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Bioleaching of the mixed oxide-sulfide copper ore by artificial indigenous and exogenous microbial community

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ABSTRACT

Bioleaching has been widely applied to recover sulfide minerals, however, little is known about the bioleaching behavior and mechanism of mixed oxide-sulfide copper ore with high-oxidative ratio. The leaching efficiency of artificial indigenous and exogenous microbial communities on a mixed oxide-sulfide copper ore with and without energy substrate was investigated. The results indicated that extra energy substrate was needed when bioleaching was applied to this kind of ore. The variation of leaching parameters caused by indigenous community was inferior to that caused by exogenous ones due to its lower activity. The leaching efficiency of sulfide part induced by indigenous strains was superior to that induced by exogenous ones, due to the stronger colonization ability of indigenous strains. It verified that both microbial activity and colonization ability were important properties affecting leaching efficiency. Sulfur oxidizers, which can oxidize sulfur or polysulfide formed on mineral surface and decrease leachate pH, played a more important role than ferrous oxidizers in bioleaching of high-oxidative oxide-sulfide ore.

1. Introduction

As non-renewable mineral resources are exploited and depleted unceasingly, it is becoming inevitable to make efficient utilization of low grade and complex refractory ores. Partially oxidative sulfide copper ore is an important copper bearing mineral. A traditional method for the recovery of this kind of mineral is sulfuration flotation, which is difficult in maintaining the flotation indexes due to oxidation rate fluctuation of raw ore (Ou and Yin, 2011). Bioleaching has been illustrated as an effective way in recovering partially oxidative sulfide ore, and different to traditional treatment of completely sulfide minerals, external energy source was sometimes needed during the bioleaching procedure (Chen et al., 2011; Li et al., 2014; Nguyen et al., 2015).

More than 40 types of bioleaching microbial species have been discovered (Baba et al., 2011). The dominant strains are iron-oxidizing species including *Leptospirillum ferriphilum* (*L. ferriphilum*) and *Ferroplasma thermophilum* (*F. thermophilum*), sulfur-oxidizing species like *Acidithiobacillus caldus* (*A. caldus*) and iron/sulfur-oxidizing species including *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) and *Sulfobacillus thermosulfidooxidans* (*S. thermosulfidooxidans*). Previous studies mainly focused on the aspect of isolating or domesticating microorganisms with higher leaching ability and exploring their leaching behavior and mechanism (Feng et al., 2012; Giaveno et al., 2013; Rawlings and Johnson, 2007; Watling et al., 2008). It has been demonstrated that the main role of iron oxidizers was to generate ferric iron to dissolve sulfide minerals, but the iron oxidizers would also lead to accumulation of elemental sulfur or polysulfide on the mineral surface. The accumulation on the mineral surface acted as a barrier against the diffusion of oxygen or Fe (III) ions and inhibited the complete dissolution of minerals. Different to the iron oxidizers, sulfur oxidizers can remove elemental sulfur or polysulfide and decrease pH value due to their ability of oxidizing elemental sulfur to sulfuric acid (Rohwerder et al., 2003). As an effective way to improve the sulfide leaching efficiency, sulfur and iron-oxidizing microorganisms are often mixed and inoculated into leaching systems by means of their cooperative bioleaching (Li et al., 2014; Xia et al., 2008; Zeng et al., 2010). The comparison of community structures of free or attached microorganisms, bacteria or archaea, and some consortia constructed under different inoculation conditions has been described in detail to reveal the community assembly rules (Akinci and Guven, 2011; Feris et al., 2003; Fuhrman, 2009; Lucheta et al., 2013; Watling et al., 2013; Zeng et al., 2010). It has been reported that the consortia of mesophiles and moderate thermophiles cultivated at 35–45 °C tend to be dominated by the iron-oxidizing *L. ferriphilum* and the sulfur-oxidizing *A. caldus* (Rawlings and Johnson, 2007).

Employing native microorganisms is another effective way to improve leaching efficiency. Numerous researchers have obtained higher

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leaching efficiency by using indigenous microorganisms (Bryan et al., 2011; Giaveno et al., 2007). For example, empirical evidence suggested that an indigenous microbial community was superior to the designed consortia when tested on a cobaltiferous pyrite due to the high potential adaptation to the mineral surface of indigenous organism which was called microbial colonization (Bryan et al., 2011; Chiume et al., 2012). However, little is known about the leaching efficiency of the community constructed by the same species from different locations on oxide-sulfide copper ore with high-oxidative ratio.

This work was the first investigation conducted on the leaching efficiency of artificial indigenous and exogenous microbial communities on a highly oxidized and low-grade oxide-sulfide copper ore (oxidation ratio 89.9%). The influence of energy substrate on the bioleaching of high-oxidative oxide-sulfide copper ore and its mechanism was clarified. Furthermore, the microbial properties of colonization and activity which was correlated with leaching efficiency were compared between indigenous and exogenous cultures, according to the leaching results and the community structures.

2. Materials and methods

2.1. Mineral components

Mineral sample was collected from Mulyashi, Zambia's Luanshya Copper Mines and ground to less than 74 μm in diameter. The chemical components of the ore sample were Cu 1.53%, Co 0.024%, Fe 3.48%, S 0.092%, P 0.053%, Mn 0.09%, CaO 1.14%, MgO 5.16%, K_2O 8.56, Na_2O 0.31%, Al_2O_3 15.13% and SiO_2 57.32%. Copper phase composition analysis by chemical phase analysis showed that the ore belong to high-oxidative oxide-sulfide ore with an oxidation ratio of 89.9% (Table 1). The X-ray diffraction analysis of the sample showed the main gangue minerals were feldspar, quartz, mica and pyrophyllite. The mineral samples were sterilized by ultraviolet.

2.2. Microbial strains and co-culture communities

The typical bioleaching strains in the exogenous consortia were isolated from Acid Mine Drainages (AMD) from Dexing copper mine (Jiangxi province, China) and were identified by 16S rDNA. The strains including *A. ferrooxidans*, *S. thermosulfidooxidans*, *L. ferriphilum*, *F. thermophilum* and *A. caldus* were mixed in a ratio of 1:1:1:1:1 after cultured respectively in 9 K base medium (Ma et al., 2017). Similarly, five corresponding species isolated from AMD of Zambia's Luanshya Copper Mines were treated in the same way to reconstitute an indigenous co-culture system. Comparison of the activity among the five indigenous and exogenous strains in pure culture was carried out in base medium with 44.7 g/L $FeSO_4$ or 10 g/L S^0 as energy substrate.

2.3. Microbial attachment on the sulfide mineral surface

Chalcopyrite was used as a typical sulfide mineral to evaluate the microbial attachment ability. Attachment experiments were performed in 100-mL shake flasks, each containing 25 mL iron-free 9 K medium and 5 g of chalcopyrite at pH 2.0. Another 25 mL iron-free 9 K medium inoculated with 2×10^9 cells/mL indigenous or exogenous microorganism was added to each culture, and the final bacterial concentration in the culture was 10^9 cells/mL. Incubation was carried out at 35 °C and 175 rpm. Cell numbers were determined at 0, 5, 10, 20, 30, 60, 90, 120 and 180 min by blood cell counting chambers (XB- K-25, QiuJing, Shanghai, China) under a phase contrast microscope (BX-41TF, Olympus, Tokyo, Japan). The number of cells attached to the mineral surface was calculated by the difference between the number of initial inoculated cells and the number of free cells.

Table 1
Copper phase compositions of the oxide-sulfide copper ore.

Phase	Mass fraction/%
Free oxidized copper	56.68
Conjunction oxidized copper	33.22
Secondary copper sulfide	5.47
Primary copper sulfide	4.63
Total	100.00

2.4. Leaching experiments

Indigenous and exogenous communities were added to the leaching pulp with or without the additional energy substrate. The energy substrate contained 4.47 g/L $FeSO_4$ and 1 g/L S^0. The bioleaching experiment was carried out in 500-mL shake flasks containing 250 mL 9 K medium with an initial pH of 2.0 and 5% pulp density (w/v). Before inoculation, pH was controlled at 2.0 every 12 h by the dropwise addition of 18 M H_2SO_4. Each culture was inoculated with 6×10^6 cells/mL mixed microorganisms after the pH value stabilized about 72 h later. Experiments were carried out at 35 °C, 175 rpm. All tests were conducted in triplicate.

2.5. Measurement of physicochemical factors

Samples of each group collected at regular intervals were measured for relevant physicochemical parameters. Cell density was measured by hemacytometer (XB-K-25, QiuJing, Shanghai, China). Redox potential (Eh, Ag^0/AgCl reference) and pH were measured by a digital pH/ORP meter (pHSJ-4A, Leici, Shanghai). Copper concentration in leachate was monitored by colour reaction with *bis*(cyclohexanone) oxalyldihydrazone (Chimpalee et al., 1995). Leaching residues of each group were filtered, washed and dried after the leaching experiment. The residues were weighed and oxide/sulfide copper phase content were analysed by chemical phase analysis for subsequent calculation of leaching efficiency. The chemical phase analysis was carried out by a combination of stepwise extraction and atomic absorption spectroscopy (PinAAcle 900F, PerkinElmer, USA).

2.6. Community structure analysis

Microbial samples at day 2, 6, 10 and 14 during the leaching experiment were collected. Free and attached microorganisms were harvested by filtration and centrifugation (12,000 × *g* for 20 min). Attached cells were collected from ore by repeated vortex and resuspension beforehand. Total DNA was extracted using the TIANamp® Bacteria DNA kit (Tiangen, Beijing, China) following the manufacturers' instructions immediately for RT-qPCR. Primers for RT-qPCR for different species were designed by Primer Premier 5.0 (Table 2) and synthesized by BioSune (BioSune Biological Engineering Technology & Services Co. Ltd., Shanghai, China). The RT-qPCR was carried out with iCycler iQ Real-time PCR detection system (Bio-Rad Laboratories Inc., Hercules, USA). All tests were conducted in triplicate.

Table 2
The designed primers used for RT-qPCR.

Target species	Primer names	Primer sequences (5′-3′)	Amplicon length (bp)
A. ferrooxidans	rus-S	ACAAGGGATTCGGTCATAGTTT	153
	rus-A	CCGTCGGATGCCAGGTAAA	
L. ferriphilum	forB-S	GAGTATGCGATTCCGACACCA	99
	forB-A	TGGCTCAAGGGATTCAAGGTA	
A. caldus	soxX1-S	CAGTATTCCACCCATCAACG	114
	soxX1-A	ACTCCACCTGGCAAGACAT	
S. thermosulfidooxidans	DoxA-S	CCCAGACACCTACGGCAACTT	267
	DoxA-A	ACATCTTCCACGGTCACAACG	
F. thermophilum	coDH-S	TTGAGGGACGAACTTGGTTTA	168
	coDH-A	CAGGGTCATTGCTTTCTATTGTT	