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The adverse effect of disrupted water-borne bacteria cells on flotation

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ABSTRACT

Existing studies have demonstrated the potential threat of bacteria to copper and gold flotation when mineral processing operations use bacteria-laden water, such as treated sewage effluent, as an alternative water source. Once being introduced into a mine water system, water-borne bacteria may experience cell disruption causing the release of constituent organic molecules into process water. However, little is known about the potential impact of these organic molecules released from disrupted bacteria cells on flotation performance. In this study, using the same representative system as previously used with intact cells, we investigated the effect of disrupted cells on flotation. This representative system consists of *E. coli* cells disrupted by sonication (also called lysed cells) as the model bacterium and three copper-containing mineral systems of increasing complexity as the ore model. It was found that the disrupted cells had a negative effect on copper flotation, which tended to weaken as ore composition became more complex. The negative effect of the disrupted cells on copper flotation was more pronounced than that of the intact cells. Flotation of gold and pyrite was found to be depressed as well possibly due to the preferential adhesion of the lysed cells on pyrite. Findings in this study enhance understanding and management of the risk posed by bacteria-laden water which is being increasingly used as an alternative water source for mineral processing.

1. Introduction

Water sources that contain a variety of bacteria at varying concentrations, such as treated effluent, are being increasingly accessed by the mining industry as alternative water sources to minimize freshwater withdrawal (Brereton et al., 2008; Schumann et al., 2003; Slatter et al., 2009). Bacteria can exist in these water sources as intact cells which maintain their structural intactness, either viable or dead. Bacterial growth is sensitive to a range of physical, chemical and biological factors in the surrounding environment, with different optimal conditions for different types of bacteria (Adamberg et al., 2003; Leroi et al., 2012). When the surrounding conditions are beyond the range that a bacterial cell can grow, intact cells undergo cell disruption or cellular breakdown, a process called bacterial lysis (Geciova et al., 2002; Lewis, 2000). Examples of physical factors that can induce cell disruption are the exposure to temperatures beyond the growth temperature range (Hagen et al., 1964; Weiser and Osterud, 1945), and to mechanical forces, such as grinding with kieselguhr and sand (Kelemen and Sharpe, 1979). Cell disruption by high concentrations of monovalent cations, detergents, or chelating agents is an example of chemically induced cell lysis (Tsuchido et al., 1995). UV radiation applied to disinfect water and wastewater is an example of cell disruption by photobiological factor (Chang et al., 1985). Cell disruption by any means would result in the release of a range of organic molecules contained in bacteria cells, such as polysaccharides, proteins, and DNA (Madigan and Martinko, 2006).

Under the context of using bacteria-laden water for flotation, bacteria cells may be disrupted in different components of a mine water system due to physical, chemical, and biological factors associated with these components. According to a published framework (Liu et al., 2013c), these factors can be considered from within a concentrator (also called internal factors) and outside a concentrator (also called external factors). Within a concentrator, cell disruption may occur in grinding mills due to the mechanical forces exerted by grinding media and ore on bacterial cells; in the flotation circuits, cell disruption may occur due to exposure of the bacterial cells to the harsh conditions beyond its tolerance limits, such as high pH and the addition of chemicals. The disrupted cells may be immediately brought back to the concentrator by water internal reuse, where water is recovered and reused without passing through tailings storage facilities. Cell disruption may also occur outside a concentrator due to exposure to chemicals and UV in water and tailings storage facilities. These disrupted cells can be brought back to the concentrator through water external reuse, where water is recovered from tailings storage facilities and then reused in the concentrator.

To understand the potential risk of water-borne bacteria to flotation performance, we have previously studied the effect of intact cells on flotation (Liu et al., 2013a). That study was carried out in a representative

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system consisting of *E. coli* in intact form as the model bacterium and three copper-containing mineral systems of increasing complexity as the ore model, i.e., high-purity chalcopyrite, simulated ore with controlled gangue minerals, and porphyry copper-gold ore. The intact cells were found to have a negative effect on copper and gold flotation (Liu et al., 2013b). Compared with the case of intact cells, there is a lack of knowledge on the potential impact on flotation associated with organics released from disrupted cells. To understand this, we investigated the effect of the lysed cells on flotation using the same representative system. Flotation tests were done in two ways: by addition of deliberately disrupted cells by sonication to flotation; and by addition of the intact cells to the grinding step prior to flotation. The latter was done because grinding is an integral step in mineral processing, where cell disruption is likely to occur.

2. Experimental

2.1. Materials

2.1.1. Bacterial strain

The model bacterial strain used was the same as previous experiments (Liu et al., 2013a). A pure culture of *E. coli* strain K12 was cultured in LB medium. The cell pellets were washed and suspended in a PBS solution (phosphate buffer saline). The bacterial cell concentration was measured by optical density (OD_{600}) using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific). A 5 mL *E. coli* cell suspension was sonicated on ice with a Bronson Sonifier 250 in a 30-second pulse at setting 4, followed by a 30-second rest. The cells were lysed by four rounds of sonication.

2.1.2. Mineral samples and reagents

The three types of ore used, i.e., high-purity chalcopyrite, simulated ore with controlled gangue, and porphyry copper-gold ore, were prepared according to the same procedures as outlined in the previous work (Liu et al., 2013a). High-purity chalcopyrite (90%) and pyrite (95%) were crushed with a roll crusher. The crushed samples were used to prepare the simulated ore, which was a mixture of 24% chalcopyrite, 33% quartz, and 43% pyrite. The copper-gold ore, referred to as the real ore in this study, was from a copper-gold porphyry deposit (0.35% copper, 0.74 ppm gold). Just prior to flotation, the high-purity chalcopyrite and the simulated ore were dry-ground with a pulverizer to a particle size of D_{80} of 150 μm. The real ore was wet-ground using a rod mill to a particle size of D_{80} of 106 μm. All chemicals used were of analytical grade.

2.2. Flotation test procedure

Flotation tests with the three types of ore were conducted as per the procedure described previously (Liu et al., 2013a). Baseline tests were carried out using distilled water without the addition of the lysed cells. To test the effect of the lysed cells on flotation, different concentrations of *E. coli* cells were added either in the flotation step as lysed cells or in the grinding step as intact cells.

For the flotation of the high-purity chalcopyrite and the simulated ore, 100 g of ore samples was mixed with 500 mL of distilled water to form slurries. The slurry was transferred to a 1.5 L flotation cell with the impeller speed set at 750 rpm. The pulp pH was the natural pH of the ground sample, which was approximately at pH 8.0. Sodium ethyl xanthate was added as the collector to a concentration of 200 g/t for the high-purity chalcopyrite and 50 g/t for the simulated ore. The frother used was MIBC (methyl isobutyl carbinol), with a concentration of 200 g/t for both samples. Air flow rate was maintained at 5 L/min for the high-purity chalcopyrite and 3 L/min for the simulated ore. Concentrates were collected at 1, 2, 4, and 8 min.

For the real ore flotation, 1 kg of the ore sample was ground with 500 mL of distilled water to a particle size of D_{80} of 106 μm. The slurry was transferred to a 2.5 L flotation cell with the impeller speed set at 1000 rpm. The pulp pH was the natural pH of the ground ore, which was approximately pH 8.3. AEROPHINE® 3418A and PAX (potassium amyl xanthate) were added as collectors to a concentration of 10 g/t and 20 g/t, respectively. MIBC was added as the frother to a concentration of 35 g/t. Air flow rate was maintained at 3 L/min. Concentrates were collected at 1, 3, 5, and 10 min. To test whether cell disruption could occur in the grinding step, the intact cells were added into the grinding mill, the slurry from which was used for the subsequent flotation process.

The concentrates and tails were filtered, dried in an oven at 70 °C overnight and weighed for the analysis. Samples were analyzed for copper by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES), and for gold by Atomic Absorption Spectroscopy (AAS). Flotation tests were carried out with three repeats of each for the high-purity chalcopyrite and in duplicate for the simulated and real ore. The standard errors were calculated and presented as error bars in all charts.

3. Results and discussion

3.1. Flotation of the high-purity chalcopyrite

Fig. 1(A) shows the mass recovery of the high-purity chalcopyrite as a function of time at different concentrations of the lysed cells. The lysed cells had a negative effect on chalcopyrite flotation. The magnitude of the effect depends on the concentration of the lysed cells, with a lower mass recovery at a higher concentration of the lysed cells. Fig. 2(B) compares the results of the lysed cells with those of the intact cells. It showed that the lysed cells had a much stronger adverse effect than the intact cells did. This might be attributed to the bacterial cell disruption causing the release of large amounts of organic molecules, which consist of 15% protein, 7% nucleic acids, 3% polysaccharides, and 2% lipids and metabolites, with the remaining 73% being water (Watson, 2003). These organics could have absorbed onto mineral particle surfaces and covered larger surface area than the intact cells did, leading to a stronger effect. The cumulative recovery data R (%) over time t (min) were then fitted to the classic first order kinetic model of flotation $R = R_{max}(1 - e^{-kt})$, from which flotation rate constant k (min^{-1}) and maximum recovery R_{max} were derived (Asghar et al., 2015; Yianatos et al., 2006). Fig. 1(C) shows that the flotation rate constant k and maximum recovery R_{max} decreased as the concentration of disrupted bacteria cells was increased, indicating that the disrupted bacteria cells negatively affected flotation kinetics as well.

3.2. Flotation of the simulated ore

High-purity chalcopyrite is expected to act differently from mineral mixtures where preferential adhesion of bacteria cells and associated organic molecules to specific minerals might occur. For example, *E. coli* cells preferentially adsorbed onto pyrite over chalcopyrite (Liu et al., 2013a), and *B. subtilis* and *M. phlei* adhered to dolomite more readily than onto apatite (Zheng et al., 2001). There are evidences in the literature showing the selective adsorption of protein and nucleic acids on mineral surfaces (Cleaves et al., 2011; Wang et al., 2012). In this study, flotation tests were carried out using a simulated ore with a defined gangue composition to understand the effect of the lysed cells on flotation of mineral mixtures.

Fig. 2(A) shows the copper recovery as a function of time at different concentrations of the lysed cells for the simulated ore. The flotation of chalcopyrite in the simulated ore was depressed in the presence of the lysed cells. A higher concentration of the lysed cells corresponded to a lower copper recovery. Comparisons were made between the high-purity chalcopyrite and the simulated ore and between the lysed cells and the intact cells by calculating the relative recovery decrease, defined as the percentage decline in final recovery based on the recovery of the baseline test. The higher the absolute value of the relative recovery