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Effect of two kinds of amino-acids on bioleaching metal sulfide①

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Abstract: By adding suitable amount of amino acid L-cysteine to acidic solution in contact with sphalerite or pyrite, the activity of *Thiobacillus Ferrooxidans* is largely enhanced. But, at comparable higher concentration of L-cysteine, a deleterious effect on bacterial activity was found, which can be due to the toxic effect of this amino acid at higher concentrations to microbes. The addition of L-methionine would be great inhibition to the bioleaching no matter how much it was applied, which indicates that L-methionine is harmful for bioleaching. The quite different effect on bioleaching between L-cysteine and L-methionine lies in that L-cysteine has a SH group which is useful in helping metal sulfide bioleaching by *Thiobacillus ferrooxidans*.

Key words: *Thiobacillus ferrooxidans*; aminoacids; bioleaching; L-cysteine; L-methionine
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1 INTRODUCTION

Thiobacillus ferrooxidans is a Gram-negative, acidophilus, chemolithotrophic bacterium which is able to derive energy for growth from the oxidation of ferrous to ferric ion and elemental sulfur or reduced inorganic sulfur compounds to sulfate using oxygen as electron acceptor. It is one of the most important microorganisms involved in bioleaching of sulphidic ores[1-4]. There are a lot of reports on the mechanism of bio-leaching of sulfide minerals. One of the major concerns is the question concerning the mechanism involved in the initial attack on the sulphide surface. It is clear that understanding this mechanism could offer new alternatives for enhancement of metal recovery by bacterial leaching. The evidence that *thiobacillus* do attach to pyrite surfaces by means of a secreted biofilm in the form of a capsule, which modifies the pyrite surface[5-7] and that sulphur particles are formed and stored during pyrite corrosion by thiobacilli[5,6], suggests that bacteria can cause mineral dissolution by means more than purely ferric electrochemical attack.

Recent studies have shown that the recovery of precious metals such as gold from sulphide ores by bacterial leaching techniques was economically improved when carbohydrates, proteins and other substances of biological origin were added to the tank reactors. Thus, for selective Cu recovery from chalcopyrite ores, the leaching media for bacteria must include a suitable nutrient supplement of potato and corn[8]. Concerning this matter, several experimental observations associated with the metabolism of *Thiobacillus ferrooxidans* such as intermediary sulphur-colloid formation, adaptive response of bacteria to pyrite via a reactive capsule relatively soluble in solutions of amino acids[9] and that polypeptides containing SH residues react spontaneously with pyrite[10], suggest that thiobiochemical groups could be involved in sulphide leaching by *Thiobacillus ferrooxidans*.

In this paper, the effect of L-cysteine and L-methionine on the bio-leaching of sphalerite and pyrite has been examined to further understand the mechanism of organic substances in bioleaching.

2 EXPERIMENTAL

2.1 Organism and cultivation

Thiobacillus ferrooxidans was isolated from Chengmenshan Mine which then was cultured in 9 K medium. When the culture reached the exponential phase of growth (about two days of cultivation), it was centrifuged at 3 000 g for 10 min, and the bacterial sediment was washed three times with the same medium without ferrous ions (buffer). The bacterial suspension was adjusted to 50 000 cells/μL (Cells count were determined microscopically with a Neubauer counting chamber) and

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saved as inoculum for the leaching experiments throughout this work.

The reactors were 250 mL Erlenmeyer flasks mounted to a HZQ-C constant temperature vibrator agitated at 200 r/min. The pH of 9 K minimal medium was adjusted to 2.0 with 1 mol/L H_2SO_4. 10 g sample of dry mineral was added to the bioreactors to serve as the growth substrate for autotrophic cells each time. The initial liquid volume of each reactor was 100 mL, with 1 mL removed for each sample. Sterility was ensured by autoclaving the flasks and liquid solutions at 121 ℃ for 25 min, covering the opening with cotton plugs, and sampling the reactors respectively. Bioreactors were confirmed to maintain dissolved O_2 concentrations in excess of 6 mg/L during the experimented periods.

2.2 Preparation of sulfide minerals

The sphalerite was prepared by grinding and sieving to a particle diameter between 425 and 832 μm, which contains 47.40% Zn^{2+}, 17.17% total iron and 34.43% S. Pyrite was grinded and sieved to below 0.075 mm which contains 62.37% total iron and 28.98% S. These two kinds of minerals were got from Fankou lead and zinc ore in China.

2.3 Measurement of oxidizing activity

The quantum of zinc leached was measured by atomic adsorption method[11]. The total concentration of iron in solution was determined by using the titration for ferrous ion once the iron was reduced to the ferrous state with stannous chloride, and the concentration of ferrous ion in solution was determined by titration with potassium dichromate with sodium diphenylamine sulfonate as the indicator[12].

3 RESULTS

3.1 Effect of L-cysteine on sphalerite bio-leaching

The dissolved zinc ion from sphalerite by bioleaching in the presence and absence of L-cysteine as a function of leaching time is given in Fig. 1, which shows the relationship between L-cysteine and bioleaching of sphalerite on time.

It can be seen that the leaching rate, i.e. the dissolved zinc ion increases with the leaching time. In the presence of L-cysteine with concentration of less than 0.4 g/L, the bio-leaching of sphalerite is greatly improved. However, when the concentration of L-cysteine in the leaching solution is over 0.6 g/L, the bioleaching of sphalerite is greatly inhibited.

3.2 Effect of L-cysteine on pyrite bio-leaching

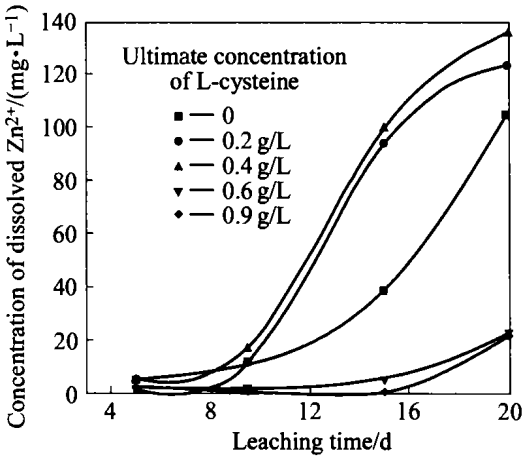

Fig. 1 Effect of L-cysteine on sphalerite bio-leaching
(Incubation concentration: 10^5 cells/mL; density of solids: 10 g/L)

Fig. 2 shows the dissolved Fe^{2+} ion from pyrite by bio-leaching as a function of leaching time. According to Fig. 2, the bio-leaching efficiency of FeS_2 is greatly increased. As leaching time increases, similar to sphalerite, effect of L-cysteine on the bio-leaching of pyrite also exhibits different behaviors depending on the concentration. When the added concentration of L-cysteine is up to 2.0 g/L, the bioleaching rate of FeS_2 is enhanced compared with that without L-cysteine. However when the L-cysteine is above 2 g/L, the leaching rate of FeS_2 is decreased, indicating that if L-cysteine is excessive, it will be harmful to bio-leaching.

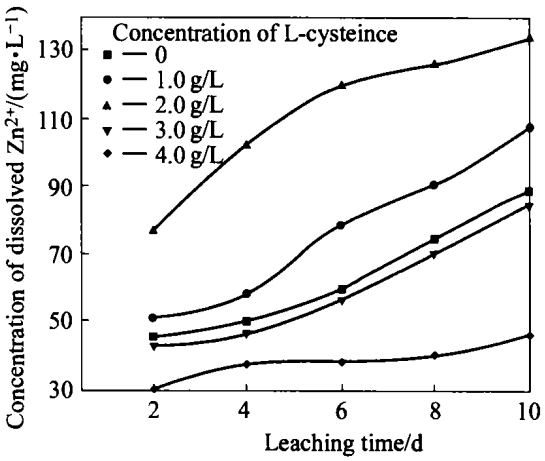

Fig. 2 Effect of L-cysteine on pyrite bio-leaching
(Incubation concentration: 10^5 cells/mL; density of solids: 10 g/L)

3.3 Effects of L-methionine on FeS_2 bio-leaching

Fig. 3 shows the relationship between bacterial leaching pyrite and the concentration of L-methionine added to the culture system. It is evident that L-methionine can greatly inhibit the bio-leaching of pyrite. The more L-methionine is added, the lower

leaching rate can be obtained, which means that L-methionine is poisonous to the *Thiobacillus ferrooxidans* cells.

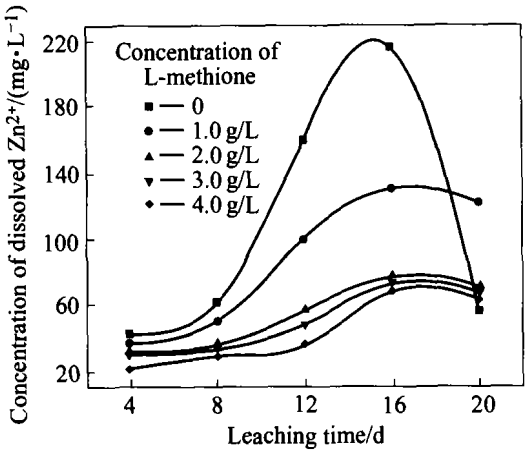

Fig. 3 Effect of L-methionine on pyrite bio-leaching
(Incubation concentration: 10^5 cells/mL; density of solids: 10 g/L)

4 DISCUSSION

The increase in sphalerite and pyrite bioleaching rate in the presence of L-cysteine is entirely coincident with the previous phototrochemical and electrochemical results on the cysteine-pyrite interaction[13]. These studies have showed that complex formation of cysteine with both iron and sulphur surfaces species with the formation of FeS-R and FeS-S-R brides (S-R, cysteine) was verified.

Previous study of bacterial interaction with different synthetic sulfide species in flasks yields the following global dissolution mechanism[14]:

$$MeS+2X+2O_2 \longrightarrow Me^{2+}+2X+SO_4^{2-} \quad (1)$$

where X is H^+, Fe^{3+} or Y^++H^+, acting as catalysts.

The bacteria thus seem to operate by chemical species which interact with the sulfide mineral to break chemical bonds and allow the bacteria to obtain energy. These species are subsequently recycled so that the entire process could be considered as a catalytic one. The catalytic species are either protons(H^+), Fe^{3+}-species, or a biochemical species which is interacting with the sulfide on the basis of thiol-chemistry leading to a product (polysulfide), which is able to transport sulfur to the bacterial cell.

$$MeS+(Y^++H^+)\xrightarrow{\text{bacteria}}Me^{2+}+Y-SH \quad (2)$$

where Y may, for example, be the thiolcysteine.

According to Tributsch et al[14], it is interestingly that , sulfide degradation by *Thiobacillus ferrooxidans* works with large gap semiconductors like zinc sulfide (E_g=3.67 eV) as well as with sulfides of low energy gap such as iron disulfide (E_g= 0.95 eV). Scheme comparing electronic structures of ZnS, FeS_2 and CdS is shown in Fig. 4, which provide different challenges for bacterial attack. The energetic position of the final electron acceptor (oxygen) and of Fe^{2+}, used as electron carrier, as well as the energetic position of electrons on sulfur species are indicated. As is possible because bacterial activity typically occurs in an acid environment (pH=1.5-3.5) where protons can disrupt chemical bonds in the sulfide interface, thus shifting electronic states from the valence band into the forbidden energy gap, where Fe^{3+} ions can assist in extracting electrons and breaking surface bonds. This will help to further push the surface reaction towards the formation of H_2S and HS^- where the bacteria can use as an energy source.

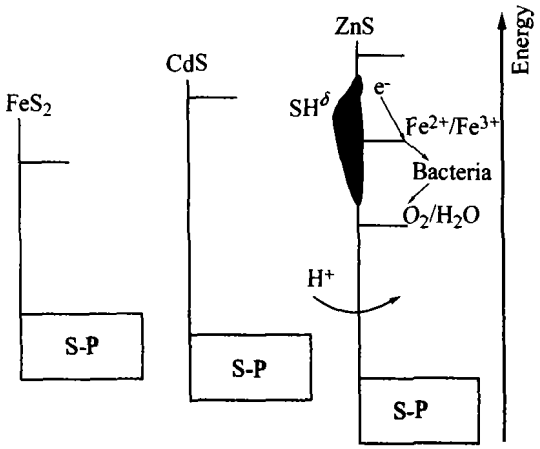

Fig. 4 Scheme of electronic structures of ZnS, FeS_2 and CdS

As shown in Fig. 4[14], which visualizes very different electronic structures that are possible in metal sulfides. ZnS has a very large gap so that direct electron extraction with Fe^{3+} is not possible. FeS_2 is characterized by a valence band derived from iron states while CdS has a valence band formed of sulfur states. Electron extraction from a sulfide with a sulfur valence band can lead to a break up of chemical bonds and consequently to a disintegration of the sulfide interface. However, extraction of electrons from sulfide with a valence band derived from metal states(like FeS_2) may not directly lead to a disruption of chemical bonds which should make the dissolution mechanism more complicated. In this case, as Tributsch et al[13], has shown that the thiol species interacting with the sulfide will play a dominant role in the disruption of the interface and the transport of the sulfur species to the bacterial cell with which the results of this paper is coincide since L-cysteine is a kind of thio species. Fig. 5 indicates the assumed role of L-cysteine in bio-leaching[15]. Two cysteine

molecules react with pyrite, made up of interfacial [Fe(Ⅱ), S] and bulk FeS_2 (Reaction(1)). The interaction disrupts the structure and leads to the formation of an iron-cysteine complex, a cysteine-pyrite complex (bonded via a sulphur bridge) and an interfacial SH group (Reaction (2)). The cysteine-pyrite complex reorganizes and leads to the liberation of an iron-sulfur-cysteine complex. This complex is the supposed chemical energy carrier (Reaction(2)) for *Thiobacillus ferrooxidans* with a cyclic turnover of cysteine and Fe(Ⅲ) (Reaction(4)).

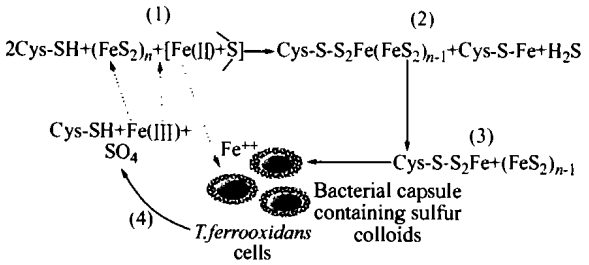

Fig. 5 Scheme for role of L-cysteine in bio-leaching

5 CONCLUSIONS

It is found that L-cysteine can greatly increase the bio-leaching speed of sulfide such as sphalerite and pyrite when being added in suitable quantum. The general properties of several sulphydryl compounds like L-cysteine as promoting agents in sulfide leaching by *Thiobacillus ferrooxidans* means a practical application in bio-hydrometallurgical operation. Till now, only *Thiobacillus ferrooxidans* cells have shown synergistic response to L-cysteine, but it may be that some other bacteria are also be able to utilize surface active regents like cysteine with —SH.

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