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Bioflotation: Bacteria-Mineral Interaction for Eco-friendly and Sustainable Mineral Processing

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Abstract

In the current study, the action of two bacteria capable of producing biosurfactants and oxidizing iron (Fe) and sulfur (S), namely *Bacillus pumilus* SKC-2 and *Alicyclobacillus ferrooxydans* SKC/SAA-2, was investigated with respect to their ability in possessing dual-function as either bio-collector or depressant for the development of sulfide bioflotation processes. Both bacterial strains were able to produce high amounts of biosurfactants interacted with pyrite that had an important role in their adhesion on the surface of pyrite as well as the change of pyrite surface properties. Over the course of the experiments, the pH of the solutions gradually decreased to ~3, indicating the active oxidation of pyrite minerals by bacteria. The growth of both bacterial strains resulted in the generation of biosurfactants as represented by the decrease of the surface tension of the solutions and the increase of the contact angle of the pyrite surfaces as a function of time. However, the contact angle of pyrite surfaces gradually decreased after 5 days of incubation until the experiments terminated on 30 days. Scanning electron microscopy equipped with energy dispersive X-ray spectroscopy (SEM-EDS) and Fourier transform Infrared (FTIR) analyses also confirmed the role of both bacterial strains in changing the pyrite surface properties to be more hydrophobic or more hydrophilic depending on the time of incubation. These results indicate that the changes of pyrite surface properties are clearly as the results of bacterial action, likely serving as both bio-collector or bio-frother and depressant that would be very applicable for flotation processes. These results increase our knowledge on the interactions in pyrite-bacteria complexes and could potentially be a very useful result with real exploitable value for those working on sulfide bioflotation processes.

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Keywords: Biosurfactant; S-Fe-oxidizing bacteria; Bioflotation

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1. Introduction

Interactions between minerals and microbes are of some significance for the development of eco-friendly, low-cost and sustainable mineral processing technologies. Since worldwide reserves of high-grade ores are diminishing, the development of metal recovery from low-grade, lower value mineral ores based on the activity of microbes is highly needed. Bioflotation is a biotechnology by employing microbes, in particular bacteria, involving the adhesion of the bacteria on the minerals that meets the industrial needs for more selective and environmentally friendly mineral separation[1,2,3,4,5]. By adhering to the surface of minerals, the bacteria essentially change the surface characteristics of the minerals as a result of interaction of microbes and mineral surfaces followed by active oxidation/reduction of mineral elements by bacteria.[6, 7, 8] The microbial metabolites and activities derived from microbe-mineral interaction bring about the increased efficiency of flotation that depends on the chemical characteristics of mineral surfaces and thus affects the beneficiation process.[1-7]

Utilization of the bacteria in flotation is quite new, especially bacteria producing biosurfactants that are also capable of oxidizing iron (Fe) and sulfur (S). Hence, in this work, the batch bacterial growth experiments for utilizing the bacteria capable of producing biosurfactants and oxidizing iron and sulfur were conducted in direct contact with pyrite under aerobic conditions. Changes in pyrite surface properties as represented by surface tension and contact angle was determined. The interaction of bacterial cells with pyrite was studied by Fourier transform Infrared (FTIR) Spectroscopy. The aggregate behavior of bacterial cells and pyrite particles was observed by scanning electron microscopy (SEM), while the nature of these aggregates was analyzed by energy-dispersive spectroscopy (EDS). These results would be very helpful and give significant insights into the enhancement of bioflotation of sulfide minerals. In this aspect the bacteria used in this study might be applicable for the flotation bioreagents for creating environmentally friendly mineral processing.

2. Materials and Methods

2.1. Pyrite samples

The pyrite used in this study was obtained from Singajaya, Garut, West Java Province, Indonesia and was ground to obtain the grain size of - 200 + 400 mesh. An X-ray powder diffractrometery (XRD) analysis showed the high purity of pyrite (FeS_2) and no other sulfide minerals were detected (Fig. 1a). In addition, ED-XRF analysis of pyrite sample revealed that its chemical composition was SiO_2 (1.63 wt.%), Al_2O_3 (2.29 wt.%), TiO_2 (0.009 wt.%), Fe_2O_3 (42.03 wt.%), MgO (0.056 wt.%), CaO (0.068 wt.%), Na_2O (0.01 wt.%), K_2O (0.013 wt.%), P_2O_5 (0.009 wt.%), SO_3 (53,81 wt.%), Cr_2O_3 (0.038 wt.%), CuO (0.036 wt.%), NiO (0.003 wt.%), ZnO (0.004 wt.%).

2.2. Bacterium and growth medium

The bacteria *Bacillus pumilus* SKC-2 and *Alicyclobacillus ferrooxydans* SKC/SAA-2used in this study were isolated from the South Sulawesi laterite mineral sand an enrichment culture of crude oil, respectively, which have the abilities of producing biosurfactants and oxidizing iron and sulfur. The growth medium used was the modified Luria–Bertani (LB) medium (l^{-1}: 10 g peptone; 5 g yeast extract; 10 g NaCl, supplemented with 0.5 g $Na_2S_2O_3.5H_2O$ and 0.25 g $FeSO_4.7H_2O$).The photomicrograph of bacterial cells of the strains are shown in Fig. 4b (*Bacillus pumilus*) and Fig. 5b (*Alicyclobacillus ferrooxydans*).

2.3. Batch experimental systems

Batch bacterial experiments were conducted in triplicate in sterile 500 ml Erlenmeyer flasks containing 300 ml of growth medium supplemented with 10% (vol/vol) inoculum of *Bacillus pumilus* or *Alicyclobacillusf*errooxidans and 25% (wt. /vol) pyrite at 25% pulp density. Cultures were incubated with agitation (150 rpm) at 30°C for 4 weeks under aerobic conditions. The hydrophobic properties (as measured by surface tension and contact angle) and pH in solution of each culture were monitored periodically, and separate sets of samples were made up and prepared for analyses by scanning electron microscopy- energy dispersive X-ray spectroscopy (SEM-EDS) and Fourier transform infrared (FTIR). The sample fixation and preparation for SEM imaging and FTIR have been described earlier.[9]

3. Results and Discussion

Bacterial growth and activity in experimental systems tended to reduce the pH of the solutions that gradually decreased to ~3 (Fig. 1b). The reduction was presumably facilitated by bacterial role as a result of the acceleration of pyrite oxidation by oxidizing iron and sulfur that generate H_2SO_4, thereby lowering the pH of the aqueous solutions [8]. The bacterial activity in experimental systems also resulted in a significant change in the hydrophobicity nature of pyrite after interaction with the bacteria as determined by contact angle and surface tension measurements (Figs. 1c, 1d). Over the course of the experiments, the surface tension of the suspensions in experimental systems generally decreased with time; from an initial value 67.5 mN/m to 51.6 mN/m for *B. pumilus* and to 55.7 mN/m for *A. ferrooxidans* (based on average values obtained from batch cultures run in triplicate) (Fig. 1c). This decrease may be due to the biosurfactant production by the strain, thus supporting more evidence for the capacity of the strain to be a frothing agent. [4, 5, 6] As the surface tension decreased, the contact angle values (°) increased with time that demonstrated a larger hydrophobic nature, and subsequently decreased at ~5 days of incubation that exhibited a larger hydrophilic nature (Fig. 1d). The increase and decrease of contact angle value observed in the measurements of pyrite contact angle after the interaction with the strains could be attributed to bacterial cells adhesion and/or metabolic products interactions onto pyrite surface surface.[3, 4] Likewise, the contact angle measurements showed a higher adhesion of the cell at pyrite surface, demonstrating a preferential adhesion of the strains at pyrite minerals.

Scanning electron microscopic observations of pyrite-cell complex revealed the network formation of cells with aggregates of pyrite particles after 30 days of reaction (Figs. 2, 3).Bacterial cells were covered with large aggregates of fine-grained pyrite particles attaching to their cell walls. Most of the complexes consist of highly dispersed pyrite-encrusted bacterial cells associated with aggregatedpyrite. The filaments observed within the pyrite particles and bacterial cells are indicative of organic exudates around the bacterial cell surface. The formation of filaments is attributed with microbial lysis or exudate residues or bacterial extracellular polymeric substances (EPS).[9] SEM-EDS spectra of the complexes showed the high contents of C, O, S and Fe and low contents of Si and Ca (for *B. pumilus*) and Al (for *A. ferrooxidans*). Furthermore, the organic compounds such as EPS in association with pyrite are considered responsible for changing the pyrite surface properties, thus becoming more hydrophilic or hydrophobic as a function of time (Figs. 1c, 1d). This is due to their high functionality (e.g., carboxylic acids) as also confirmed by FTIR spectra (Figs. 4, 5). There was a broad band centered around 3376 - 3426 cm^{-1} that may be attributed to hydrogen-bonded (O-H) hydroxyl groups, N-H amine groups. The absorption bands in the range 1610 –1652 cm^{-1} may be assigned alkene (C=C), carbonyl (C=O), C=N groups and Fe-phosphate. The bands in the region 1399 cm^{-1} arise due to the surface interaction of pyrite and bacteria, band C-H vibration from metals. Four results obtained by FTIR analyses are especially interested in the peaks at 1536 cm-1 (C-N-H and amides resulting from bacterial cell proteins) and at 1028 cm^{-1} up to 1104 cm^{-1}, the bands assigned to complex vibration modes of polysaccharides, C-O ester or carboxylate acid vibrations (resulting from bacterial cells for samples on day 7, 14 and 30) and Si-O stretching (for samples at the onset of the experiment and starting pyrite).More interestingly, the peaks at 741-797 appeared for the samples after interacted with bacteria (7, 14 and 30 days of incubation). The bands are attributed to iron oxides as a result of the bacterial action in oxidizing iron and sulfur, thus providing more evidence for the pyrite oxidation due to the bacterial activity. This obviously confirmed the role of both bacteria in serving as bioflotation depressants.

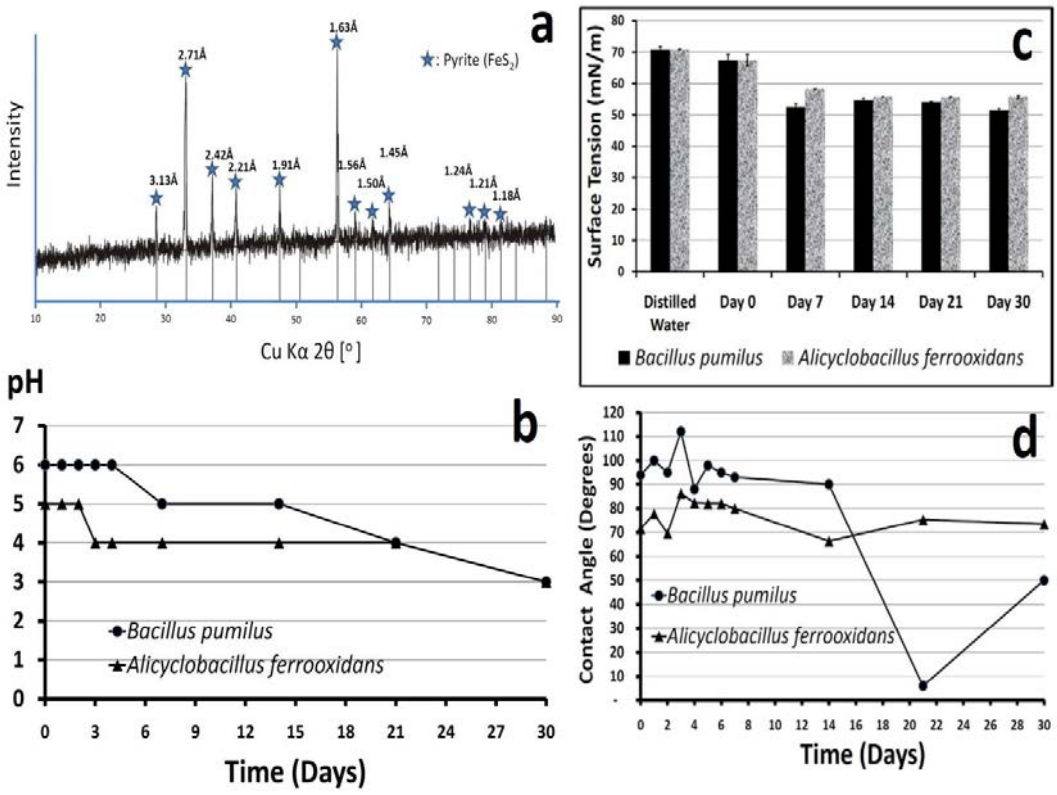

Fig. 1. (a) X-ray powder diffraction analysis of pyrite mineral (FeS_2) used in this study; (b) pH changes in experimental systems inoculated with *Bacillus pumilus* or *Alicyclobacillusf*errooxidans over a 30-day period of the experiment; (c) Surface tension (mN/m) and pH of suspensions in experimental systems inoculated with *Bacillus pumilus* or *Alicyclobacillusf*errooxidans over a 30-day period of the experiment; (d) Contact angle of pyrite before and after interaction with *Bacillus pumilus* or *Alicyclobacillusf*errooxidans over a 30-day period of the experiment. Error bars represent standard deviations based on the averages obtained from batch cultures run in triplicate.

The bacterial strains produce EPS or biosurfactants (surface-active materials) that enhance contact between bacteria and pyrite, thereby enhancing the change of pyrite surfaces. In addition, the abilities of bacteria in oxidizing iron and sulfur also supported their changes as being more hydrophilic or hydrophobic. Based on the SEM-EDS and FTIR analyses, it is also possible that direct contact between cells and pyrite may also create microenvironments (pyrite-bacteria complexes) where pH conditions favor bacterial EPS production, the formation of biofilm matrix, and the formation of iron oxides and hydroxides which might change the chemistry and properties of pyrite surfaces.[8,9]

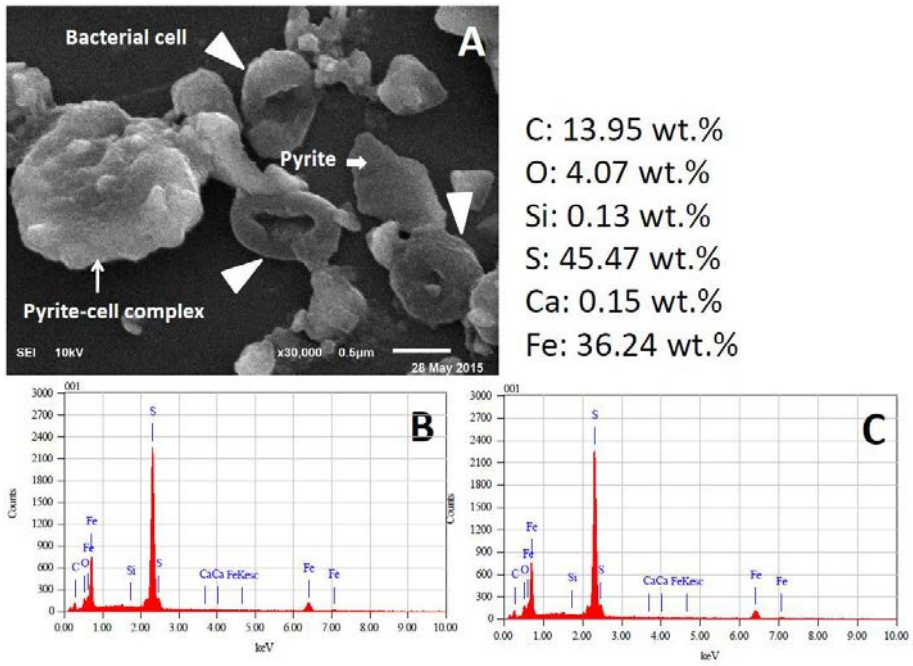

Fig. 2. SEM image (A) and associated EDS spectra; (B, C) of the pyrite-bacterial cell complex in experimental systems inoculated with *Bacillus pumilus* after 30 days of the experiment. Bacterial cells are interacted with an intact pyrite in connection with aggregated pyrite showing the attractive network formation between pyrite and bacterial cells where abundant bacterial cells are clearly seen. The arrows indicate bacterial cells, pyrite particles and pyrite-cell complex.

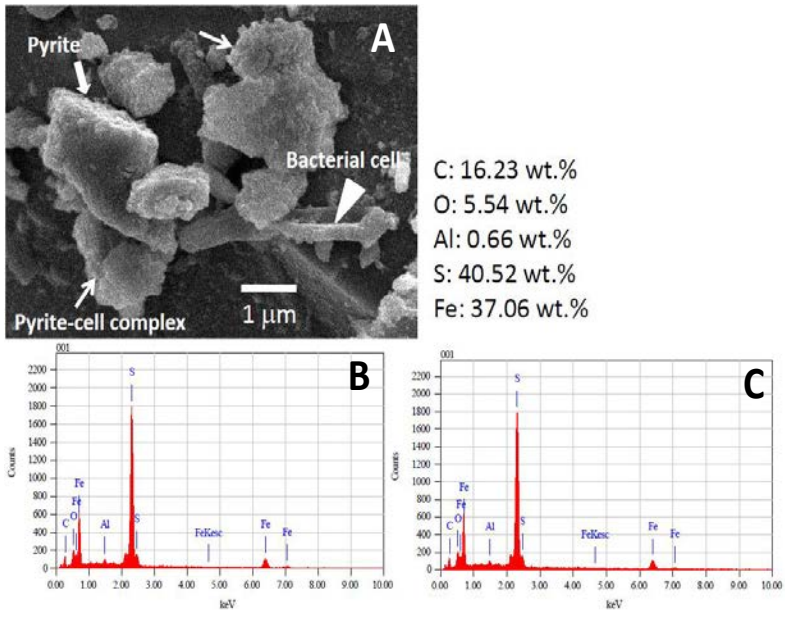

Fig. 3. SEM image (A) and associated EDS spectra; (B, C) of the pyrite-bacterial complex in experimental systems inoculated with *Alicyclobacillusferrooxidans* after 30 days of the experiment. Extracellular polymeric substances (EPS) produced by bacterial cells appears to be a cell aggregation along with the pyrite, where pyrite particles become embedded in the biofilm matrix. The arrows indicate bacterial cells, pyrite particles and the pyrite-cell complex.

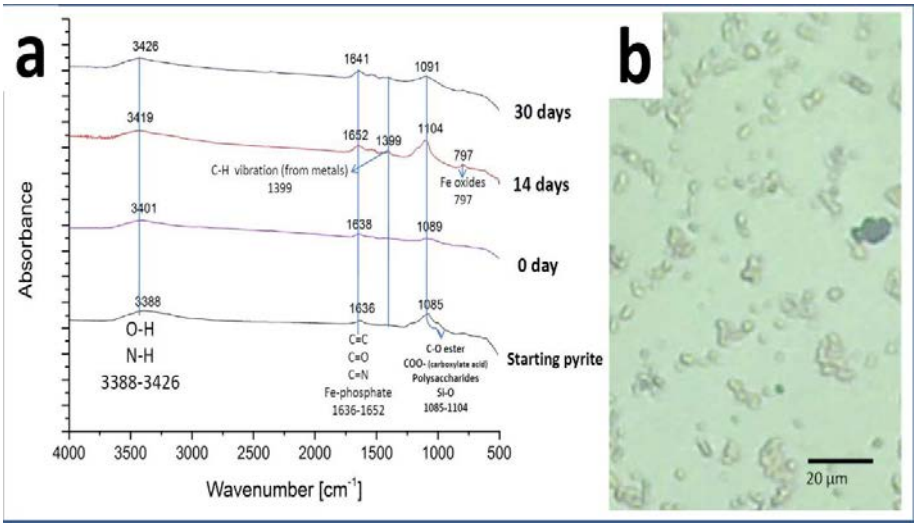

Fig. 4. Fourier transform infrared (FTIR) spectra of starting pyrite, the pyrite-bacterial complexes in experimental systems inoculated with *Bacilluspumilus* at the onset of the experiment and after 14 and 30 days of the experiment (a). Photomicrograph of bacterial cells of *Bacillus pumilus*grown on LB agar supplemented with 0.5 g/L $FeSO_4.7H_2O$ and 0.5 g/L $Na_2S_2O_3.5H_2O$ for 24 h at 25ºC (b).

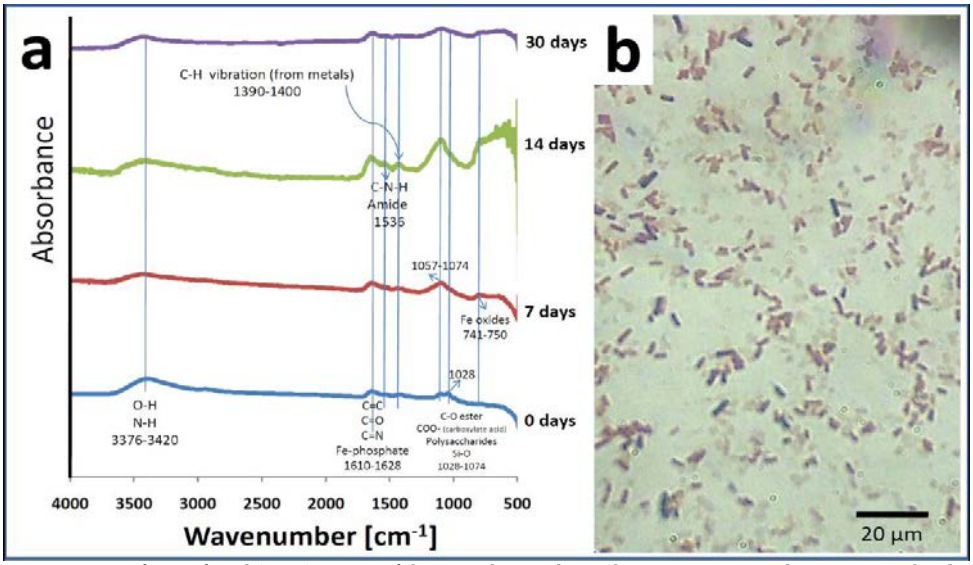

Fig. 5. Fourier transform infrared (FTIR) spectra of the pyrite-bacterial complexes in experimental systems inoculated with *Alicyclobacillusferrooxidans* at the onset of the experiment and after 7, 14 and 30 days of the experiment. Photomicrograph of bacterial cells of *Alicyclobacillusferrooxidans* grown on LB agar supplemented with 0.5 g/L $FeSO_4.7H_2O$ and 0.5 g/L $Na_2S_2O_3.5H_2O$ for 24 h at 25ºC (b).

4. Conclusions

The present study reveals that the bacteria *Bacillus pumilus* SKC-2 and *Alicyclobacillus ferrooxydans* SKC/SAA-2 were able to change the surface chemical properties of pyrite due to biosurfactant production as shown by its high surface activity and frothability for pyrite. Owing to their capability of oxidizing iron and sulfur, the bacteria are also

applicable for serving as depressants. It is concluded that the usage of these strains are promising for environmentally friendly bioreagents, thus being applicable for the eco-friendly development of the bioflotation of sulfide minerals.

Acknowledgements

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