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Thermophilic archaeal community succession and function change associated with the leaching rate in bioleaching of chalcopyrite

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HIGHLIGHTS

- A first study on community succession and function associated with chalcopyrite leaching rate.
- *Sulfolobus metallicus* was most sensitive to the environmental change.
- *Acidianus brierleyi* showed the best adaptability and highest sulfur oxidation ability.
- The bioleaching rate correlated closely with the consortium function embodied by *soxB* gene.

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ABSTRACT

The community succession and function change of thermophilic archaea *Acidianus brierleyi*, *Metallosphaera sedula*, *Acidianus manzaensis* and *Sulfolobus metallicus* were studied by denaturing gradient gel electrophoresis (DGGE) analysis of amplifying 16S rRNA genes fragments and real-time qPCR analysis of amplifying sulfur-oxidizing *soxB* gene associated with chalcopyrite bioleaching rate at different temperatures and initial pH values. The analysis results of the community succession indicated that temperature and initial pH value had a significant effect on the consortium, and *S. metallicus* was most sensitive to the environmental change, *A. brierleyi* showed the best adaptability and sulfur oxidation ability and predominated in various leaching systems. Meanwhile, the leaching rate of chalcopyrite closely related to the consortium function embodied by *soxB* gene, which could prove a desirable way for revealing microbial sulfur oxidation difference and tracking the function change of the consortium, and for optimizing the leaching parameters and improving the recovery of valuable metals.

1. Introduction

The mesophilic microorganisms have been widely used in the hydrometallurgical field in the past several decades (Rawlings, 2002). However, increasing number of researchers have been interested in using moderately and/or extremely thermophilic organisms to improve the dissolution rate of valuable metals (Konishi et al., 2001; Qin et al., 2013; Wang et al., 2012), due to exothermic reaction during bioleaching that results in temperature increasing to 50 °C or greater in the pregnant leach solution (Riekkola-Vanhanen, 2007). As reviewed in the literatures (Konishi et al., 2001; Schnell and Rawlings, 1997), the thermophiles, including *Acidianus brierleyi*, *Acidianus manzaensis*, *Metallosphaera sedula* and *Sulfolobus metallicus*, have inherent advantages for industrial applications of metal extraction from various sulfide minerals when the temperature rises over 60 °C. In our previous work, the result was also confirmed (Zhu et al., 2011).

Previous studies had mainly focused on the aspects of leaching behavior and leaching mechanism of thermophilic archaea, while few information about their community succession and function change in the bioleaching of sulfide minerals had been revealed (Qiu et al., 2008). Various kinds of microbe shows distinct metabolic activity at different conditions, the environmental factors exercise great impact on the structure and function of microbial community (Li et al., 2011). Moreover, the current methods of characterizing the microbial metabolic activity, such as pH value, sulfate ion concentration and cell concentration are often affected by many factors, and it is hard to accurately reflect the role of a specific species of microbe. However, this information is very important for optimizing the leaching parameters and improving the recovery of valuable metals, therefore, it is necessary to find a desirable method to efficiently characterize the role of various microbes at different conditions during bioleaching processes.

In some species of bacteria, sulfur oxidation is catalyzed by sulfur-oxidizing (Sox) enzyme systems (Chen et al., 2007). Sox is a well-characterized multi-enzyme system for thiosulfate oxidation, and is capable of oxidizing various reduced sulfur compounds

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(hydrogen sulfide, elemental sulfur, thiosulfate, and sulfite) to sulfate (Harada et al., 2009). As one of the most important member of Sox multi-enzyme system, SoxB is not only essential for thiosulfate oxidation but also responsible for sulfide oxidation (Azai et al., 2009), and its detection in a sulfur-oxidizing microbe might be used as an indicator for the presence of components of the Sox enzyme system and a first indication for evaluating some microbial function in sulfide oxidation (Pandey et al., 2009). The Sox enzymes of mesophilic and neutrophilic bacteria have been found and well characterized. But little information is available from the acidophilic bacteria and archaea that widely used in bioleaching (Chen et al., 2007). The partial *soxB* gene has been detected in cloning of the terminal oxidase genes of *M. sedula* (Auernik et al., 2008; Kappler et al., 2005). Interestingly, we have found in our previous research that there was an identical related sulfur-oxidizing *soxB* gene existed in thermopiles *A. brierleyi*, *A. manzaensis*, *M. sedula* and *S. metallicus*, and confirmed *soxB* gene of four thermophilic archaea could oxidize sulfur with similar physiological basis.

At present, many techniques have been used in the analysis of microbial community structure (Johnson, 2001), of which the denaturing gradient gel electrophoresis (DGGE) technique is a fingerprint identification technology that widely applied in dynamic analysis of microbial community diversity, but it cannot provide the cell growth profiles and information of functional gene expression (Xing and Ren, 2006). Real-time qPCR is a reliable technology to study the microbial community in bioleaching environments. Though real-time qPCR cannot distinguish some microorganisms with very high similarity, it is a useful tool to assist microbial function analysis, and to further understand microbial leaching capacity (Chen et al., 2009). Therefore, by combining the DGGE method and real-time qPCR technique that monitor the changes of community succession and function of the consortium, further understanding to the contributions of each specific species to the bioleaching, and the critical role of functional gene in bioleaching of chalcopyrite could be obtained.

In this paper, a predefined thermophilic archea consortium consisting of *A. brierleyi*, *M. sedula*, *A. manzaensis* and *S. metallicus* was used to leach chalcopyrite. DGGE method was used to investigate the thermophilic archaeal community succession, and real-time qPCR technique was applied to monitor the expression level of *soxB* gene, which was used to characterize the function change of the consortium during bioleaching of chalcopyrite. Finally, the relationship between the function change of the consortium and the leaching rate of chalcopyrite was analyzed and the effect of environmental factors was studied.

2. Methods

2.1. Microorganisms and culture media

Four thermophilic archea used in this work were *A. brierleyi* (JCM 8954), *M. sedula* (YN 23), *A. manzaensis* (YN 25) and *S. metallicus* (YN 24), which were conserved by the Key Laboratory of Biometallurgy, Ministry of Education of China, Central South University, China. The basal medium used in this study contained 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 $Ca(NO_3)_2$ with 0.2 g/L yeast extracts.

2.2. Mineral samples

The mineral samples were collected from Dexing in Jiangxi province, China. The mineral consisted of chalcopyrite (80%), pyrite (5%), quartz (5%) and others (5%). The main contents of mineral were (mass fraction): Cu 32.02%, Fe 30.90% and S 22.65%. The mineral was grinded to fine powder with the size less than 75 μm.

2.3. Bioleaching experiments

Bioleaching experiments were carried out with the consortium in 250 mL Erlenmeyer flasks at 65 °C with initial pH (1.0, 1.5, 2.0) and with initial pH 1.5 at temperature (55, 65 °C) on a rotary shaker at 180 rpm. The flasks contained 100 mL of basal medium supplemented with 10 g/L chalcopyrite as the sole energy source. The initial cell density was approximately 1×10^8 cells/mL. The initial pH of the culture was adjusted with diluted sulfuric acid. All experiments were performed in triplicate at the same conditions and the abiotic control was also run. Evaporated water was compensated by additional distilled water. Samples were taken at regular intervals to analyze the cell density, copper concentration, community succession and function change of the consortium during bioleaching experiments.

The cell density was determined by direct counting with a Neubauer chamber counter. Copper concentrations in solution were determined by atomic absorption spectrophotometry. The community succession and function change of the consortium were analyzed with the methods described in Sections 2.4 and 2.5.

2.4. Community structure analysis

2.4.1. Preparation of DNA

In order to sample with sufficient representation, the free and attached microorganisms of three parallel flasks were used to extract the DNA every 2 days. The separation method of attached microorganisms was described by Zeng et al. (2010). The leaching solution of samples was allowed to settle for 1.5 h and the supernatant was decanted. Then the pellets were re-suspended with 10 mL Milli Q water in a 50 mL centrifuge tube. After this, 1 g of glass beads with a diameter of 0.2 mm was added into the tube. The tube was pressed on a vortexer for 8 min of vigorous vortexing. Then about 36 mL Milli Q water was added into the tube and the vortexing continued for 2 min. After this, the 50 mL mixture was centrifuged at 2000*g* for 2 min to separate the ore residue and solution. About 0.1 mL of the supernatant was used to count the cell number under the optical microscope and the others were transferred to a 250 mL flask. The ore residue was used again, underwent vigorous vortexing for 10 min and centrifugation at 2000*g* for 2 min, followed by cell collection and counting. After this, the remaining ore residue was then washed repeatedly until no bacteria remained. Solutions washed from the pellets were mixed together with the supernatant, and centrifuged at 10000*g* for 10 min at 4 °C to collect the cell.

Total DNA was extracted from the cell pellet of previous collection by washing twice in 10 mM Tris containing 10 mM EDTA pH 8.5. Subsequent steps for DNA extraction were described previously (Zhou et al., 2007). The crude DNA was purified by using Wizard plus SV Minipreps DNA purification system (Promega Corporation, USA) and stored at −20 °C.

2.4.2. Primers and PCR amplification for DGGE

The 16S rRNA gene fragments were amplified with primers ARCH 344F-GC (5′-ACGGGGTGCAGGCGCGA-3′) and ARCH 915R (5′-GTGCTCCC CCGCCAATTCCT-3′), which are for universal primers for conserved archaeal 16S rRNA (Casamayor et al., 2002). A 40-bp GC-rich sequence (GC-clamp: 5′-CGCCCGCCGCGCCCCGCGCCCGTC CCGCCGCCCCCGCCCG-3′) was attached to the 5P end of primer 344F-GC to prevent complete melting of the DNA fragments during the DGGE analysis. The 16S rRNA gene fragments (around 600 bp) suitable for subsequent denaturing gradient gel electrophoresis (DGGE) analyses were obtained by PCR as previously described (Karr et al., 2005). PCR amplification reactions were performed with the reagents supplied with T-Gradient ThermoBlock (Biometra, Germany). A hot start PCR was performed at 95 °C for 5 min