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Bioleaching of a low-grade nickel–copper sulfide by mixture of four thermophiles

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HIGHLIGHTS

- A first study applying mixture of thermophiles in nickel–copper sulfide bioleaching.
- A first study on community succession during nickel–copper sulfide bioleaching.
- L-Cysteine contributed to higher ORP, zeta potential, cell amount and lower pH value.
- Ni recovery was always higher than that of Cu and L-cysteine promoted them both.
- Without L-cysteine, *S. m* dominated while *A. m* dominated when L-cysteine added.

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ABSTRACT

This study investigated thermophilic bioleaching of a low grade nickel–copper sulfide using mixture of four acidophilic thermophiles. Effects of 0.2 g/L L-cysteine on the bioleaching process were further evaluated. It aimed at offering new alternatives for enhancing metal recoveries from nickel–copper sulfide. Results showed a recovery of 80.4% nickel and 68.2% copper in 16-day bioleaching without L-cysteine; while 83.7% nickel and 81.4% copper were recovered in the presence of L-cysteine. Moreover, nickel recovery was always higher than copper recovery. L-Cysteine was found contributing to lower pH value, faster microbial growth, higher Oxidation–Reduction Potential (ORP), higher zeta potential and absorbing on the sulfide surfaces through amino, carboxyl and sulfhydryl groups. X-ray Diffraction (XRD) patterns of leached residues showed generation of S, jarosite and ammoniojarosite. Denaturing Gradient Gel Electrophoresis (DGGE) results revealed that L-cysteine could have variant impacts on different microorganisms and changed the microbial community composition dramatically during nickel–copper sulfide bioleaching.

1. Introduction

The worldwide growing demand for nickel and copper has generated interest in the development of new processes suitable for low grade complex ores (Watling, 2008). Bioleaching has been proved to be an effective, low-cost and eco-friendly way to process many sulfide ores which are difficult to process by conventional techniques (Mishra et al., 2005). Mesophilic microorganisms have been widely applied in bioleaching processes in the past decades (Rawlings, 2002). However, increasing interests are showing in using thermophiles to improve the dissolution rate of valuable metals (Brierley and Brierley, 2013), since thermophiles can tolerate high temperature (50 °C or greater) in the pregnant leach solution derived from the exothermic reaction during bioleaching (Zhu et al., 2013), and the reaction rates increase with increasing temperature (Pradhan et al., 2008). Using thermophiles was also found to reduce or hinder passivation during primary metal sulfide (such as chalcopyrite) bioleaching (Jordan et al., 2006).

However, most previous studies on nickel–copper sulfide bioleaching still focused on mesophiles (Watling, 2008), and few data related to thermophilic microbes are currently available. Moreover, the rest several studies using thermophiles only applied a single species, while microbial analyses of practical bioleaching plants have shown that, in all cases so far reported, multiple species are involved in (Rawlings and Johnson, 2007). Increasing interests are transferring to the study of metal sulfide bioleaching by mixed cultures since mixed cultures are usually considered more efficient at oxidizing minerals than single strains (Okibe and Johnson, 2004). Mixed cultures are usually comprised of more than one individual that could oxidize either iron or sulfur (or both), and which could either fix CO_2 or use organic carbon, and such

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diversity is important in a stable and effective mineral bioleaching consortium (Rawlings and Johnson, 2007). Strains possessing different physiological properties can cooperate to response better to environmental changes during bioleaching. When using mixed culture in bioleaching, understanding the microbial community shifts during the bioleaching process has been considered as key to advancing bioleaching performance (Rawlings, 2002). Various kinds of microbe show distinct metabolic activity at different conditions thus environmental changes will exercise great impact on the structure and function of microbial community.

Studies have revealed that increasing activity of bacteria or changing the surface property of minerals could improve the bioleaching performance (Rojas-Chapana and Tributsch, 2000). Certain substances of biological origin have been investigated in order to realize this goal in an economical and environmentally safe manner (Ghosh et al., 2012; Hu et al., 2004; Puhakka and Tuovinen, 1987; Rojas-Chapana and Tributsch, 2000). According to these reports, the addition of L-methionine, L-aspartic acid, L-glutamic acid, L-histidine, L-serine or yeast extract did not improve bioleaching or just improve the bioleaching in ways through which the effects of them were limited, while L-cysteine could be a potential regent among the tested organic compounds. Adding suitable amount of L-cysteine was found to improve the bioleaching of pyrite and sphalerite by *Acidithiobacillus ferrooxidans* (Hu et al., 2004; Rojas-Chapana and Tributsch, 2000), and the bioleaching of nickel-copper sulfide by *Acidithiobacillus caldus* (He et al., 2009) or *Acidianus manzaensis* (He et al., 2011). It has also been found to play important role in bioleaching at other aspects. For example, the extracellular hydrophilic peptides abundant of cysteine residues was thought to play a very important role in sulfur activation by using their thiol groups to bond sulfur (Zhang et al., 2008). Zhu et al. (2008) used cysteine as one simulative component of EPS of leaching bacteria and found it important in bacterial attachment to sulphide surface. Rojas-Chapana and Tributsch (2000) speculated that the active group sulfydryl (–SH) of cysteine participated in binding process with pyrite with bonds formation similar to those present in ferredoxin complexes of biological membrane, and consequently disrupt chemical bond of ferrous sulfide and release iron sulfur clusters. Study by Wang et al. (2010) proved chemisorption of cysteine on pyrite surface and found pyrite more susceptible to be oxidized even at lower potential with addition of cysteine. However, the mechanism related to L-cysteine remains unclear especially when multiple species are involved in. Understanding the mechanism involved in this process will help in searching for cheaper additives to enhance bioleaching rate. Thus it would be of fundamental significance and practical importance to see whether L-cysteine can also improve the bioleaching performance in the case of mixed multiple thermophilic species used as inoculation and if L-cysteine has variant effects on different species during bioleaching process from the aspect of microbial community compositions.

In this study, nickel–copper sulfide bioleaching was investigated using four mixed thermophiles as inoculation (*Acidianus brierleyi*, *A. manzaensis*, *Metallosphaera sedula* and *Sulfolobus metallicus*), and the effects of L-cysteine were further evaluated. The objectives of this study were to find out the effects of 0.2 g/L L-cysteine on the bioleaching performance when mixed multi-species was used as inoculation.

2. Methods

2.1. Ore sample

Nickel–copper sulfide ore was prepared by crushing and screening to particles in the size range 0.074–0.147 mm. A representative sample of the ore was pulverized for chemical and mineralogical analysis. Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) showed Ni (3.77%), S (35.04%), Fe (56.70%) and Cu (1.32%) as the major elements and X-ray diffraction (XRD) analysis revealed that the ore was comprised primarily of pyrrhotite, chalcopyrite, pentlandite and sulfur. The mineral samples were sterilized by steam autoclaving (Karan et al., 1996) at 121 °C for 30 min to ensure the absence of external bacterial contamination.

2.2. Microbes and culture conditions

Four thermophiles used in this study were *A. brierleyi* JCM 8954, *A. manzaensis* YN25, *M. sedula* JCM 9185 and *S. metallicus* JCM 9184, among which *A. manzaensis* YN25 was isolated from an acidic hot spring sample in Tengchong, Yunnan Province, Southwest China and conserved by the Key Laboratory of Biohydrometallurgy, Ministry of Education of China, Central South University, China. And the other three strains were purchased from Japan Collection of Microorganisms (JCM).

These strains were grown in 9 K basal medium (3 g/L $(NH_4)_2SO_4$, 0.1 g/L KCl, 0.5 g/L K_2HPO_4, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.01 g/L $Ca(NO_3)_2$, 44.7 g/L$Fe_2SO_4{\cdot}7H_2O$) with sulfur (10 g/L) and yeast extract (0.2 g/L) as energy source.

2.3. Mixed culture bioleaching experiments

Cells of four species were collected respectively by centrifuging at later log phase and suspended in sterile basal salt medium (pH 1.5) to an initial density of 4×10^8 cells/mL to serve as inoculum. All bioleaching trials were carried out in triplicate in 250 mL flasks containing 90 mL 9 K basal medium (pH 1.5) with 2% wt/vol nickel–copper sulfide and inoculated with 10 mL of inoculum as described above, except for abiotic control flasks which were supplemented with 10 mL of sterile basal salt medium (pH 1.5). Experiments were conducted in the absence and presence of 0.2 g/L of L-cysteine. The cultures were incubated at 65 °C, shaken at 180 rpm. The experiments lasted for 16 days. Water evaporation during bioleaching was compensated for with sterilized distilled water.

2.4. Analytical methods

The progress during bioleaching process was monitored by sampling every two days. Each sampling would remove three identical flasks. Ni and Cu concentrations in solution were determined using atomic absorption spectrometry; the pH value and ORP in the leaching solution was measured with a pH-meter (PHSJ-4A); the density of microbes was monitored by microscopic counting method using a counting chamber and a general microscope (Olympus CX31); cells were also harvested by centrifugation and nucleic acid was extracted for monitoring microbial community successions by 16S rRNA-based PCR–DGGE. The chemical compositions of the bioleached residues after 16 days bioleaching were analyzed by X-ray diffraction (XRD) analysis.

2.5. Preparation of DNA

Harvest of cells from experimental samples and extraction of nucleic acids were done according to the procedure described in He et al. (2010). Harvested cells were mixed with 13.5 mL extraction buffer (0.1 M phosphate, pH 8.0, 0.1 M EDTA, 0.1 M EDTA, 1.5 M NaCl, and 1% CTAB) and 50 μL protenase K (10 mg/mL) in a 50 mL centrifuge tube, then incubated at 37 °C for 30 min. 1.5 mL of 20% SDS was added and mixed gently, then incubated at 65 °C for 2 h. The mixture was centrifuged and the supernatant was transferred into a new 50 mL centrifuge tube. The crude DNA