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Influence of Indigenous Mixotrophic Bacteria on Pyrite Surface Chemistry: Implications for Bioflotation

EDY SANWANI[1], NUSLIA BAYANGKARA LAMANDHI[1], HALIMATUL HUSNI[1], SITI KHODIJAH CHAERUN[1,2*], FIKA ROFIEK MUFAKHIR[3], AND WIDI ASTUTI[3]

[1]Department of Metallurgical Engineering, Faculty of Mining and Petroleum Engineering, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia;
[2]Geomicrobiology–Biomining & Biocorrosion Laboratory, Microbial Culture Collection Laboratory, Biosciences and Biotechnology Research Center (BBRC), Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia;
[3]Research Unit for Mineral Technology, Indonesian Institute of Sciences, Jalan Ir. Sutami KM. 15, Tanjung Bintang, Lampung Selatan 35361, Indonesia.

Given the low-cost and eco-friendly method, biotechnology has been widely utilized in industries as an alternative for physical and chemical processes, including in the biomining process (e.g., bioflotation and biobeneficiation). However, the use of biochemical reagent, which is selective for certain minerals, has not been well studied. This research was aimed to investigate the potential use of biosurfactant-producing mixotrophic bacteria as an alternative to chemical reagents during bioflotation and biobeneficiation process. Thirteen bacterial strains were investigated for their ability to produce biosurfactants and their effects on the surface properties of pyrite minerals. Bacteria-pyrite interaction experimental results showed that pyrite surface properties became more hydrophilic in the experimental systems inoculated with bacteria adapted with pyrite for 48 h than that without bacterial adaptation to pyrite, which was evidenced by the decrease in the contact angle of pyrite minerals by up to 50%. This evidence was also confirmed by the highest emulsifying index value (51.6%) attained during the bacteria-pyrite interaction. Hence, these bacteria can potentially be applied to selective flotation as pyrite depressants.

Key words: bioflotation, biosurfactants, contact angle, mixotrophic bacteria, pyrite

Dengan mempertimbangkan metode yang lebih murah dan ramah lingkungan, proses bioteknologi telah banyak digunakan dalam industri sebagai alternatif untuk proses fisika dan kimia, termasuk dalam proses biomining (seperti bioflotasi dan biobenefisiasi). Namun, penggunaan bioreagen yang selektif untuk mineral tertentu belum diteliti dengan baik. Penelitian ini bertujuan untuk menyelidiki potensi penggunaan bakteri mixotrofik penghasil biosurfaktan sebagai alternatif pengganti reagen kimia selama proses bioflotasi dan biobenefisiasi. Tiga belas strain bakteri diteliti karena kemampuan mereka dalam memproduksi biosurfaktan dan mempengaruhi sifat permukaan mineral pirit. Hasil percobaan interaksi bakteri-pirit menunjukkan bahwa sifat permukaan pirit menjadi lebih hidrofilik dalam sistem percobaan yang diinokulasi dengan bakteri yang diadaptasi dengan pirit selama 48 jam dibandingkan tanpa adaptasi bakteri terhadap pirit, yang dibuktikan dengan penurunan sudut kontak mineral pirit hingga 50%. Bukti ini juga dikonfirmasi oleh nilai indeks pengemulsifikasi tertinggi (51,6%) yang dicapai selama interaksi bakteri-pirit. Oleh karena itu, bakteri ini berpotensi dan dapat dijadikan sebagai alternatif bioreagen flotasi yang selektif sebagai depresan pirit.

Kata kunci: bakteri mixotrof, bioflotasi, biosurfaktan, pirit, sudut kontak

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Nowadays, biotechnology is being developed in various industries in the world, especially in biomining and biobeneficiation. One of the interesting topics that has been widely discussed and developed is bioflotation in the last few decades. This is a major trend to carry out environmentally friendly mineral processing. Bioflotation is the process of separating valuable minerals from the gangue minerals by using bioflotation reagents, such as biosurfactants (Kyzas and Matis 2019). The use of biosurfactants as flotation bioreagents is a promising alternative to green flotation (bioflotation) because the biosurfactant is degradable, not toxic, and especially it brings out from the renewable resources (Lima *et al.* 2019; Silva *et al.* 2019).

Biosurfactants are produced from amphiphilic microorganisms consisting of hydrophilic and hydrophobic molecular groups that function to reduce surface tension (Almeida *et al.* 2019). Several studies have been conducted to show the use of biosurfactants as an alternative to environmentally friendly flotation reagents and continue developing to this day. Sanwani *et al.* used biosurfactants of the bacterium

*Corresponding author: Phone: +62-22-2502239; Fax: +62-22-2504209; Email: skchaerun@metallurgy.itb.ac.id

Pseudoclavibacter sp. to increase the hydrophobicity of chalcopyrite so that it can separate chalcopyrite from copper sulfide ores (Sanwani *et al.* 2017). The results of this study showed that the hydrophobicity of chalcopyrite mineral increased from 30 to 73. Another study conducted by Merma *et al.* showed that the flotability of hematite increased as high as 90% based on the increase in biosurfactant concentration at acidic pH (Merma *et al.* 2018). In addition, there are still many studies conducted to study the effect of biosurfactants produced by microorganisms on the flotation process. These studies showed that the biosurfactant can separate minerals selectively (such as the separation of galena from sphalerite, chalcopyrite from pyrite, quartz from kaolinite, hematite from quartz, and many others) and can increase the flotability of valuable minerals (Hosseini *et al.* 2020; Huang *et al.* 2019; Kim *et al.* 2015; Consuegra *et al.* 2020; Martín *et al.* 2020).

Therefore, further research is needed to evaluate and optimize the use of biosurfactants in changing the surface properties of minerals so that later they can completely move flotation chemical reagents to be more environmentally friendly. This research was conducted to seek some alternative flotation reagents from the biosurfactants produced by various bacterial strains and their effects on the surface properties of pure pyrite. The results of this study may provide useful information on more environmentally friendly flotation reagents that are selective for certain minerals, namely pyrite.

MATERIALS AND METHODS

Selection of Potential Bacteria. Screening of bacteria that can produce biosurfactants is important to take on (Panjiar *et al.* 2017) because not all indigenous microbial isolates produce a lot of biosurfactants. Hence, it is necessary to select microorganisms properly to find out the microorganisms that produce optimum biosurfactants.

Bacterial Inoculation. Thirteen bacterial strains of the genera *Bacillus* spp., *Paenibacillus* sp., *Pseudoclavibacter* sp., *Morganella* sp., and *Citrobacter* spp. that had the ability to produce biosurfactants were evaluated to determine their potential as bioreagents in the flotation process. These pure bacterial strains were inoculated into a 250 ml Erlenmeyer flask containing SKC-broth medium (molasses in seawater) that had been sterilized in an autoclave for 20 min at 121 °C and 1.5 atm (Arslan 2019). After that, the bacterial cultures were placed on a gyratory shaker and incubated for 60 hours under aerobic conditions by shaking at a rotation speed of 180 rpm. Biosurfactants produced by bacteria were observed by measuring the emulsifying activity index (EI), which was sampled periodically every 20 hours.

Emulsifying Index (EI) Measurement. A small amount of the incubated bacterial culture (2 ml) was taken using a micropipette, then put into a test tube. A buffer solution (2 ml) and coconut oil (1 ml) were added to the test tube. After that, the solution in the test tube was stirred gradually for 2 minutes using a vortex at high speed –(Dikit *et al.* 2019; Campos *et al.* 2019). The optical density of the solution was measured using a UV-VIS spectrophotometer (recorded as A0), held out for 1 hour, and then the optical density of the solution was measured again (recorded as A1). The emulsifying index (EI) was calculated using the following formula:

$$EI\ (\%) = \frac{A1}{A0}\, x\, 100\%$$

Batch Experimental Procedure. Pure pyrite was used to study the interaction of bacteria with pyrite for evaluating pyrite surface properties. Pyrite was comminuted by the crushing and grinding tools to obtain a size fraction of -75 + 45 µm and passing 80% (P_{80}) at 75 µm. Fine particles are used to maximize the interaction between mineral particles and air bubbles during aeration and also to obtain the optimum recovery (Kim et al. 2017; La Vars 2018). The mineralogical characterization of pyrite minerals was analyzed by X-ray diffraction (XRD) shown in Figure 1, showing the purity of pyrite of about 99.8%. Before being used in the bacteria-pyrite interaction experiment, pure pyrite was washed with a low acid solution (0.05M HNO_3) and then washed with deionized water two times to ensure that the particles were not oxidized (Parthasarathy et al. 2014). The bacteria-pyrite interaction experiments were run in duplicate in 250 ml Erlenmeyer flasks containing the SKC-broth medium for 48 hours. The flasks were then inoculated with bacteria (herein *Citrobacter freundii* strain SKC-4 or *Bacillus altitudinis* strain SKC/S-8), which were previously adapted to 20% w/v pure pyrite for 48 hours (designated "adapted"). In comparison, a control experiment was also conducted, which was identical to "adapted" but inoculated with bacteria without adaptation to pyrite (designated "non-adapted"). After 48 hours, the bacterial cultures were taken, and the separation of solid matter (herein pyrite) from solutions was performed using a centrifuge for 20

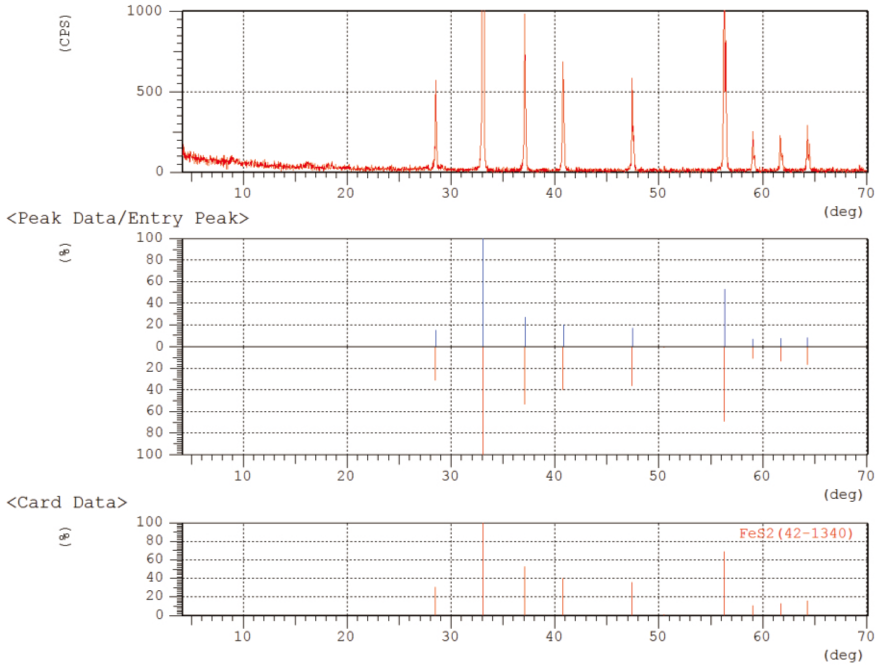

Fig 1 Characterization of pure pyrite used in this study using X-ray powder diffraction (XRD).

minutes. The separated solid was then put into the desiccator for 48 hours to obtain dried pyrite, and then a water drop test was performed to measure the contact angle of pyrite for evaluating its surface properties (Sharma 2001; Sharma *et al.* 2001). The images obtained from the water drop test were measured with the ImageJ application to find out the contact angle values(Williams *et al.* 2010).

RESULTS

Emulsifying Index (EI). Figure 2 shows the emulsifying index of all bacterial strains as a function of time. Emulsification index (EI) of four strains of the genus *Bacillus* (*Bacillus velezensis* strain SKC/S-2, *Bacillus velezensis* strain SKC/S-4, *Bacillus velezensis* strain SKC/S-5 and *Bacillus nitratireducens* strain SKC-2a) showed to increase from the onset of the incubation to 40 hours of incubation time. Two other species of the genus Bacillus, namely *Bacillus altitudinis* strain SKC/S-8 and *Bacillus aryabhattai* SKC-5, showed a decrease in EI after 20 hours, and a slight increase of EI was again observed after 40 hours. The exponential phase of almost all bacterial strains began after 20 hours of incubation time and followed by the stationary phase after 40 hours of incubation time. Correspondingly, bacteria of the genus *Paenibacillus*, namely *Paenibacillus pasadenensis* strain SKC/S-7 and the genus *Morganella*, namely *Morganella psychrotolerans* strain SKC/S-6, showed a similar EI pattern to the genus *Bacillus*. However, *Paenibacillus pasadenensis* strain SKC/S-3 showed an increase in EI up to 40 hours of incubation and then increased significantly after 40 hours. *Pseudoclavibacter* sp. strain SKC/La and *Citrobacter freundii* strain SKC-4 showed a continuous increase from the beginning to the end of the incubation time, whereas *Pseudoclavibacter* sp. strain SKC/Ls and *Citrobacter murliniae* strain SKC-3 showed a decrease in EI after 20 hours of incubation time. In this study, *Citrobacter freundii* strain SKC-4 had the highest EI value of 51.64% and continued to rise up to the end of incubation time. This bacterium was hereafter used in subsequent experiments to evaluate the effect of bacteria on pyrite surface properties. In comparison, the bacterial strain that had the lowest EI value was also chosen for the next experiment, namely *Bacillus altitudinis* strain SKC/S-8.

Contact Angle Measurement for Pyrite Surface Properties. Contact angle measurement was performed to evaluate the effect of the biosurfactants generated by the two selected bacteria on pyrite surface properties (i.e., the hydrophobicity and hydrophilicity of pyrite). The pyrite contact angle values before and after interacting with bacteria were shown in Fig 3. *Citrobacter freundii,* which produced the highest biosurfactant, led to a pyrite surface contact angle value of 114°, which had a similar value to *Bacillus altitudinis*

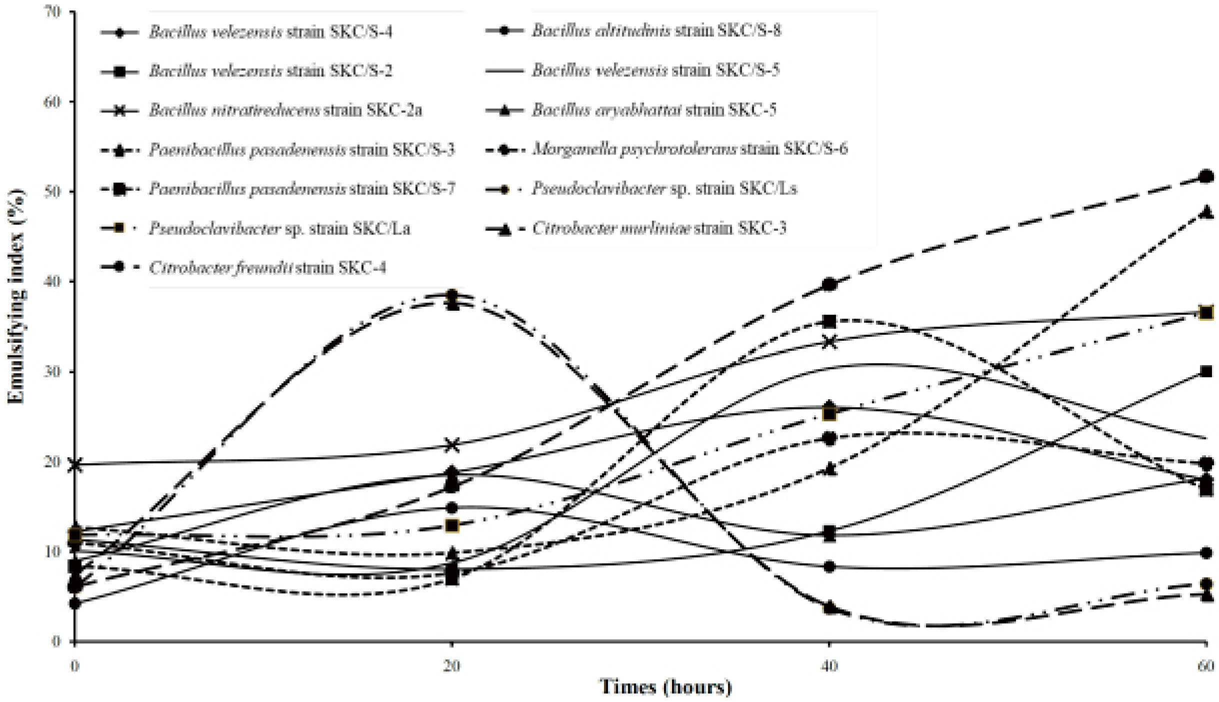

Fig 2 The emulsifying index of all bacterial strains as a function of time.

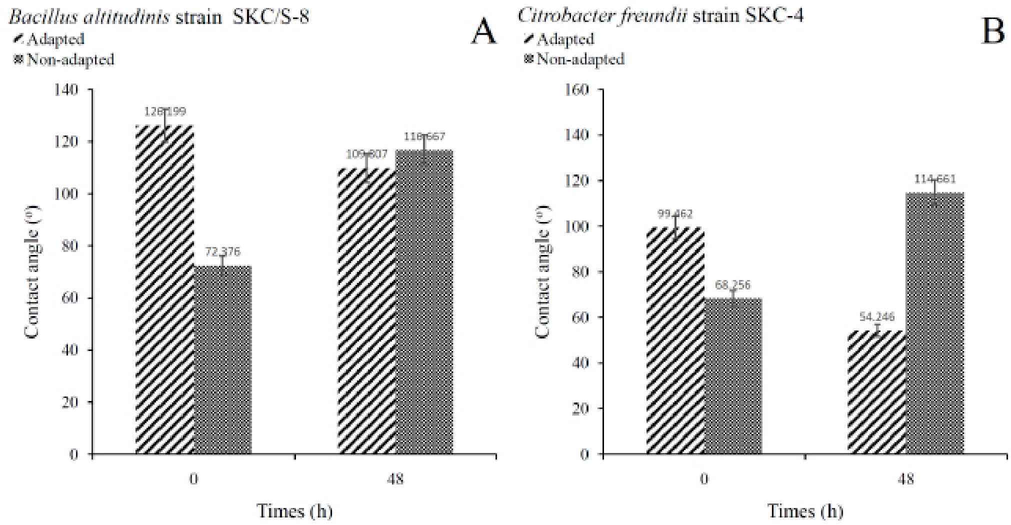

Fig 3 The contact angle values of pyrite before and after interacting with bacteria.

(116°). Both bacterial strains were not adapted to pyrite before the bacteria-pyrite interaction experiment, which showed an increase in contact angle after 48 hours of the experiment. Other experiments carried out by adapting both bacteria with pyrite for 48 hours during the bacteria-pyrite interaction showed different effects. After 48 hours, the experimental systems with *Citrobacter freundii* showed a decrease in contact angle value of up to 50%. In contrast, the experimental system with *Bacillus altitudinis* did not have a significant effect.

DISCUSSION

Bioflotation process is one of the physicochemical methods that involve microorganisms in separating impurities from valuable minerals. This process utilizes certain mineral surface properties to attach to air bubbles and float together to the surface. The utilization of bacteria as bioreagent is needed in the separation of impurities from low-grade valuable minerals or complex minerals. The microorganisms produce biosurfactants, which can be an alternative to flotation bioreagents. Bacteria and their metabolic substances require contact time with certain minerals. When bacterial growth reaches an optimum time, the bacteria then release biosurfactants and form biofilms and then attach to certain mineral surfaces. Biosurfactants produced by bacteria depend on energy sources in their growth medium. Bacteria used in this study obtained their carbon source from molasses. Molasses has been reported to be the optimal carbon source for producing more biosurfactants compared to other carbon sources such as glucose and palm oil (Dikit *et al.* 2019). The amount of biosurfactant produced by bacteria is proportional to the value of the emulsifying index. Emulsifying indexes change over a certain period of time. This behavior seems to be influenced by bacterial growth, where bacterial growth experiences the lag phase, the exponential phase, and the stationary phase. In the exponential phase, bacteria grow significantly so that biosurfactant production increases. However, the most active bacteria occur in the stationary phase so that the amount of biosurfactant produced continues to increase (Abbasi *et al.* 2012; Amaral *et al.* 2006). Bacterial strains that show a high EI value suggest that the bacteria can produce more biosurfactants than other bacterial strains.

Bacteria that produce optimum biosurfactants were tested in a batch experiment that interacted with pyrite minerals. This experiment was carried out to evaluate the effect of bacteria and their biosurfactants on changes in the surface properties of pyrite minerals characterized by the contact angle values. The adaptation of bacteria with certain minerals at given incubation time, especially pyrite, in this study may allow biosurfactants to be produced by the bacterium to work more selectively against certain minerals. The results of this study are very prospective to be applied to the sulfide mineral bioflotation with pyrite as the gangue mineral. This result provides information on the use potential of this bacterium as depressants for pyrite minerals.

As conclusion, microorganisms can produce heteropolar biosurfactants that are hydrophobic as well as hydrophilic. The bacteria producing biosurfactants can be more useful for the metallurgy industry, one of which is as an alternative to environmentally friendly flotation reagents. However, not all microorganisms produce biosurfactants that have the same ability, so that it is necessary to choose the appropriate microorganisms to be utilized in a particular process. In this study, *Citrobacter freundii* can produce more biosurfactants than other bacterial strains. The biosurfactants produced by the bacterium are also beneficial, thus decreasing the hydrophobicity of pyrite minerals or increasing the hydrophilicity. The biosurfactant generated by this bacterium is expected to be an alternative flotation reagent that can be used as a depressant for pyrite minerals.

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