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Comparsion of bioleaching of copper sulphides by *Acidithiobacillus ferrooxidans*

Kaibin Fu[1,2*], Hai Lin[2], Deqiang Luo[1], Wufei Jiang[1] and Ping Zeng[1]

[1]Key Laboratory of Solid Waste Treatment and Resource Recycle, Ministry of Education, Southwest University of Science and Technology, Mianyang 621010, China.
[2]School of Civil and Environmental Engineering, University of Science and Technology Beijing, Beijing 100083, China.

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Bioleaching of copper sulphides were investigated. The results show that the copper extraction from djurleite, bornite, covellite and chalcopyrite under the optimal conditions were 95.12, 84.5, 54.1, and 18.33%, respectively. A ranking for the bioleaching of copper sulfides was obtained: djurleite > bornite > covellite > chalcopyrite. A determination of the extent of adsorption of *A. ferrooxidans* on the surface of the minerals showed that the cell density of *Acidithiobacillus ferrooxidans* on djurleite was the highest. The order of adsorption on the copper sulphides was: djurleite > bornite > covellite > chalcopyrite. The results suggest a difference in affinity of bacteria for different copper sulphides. The higher the leaching rate of copper sulphides, the greater the density of bacteria absorbed on the surface of minerals.

Key words: Bioleaching, copper sulphides, *Acidithiobacillus ferrooxidans*, cell density, copper extraction.

INTRODUCTION

Bacterial heap leaching of low-grade copper sulphides is a developing technology. Bioleaching has been applied successfully to the extraction of copper from secondary sulphide minerals, such as chalcocite, at a number of operations worldwide (Watling, 2006). However, heap bioleaching of the refractory primary copper sulphide, chalcopyrite, is still a major challenge due to slow kinetics and poor extraction (Zhou et al., 2009). A study at Billiton Process Research showed bioleaching of secondary sulphide minerals with adapted mesophilic bacterial cultures is technically feasible. The preferential order in which sulphide minerals are leached by such mesophilic cultures is: Chalcocite>bornite>cubanite>covellite>pyrite> enargite> carrolite>> chalcopyrite (Dew et al., 1999). Regrettably, the reason for difference of bioleaching of sulphide minerals was nott analyzed.

Rodríguez et al. (2003) confirmed that a relationship exists between attachment and mineral dissolution rates. The bioleaching process can be divided into three stages. An initial stage with extensive bacterial attachment to the minerals is of major importance in order to obtain high dissolution rates. In a second stage, the bacterial attachment diminishes due to the saturation of the surface by the attached cells. Finally, in a third stage, a balance between free and attached cells is reached, giving rise to a cooperative mechanism (Sand et al., 1995; Rojas-Chapana and Tributsch, 2004). Attached cells play important roles in the whole bioleaching process.

The present work is a comparative study of the bioleaching of several copper sulfide minerals, djurleite ($Cu_{31}S_{16}$), bornite (Cu_5FeS_4), covellite (CuS), chalcopyrite ($CuFeS_2$). Experiments were conducted to characterize the adsorption

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*Corresponding author. E-mail: fukaibin@126.com. Tel: +86-15884633446.

Abbreviations: XRD, X-ray diffraction; **AAS,** atomic absorption spectrometry; **ORP,** oxidation reduction potential; **SEM,** scanning electron microscopy.

Table 1. Chemical analysis of copper sulphides.

Mineral	Cu (%)	Fe (%)	S (%)	Purity (%)
Djurleite	71.94	1.22	20.53	90.65
Bornite	50.17	10.67	24.38	79.22
Covellite	60.26	3.20	33.52	90.48
chalcopyrite	27.88	28.29	32.36	80.62

of *Acidithiobacillus ferrooxidans* on copper sulphides as functions of time and cell concentration. A relationship between attachment and mineral dissolution rates will be discussed.

MATERIALS AND METHODS

Minerals

Four copper sulphides were used in the experiments. These minerals included djurleite, bornite, covellite and chalcopyrite. They were supplied by the Bofang copper mine in Hunan province, the Dongxiang copper mine in Jiangxi province, the Zijinshan copper mine in Fujian province, and the Dexing copper mine in Jiangxi province, respectively. The chemical analysis of the copper sulphides is shown in Table 1.

X-ray diffraction (XRD) analysis showed that djurleite ($Cu_{31}S_{16}$) contained small amounts of chacocite, bornite and quartz; bornite (Cu_5FeS_4) contained small amounts of chalcopyrite, pyrite (FeS_2), helvite, and quartz; covellite (CuS) contained small amounts pyrite and enargite; chalcopyrite ($CuFeS_2$) contained small amounts of pyrite and quartz. The handpicked samples were ground in a porcelain ball mill to a particle size of less than 74 μm, then stored under nitrogen in a sealed container. The specific surface area of djurleite, bornite, covellite and chalcopyrite were 302.67, 818.93, 1319.34 and 1362.47 cm^2/g, respectively.

Bacteria

An *A. ferrooxidans* (*Atf.6*) strain used in this study was isolated from a copper mine in Daye (China), and then screened through different stages of adaptation. Bacteria were grown in optimized 4.5 K medium (2.0 g/L $(NH_4)_2SO_4$, 0.25 g/L K_2HPO_4, 0.25 g/L $MgSO_4·7H_2O$, 0.1 g/L KCl, and 22.0 g/L $FeSO_4·7H_2O$) (Fu et al., 2011) and harvested from the late logarithmic phase of growth. The optimal cultivation conditions of *Atf.6* were temperature of 30°C, pH of 2.0, and a rotation speed of 160 rpm.

Bioleaching experiments

Bioleaching experiments were performed in 250 mL flasks, at pulp density of 20 g/L. When the pH was adjusted to 2, nutrients and inoculum were added to the erlenmeyer flask. The flasks were kept at 30°C and shaken at 160 rpm. The number of viable bacteria, pH, redox potential, and copper ion concentration in the leaching solution were determined at certain intervals. During the leaching process, distilled water was supplied to compensate for evaporation, and to maintain a solution volume of 100 mL in the flasks. Finally, the mineral residues were washed with Milli-Q water, and vacuum freeze-dried.

Adsorption studies

The bacterial cultures were fully grown in a sterile modified medium (for 60 h) and filtered through a Whatman No. 1 filter paper to remove all of the insoluble compounds. The filtrate was centrifuged at 10,000 rpm for 10 min, washed several times with distilled water, and then dispersed in distilled water at pH of 2.0 (Jia et al., 2008). Attachment experiments were carried out in 50 mL KCl solution with an ionic strength of 0.001 mol/L, containing $2.0×10^{10}$ cells and 0.5 g mineral (50 μm), on a rotary shaker (120 rpm) at 30°C.

The number of attached cells was calculated by subtraction of the remaining planktonic cells from the total numbers of cells inoculated.

Analytical methods

The concentration of dissolved copper ions in the leaching solution was analyzed by atomic absorption spectrometry (AAS). The ferrous iron was determined by titration with potassium dichromate (K_2CrO_7). The pH value and redox potential were measured by S20 Seven easy pH/Eh process controller. The bacterial number was determined by hemocytometer under ZBM-300E biological microscope.

SEM observations

SEM studies were carried out to observe bacterial cells attachment to mineral surfaces. After the adsorption equilibrium of *A. ferrooxidans* on copper sulfides, the mineral samples were gently washed twice with distilled water to remove loosely attached bacteria.

The samples were then vacuum freeze-dried and coated with carbon under vacuum. Images were obtained using a LEO-1450 model scanning electron microscope.

RESULTS AND DISCUSSION

Bioleaching of copper sulphides

Inoculum

The media contained 0 g/L of Fe^{2+}. The effect of cell concentration in the inoculum on the copper extraction from the copper sulphides is shown in Figure 1. When the pH was adjusted to 2, the copper extraction from djurleite, bornite, chalcocite, and chalcopyrite reached 36.45, 18.38, 9.92, and 0.34%, respectively. Figure 1 shows the effect of cell concentration on copper extraction of copper sulphides. In the initial stages of leaching, copper extraction increased with the increase of inocula. Generally, within the range of a cell concentration, as bacterial inocula increases, more bacterial cells were adsorbed on the mineral surface. Rapid dissolution rate depends on adsorption of large numbers of bacteria on the surface of metal sulphide mineral, which results in the release of Fe^{2+} through "contact" bioleaching (Pich otero et al., 1995;

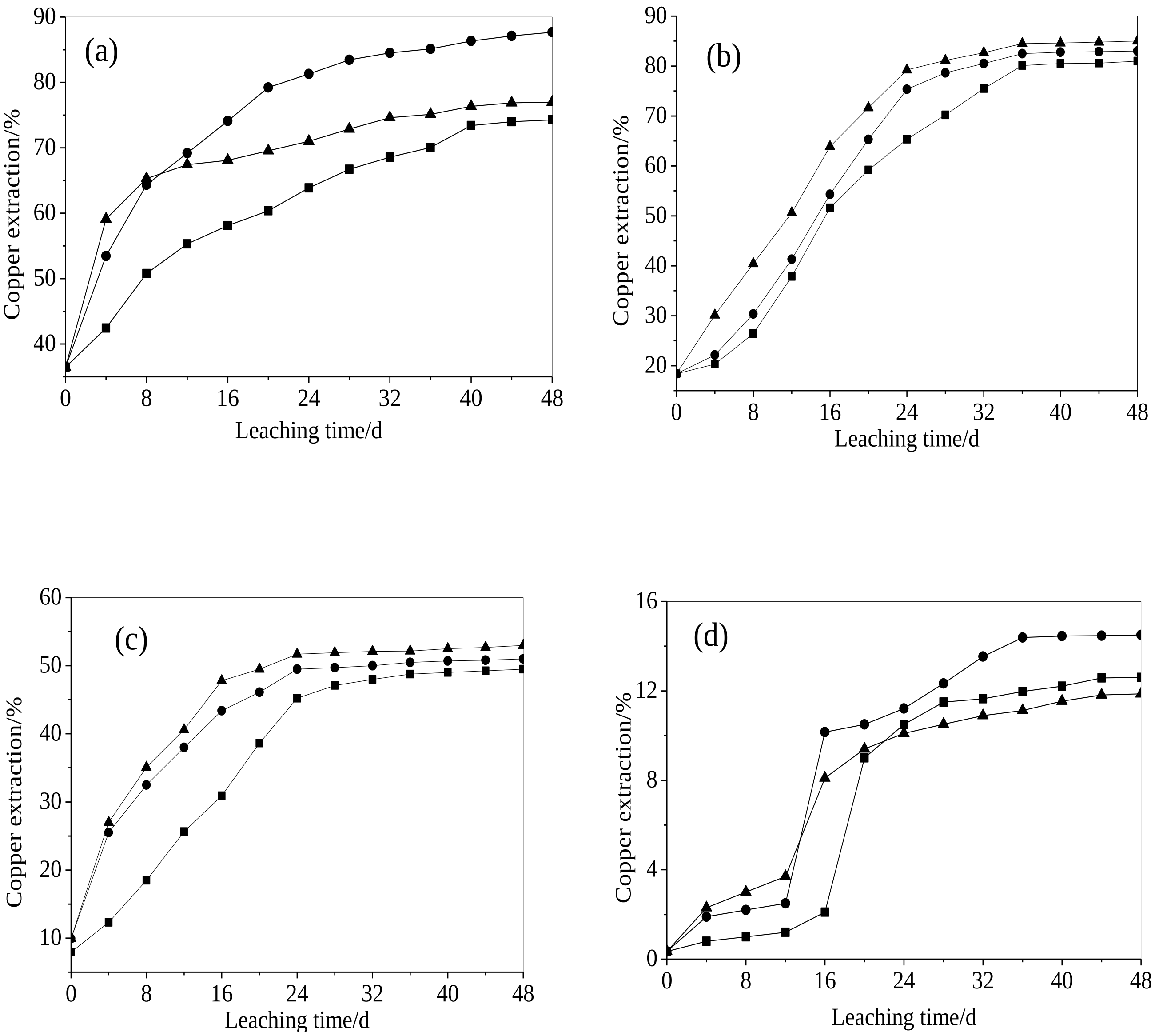

Figure 1. Effect of cell concentration in inocula (■, 0.5×10^8 cells; ●, 1.0×10^8 cells; ▲, 1.5×10^8 cells) on copper mobilization from djurleite (a), bornite (b), covellite (c), and chalcopyrite (d).

Rodríguez et al., 2003). As the duration of leaching lengthened, the differences in leaching rate at different inoculum sizes became smaller. Copper extraction from djurleite with inoculum of 1.0×10^8 cells was greater than that with an inoculum of 1.5×10^8 cells from day 10 to day 48. More copper was extracted from chalcopyrite with an inoculum of 0.5×10^8 cells than that with 1.5×10^8 cells. The optimum inoculation quantity is beneficial to bioleaching of copper sulphides. The maximal copper recovery of djurleite and chalcopyrite were achieved when the inoculation amount is 1.0×10^8 cells. After 48 day of bioleaching, the copper dissolution of djurleite and chalcopyrite were 87.67 and 14.5%, respectively. The maximum copper extraction of bornite and covellite were 85 and 53%, respectively, when the inoculation amount is 1.5×10^8 cells. The results present in Figure 1 indicated the preferential order of mineral bioleaching: djurleite> bornite> covellite> chalcopyrite.

Initial ferrous ion concentration

Other than the bacterial inoculum, the initial ferrous iron concentration is an important parameter in the bioleaching of copper sulphides (Van et al., 2010; Kinnunen et al., 2006). The effect of the initial ferrous ion concentration on the bioleaching of copper sulphides is shown in Figure 2. Substantial work with regards to the mechanism of bioleaching has been done with *A. ferrooxidans*. Since the discovery of this bacterium in acid mine drainage, two bio-oxidation mechanism have been discussed: the direct

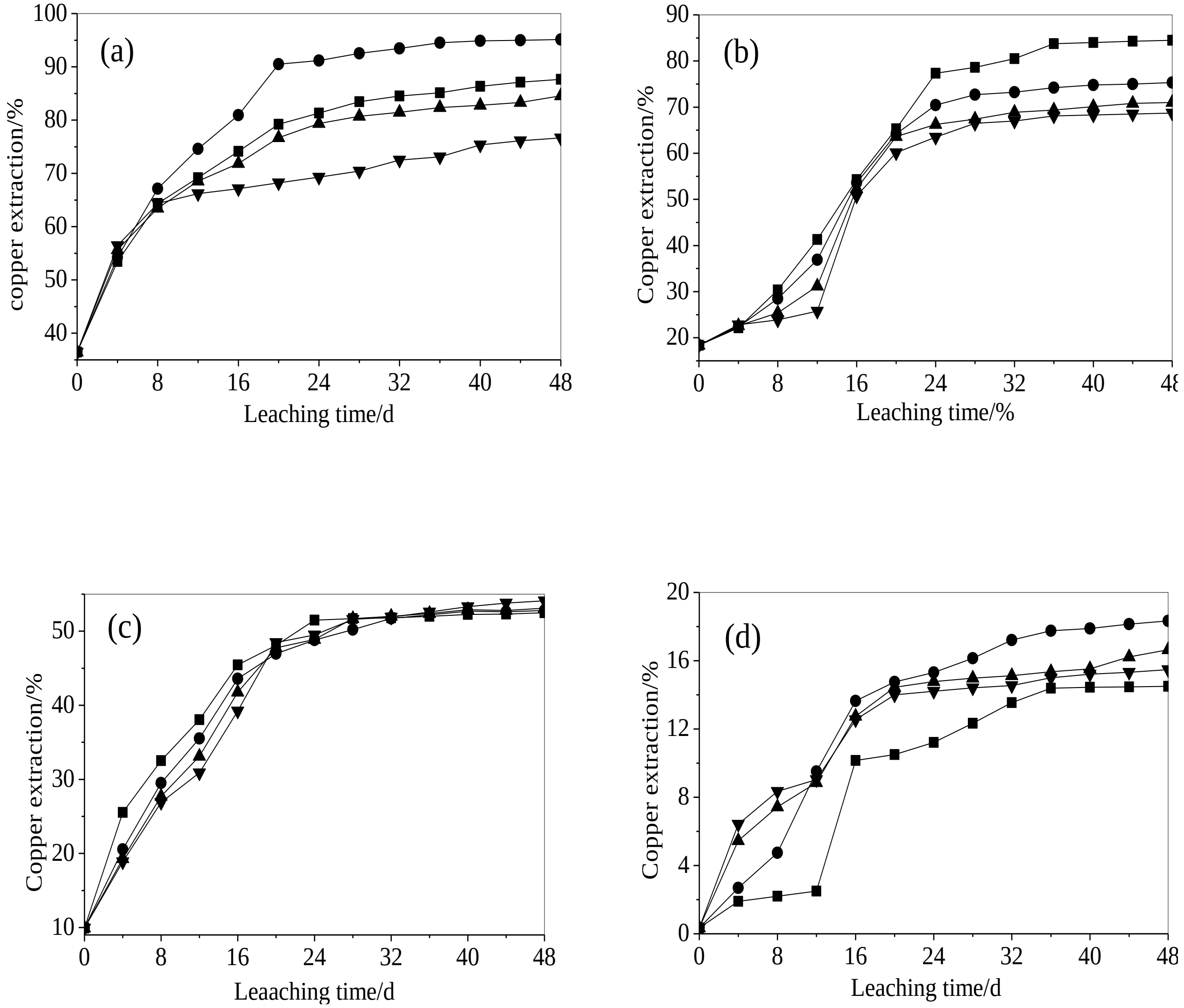

Figure 2. Effect of [Fe^{2+}]$_{initial}$ (■, 0g/L ; ●, 1.5g/L ; ▲, 2.5g/L ; ▼, 4.5g/L) on bioleaching of djurleite (a), bornite (b), covellite (c), and chalcopyrite (d).

one and the indirect one (Schippers and Sand, 1999; Colmer and Hinkle, 1947). The direct mechanism assumes the action of a metal sulfide-attached cell oxidizing the mineral by an enzyme system with oxygen to sulfate and metal cations. In contrast, the indirect mechanism basically involving the oxidation of the metal sulphide by Fe^{3+} produced by the bacteria in the oxidation of Fe^{2+}. In the course of metal sulphide oxidation by Fe^{3+}, Fe^{2+} and elemental sulfur (S_8) are generated. These products are then biologically oxidized to Fe^{3+} and sulfate (Sand et al., 2001; Tributsch, 2001). Accordingly, the bioleaching of copper sulphides are summarized by the following equations (Fu et al., 2013; Ahmadi et al., 2012):

$$Cu_{31}S_{16} + 31Fe_2(SO_4)_3 \rightarrow 31CuSO_4 + 62FeSO_4 + 16S \quad 1$$

$$Cu_{31}S_{16} + 15H_2SO_4 + 79/2O_2 \xrightarrow{bacteria} 31CuSO_4 + 15H_2O \quad 2$$

$$CuS + 2O_2 \xrightarrow{bacteria} CuSO_4 \quad 3$$

$$CuS + Fe_2(SO_4)_3 \longrightarrow CuSO_4 + 2FeSO_4 + S \quad 4$$

$$Cu_5FeS_4 + 9O_2 + 2H_2SO_4 \xrightarrow{bacteria} 5CuSO_4 + FeSO_4 + 2H_2O \quad 5$$

$$Cu_5FeS_4 + 6Fe_2(SO_4)_3 \longrightarrow 5CuSO_4 + 13FeSO_4 + 4S \quad 6$$

$$CuFeS_2 + 4O_2 \xrightarrow{bacteria} CuSO_4 + FeSO_4 \quad 7$$

$$CuFeS_2 + 2Fe_2(SO_4)_3 \longrightarrow CuSO_4 + 5FeSO_4 + 2S \quad 8$$

$$4FeSO_4 + O_2 + 2H_2SO_4 \xrightarrow{bacteria} 2Fe_2(SO_4)_3 + 2H_2O \quad 9$$

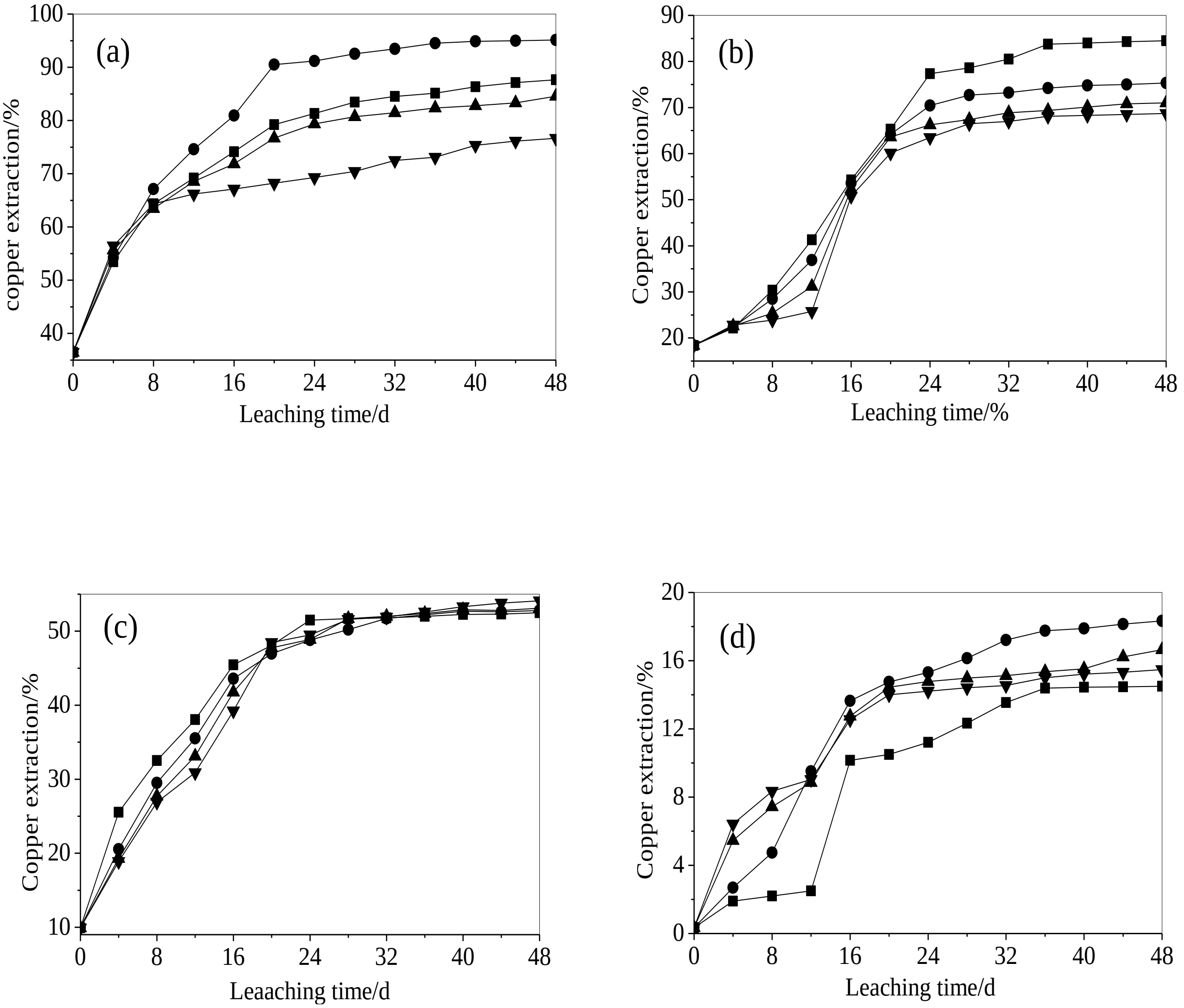

Figure 2. Effect of $[Fe^{2+}]_{initial}$ (■, 0g/L ; ●, 1.5g/L ; ▲, 2.5g/L ; ▼, 4.5g/L) on bioleaching of djurleite (a), bornite (b), covellite (c), and chalcopyrite (d).

$$2S + 3O_{2(aq.)} + 2H_2O \xrightarrow{\text{bacteria}} 2SO_4^{2-} + 4H^+ \qquad 10$$

Figure 2 shows copper dissolution was initially fast, but slowed down rapidly after approximately 24 day. Copper extraction inceased with an increase in initial ferrous iron concentration. The results indicate the oxidizing action of Fe^{3+} played a leading role in bioleaching of copper sulfides. In the lag phase, copper sulfides were oxidized by Fe^{3+} (Equations 1, 4, 6 and 8). After a short lag phase, the bacteria adapted to copper ores environment, and multiplied rapidly. *A. ferrooxidans*, together with Fe^{3+}, facilitates dissolution of copper sulfides. With the dissolution of minerals, the excess of iron ions has a negative effect on the copper dissolution rate. Jarosite formed gradually, and coated on the surface of the remaining unreacted ore. This jarosite precipitation restricted the leaching of the sulphide mineral. The ferrous ion, as energy source of *A. ferrooxidans* plays a very important role in extracting copper and will affect the recovery of copper. The apropriate initial ferrous ion concentration is in favor of dissolution of copper sulfides. After 48 day of bioleaching, when the initial ferrous ion concentration was 1.5 g/L, the maximal copper dissolution of djurleite and chalcopyrite achieved were 95.12 and 18.33%, respectively. The ferrous ion would hinder the dissolution of bornite, the bornite dissolution was 84.5% at 0 g/L of the initial ferrous ion concentration. The covellite dissolution was 54.1% at 4.5 g/L of $[Fe^{2+}]_{initial}$. The preferential order of bioleaching of copper sulfides still is: djurleite> bornite> covellite> chalcopyrite.

Bioleaching under optimal conditions

Based on the results determined in the previous tests, the optimum conditions of bioleaching of djurleite and chalco-

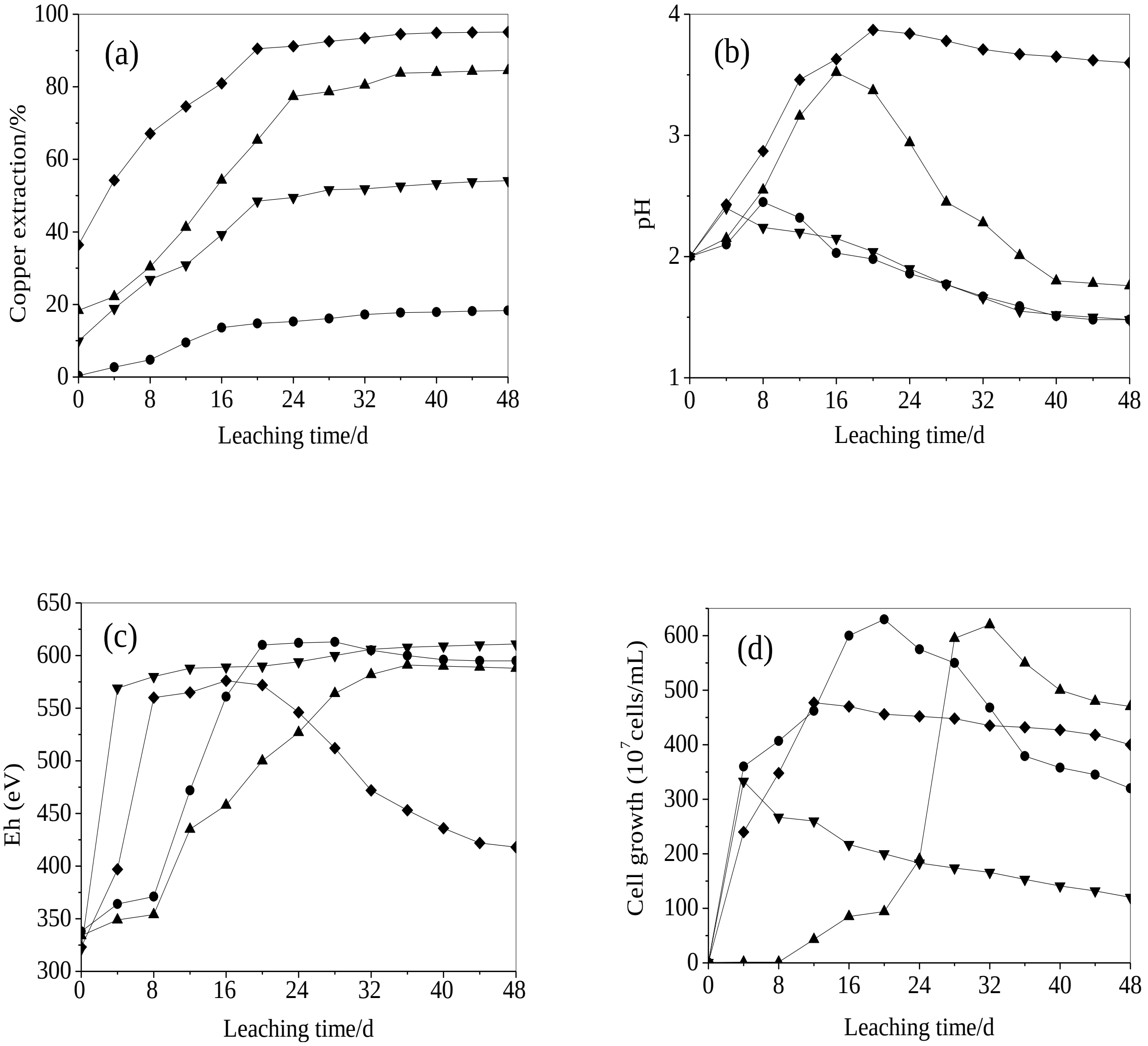

Figure 3. Bioleaching of djurleite (◆), bornite (▲), covellite (▼), and chalcopyrite (●) under the optimal conditions. Variations of copper extraction (a), pH (b), Eh (c) and cell growth (d).

pyrite were determined as follows: An inoculum of 0.5×10^8 cells, and the initial ferrous ion concentration of 1.5 g/L. The optimal inoculum of bornite and covellite were 1.5×10^8 cells. The appropriate initial ferrous ion concentration of bornite and covellite were 0 and 4.5 g/L, respectively. The bioleaching of copper sulfides under the optimal conditions is shwon in Figure 3. Figure 3a shows the copper extraction from djurleite, bornite, covellite and chalcopyrite were 95.12, 84.5, 54.1, and 18.33%, respectively. The ranking for the bioleaching of copper sulfides is obtained: djurleite> bornite> covellite> chalcopyrite. The results are in good agreement with those obtained by Dew et al. (1999).

Figure 3b shows the change of pH during the bioleaching of copper sulfides. The pH in the bioleaching of covellite and chalcopyrite was lower than 2.5, as the leaching reaction progressed, the pH decreased, low pH resulted in high ratio of [Fe^{3+}] to [Fe^{2+}] (Figure 3c). The oxidation reduction potential (ORP) in the solution of covellite and chalcopyrite was higher than 550 eV. Sandstrom et al. (2005) reported chalcopyrite leached rapidly at the lower potential, dropping at the higher redox value. The bioleaching experiments of Third et al. (2002) showed that the dissolution of chalcopyrite was inhibited by redox potentials> 420 mV. High [Fe^{3+}]/[Fe^{2+}] might hinder the dissolution of covellite and chalcopyrite. The pH in bioleaching of djurleite and bornite were higher than that in bioleaching of covellite and chalcopyrite after 8 day of bioleaching. The pH of djurleite bioleaching was higher than 3.0 after 9 day (Figure 3b). The ORP in the solution of djurleite gradually decreased after 20 day of bioleaching (Figure 3c), and was 418 eV at the end of bioleaching. *A. ferrooxidans* obtains energy from the simultaneous oxidation of Fe^{2+} and elemental sulfur, their growth rate is given by the following equation (Vilcaez and Inoue 2009):

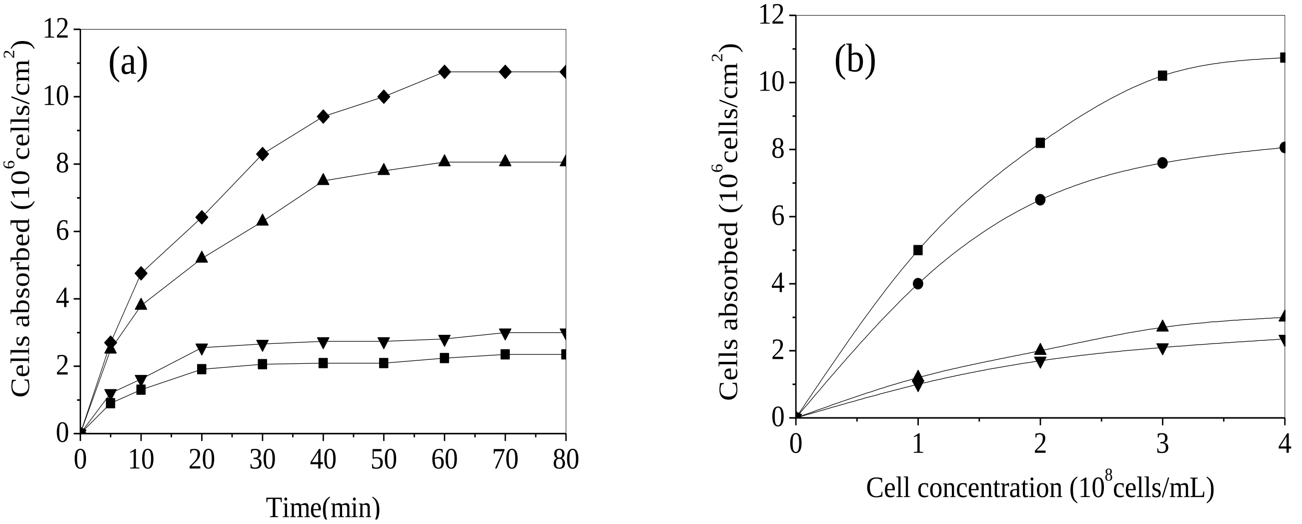

Figure 4. Adsorption of *A. ferrooxidans* on djurleite (◆), bornite (▲), covellite (▼), and chalcopyrite (●). A, Time; B, cell concentration.

$$\frac{d[N]}{dt} = \mu_{Fe}\frac{\left[Fe^{2+}\right]}{K_{Fe}+\left[Fe^{2+}\right]}\left[N\right] + \mu_S\frac{\left[S^0\right]}{K_S+\left[S^0\right]}\left[N\right] \qquad 11$$

Where, N is the biomass concentration, μ_{Fe} is the specific growth coefficient of mesophiles in the oxidation of iron, K_{Fe} is the half-saturation coefficient of the oxidation of iron, μ_S is the specific rate coefficient in the oxidation of elemental sulfur by mesophiles, K_S is the saturation coefficient in the oxidation of elemental sulfur.

When the initial ferrous ion concentration was 0 g/L, *A. ferrooxidans* obtained energy from the oxidation of copper sulphide, the lag phase of bacteria in solution of bornite lengthened (Figure 3d). The initial ferrous ion concentration in the solution of djurleite, chalcopyrite and covellite were 1.5, 1.5 and 4.5 g/L, respectively. *A. ferrooxidans* obtained easily energy from the oxidation of Fe^{2+} to Fe^{3+}. The lag phase of *A. ferrooxidans* in the solution of djurleite, chalcopyrite and covellite shortened. The appropriate initial ferrous ion is beneficial to the bacterial growth. The cell concentration in the solution of covellite decreased after 4 days of bioleaching. This might be atttibuted to the low pH value. Deveci et al. (2004) reported that the increase in the acidity to pH 1.2-1.4 led to a decrease in the oxidising activity of bacteria, indicating the inhibitory effect of increased acidity on bacteria.

Adsorption experiment

The adsorption of *A. ferrooxidans* on copper sulphides at pH=2.0 is shown in Figure 4. Figure 4a is the adsorption curve of *A. ferrooxidans* on copper sulphides. Figure 4b is the adsorption isotherm of *A. ferrooxidans* on copper sulphides. Figure 4a shows the adsorption quantity increased with the extension of time. The adsorption equilibrium of *A. ferrooxidans* on covellite and chalcopyrite were attained after 20 min. However, the adsorption equilibrium of *A. ferrooxidans* on djurleite and bornite were attained after 60 min. As for as the four copper sulphides, the cell density of *A. ferrooxidans* on djurleite was the highest. The order of adsorption of cells on copper sulphides is: djurleite> bornite> covellite> chalcopyrite. The order is in accordance with their copper dissolution rate.

The shape of adsorption isotherms enables characterization of adsorption process. Adsorption isotherms have been classified by Giles et al. (1960) into four main groups: L, S, H, and C. According to the classification, the isotherm of adsorption of *A. ferrooxidans* on copper sulphides displayed an L curve pattern (Figure 4b). The L shape of the adsorption isotherms means that there is no strong competition between solvent and the bacteria to occupy the copper sulphides.

As can be seen from Figure 4b, the values of experimental maximum adsorption of *A. ferrooxidans* on djurleite, bornite, covellite, and chalcopyrite were about 10.74×10^6, 8.06×10^6, 3.0×10^6, and 2.35×10^6 cells/cm^2, respectively. Several mathematical models can be used to describe experimental data of an adsorption isotherm. The equilibrium data in Figure 4b were modeled with the Langmuir and Freundlich models, respectively. The linearized equations of adsorption isotherm of *A. ferrooxidans* on copper sulphides and the corresponding correlation coefficient are shown in Table 2.

Based on Table 1, the Langmuir isotherm was more applicable to the experimental data than the Freundlich adsorption isotherm because of the higher values of correlation coefficient (R^2). The coefficients of correlation of the Langmuir adsorption isotherm are high (≥0.9944), which shows a good linearity. The result shows the ad-sorption of *A. ferrooxidans* on copper sulphides is mono-layer adsorption. This was verified by the SEM image of adsorption of *A. ferrooxidans* on copper sulphides (Figure 5).

Table 1. Chemical analysis of copper sulphides.

Mineral	Cu (%)	Fe (%)	S (%)	Purity (%)
Djurleite	71.94	1.22	20.53	90.65
Bornite	50.17	10.67	24.38	79.22
Covellite	60.26	3.20	33.52	90.48
chalcopyrite	27.88	28.29	32.36	80.62

Table 2. Adsorption isotherm of *A. ferrooxidans* on copper sulphides.

Mineral	Langmuir adsorption isotherm		Freundlich adsorption isotherm	
	Linear form	R^2	Linear form	R^2
Djurleite	$1/q_e=0.040/C_e+0.018$	0.9947	$\lg q_e=0.53\lg C_e+4.95$	0.963
Bornite	$1/q_e=0.013/C_e+0.011$	0.9944	$\lg q_e=0.44\lg C_e+6.08$	0.9646
Covellite	$1/q_e=0.042/C_e+0.013$	0.9983	$\lg q_e=0.65\lg C_e+4.07$	0.9895
Chalcopyrite	$1/q_e=0.048/C_e+0.017$	0.9976	$\lg q_e=0.59\lg C_e+4.41$	0.9803

q_e, Equilibrium adsorption quantity, cells/cm^2; C_e, equilibrium concentration of bacterial cells, cells/mL; R, correlation coefficient.

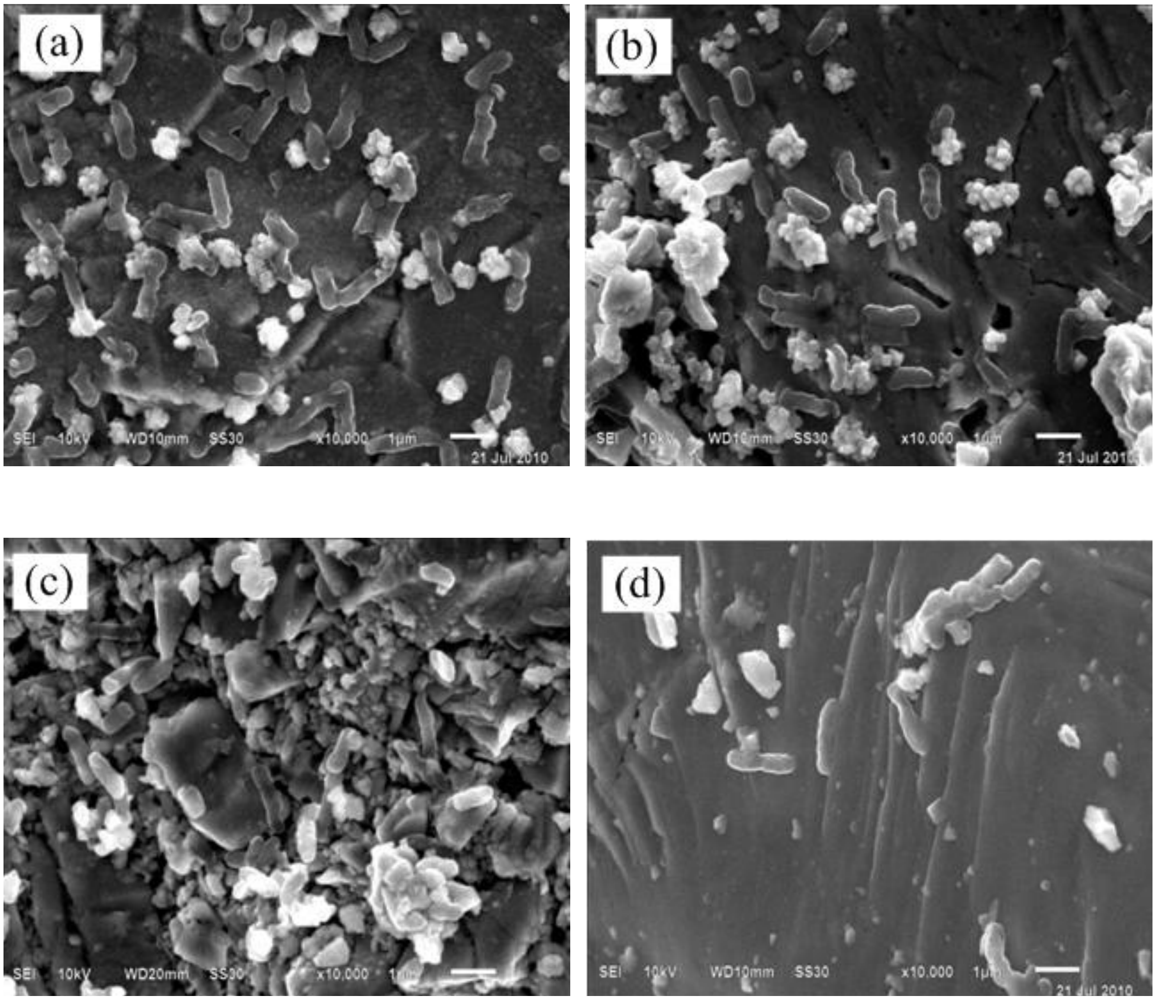

Figure 5. SEM image of adsorption of *A. ferrooxidans* on djurleite (a), bornite (b), covellite (c), and chalcopyrite (d).

Scanning electron microscopy (SEM) photomicrographs in Figure 5 shows patterns of adherence of *A. ferrooxidans* on djurleite, bornite, covellite, and chalcopyrite, respectively. Figure 5 shows an obvious difference in the adsorption density of bacteria on copper sulphides. This may be associated with the distribution and the atom number proportion of Cu, Fe, and S on the surface of copper sulphides (Sanhueza et al., 1999). The adsorbed bacteria on the surface of djurleite, bornite, and covellite were much more evenly distributed, and were mainly present as isolated bacteria. Attached bacteria on the surface of chalcopyrite were unevenly distributed, and present as short-string-like chains with two or three bacteria in few zones.

The values of surface density of attached bacteria were calculated from the population of bacteria observed in the SEM micrographs (Figure 5), and their order is: djurleite> bornite> covellite> chalcopyrite. The results suggest that the high density of *A. ferrooxidans* on the surface of a mineral and the evenly distributed the bacteria would be beneficial to each of the mineral surface. A direct enzymatic attack on the mineral surface could initiate the leaching reaction (Vilcáez and Inoue, 2008).

Conclusions

The bioleaching of copper sulphides showed that copper extraction from djurleite, bornite, covellite and chalcopyrite under the optimal conditions were 95.12, 84.5, 54.1, and 18.33%, respectively. The ranking for the bioleaching of copper sulfides is obtained: djurleite> bornite> covellite> chalcopyrite.

The adsorption of *A. ferrooxidans* on copper sulphides is monolayer adsorption. After the saturation of the surface by the attached cells, the values of adsorption density of *A. ferrooxidans* on djurleite, bornite, covellite, and chalcopyrite were about 10.74×10^6, 8.06×10^6, 3.0×10^6, and 2.35×10^6 cells/cm^2, respectively. The cell density of *A. ferrooxidans* on djurleite was the highest. The order of adsorption on copper sulphides is: djurleite> bornite> covellite> chalcopyrite. It seems that cells adsorbed by the minerals are beneficial to bioleaching ofcopper sulphides. The results suggest that the high density of *A. ferrooxidans* on the surface of a mineral and the evenly distributed the bacteria would be beneficial to each of the mineral surface. On the other hand, the higher the leaching rate of copper sulphides, the greater the density of bacteria absorbed on the surface of minerals.

ACKNOWLEDGEMENTS

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