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Bioleaching of chalcopyrite by a moderately thermophilic culture at different conditions and community dynamics of planktonic and attached populations

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ABSTRACT

A moderately thermophilic culture was used to bioleach chalcopyrite. It showed a good performance in copper extraction. In order to further improve efficiency of chalcopyrite bioleaching, effects of pH control and redox potential (ORP) control on chalcopyrite dissolution were investigated. The community dynamics of planktonic and attached populations were also monitored during bioleaching of chalcopyrite at different conditions. The copper extraction was improved by controlling pH or ORP, especially in the final stage of the bioleaching. The maximal growth rate of microorganisms was up to 0.94 generations/day when the pH was controlled in the range of 1.40–1.85. The ORP controlled at 420 ± 20 mV caused a reduced jarosite formation. Community dynamics analyses show that the pH control and the ORP control had significant effects on community dynamics of planktonic and attached moderate thermophiles. The species contained in the culture showed different succession trends compared with each other, not only in the leachate but also on the mineral surfaces. The pH control was not favorable for the attachment of microorganisms. It can also be found that succession of attached cells is significantly different from the community dynamics for their planktonic counterparts.

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1. Introduction

Bioleaching of sulfide minerals continues to attract global attention due to its unparalleled advantages, such as low capital investment, environment friendly, mild reaction and low energy consumption. The technology has been widely employed to extract gold, copper, cobalt, nickel, zinc, uranium, etc. (Petersen and Dixon, 2006). However, bioleaching of chalcopyrite on an industrial scale is still immature due to slow kinetics and poor dissolution, although many laboratorial and pilot scale processes have been tested to enhance copper extraction from chalcopyrite (Dreisinger, 2006; Watling, 2006). Several hypotheses have been proposed to account for these phenomena, such as formation of passivation layers on the surfaces, very stable structural configuration of chalcopyrite, and high lattice energy (Dutrizac, 1981; Marhual et al., 2008). The optimization of process by operating parameters is one of classical approaches to improve performances of chalcopyrite bioleaching. It has been shown that pH and redox potential (ORP) have significant effects on chalcopyrite bioleaching (Plumb et al., 2008; Pogliani and Donati, 2000; Third et al., 2002).

In addition, it is believed that microorganisms sharing the same environment during bioleaching interact with each other and variations of physical and chemical parameters have significant effects on microbial community structure and dynamics (Johnson, 1998, 2001). However, the data on microbial populations in bioleaching systems are very limited and most of previous studies did not investigate the relationship between bioleaching performance, microbial community dynamics and changes in process operating conditions (d'Hugues et al., 2008; Okibe et al., 2003). Furthermore, in most cases, studies of community dynamics or diversity during bioleaching were mainly focused on planktonic microorganisms. However, attached microorganisms on the mineral surfaces also play important roles in dissolution of sulfide minerals, and microenvironments confronted by attached and planktonic cells are different from each other (Gautier et al., 2008; Sand and Gehrke, 2006). Thus, to obtain a complete comprehension, it is also very important to investigate how the attached community on the mineral surfaces develops during bioleaching of sulfide minerals.

The aim of the present work was to investigate the effects of pH and ORP on copper extraction and community dynamics during bioleaching of chalcopyrite by a moderately thermophilic culture. The real-time

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quantitative PCR technology was used to monitor community dynamics of predominant species suspended in the leachate and attached to the mineral surfaces.

2. Materials and methods

2.1. The moderately thermophilic culture

The moderately thermophilic culture used in this study was as described by our previous study (Wang et al., 2014). It consisted of three species, including *Acidithiobacillus caldus*, *Sulfobacillus acidophilus* and *Ferroplasma thermophilum*.

2.2. Mineral components

The chalcopyrite used in this study was obtained from the Dong Shengmiao Copper Mine in Inner Mongolia, China. X-ray diffraction (XRD) analysis shows chalcopyrite (60%, w/w), sphalerite (15%) and pyrrhotite (10%) as the major components and galena (5%) and quartz (3%) as the minor ones. The main chemical composition of the concentrate is (w/w) 18.97% Cu, 24.20% Fe, 28.17% S, 4.03% Pb, 5.00% Zn and 0.34% Ag.

2.3. Bioleaching experiments

Bioleaching experiments were carried out in a laboratory scale jacketed stirred-tank reactor with a total volume of 12.5 L. Experimental conditions were as follows: work volume, 8 L; pulp density, 10% (w/v); agitation rate, 200 rpm; temperature, 45 °C; air flow, 200 mL/h/L; initial cell density, 1×10^7 cells/mL. To investigate the effects of pH on copper extraction and community dynamics, the pH was maintained in the range of 1.40–1.85 by adding 9 M H_2SO_4 or 10 M NaOH. To evaluate the effects of ORP on copper extraction and community dynamics, the ORP was controlled at 420 ± 20 mV by adjusting agitation rate and air flow. Bioleaching of chalcopyrite without pH control and ORP control acted as control experiment.

The reactor was monitored at regular intervals for pH, ORP, cell density, dissolved oxygen (DO), iron concentration and copper extraction. The loss of water and taken sample from the bioleaching system was compensated by adding water and fresh medium, respectively. Community dynamics was analyzed every five days by real-time quantitative PCR (qPCR).

2.4. Physicochemical analysis

The concentrations of copper and total iron were measured by atomic absorption spectrometry. The ferrous iron concentration was determined by titration with potassium dichromate. The ferric iron concentration was determined by subtracting the ferrous iron from the total iron. The cell density was determined by direct counting using optical microscope with Tween-20 as described by Marhual et al. (2008). And the pH meter was adopted to measure pH value. The DO was determined by a polarographic oxygen probe. The ORP value was measured using a platinum electrode with an Ag/AgCl reference electrode. The residues after leaching were analyzed by XRD.

2.5. Community dynamics analyses by qPCR

Leachate (50 mL) containing mineral particles and microorganisms was centrifuged at 3000 × *g* for about 5 min and then the liquid phase was transferred into a new centrifuge tube. The attached cells of particulate phase were removed as described by Zammit et al. (2011). After that, DNA extraction of attached and planktonic cells, and procedures for the detection of specificity of primers and for qPCR were as described by Zhang et al. (2009). The primers for corresponding species used in this study were as listed in Table 1. Variance analysis and principal component analysis were performed by SPSS version 20.0.

3. Results and discussion

3.1. Bioleaching of chalcopyrite by a moderately thermophilic culture at controlled pH

It is believed that pH has a significant effect on the iron(II) ion-oxidizing capacity of acidophiles (Plumb et al., 2008). Most iron-oxidizers for bioleaching prefer to grow at pH 1.5–2.0 (Johnson and Hallberg, 2003). However, bioleaching of chalcopyrite is characterized by steep pH change (from higher than 2.0 to lower than 1.0). Furthermore, ferric iron is the major oxidizing agent for chalcopyrite dissolution (Sand et al., 2001). Thus, it is necessary to control pH at specific range for improving chalcopyrite bioleaching kinetics. In this study, the pH was maintained in the range of 1.40–1.85 by adding 9 M H_2SO_4 or 10 M NaOH.

It can be found from Fig. 1 that the pH controlled at 1.40–1.85 could avoid dramatic pH change, which was beneficial to the growth of microorganisms. As can be seen in Fig. 2, there was no obvious lag phase in the growth of microorganisms when the pH was controlled and cell density was up to 1×10^8 cells/mL on day 4, while the growth of microorganisms was still in the lag phase when the pH was not controlled. The growth rate was only 0.58 generations/day when the pH was not adjusted in the first 6 days compared to 0.94 generations/day at controlled pH. However, the growth rate began to decline at a very high rate from day 7 at controlled pH and the growth of microorganisms went into stationary phase at that point in time. Furthermore, cell density declined from about 9×10^8 cells/mL on day 15 to 5×10^8 cells/mL at the end of the run when the pH was controlled.

Although microorganisms grew well when the pH was controlled, copper extraction did not increase greatly in the initial stage compared with that at uncontrolled pH (Fig. 3). This was probably related to high sulfur-oxidizing activity, which could be confirmed by variation of acid consumption in the initial stage (Fig. 1). Acid consumption increased significantly in the first 5 days. After that, acid consumption decreased, and it was no longer necessary to add H_2SO_4 after day 6. It is believed that acid production exceeded acid consumption, which resulted from microbial sulfur oxidation. However, sulfur oxidation cannot contribute to dissolution of sulfide minerals directly (Dopson and Lindstrom, 1999). In addition, there was no significant difference in ORP between

Table 1
The primers used in this study for analyses of community dynamics.

Primers[a]	Target species	Primer sequences (5′–3′)	Target gene	Expected amplicon length
NR-R2[b]	–	AGCTGRCGACRRCCATGCA	16S rRNA gene	–
Acaldus-P1	*A. caldus*	TTGGCGCCTTAGGTGCTGA		239 bp
Fer-P1	*F. thermophilum*	CCCACTTTGATGTTGCTTTTCCG		248 bp
Sacid-F	*S. acidophilus*	ACGTAGCGGTTTTCAGCC		244 bp
Sacid-R		GACACCTCGTATCCATCGTTTAC		

[a] NR-R2 and Acaldus-P1 were referenced by Liu et al. (2006). Fer-P1, Sacid-F and Sacid-R were referenced by Zhang et al. (2009).
[b] Universal primer for *A. caldus* and *F. thermophilum*.

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