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Biofilm forming and leaching mechanism during bioleaching chalcopyrite by *Thiobacillus ferrooxidans* ①

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Abstract: The mechanism of attachment and leaching of *thiobacillus ferrooxidans*(*T.f.*) on chalcopyrite were studied. The shaking flasks with bacteria were observed by SEM. The process of *T.f* attached to the surface of the mineral sample and the biofilm forming were described. The promoting role of the biofilm for bioleaching was discussed. The existence of Fe^{2+} in the exopolysaccharide layer of *T.f* was demonstrated by EM(electronic microscope) cell-chemistry analysis. These results show that under the proper growth condition of bacteria, bioleaching of chalcopyrite results in the formation of complete biofilm after 2－3 weeks. There are iron ions in the outer layer polymer of *T.f.*, which provides the micro-environment for themselves, and can guaruntee the energy needed for the bacteria growth in the biofilm. At the same time, Fe^{3+} ions produced oxidize sulfide which brings about the increase of both growth rate of the bacterial and leaching rate of sulfide minerals.

Key words: bioleaching; bacterial attachment; biofilm forming; EM cell-chemistry
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1 INTRODUCTION

Fe^{2+} and sulfides can be oxidized by chemical leaching and bioleaching. Under the same condition, oxidative velocity of sulfide or ferrite in the presence of bacteria is higher than that in the presence of ferric or other chemical reagents[1]. There are many reports on bioleaching mechanism, which involved the direct function, indirect function and electro-chemistry[2-4]. The bacteria attach to mineral surface[5-7], which is the most important link in leaching process, and also is the precondition of bioleaching of mineral(especially chalcopyrite) by the direct function. The attaching rate of the bacteria to the surface of the chalcopyrite is very high, and the experiments[8] show that there are 96%－98% *thiobacillus ferroxidans* (*T.f.*) attached to chalcopyrite. In order to optimize the process of metals recover and metals withdraw, the course of the biofilm forming is observed by SEM, and the iron ion behavior in expolysaccharide layer of *T.f.*-dx (Dexing, Jiangxi, China) raised in 9K medium is studied by EM cell-chemistry.

2 EXPERIMENTAL

2.1 Mineral and bacteria

Chalcopyrite sample and acidic mine pit water (contains bacteria) were taken from Dexing Mine Jiangxi, China. The primitive cell was separated, purified and acquired from *T.f.*-dx.

2.2 Methods

2.2.1 Disposal of 9 K medium

9 K medium composed of $(NH)_4SO_4$ 30 g/L, KCl 0.1 g/L, K_2HPO_4 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $Ca(NO_3)_2$ 0.01 g/L, $FeSO_4 \cdot 7H_2O$ 44.5 g, distilled water 1 L. The initial pH 2.0 was achieved by addition of 18.0 mol/L H_2SO_4.

2.2.2 Multi-elemental analysis of chalcopyrite Cu 22.35%, Fe 26.74%, S 25.5%, Mg 0.13%.

2.2.3 Harvest cell

The cells was cultured in 9 K medium under aeration at 30 ℃ for 3 d. The cells were filtered with filter paper(No. 2) to remove the bulk of ferric precipitates and centrifuged, then collected(used for the EM Cell Chemistry analysis).

2.2.4 Method of preparing sample for SEM

Six 50 mL breakers were charged with 30 mL (pH 2.0) 9 K(no iron) medium respectively. 3 mL 3×10^8/mL *T.f.*-dx and 5－6 chalcopyrite with volume of 5 mm^3 were added respectively. Then they were cultured in a oscillating culture box at 30 ℃

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with rate of 180 r/min. The sampling were carried on at different periods and through the natural dry and ion sputtering. The samples were observed by SEM (S-570, KYKY-2800).

2.2.5 EM Cell Chemistry[9] (Perls reaction) analysis

Based on Turnbull reaction, a yellow ammonium sulfide can convert Fe^{3+} into Fe^{2+}, then the latter can again combinate with ion of ferrous cyanide and negative charge to form a blue indissoluble resultant:

$$3Fe^{2+} + 2K_3[Fe(CN)_6] \rightarrow Fe_3[Fe(CN)_6]_2 + 6K^+$$

$K_3[Fe(CN)_6]$ reaction solution was obtained by adding 1 mL 2% $K_3[Fe(CN)_6]$ solution in 50 mL 1% HCl.

The cell was fixed for 1 h with 2%–4% osmic acid(pH 7.2–pH 7.4) at 4 ℃, then was cleaned with phosphoric acid buffer(PAB). The cell was put into $K_3[Fe(CN)_6]$ solution, soaked for 40–60 min at room temperature, washed with PAB(pH 7.2–pH 7.4) for 30–60min; dewatered with gradient alcohol, embeded with Epon812 and cut into super-thin slices. For comparison, the sample preparation was the same besides it was not soaked with $K_3[Fe(CN)_6]$.

3 RESULTS

3.1 SEM images

The process of the cells swarming into the surface of minerals is divided into 4 stages obviously. In the stage Ⅰ, by spreading, delivering and active moving, the bacteria transfer to the mineral surface. In the stage Ⅱ, the bacteria adhere to the mineral, which is dominated by hydrophobic and electrostatic interaction between bacteria and mineral surface. In the stage Ⅲ, the bacteria attached at the mineral surface form some special construction such as the cilia, or secreted polymer as polysaccharide, more firmly attached to the mineral surface. After shaking breaker for 3 d(Fig. 1(a)), it was observed that a few scattering *T.f* distributed on the surface of the chalcopyrite. In the stage Ⅳ, the formative colonies gradually expand and form biofilm on the mineral surface. After shaking breaker for 10 d. The secretion of bacterial surface increases, many *T.f* gathers and gradually expands to surrounding area(Fig. 1(b)). After shaking breaker for 17 d, more complete biofilm was formed preliminarily at most of the surface of the mineral, and the substance exchanging passageway is also very obvious(Fig. 1(c)).

These results are obtained at the situation that the biofilm was not formed completely. After the biofilm is formed completely, the leaching bacteria are located on the side of the solution so that they are difficult to get into touch with the chalcopyrite directly. Although the leaching rate is increased continuously in some degree in this case, it is only contributed to the bacteria in indirect way, because the iron ions can pass through interval of the biofilm and reach the mineral surface easily. In this way, the chalcopyrite is continuously oxidized chemically. But the cells are difficult to pass the biofilm to adhere to surface directly. Only a few cells attach to the surface of mineral and are bioleached continuously during the process that the biofilm is not formed completely.

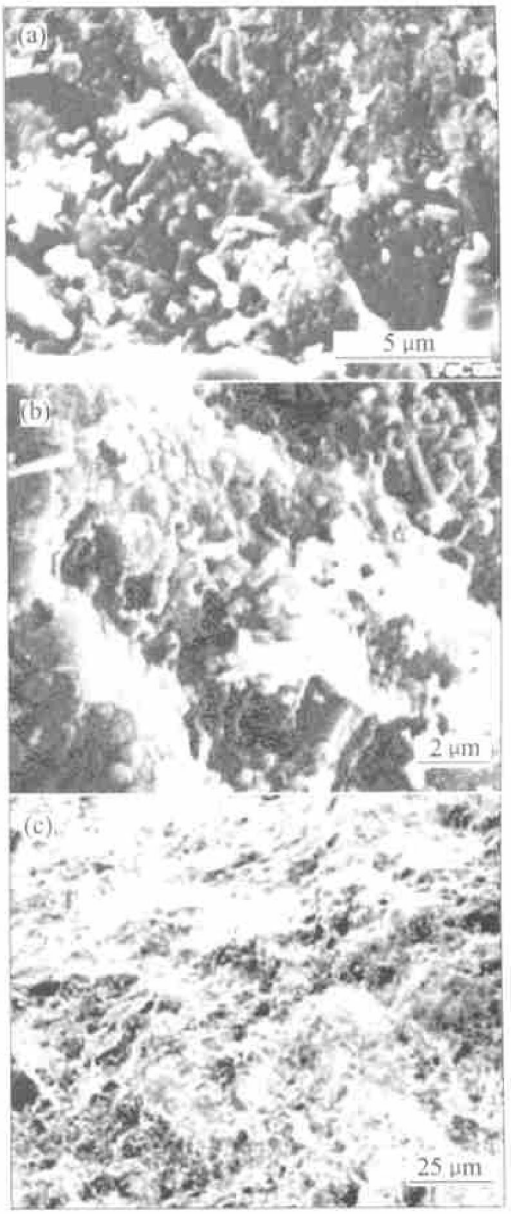

Fig. 1 SEM images of *T.f* attached to face of chalcopyrite and biofilm formed
(a) —Leaching $CuFeS_2$ for 3 d;
(b) —Leaching $CuFeS_2$ for 10 d;
(c) —Leaching $CuFeS_2$ for 17 d

3.2 Results of EM Cell Chemistry analysis

In Fig. 2, there are some bacteria. Each has Fe^{2+} resultant in very tiny particles around the polymer layer, and the distribution of the tiny particles is even. In Fig. 3, the bacteria is in their division

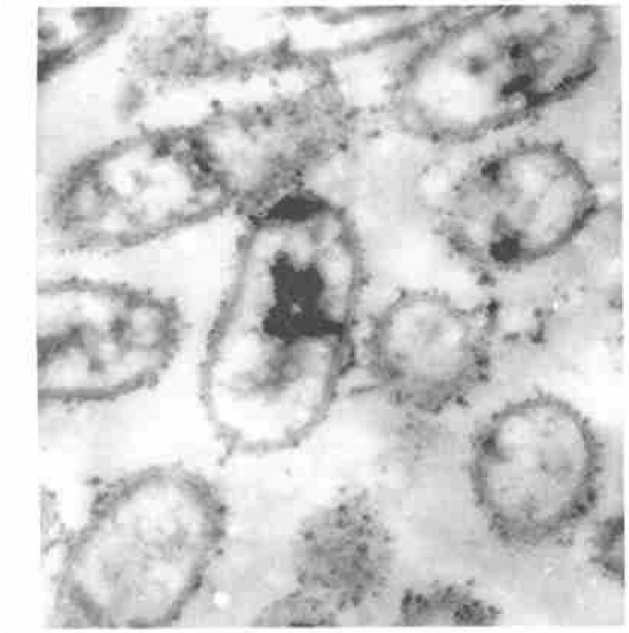

Fig. 2 Morphology of iron ion in exopolysaccharide layer of cells

phase. Around outer-membrane of the two bacteria (include the hollow part between the two bacteria) the stronger positive reaction appears. In Fig. 4, a high electron density in the polymer layer of single bacterium is displayed. The above results express that Fe^{2+} exists in the extracellular polymeric substances of *T. f*-dx. It is generally accepted that the main place in which the bacteria oxidize Fe^{2+} into Fe^{3+} is in its inner membrane. However no higher electron density in the inner membrane can be observed. An intact bacterium (with solid cell wall) is fixed (or killed) by osmic acid firstly, which turns the movable dynamic system into the unmovable and stable colloid. In this case, the cell wall of the bacteria that soaks in $K_3[Fe(CN)_6]$ solution cannot be penetrated by the solution so that the Fe^{2+} cannot be acted and form crystal further (ultracutting is finished after the course of preparing sample). Comparing the EM Cell Chemistry experimental results with that of Fig. 5, it is obvious that they are entirely different.

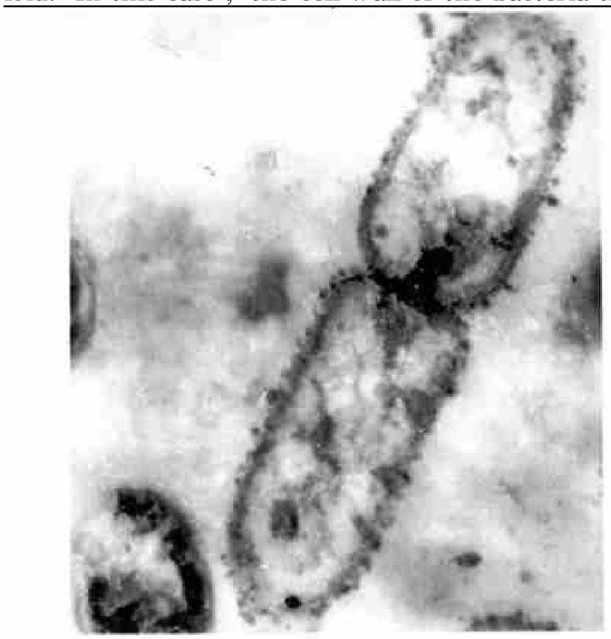

Fig. 3 Morphology of iron ion in exopolysaccharide layer of division-cell

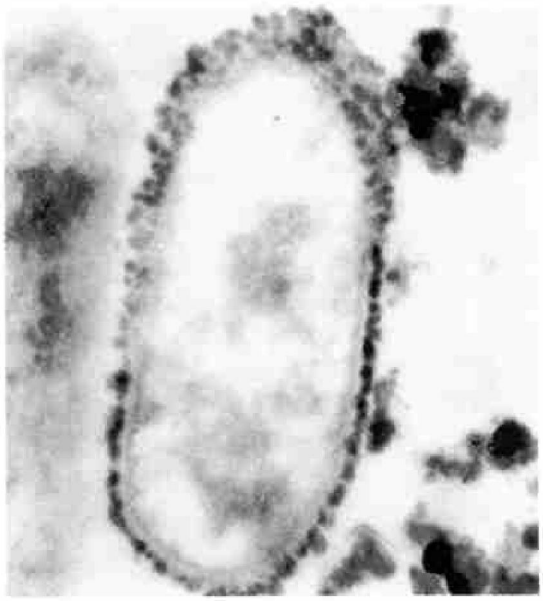

Fig. 4 Morphology of iron ion in exopolysaccharide layer of single cell

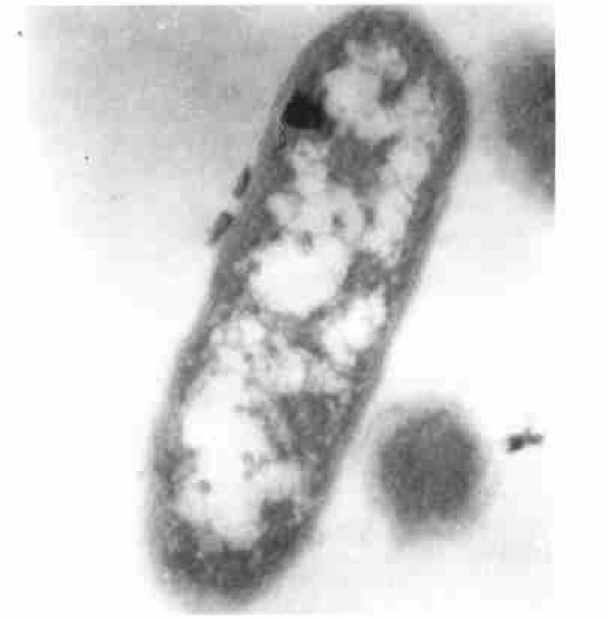

Fig. 5 Contrast of EM Cell Chemistry analysis result without handling with $K_3[Fe(CN)_6]$ reaction solution

In the direct mechanism, it is generally accepted that the enzyme plays an important role. During the bioleaching, the bacteria secrete oxidise of sulfur or iron and then catalyze the dissolution and oxidation of sulfur or iron from the lattice of mineral.

Recently research[10] discovered that, physiology ecosystem of the bacteria has the inside provision for

the bioleaching mechanism, but this kind of internality is influenced by the different exterior term, mainly by the difference of the mineral. The physiologic characteristics of bacterial oxidation decide the different mechanism in leaching mineral sulfide: *T. f* plays mainly direct function as well as slow indirect function in bioleaching of chalcopyrite.

4 DISCUSSION

The colonization of a surface by bacteria is visualized as described in following four distinct steps. In the first step bacteria are transported to the surface by diffusion, convection or active movement. The next step is the initial attachment or adhesion of the bacteria to the surface. This process is thought to be dominated by physicochemical forces such as the electrostatic and hydrophobic forces between particles and surfaces. The terms, initial attachment and adhesion, are used interchangeably to describe this second step. The attachment of bacteria to the surface is the third step. Bacteria adhered to the surface may develop special structures such as fibrils, or secrete polymers such as polysaccharides to become more firmly attached to the surface. The fourth and final process is the colonization of the surface by the formation of microcolonies and biofilms. The bacteria and their secretion stablely increase on the surface, which results in the biofilm formation. Crundwell[11] used co-focusing microscope to study the swarm of iron-oxidation mixed bacteria on the polishing thin slice of pyrite. He has observed the micro-colony in 3 – 4 d. The thin slice was completely covered by the biofilm after 13 d. The thickness of biofilm is about 30 – 50 μm. A layer of iron hydroxide was formed under the biofilm and the bacteria were crowded by the side of the solution. He proposed that iron ion was circulated among the bacteria in the biofilm and pyrite mineral, and suggested a model(Fig. 6(a)).

Sand et al[12] also gave a similar model(Fig. 6(b)), suggested that the iron ions circulate between lipopolysaccharide layer of single bacterium and the surface of pyrite, instead of the colony. Sand's model is supplementary to Crundwell's model. These models showed that there is an increase of the number of bacteria attached on the mineral surface during leaching and they keep at the higher redox potential in the solution, which will raise leaching rate of sulfide mineral. In the natural environment, the course of the biofilm formation should be more complicated. After attaching to the mineral surface, the bacteria will grow up, reproduce and form micro-colony, then gradually form the biofilm. Afterwards, the free bacteria of the same or different kind keep attachment to the solid, which brings about the environment of the bacteria growing jammed and the metabolite piling up. Under the living competing pressure, through starting and regulating of signal system, the bacteria secret exopolysaccharide and slightly shift on the solid surface at the same time, which cause the biofilm area to gradually enlarge. However, there are gaps between the bacteria colonies. They play a passageway role and are convenient for circulating of the oxygen and other component. The bacteria can create micro-environment for themselves in the biofilm, in which the concentration of both Fe^{2+} and Fe^{3+} gets high. The iron-bacteria can obtain more energy through oxidizing more Fe^{2+} ions by iron-oxidation system, and can make both the growth rate of the bacteria and dissolving rate of the mineral sulfide increase. Under aerobic atmosphere, energy requirement for growth of the iron oxidation bacteria *T. f* and *L. f* can be met by oxidizing Fe^{2+} into Fe^{3+}. Membrane protein, Fe(II) oxidise, cytochrome C_{552}, rusticyanin and aa3-model cytochrome C oxidise in the path of iron-oxidation are separated and identified. The sequence of amino acid of rusticyanin was determined[13, 14] and Fe(II) oxidoreductase was determined and cloned[15].

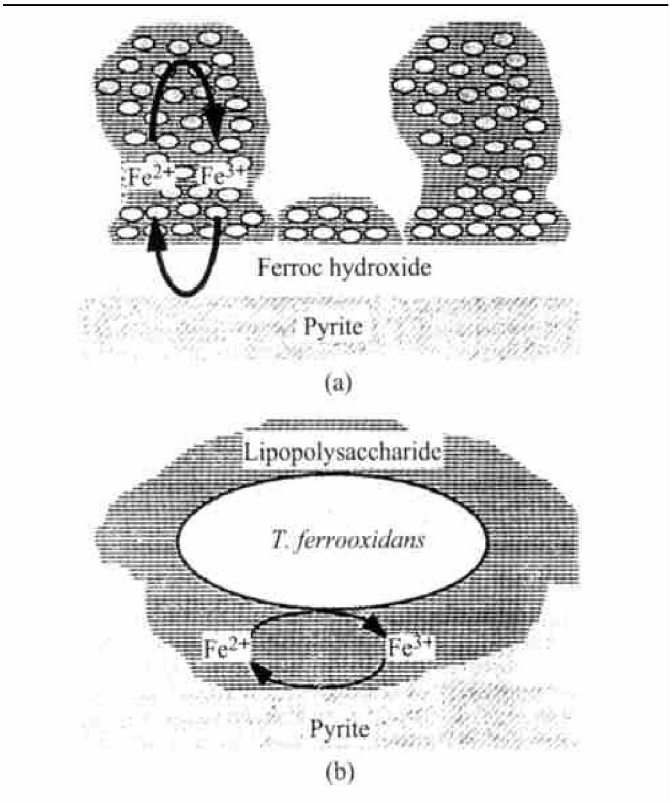

Fig. 6 Models of bioleaching mechanism
(a) —Crundwell's model;
(b) —Sand's model

5 CONCLUSIONS

1) Under the proper growth condition of the bacteria, bioleaching of chalcopyrite for 2 - 3 weeks will produce complete biofilm.

2) There are some pores or passages in the biofilm, which are convenient for the circulation of oxygen and other composition.

3) The results of EM Cell Chemistry experiment further prove that there are iron ions in the outer layer polymer of *T.f.* which provides the microenvironment for themselves, and can guarantee the energy needed for the bacteria growth in the biofilm. The more the ferrous oxidise, the more the amount of energy can be obtained. At the same time, the Fe^{3+} ions produced again oxidize mineral sulfide and are reduced to Fe^{2+}, which results in the increase of Fe^{2+} and Fe^{3+} concentrations in the micro-environment. This brings about the increase of both growth rate of the bacteria and leaching rate of mineral sulfide .

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