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Surface Characteristic of Pyrrhotite Bio-oxidized by Acidithiobacillus Ferrooxidans

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Abstract. This paper deals with the surface characteristic of pyrrhotite bio-oxidized by *Acidithiobacillus ferrooxidans*. Large amounts of jarosite and element sulfur were determined in the bio-oxidation processe of pyrrhotite. More complicatedly, biofilm exists on the surface of pyrrhotite. This type of structured community of *A. ferrooxidans* was enclosed in the extracellular polymeric substances (EPS), and covered with the deposition generated in the bio-oxidation processe of pyrrhotite.

Introduction

One of the focuses in geomicrobiological studies is microorganism-mineral interaction, which involves the promotion of microbial activities to mineral weathering and the control of microorganism over mineral formation[1]. The bacteria, including sulfur oxidization bacteria (e.g., *Acidithiobacillus thiooxidans*, *Thiobacillus caldu*) and ferrous iron oxidization bacteria (e.g., *Leptospirillum ferrooxidans*, *Ferroplasma acidarmanus*)[2-3], have been extensively examined for their interactions with sulfide minerals. Among them, *Acidithiobacillus ferrooxidans* is highlighted owing to its ability to oxidize Fe2+ ions, elemental sulfur, hydrogen and hydrogen sulfide in acidic solution.

Many factors were considered in previous studies, e.g., specific surface area of mineral, structural character of mineral, temperature, pH, dissolved oxygen, rate of bacterium metabolism, and attachment of bacterium to mineral surface, the last of which favors an indirect mechanism. Previous results indicated the positive correlation between oxidation rate of sulfide mineral and dissolved oxygen, concentration of ferric, and the number of bacteria on surface of sulfide minerals[4], and further established the rate equation of bio-oxidation on sulfide minerals. Although such studies have made some progress, issues remain regarding mineral oxidation mechanism[5-6]. Fowler et al obtained similar results in the pyrite and sphalerite leaching experiments in spite of absence or presence of bacteria under the constant concentrations of Fe^{2+} and Fe^{3+}, pH and Eh in solution. Therefore, they disagreed the direct mechanism, and suggested that the only role of the bacteria under the conditions is to make the pH at the surface of the minerals increase[7-8]. Significantly, 1.0g/L ferrous ion was maintained all along in the experimental solution of Fowler et al. Lilova et al., however, claimed that it is necessary to discuss the direct mechanism under iron-free conditions[9]. Thus, there is still a debate regarding whether the direct mechanism really exists. In addition to the study on solution chemicals, analyses of the mineral surface before and after reaction may provide important information on the chemical change of reaction interfaces. Presently, studies of *A. ferrooxidans* on sulfide surface and surface morphological changes of mineral have started to receive attention in China. For instance, in a study on the surface morphological change of pyrite before and after oxidation, Lu et al suggested that a direct mechanism might have played an important role in the bio-oxidation of pyrite[10]. With modern biological microscopic approaches and surface morphological and in-situ chemical analyses (scanning electron microscope, transmission electron microscope), we will present in this paper surface characteristic of pyrrhotite during bacterium-assisting oxidation to better understand the bio-oxidation processes of pyrrhotite.

Experimental

A. Preparation of mineral samples

The pyrrhotite samples used for this study were ground in an agate mortar to the size of -80 meshes. The mineral grains were marinated in distilled ethanol for two hours and then rinsed twice with deionized water, and dried in a vacuum drying incubator at 40℃.

B. Bacterial strain and media

A. ferrooxidans was cultured in 9K medium that contained per liter: mineral salts medium [3g $(NH_4)_2SO_4$, 1g KCl, 0.5g K_2HPO_4, 0.5g $MgSO_4.7H_2O$, 0.001g $Ca(NO_3)_2$], and 44.2g $FeSO_4.7H_2O$. The pH was adjusted to 2.0 with H_2SO_4. *A. ferrooxidans* was subcultured thrice before inoculation for experiments. The cultures were filtered by Whatman17 filter paper to remove the suspended solid material. The cells were then harvested from filtrate by centrifugation (2376×g) to eliminate residual ferric ion and washed twice with H_2SO_4 solution of pH 2.00.

C. Bioleaching experiments

The experiments were conducted in a 250mL Erlenmeyer flask. All the reactors contained 4 g of sterilized pyrrhotite per 100 mL of mineral salts medium. Bio-oxidation was inoculated with an active growing culture in order to get an initial population of 4×10^7 cells/mL, and initial pH was adjusted to 2.0 with H_2SO_4.

D. Analytical methods

Measurements of ion concentration: During the experiment, the concentration of total iron ion (Fe^{3+} and Fe^{2+}) was measured with PE Plasma 2000 ICP-AES.

Surface analysis: Mineral particles were observed using a Dutch FEI Quant 400 Environmental Scanning Electron Microscope (SEM). In a low vacuum mode, the mineral particle samples, without any surface processing (spraying), were directly observed.

Transmission electron microscope observation of bacteria: The suspending bacteria collected by centrifugation (2376×g) from the solution with 39-days-bio-oxidation of pyrrhotite were concreted by 0.4% agar solution, and cut into 0.15cm x 0.45cm slices. These slices were then fixed with 2% glutaraldehyde for 2 h, and washed by PBS thrice for 10 min each time. Subsequently, samples were immersed in 1% osmic acid at 4ºC for 2 h, and then were stepwise dehydrated in ethanol with concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% for 10 min respectively. After that, samples were transferred through epichlorohydrin and embedded in epoxy resin. Finally, samples were cut into ultrathin section and dyed with uranyl acetate and lead citrate. The inner structure of bacteria and the relationship of mineral were observed under a Holland FEI Tecnai 12 Transmission Electron Microscope with an accelerating voltage of 100kV.

Reaction product analysis: After pyrrhotite was bio-oxidized for 39 days, solid minerals were collected, dried in a vacuum drying incubator at 60℃, and qualitatively analyzed using a Rigaku D/max-1200 X-ray Diffractometer.

Results and Discussion

A. Solid product identification

The deposition generally exists all through the bio-oxidation process of pyrrhotite. With 3-days-bio-oxidation of pyrrhotite, yellowish deposition appears in both reaction solutions, but the deposition changed to yellow brown after day 6. X-ray diffraction analysis revealed that the deposition phases include jarosite and elemental sulfur (Fig. 1). With 39-days-bio-oxidation of pyrrhotite, SO_4^{2-} mol concentration decreased by 4.67 mM (Table 1). Therefore, in the bio-oxidation process of pyrrhotite, not only all the SO_4^{2-} is generated in the elemental sulfur oxidation, but also a part of it in the former solution has become jarosite deposition. Although the total Fe ion mol concentration finally measured is just 16.66 mM (Table 1), it can indicate that the Fe ion concentration generating in reaction is much higher than this value. After all the results are considered, it is concluded that the bio-oxidation rate of pyrrhotite is the high.

B. Surface characteristic of pyrrhotite after bio-oxidation

Fig. 2A shows that the pyrrhotite surface has been covered by a smooth film but some part has fallen off with-15-days-bio-oxidation. On the spot where the film fell off, honeycomb holes can be found (Fig. 2B). The form of honeycomb may be directly related to the attachment of bacteria. As shown in Fig. 3A, with 15-days-bio-oxidation of pyrrhotite, some film-like substances exist among the mineral grains. The thickness of such substances is about 2-5 um, representing the film falling off the grain surface (Fig. 3B). A large amount of bacteria can be found on the backside of the film (Fig. 3D). After 39 days, most of the films covering the pyrrhotite surface have also fallen off and the pyrrhotite surface becomes rough and much holey (Fig. 3C).

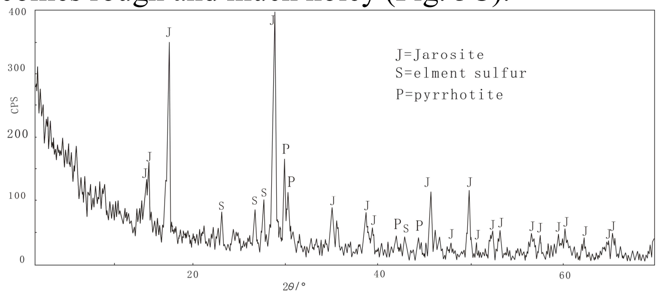

Figure 1. X-ray diffractometer analysis of solid mineral of pyrrhotite with 39-days-bio-oxidation.

Table 1. Time dependence of ion concentration in the bio-oxidation processes of pyrrhotite

Time (days) / ion concentration	Total Fe(mM)	SO_4^{2-} (mM)
0	0	38.90
3	5.33	33.55
6	5.42	30.61
9	3.42	32.51
12	9.60	34.16
15	3.55	33.70
18	4.54	35.64
22	5.95	37.28
26	6.56	25.55
30	8.08	23.49
34	10.89	24.37
39	16.66	34.23

C. Evidence of the existence of biofilm on pyrrhotite surface

Current definition of a bacterial biofilm is that a structured community of bacterial cells is enclosed in a self-produced polymeric matrix and attaches to an inert or living surface[11]. According to this definition, biofilm should meet at least the following two conditions: (1) bacteria attach to some interface or surface; (2) bacteria are wrapped by the polymeric matrix produced by themselves. Fig. 9D shows that some bacteria and floccules concentrated on the backside of film. For proving whether it is biofilm formed by A. ferrooxidans, further researches were carried out with TEM. Fig. 4A shows the characteristics of the centrifugally collected suspending bacteria in the solution with 39-days-bio-oxidation. The bacteria take the shape of brevibacterium (the length difference is mainly decided by the angle of slicing), and bacteria contour is in focus. As Fig. 4B and C show clearly, the bacterium in the solid mineral formed after bio-oxidation of pyrrhotite is wrapped by EPS, obviously different from the suspending bacteria shown in Fig. 4A. The bacterium is also surrounded by rhombi-structured bulk crystals with side length less than or close to 200nm, with part of the crystals around the bacteria. Fig. 4D further shows the characteristics of such crystals in large amount. Through SEM study, Zhu et al found that *A. ferrooxidans* may promote quick and perfect crystallization of jarosite by increasing the supply rate of Fe^{3+} in the microzones and finally form very regular rhombi[12]. As we can learn from the comparison of above-mentioned studies, crystals shown in Fig. 4B-D are jarosites formed under the effect of *A. ferrooxidans* in the bio-oxidation processes of pyrrhotite. Based on the characteristics shown in Fig. 3D and Fig. 4B and C, it can be confirmed that the community of bacteria and floccules on the backside of film is the biofilm formed by *A. ferrooxidans*. Since the steady deposition of jarosites and element sulfur on the mineral surface, it made the biofilm completely covered by deposition. Thus, biofilm was embedded between mineral surface and deposition.

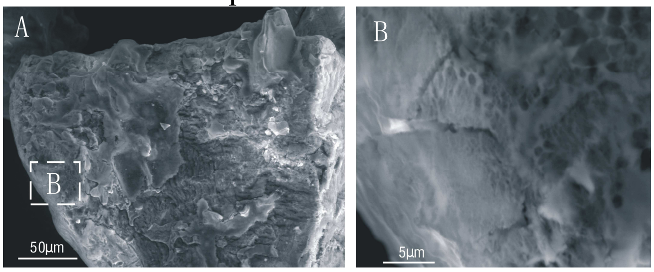

Figure 2. SEM images of pyrrhotite surface with 15-days-bio-oxidation

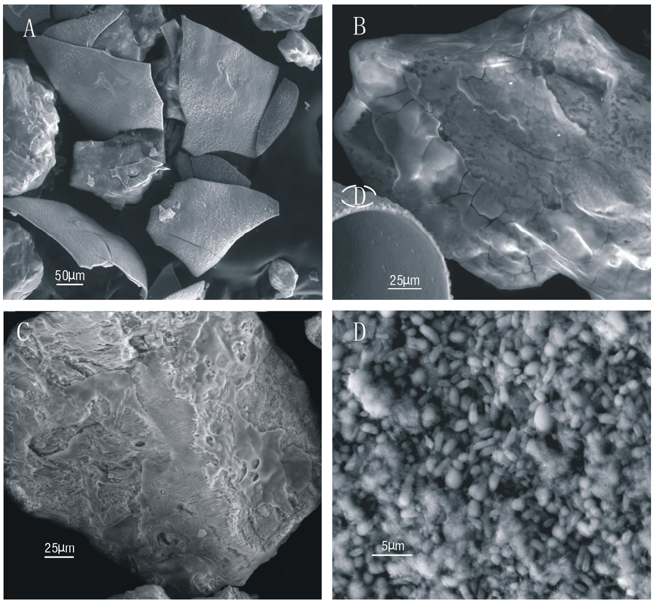

Figure 3. Surface characteristics of pyrrhotite (A, B, D, SEM images of pyrrhotite surface with 15-days-bio-oxidation; C, SEM image of pyrrhotite surface with 39-days-bio-oxidation).

Costerton recognized three stages in the formation of biofilm[13]: ①the attachment of bacteria to the medium surface; ②the former bacteria attaching to the surface become firmer under the interaction of the EPS layer produced by the bacteria themselves, and the basic structural unit of biofilm, microcolony with mush-room-like shape, comes into being; and ③the biofilm with highly organizational structure finally occurs. The mature biofilm is heterogenic, and there are many channels around the microcolonies for water transfer, from which nutrient, enzyme and metabolite can be transferred, so the biofilm would not restrain the oxidation of pyrrhotite. Moreover, the biofilm lies between the mineral surface and the deposition that covers the surface of sulfides, even integrating with the upper deposition, which would fall off from the mineral surface during the bio-oxidation. The generation of biofilm is a dynamic process. Because the bio-oxidation is always in a stirring status, the fall-off of biofilm and upper layer deposition as a whole may be not only caused by physics but also by the death of the attachment bacteria.

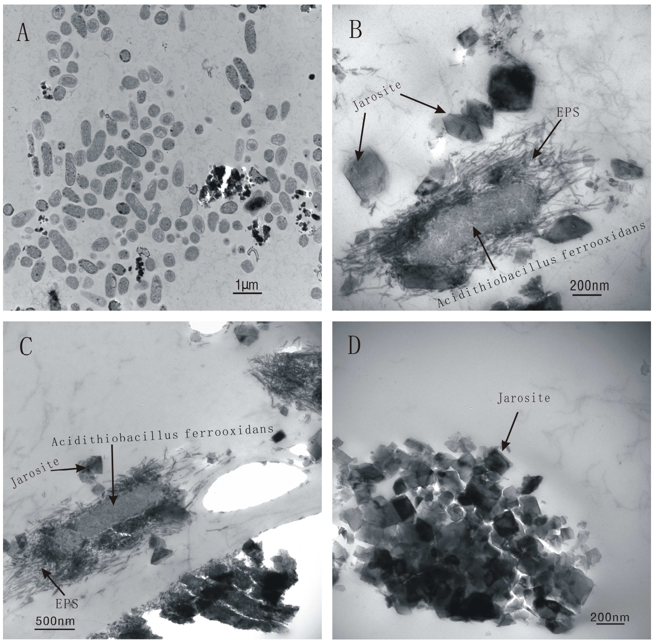

Figure 4. A, TEM image of suspension bacteria collected by centrifugation from the solution with 39-days-bio-oxidation of pyrrhotite; B, C, D, TEM images of solid minerals of pyrrhotite with 39-days-bio-oxidation.

Conclusions

The bio-oxidation processe of pyrrhotite was studied with various methods of mineralogy and biology. Experimental results show that the attachment of *A. ferrooxidans* to the mineral surface exists in the bio-oxidation processes. Large amounts of jarosite and element sulfur were determined in the bio-oxidation processe of pyrrhotite. Biofilm exists on the surface of pyrrhotite. This type of structured community of *A. ferrooxidans* is enclosed in the extracellular polymeric substances (EPS), and covered with the deposition generated in the bio-oxidation processe of pyrrhotite.

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