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Study of Low-Grade Zinc Sulphide Ore Bioleaching Using *Acidithiobacillus ferrooxidans*

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In this work, a comprehensive modelling study of the zinc sulphide bioleaching with *Acidithiobacillus ferrooxidans* was investigated. The effects of particle size, pulp density, stir time, pH and ferrous iron on the bioleaching of zinc were evaluated. Experimental results show that the strains isolated from acid mineral drainage and cultivated in 9/2K medium resulted in high metabolic activities. The bioleaching rates of zinc decreased with the increasing of ore sizes and highest leaching rate of zinc was achieved at the ore size 0.14 mm, pulp density 10 %, stir time 9 days, pH value 1.5-2.0 and 9/2 K medium.

Key Words: Bioleaching, Zinc suphfide, Metabolic activities, *Acidithiobacillus ferrooxidans*.

INTRODUCTION

Zinc demand has increased significantly since 2005. Over the last 25-30 years, the zinc industry has moved away from traditional pyrometallurgy to hydrometallurgy. Nowadays, about 90 % of the world's total zinc is produced through the extraction of zinc from sphalerite by roast-leach-electrowinning (RLE) and pressure hydrometallurgy[1,2].

Previous research shows that the higher bioleaching efficiency was achieved using the adapted *Acidithiobacillus ferrooxidans* on bioleaching of pyrite[3]. Moreover, the adapted *Acidithiobacillus ferrooxidans* increased the dissolution rate of marmatite[4]. There was significant difference in bioleaching rate between unadapted and adapted bacteria. In addition, some differences of copper accumulation and distribution in adapted and unadapted cells also existed, but this was not one of the key factors that affected their bioleaching rates[5].

Yelloji *et al*.[6] investigated the growth and oxidation of *Acidithiobacillus ferrooxidans* on high purity sphalerite[6]. The reaction during the bioleaching process in these two kinds of minerals could be expressed as:

$$ZnS + 2O_2 \rightarrow ZnSO_4 \text{ (general reaction)} \quad (1\text{-}1)$$

or

$$ZnS \rightarrow Zn^{2+} + S + 2e \text{ (sphalerite in anodic dissolution)} \quad (1\text{-}2)$$

$$S + 4O + 4e \rightarrow SO_4^{2-} \text{ (bacteria oxidation of sulfur elements)} \quad (1\text{-}3)$$

$$O_2 + 4H^+ + 4e \rightarrow 2H_2O \text{ (oxygen cathodic reduction in the inert mineral surface)} \quad (1\text{-}4)$$

For galena, the general reaction could be expressed as:

$$PbS + 2O_2 \rightarrow PbSO_4 \quad (1\text{-}5)$$

The electric dipole function exist in the bioleaching of mixed sulphide (pyrite, chalcopyrite, galena and sphalerite), and the surplus potential was measured. According to the measured potential, the bacteria are most suitable for sphalerite bioleaching process which is suitable for pyrite leaching[7]. There are many papers show that zinc would be dissolved first during the mixed bioleaching process of pyrite, chalcopyrite, galena and sphalerite[8-10].

Two similar mechanisms have been proposed to leach zinc sulphides: (1) Direct atmospheric leaching in which zinc sulphide concentrates are leached directly with a ferric iron solution (produced during the leaching step of roast-leach-electrowinning process); (2) Pressure leaching that adopts a similar approach except that leaching is carried out in autoclaves. Conversely, the direct atmospheric leaching process requires about 24 h for leaching and therefore larger reactors are required as compared to pressure leaching plants[11].

A wide variation range of ferric iron and ferrous iron concentration was observed in previous work and the bioleaching mechanism related with ferrous and ferric iron concentration[12]. Consequently, it is difficult to determine whether the direct or indirect mechanism dominated the bioleaching process owing to the instability of ferrous and ferric iron concentrations. The growth of bacteria and the dissolution process was also affected by many influences, such as temperature, pH, nutrients, O_2 and CO_2, solid ratio and metal

TABLE-1
COMPOSITIONS OF LOW-GRADE ZINC SULPHIDE ORE

Element	Zn	Fe	S	Pb	As	Mn	Ti	CaO	MgO	SiO_2	Ag	Ni	Co	Sb	Al_2O_3
Contents (%)	7.02	4.81	7.67	0.97	<0.1	<0.5	0.18	9.32	0.22	53.81	0.0380	0.001	0.01	0.01	2.44

TABLE-2
MINERAL COMPOSITIONS OF ZINC AND IRON IN LOW-GRADE ZINC SULPHIDE ORE

Zinc mineral	Zinc silicate	Zinc carbonate	Zinc sulphide	Gahnite and others	Total zinc
Zinc contents (%)	0.28	1.20	5.62	0.074	7.17
Distribution rates (%)	3.91	16.74	78.38	1.03	100
Iron mineral	Magnetite	Carbonate	Silicate	Hematite and others	Total Iron
Iron contents (%)	0.35	0.10	0.40	0.30	4.63
Distribution rates (%)	7.56	2.16	8.64	6.48	100

toxicity[13-15]. Thus, further studies are necessary to investigate bioleaching process of zinc sulphide ore.

The aim of this paper is to comparative study of the zinc bioleaching using adapted and unadapted strains and also to ascertain the influences involved in the bioleaching of low-grade zinc sulphide using the selected strains.

EXPERIMENTAL

The zinc sulphide ore obtained from Lanping, Yunnan Province in China was used, whose chemical compositions were shown in Table-1. Silica, CaO and Zn are contained in the minerals, pyrite is also contained. So this mineral is a typical low-grade zinc sulphide ore.

Phase analyses of zinc sulphide ore (Table-2), the zinc sulphide ore used in the experiment mainly exists as sulphide and its oxidation rate is 21.62 %. Therefore, this ore can represent the typical low grade zinc sulphide ore. In addition, iron also exists as pyrite in the minerals, it indicates that this low grade ore is sphalerite.

Microorganisms: Bacterium were widely collected and isolated: copper mine caves ($1^{\#}$, 2004); zinc ore caves ($2^{\#}$, 2005); still acid mineral drainage ($3^{\#}$, 2006); flowing acid mineral drainage ($4^{\#}$, 2006). The bacteria were grown in the medium with compositions: $(NH)_2SO_4$ 0.3 g L^{-1}, KCl 0.1 g L^{-1}, K_2HPO_4 0.1 g L^{-1}, $MgSO_4{\cdot}7H_2O$ 0.5 g L^{-1}, $Ca(NO)_2$ 0.01 g L^{-1}. Besides, $FeSO_4{\cdot}7H_2O$ 44.8 g L^{-1} was included in 9 K medium, similarly, $FeSO_4{\cdot}7H_2O$ of 22.4 g L^{-1} and 0 g L^{-1} were contained in 9/2 K and 0 K medium, respectively. *Acidithiobacillus ferrooxidans* were incubated in 250 mL Erlenmeyer flasks each containing 100 mL of the medium and 20 % (volume fraction) inoculum, on a rotary shaker at 200 rpm at 30 ºC. The initial pH of the cultures was adjusted to 2 using 0.5 mol L^{-1} H_2SO_4. In order to adapt the experimental environment, the bacteria were subcultured in the medium for once a week. After one month, minerals was added into the medium to domesticate the bacteria and the addition of minerals was in turns with 5, 10, 15, 20 and 25 %.

Metabolic activity of bacteria: Metabolic activity of the bacteria is the key restriction for mineral bioleaching, the growth of bacteria and biological activity were inhibited by the toxic components and disadvantage environment and the bioleaching rates were decreased due to the low metabolic activity[16]. These experiments were confirmed to determine oxidation rates by ferrous iron concentration. In terms of this principle: high metabolic activity increased with the oxidation rates, and then the leaching efficiency was improved.

5 mL of the domesticated strains($1^{\#}$, $2^{\#}$, $3^{\#}$, $4^{\#}$) was incubated into 250 mL flasks, containing 95 mL of 9 K medium at pH 1.5. The ferrous iron concentration in the solution was determined by potassium dichromate titration.

Stir bioleaching of low-grade zinc sulphide ore: The strain $3^{\#}$ was used to investigate the stir leaching experiments. Bioleaching experiments were carried out in 9/2 K medium of 200 mL in 250 mL shake flasks, with 20 % inoculation of strain $3^{\#}$, pulp density 10 % (w/v). The flasks were incubated in a constant-temperature shaker at 150 rpm and temperature 35 ºC.

Analytical methods: The amount of zinc released in the bioleaching and total iron in solution were measured with an atomic absorption spectrophotometer (Zí8000 PE). Ferrous iron concentrations in solution were determined regularly by titration with potassium dichromate. Ferric iron concentrations were assessed by the difference between total iron and ferrous iron concentrations. The pH was also measured with a pH meter (PHS-3C).

RESULTS AND DISCUSSION

Bacterial cultivation: Initially, the bacteria were incubated in 9 K medium, when the culture time was 12-24 h, the bacteria achieved high activity and the cell number reached 10^9 cell /mL. Meanwhile, the highest oxidation rate was also achieved. After the bacteria were cultured for 3 generations, the bacteria adapted the environment well. And then, reduced the addition of $FeSO_4$, the initial 9 K medium was replaced by 9/2 K and 0 K medium, respectively. The results shows that 9/2 K medium is most suitable for the bioleaching process.

In addition, bacteria was observed with optical microscope and electron microscope in the cultivated and domesticated process, *Acidihiobacillus ferrooxidan* was the primary strain, and also mixed with some crossbred bacteria (Fig. 1).

Metabolic activity of bacteria: Strains ($1^{\#}$, $2^{\#}$, $3^{\#}$, $4^{\#}$) were cultured in the 9/2 K medium, the oxidation rates of each strain were shown in Fig. 2. The results indicated that strain $1^{\#}$ needs 2.5 days to oxidize ferrous iron entirely in the 9/2 K medium. However, 4 days, 12 days and 30 days were needed on the second, third and fourth generation, respectively. Because the bacteria aged quickly, so the bacteria are not suitable for the leaching experiment. On the other hand, the strain $2^{\#}$ needs 6 days to oxidize ferrous iron entirely in the 9/2 K medium. And 2 days were needed on the second and third generation. Moreover, only 8 h was needed on the fourth generation. It indicated that the bacteria growing and breeding quickly, also it would not be degenerated after keeping for a long time. Thus, these bacteria can be used for the bioleaching process. Similarly, the strain 3# needs 8 days to oxidize ferrous iron entirely in the 9/2 K medium. However, the oxidation rate reached 100 % only need 1.5 days on the second and third generation. The reason for the need of bacteria on the first generation need so much time is probable that the bacteria in the sample water was a small quantity, after cultured in the leaching solution for several generations, the bacteria adapted the leaching environment well. These bacteria are also suitable for the bioleaching process. However, compared with the former strains, strain 4 # need much more time in the third and fourth generation, it inferior to the strain in the first generation and it is not suitable for zinc sulphide ore leaching experiment.

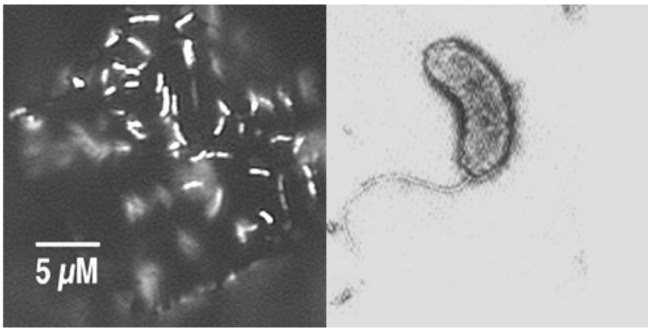

Fig. 1. SEM of *Acidithiobacillus ferrooxidans*

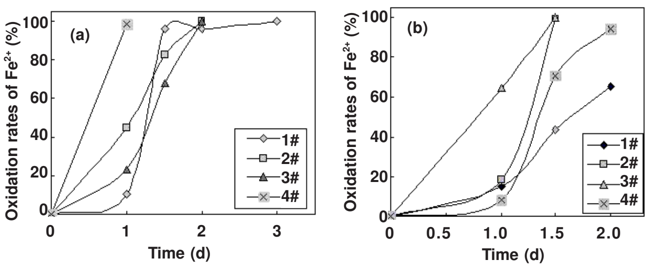

Fig. 2. Comparison of the oxidation rates of different strains on the (a) second and (b) third generation

With the higher adaptation and oxidation abilities of strain 3#, it was used in the later experiments. The shortage of this experiment is that Fe^{2+} concentration in the leaching solution was determined as the oxidation ability of the bacteria. However, S and S^{2-} were contained in the zinc sulphide ores, so it is not aim at the real leaching process.

Stir bioleaching of low-grade zinc sulphide ore

Zinc dissolution of at different particle sizes: Fig. 3 shows that the initial zinc dissolution was almost the same. As the experiments continue, when the initial particle size was 0.14-1.0 mm, the leaching rates of zinc were decreased with the increasing of ore size. However, when the particle size was 0.09-0.14 mm, the leaching rates increased with the increasing of ore size. So it has the highest extraction at ore size of 0.014 mm.

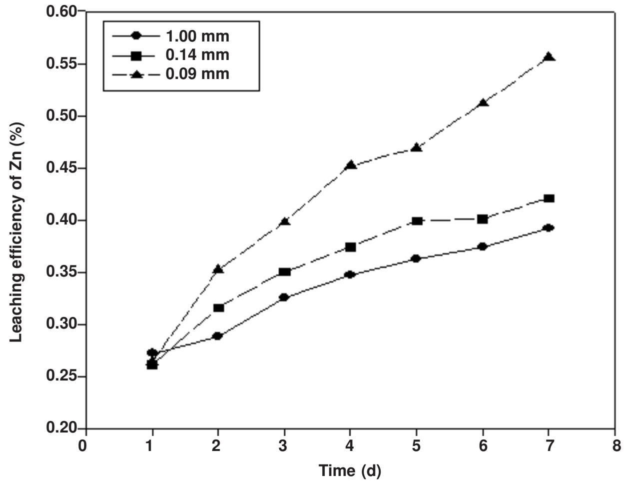

Fig. 3. Bioleaching efficiency at different initial particle sizes

Evidently, previous analysis indicated that smaller particle size was suitable for bioleaching process. However, the experimental results were inconsistent with the analytical results. In the whole bioleaching process, the inoculated bacteria were almost the same, so the bacteria contacted with the surface of zinc sulphide were also equally. Thus, the leaching rates should be the same or almost the same, theoretically, which is according to the direct mechanism. Meanwhile, for the smaller ore size, Fe^{3+} concentrations in solution were higher than other two groups of larger sizes. However, it was not correctly fit with the experiment results, so it demonstrated that the direct mechanism played leading role in zinc bioleaching process, and it also cooperated with the indirect mechanism.

The Fe^{2+} concentration in solution was less than 0.5 g L^{-1} at different particle sizes and Fe^{3+} as the manly phase exists in the leaching solution. The concentration differences of zinc and iron were shown in Fig. 4. As it can be seen, the concentration differences of zinc have no relation with concentrations difference of iron, it indicated that the function of iron in the leaching process zinc sulphide ore was not obviously, the increasing of ferric iron concentration did not improved the leaching rate of zinc, which demonstrated that bioleaching of zinc sulphide ore was the result of direct mechanism in other sense.

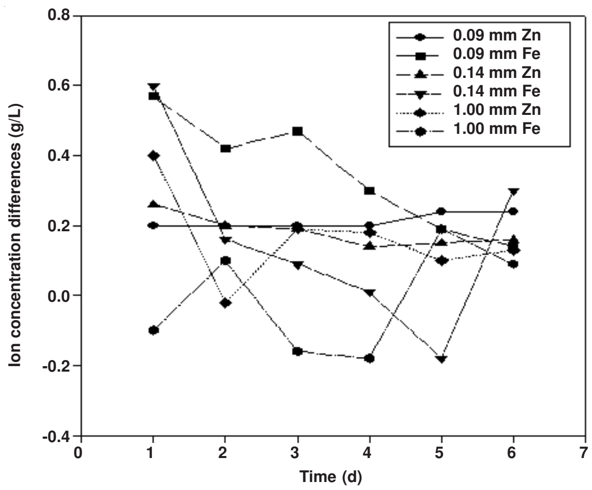

Fig. 4. Variations of zinc and iron ion concentration differences during the bioleaching process

Effect of pulp density: Eccleston and Kelly[17] put forward an empirical formula to explain the relationship between bioleaching of zinc sulphide and pulp density.

$$V = \frac{V_m \times S}{(K_S + S)}$$

where V is the leaching rate (g/L h); V_m is the maximum leaching rate; K_s is a michaelis constant, it characterize the adsorption level of the bacteria to mineral surface (g L^{-1}). Getting of the reciprocal on both sides of the formula:

$$V = \frac{K_S}{V_m} \times \frac{1}{S} + \frac{1}{V_m}$$

The effect of pulp density on bioleaching was shown in Fig. 5. As it can be seen from the results, the leaching rates of zinc were decreased with the increasing of pulp density and highest efficiency was achieved at the pulp density of 10 % (w/v). However, the total zinc increased in oppositely, it demonstrated that the bacteria have lower activity at higher pulp density. In actual operate process, with the increasing of the pulp density, the solution in agitator stir become sauces thicken, this lead to metabolic activity and quantity of bacteria decreased significantly.

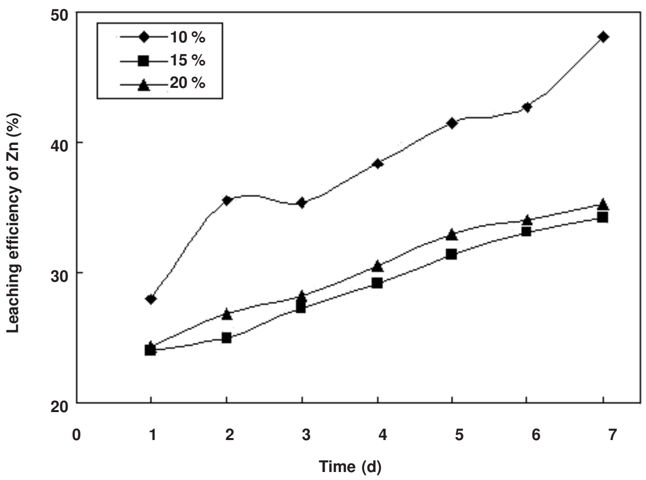

Fig. 5. Effect of pulp density on bioleaching rates

Effect of initial ferrous iron concentrations: As shown in Fig. 6, the leaching rates in 9 K and 9/2 K mediums were nearly the same (48 %) on the 7 days, but it superior to the 0 K medium (36.5 %) obviously. It demonstrated that the metabolic activity and metal ion tolerance of the bacteria are different in different medium. And for zinc sulphide bioleaching process, 9 K and 9/2 K mediums are the best choice. On the other hand, the leaching rates of zinc increased with the Fe^{2+} concentrations in the medium. It demonstrated that the leaching rate related little with the Fe^{2+} concentration, on the other hand, it also indicated that bioleaching of zinc sulphide was the result of direct mechanism, because the Fe^{2+} in the solution is the many influence to the leaching rate according to the indirect mechanism.

Effect of stir time: During the stir bioleaching, the particles collided with each other and resulted in a number of bacteria death. Meanwhile, the breeding bacteria were not considerate in the whole bioleaching. So the breeding bacteria were regarded nearly the same quantity as the death quantity. The effect of the stir time on the leaching rates was shown in the Fig. 7. The results showed that the leaching rates increased in the first nine days and kept constant (approximately 53 %) till the end of the experiment. The reason is probable that the lacking of nutrient substance and resulted in the bacteria death and lower metabolic activity. It may also attribute to the adsorption of the oxidation product (sulfur or hydrolysis sediment) to the surface of zinc sulphide and then prevent the bacteria attacking.

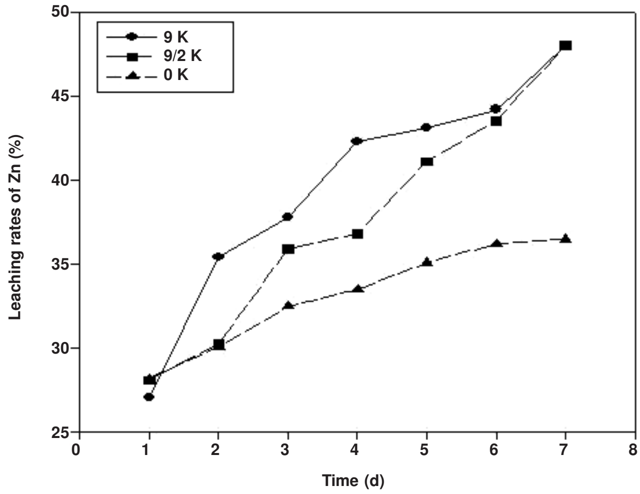

Fig. 6. Effect of cultures on bioleaching rates

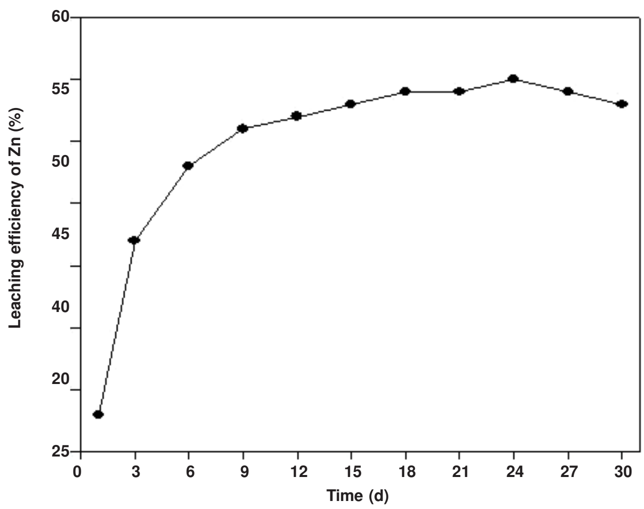

Fig. 7. Effect of stirring time on bioleaching rates

Effect of pH values: The leaching rates of zinc were relative low while pH was below 1 or above 3 (Fig. 8). This result matched well with microscopic examination that a small number of cells were observed when the pH in this range. However, when the pH was 1.5-2.0, highest leaching rates was achieved. On the other hand, considering of the iron precipitation, pH should also be controlled between 1.5 and 2.0.

In the bioleaching process, the iron concentration decreased to a very low level (under 0.5 g L^{-1}) when the pH was ranging from 2.0 to 2.5. However, the leaching rates of zinc were almost the same compared with the pH in the range of 0.5-1.0. And when the pH below 1.0, the iron concentration was much higher compared with the pH was in the range of 2.0-2.5. It also demonstrated that Fe^{3+} related little with the zinc sulphide bioleaching and the direct mechanism plays leading role in bioleaching process.

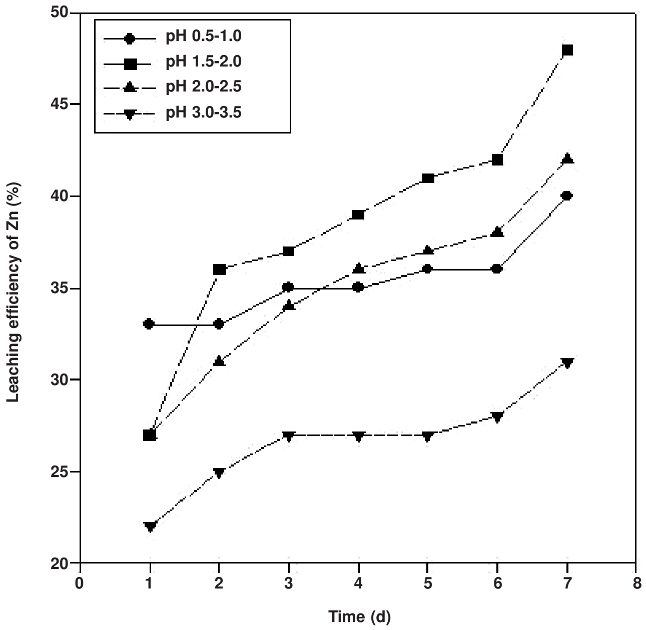

Fig. 8. Effect of pH values on bioleaching rates

Conclusion

A comprehensive bioleaching condition was obtained in this test and the bacteria suitable for zinc sulphide were isolated. These results will help to improve the bioleaching of zinc sulphide ore. In particular, the indirect mechanism proposed in the test was examined repeatedly. Nevertheless, the effect of O_2, CO_2 and other limitations were not considerate in this text. And further tests using stirred tank reactors should be investigated.

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