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Bacterial Leaching of an Indonesian Complex Copper Sulfide Ore Using an Iron-Oxidizing Indigenous Bacterium

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The bioleaching of an Indonesian complex copper sulfide ore was studied in shake flasks over a period of 14 days using an iron-oxidizing indigenous bacterium at room temperature (28 °C) and various pulp densities (5% and 20%). The bioleaching suspensions were periodically analyzed for Cu and Fe concentrations as well as Eh, pH and DO values. Cu bioleaching efficiencies at 5% pulp density were higher than those at 20% pulp density, which correlated with Fe concentration in solution. Over a period of 14 days, the pH of bioleaching suspension was in the range of 5 ~ 9, indicating that Cu bioleaching was greatly influenced not only by proton H^+ dan ferric ion but also by extracellular polymeric substances (EPS) generated by the bacterium. The current study may improve our better understanding on the bacterial action for bioleaching of complex copper sulfide ores that remains debated so far as refractory ores.

Key words: biohydrometallurgy, bioleaching, complex copper sulfides, chalcopyrite, iron-oxidizing bacteria

Bioleaching bijih sulfida tembaga komplek dari Indonesia diteliti pada percobaan menggunakan labu yang digoyang selama 14 hari dengan memanfaatkan bakteri indigen pengoksidasi besi yang dilakukan pada suhu ruang (28 °C) dan variasi pulp density ((5% and 20%). Suspensi *bioleaching* diamati dan diukur secara periodik yaitu konsentrasi Cu dan Fe serta Eh, pH dan DO. Hasil penelitian menunjukkan bahwa efisiensi *bioleaching* Cu pada 5% *pulp density* lebih besar dibandingkan dengan efisiensi *bioleaching* Cu pada 20% *pulp density* dimana nilainya berkorelasi positif dengan konsentrasi Fe total yang terlarut. Selama 14 hari percobaan bioleaching, nilai pH suspensi *bioleaching* berkisar 5~9 yang menunjukkan bahwa bioleaching Cu terjadi tidak hanya disebabkan oleh proton H^+ dan konsentrasi Fe (III) yang terlarut tapi juga sangat dipengaruhi oleh *extracellular polymeric substances* (EPS) yang diproduksi oleh bakteri. Hasil penelitian ini mungkin meningkatkan pemahaman kita tentang peranan bakteri dalam melakukan *bioleaching* bijih sulfida tembaga komplek yang masih merupakan perdebatan sampai saat ini sebagai bijih yang sangat sulit untuk di-*leaching* atau di-*bioleaching*.

Kata kunci: bakteri pengoksidasi besi, bijih sulfida tembaga komplek, biohidrometalurgi, *bioleaching*, kalkopirit

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It is well known that bioleaching is a low-cost, effective, environmentally friendly technology that has been applied worldwide to recover metals from both high-grade and low-grade ores as well as tailings, mine wastes and urban solid wastes. The most challenging application of the bioleaching is for processing complex copper ores which contain both copper sulfides and oxides. Owing to the complexity in the mineralogical characteristics of the ores, most efficient metal extraction from the ores can be achieved through bioleaching method. Some researchers have studied the bioleaching of complex copper sulfide ores and have found that the primary sulfide minerals of copper have been difficult to leach for the purpose of direct copper extraction (in particular chalcopyrite, $CuFeS_2$) due to passivation phenomena under a variety of oxidative leaching conditions (Dreisinger 2006; Watling 2006; Arce and González 2002; Herrera *et al*. 1989).

Correspondingly, many trials have been conducted to bioleach copper and overcome the passivation of chalcopyrite using different genera of prokaryotes (Wang *et al*. 2016; Zhao *et al*. 2015a; Zhao *et al*. 2015b; Gu *et al*. 2013; Zhao *et al*. 2013; Yu *et al*. 2011; Qiu *et al*. 2005; Bevilaqua *et al*. 2002). Those studies employed chemolithotrophs such as *Acidithiobacillus ferrooxidans* (Qiu *et al*. 2005; Zhao *et al*. 2013),

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Acidithiobacillus thiooxidans (Qiu *et al.* 2005), *Leptospirillum ferriphilum* (Wang *et al.* 2016; Zhao *et al.* 2015a; Zhao *et al.* 2015b; Gu *et al.* 2013), *Acidithiobacillus caldus* (Wang *et al.* 2016; Zhao *et al.* 2015b), and *Sulfobacillus thermosulfidooxidans* (Wang *et al.* 2016). As a matter of fact, naturally occurring complex ores are always found in association with not only inorganic matter but also organic matter. Hence, the mixotrophic bacteria which are capable of using both organic and inorganic matters are needed to be employed in bioleaching processes, since chemolithotrophs are very sensitive to organic matters. In addition to the mechanism of bioleaching copper sulfides, microbially produced extracellular polymeric substances (EPS) which facilitates the attachment of microbes to the mineral surface can sequester metals (herein Fe and Cu) into soluble metal-ligand complexes by chelation, thus enhancing copper dissolution (Sand and Gehrke 2006). Therefore, the current work studied the bioleaching of complex copper sulfide ores from Indonesia using an iron-oxidizing indigenous bacterium capable of oxidizing iron and producing EPS in order to provide a better understanding of bioleaching copper sulfides that are recalcitrant to bioleaching.

MATERIALS AND METHODS

Ore Samples. Complex copper sulfide ore was obtained from the Sungai Mak copper deposits (Gorontalo, Indonesia) and was ground to obtain a particle size of d_{80} = 75 µm. An X-ray powder diffractometry (XRD) analysis showed that the ore contained quartz (as the dominant mineral), muscovite, kaolinite, and alunite with various forms of copper minerals including covellite, chalcocite, chalcopyrite, cuprite/chalcotrichite, digenite, azurite, malachite, and chalcanthite.

Bacterium and Growth Medium. A mixotrophic bacterium used in this study was isolated from an Indonesian mine site (identified as *Alicyclobacillus* sp.), which has the abilities of oxidizing iron and sulfur and producing a high amount of extracellular polymeric substances (EPS) (Mubarok *et al.* 2017). The growth medium used was the modified Luria–Bertani (LB) medium, containing 10 g L^{-1} peptone, 5 g L^{-1} yeast extract, 10 g L^{-1} NaCl, supplemented with 0.5 g L^{-1} $Na_2S_2O_3.5H_2O$, and 0.25 g L^{-1} $FeSO_4.7H_2O$. The modified LB medium was used in this study to provide organic carbon source in addition to iron and sulfur for bacterium in order to produce a high amount of EPS since the bacterium was more favorable to generate EPS under high organic carbon concentration (unpublished data). The photomicrographs of bacterial cells and its colonies are shown in Fig 1A and 1B.

Experimental Procedure. Bioleaching experiments were carried out in duplicate in sterile 300 ml Erlenmeyer flasks containing 150 mL of the modified LB medium supplemented with 10% (vol vol^{-1}) inoculum of the bacterium and complex copper sulfide ores (a particle size of d_{80} = 75 µm) at temperature of 28 °C. They were subsequently incubated for 14 d at a rotation speed of 180 rpm under various bioleaching parameters of solid to liquid ratio (pulp density) (5% and 20%), NaCl addition (10 g L^{-1}) to prevent passivation. The pH, Eh (redox potential) and DO (dissolved oxygen) in bioleaching suspension were monitored periodically, and the solution (2 mL) was removed for measuring dissolved copper and iron concentrations by using atomic absorption spectrophotometer (AAS). The data are presented as the averages obtained from the duplicate experiments. EPS production by the bacterium during bioleaching was assayed by determining the emulsifying activity index (EI, %) as described by Francy *et al.* (1991) with modification, which estimated the ability of EPS to emulsify liquid hydrocarbons such as coconut oil, corn oil, and kerosene.

RESULTS

Physicochemical Characteristics of Bioleaching Suspension. Figures 2A-C depict the physicochemical characteristics (pH, redox potential, and dissolved oxygen) of bioleaching suspension over a 14-day bioleaching experiment. The pH values of suspension tended to increase over time from ~5 to ~9 (Fig 2A), indicating that there was H^+ consumption during bioleaching processes. As the pH increased, the redox potential (Eh) values in bioleaching suspension also tended to increase from 248 mV (vs. SHE) to 403 mV (for 5% pulp density) and from 365 mV to 488 mV (for 20% pulp density) (Fig 2B). This suggests that the bacterium creates a certain potential environment that greatly affects the leaching of copper species, since the leaching of each copper species is largely dependent on the electrochemical potential of the leaching solution. In addition, the dissolved oxygen levels over 14 days of bioleaching experiment were in the range of 7.1~8.3 mg L^{-1} (Fig 2C), indicating a sufficient O_2 supply for viability and activity of leaching microbes.

Copper and Iron Bioleaching. Figures 3A-B represent the dissolution of copper and iron in solution at pulp density of 5% and 20% over a 14-day bioleaching experiment. The copper bioleaching efficiencies from the ores increased rapidly during the first 1 d and subsequently increased slightly for another 13 d, which achieved the extraction levels of ~31% (for 5% pulp density) and ~27% (for 20% pulp density) (Fig 3A). These increases were concomitant with an increase in iron bioleaching efficiencies (Fig 3B). For 5 % pulp density, iron dissolution (with a maximum iron extraction of 4%) increased rapidly during the first 2 d and subsequently decreased rapidly for the remaining bioleaching time. Iron dissolution for 20% pulp density also increased for the first 3 d, subsequently remained relatively constant for another 4 d and finally decreased up to the end of bioleaching experiment, which reached levels of only 0.9%. Compared to copper extraction, the iron dissolution was very low.

EPS Production during Bioleaching. Figure 4 represents the EPS generated by an iron-oxidizing indigenous bacterium over a period of 7 days. Three hydrocarbons (coconut oil, corn oil and kerosene) were evaluated for EPS production, which was represented as emulsifying activity index (EI). It appeared that EPS production tended to increase over time, thus providing the evidence of EPS generation during bioleaching processes.

DISCUSSION

A variety of biological and chemical leaching processes have been studied by many researchers for enhancing copper leaching from complex copper sulfides as well as overcoming the passivation of chalcopyrite. One of the biological leaching processes employed is by the application of thermophilic prokaryotes to increase the solubilization of copper more efficiently (Plumb *et al.* 2002). However, high-temperature bioleaching process suffers from high electrical energy and capital cost. In addition, most studies on copper bioleaching utilize chemolithotrophs that are very sensitive to organic matters. Since complex copper sulfides are always associated with organic compounds, the indigenous bacterium employed in the present study grows well at room temperature (20 ~ 40 °C) and belongs to mixotrophic group, which is capable of utilizing both organic and inorganic compounds as well as oxidizing iron and producing EPS. By having such capacities, the bacterium benefits copper bioleaching from complex copper ores - both for accelerating copper dissolution and for preventing passivation phenomenon.

During the bioleaching process, passivation layers formed by the jarosite precipitation on the mineral surface are thought to cause slow copper dissolution, and the passivation layer hinders greater copper extraction by restricting the flow of bacteria, nutrients, oxidants, and reaction products to and from the mineral surface (Zhang *et al.* 2009; Watling 2006; Stott *et al.* 2000). Depending on the pH, redox potential (Eh), temperature, ionic composition, and Fe(III) concentration, the solubility of ferric iron is controlled by precipitation of schwertmannite and jarosite (Bevilaqua *et al.* 2002; Bigham *et al.* 1996). From the results of the present study, the Eh continued to rise during the bioleaching period (Fig 2B), indicating continued bacterial ferrous ion oxidation to ferric ion. This high ferrous-oxidizing activity of the bacterium in this study caused a steady increase in the Eh over time, thus generating the amount of ferric ion in solution (Fig 3B). The copper was leached rapidly from the ore during the first 1 d (Fig 3A) which was concomitant with a high iron dissolution at that period for any pulp densities (Fig 3B), suggesting that ferric ions enhanced copper dissolution. However, since the pH of bioleaching suspension was higher than 5 over time (Fig 2A), ferric iron was in insoluble form as precipitates, bringing about passivation which in turn resulted in low copper bioleaching (only a maximum copper extraction of ~31%) (Fig 3A). Moreover, the bacterium used in this study is able to produce a huge amount of EPS grown in LB medium, thus the EPS enables insoluble ferric iron to be in soluble form, which in turn help contribute to the enhancement of copper dissolution (Fig 3A). Copper dissolution rate is strongly dependent on the reduction potential (Eh) in solution and this parameter is far more influential than the number or activity of bacterial cells (Third *et al.* 2000), where maximum chalcopyrite dissolution has been reported to occur at about 650 mV (vs. SHE) (Kametani and Aoki 1985). Since the Eh values of bioleaching suspension in this study ranged from 206 to 488 mV (vs. SHE) (< 650 mV), the passivation phenomena in this study could be reduced.

In conclusion, the present study has shown that an iron-oxidizing bacterium indigenous to a mine site of Indonesia is capable of bioleaching copper from a complex copper sulfide ore through three roles - creating the reduction potential (Eh) in solution which is favourable for copper leaching, generating ferric ions at a suitable rate that are needed for continued leaching of the ores, and producing EPS to concentrate ferric ions by complexation at the mineral surface

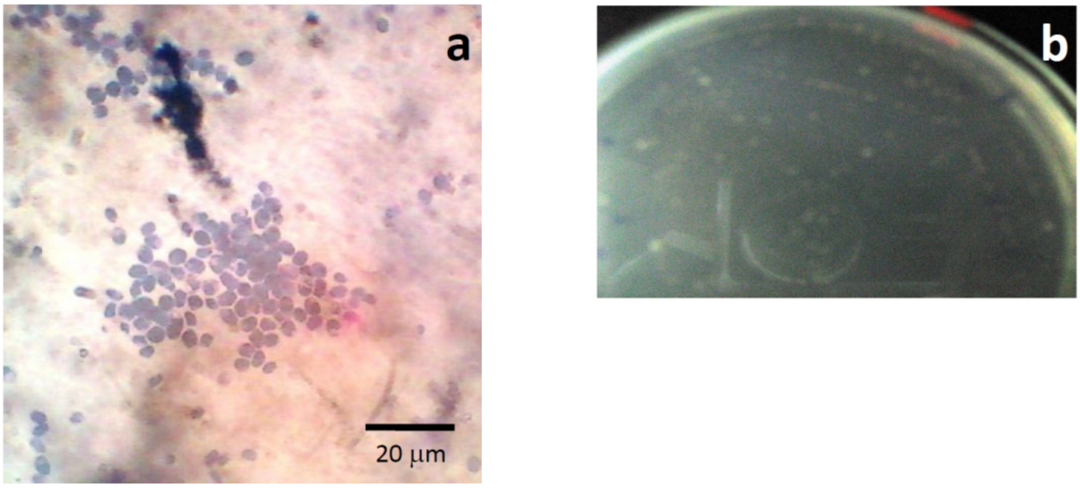

Fig 1 Photomicrographs of bacterial cells of an iron-oxidizing bacterium (identified as *Alicyclobacillus* sp.) (A) and its colonies (B).

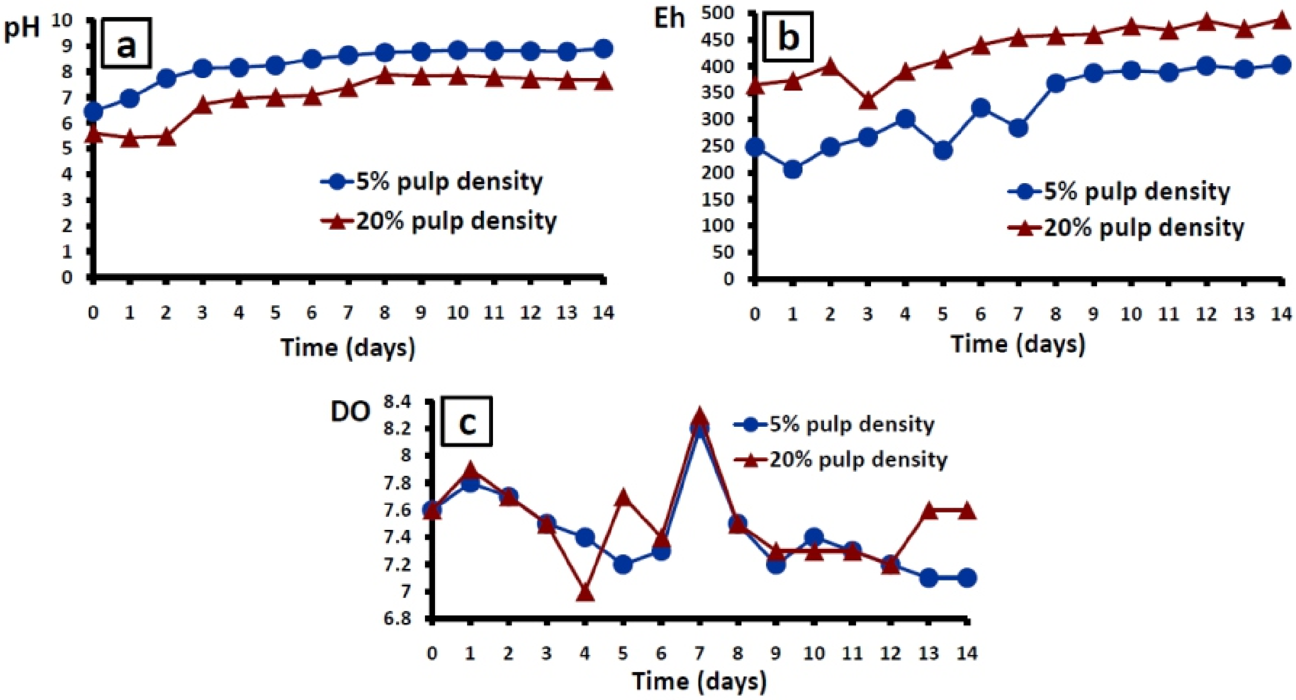

Fig 2 The values of pH (A), redox potential (Eh) in mV (vs. SHE) (B) and dissolved oxygen (DO) in mg L^{-1} (C) of bioleaching suspension containing an iron-oxidizing indigenous bacterium at various pulp densities (5% and 20%) over a period of 14 days.

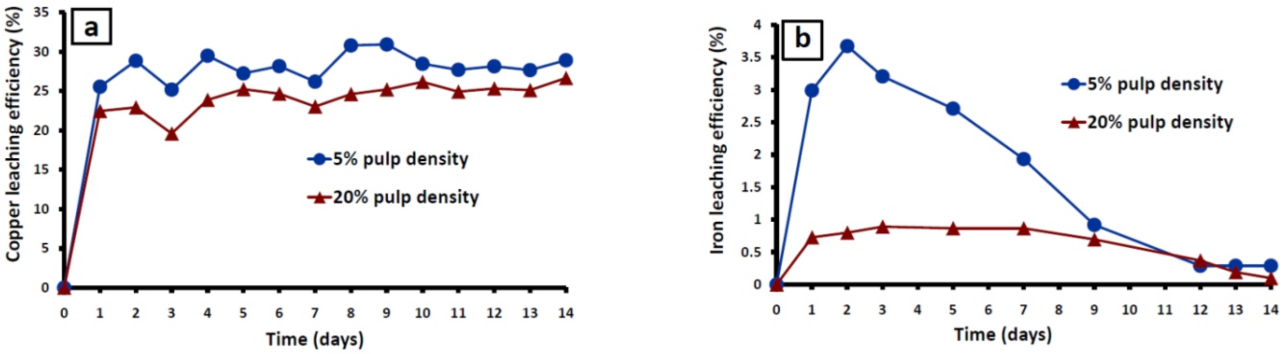

Fig 3 Copper (A) and total iron (B) dissolution (%) by an iron-oxidizing indigenous bacterium at various pulp densities (5% and 20%) over a period of 14 days.

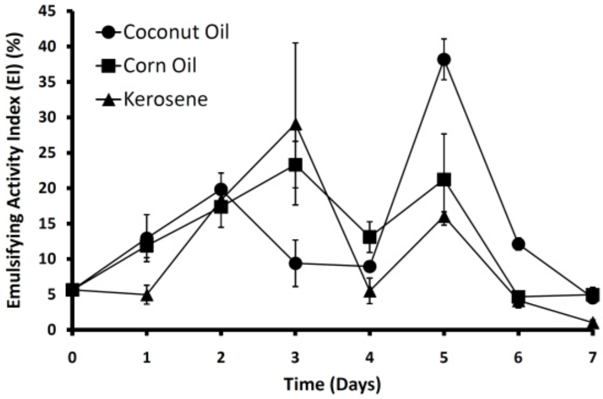

Fig 3 EPS (extracellular polymeric substances) production by an iron-oxidizing indigenous bacterium as represented by emulsifying activity index (EI) over a period of 7 days. Error bars represent standard deviation from the mean (n = 2-3).

which thus allow an oxidative attack on the sulfide as well as to sequester metals (herein Cu) into soluble metal-ligand complexes by chelation. These in turn result in copper dissolution. The results obtained from this study may thus contribute to the development of the bioleaching technology for processing complex copper sulfide ores as refractory ores.

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