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Enhancement in copper extraction from chalcopyrite by re-inoculation of different acidophilic, moderately thermophilic microorganisms

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ABSTRACT

The feasibility and optimization of strategies of microbial re-inoculation were investigated to enhance chalcopyrite bioleaching. The application of microbial re-inoculation consisted of the re-addition of cultures of *Acidithiobacillus caldus*, *Ferroplasma thermophilum* or *Leptospirillum ferriphilum* (with different inoculum concentrations) into the two defined different bioleaching systems that separately represented the early and middle stages of chalcopyrite bioleaching. Changes in the bioleaching performance and microbial community structure after re-inoculation were compared to that in the control bioleaching experiment without re-inoculation. Results of re-addition of pre-grown microbial cultures into the early stage indicated that the re-inoculated strain survived and/or grew in the leaching environments and meanwhile synergistically promoted the growth of other microorganisms, then accelerated the total iron/sulfur oxidation compared to the unamended control. Finally, all these factors resulted in a significant enhancement of copper extraction. Moreover, a higher re-inoculation concentration exerted a more significant improvement in copper leaching from chalcopyrite. In all experiments, re-inoculation with *L. ferriphilum* into the early stage showed the best enhancement in copper leaching, which significantly shortened the incubation time and improved the maximum copper extraction. Re-inoculation with iron or sulphur oxidisers into the middle stage could not or slightly improve the final leaching level of copper. These results demonstrate that re-inoculation can be a useful step to improve the bioleaching kinetics and level of chalcopyrite; however its efficacy is influenced by the functional strain selection and procedures of culture re-inoculation (including re-addition time and inoculum concentration).

1. Introduction

Chalcopyrite ($CuFeS_2$) is the most important copper source in the world, which accounts for approximately 70% of the known copper reserves in the lithosphere (Wang, 2005). However, the mineral is also known to be the most refractory copper-bearing sulfide. Bioleaching as an emerging alternative to other conventional technologies has attracted global attention due to its low capital investment, environment friendly, mild reaction and higher recovery. The technology has been widely employed to extract gold, copper, cobalt, nickel, zinc, uranium, etc. from secondary and low-grade ores (Brierley and Brierley, 2013; Watling, 2006). Nevertheless, bioleaching of chalcopyrite on an industrial scale is not successful (Watling, 2006) due to the slow kinetics and poor dissolution (Hackl et al., 1995; Khoshkhoo et al., 2014a; Munoz et al., 1979; Nazari et al., 2011; Pinches et al., 1976; Sand et al., 2001; Warren et al., 1982). Therefore, there is a strong need to develop new and efficient ways to improve copper extraction from chalcopyrite (Ahmadi et al., 2011; Feng et al., 2014; Johnson et al., 2008; Nazari et al., 2011).

Various methods have been proposed to improve chalcopyrite biodissolution, such as elevating the bioleaching temperature with inoculum of mixed moderately thermophilic cultures (their average optimum temperature is 45 °C), adding a 'catalyst' like pyrite, Ag^+ or $Cl^-$to alter the electrochemical behaviour of chalcopyrite or maintaining a low redox potential in bioleaching system (Ahmadi et al., 2010; Johnson et al., 2008; Khoshkhoo et al., 2014b; Nazari et al., 2011; Wang et al., 2014; Zhou et al., 2009). In these optimised systems, an enhancement of copper extraction has been demonstrated in comparison to the control bioleaching process.

In the present study, a new strategy of 'microbial re-inoculation' was introduced to strengthen the bio-oxidation of copper sulfide (e.g. chalcopyrite). The operation of re-inoculation is to re-add pre-grown specific indigenous or allochthonous wild type cultures into a bio-treatment system to improve the catabolism of specific compounds, e.g. diesel oil in the contaminated soil, 17β-estradiol in wastewater, or some pharmaceuticals from sewage sludge (Bento et al., 2005; Iasur-Kruh et al., 2011; Rodríguez-Rodríguez et al., 2014). Some previous studies have indicated that the re-inoculation of biomass enhanced the removal of toxic or

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recalcitrant compounds as well as changed the composition of the naturally occurring microbial biota in a system (Iasur-Kruh et al., 2011; Wen et al., 2013). However, the data on the enhancement of chalcopyrite bio-oxidation by the re-inoculation of different cultures are very limited. And the relationship between bioleaching performance, microbial community dynamics and treatments of microbial re-inoculation are also poorly understood.

Thus, to obtain a complete understanding, the present study carried out a study of microbial re-inoculation to improve the bioleaching process of chalcopyrite. *Acidithiobacillus caldus* s2, *Ferroplasma thermophilum* L1 or *Leptospirillum ferriphilum* YSK was used as the re-inoculum, which are often present in significant numbers in mineral leaching operations at 45–50 °C (Johnson, 2008; Okibe et al., 2003; Zeng et al., 2010). Moreover, the optimization of re-inoculation strategies (including strain selection and re-inoculation concentration and time) for improving copper leaching was separately investigated.

2. Material and methods

2.1. Mineral

The sample of flotation chalcopyrite concentrate was provided by the Dong sheng-miao Mining Company in Inner Mongolia, China, and mainly consisted of 60% chalcopyrite, 10.1% pyrrhotite, 15% sphalerite and 5% galena. A major elemental analysis of the copper concentrate yielded the following result: Cu 20.21%, Fe 24.20%, S 28.17%, Pb 4.03%, Zn 5%, and Ag 0.34%. The ore sample as supplied was a fine powder and passed through a sieve with a pore size of 75 μm before bioleaching experiments.

2.2. Microorganisms and growth conditions

Three moderately thermophilic acidophiles were used, including one sulfur-oxidising bacterium *At. caldus* s2 (Qiu et al., 2007), one iron-oxidising bacterium *L. ferriphilum* YSK (Gao et al., 2007) and one chemomixotrophic archaeon *F. thermophilum* L1 (Zhou et al., 2008). They were isolated from an extremely acid mine or coal drainage site by our laboratory. Pure cultures of *L. ferriphilum* YSK and *At. caldus* s2 were cultured in modified basal salt medium (Silverman and Lundgren, 1959) with initial pH 1.5 and supplemented with $FeSO_4 \cdot 7H_2O$ (30 $g \cdot L^{-1}$) or sulfur (10 $g \cdot L^{-1}$) as the energy source, respectively. *F. thermophilum* L1 was grown chemomixotrophically with 20 $g \cdot L^{-1}$ $FeSO_4 \cdot 7H_2O$ and 0.02% (w/v) yeast extract in basal salt medium (acidified to initial pH 1.0 with 50% (v/v) H_2SO_4) (Silverman and Lundgren, 1959).

2.3. Bioleaching experiments

All bioleaching experiments were carried out in 500-ml shake flasks containing 200 ml modified basal salt medium (Silverman and Lundgren, 1959) and 2% (w/v) flotation chalcopyrite concentrate. And the initial pH was 1.5. Flasks were previously sterilized by autoclaving for 25 min at 121 °C and 103.4 kPa. Then, all flasks in the sterile condition were inoculated with a mixed culture of the prepared pure type strains of *At. caldus* s2, *L. ferriphilum* YSK and *F. thermophilum* L1. The final cell density for each strain was around 1.0×10^7 cells·ml^{-1}. Finally, these cultures were incubated on a rotary shaker at 180 rpm and 45 °C for about 24 days.

All flasks were separated into two main series that were subjected to re-inoculation treatment or not: Series 1 (the control bioleaching experiment, labelled as 'Control') lacked re-inoculation but instead was supplemented with an equal volume of sterile nutrition solution (pH 1.5); Series 2 (bioleaching groups with re-inoculation treatment) was re-inoculated with different strains into the bioleaching process of chalcopyrite. The influences of re-inoculation time, inoculum concentration and strain selection on chalcopyrite dissolution were separately researched. Based on the pre-test result of copper extraction in the control bioleaching experiment (Fig. 1), re-inoculation time influence was tested, respectively, at the early copper leaching phase (e.g., on the 5th day) featuring a lower chalcopyrite dissolving rate and at the middle stage (e.g., on the 13th day) with an exponential increase in copper recovery. The addition doses of cultures were separately studied at a concentration that was 1/3 or 1/1 times as much as cells (1.79×10^8 cells·ml^{-1}) on the 5th day and 1/10 or 1/3 times as much as cells (approx. 4.85×10^8 cells·ml^{-1}) on the 13th day in the control bioleaching experiment. Meanwhile, *At. caldus* s2, *F. thermophilum* L1 and *L. ferriphilum* YSK were separately used as the re-inoculum.

In the re-inoculation series of chalcopyrite bioleaching, the flasks were further separated into three main groups according to the selection of re-inoculation strain: Group 1 (bioleaching group with re-inoculation of *At. caldus*) was re-inoculated with different numbers of *At. caldus* s2 cells on the 5th (labelled as 'Ac-1/3' and 'Ac-1/1') or 13th day (labelled as 'Ac-M1/10' and 'Ac-M1/3'); Group 2 (bioleaching group with re-inoculation of *F. thermophilum*) was carried out by adding different concentrations of *F. thermophilum* L1 on the 5th (labelled as 'Ft-1/3' and 'Ft-1/1') or 13th day (labelled as 'Ft-M1/10' and 'Ft-M1/3'); Group 3 (bioleaching group with re-inoculation of *L. ferriphilum*) was done by re-addition of different amounts of *L. ferriphilum* YSK on the 5th (labelled as 'L.f-1/3' and 'L.f-1/1') or 13th day (labelled as 'L.f-M1/10' and 'L.f-M1/3'). Unless otherwise stated, all experiments were performed in triplicate, with results reported as means ± standard deviations (SD, n = 3). Sterile distilled water was added into the flasks to compensate for evaporation losses.

Throughout the entire experimental processes, three pure strains were sub-cultured in their proper medium. Cultures were collected at their exponential growth phase by centrifugation at 12,000 ×*g* for 15 min. The collections were washed 3 times with a sterilized sulfuric acid solution (pH 2) to remove any iron precipitates or other compounds (Third et al., 2000), then re-suspended in the 4–5 ml sterile nutrition solution (pH 1.5). Finally, the concentrated microorganisms were used as the re-inocula into the bioleaching experiments.

Samples were withdrawn at regular intervals in the sterile environment and analysed for copper extraction and iron dissolution. The leached solid residues were filtered and dried using a freeze drier for X-ray diffraction (XRD) analysis. For the microbial community analysis, flasks were thoroughly mixed; then slurry samples were immediately taken out under aseptic conditions and kept frozen in sterile tubes at −20 °C.

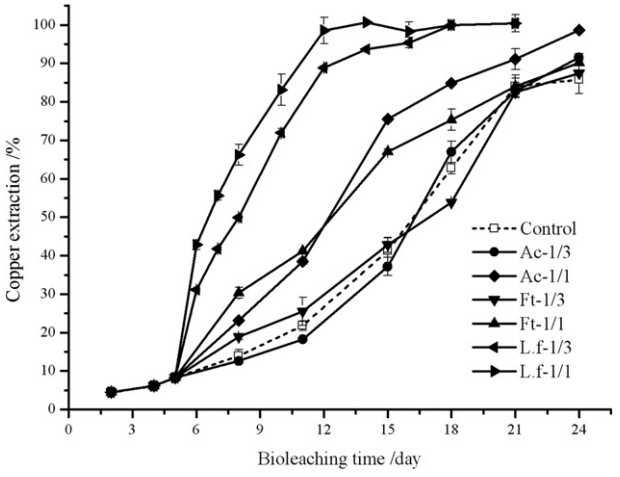

Fig. 1. Copper extraction over time in the different bioleaching tests ('Control' — bioleaching experiment without re-inoculation; 'Ac-1/3 or Ac-1/1', 'Ft-1/3 or Ft-1/1' and 'L.f-1/3 or L.f-1/1' — Bioleaching groups with re-addition of different inoculum concentrations of *At. caldus* s2, *F. thermophilum* L1 or *L. ferriphilum* YSK on the 5th day, respectively).