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Novel integration strategy for enhancing chalcopyrite bioleaching by *Acidithiobacillus* sp. in a 7-L fermenter

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HIGHLIGHTS

- Additional energy substrate was used for shortening SAG phase.
- Three-stage pH control was employed for weakening jarosite formation.
- Fed-batch strategy was determined for decreasing mineral substrate inhibition.
- With the novel and integration strategy, the efficiency was improved by 52.8%.
- The domination course of master strain was accelerated via the integration strategy.

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ABSTRACT

An integrated strategy (additional energy substrate-three stage pH control-fed batch) was firstly proposed for efficiently improving chalcopyrite bioleaching by *Acidithiobacillus* sp. in a 7-L fermenter. The strain adaptive-growing phase was greatly shortened from 8 days into 4 days with the supplement of additional 2 g/L Fe^{2+} + 2 g/L S^0. Jarosite passivation was effectively weakened basing on higher biomass via the three-stage pH-stat control (pH 1.3–1.0–0.7). The mineral substrate inhibition was attenuated by fed-batch fermentation. With the integrated strategy, the biochemical reaction was promoted and achieved a better balance. Meanwhile, the domination course of *A. thiooxidans* in the microbial community was shortened from 14 days to 8 days. As the results of integrated strategy, the final copper ion and productivity reached 89.1 mg/L and 2.23 mg/(L d), respectively, which was improved by 52.8% compared to the uncontrolled batch bioleaching. The integrated strategy could be further exploited for industrial chalcopyrite bioleaching.

1. Introduction

While worldwide copper demand is growing by around 3% per year, the minerals industry is increasingly faced with the need to process the waste and low-grade ores (Wang, 2005; Behera et al., 2012; Watling et al., 2014). Concerned on the investment, operating cost and pollution issues, bioleaching is recognized as a green and economical technology for recovering these discarded minerals (Suzuki et al., 1999; Behera et al., 2011; Vera et al., 2013). More than 20% of the world's copper has been produced by this green technology associated with solvent extraction-electrowinning in 2010 (He et al., 2010; Watling et al., 2010). However, as the most abundant copper-bearing minerals, the industrial chalcopyrite ($CuFeS_2$) bioleaching is not successful because of the extreme low-grade and complex-composition (Wang, 2005; Marhual et al., 2008; Africa et al., 2013). Therefore, research directed at improving chalcopyrite is attracting increased attention.

In the previously work, various operating methods such as pH control, optimization of key chemical ion, and other more optimal conditions have been individually investigated for improving this bioprocess (Rawlings, 2002; Kinnunen et al., 2006; Liang et al., 2012). However, most studies focused on the effects of individual operating condition on the whole bioprocess. To date, the main reasons of low-efficiency in chalcopyrite bioleaching such as the time-consuming cycle, jarosite passivation, and mineral substrate inhibition were still not well solved. It was reported that about 10 days was consumed in the lag phase, while the whole bio-cycle was only 40 days (Zeng et al., 2010). The bioleaching efficiency was also significantly inhibited by the massive iron precipitation from the middle bioleaching stage (Stott et al., 2000; Leahy and Schwarz, 2009). Meanwhile, the effects of substrate inhibition under excessive high pulp density also contributed to lower efficacy (Marhual et al., 2008). Moreover, owing to the ever-changing chemical

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parameters along with bioprocess, the optimal condition for bioleaching in different stages was not a constant (Dopson and Lindström, 2004). Sequentially, the microbial community dynamics was also various with the above fluctuation, which should be considered (He et al., 2008; Yang et al., 2014). Therefore, it is crucial to provide an integrated strategy for overcoming the above problems, especially basing on the biochemical parameters in different stages.

In our previous work, an extremely acidophilic strain *A. thiooxidans* ZJJN has been isolated and applied in chalcopyrite bioleaching (Feng et al., 2012b). The 16S rRNA-based sandwich hybridization assay was also established for quantifying *Acidithiobacillus ferrooxidans* in bioleaching (Feng et al., 2012a). Meanwhile, an integrated system (stable pH 1.3, 2.0 mg/L silver ion, and 2.5 mg/L chloride ion) by *Acidithiobacillus* sp. was employed for decreasing the formation of jarosite and S^0 membrane (Feng et al., 2013). This work aims to establish an efficient strategy for further enhancing the chalcopyrite bioleaching. First, the additional energy substrate such as Fe^{2+} and S^0 was supplemented for shortening the strain adaptive-growing phase. Then, a three-stage pH-stat control strategy was proposed for weakening jarosite passivation. The mineral substrate inhibition was further decreased by the fed-batch fermentation. Moreover, the efficiency of integrated strategy was finally proved in a 7-L fermenter and the microbial community dynamics were also investigated.

2. Methods

2.1. Strain, media and culture condition

A. ferrooxidans CUMT-1 was kindly presented by China University of Mining and Technology, Xuzhou, Jiangshu, China. *A. thiooxidans* ZJJN-3 was isolated and identified from the largest-scale commercial bio-heap in Zijinshan Copper Mine, Longyan, Fujian, China (Feng et al., 2012b). The strain (M2012104) was deposited in the China Center for Type Culture Collection (CCTCC), Wuhan, China. The main bacterial traits were listed in Table 1. *A. ferrooxidans* was cultured in 9 K media and the basal salts were listed as follow (g/L): $(NH_4)_2SO_4$ 3.0, K_2HPO_4 0.5, $MgSO_4{\cdot}7H_2O$ 0.5, KCl 0.1, $Ca(NO_3)_2$ 0.01. 44.7 g/L $FeSO_4{\cdot}7H_2O$ was added as energy substrate. *A. thiooxidans* was cultured in Starkey media and the basal salts were listed as follow (g/L): $(NH_4)_2SO_4$ 0.3, KH_2PO_4 3.5, $MgSO_4$ 0.5, $CaCl_2{\cdot}2H_2O$ 0.25. 10.0 g/L elemental sulfur was added as energy substrate. Trace element was added of 9 K and Starkey media was listed as follow (mg/L): Na_2SO_4 50, $FeCl_3{\cdot}6H_2O$ 11.0, H_3BO_3 2.0, $MnSO_4{\cdot}H_2O$ 2.0, $ZnSO_4{\cdot}7H_2O$ 0.9, $Na_2MoO_4{\cdot}2H_2O$ 0.8, $CoCl_2{\cdot}6H_2O$ 0.6, $CuSO_4$ 0.5, Na_2SeO_4 0.1. All basal mediums were sterilized by an autoclaves sterilizer (LS-B50L, Hezi, China) at 0.1 M Pa (about 121 °C) for 20 min. Then the mediums were adjusted to initial pH 2.0 with 6 M HCl under sterile condition. The strains were adapted in 2.0% (w/v) pulp density with the low-grade chalcopyrite at 30 °C and 170 rpm. Strain was inoculated into another fresh media once a month.

Table 1
The main characteristics of strains used in the study.

Speices	Strain	Energy type	Optimal T/pH	Description and source
Acidithiobacillus ferrooxidans	CUMT-1	Ferrous and sulfur oxidizer	30–35 °C, pH 1.8–2.5	Acid mine drainage of coal ore, Yanzhou, Jiangsu, China
Acidithiobacillus thiooxidans	ZJJN-3	Sulfur oxidizer	28–30 °C, pH 0–2.0	Zijinshan copper mine, Longyan, Fujian, China

2.2. Mineral composition and preparation

The mineral sample was collected from a secondary sulfide heap of Dongguashan copper mine, Tongling, Anhui, China. The main compositions of the secondary sulfide ore were chalcopyrite, pyrite, pyrrhotite, and magnetite. The detailed elements contents of mineral sample were assayed by the atomic absorption spectrometry as follow (%): Cu 1.01 ± 0.06, S 12.9 ± 0.12, Fe 33.2 ± 0.45, Ca 3.77 ± 0.23, Mg 3.66 ± 0.25, Al 1.41 ± 0.13, Mn 0.052 ± 0.01, Zn 0.051 ± 0.01, Ni 0.036 ± 0.006, Pb 0.025 ± 0.006, As 0.0038 ± 0.001. Other element contents, such as Ag, Au, Co, Cd, and Hg, were below the detectable limit (<0.0002%). The particles diameter of mineral sample was <48 μm via being sieved through a 300-mesh grid. The main particle-size distribution was listed as follow: <48 μm (100%), <45 μm (77.5%), <38 μm (25.6%), and <25 μm (10.2%). The mineral sample was previously washed by 2 M HCl, distilled water, and pure ethanol before the bioleaching experiments. Then the ore sample was dried and reserved in a vacuum desiccator at room temperature.

2.3. Bioleaching experiment

2.3.1. Operation condition in shake-flask

100 mL basal salts media (50 mL Starkey media and 50 mL 9 K media) was added into the 500 mL shake flask. The cell densities of *A. ferrooxidans* and *A. thiooxidans* were controlled at 2.0×10^7 cells/mL, respectively, after being inoculated. Then, 2% pulp density was controlled by adding mineral sample, except the fed-batch fermentation. The flasks were shaken at 30 °C and 170 rpm. The loss of evaporation was balanced by 2.0 ml sterile water once a day.

The first series of bioleaching experiments were designed at five energy substrate strategies: uncontrolled batch bioleaching, 2 g/L Fe^{2+}, 2 g/L S^0, 2 g/L Fe^{2+} + 2 g/L S^0, and 4 g/L Fe^{2+} + 4 g/L S^0, respectively. The second series of bioleaching experiments were designed at four pH control strategies: pH-stat 1.3, pH-stat 0.7, pH-stat 1.3 (0–8 d)-0.7 (8–40 d), and pH-stat 1.3 (0–8 d)-1.0 (8–14 d)-0.7 (14–40 d). The pH of these systems was monitored and revised each 24 h. The third series of bioleaching experiments were designed at two feeding strategies. 1 g mineral sample was added into each system before inoculation. The other mineral was fed as follow. Model A: 1 g mineral sample was added at 8 day; model B: 0.25 g mineral sample was fed at 8 d, 10 d, 12 d, and 14 d, respectively. All experiments were performed in triplicate with three flasks per treatment.

2.3.2. Operation condition in 7-L fermenter

The integrated fermentation strategy was verified in a series of 7-L stainless-steel fermenters (NBS BioFLO-415, Connecticut, USA). 4 L basal salts media (2 L Starkey media and 2 L 9 K media) was added into the bioreactor. The cell densities of *A. ferrooxidans* and *A. thiooxidans* were controlled at 2.0×10^7 cells/mL, respectively, after being inoculated. The bioreactor was controlled at 30 °C and 400 rpm. The air flow rate was 1.6 L/min. The stable pH in different stages was adjusted by 6 M HCl and 6 M NaOH. pH, temperature, air flow rate and impeller speed was monitored and recorded using Advanced Fermentation Software from New Brunswick Scientific Co. Inc.

The integrated fermentation strategy was carried out as follow. The 2 g/L Fe^{2+} + 2 g/L S^0 was added as energy substrate after inoculation. The three-stage pH controlled was stable pH-stat 1.3 (0–8 d)–1.0 (8–14 d)–0.7 (14–40 d). The mineral sample was fed as model B in Section 2.3.1. Meanwhile, uncontrolled batch bioleaching was carried out as blank control in another 7-L fermenter. The experiments were also performed in triplicate with three fermenters per treatment.