biochem Eng, 1977, 6: 1−37.

[15] YAHYA A, JOHNSON D B. Bioleaching of pyrite at low pH and low redox potentials by novel mesophilic gram-positive bacteria [J]. Hydrometallurgy, 2002, 63(2): 181−188.

[16] BRIERLEY C L. Bacterial succession in bioheap leaching [J]. Hydrometallurgy, 2001, 59(3): 249−256.

[17] Schippers A, Sand W. Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur [J]. Appl Environ Microbiol, 1999, 65(6): 319−321.

(Edited by YUAN Sai-qian)