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Sulfur oxidation activities of pure and mixed thermophiles and sulfur speciation in bioleaching of chalcopyrite

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ABSTRACT

The sulfur oxidation activities of four pure thermophilic archaea *Acidianus brierleyi* (JCM 8954), *Metallosphaera sedula* (YN 23), *Acidianus manzaensis* (YN 25) and *Sulfolobus metallicus* (YN 24) and their mixture in bioleaching chalcopyrite were compared. Meanwhile, the relevant surface sulfur speciation of chalcopyrite leached with the mixed thermophilic archaea was investigated. The results showed that the mixed culture, with contributing significantly to the raising of leaching rate and accelerating the formation of leaching products, may have a higher sulfur oxidation activity than the pure cultures, and jarosite was the main passivation component hindering the dissolution of chalcopyrite, while elemental sulfur seemed to have no influence on the dissolution of chalcopyrite. In addition, the present results supported the former speculation, i.e., covellite might be converted from chalcocite during the leaching experiments, and the elemental sulfur may partially be the derivation of covellite and chalcocite.

1. Introduction

In contemporary era, the research and development of bioleaching have been always focused on achieving effective recovery of valuable metals by improving the efficiency of bioleaching microorganisms (Brierley, 2008), which is related to sulfur oxidation activities of sulfur-oxidizing microbes and the speciation of intermediate compounds formed during bioleaching processes (Akcil et al., 2007; Jordan et al., 2006; Vilcáez et al., 2008; Xia et al., 2010a).

Currently, the mesophilic microorganisms are widely used in the hydrometallurgical field such as secondary metal sulfides leaching and gold ores pretreatment (Brierley, 2003). However, the copper extraction yields in bioleaching of chalcopyrite, the most widespread and refractory primary metal sulfide mineral, with mesophilic microorganisms are still unsatisfiable (Batty and Rorke, 2006; Marhual et al., 2008). In contrast, thermophilic microorganisms, mainly from the *Sulfolobus*, *Acidianus, Metallosphaera* and *Sulfurisphaera* (Orell et al., 2010), because of the inherent advantages of tolerating the high temperature derived from a highly exothermic reaction of biooxidation (Acevedo and Gentina, 2007), may significantly improve the leaching kinetics, accelerate the reaction rate and shorten the leaching cycle (D'Hugues et al., 2002; Rawlings, 2004). In addition, thermophilic microorganisms are capable of dissolving the highly refractory ores such as chalcopyrite, which are difficult to attack by using mesophilic microorganisms (Javier et al., 2009; Pradhan et al., 2008; Rawlings, 2004), so that they are considered as one of the major contribution promoting the industrialization of bio-hydrometallurgical technology (García-Balboa et al., 2010).

In an industrial scale, both in heaps and in tank reactors, the effective dissolution of metal sulfide is actually conducted by the cooperation of different microoganisms (He et al., 2010; Mutch et al., 2010; Williams and Cloete, 2008), where the synergistic action may inspire a full potential of bacteria to promote the metal dissolution rate (Pradhan et al., 2008).

The advantages of extreme thermophiles have already intrigued great interest of researchers, with a series of literatures issued on the bioleaching kinetic and leaching mechanisms (Auernik et al., 2008; Rubio and Gardia, 2002; Vilcáez et al., 2008). However, compared with the mesophiles, there is still lack of understanding to the bioleaching behaviors and sulfur oxidation activities of thermophiles, especially with mixed cultures. Therefore, further study on such subjects related to the pure and mixed thermophiles is important to understand the mechanisms of thermophilic bioleaching and devise an efficient way to enhance the copper yield in chalcopyrite bioleaching.

In bioleaching process, the sulfur in sulfide is stepwise oxidized to sulfuric acid, companied with complex sulfur species such as elemental sulfur, jarosite, etc. (Sandström et al., 2005; Sasaki

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et al., 2009). It was reported that the nature of the intermediate compounds could also vary at different conditions including microorganisms. Vargas et al. (2008) found there were thiosulfate, sulfite, sulfo-hydrosulfate, hydrosulfate and sulfate in chalcopyrite bioleaching at 70 °C with *S. metallicus*, while Sasaki et al. (2009) found potassium jarosite, ammonium jarosite, covellite and elemental sulfur in addition with the evidence of potassium jarosite formed prior to ammonium jarosite with mesophile *Acidithiobacillus ferrooxidans*. In the previous studies, we found covellite formed during the leaching process by extreme thermophilic microorganism *Acidians manzaensis* (He et al., 2009), comparing with chalcocite during the leaching process by *Sulfobacillus thermosulfidooxidans* (Xia et al., 2010a). The reason needs to be further studied.

Consequently, in this study we attempt to compare the sulfur-oxidizing activities of pure and mixed thermophiles consisting of *Acidianus brierleyi*, *Metallosphaera sedula*, *A. manzaensis* and *S. metallicus* and investigate the sulfur speciation of mineral surface, to promote the understanding of the synergistic effect of the thermophiles as well as bioleaching mechanism by the mixed thermophilic culture, and to devise an efficient way to enhance the yield of chalcopyrite bioleaching.

2. Methods

2.1. Microorganisms and culture media

The four pure thermophilic archaea used in the present work were *Acidianus brierleyi* (JCM 8954), *M. sedula* (YN 23), *A. manzaensis* (YN 25) and *S. metallicus* (YN 24), among which *A. brierleyi* was purchased from Japan Collection of Microorganisms (JCM), and the other three strains were isolated from several acidic hot spring mine samples in Tengchong, Yunnan Province, Southwest China and conserved by the Key Laboratory of Biometallurgy, Ministry of Education of China, Central South University, China. The mixed culture was inoculated with approximately equal numbers of cells from the four pure strains.

Basic media used in this study contains 3.0 g/L $(NH_4)_2SO_4$, 0.5 g/L K_2HPO_4, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.1 g/L KCl, 0.01 g/L $Ca(NO_3)_2$ and 10 g/L S, with 0.2 g/L yeast extracts.

2.2. Mineral samples

The mineral samples used in these experiments were provided by School of Minerals Processing and Bioengineering, Central South University, China. The main contents of mineral were (mass fraction): Cu 34.63%, Fe 25.35% and S 30.45%. The mineralogical compositions tests (XRD) indicated that chalcopyrite ($CuFeS_2$) as the main mineral phase, and galena (PbS) and calcite ($CaCO_3$) as the minor phases. The mineral was grinded to fine powder and then passed through a sieve of – 200 mesh, which guaranteed the diameter of the mineral powder obtained was less than 75 μm.

2.3. Bioleaching experiments

Before the leaching experiments, the strains used in the present study were initially activated in 500 mL Erlenmeyer flasks with 200 mL basic medium supplemented with 1% S as the sole energy source on a rotary shaker at 65 °C, respectively. The initial pH of the culture was firstly adjusted to 2.0 with diluted sulfuric acid, which was gradually adapted to the modified media with an initial pH of 1.5 and supplemented with 1% chalcopyrite as the sole energy source. Bioleaching experiments were carried out in the same conditions with activation, except to change pulp density to 2% (w/v).

The strains used in all leaching tests were previously cultivated to their exponential growth phase, and then harvested by filtration (0.45 mm pore size) and centrifugation (10,000 rpm), washed twice with sterilized distilled water (pH 1.5), and resuspended in fresh leaching solution. In the pure culture experiment, the cell concentration was about 1×10^7 cells/mL. The mixed culture was inoculated with approximately equal amounts of the four pure strains to get the same final concentration. The abiotic controls were also implemented with the same medium and conditions.

All experiments were performed in triplicate at the same conditions. Evaporated water was compensated by additional distilled water based on weight loss.

2.4. Analyses

During leaching experiments, sample solutions were taken out at the same intervals to detect the cell concentration, sulfate ions, copper and iron ions, and pH and Eh values. The cell concentration was determined by direct counting with a Neubauer chamber counter. The pH and Eh values in the leaching solutions were measured with a pH-meter (SJ-4A) and a platinum electrode with an Ag/AgCl reference electrode, respectively. Sulfate ions were quantified by a barium sulfate turbidimetric colorimetric method (Ding, 2007). Copper and total iron concentrations in solution were determined by atomic adsorption spectrophotometry (Olson, 1991).

In order to investigate leaching products and the sulfur speciation on mineral surface, both the original mineral samples and leaching residues at different time during bioleaching process were characterized by X-ray diffraction (XRD), Raman spectrometry and sulfur K-edge X-ray absorption near edge structure spectroscopy (XANES). All of the standard compounds for XRD, Raman spectrometry and XANES and the data calculated were obtained as described by He et al. (2009) and Xia et al. (2010b) and the instrumental details, calibration and samples preparation for XANES tests were performed as described by Xia et al. (2010a).

3. Results and discussion

3.1. Sulfur oxidation activities of pure and mixed thermophilic archaea

Sulfur oxidation activities of the pure and mixed thermophilic archaea were characterized in terms of the cell concentration, pH and sulfate concentration during bioleaching, and the results are presented in Fig. 1a–c.

As shown in Fig. 1a, the mixed culture, compared with the pure culture, grew better with longer log and stationary phases, peaking at 7.67×10^7 cells mL^{-1} at day 12, in accordance with the result of Zhou et al. (2009). The reason for the growth advantages that mixed culture possessed has been presented by Gomez et al. (1996) and Hackl et al. (1995) who considered that the mixed culture could better harness the growth factors in the media.

In Fig. 1b, it can be seen that the pH fluctuated through the whole process. At beginning, all pH values maintained a rise trend. After that, the pH values dropped dramatically. These data show that all leaching processes apparently began with the acid consumption reflected by an initial increasing of the pH, followed by the acid production reflected by a decrease of the pH, which was corresponding with the results of previous studies (Zhou et al., 2009). The acid consumption may be mainly related to the proton attack to the mineral, and the acid production may mainly result from the bacterial sulfur oxidation (Rawlings, 2005; Vilcáez et al., 2008).

Fig. 1c shows that the sulfate ions concentration of the mixed culture rose dramatically to 19.2 g/L at day 2, and moderately increased to peak value of 19.87 g/L at day 4, followed by rapid