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Bioleaching of arsenopyrite by mixed cultures of iron-oxidizing and sulfur-oxidizing microorganisms

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HIGHLIGHTS

- Arsenopyrite was leached by mixed cultures of iron- and sulfur-oxidizing thermophiles.
- The two kinds of mixed cultures showed different bioleaching performance.
- *A. caldus* could eliminate the sulfur passivation layer on the arsenopyrite surface.
- The community structure succession during bioleaching of arsenopyrite was analyzed.

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ABSTRACT

Arsenic is a critical environmental pollutant associated with acid mine drainage. Arsenopyrite is one of the major arsenic sulfide minerals whose weathering lead to the contamination of arsenic. In this study, the leaching behaviors of arsenopyrite by two mixed cultures of iron-oxidizing and sulfur-oxidizing microorganisms (*Ferroplasma thermophilum* and *Acidithiobacillus caldus*, *Sulfobacillus thermosulfidooxidans* and *Acidithiobacillus caldus*) were investigated, accompanying with community structure analysis of free microorganisms. The ratio of *F. thermophilum* to *A. caldus* of 1/1 showed a more favorable effect on the arsenic leaching than other ratios, and *F. thermophilum* played a dominant role in the solution all the leaching time. While adding *A. caldus* in the *S. thermosulfidooxidans* bioleaching system, the dissolution of arsenopyrite was suppressed. Notably, when the ratio of *S. thermosulfidooxidans* to *A. caldus* was 2/1, the arsenic extraction was accelerated at the early stage, but later it slowed down. The reason was because *A. caldus* was the predominant species at the later stage which made the redox potential decrease faster. XRD demonstrated that the proper addition of *A. caldus* could eliminate the sulfur passivation and promote the leaching in a degree. These studies are helpful to evaluate the environmental impact of arsenic.

1. Introduction

Acid mine drainage (AMD) is one of the challenging environmental problems in the world but highly concerned. AMD polluted waters are generated by the biochemical oxidation of metal sulfides in mine wastes, which contributes to the dissolution of ores and release of sulfates, protons, and toxic heavy metal ions in soil solutions (Pagnanelli et al., 2008; Lefebvre et al., 2012). Arsenic, as the 20th most abundant element in the earth's crust, is one of the main AMD pollutants which is detrimental to environment and most living organisms (Cheng et al., 2009). Arsenic is always associated with some sulfide ores including gold, copper and iron. The weathering of arsenic-bearing sulfide ores releases considerable amounts of arsenic into ambient conditions (Zhang et al., 2015). Microorganism plays an important role in the weathering of arsenic-bearing sulfides and the mobility of arsenic which control the geochemical processes of arsenic (Cheng et al., 2009). An in-depth study on the bioleaching behaviors of arsenic-bearing sulfides is required in order to assess the arsenic pollution to environment.

Arsenopyrite is one of the major arsenic-bearing sulfide minerals. It is also one of the most important gold-bearing minerals. Gold particles are often encapsulated in arsenopyrite. Biooxidation pretreatment has been proved to be effective for rendering the contained gold liberated for subsequent process (Li et al., 2009). Consequently, apart from assessment of arsenic environmental

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impact, it is also of great guidance for improving the gold recovery to investigate the bioleaching behaviors of arsenopyrite. Previous studies have found that elemental sulfur, jarosite, iron hydroxides, amorphous ferric arsenate/scorodite, ferric phosphate are possible intermediate and secondary products during the biooxidation of arsenopyrite (Tuovinen et al., 1994; Corkhill et al., 2008; Henao and Godoy, 2010; Fomchenko and Muravyov, 2014; Zhu et al., 2014). But in most cases, amorphous arsenate/scorodite are not detected, because ferric ions prefer oxidizing arsenopyrite to oxidizing As(III) (Barrett et al., 1993), and As(III) is the major valence state of arsenic in the solution. Only in the presence of pyrite or chalcopyrite, the transformation of As(III) to As(V) can be achieved (Barrett et al., 1993; Wiertz et al., 2006).

Since biooxidation is an exothermic reaction, high operation temperature is favorable to reduce the cost of cooling and accelerate the leaching (Rahaman et al., 2008). Hence thermophiles present a great potential in the biooxidation of sulfide ores. In recent years, thermophiles have been widely applied to bio-oxidation of chalcopyrite and pyrite, and a lot of remarkable achievements have been gained (Mikkelsen et al., 2007; Zhou et al., 2009; Zeng et al., 2010; Chang-Li et al., 2012; Qin et al., 2013). It is known that mutual effect occurs between different kinds of microorganisms in many cases (Johnson, 1998). Studies showed that mixed cultures of iron-oxidizing and sulfur-oxidizing microorganisms helped to accelerate the leaching rate, avoid surface passivation, and then improve copper extraction during bioleaching of chalcopyrite (Zeng et al., 2010; Zhu et al., 2011; Gu et al., 2013). However, few studies have been devoted to the bioleaching behaviors of arsenopyrite by promising thermophiles, especially by the mixed cultures of iron-oxidizing and sulfur-oxidizing thermophiles.

Bioleaching is a complex process which is related to biological, chemical and physical interactions. Temperature, pH, $c(Fe^{3+})/c(Fe^{2+})$ and dissolved oxygen, carbon dioxide, sulfate and metal ions are all the factors influencing the community structure of microorganisms (Yu et al., 2014). It is critical to understand the ecological laws of microorganisms during bioleaching of minerals. At present, many molecular phylogenic techniques have been widely used to community structure analysis in bioleaching systems (Zhou et al., 2009). Real-time PCR is an effective and rapid method to study the microbial community and to further understand microbial function in bioleaching systems, though it is difficult to distinguish some bacteria very accurately (Bowei et al., 2009; Zhang et al., 2009).

Ferroplasma thermophilum, *Acidithiobacillus caldus* and *Sulfobacillus thermosulfooxidans* are three kinds of promising moderate thermophiles involved in the biooxidation of minerals. *F. thermophilum*, a ferrous iron-oxidizing archaea, was isolated from a chalcopyrite-leaching bioreactor (Zhou et al., 2008). Additionally the cells lack typical cell wall and cannot survive in high pulp density which contributes to strong stirring shears (Zhou et al., 2009). *A. caldus* is a kind of sulfur-oxidizing bacteria which can use elemental sulfur and sulfur-containing compounds as energy sources. *S. thermosulfidooxidans* can oxidize both sulfur and ferrous iron. All these three kinds of microorganisms exhibit excellent arsenic resistance as reported in previous studies (Golyshina and Timmis, 2005; Watkin et al., 2009; van der Merwe et al., 2010; Golyshina, 2011). In this study, the bioleaching behaviors of arsenopyrite were investigated in shake flasks by the two mixed cultures of iron-oxidizing and sulfur-oxidizing moderate thermophiles: *F. thermophilum* and *A. caldus*, *S. thermosulfooxidans* and *A. caldus*. Real-time PCR was used to quantify the community structure of free microorganisms in the bioleaching solution. These studies are pretty meaningful for understanding the interaction of the thermophiles as well as the bioleaching mechanism by mixed cultures of iron-oxidizing and sulfur-oxidizing microorganisms. On the other hand, more information is provided for the estimation of arsenic pollution to environment and biooxidation pretreatment of refractory gold ores.

2. Materials and methods

2.1. Mineral samples

The mineral arsenopyrite used in the study was obtained from Inner Mongolia, China. The samples were prepared by hand-sorting to remove the gangue minerals, crushing, grinding and dried-sieving to 0.037–0.074 mm. The chemical analysis showed that the samples contained Fe 33.92%, As 44.60%, S 19.63 (96.63% FeAsS). The X-ray diffraction pattern (Fig. 1) showed the main mineral phase was arsenopyrite.

2.2. Microorganisms and culture media

The three moderate thermophiles strains used in this study were provided by the Key Lab of Biometallurgy of Ministry of Education, Central South University, China. All strains were cultured aerobically in the 9 K medium consisting of $(NH_4)_2SO_4$ (3 g/L), K_2HPO_4 (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), $Ca \cdot (NO_3)_2$ (0.01 g/L), $KCl \cdot$(0.1 g/L) (Gu et al., 2013). Other culture conditions of the three strains were shown in Table 1. The rotating speed of the incubator shaker was set as 165 r/min. Cells were obtained through filtrating by filter paper allowing cells to get through the suspended solid materials. The suspension containing cells was successively centrifuged (9000 r/min) for 20min and suspended with diluted H_2SO_4 for three times to remove residual ions from the medium. The collected cells were then suspended in 9 K medium without any energy sources for experiments.

2.3. Bioleaching tests

All bioleaching tests were performed in 250 mL Erlenmeyer flasks with 100 mL iron-free 9 K medium. 1 g arsenopyrite was added to each flask, and the initial cell density was 1×10^7 cells/mL. The rotating speed and temperature of the rotary shaker were 165 r/min and 48 °C, respectively. The initial pH was adjusted to 1.0 and 1.6 with 20% H_2SO_4. All the experiments were triplicated at the same condition.

Leaching parameters of pH, redox potential (*vs* SCE), free cell density and arsenic concentration were analyzed at a fixed interval of time. The average values of the parallel experiments were used to plot the results. The weight of every flask was weighted before measuring. Evaporation loss was compensated by adding pH = 1.0/1.6 water. The sampling loss was supplemented by the same volume of basal 9 K medium.

The PHS-3C type pH meter was used to measure the solution pH values. The redox potential (*vs* SCE) was measured in the potential profile of PHS-3C type pH meter by using a platinum electrode with an $Hg/HgCl_2$ reference electrode. The cell density was determined microscopically by direct cell counting, using haemacytometer. Arsenic concentration was analyzed by ICP-AES method (IRIS Intrepid II XSP, Thermo Fisher, USA). At the end of leaching experiments, the residues were filtered from leaching solution through filters, and rinsed with pH = 1.0/1.6 water and distilled water, then air dried to send for XRD analysis. XRD tests were conducted using a X-ray diffractometer (Model D/Max2500 PC) with Cu K_α radiation (λ = 1.54056 Å) in the range of 2θ from 5° to 75°.