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Short Communication

The bioleaching feasibility for Pb/Zn smelting slag and community characteristics of indigenous moderate-thermophilic bacteria

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ABSTRACT

The feasibility of recovering metal values and removing hazardous elements from the Pb/Zn smelting slag using bioleaching technique were studied through a flask experiment, and the community characteristics of the indigenous moderate-thermophilic bacteria in this bioleaching system were also analyzed through a culture-independent restriction fragment length polymorphism (RFLP) of 16S rRNA genes approach. The results show that more than 80% of Al, As, Cu, Mn, Fe and Zn in the Pb/Zn smelting slag were leached at 65 °C, pH 1.5, pulp density 5%, but only about 5% of Pb. Phylogenetic analysis revealed that the bacteria in the bioleaching system mainly fell among *Firmicutes*, *Gammaproteobacteria* and *Betaproteobacteria*, and the dominant bacteria are affiliated with *Bacillus* spp., *Sporosarcina* spp. and *Pseudomonas* spp.

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1. Introduction

With the rapid development of metallurgical industries, especially the primary and secondary non-ferrous processing industries, a variety of metals-containing solid wastes such as slag, ash, sludge and residues have greatly increased (Agrawal et al., 2004; Liu et al., 2008). Heavy metals and other toxic elements in these residues, including As, Cd, Cr, Ni, Pb, Cu, Hg and Zn, are considered harmful to the environment. Recovering metal values and removing the hazardous elements from the wastes are important not only for saving metal resources but also for protecting the environment (Agrawal et al., 2004). Bioleaching technology, which is based on the ability of microorganisms to transform solid compounds into soluble and extractable elements that can be recovered, has been rapidly developed in recent decades for its advantages, which include mild reaction condition, low energy consumption, simple process, low environmental impact and being suitable for low grade mine tailings and residues. However, there are few studies on the application of this innovative technique for recovering metals and reducing the toxicity of metal-containing residues (Guo et al., 2008), especially for the smelting slag with low concentrations of metals.

As is known, the bacteria in the bioleaching system play a key role in the solubilization of metals. Thermophiles are thought to make bioleaching more efficient for the process to run at higher temperatures which result in a faster reaction rates (Cancho et al., 2007). Unfortunately, many dominant indigenous microorganisms, especially thermophiles in these process, which are involved in the mobility of metals through their oxidation, reduction, accumulation and their metabolites, cannot be cultured using current culture-based methods, and therefore our understanding of these bacterial communities is limited. However, the development of modern molecular biology techniques makes it possible to examine the unculturable microbial communities in these processes.

In China, millions of tons of smelting slag from Pb/Zn processing and refining industries have been identified as hazardous solid waste according the Chinese national list of dangerous waste by the Ministry of Environmental Protection and National Development and the Reform Commission of the People's Republic of China in 2008. In order to find a technical and economical acceptable technology to deal with the Pb/Zn smelting slag and potentially reclaim metal resources, in this study, a flask experiment was carried out to study the feasibility of recovering metal values and removing hazardous elements from the Pb/Zn smelting slag by bioleaching. Moreover, a culture-independent RFLP separation of 16S rRNA genes approach was applied to reveal the community characteristics and the dominant bacteria in the bioleaching system.

2. Methods

2.1. Characteristics and mineralogy of Pb/Zn smelting slag

The Pb/Zn smelting slag was collected from a Pb/Zn metallurgical industrial site in Hunan Province of China. The elements

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content of Al, As, Cu, Fe, Mn, Pb and Zn in the dried slag were 28750 mg/kg, 4587 mg/kg, 13115 mg/kg, 282210 mg/kg, 40525 mg/kg, 5740 mg/kg, and 28378 mg/kg, respectively. The results from the X-ray diffraction analysis show that the metals, As, Cu, Mn, Pb and Zn together with the Fe, Si, Al and Ca, mainly formed the complicated amorphous structures after quenching from the smelting process with high temperature (1100–1300 °C). A few crystals, such as single iron, iron oxides and sulfide, zinc sulfide, manganese fayalite, vuagnatite, copper sulfide and quartz are trapped within the amorphous oxides. There are also 2.85% of total S and about 17% of coke residues in the slag for the incomplete combustion.

2.2. Microorganisms and bioleaching experiment

The bacteria were collected from an old Pb/Zn smelting slag dumping site in Hunan Province of China. The bacteria had been cultured in 100 mL culture medium with 5 g Pb/Zn smelting slag at the temperature of 65 °C and gradually decrease the pH to 1.5 in order to adapt to the stress conditions. The culture medium includes $(NH_4)_2SO_4$ 0.5 g/L, NaCl 0.2 g/L, $MgSO_4 \cdot 7H_2O$ 0.3 g/L, KH_2PO_4 0.2 g/L, $Ca(NO_3)_2 \cdot 4H_2O$ 0.07 g/L and 2 g/L yeast extract used as carbon source. The bacteria were cultured over 9 months by continuously transferring per fortnight and the morphologic observation for bacteria through the light microscope (NOVEL XSZ-N107) was conducted in order to understand the growth stage of bacteria over period of culturing. The indigenous moderate-thermophilic bacteria at the stationary growth stage were centrifuged ($\times$10000g), washed and then suspended in the culture medium (pH 1.5) as inoculums for the bioleaching experiment.

Bioleaching experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL culture medium and 5 g Pb/Zn smelting slag at pH 1.5 (Guo et al., 2008), and 10% (v/v, 1×10^7 cell/mL) of active bacteria enrichment described above as inoculums. The Erlenmeyer flasks were placed in a rotary shaking incubator at 65 °C with a stirring rate of 120 rpm (Guo et al., 2008). Deionized water was added to the Erlenmeyer flasks to compensate for evaporation losses. Control experiments were carried out without inoculums and under sterile conditions. The acidity was adjusted to pH 1.5 by adding 1:1 (v/v) sulfuric acid solution per 24 h. Samples were taken at 24 h intervals and all of Al, As, Cu, Fe, Mn, Pb and Zn in the leachates were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Intrepid II XSP, USA).

2.3. Restriction fragment length polymorphism (RFLP) for microbial community

2.3.1. DNA extraction and purification

The leachate was collected from 20 flasks (containing 100 mL culture medium and 5 g slag per Erlenmeyer flask) at stationary growth phase through centrifugation ($\times$3000 r/min). The solid residues were washed twice with the culture medium, centrifuged again and the supernatant was combined with the previous leachate. Then the combined leachates were separated by centrifugation ($\times$10,000 r/min) and about 5 g pellet which contains a large amount of bacteria was collected. The bulk community DNA was extracted from the biomass using a protocol described by Zhou et al. (1996). Through the combination of grinding, freezing and thawing, and sodium dodecyl sulfate treatments, it was hoped all bacteria in the sample would be effectively lysed. The crude DNA was run on a 1.0% agarose gel then purified by E ZNATM Gel Extraction Kit according the manufacturer's instruction (Yin et al., 2008).

2.3.2. PCR amplification and cloning of 16S rRNA genes

The extracted DNA was used as template for PCR amplification of the 16S rRNA genes. The PCR amplification and cloning of 16S rRNA genes following the protocol was described by Yin et al. (2008). The reverse primer was the universal 1492R (5′-GGTTACCTTGTTACGATT-3′) and the forward primer was the bacteria universal 27F (5′-AGAGTTTGATCCTGGCTCAG-3′). About 100 clones were randomly selected from each insert-positive clone. The inserted fragments were amplified with vector M13 primer, and then were screened by digestion with 1 U each of the 4-base-specific restriction end nucleases HinPI and MspI in 1$\times$ buffer (New England Biolabs) overnight at 37 °C. The resulting RFLP products were separated on a 3% agarose gel. Bands were visualized by staining with ethidium bromide and UV illumination. Jaccard coefficients were used for all pairwise comparisons of the RFLP banding patterns and dendrograms were constructed with the outweighed pair group mean average method in Molecular Analyst (Version 1.1, Bio-Rad). RFLP banding patterns were identified and a representative clone was selected for nucleotide sequence determination (Yin et al., 2008).

2.3.3. Phylogenetic analysis and nucleotide sequence accession number

Phylogenetic affiliations of the partial sequences were initially estimated using the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/BLAST/). Then, 16S rRNA gene sequences were selected for alignments using ClustalW. The final phylogenetic tree was constructed by the DNA distance program Neighbor-Joining with Mega 3 (Kumar et al., 2004). All the 16S rRNA gene sequences in this study have been submitted to GenBank with accession numbers from FJ005055 to FJ005065.

2.4. Statistical methods

The rarefaction analysis was performed with SigmaPlot software. An exponential model

$$y = a \times [1 - \exp^{(-bx)}]$$

was used with SigmaPlot 8.0 nonlinear regression software to fit the clone distribution data.

3. Results and discussion

3.1. Metal leachability from Pb/Zn smelting slag through bioleaching

The leachability of Al, As, Cu and Zn in bioleaching system was significantly improved. More than 80% of metals, including 82–84% of Al, 85–91% of As, 86–88% of Cu and 95–97% of Zn, were leached from the Pb/Zn smelting slag within 6 days, but, only 33–35% of Al, 20–25% of As, 20–30% of Cu and 46–50% of Zn were mobilized in the control (Fig. 1). About 85–95% of Mn and 90–95% of Fe were leached by bioleaching, but, only about 10–15% more than the control. Most of Fe and Mn can be also effectively mobilized by acid. However, the leachability of Pb was low (4–5%, Fig. 1) due to the solubilized Pb forming $PbSO_4$ ($Ksp = 1.62 \times 10^{-8}$) with sulfate (Guo et al., 2008). The results indicated that bacteria in the bioleaching system can greatly enhance the solubilization of Al, As, Cu, Fe, Mn and Zn. Meanwhile, the moderate-thermoacidophiles can grow in the extremely acidic (pH 1.5) metal-containing leachates (As more than 200 mg/L, Cu more than 500 mg/L, Mn more than 2032 mg/L, Fe more than 12,000 mg/L, Zn more than 1300 mg/L, respectively), and show they have strong tolerance to the high acidity, high metals toxicity, high temperature in the bioleaching system for the Pb/Zn smelting slag. Bioleaching with the indigenous moderate-thermophilic bacteria is a promising technique to recover metals and removal the hazardous elements from the Pb/Zn smelting slag.

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