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Bioleaching of chalcopyrite by pure and mixed cultures of *Acidithiobacillus* spp. and *Leptospirillum ferriphilum*

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Abstract

Bioleaching is an economical method for the recovery of metals that requires low investment and operation costs. Furthermore, it is generally more environmentally friendly than many physicochemical metal extraction processes. The bioleaching of chalcopyrite in shake flasks was investigated with pure and mixed cultures of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Acidithiobacillus caldus*, and *Leptospirillum ferriphilum*. The mixed cultures containing both iron- and sulfur-oxidizing bacteria were more efficient than the pure culture alone. The presence of sulfur-oxidizing bacteria positively increased the dissolution rate and the percentage recovery of copper from chalcopyrite. Mixed cultures consisting of moderately thermophilic *L. ferriphilum* and *A. caldus* leached chalcopyrite more effectively than mesophilic *A. ferrooxidans* pure and mixed cultures. The decrease of the chalcopyrite dissolution rate in leaching systems containing *A. ferrooxidans* after 12–16 days coincided with the formation of jarosite precipitation as a passivation layer on the mineral surface during bioleaching. Low pH significantly reduces jarosite formation in pure and mixed cultures of *L. ferriphilum* and *A. caldus*.

Keywords: *Acidithiobacillus*; Bioleaching; Chalcopyrite; *Leptospirillum*; Jarosite

1. Introduction

The solubilization of metals by the application of microorganisms from the mineral ores and the subsequent recovery of metals from solution are referred to as bioleaching (Rohwerder et al., 2003). This is an economical method for the recovery of metals from minerals, especially low-grade ores, overburden, and waste from current mining operations which requires moderate capital investment with operating cost (Watling, 2006). Furthermore, bioleaching is generally more environmentally friendly than conventional metal recovery processes such as concentration and smelting. Those physicochemical metal extraction processes generate sulfur dioxide, a toxic emission that is increasingly the target of regulatory legislation (Stott et al., 2000; Olson et al., 2003).

Microorganisms are important in metal recovery from sulfide ores, particularly in large-scale heap or tank aeration processes for commercial application. In processes that operate from ambient temperatures to about 35–45 °C, the most important bacteria are iron- and sulfur-oxidizing *Acidithiobacillus ferrooxidans*, sulfur-oxidizing *Acidithiobacillus thiooxidans* and *Acidithiobacillus caldus*, and iron-oxidizing *Leptospirillum* spp. (*Leptospirillum ferriphilum* and *Leptospirillum ferrooxians*) (Coram and Rawlings, 2002; Fouchera et al., 2003; Okibe et al., 2003; Rawlings, 2005). It has been shown that *L. ferrooxians* outnumber *A. ferrooxidans* and *A. caldus* exceed *A. thiooxidans* in the bioreactors (Lawson, 1997; Rawlings et al., 1999; Okibe et al., 2003); *Leptospirillum* species and *A. caldus* have been recognized as the dominant microbes in bioreactors processing mineral ores that are operating at 45–50 °C (Rawlings et al., 1999). However, *L. ferriphilum*, a newly found iron-oxidizing bacterium (Coram and Rawlings, 2002), has not been well studied.

Chalcopyrite ($CuFeS_2$) is both the most abundant and the most refractory of the copper sulfides. Bioleaching of

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chalcopyrite is the key industry target. The main problem hindering commercial application of chalcopyrite bioleaching is the slow dissolution rate. The polysulphides, elemental sulfur layer and the iron-hydroxy precipitate layer, such as jarosite on the mineral surface, are thought to be the cause of slow dissolution (Fowler and Crundwell, 1999; Klauber et al., 2001). The insoluble reaction products hinder greater copper extraction by restricting the flow of bacteria, nutrients, oxidants, and reaction products to and from the mineral surface (Stott et al., 2000; Watling, 2006).

The objectives of the current study were: (1) to compare the rates of chalcopyrite dissolution in shake flasks by pure and mixed cultures of *A. ferrooxidans*, *A. thiooxidans*, *A. caldus*, and *L. ferriphilum*, and (2) to investigate the role of jarosite precipitation in the bioleaching of chalcopyrite. The chalcopyrite bioleaching mechanisms of the above four bacterial species were looked at.

2. Materials and methods

2.1. Culture media and microorganisms

A. ferrooxidans type strain ATCC23270 (AF465604) was purchased from ATCC, *A. thiooxidans* (DQ508105), *A. caldus* (DQ256484) and *L. ferriphilum* (DQ343299) used in these experiments were isolated and conserved by our laboratory.

A. ferrooxidans and *L. ferriphilum* were grown in medium 9 K with an initial pH of 2.0 and 1.6, respectively. *A. caldus* and *A. thiooxidans* were maintained in Starky basal salt medium (Starky, 1935) (pH 2.5) with sulfur as the energy source. The 9 K medium (in grams per liter): $(NH_4)_2SO_4$ (3), KCl (0.1), K_2HPO_4 (0.5), $MgSO_4 \cdot 7H_2O$ (0.5), $Ca(NO_3)_2$ (0.01), $FeSO_4 \cdot 7H_2O$ (44.7). Starky-S medium: $(NH_4)_2SO_4$ (3), KH_2PO_4 (3), $MgSO_4 \cdot 7H_2O$ (0.5), $CaCl_2 \cdot 2H_2O$ (0.25), S (10).

2.2. Minerals

The mineral sample used in the experiments contained about 95% chalcopyrite and 5% galena. Chemical analyses showed the ore contained 23.20%Fe, 31.91% Cu, and 27.14% S. The mineral was crushed, and then passed through a sieve with a pore size of 75 μm. Thin polished slabs of chalcopyrite were prepared by attaching the mineral to the glass slides, cutting thin sections, and then polishing the exposed faces. Several $\approx 2 \times 2 \times 1\text{-mm}^3$ blocks were produced from the slab. The mineral blocks were then washed with acetone and ethanol (Mcguire et al., 2001).

2.3. Bioleaching experiments

Bioleaching tests were carried out in 250 ml flasks containing 100 ml medium. The 9 K basal salts medium without sulfur or iron was used in the sulfide minerals bioleaching experiments. The mineral concentration was 2% (wt/vol). The experiments were carried out in duplicate.

Cells were harvested by centrifugation and washed twice in distilled water adjusted to pH 2.0 with sulfuric acid. The cells were then suspended in basal salts medium without energy sources. Bioleaching experiments at 30 °C with initial pH 2.0 were performed using the following five inocula: (1) *A. ferrooxidans*; (2) *A. thiooxidans*; (3) *A. ferrooxidans* and *A. thiooxidans*; (4) *A. ferrooxidans* and *A. caldus*; (5) *A. ferrooxidans*, *A. thiooxidans*, and *A. caldus*. The cell density of each microorganism in culture medium after inoculation was about 1×10^7 cells/ml. Bioleaching experiments at 40 °C with initial pH 1.6 were performed using the following five inocula: (1) *L. ferriphilum*; (2) *A. caldus*; (3) *L. ferriphilum* and *A. caldus* (in the bacterial population ratio of 1:2); (4) *L. ferriphilum* and *A. caldus* (in the bacterial population ratio of 1:1); and (5) *L. ferriphilum* and *A. caldus* (in the bacterial population ratio of 1:0.5). In the pure culture of *L. ferriphilum* and the mixed culture, the cell density of *L. ferriphilum* after inoculation was the same (about 1×10^7 cells/ml). In the pure culture of *A. caldus*, the cell density after inoculation was about 1×10^7 cells/ml. The abiotic controls were also designed.

For the experiments with abiotic controls at 40 °C, pure culture *L. ferriphilum* and mixed cultures of *L. ferriphilum* and *A. caldus* (1:1), several blocks of the appropriate polished mineral were supplemented with the crushed mineral.

Aliquots of leachate were sampled and copper concentration was determined by atomic absorption spectrometry within 32 days of incubation. The ferrous iron concentration was determined by titration with potassium dichromate ($K_2Cr_2O_7$). The structural morphology of polished mineral blocks was examined by scanning electron microscopy. The leached residues were filtered and dried using a freeze drier, and their chemical composition was analyzed by X-ray diffraction. The proportion of the elements in the leached residues was determined by atomic absorption spectrometry.

3. Results

3.1. Bioleaching of chalcopyrite by pure and mixed cultures of A. ferrooxidans, A. thiooxidans, and A. caldus

Comparison of chalcopyrite leaching at 30 °C by pure and mixed cultures of *A. ferrooxidans*, *A. thiooxidans*, and *A. caldus* is shown in Fig. 1. *A. ferrooxidans* pure and mixed culture oxidized chalcopyrite more actively than pure culture of *A. thiooxidans* and abiotic controls. As anticipated, the pure culture of *A. thiooxidans* was ineffective in leaching chalcopyrite, with copper concentrations (about $0.61\,g\,l^{-1}$) being little higher than the abiotic controls ($0.23\,g\,l^{-1}$). Copper concentrations after 32 days of incubation reached $2.19\,g\,l^{-1}$ in pure culture of *A. ferrooxidans*, $2.47\,g\,l^{-1}$ in mixed culture of *A. ferrooxidans* and *A. thiooxidans*, $2.32\,g\,l^{-1}$ in mixed culture of *A. ferrooxidans* and *A. caldus*, and $2.60\,g\,l^{-1}$ in mixed cultures of the three bacterial species, which oxidized the mineral most effectively. The addition of sulfur-oxidizing bacteria significantly increased the percentage of copper

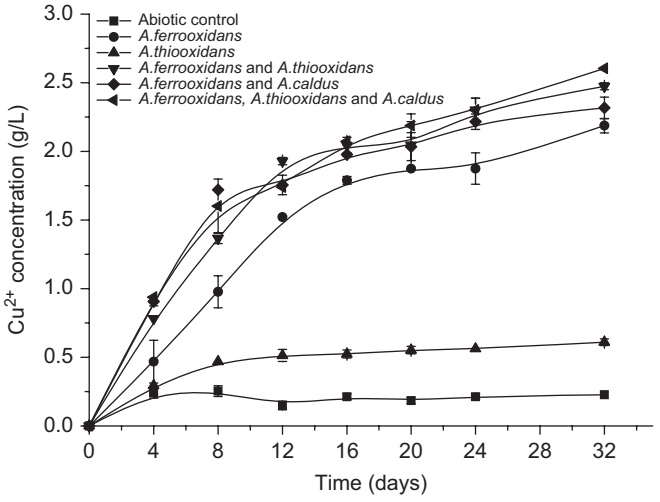

Fig. 1. Copper concentrations of chalcopyrite leached by pure and mixed cultures of *A. ferrooxidans*, *A. thiooxidans*, and *A. caldus* at pH 2.0 and 30 °C.