Minerals Engineering 110 (2017) 179–184

Contents lists available at ScienceDirect

Minerals Engineering

journal homepage: www.elsevier.com/locate/mineng

Selective bio-flotation of sphalerite from galena using mineral – adapted strains of *Bacillus subtilis*

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ARTICLE INFO

Keywords:
Bioflotation
B. subtilis
Mineral adaptation
Sphalerite
Galena

ABSTRACT

In this study, the effect of adaptation of *B. subtilis* to sphalerite and galena with respect to the selective flotation of sphalerite from a sphalerite-galena synthetic mixture has been examined. The changes in the surface charge of the mineral and the bacterium, cell wall components and the profiles of secreted proteins are discussed. The protein profiles of the unadapted and adapted cells are found to differ distinctly, both qualitatively and quantitatively, with impact on the selective flotation of sphalerite and galena. Electrokinetic measurements show shifts in the iso-electric points of not only *B. subtilis* but also of the chosen minerals after adaptation. Additionally, the zeta potential of *B. subtilis* is found to become less negative after bacterial adaptation to the chosen sulfide minerals, while it showed an increase in the magnitude of surface negative charge after various enzymatic treatments. The change in the surface morphology and cell wall components such as phosphate, uronic acid and acetylated sugars of the bacterial species during adaptation to sphalerite and galena has been assessed. Selective flotation tests on a synthetic mixture of galena and sphalerite confirm that sphalerite can be preferentially floated from galena in the presence of the insoluble fraction of lysed *B. subtilis* cells initially adapted to sphalerite, with a high selectivity index.

1. Introduction

A growing demand for minerals across the world, coupled with the depletion of high-grade ores over the years, has culminated in the exploitation of lean-grade ores with complex mineralogy. Additionally, some of the chemical reagents used in existing flotation processes viz., cyanides, chromates etc., have caused concern on environmental grounds. Recent developments in biotechnology have opened up possibilities for the utilization of microorganisms in mineral beneficiation as flotation collectors, depressants and activators. The application of microorganisms for bioflotation and bioflocculation has been critically reviewed (Rao and Subramanian, 2007; Dwyer et al., 2012). Detailed surface chemical studies on sphalerite and galena using *Paenibacillus polymyxa* and *Acidithiobacillus thiooxidans* for the selective separation of sphalerite from galena has been reported (Santhiya et al., 2001a, 2001b, 2001c, 2002). The adhesion of *Bacillus subtilis* and *Mycobacterium phlei* onto dolomite and apatite with respect to the anionic flotation of apatite from dolomite has been studied (Zheng et al., 2001). Gram-positive bacteria are very promising candidates for the separation of minerals because their distinctive cell wall architecture contains a thick peptidoglycan layer which harbours several functional groups of interest vis-à-vis metals. Hence, the growth of bacteria in the presence of different metal ions/minerals leads to dynamic changes, both qualitative and quantitative, in its cell surface as well as extracellular secretions viz., polysaccharides, proteins and enzymes (Reed, 1987; Chudobova et al., 2015). Thus, adaptation of bacteria to minerals can bring about several physico-chemical changes on the bacterial cell and mineral surfaces. The adapted state may involve changes in the bacterial cell morphology, compositional change in the secreted proteins and polysaccharides as well as the surface charge.

B. subtilis is a rod shaped, neutrophilic, Gram positive, mesophilic bacterium. It is found abundantly in soil and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions (Madigan et al., 2010). The cell surface is made up of many components like peptidoglycan, teichoic and teichuronic acids, surface proteins, polysaccharides and polypeptides which facilitate the microbe-mineral interactions. In this communication, the adaptation of *B. subtilis* to either sphalerite or galena has been studied with respect to the selective flotation of sphalerite from a sphalerite-galena synthetic mixture. Additionally, adaptation induced changes in cell surface charge, cell wall composition and the profiles of secreted proteins are discussed.

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http://dx.doi.org/10.1016/j.mineng.2017.05.002
Received 7 January 2017; Received in revised form 19 April 2017; Accepted 11 May 2017

2. Materials and methods

2.1. Minerals

Mineral samples of sphalerite and galena were obtained from Wards Natural Science Establishment, USA and Alminrock Indscer Fabriks, Bangalore, India respectively. The samples were subjected to dry grinding in a porcelain mill. The ground sample was then dry screened and the $d_{50} \sim 5$ μm size fraction was used in the electrokinetic studies and (−150 + 105 μm) size fraction of the minerals was used in the flotation tests.

2.2. Bacterial strain and growth conditions

Bacillus subtilis (NCIM 2063) obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India was cultured using the modified Bromfield medium as described elsewhere (Vasanthakumar et al., 2013). The bacterial cell number was enumerated by microscopic counting using a Petroff–Hausser counter under a phase contrast microscope. The culture was centrifuged at 10,000 rpm for 15 min at 4 °C. The cell pellet was washed twice and resuspended in 0.1 M of sodium phosphate buffer at pH 8.0. For adaptation studies, the bacteria were grown in modified Bromfield medium in the presence of either sphalerite or galena mineral at 2% pulp density. The minerals were sterilized separately before use in the adaptation studies. The subculturing of the adapted bacteria was carried out after every 48 h for ten cycles.

2.2.1. Protein precipitation procedure

Bacterial cultures grown for 48 h were centrifuged at 10,000 rpm for 15 min at 4 °C to separate the cells from the extracellular materials. Proteins present in the cell-free metabolite were obtained by precipitation with ammonium sulphate at a saturation level of 80%. The protein precipitate was dissolved in a minimum volume of 0.1 M sodium citrate buffer at pH 4.5 and dialyzed against the same buffer. Protein yields were estimated using A_{280} nm method.

2.2.2. Thermolysis procedure

Bacterial cells were resuspended in 0.1 M sodium phosphate buffer at pH 8.0 and boiled at 100 °C for 30 min. The thermolysed cells were cooled and centrifuged at 15,000 rpm for 30 min at 4 °C to obtain the soluble and insoluble fractions.

2.2.3. Selective flotation test procedure

One gram of the mineral mixture (sphalerite and galena in the ratio of 1:1) was pulped in 200 ml of 0.1 M sodium phosphate buffer containing 1×10^9 cells/ml of bacterial cells or thermolysed cells or soluble fraction or insoluble fraction and interacted for 30 min at room temperature. After interaction, the conditioned minerals were transferred to a modified Hallimond tube (Fuerstenau et al., 1957). Flotation of the mineral was carried out as per the procedure detailed elsewhere (Vasanthakumar et al., 2013). The settled and floated fractions were separated, dried and weighed. The amount of lead and zinc in the concentrate and tailing fractions was analyzed by Atomic Absorption Spectroscopy (AAS) (M series, Thermo Electron Corporation) and the percent recoveries were calculated. Selectivity Index was calculated according to Gaudin's formula (Gaudin, 1957).

2.2.4. Electrokinetic measurements

Electrophoretic measurements were carried out using Zetasizer Nano ZS90, from Malvern Instruments Limited, Worcestershire, UK. Zeta potentials of the chosen minerals as well as that of the unadapted or mineral adapted bacteria were measured from pH 2 to 10 with an interval of one pH unit. In addition, the zeta potential of mineral adapted bacteria after various enzymatic treatments as described elsewhere (Vasanthakumar et al., 2013) was measured.

2.2.5. SEM analysis

The morphology of sphalerite and galena adapted bacterial cells was observed using a FEI Sirion, high resolution electron microscope from Icon Analytical Equipment Pvt. Ltd., Mumbai, India. After vacuum drying, the samples were made conducting by gold sputtering coating using a JEOL ion sputtering device.

2.2.6. Isolation and estimation of bacterial cell wall components

The phosphorus, N-acetyl glucosamine and uronic acid content of isolated bacterial cell walls was determined as per the protocol described elsewhere (Chen et al., 1956; Reissig et al., 1955; Dutton, 1966).

3. Results and discussion

3.1. Selective flotation in the presence of adapted cells

The bacterial cell population or its components, interaction period with minerals, mineral surface coverage through bacterial adhesion etc. were set as controlling factors which determine mineral surface hydrophobicity with reference to flotation. The selective flotation recovery of sphalerite from a sphalerite-galena mineral mixture in the presence of intact or thermolysed *B. subtilis* cells after 10 cycles of adaptation to either sphalerite or galena is shown in Fig. 1. The intact sphalerite adapted bacteria show a higher selectivity index (4.1) vis-à-vis intact galena adapted bacteria (1.1). Thermolysis disrupts bacterial cellular structure (Russell and Harries, 1968) and leads to denaturation of ribosomes (Lee and Kaletunç, 2002), inactivation and aggregation of various cellular proteins (Lepock, 2005), RNA splicing (Richter et al., 2010) and the breakage of the genomic DNA (Kim et al., 2008). Thermolysed bacterial cells enhance the selective flotation of sphalerite from the mineral mixture. This characteristic feature is observed in the unadapted as well as the adapted state. Thermolysed bacterial cells can be separated into soluble and insoluble fractions. The maximum selectivity index (20.4) is obtained in the presence of the insoluble fraction of thermolysed sphalerite adapted bacteria. A diametrically opposite behaviour of the two adaptations towards galena flotation is observed. This shows that sphalerite adapted cells depress galena flotation, while galena adapted cells enhance the flotation recovery of galena from the mineral mixture. Despite this differential behaviour of adaptation towards flotation of galena, sphalerite recovery is consistently higher than that of galena in all conditions. In general, the picture that emerges is that thermolysed cells of bacteria after adaptation to sphalerite result in better selective flotation of sphalerite from the synthetic mineral mixture and consequently a higher value of selectivity index. Thus, prior adaptation of bacteria to a mineral enhances the flotation recovery of that mineral, compared to the unadapted bacteria.

The charged/hydrophilic surface of such bio-macromolecules could interact with the surface of the mineral to be floated and expose its hydrophobic surface to the aqueous medium. In the present case, this induced hydrophobicity could aid the flotation of the sphalerite mineral. The amphipathic nature of DNA, with the phosphate bearing hydrophilic surface on one side and the aromatic base containing hydrophobic surface on the other side, appears to fulfill the above criteria very well. Additionally, the distance between the interphosphate anionic species of DNA matches closely with the translational distance between adjacent zinc atoms of sphalerite rather than that of lead atoms in galena. Hence, the preferential flotation of sphalerite rather than galena is facilitated. Simultaneously, it can be expected that the non-amphipathic (charged and hydrophilic) macromolecules present in the grossly fractionated biomass bind the mineral to be depressed, i.e., galena. Thus, a combination of activator and depressant bio-macromolecules bring about selective flotation.