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Bioflotation of malachite using different growth phases of *Rhodococcus opacus*: Effect of bacterial shape on detachment by shear flow

Gahee Kim [a], Kyuhyeong Park [a], Junhyun Choi [a], Allan Gomez-Flores [a], Yosep Han [a], Siyoung Q. Choi [b,*], Hyunjung Kim [a,*]

[a] Department of Mineral Resources and Energy Engineering, Chonbuk National University, 567, Baekje-daero, Deokjin-gu, Jeonju, Jeonbuk 556-756, Republic of Korea
[b] Department of Chemical and Biomolecular Engineering, KAIST, Daejeon 305-701, Republic of Korea

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ABSTRACT

We study the effects of the growth phase of *Rhodococcus opacus* on the floatability of malachite. We find that bacteria in stationary phase show more than two-fold higher floatability than bacteria in mid-exponential phase. To understand the mechanism of this higher floatability for the stationary phase, we examine bacteria surface properties such as zeta potential and contact angle. Surprisingly, all bacteria surface properties are nearly the same; moreover, the amounts of bacteria adsorbed onto malachite are also identical. Despite bacteria's similar surface properties, we discover that the amount of bacteria detached from malachite during the mixing process in flotation is larger for the mid-exponential phase than for the stationary phase. We attribute this to hydrodynamic shear stress due to the fluid flow established in mineral mixing. Furthermore, we observe that bacteria–bacteria interactions are far more significant for the mid-exponential phase, leading to end-to-end aggregation and thus allowing unique loosely-packed structures on the malachite surface to form. Such loose structures of bacteria on the mineral are highly susceptible to fluid flow and can be easily detached from the mineral surface. Our findings suggest that due to the relatively large size of the bacteria, it is crucial to consider the detachment of adhered bacteria on the mineral surface due to fluid flow during mixing, which is easily ignored in typical flotation processes.
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1. Introduction

Separating desired minerals from natural ores has been one of the fundamental engineering issues of the last century. To separate minerals, various techniques have been developed, including many different types of flotation and chemical leaching (Ahmed et al., 2007; Houot, 1982, 1983; Matis, 1994; Teague et al., 1998). Of the various techniques, flotation has been one of the most commonly used techniques because it is cheap and efficient (Houot, 1982, 1983; Matis, 1994). However, despite the many advantages of flotation, the hazardous chemicals used in the flotation processes for many minerals limit further development of this process for other minerals and more widespread usage of this process in general. For example, malachite and other oxide Cu ores involve a series of environmentally hazardous reagents such as sodium sulfide, ammonium sulfide, and xanthate (Gottofrey et al., 1988; Guo et al., 2010; Pearse, 2005; Warenycia et al., 1989; Webb et al., 1976). Accordingly, many previous attempts have been made to replace hazardous materials in the flotation process (Dwyer et al., 2012; Merma et al., 2013). Instead of using harmful chemicals, bioflotation has used biological organisms to replace toxic collectors; it has been shown that bioflotation can be an alternative solution to avoid environmental and safety issues even though it has been only successful in the lab to date (Dwyer et al., 2012; Merma et al., 2013; Rao and Subramanian, 2007; Rao et al., 2010; Vilinska and Rao, 2008).

Microorganisms (e.g. bacteria, algae, fungi, and yeast) are typically used for bioflotation because of their abundance in nature (Rao and Subramanian, 2007; Rao et al., 2010; Solozhenkin et al., 2003; Vilinska and Rao, 2008). It is relatively easy to multiply the number of any bacteria, and so bioflotation can also be cost effective. In particular, hydrophobic bacteria have been intensively used as collectors because they facilitate mineral adsorption to a gas/liquid interface (Dwyer et al., 2012; Rao and Subramanian, 2007). Furthermore, some bacteria can improve adhesion to specific minerals, thus allowing *selective* flotation to be performed (Botero et al., 2008; Dahlbäck et al., 1981; de Mesquita et al., 2003; El-Midany and Abdel-Khalek, 2014; Hosseini et al., 2005; Merma et al., 2013; Ohmura et al., 1993; Patra and Natarajan, 2004a,b; Subramanian et al., 2003; Yang et al., 2013; Zheng et al., 2001). These previous studies strongly suggest that the various types of surface chemistry of bacteria can be used to achieve effective bioflotation for a myriad of minerals.

While surprisingly many capabilities of bacteria in the flotation process have been discovered and studied, bacterial adhesion and detachment mechanisms on minerals still remain elusive. Bacteria surface interaction with minerals is fundamentally different from that of

* Corresponding author.
E-mail addresses: sqchoi@kaist.ac.kr (S.Q. Choi), kshjkim@jbnu.ac.kr (H. Kim).

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surfactants: surface–surface interaction becomes more important for micron-sized bacteria, while surfactant interaction with minerals is mostly point–point interaction. In other words, nanometer-sized typical collectors are attached to a mineral surface at the point either chemically or physically whereas bacteria collectors are attached by surface–surface interactions. These fundamentally different types of interaction from a typical flotation process require thorough examination of bacteria–mineral interaction. To date, numerous studies have focused on the roles of individual bacteria in the flotation process (Botero et al., 2008; Calfa and Torem, 2008; Cayllahua and Torem, 2011); however, as far as we know, the effect of the growth phase on floatability has never been investigated, even though it is known that bacteria surface chemistry (e.g. size, functional groups, extracellular polymeric substances, hydrophobicity, and charge density) can differ according to bacteria growth phase (Bayne-Jones and Adolph, 1932; Daughney et al., 2001; Ghosh et al., 2006; Hong and Brown, 2006; Jana et al., 1999; Marcus et al., 2012; Pohlmann-Dietze et al., 2000; Soon et al., 2011; Walker et al., 2005a,b).

In this study, we investigated the effect of the bacteria growth phase on the floatability of malachite. We found that bacteria in the stationary phase show floatability roughly two times higher than that of bacteria in the mid-exponential phase. Despite this huge difference in floatability, the bacteria surface properties are remarkably similar: bacteria in both phases show the same electrostatic properties, as determined by their zeta potential measurements; the two types of bacteria have the same adhesion ability onto malachite. However, differences present themselves due to the detachment process in the agitation and mixing of minerals. We attribute such differences to the shear-induced detachment process in the boundary layer near the surface of the minerals, which results in differences in size, and the differences are also due to the distinct structure formation on the malachite due to sparse end-to-end aggregation of the bacteria themselves. Based on experimental observations, we provide a simple model that can show how shear detachment force depends on the size of the bacteria in the presence of flow.

2. Materials and methods

2.1. Minerals and bacteria

Sphere-shaped malachite ($Cu_2(OH)_2CO_3$) with 55% minimum content of Cu was purchased from Junsei Co. (Japan). Two sieves with −270 and +325 mesh were used to separate from the rest for obtaining only the malachite particles with diameters ranging from 45 μm to 53 μm; only the separated malachite was used in this study.

Rhodococcus opacus (*R. opacus*), one of typical soil bacteria, was obtained from the Korean Collection for Type Cultures (KCTC 9811); it was sub-cultured multiple times to increase the bacteria activity in the liquid–solid medium before culturing in agar medium. *R. opacus* was grown until the formation of a mid-exponential and a stationary phase in glucose–asparagine medium, which is composed of 10 g of glucose, 1.0 g of L-asparagine, 0.5 g of K_2HPO_4, and 2.0 g of yeast extract in 1 L deionized water with the pH of 7.3. The medium was autoclaved for 20 min at 121 °C before usage. All other chemical reagents for the medium were obtained from Sigma-Aldrich except for glucose (Duksan Pure Chemicals Co., Ltd.) and yeast extract (BD Biosciences).

R. opacus were grown to the mid-exponential phase (12 h) and the stationary phase (48 h) at 30 °C, 200 rpm in an incubator. Once cells were grown to the specific phase, bacteria pellets were collected by centrifugation (1580MGR, Gyrozen, South Korea) at 4000 *g* force, at 4 °C for 15 min; then, the supernatant was gently removed. In order to remove any unnecessary residue on the surface of the collected cells, they were washed with 10 mL of pre-autoclaved 0.1 mM NaCl solution and vortexed for 30 s. After vortexing, cell suspension was centrifuged at 4000 *g*-force, at 4 °C for 15 min, and then resuspended in 10 mL of 0.1 mM NaCl solution. After at least three iterations of these washing steps, cells were finally resuspended in 0.1 mM NaCl solution. Bacteria stock solution was used for all the experiments in this study.

2.2. Characterization of malachite and bacteria

2.2.1. Zeta potential

In order to characterize the electrokinetic properties of *R. opacus* and malachite, the electrophoretic mobility was measured by ELS-Z (Otsuka, Hirakata, Japan); the electrophoretic mobility was converted to the zeta potential using Smoluchowski's equation (Overbeek, 1946). Since the separated malachite was still too big for this measurement, it was suspended in DI water (Merck Millipore, Molsheim, France) and ground at 700 rpm for 4 h using an attrition mill (Korea Material Development Co., Siheung, South Korea) with an addition of 5 mm alumina ball as grinding media to make a measurable size distribution (3–5 μm) of ELS-Z. A small amount of ground malachite was sampled and resuspended in 1 mM NaCl solution; electrophoretic mobility was measured for various levels of pH. Samples of *R. opacus* in the mid-exponential and stationary phases were also suspended in 1 mM NaCl solution and their zeta potential was measured. pHs of the bacteria and of the malachite suspensions were adjusted to the desired values using 0.1 M HCl and 0.1 M NaOH (Fisher Scientific).

2.2.2. Contact angle measurements

The contact angles of *R. opacus* during each growth phase were determined using a goniometer (KRÜSS, Hamburg, Germany). *R. opacus* was collected by suction filtration on a 0.45 μm pore size cellulose acetate membrane filter (ADVANTEC, Tokyo, Japan) (van Oss, 1975; van Oss and Gillman, 1972). After filtration, the collected quantity of bacteria was dried for 20 min in a desiccator and bacteria contact angle was measured within 30 min (van Oss, 1994). Drop volume was fixed at 0.2 μL using a syringe with a needle diameter $\Phi = 0.212$ mm (NE60, KRÜSS, Germany). This contact angle measurement was repeated at least 10 times for each growth phase of the bacteria.

2.3. Microflotation

The flotation was conducted using a custom-made Hallimond tube (Somasundaran and Zhang, 2006) for 5 min with 340 rpm agitation (PC-410D, Corning Life Sciences, Mexico). 99.999% purity nitrogen gas was injected with a fixed flow rate of 30 mL/min into the 1 mM NaCl solution. The flotation experiments were conducted at 2×10^7, 5×10^7, 1×10^8, and 2.5×10^8 cells/mL of bacteria concentration at each growth phase. Both the concentrate and the tailing resulting from the flotation were dried at 45 °C in a dryer (JISICO Co., LTD., Seoul, South Korea) for 24 h; then, flotation efficiency was calculated according to the weight ratio of the concentrate to the total mineral.

2.4. Bacterial adhesion test

Various concentrations of *R. opacus* suspension, 2×10^7, 5×10^7, 1×10^8, and 2.5×10^8 cells/mL were first made in the 1 mM NaCl solution. 10 mL of each concentration of *R. opacus* suspension was added to 1 g of malachite in a centrifuge tube and pH was adjusted to 6. Then, the bacteria-malachite suspension was rotated at 40 rpm (RT-10, DAIHAN Scientific, South Korea) for 10 min. After settling the suspension of bacteria-malachite by gravity for 3 min, the remaining amount of bacteria in the supernatant was transferred to a sterilized centrifuge tube followed by vortexing and determined in the counting chamber (Bruker–Turk, MARIENFELD, Germany) (Strober, 2001). The amount of *R. opacus* adhering to the malachite was calculated using a well-known Eq. (1) (Akar and Tunali, 2006),

$$\Gamma = (C_0 - C) \times \frac{V}{m}, \tag{1}$$

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