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On the fundamentals aspects of hematite bioflotation using a Gram positive strain

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ABSTRACT

The use of microorganisms and/or their metabolic products is becoming an attractive alternative in mineral processing. In that sense, this research deals with the use of the hydrophobic gram positive *Rhodococcus erythropolis* bacteria as a possible substitute of synthetic reagents used in hematite flotation. Bacterial growth experiments, using culture media of tryptic soy broth (TSB) and yeast and malt extract with glucose (YMG) separately, showed a greater bacterial density for the first one. In addition, it was observed that the isoelectric point (IEP) of hematite was shifted after biomass interaction, suggesting that the bacterial cells were adhered onto the mineral surface. Moreover, bacterial adhesion was higher at acidic pH, which also suggests an electrostatic attraction between the mineral surface and the biomass at this pH range. Microflotation tests were carried out in a modified *Hallimond* tube achieving a maximum hematite floatability of 83.86% at pH 6. Finally, the kinetics of the process followed a second order model.

1. Introduction

The production of bioreagents capable of a selective separation in mineral processing has been the objective of several studies. The most important advantages for the use of these bioreagents can be: their selectivity for a specific mineral and environmental impact reduction caused by conventional reagent (Santhiya et al., 2001; Chandraprabha and Natarajan, 2006; Patra and Natarajan, 2008; Gericke and Govender, 2011; Merma et al., 2013; Lopez et al., 2015). Several studies have proved that microorganism (dead and alive) and their metabolic products can act as surface modifiers, collectors, depressants and even as dispersing agents in flocculation and flotation processes (Dubel et al., 1992; Deo and Natarajan, 1997; Zheng et al., 2001; Sharma et al., 2001; Mesquita et al., 2003; Hosseini et al., 2005; Farahat et al., 2008). The principal function of a microorganism in mineral processing is related to the non-polar (hydrocarbon chain) and polar (carboxyl, phosphate, hydroxyl) functional groups present in its cell wall, which confer properties that render them capable of act as bioreagents. These functional groups can interact with the mineral surface, conferring amphipathic characteristics in the same way as the reagents traditionally used in mineral processing (Mesquita et al., 2003; Dwyer et al., 2012).

Most of the works dealing with mineral bioflotation focuses primarily on its fundamental aspects (Mehrabani et al., 2010; Khoshdast et al., 2012; Elmahdy et al., 2013), understanding the interaction mechanisms between the bioreagent and the mineral surface (Deo and Natarajan, 1998; Natarajan and Deo, 2001). These interactions can include: relation hydrophobicity/hydrophilicity in the mineral surface, and adhesion/adsorption of the bioreagent/metabolic product onto the mineral surface. The literature review shows that many microbial strains have been developed as collectors for hematite, showing their potential use as bioreagents. Most studied bacteria are: *Mycobacterium phlei* (Dubel et al., 1992; Yang et al., 2007), *Bacillus polymyxa* (Deo and Natarajan, 1997; Shashikala and Raichuur, 2002), *Paenibacillus polymyxa* (Deo and Natarajan, 1998), *Desulfovibrio desulfuricans* (Prakasan and Natarajan, 2010), *Rhodococcus opacus* (Natarajan and Deo, 2001; Mesquita et al., 2003; Merma et al., 2013; Kim et al., 2015), *Bacillus subtilis* (Sarvamangala and Natarajan, 2011) and *Rhodococcus ruber* (Lopez et al., 2015).

Rhodococcus erythropolis, is a Gram positive nonpathogenic microorganism found widely in nature in a large variety of sources including soils, rocks, boreholes, groundwater, marine sediments, animal dung, the guts of insects and from healthy and diseased animals and plants (Bell et al., 1998; Carla and Manuela, 2005). This strain contains a large set of enzymes that allows it to carry out an enormous number of bioconversions and degradations, performing different reactions such as: oxidations, dehydrogenations, epoxidations, hydrolysis, hydroxylations, dehalogenations

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and desulfurizations (Carla and Manuela, 2005). In addition, it has been widely used for bioremediation of oil-contaminated water and soil (Liu and Liu, 2011; Carla, 2012) and it was recently studied in mineral biotechnology, as a collector reagent for hematite from iron ores (Yang et al., 2013).

As mentioned in previous researches, the use of microorganisms as bioreagents could be a promising solution to the current problems in mineral flotation, such as, the need to achieve lower operating costs in the processing of low grade ores, mineral selectivity in the processing of fine and ultrafine mineral particles and to the constant quest of reagents that attends the rigorous specifications for production of concentrates and stricter environmental legislation (Gericke and Govender, 2011; Lopez et al., 2015; Ramos-Escobedo et al., 2016).

Therefore, the review of the state-of-the-art of mineral bioflotation requires fundamental studies that show the relevant potential use of biomass as biocolletors and/or biofrothers in the flotation of minerals, focusing in the bioadhesion process as one of the most important steps in mineral bioflotation. Against this background, this paper provides a study based on the fundamental aspects of hematite bioflotation with the main objective to evaluate the behavior of the *R. erythropolis* strain onto the hematite surface and its potential use as a biocollector and/or biofrother.

2. Materials and methods

2.1. Sample preparation

The hematite sample, purity of 94%, was provided by local supplier from Araçuaí in Minas Gerais. The samples were jaw-crushed and dry-screened to −3 mm. These samples were then dry-ground in a porcelain mortar and wet-screened for obtaining the desired size fractions (Table 1).

2.2. Microorganism, media and growth

Rhodococcus erythropolis was obtained from the CBMAI – UNICAMP. The microorganism was cultivated in tryptic soy broth (TSB) culture medium and yeast and malt extract with glucose (YMG) culture medium. The TSB culture medium was composed of pancreatic digest of casein 17.0 g/l, papaic digest of soybean 3.0 g/l, dextrose 2.5 g/l, sodium chloride 5.0 g/l and dipotassium phosphate 2.5 g/l. While the YMG culture medium was composed of glucose 10.0 g/l, peptone 5.0 g/l, malt extract 3.0 g/l and yeast extract 3.0 g/l.

Stocks of the bacteria were prepared using a solid medium in Petri plates and saving them in a refrigerator at 4 °C to later be inoculated in liquid medium. After the bacterial inoculation in liquid medium, the flasks were disposed on a rotary shaker, maintained at 150 rpm and 28 °C until it reaches its maximum concentration. Thereafter, the bacterial suspension was centrifuged and the obtained biomass was twice washed with deionized water, then the cells were re-suspended in a 10^{-3} M NaCl solution. Finally, the bacterial concentrate was autoclaved in order to avoid further bacterial development.

Table 1
Experiments and particle size range.

Experiments	Size
Zeta potential	<38 μm
Adsorption	(−53 + 38 μm)
Flotation	(−150 + 106 μm)
	(−106 + 75 μm)
	(−53 + 38 μm)

For the measurement of cell concentration in suspension, the dry weight method was adopted, which was determined by the difference between the final weight and the initial weight, expressed in g/l.

2.3. Quantification of carbohydrates and proteins presented in the bacterial concentrate

There are several methods to establish the composition of the cell wall, some of them using methods such as X-ray diffraction, infrared spectroscopy and biochemical analysis. The composition of the carbohydrates presented in the bacterial concentrate was determined by the anthrone method (proposed by Yemm and Willis, 1954) using glucose as a standard reagent and a standard curve of absorbance (620 nm) × carbohydrates (mg/ml). While proteins associated to the bacterial concentrate was determined by the biuret method (Stickland, 1951) using casein as standard and making a standard curve of absorbance (540 nm) × proteins (mg/ml).

2.4. Zeta potential measurements

Zeta potential measurements for the mineral sample and the microorganism were carried out on the micro-electrophoresis apparatus "Zeta Meter System 4.0". The concentration of the cell suspension was 50 mg/l and NaCl 10^{-3} M was used as indifferent electrolyte, while for mineral samples a concentration of 70 mg/l was used, pH was adjusted using diluted HCl and NaOH solutions. The evaluation of the zeta potential profiles of cells was carried out before and after interaction between the bacterial cells and the mineral sample.

2.5. Adhesion experiments

All bioadhesion experiments were carried out in a duplicate run basis, at 25 °C. The suspensions were prepared by mixing 0.25 g of the mineral sample and known quantities of the bacteria cells (50, 100, 150 and 200 mg/l) in 35 mL of NaCl 10^{-3} M solution, the suspension was agitated on a rotary shaker at a constant shaking of 150 rpm, during 5 min to ensure that equilibrium was reached. The studies were performed as a function of pH (range 2.0–9.0). The concentration of the bacteria was determined using the optical density method in a UV-1800 Shimadzu spectrophotometer, at a wavelength of 620 nm.

The amount of bacterial adhered was calculated by:

$$q = \frac{V(C_0 - C_f)}{M} \quad (1)$$

where C_0 and C_f are the initial and equilibrium concentrations of the bacterial (g/l) respectively, M is the weight of mineral (g) and V is the solution volume (l).

2.6. SEM analysis

The analysis of scanning electron microscopy (SEM) was used to observe *R. erythropolis* and its adsorption onto hematite. The *R. erythropolis* was dehydrated in graded series of acetone and air dried under vacuum; while for the hematite sample after adsorption, it was carefully washed with deionized water to remove loosely attached bacteria and subsequently it was dried. Before analysis, *R. erythropolis* and hematite samples were gold coated under vacuum using a Bal-Tec SCD 050 device and then they were immediately placed on the FEI Quanta 400 scanning electron microscope to get the images.