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Effects of dissolved oxygen and carbon dioxide under oxygen-rich conditions on the biooxidation process of refractory gold concentrate and the microbial community

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ABSTRACT

Biomining microorganisms obtain energy from the oxidation of reduced sulfur and iron (II) by using dissolved oxygen (DO) as the electron acceptor. Carbon dioxide, the carbon source for biomining microorganisms, is essential for biooxidation. However, to date, no published reports exist regarding the effect of reactive oxygen species (ROS) and the CO_2 content in an oxygen-rich condition (when oxygen is sufficient) on the biooxidation process. In this study, a microbial community was used to oxidize refractory sulfide gold concentrate in a 1.5 L experimental stirred tank reactor. The effects of DO in a slurry and the CO_2 content in the intake gas on the biooxidation process, bacterial growth and microbial community were investigated. It was found that the biooxidation efficiency increased at first and then decreased as the DO level elevated, while the content of ROS significantly increased within the bacteria cells. Under an oxygen-rich condition, the biomass increased as the CO_2 content increased, while the biooxidation efficiency first increased and then decreased. These changes revealed that the oxidation activity of biomining microorganisms was inhibited by a high CO_2 content and that bacterial growth and energy utilization were decoupled. *Leptospirillum ferriphilum-like* bacteria and *Sulfobacillus thermosulfidooxidans* were the dominate strains in the experiment. As the DO increased, the relative proportion of *L. ferriphilum-like* bacteria in the bacteria community first increased and then decreased, while *S. thermosulfidooxidans* showed the opposite trend. With an increasing CO_2 content in the intake gas, the relative proportion of *S. thermosulfidooxidans* increased, while that of *L. ferriphilum-like* bacteria decreased.

1. Introduction

Bioleaching has been proven to be an effective, low-cost and eco-friendly method to process sulfide concentrates that are difficult to process by conventional techniques (Mishra et al., 2005). The commercial bioleaching process of refractory sulfide gold concentrates has been established. Mesophilic microorganisms have been widely applied in bioleaching processes in the past few decades (Rawlings, 2002). In the bioleaching process of refractory sulfide gold concentrate, iron- and sulfur-oxidizing bacteria obtain energy by oxidizing reduced sulfur and iron (II) and, simultaneously, transfer the electrons to oxygen through the electron transport chain. Thus, an adequate oxygen supply is essential for this process. Research regarding the effective range of the DO concentration for the biooxidation process has been carried out. For example, de Kock et al. found that oxygen limitation did not occur at DO concentrations higher than 1.5 ppm when archaea were used to oxidize metal sulfides (de Kock et al., 2003). Some studies also found that biooxidation rates increased as DO levels increased (Gleisner et al., 2006). However, it is well known that biooxidation strains live in a low pH environment and that high concentrations of soluble metals and high oxygen consumption are apt to produce reactive oxygen species (ROS) Cárdenas et al., 2012. Recent studies showed that ROS could be harmful to the bioleaching process (Jones et al., 2011). Additionally, it was also found that the content of ROS inside cells of *Streptococcus zooepidemicus* increased as DO increased during the hyaluronic acid (HA) fermentation process (Duan et al., 2009); this increase was more harmful to the strains than ROS outside the cell. Therefore, it was speculated that the effect of ROS produced from oxygen-rich aeration might be more prominent than the normal fermentation process in the biooxidation process. Unfortunately, no report with this result has been published.

Biomining microorganisms transform carbon dioxide into organic matter as a result of consuming energy obtained from the oxidation of concentrates. Thus, the biooxidation rates were

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closely related to the supply rates of CO_2. Numerous studies have been published regarding the impact of carbon dioxide on the biooxidation efficiency. For instance, Bryan et al. cultured *Acidithiobacillus ferrooxidan* and *Leptospirillum ferriphilum* in a Fe_{Liq} medium (Bryan et al., 2012). They found that both the maximum specific growth rate and the maximum amount of biomass were obtained under the condition when the CO_2 content in intake air was lower than that of normal air. While de Kock et al. found that intake gas with CO_2 concentrations (v/v) from 7% to 17% promoted the biooxidation efficiency when 9 K medium with 3 g/L sodium tetrathionate ($Na_2S_4O_6{\cdot}2H_2O$) as energy source was used to culture *Sulfolobus* sp. (de Kock et al., 2003), d'Hugues et al. discovered that a CO_2 concentration increase from 1% to 2% had no impact on the oxidation process of chalcopyrite concentrate with extreme thermophilic bacteria (d'Hugues et al., 2001). However, it was difficult to compare their results because of the differences in strains, ores, reactors, operating conditions, and so on.

Theoretically, the CO_2 demand of bioleaching bacteria should be increased under oxygen-rich conditions. However, published reports regarding the impact of CO_2 on the biooxidation efficiency under oxygen-rich conditions are rare.

Many researchers found that a mixed culture could accelerate the rate of the bioleaching process. The species and quantity of microorganisms in commercial CSTR reactors were greatly affected by the operating parameters (Okibe et al., 2003). Therefore, it was necessary to understand the role of each strain in the biooxidation process and their relationships under different operating parameters (Akcil et al., 2007). However, the influence of ROS and the CO_2 content under an oxygen-rich condition in a microbial community structure has scarcely been reported.

Therefore, in this study, a mixed bioleaching culture that mainly consists of *L. ferriphilum-like* bacteria and *Sulfobacillus thermosulfidooxidans* was used for the biooxidation of refractory sulfide gold concentrate. The influences of ROS and the CO_2 content in intake gas under oxygen-rich conditions on bacteria growth as well as the concentrate oxidation process were explored. A high-throughput DNA sequencing technique was adopted to study the community structure. The mechanisms of microbial activity and mineral oxidation were investigated to gain a better understanding and optimization of biooxidation operations.

2. Materials and methods

2.1. Sulfide material

The high-grade refractory gold concentrates used in the experiments were kindly supplied by Axi Gold Mine Co. Ltd., Xinjiang, P. R. China, containing 45 ± 1.0 g/t Au, 60 ± 1.2 g/t Ag, 30 ± 0.5% Fe, 28 ± 0.3% S and 3 ± 0.1% As. X-ray diffraction (XRD) analysis showed that the major components of the samples were pyrite and quartz, and the sample was sieved to obtain a particle size distribution between 63 μm and 90 μm.

2.2. Microorganisms and the culture medium

A mixed microbial culture was provided by Axi Gold Mine Co. Ltd. The predominant organisms were studied by analyzing the V4 region of 16S rDNA using a high-throughput DNA sequencing technique. The results revealed the presence of bacteria that were mainly affiliated with *L. ferriphilum-like* bacteria and *S. thermosulfidooxidans*.

The iron-free medium (0 K media) used for the bacterial culture was developed from the previously reported 9 K media (Silverman and Lundgren, 1959). The media formulation consisted of (in g/L) $(NH_4)_2SO_4$: 3.0, KCl: 0.1, K_2HPO_4: 0.5, $MgSO_4{\cdot}7H_2O$: 0.5 and $Ca(NO_3)_2$: 0.01. All chemical reagents were of analytical grade. When used, 10 wt.% gold concentrate was added in the 0 K media to serve as an energy source.

2.3. Experimental procedures

Experiments regarding the effects of DO on the biooxidation process were carried out in 1.5 L STRs with 900 ml of 0 K medium, 100 ml of the inoculated bacterial culture and 100 g of the concentrates as the energy source. The agitation was provided by a six-bladed 45° downward pitched blade turbine (PBT) impeller. A sparger was situated below the bottom impeller. The dry air aeration rate was measured by a gas rotameter and controlled by a valve attached to the rotameter. The aeration rate was adjusted from 0.02 to 3 L/min (the same air flow rates were maintained to ensure the same CO_2 concentration), and the appropriate amount of oxygen was supplemented to stabilize the DO concentration at 2.51 ppm, 3.75 ppm, 5.01 ppm or 7.51 ppm during the biooxidation process. The environmental conditions were maintained at 41 °C, with an initial pH of 1.4, and the impeller speed in the STRs was 300 rpm. No pH control was used during the process.

Experiments regarding the effects of the CO_2 concentration on biooxidation under oxygen-rich conditions were also performed in batches in three 1.5 L STRs. During the experimental process, dissolved oxygen was controlled under an oxygen-rich condition (DO 3.75 ppm) by adjusting the air flow rate from 0.02 to 3 L/min and by appropriately supplying a small amount of pure oxygen. Three experimental reactors were run simultaneously. The first reactor was bubbled with normal air. Air mixed with 5% CO_2 was introduced into reactor 2, and the third reactor was fed with air mixed with 10% CO_2. The other conditions were the same as described above. The pH throughout the biooxidation process was controlled between 1.2 and 1.5 by the dropwise addition of a NaOH solution.

2.4. Analytical methods

The pH and DO concentration of the suspension were measured by a pH meter (Mettler model FE20) and a DO meter (OXYFERM 225, Hamilton). Mineral dissolution was determined by measuring the iron concentration in the solution. The concentration of ferrous iron was determined by titration with potassium dichromate in the presence of the indicator N-phenylanthranilic acid (Vogel, 1961). The ferric iron concentration was determined by titration with EDTA at pH 2 in the presence of the indicator sulfosalicylic acid (Davis and Jacobsen, 1960). The total iron concentration in the liquid phase was the summation of the ferric and ferrous iron concentrations. The oxygen uptake rate (OUR) was measured using the dynamic gas-out/gas-in method (Bandyopadhyay et al., 1967). Because the vast majority of bacteria were adsorbed on the concentrate (only approximately 4×10^6 cell/ml was observed in solution under a microscope that accounted for approximately 1% of the cells attached to the concentrate), the amount of cells was measured by the Coomassie Brilliant Blue method (Bradford, 1976). A 0.2 g concentrate was boiled with 3 ml of a 0.2 N NaOH solution, and a 1 ml digestive solution was sent for detection. ROS was detected using the DCFH method (Farrell et al., 2011) by adding 6 μl of DCFH-DA to 5 ml of pulp and then collecting the bacteria after processing the concentrates with trypsin to release some bacteria. Exopolysaccharides (EPS) were analyzed using the colorimetric assay (phenol/sulfuric acid method) employed by d'Hugues et al. using glucose as the standard (d'Hugues et al., 2008). The microbial community structure was detected by sequencing the V4 region of 16S rDNA using the high-throughput sequencing technique on the Illumina Miseq platform (Shanghai Personal Biotechnology Co., Ltd.) after extracting