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Spatial variation of microbial community structure in the Zijinshan commercial copper heap bioleaching plant

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ABSTRACT

The distribution and diversity of microbial community in Zijinshan commercial non-aerated copper heap-bioleaching system operated at pH 0.8 for three years were investigated. In this research, 12 ore residue samples and 3 liquid samples were collected, the microbial community structure of heap was investigated by clone libraries, key acidophiles were assayed by real-time PCR, and physicochemical characteristics of the samples were also analyzed. 27 OTUs were obtained from 16S rRNA libraries, among them, bioleaching microorganism accounted for 40.7% of OTUs and 95.6% of clones. The species which had the ability to oxidize reduced inorganic sulfur compound accounted for 52.9% of clones and to oxidize Fe^{2+} accounted for 42.7% of clones. The other remaining OTUs accounted for 59.3% of OTUs and 4.4% of clones, most of which belong to heterotrophic and facultative bacteria. These bacteria could use organic compounds of the heap system and decrease the inhibition of organic compounds to autotrophic bacteria. In the heap, *Acidithiobacillus* mainly located in the inner and higher sites of ore heap, *Leptospirillum* mainly located in the outer and lower sites of ore heap, and heterotrophic and facultative microbes mainly located in the middle of the ore heap. There was higher leaching efficiency in the core of the heap, this might be related with the higher temperature of the heap core and higher diversity of microorganism community.

1. Introduction

Biohydrometallurgy has been employed to economically extract metal from certain sulfide minerals over half a century (Brierley and Brierley, 2013). In the recent two decades, biohydrometallurgy developed rapidly and achieved in industrial applications for extraction of copper, gold, nickel, zinc, cobalt, uranium, etc., the production of copper by bioleaching technology accounted for 15% of world production (Akcil, 2004).

In commercial copper heap bioleaching plant, finely crushed material (typically 80% smaller than 10 mm) usually agglomerated, had relative high solution pH (around 1.8), relative low heap temperature (12–27 °C), and usually aerated (Domic, 2007; Ruan et al., 2013; Schnell, 1997; Watling, 2006). By using such operation parameter, the total leach duration is typically 10–18 months, and 80–85% copper extraction can be commonly obtained (Domic, 2007). In China, the first commercial plant of bio-heap leaching with a copper cathode production capacity of 10,000 t/yr at the Zijinshan Copper Mine started operation by the end of 2005 (Ruan et al., 2006). Unlike other copper commercial heap bioleaching practice, the crushed ore with coarse particle size typically 80% smaller than 40 mm was directly stacked to the permanent heap by truck without agglomeration, and aeration was not applied to the heap (Ruan et al., 2011). Zijinshan copper ore contains high pyrite (5.8%), which releases heat and iron when pyrite is oxidized; the multi-lift heap configuration was used to keep heat inside the heap. In the past few years, a heap bioleaching system was established at Zijinshan, which had distinct low solution pH (pH 0.9–1.1), high iron concentration (~50 g/L), elevated temperature (45–60 °C) in the leaching solution, and more than 60 °C inside the heap, low redox potential (Ag/AgCl electrode value is between 700 and 760 mV, which was lower than the other commercial copper heap) (Ruan et al., 2011). Although the operation parameters of Zijinshan heap bioleaching system differ a lot when

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comparing with other commercial copper heap bioleaching practice, the recovery of copper (80%) makes it a unique microbial ecology research project.

Microbial communities researches focused on heap bioleaching plants were reported previously (Demergasso et al., 2005; Halinen et al., 2012; He et al., 2007; Watling et al., 2014; Xie et al., 2007). The application of molecular biology techniques, especially 16S ribosomal RNA (16S rRNA) clone library, has made significant progress in the field of microbial ecology (Amann et al., 1995). In this paper, by using 16S rRNA clone library technique and real-time polymerase chain reaction (RT-PCR) quantitative analysis, community structure in the ore residue of different vertical, horizontal locations and liquid samples from different solution pond were analyzed and compared. Functional groups in the heap were discussed and correlated to the bioleaching condition.

2. Materials and methods

2.1. Heap description and mineralogy

Zijinshan Copper Mine located in the southeast of China, and the heap bioleaching plant is now the largest bioleaching plant in China. From 2008 year, copper ore with an average copper grade of 0.5% were heaped up to width of 44 m and height of 8 m for bioleaching, the leaching cycle was 180 d, after every leaching cycle of 180 d, covered 8 m copper ore on the original ore heap for another leaching cycle of 180 d, until the height of ore heap reached 24 m.

2.2. Sample preparation, DNA extraction and 16S rRNA gene clone library construction

Flowchart of bioleaching system and sampling sites were shown in Fig. 1, which include ore heap, raffinate solution (RS) pond, spray solution (SS) pond and pregnant leach solution (PLS) pond, the organic solvents were removed in the "treatment" step when raffinate solution was pumped to the spray solution pond. The heap was dug along the trapezoid section by an excavator, samples were collected from section (under new exposed surface 10 cm) with a sterilized container for each samples. Nine wet ore residue samples were obtained and three liquid samples were taken from RS pond, SS pond and PLS pond respectively, the sampling sites were showed in Fig. 1. Added 1 L double distilled water (pH 2.0) to 500 g ore residue sample, stirred 500 r/min for 5 min using a stirrer, filtered with a filter paper and collected the water, repeat this process two times, nearly 3 L water collected from washing process were filter with 0.02 μm membrane, the resulting pellets was subjected to microbial analysis.

Total DNA was extracted according to the reference (Oved et al., 2001). 16S rRNA genes were amplified by PCR with general primers 27f and 1492r for bacteria (Lane, 1991) and arch21f and 1492r (DeLong, 1992) for archaea. The amplified 16S rRNA genes were inserted into pGEM-T easy vectors (Promega, WI, USA) and transformed into *Escherichia coli* JM109 to construct clone library. At least 100 clones from each clone library were sequenced by Sangon Corp. (Shanghai, China).

2.3. Clone library analysis

Chimera detection and sequences blast were analyzed using the RDP site and NCBI database respectively. Alignments of 16S rRNA gene sequences were performed with the CLUSTAL_X program (Thompson et al., 1997). A phylip-generated distance matrix was used as the input file to distance-based operational taxonomic unit (OTU) and richness (DOTUR) (Schloss and Handelsman, 2005). Rarefaction analysis was applied to estimate whether the library had been sequenced enough (Chao, 1984). A maximum likelihood phylogenetic tree was constructed based on evolutionary distances that were calculated with the Kimura two-parameter model using MEGA6.06. Samples similarity tree and samples community composition were analyzed by mothur software (Oved et al., 2001).

2.4. Real-time PCR quantative analysis

Real-time PCR based on SybrGreen I was performed according to the manufacturer's instructions (SYBR Primix Ex Taq, RR420, TAKAKA) with Rotor-Gene 6000 (Corbett Research), all tests were conducted in triplicate. For the quantification of *Acidithiobacillus* sp., *Leptospirillum* sp., *Sulfobacillus* sp., *Ferroplasma* sp., primer pairs were referenced by Zammit et al. (2008). To convert DNA copy

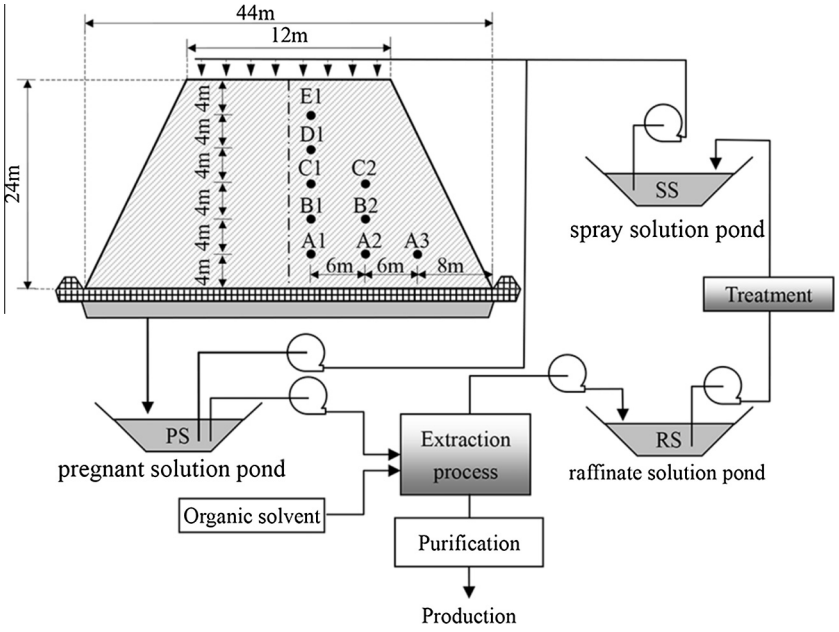

Fig. 1. Flowchart of bioleaching system and sampling sites.