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Utilization of *Bacillus pumilus* and *Citrobacter youngae* as Flotation Bioreagents in the Microflotation of Chalcopyrite, Pyrite, and Silica

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To date, relatively toxic and expensive chemical reagents are routinely used in the flotation of sulfide and oxide minerals in mining and metallurgical industries. To establish a more environmentally friendly flotation process, alternative flotation reagents have been explored extensively by using microbes and their metabolic products such as biosurfactants or EPS (extracellular polymeric substances). Hence, the present work focused on the application of the mixotrophic bacteria capable of both producing biosurfactants and oxidizing iron and sulfur (herein *Bacillus pumilus* strain SKC-2 and *Citrobacter youngae* strain SKC-4) as flotation bioreagents in the microflotation of chalcopyrite ($CuFeS_2$), pyrite (FeS_2), and silica (SiO_2). Laboratory microflotation tests using both bacterial strains as bioreagents were evaluated as a function of conditioning time, pH, and bacterial cell concentration. Experimental evidence indicated that the chalcopyrite recoveries could be achived using both bacterial strains but its better recovery was obtained with the bacterium *Citrobacter youngae* as bioreagents. In addition, the flotability of chalcopyrite was greater than that of pyrite or silica, indicating that both bacterial strains can function not only as collector for chalcopyrite but also as depressant for pyrite and silica. The findings of this study thus suggest the possible application of both bacterial strains as flotation bioreagents in order to establish a more eco-friendly mineral processing.

Key words: bioflotation, biosurfactant-producing mixotrophic bacteria, chalcopyrite, flotation, iron-sulfur-oxidizing bacteria, microflotation, pyrite, silica

Sampai saat ini reagen flotasi kimia yang relatif toksik dan mahal digunakan secara rutin dalam flotasi mineral oksida dan sulfida dalam industri pertambangan dan metalurgi. Dalam rangka menciptakan proses flotasi yang lebih ramah lingkungan, maka reagen flotasi alternatif telah banyak diteliti dan dikembangkan, yaitu dengan menggunakan mikroba dan produk metabolitnya seperti biosurfaktan atau EPS (*extracellular polymeric substances*; biosurfaktan berat molekul besar). Dalam penelitian ini, bakteri mixotrof yang dapat memproduksi biosurfaktan dan dapat mengoksidasi besi dan sulfur yaitu *Bacillus pumilus* galur SKC-2 and *Citrobacter youngae* galur SKC-4 digunakan sebagai bioreagen dalam proses mikroflotasi mineral kalkopirit ($CuFeS_2$), pirit (FeS_2), dan silika (SiO_2). Parameter flotasi yang diamati yaitu waktu pengkondisian, pH, dan konsentrasi sel bakteri. Hasil penelitian menunjukkan bahwa kedua bakteri tersebut dapat melakukan flotasi kalkopirit dengan baik dengan perolehan pirit dan silika yang rendah, dimana perolehan kalkopirit terbaik terjadi dengan menggunakan *Citrobacter youngae* sebagai bioreagen flotasi. Hal ini menunjukkan bahwa kedua bakteri tersebut mempunyai potensi yang besar untuk digunakan sebagai bioreagen flotasi dalam rangka menciptakan proses mineral yang lebih ramah lingkungan.

Kata kunci: bakteri mixotrof penghasil biosurfaktan, bakteri pengoksidasi besi dan sulfur, bioflotasi, flotasi, kalkopirit, mikroflotasi, pirit, silika

The development of a more economical and environmentally friendly flotation process is highly needed to create green industries in mineral processing. Since until now the relatively toxic and expensive chemical flotation reagents are commonly employed in the flotation process of sulfide and oxide minerals, the efforts should be conducted to enhance the flotation performance by gaining low-cost and eco-friendly flotation reagents. In principle, the flotation process is a physicochemical process based on exploiting the differences in surface properties between the desired, valuable minerals and undesired gangue minerals (Bradshaw *et al*. 2005; Didyk and Sadowski 2012; Subramanian *et al*. 2003; Yüce *et al*. 2006). Reagents that function as collectors, frothers, and depressants used in the flotation process have roles in manipulating the pulp chemistry and enhancing the differences in mineral surface hydrophobicity to facilitate the separation (Reyes-Bozo *et al*. 2014; Wiese *et al*. 2006,

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2007, 2008, 2010). To date, the most commonly used flotation reagents include high-cost, relatively hazardous chemical reagents that have raised concern with respect to environmental issues.

Therefore, numerous studies have been carried out to discover more benign flotation reagents for achieving lower operating costs as well as gaining green mineral processing techniques. One of such flotation reagents is by using microbes and their metabolic products that have over the years been evaluated in the flotation of minerals (Chandraprabha *et al*. 2004; Chandraprabha *et al*. 2005; Chandraprabha and Natarajan 2006; de Mesquita *et al*. 2003; Diaz-López *et al*. 2012; Hosseini *et al*. 2005; Merma *et al*. 2013; Patra and Natarajan 2006; Pecina-Treviño *et al*. 2012; Santhiya *et al*. 2000; Santhiya *et al*. 2001; Sarvamangala *et al*. 2013; Vilinska and Rao 2008). Since the microbial cellular surfaces and their metabolic products are characterized by functional non-polar groups such as hydrocarbon chains and polar groups (carboxyl, hydroxyl, phosphates) which correspond to the characteristics of surfactant molecules, they can act as flotation bioreagents and induce hydrophobic properties once they have adhered to the mineral surfaces by either directly or indirectly modifying them (Botero *et al*. 2008; Govender and Gericke 2011). However, from a microbiological standpoint, the microbes involved in the flotation of metals-bearing minerals are emphasized on heterotrophic bacteria alone or chemolithotrophic bacteria alone and the action of mixotrophic bacteria as flotation bioreagents has rarely been studied. These mixotrophic bacteria have to be taken into consideration because they would be beneficial to the flotation bioreagents as being functioned to modify the mineral surfaces to be either more hydrophilic or hydrophobic. By having a dual-function, the bacteria may potentially be advantageous for the improvement of the flotation processes. Therefore, the objectives of the current work were to investigate the application of the two mixotrophic bacteria (i.e. *Bacillus pumilus* strain SKC-2 and *Citrobacter youngae* strain SKC-4) capable of both generating biosurfactants and oxidizing iron and sulfur as bioreagents in the microflotation of chalcopyrite, pyrite, and silica as a function of conditioning time, pH, and bacterial cell concentration. Thus, the findings of this study may improve a better understanding on the behaviour of microbial usage as bioflotation bioreagents which thus contribute to the overall flotation performance.

MATERIALS AND METHODS

Mineral Samples. The silica and pyrite samples used in this study were obtained from Karangnunggal (Tasikmalaya) and Singajaya (Garut), respectively, where both are located in West Java Province, Indonesia. Chalcopyrite samples were kindly provided by PT Freeport Indonesia. ED-XRF analysis of the samples determined their chemical composition as summarized in Table 1. An X-ray powder diffractrometery (XRD) analysis showed a high purity of the samples (data not shown). The mineral samples were ground to obtain the grain size of -75+38 μm that was used for microflotation studies. The minerals were stored in a desiccator under nitrogen atmosphere before being used to prevent the oxidation of both chalcopyrite and pyrite samples.

Bacteria and Growth Medium. The bacterial strains used in this study were the Gram-positive bacterium *Bacillus pumilus* strain SKC-2 and the Gram-negative bacterium *Citrobacter youngae* strain SKC-4, which have the abilities of producing biosurfactants and oxidizing iron and sulfur. They are mixotrophic bacteria, which were isolated from a crude oil-enriched culture and from the hot spring water of the Domas crater (at Tangkuban Perahu, Bandung, Indonesia), respectively. 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 photomicrographs of bacterial cells of the strains and their colonies are shown in Fig 1A~D. Bacterial cultures used in microflotation test were prepared by growing the strains in sterile 500 mL Erlenmeyer flasks containing 300 mL of growth medium supplemented with 10% (vol/vol) inoculum *B. pumilus* strain SKC-2 or *C. youngae* strain SKC-4. Cultures were incubated with agitation (150 rpm) at 30 °C for 4 days under aerobic condition until their growth reached 70-80% of the logaritmic growth phase. Subsequently, they were used in the microflotation tests and bacterial cell concentrations (CFU mL^{-1} or CFU g^{-1}) used for varying conditioning time were determined by a serial dilution-agar plating procedure (Chaerun *et al*. 2013).

Laboratory Microflotation Experiments. The microflotation of the mineral samples was performed using a modified Hallimond tube (Feasby 1966) equipped with a magnetic stirrer as agitator and a flowmeter for measuring and adjusting the flow of nitrogen gas (Fig 2). Nitrogen gas was flown to the Hallimond tube to prevent the oxidation of mineral

Table 1 Elemental composition (wt.%) of chalcopyrite, pyrite and silica used in this study

Element	Chalcopyrite	Pyrite	Silica
Na	n.d.	0.004	0.002
Mg	n.d.	0.034	n.d.
Al	4.29	0.61	0.34
Si	12.6	0.76	**43.35**
P	n.d.	0.003	0.004
S	**5.1**	**21.6**	n.d.
K	4.76	0.005	0.01
Ca	0.72	0.05	0.09
Ti	0.24	0.005	0.01
V	0.04	n.d.	n.d.
Cr	0.098	0.013	n.d.
Mn	0.068	n.d.	0.002
Fe	**53.3**	**14.7**	0.08
Ni	0.1	0.002	n.d.
Cu	**15**	0.03	n.d.
Zn	n.d.	0.003	0.002
Rb	0.38	n.d.	n.d.
Mo	3.4	n.d.	n.d.

n.d.: not detected

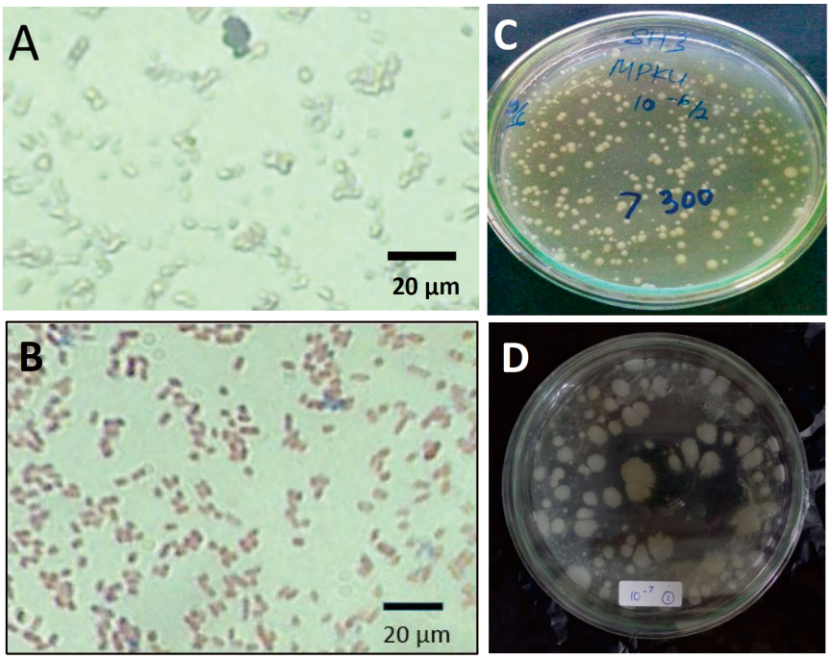

Fig 1 Photomicrographs of bacterial cells of *Bacillus pumilus* strain SKC-2 (A) and *Citrobacter youngae* strain SKC-4 (B) and the colonies of *B. pumilus* strain SKC-2 (C) and *C. youngae* strain SKC-4 (D).

samples (in particular chalcopyrite and pyrite). The agitation speed of magnetic stirrer was 700 rpm at the flow rate of 45 mL min^{-1}. An amount (1.0 g) of each mineral (chalcopyrite or pyrite or silica) was added to a total volume of 40 mL of suspension of bacterial cell concentrations (containing 10 mL bacterial culture grown in LB broth and 30 mL distilled water) at different values of pH (3, 5, 7, and 10), which were adjusted with diluted H_2SO_4 and NaOH solutions, with varied bacterial cell concentrations of $2.8x10^9$, $5.6x10^9$, $8.4x10^9$, and 11.2 $x10^9$ CFU g^{-1} mineral. The mineral was conditioned with the bacterial suspension inside the Hallimond tube under constant stirring for 15, 30, 60, and 120 min, and then the mineral flotation tests were carried out using nitrogen gas at a flow rate of 45 mL min^{-1} for 5 min. All experiments were conducted under room temperature (25-30 °C). The floated and tailing samples were collected separately, filtered, dried, and weighed. The flotability or flotation recovery efficiency was then calculated as the ratio of floated and non-floated mineral amounts and the total weighed sample.

RESULTS

The flotation process parameters assessed in the laboratory microflotation tests in a Hallimond tube in this study included: (1) the assessment of both bacterial capacity (*B. pumilus* strain SKC-2 and *C. youngae* strain SKC-4) as potential bioflotation agents in the flotation recovery of minerals (chalcopyrite, pyrite and silica) with bacterial cell concentration of 2.8 x 10^9 CFU g^{-1} mineral and conditioning time of 30 min at pH 7; (2) the various conditioning times (15, 30, 60, and 120 min) in the presence of bacterial cell (2.8 x 10^9 CFU g^{-1} chalcopyrite or pyrite or silica) at pH 7 for both bacteria *B. pumilus* strain SKC-2 and *C. youngae* strain SKC-4; (3) the varying bacterial cell concentrations of *C. youngae* strain SKC-4 ($2.8x10^9$, $5.6x10^9$, $8.4x10^9$, and $11.2x10^9$ CFU g^{-1} mineral) with conditioning time of 60 min at pH 7; and (4) the varying pH values of bacterial suspension of *C. youngae* strain SKC-4 with bacterial cell concentration of $2.8x10^9$ CFU g^{-1} under conditioning time of 60 min.

Assessment of Bacterial Capacity of *B. pumilus* and *Citrobacter youngae* as Potential Bioflotation Agents. The assessment of both bacteria *B. pumilus* and *C. youngae* as potential bioflotation agents in the microflotation tests was performed for the flotation recovery of minerals (chalcopyrite, pyrite and silica) with bacterial cell concentration of 2.8 x 10^9 CFU gr^{-1} mineral and conditioning time of 30 min at pH 7. This showed that both bacterial strains had the capacity to more efficiently recover chalcopyrite (as desired valuable mineral) than pyrite or silica (as undesired gangue mineral) (Fig 3). However, the bacterium *C. youngae* was observed to have a greater chalcopyrite flotation recovery (43.7%) than *B. pumilus* (37.6%), indicating that *C. youngae* could be more applicable as biocollector for chalcopyrite. Since the recovery of pyrite was lower than that of chalcopyrite, it is also reasonable that both bacterial strains may also act as bio-depressants for pyrite.

Effect of Conditioning Times on the Mineral Flotability in the Presence of *B. pumilus* or *C. youngae.* For a better understanding of the role of both bacterial strains as bioflotation reagents in order to achieve the optimum flotation performance, the minerals were conditioned with bacterial cells of both strains in the presence of bacterial cell (2.8 x 10^9 CFU gr^{-1} chalcopyrite or pyrite or silica) at pH 7 for both bacteria *B. pumilus* strain SKC-2 and *C. youngae* strain SKC-4 for different periods of time (i.e. 15, 30, 60 and 120 min) (Fig 4A,4B). Again, it is evident that chalcopyrite exhibited more significantly flotability when conditioned with *C. youngae* (Fig 4B) than with *B. pumilus* (Fig 4A). The greater flotability of chalcopyrite than pyrite or silica for both bacterial strains was obtained at different conditioning times, that were at 30 min for *B. pumilus* and 60 min for *C. youngae*. For each conditioning time, after the minerals were conditioned with bacterial cells of *C. youngae*, the pH of the bacterial suspension was measured (Fig 4C) and showed extremely acidic suspension for pyrite (pH 2~3), slightly acidic suspension for chalcopyrite (pH 4.5~5) and slightly neutral suspension for silica (pH 6~7). This indicates that the pH also affects mineral flotation recoveries.

Effect of Varying Bacterial Cell Concentrations of *C. youngae* on the Mineral Flotability. A series of tests were conducted on chalcopyrite, pyrite and silica to determine the optimal cell concentration to be used during microflotation tests. The bacterial cell concentrations evaluated were $2.8x10^9$, $5.6x10^9$, $8.4x10^9$, and $11.2x10^9$ CFU g^{-1} mineral with conditioning time of 60 min at pH 7. There was no clear tendency in chalcopyrite recoveries at any bacterial cell concentrations. However, the flotation recoveries of all minerals were reduced with increasing bacterial cell concentration (Fig 5).

Effect of the pH Values of Bacterial Suspension of *C. youngae* on the Mineral Flotability. The

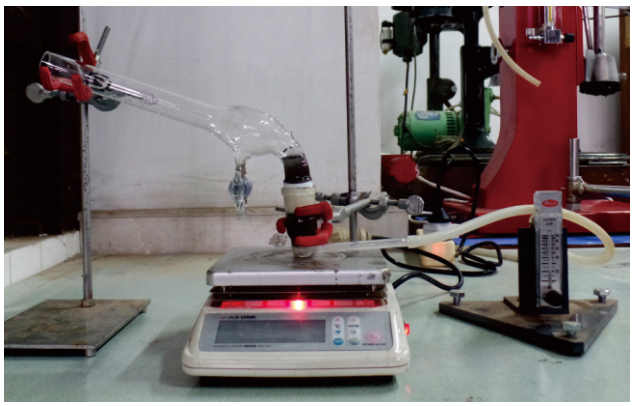

Fig 2 Photograph of a modified Hallimond Tube used for microflotation test.

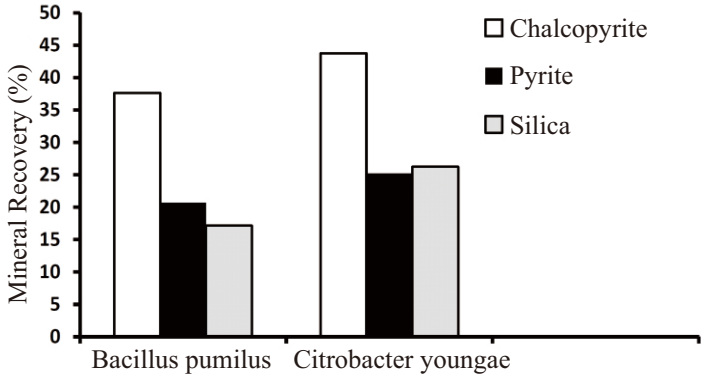

Fig 3 Flotation recovery (%) of chalcopyrite, pyrite and silica at conditioning time of 30 minutes and pH 7 with cell concentration of the bacteria *Bacillus pumilus* strain SKC-2 or *Citrobacter youngae* strain SKC-4 (2.8 x 10^9 CFU g^{-1} mineral).

flotation behaviour of chalcopyrite, pyrite, and silica was also affected by the pH of bacterial suspension of *C. youngae* with bacterial cell concentration of 2.8x10^9 CFU g^{-1} mineral under conditioning time of 60 min (Fig 6). It was observed that the bacterial strain as biocollector was more functional at low pH (for chalcopyrite), at low and high pH (for pyrite), and at neutral pH (for silica) (Fig 6).

DISCUSSION

Mineral bioflotation includes the concepts and techniques employed in mineral flotation using microbes and their metabolic products as flotation reagents of which their use has been demonstrated by many researchers in selective separation, including the selective flotation or depression of sulfides and oxides. A variety of microbes has been used in the flotation studies, including *Mycobacterium phlei*, *Paenibacillus polymyxa*, *Rodococcus opacus*, *Acidithiobacillus ferrooxidans*, *Acidhithiobacillus thiooxidans*, and *Leptospirillium ferrooxidans* (Botero *et al*. 2008; Chandraprabha and Natarajan 2006; Chandraprabha *et al*. 2005; Deo and Natarajan 1998; Raichur *et al*. 1997; Vilinska and Rao 2008). Therefore, it should be noted that the use of the bacteria *B. pumilus* and *C. youngae* in this study is the first report on their use as flotation bioreagents. Moreover, both bacteria are novel strains which were isolated from Indonesian sites. From the micro-flotation experiments in a modified Hallimond tube (Fig 3), it was obtained that both bacterial strains have the capacity for bio-collectors for chalcopyrite and bio-depressants for pyrite. Since pyrite is hydrophobic that should have floated, the ability of bacterial cells to depress pyrite may be caused by their capability in oxidizing iron and sulfur, thus forming iron hydroxides and/or elemental sulfur on the pyrite surface at neutral pH (Sanwani *et al*. 2016). This in turn makes pyrite more hydrophilic and reduce its flotation recovery.

Furthermore, the application of microbes and their metabolites as bioflotation reagents is dependent on the flotation behaviour of each mineral in the presence of these bioreagents as well as the flotation parameters such as pH, bacterial cell concentration, conditioning and flotation time. The results from this study showed that the flotability of chalcopyrite using *C. youngae* was greater than that using *B. pumilus* (Fig 4A, 4B).

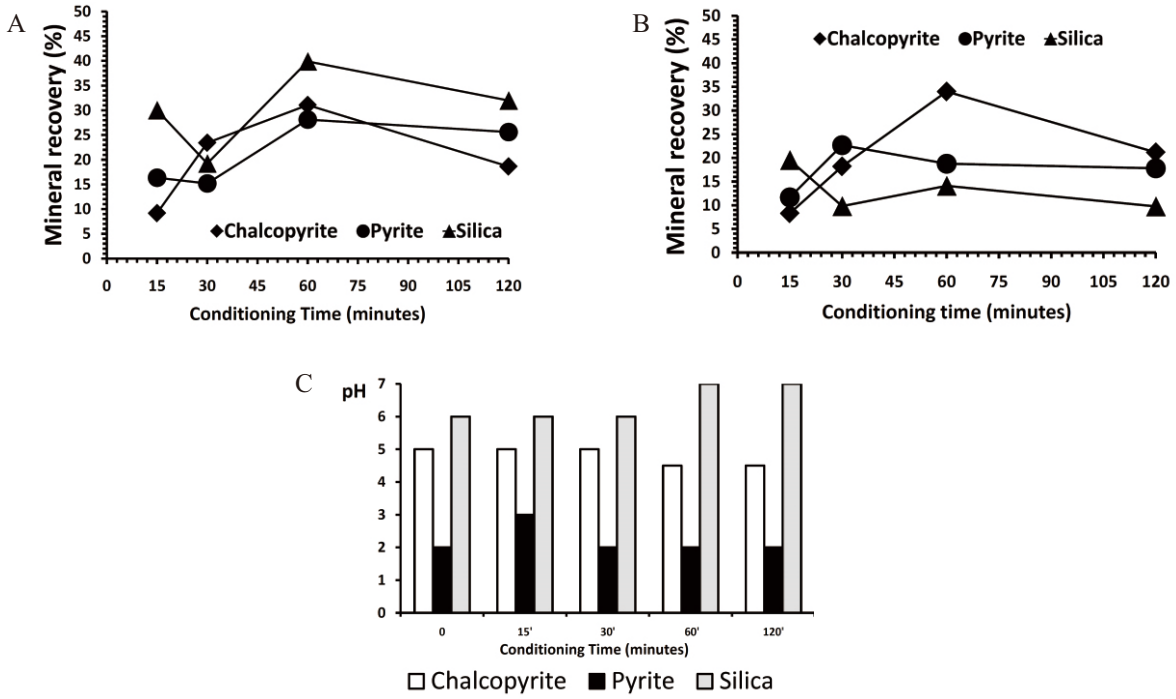

Fig 4 Flotation recovery (%) of chalcopyrite, pyrite, and silica at varying conditioning times with cell concentration of the bacterium *Bacillus pumilus* strain SKC-2 (2.8 x 10^9 CFU g^{-1} mineral) (A). Flotation recovery (%) of chalcopyrite, pyrite, and silica at varying conditioning times with cell concentration of the bacterium *Citrobacter youngae* strain SKC-4 (2.8 x 10^9 CFU g^{-1} mineral) (B). The pH values of the bacterial suspension of the bacterium *C. youngae* strain SKC-4 after chalcopyrite, pyrite, and silica were conditioned with bacterial cells of *C. youngae* in the microflotation of chalcopyrite, pyrite, and silica (C).

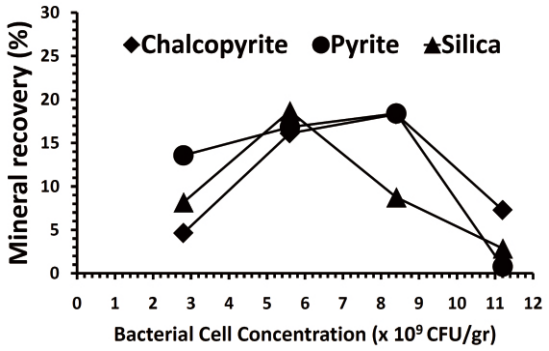

Fig 5 Flotation recovery (%) of chalcopyrite, pyrite, and silica at different bacterial cell concentrations of the bacterium *C. youngae* strain SKC-4 at pH 7.

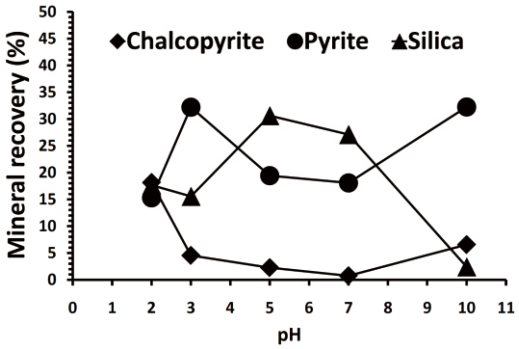

Fig 6 Flotation recovery (%) of chalcopyrite, pyrite, and silica at different pH values of bacterial suspension with conditioning time of 60 min and cell concentration of the bacterium *C. youngae* strain SKC-4 (2.8 x 10^9 CFU g^{-1} mineral).

The possible reason for this behaviour is that *C. youngae* cells may have a more natural affinity for sulfide minerals such as chalcopyrite than *B. pumilus* due to the origin of *C. youngae* isolated from sulfide-rich minerals, thus enabling it to act better in selective flotation of chalcopyrite. The results from this study also indicated that the flotability of minerals depended on pH which had the different pH values for each mineral tested (Fig 4C). This is supported by the works of Bradshaw *et al*. (2005) and Didyk and Sadowski (2012) demonstrating that pyrite is most floatable under acidic conditions and its recovery dropped with increase in alkalinity due to the changes in solubility of iron hydroxides on the pyrite surface as the pH changes, which is in contrast to the recovery of chalcopyrite remaining almost constant at all the pH studied.

In addition to the flotation recoveries in this study, the flotability of all minerals was reduced with increasing bacterial cell concentration (Fig 5). This result is consistent with the earlier observations of Govender and Gericke (2011) that the best chalcopyrite recovery was achieved at low bacterial cell concentrations, while the addition of high bacterial cell concentration resulted in a decrease in chalcopyrite recovery. Likewise, the decreased recovery of chalcopyrite due to the increased bacterial concentration in this study may be as a result of the chalcopyrite ($CuFeS_2$) particles being affected by the high viscosity from the excessive levels of EPS generated by bacterial cells in the solution (Li *et al*. 2008; Govender and Gericke 2011). The study of Govender and Gericke (2011) also reported that the presence of excessive biopolymers can result in decreased flotation capability as there would be insufficient free space for biopolymers to attach to the particle surface. From the microbial point of view, the proteins in bacterial cells essentially serve as a hydrophobic agent with higher surface hydrophobicity, and lower surface charge are related to higher dispersion and flotation tendencies (Govender and Gericke 2011). Therefore, it is suggested that the proteins of the bacteria *B. pumilus* strain SKC-2 and *C. youngae* strain SKC-4 are also likely to help in the selective separation of chalcopyrite, pyrite and silica in this study.

The present study has shown that the two biosurfactant-producing mixotrophic bacteria of *B. pumilus* strain SKC-2 and *C. youngae* strain SKC-4, which are also capable of oxidizing iron and sulfur, are promising to be potentially applied as bioreagents for the flotation of sulfide and silicate minerals. The results obtained from this study may thus contribute to the development of the flotation technology for processing sulfide and silicate mineral ores.

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