Bioresource Technology 223 (2017) 121–130

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Co-culture microorganisms with different initial proportions reveal the mechanism of chalcopyrite bioleaching coupling with microbial community succession

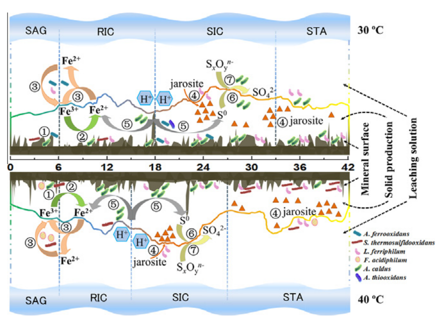
Liyuan Ma [a,b], Xingjie Wang [a], Xue Feng [a], Yili Liang [a,b], Yunhua Xiao [a,b], Xiaodong Hao [a,b], Huaqun Yin [a,b], Hongwei Liu [a,b], Xueduan Liu [a,b,*]

[a] School of Minerals Processing and Bioengineering, Central South University, 410083, China
[b] Key Laboratory of Biometallurgy of Ministry of Education, 410083, China

HIGHLIGHTS

- Effect of different initial microbial proportions on $CuFeS_2$ bioleaching was studied.
- More sulfur oxidizers lead to higher Cu recovery than more ferrous oxidizers.
- Succession of free and attached cells with different initial proportions was studied.
- A model for $CuFeS_2$ bioleaching was established coupling with community succession.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 August 2016
Received in revised form 12 October 2016
Accepted 19 October 2016
Available online 21 October 2016

Keywords:
Chalcopyrite bioleaching
Initial proportions
Microbial community succession
Iron/sulfur metabolism

ABSTRACT

The effect of co-culture microorganisms with different initial proportions on chalcopyrite bioleaching was investigated. Communities were rebuilt by six typical strains isolated from the same habitat. The results indicated, by community with more sulfur oxidizers at both 30 and 40 °C, the final copper extraction rate was 19.8% and 6.5% higher, respectively, than that with more ferrous oxidizers. The variations of pH, redox potential, ferrous and copper ions in leachate also provided evidences that community with more sulfur oxidizers was more efficient. Community succession of free and attached cells revealed that initial proportions played decisive roles on community dynamics at 30 °C, while communities shared similar structures, not relevant to initial proportions at 40 °C. X-ray diffraction analysis confirmed different microbial functions on mineral surface. A mechanism model for chalcopyrite bioleaching was established coupling with community succession. This will provide theoretical basis for reconstructing an efficient community in industrial application.

1. Introduction

The majority of copper reserves in the world are bound in the sulfide mineral chalcopyrite ($CuFeS_2$). As an economical and environmental friendly method, chalcopyrite bioleaching continues attracting much attention because non-renewable mineral resources are exploited and depleted unceasingly (Panda et al., 2015). However, the dissolution of chalcopyrite in acid solution is an extremely slow process due to its high lattice energy (Klauber, 2003; Watling, 2013). As a result, the practical application of chalcopyrite bioleaching is limited.

* Corresponding author at: School of Minerals Processing and Bioengineering, Central South University, 410083, China.
E-mail address: xueduanliu@126.com (X. Liu).

http://dx.doi.org/10.1016/j.biortech.2016.10.056
0960-8524/

Many considerable efforts have been devoted to enhance the bioleaching efficiency, including using photocatalysis to reduce ferric iron to ferrous iron as metabolic substrates (Zhou et al., 2015), controlling the redox potential to limit the formation of passivation layer (Córdoba et al., 2008; Zhao et al., 2015), or inventing new method using BACFOX bioreactor by the direct removal of surface passivation layer (Panda et al., 2013). At the same time, many researchers attempted to provide insight into the microbial community linked to different environmental stress during bioleaching (He et al., 2010; Watling et al., 2010). More than 40 types of bioleaching microbial species have been discovered in the leaching system (Edwards et al., 1999; Panda et al., 2015). According to their functional categories, acidophiles can be classified into ferrous oxidizer, sulfur oxidizer and ferrous/sulfur oxidizer (Méndez-García et al., 2015). Previous studies have demonstrated that the mixed cultures were more efficient than pure cultures on leaching performance (Li et al., 2014; Qiu et al., 2005). The dominant strains applied in different bioleaching systems were always differential owing to the differences of environmental pH, temperature and mineral properties. As a whole, it can be generally classified according to their functional categories, as *Leptospirillum ferriphilum* and *Ferroplasma acidiphilum* (ferrous oxidizer), *Acidithiobacillus caldus* and *A. thiooxidans* (sulfur oxidizer), *A. ferrooxidans* and *Sulfobacillus thermosulfidooxidans* (ferrous/sulfur oxidizer) (Chen et al., 2014; Gonzalez-Toril et al., 2003; Shahrabi-Farahani et al., 2014). The synergistic function between different type strains was beneficial for better balancing iron and sulfur metabolism (Behera et al., 2011). However, the communities used in researches were either too simple by only one or two strains (Feng et al., 2016) or too complicated by natural samples (Liu et al., 2016). The former was not credible for industrial application, and the latter usually omit the functions of rare species that indeed existed in the community. Few researches have reported the effect of initial proportions of microorganisms isolated from the same habitat on chalcopyrite leaching.

Breeding excellent strains and optimizing microbial structure were effective ways to improve the microbial function. As the dynamics of different oxidizers in a community was closely related to the process of chalcopyrite dissolution, it is meaningful to investigate the effect of different microbial proportions in a community on chalcopyrite bioleaching. Both the adsorption capacity of attached cells on mineral surface and the oxidative activity of free cells in leachate were critical to the leaching efficiency, so evaluating the free and attached microbial community succession was meaningful to reveal the mechanism of chalcopyrite bioleaching (Zeng et al., 2010). In the previous work, six strains isolated from different habitat have been combined and inoculated into chalcopyrite to access the leaching efficiency and microbial community structure roughly (Feng et al., 2015b). Herein, in order to eliminate the unexpected genetic divergence of strains from different localities, a microcosm by six typical strains isolated from the same habitat was reconstructed. The microcosm was used to reveal the mechanism of chalcopyrite bioleaching coupling with microbial community succession based on different initial microbial proportions. Considering that temperature variation displayed a great influence on microbial community (Ma et al., 2013), two temperature gradient was set at 30 °C and 40 °C.

2. Materials and methods

2.1. Minerals and microorganisms

The chalcopyrite used in this study was ground and sieved to less than 74 μm in particle diameter. Elemental composition was analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES, PS-6, Baird, USA), and mineralogical analysis was carried out by X-ray diffraction (XRD, D/Max 2500, Rigaku, Japan). The results showed that Fe (29.59%), Cu (30.74%), and S (25.73%) were the major elements, and the mineral sample mainly comprised of chalcopyrite.

Six typical bioleaching strains, according to their different types of energy utilization, were used to rebuild four artificial co-culture systems based on different initial microbial proportions. They were *A. ferrooxidans* DX-m (GenBank accession No. KX694508), *S. thermosulfidooxidans* DX-m (KX694510), *L. ferriphilum* DX-m (KX694509), *F. acidiphilum* DX-m (KX694511), *A. caldus* DX-m (KX694512) and *A. thiooxidans* DX-m (KX694513), which were all isolated from the acid mine drainage of Dexing copper mine, China. The pure culture conditions of each strain were listed in Table 1. The 9K basal medium contains of the following ingredients (g/L): $(NH_4)_2SO_4$ (3), K_2HPO_4 (0.5), KCl (0.1), $Ca(NO_3)_2$ (0.01), $MgSO_4{\cdot}7H_2O$ (0.5).

2.2. Bioleaching experiment

To avoid external bacterial contamination, the mineral samples and 100 mL 9K medium were sterilized by autoclaving (Karan et al., 1996) at 110 °C for 40 min and 121 °C for 25 min, respectively. Then, they were mixed in 250 mL shake flasks after cooling down. Acid pre-leaching was introduced until the pH was stabilized at 2.0 (adjusted by 10% H_2SO_4). The chalcopyrite bioleaching experiments were conducted with initial cells density of 6×10^6 cells/mL and pulp density of 3% (w/v). According to their different types of energy utilization, *A. ferrooxidans* DX-m (ferrous and sulfur), *S. thermosulfidooxidans* DX-m (ferrous and sulfur), *L. ferriphilum* DX-m (only ferrous), *F. acidiphilum* DX-m (only ferrous), *A. caldus* DX-m (only sulfur) and *A. thiooxidans* DX-m (only sulfur) were classified to construct four microbial groups: I, 1:1:1:1:1:1; II, 10:10:1:1:1:1; III, 1:1:10:10:1:1; IV, 1:1:1:1:10:10 (cell amount). Considering temperature variation displayed a great influence on microbial community, the four co-culture groups and one abiotic control were incubated at 30 °C and 40 °C, respectively. All ten leaching reactions were undertaken on a rotary platform at 175 rpm for 42 days. During leaching process, distilled water and 9K medium were added periodically to compensate for the evaporation loss and sampling loss. All experiments were performed in triplicate with three flasks per treatment.

2.3. Analytical methods

2.3.1. Analysis of main chemical parameters

The physicochemical parameters were measured during the chalcopyrite bioleaching. The supernatant was withdrawn every three days and was analyzed for the concentration of dissolved copper and ferrous iron using BCO spectrophotometry and byophenanthroline spectrophotometry, respectively. In addition, pH was measured by a digital pH meter (PHSJ-4A, Leici, Shanghai, China) and oxidation-redox potential (ORP) of leaching solution was measured by a platinum electrode (213-01, Leici, Shanghai, China) with an Ag/AgCl reference electrode (218, Leici, Shanghai, China). Residue samples were collected and weighed regularly for ICP-AES and copper extraction rate calculation, XRD analysis was carried out for detecting the mineral constituent.

2.3.2. Analysis of community succession in co-culture systems

The leaching sample (5.0 mL) for each of the four groups was collected and centrifuged at 2000×g for 2 min for separating supernatant from mineral precipitation. The supernatant containing free cells was transferred thoroughly. The bottom mineral sample was re-suspended using 25 mL fresh media. Then, 1 g of 0.2 mm glass beads was added and shaken on a vortexer for