Comparison of adhesion of *A. ferrooxidans* on different copper sulfides: Surface thermodynamics and extended DLVO theory

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Abstract

We investigated experimentally and theoretically the adhesion behavior of Acidithiobacillus ferrooxidans (At.f_6) *to different copper sulfides. The adsorption experiment showed cell densities of* 10.74×10^6, 8.06×10^6, 3×10^6, 4.24×10^6 *and* 2.35×10^6 *cells/cm*^{2}*, respectively, on the surfaces of djurleite, bornite, covellite and chalcopyrite. The order was: djurleite > bornite > covellite > chalcopyrite. The results indicate that* A. ferrooxidans *has high affinity to adhering to the surfaces of djurleite and bornite in the acidic region. But the thermodynamic approach predicts that there will be no attachment of* At.f_6 *to the surfaces of bornite, covellite and chalcopyrite. This discrepancy is due to the inadequate description of electrostatic interactions. The DLVO approach shows attachment of* At.f_6 *to the surfaces of djurleite, bornite and covellite, and repulsion between* At.f_6 *and chalcopyrite. The DLVO approach can explain the differences of the adhesion of* At.f_6 *to different copper sulfides.*

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Introduction

The bioleaching of low-grade copper sulfides is a developing technology, and has been applied successfully to the extraction of copper from secondary sulfide minerals (Watling, 2006; Pradhan et al., 2008; Akcil and Deveci, 2010). The bioleaching of chalcopyrite is still a major challenge (Bosecker, 1997; Brierley and Brierley, 2001; Ehrlich, 2004). A study conducted at Billiton Process Research showed the following preferential order of sulfide minerals leached by mesophilic cultures: chalcocite > bornite > cubanite > covellite > pyrite > enargite > carrolite >> chalcopyrite (Dew et al., 1999). The bacteria can solubilize the valuable metals from the orebodies by the oxidation of minerals. Bacterial adhesion plays a critical role in the bioleaching processes (Chen et al., 2008; Harneit et al., 2006). Generally, bacterial adhesion can be explained by surface thermodynamics and the extended DLVO theory, in which the adhesion energy between the bacteria and mineral is calculated as a function of the separation distance (Sharma, 1999; Bos et al., 1999; Sharma and Hanumantha Rao, 2002). These methods take into account Lifshitz-van der Waals interactions, electrostatic interactions and acid-base interac-

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tions (Busscher et al., 2010). These interactions are very well understood and formulated in mathematical equations. The most important inputs in these calculations are surface energy, surface charge, electron donation and electron acceptance on the bacterial cell surface and mineral.

Many factors affect the leaching process and leaching efficiency. Bacterial adherence to the mineral surface is a very important factor among them (Behera et al., 2011). This study is aimed at better understanding the bioleaching of different copper sulfide minerals from the perspective of the interactions between the bacteria and minerals. The adhesion behavior of *At.f6* to different copper sulfides is investigated using the approaches of surface thermodynamics and the extended DLVO theory.

Materials and methods

Mineral samples. Four copper sulfides (djurleite, bornite, covellite and chalcopyrite) were used in the experiments. Djurleite with 71.94% copper (Cu), 1.22% iron (Fe) and 20.53% sulfur (S) was obtained from the Bofang copper mine in Hunan province, bornite (50.17% Cu, 10.67% Fe and 24.38% S) was obtained from the Dongxiang copper mine in Jiangxi province, covellite (60.26% Cu, 3.20% Fe and 33.52% S) was obtained from the Zijinshan copper mine in Fujian province, and chalcopyrite (27.88% Cu, 28.29% Fe and 32.36% S) was obtained from the Dexing copper mine in Jiangxi province.

The handpicked samples were crushed with a hammer and ground in a porcelain ball mill to particle sizes of less than 74 µm, then stored under nitrogen in a sealed container. The specific surface areas of djurleite, bornite, covellite and chalcopyrite were 302.67 cm^2/g, 818.93 cm^2/g, 1,319.34 cm^2/g and 1,362.47 cm^2/g, respectively. Some samples were further ground to particle sizes of less than 5 µm for electrokinetic experiments.

Bacterial strain. A pure culture of an *Acidithiobacillus ferrooxidans* (*At.f6*) strain, isolated from the Liwu copper mine in Sichuan province in China, was used in this study. The strain was cultured in an optimized medium consisting of $(NH_4)_2SO_4$ (2.0 g/L), K_2HPO_4 (0.25 g/L), $MgSO_4 \cdot 7H_2O$ (0.25 g/L), KCl (0.1 g/L), and $FeSO_4 \cdot 7H_2O$ (22.0 g/L) (Fu et al., 2011, 2013). Cultures were grown in shake flasks under the conditions of 160 rpm and 30°C.

Contact angle measurement. Samples were prepared using the method reported by Sharma and Hanumantha Rao (2003). Between measurements, the mineral surfaces were freshly polished with paper No. 4000 and dipped in ethanol to prevent any oxidation of the surfaces, and contact angles were measured within 5-10 minutes of polishing.

For measuring the contact angle of bacteria, bacterial lawn was prepared by filtering bacterial suspension on a filter membrane, with about 800 bacterial layers depositing on the filter membrane. The filter membrane with the bacterial lawn was vacuum freeze-dried, cut into strips, and fixed with double-sided adhesive tape onto a sample holder (Farahat et al., 2009).

Table 1 — Surface tensions (mJ/m^2), as obtained from literature (Bos et al., 1999).

Liquid	γ^{TOT}	γ^{LW}	γ^{AB}	γ^+	γ^-
Water	72.8	21.8	51	25.5	25.5
Ethylene glycol	48.0	29	19.0	1.92	47.0
Glycerol	64	34	30	3.92	57.4

Polar liquids (water, ethylene glycol and glycerol) were used to measure the angles on copper sulfide surfaces and bacterial lawns. The contact angles of these three liquids on solid sulfide surfaces and bacterial lawns were measured using the sessile drop technique with an Easydrop DSA20 dynamic adsorption tester. The surface tension components of the liquids are shown in Table 1.

Adsorption studies. The bacterial cultures were fully grown in a sterile medium (for 60 hours) and filtered through a Whatman No. 1 filter paper to remove the insoluble compounds. The filtrate was centrifuged at 10,000 rpm for 10 minutes, washed several times with distilled water, and then dispersed in distilled water at a pH of 2.0 (Jia et al., 2008).

Attachment experiments were carried out in a 50-mL KCl solution with ionic strength of 0.001 mol/L, containing 2.0 × 1,010 cells and 0.5 g of mineral (-74 µm), on a rotary shaker (120 rpm) at 30°C. The number of attached cells was calculated by subtracting the remaining planktonic cells from the total numbers of cells inoculated.

Electrokinetic measurements. The electrophoretic mobilities of the copper sulfides as well as *At.f6* were measured using a Brookhaven Zeta PALS instrument. The zeta potential of bacterial cells was measured at a concentration of 4×10^8 cells/mL. All measurements were conducted at the same ionic strength (0.001 mol/L). Each sampling was taken and analyzed in triplicate (Farahat et al., 2010; Subramanian et al., 2003).

Results and discussion

Thermodynamic approach. The interfacial free energy of microbe-mineral can be described using the thermodynamic approach (Sharma and Hanumantha Rao, 2002), and compared before and after adhesion. The comparison is expressed in terms of free energy of adhesion as:

$$\Delta G_{adh} = \gamma_{sb} - \gamma_{sl} - \gamma_{bl} \quad (1)$$

where γ_{sb}, γ_{sl} and γ_{bl} are mineral-bacteria, mineral-liquid and bacteria-liquid interfacial free energies, respectively. ΔG_{adh} is negative, which means that bacterial adhesion will take place on the mineral, and adhesion is thermodynamically unfavorable when ΔG_{adh} is positive. The interfacial energies of γ_{sl} and γ_{bl} can be calculated using contact angle data measured with standard liquids with known free energies. Different approaches to converting contact angle data to surface free energies had been described previously (Sharma and Hanumantha Rao, 2002). Detailed descriptions of the various approaches can be found in the literature: equation of state approach (Neumann et al., 1974; Neumann et al., 1980), geometric mean approach (Owens and Wendt, 1969) and Lifshitz-van der Waals' acid-base approach (LW-AB) (Van Oss, 1993, 1994). In this study, the LW-AB approach was used to calculate the solid surface free energies and interfacial energies:

$$\Delta G_{abh} = \Delta G_{adh}^{LW} + \Delta G_{adh}^{AB} \quad (2)$$

$$\Delta G_{adh}^{LW} = -2\left(\sqrt{\gamma_{bv}^{LW}} - \sqrt{\gamma_{lv}^{LW}}\right)\left(\sqrt{\gamma_{mv}^{LW}} - \sqrt{\gamma_{lv}^{LW}}\right) \quad (3)$$

Table 2 — Interaction energy terms used in XDLVO approach.

Lifshitz-van der Waals (LW) interaction energy, ΔG^{LW}, mJ/m^2	
Mineral-*At.f*$_6$	$-\frac{A}{12}\left[\frac{2a(H+a)}{H(2a+H)}-\ln\left(\frac{H+2a}{H}\right)\right]\left(\frac{1}{1+1.77(2\pi H/\lambda)}\right)$
At.f$_6$-*At.f*$_6$	$-\frac{A}{12}\left[\frac{y}{x^2+xy+x}+\frac{y}{x^2+xy+x+y}+2\ln\left(\frac{x^2+xy+x}{x^2+xy+x+y}\right)\right]\left(\frac{1}{1+1.77(2\pi H/\lambda)}\right)$
Lewis acid-base (AB) interaction energy, ΔG^{AB}, mJ/m^2	
Mineral-*At.f*$_6$	$2\pi a h_0 \Delta G^{AB} \exp\left(\frac{H_0-H}{h_0}\right)$
At.f$_6$-*At.f*$_6$	$\pi a h_0 \Delta G^{AB} \exp\left(\frac{H_0-H}{h_0}\right)$
Electrostatic interaction energy (EL), ΔG^{EL}, mJ/m^2	
Mineral-*At.f*$_6$	$\pi\varepsilon_0 a(\varphi_{01}^2+\varphi_{02}^2)\left(\frac{2\varphi_{01}\varphi_{02}}{\varphi_{01}^2+\varphi_{02}^2}p+q\right)$ $p=\ln\left[\frac{1+\exp(-\kappa H)}{1-\exp(-\kappa H)}\right]$ $q=\ln[1-\exp(-2\kappa H)]$
At.f$_6$-*At.f*$_6$	$2\pi\varepsilon_0 a\varphi_{01}^2 \ln[1+\exp(-\kappa H)]$

$A = -12\pi H_0^2 G_{adh}^{LW}$; a is the radius of *At.f*$_6$, 1 µm; H is the separation distance; H_0 is the minimum separation distance between mineral and *At.f*$_6$, 6.57 Å; $x = H/(a_1 + a_2)$; $y = a_1/a_2$, 1; λ = 1,000 Å; h_0 is the correlation length of molecules in liquid, ≈ 6 Å; $k = 0.328 \times 10^{10}(I)^{1/2}m^{-1}$; φ_{01} and φ_{02} are the zeta potentials of cell and mineral, respectively, mV; ε_0 is the permittivity of the medium, 6.95×10^{-10} C^{2}J/m; I is the ionic strength, M; and κ is the double-layer thickness^{-1}.

$$\begin{aligned}\Delta G_{adh}^{AB} &= 2\left(\sqrt{\gamma_{bv}^{+}}-\sqrt{\gamma_{mv}^{+}}\right)\left(\sqrt{\gamma_{bv}^{-}}\right.\\ &\quad -2\left(\sqrt{\gamma_{bv}^{+}}-\sqrt{\gamma_{lv}^{+}}\right)\left(\sqrt{\gamma_{bv}^{-}}-\sqrt{\gamma_{lv}^{-}}\right)\\ &\quad -2\left(\sqrt{\gamma_{mv}^{+}}-\sqrt{\gamma_{lv}^{+}}\right)\left(\sqrt{\gamma_{mv}^{-}}-\sqrt{\gamma_{lv}^{-}}\right)\end{aligned} \tag{4}$$

where b, m and v indicate bacteria, mineral and vacuum.

Extended DLVO approach. Classical DLVO theory described by Verwey and Overbeek (1948) and Deryagin and Landau (1941) includes Lifshitz-van der Waals forces and the electrostatic force. Van Oss and Good (1984) later added the acid-base contribution. In the system of bioleaching of copper sulfides, bacterial adhesion is described as a balance between Lifshitz-van der Waals forces (LW), electrostatic force (EL) and acid-base interaction force (AB):

$$G_{adh}(H) = G^{EL}(H) + G^{LW}(H) + G^{AB}(H) \tag{5}$$

The mathematical formulations used for the calculations of these forces (Blessing, 1997; Hemansson, 1999; Poortinga et al., 2002; Mills et al., 1996; Oliveria, 1997) are shown in Table 2.

Surface free energy of solids and bacteria. The contact angles measured with the three liquids on djurleite, bornite, covellite and chalcopyrite, and the calculated surface free energies using the LW-AB approach are presented in Table 3. The surface energies of djurleite, bornite, covellite and chalcopyrite are 35.0, 39.6, 42.9 and 44.7 mJ/m^2, respectively. The result shows that the mineral surfaces are differently energetic. If we compare only the γ^{AB} part, the chalcopyrite surface seems more polar than the surfaces of djurleite, bornite and covellite. Chalcopyrite has higher electron-donating (γ-) part and electron-accepting (γ+) part than djurleite, bornite and covellite, so its γ^{AB} is high.

The bacterial surfaces were found to be more energetic than the mineral surfaces and hence more hydrophilic, principally due to the very high electron-donating characteristic (γ^{-} = 55.2

Table 3 — Contact angle and surface free energy data for mineral and bacterial cells.

Mineral	Contact angle, θ/(°)			Surface energy (mJ/m^2)				
	Water	Ethylene glycol	Glycerol	γ^{LW}	γ^{+}	γ^{-}	γ^{AB}	γ^{TOT}
Djurleite	75	38	60	25.6	3.0	7.4	9.4	35
Bornite	50	36	56	30.2	0.6	36.7	9.4	39.6
Covellite	38	26.5	49	33.1	0.5	48.5	9.8	42.9
Chalcopyrite	43	34	53	10.7	5.6	51.7	34	44.7
At.f$_6$	29	10	43.5	28.6	1.5	55.2	18.2	46.8

γ^{TOT}: total surface energy; γ^{LW}: van der Waals; γ^{+}: electron acceptor; γ^{-}: electron donor; γ^{AB}: acid-base.

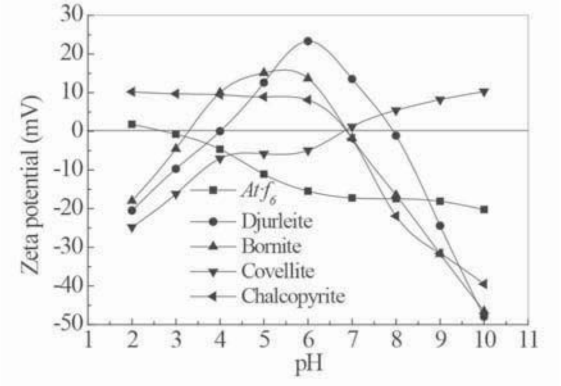

Figure 1 — Zeta potential curves of minerals and cells as a function of pH.

mJ/m^2). The surface energy of the *At.f6* is higher than those of these minerals, which is due of the fact that the γ^- component for the *At.f6* is high. But the bacteria have less γ^+ component than djurleite and chalcopyrite.

Free energy of adhesion. The free energies of bacterial adhesion on djurleite, bornite, covellite and chalcopyrite were calculated using the LW-AB approach and are shown in Table 4. The Lifshitz-van der Waals forces are attractive for the *At.f6* on djurleite, bornite and covellite. However, the van der Waals interactions between the *At.f6* and chalcopyrite are repulsive. The acid-base component is attractive for djurleite, and repulsive for bornite, covellite and chalcopyrite. The total free energy of adhesion of the *At.f6* to djurleite is negative. However, the ΔG_{adh}^{TOT} of adhesion of the *At.f6* to bornite, covellite and chalcopyrite are positive. The thermodynamic approach predicts that there will be no adhesion of the *At.f6* on bornite, covellite and chalcopyrite. This may be because the *A. ferrooxidans* strain is hydrophilic, and bacterial cells prefer to stay in the aqueous phase rather than attach themselves to these mineral surfaces. The djurleite exhibits higher acid-base attraction compared with other minerals due to the fact that djurleite has low electron-donation character (γ^-) and electron-accepting character (γ^+). The *At.f6* has very high electron-donation character (γ^-), and hence it is attracted by djurleite.

Zeta potential. The surfaces of *At.f6* cells are charged due to the presence of functional groups, such as carboxyl (-COOH), amino (-NH$_2$) and hydroxyl (-OH), originating from the cell wall components of lipopolysaccharides, lipoprotein and bacterial surface proteins (Fu, 2012). The zeta potentials of minerals and cells as a function of pH are shown in Fig.1.

The isoelectric point (IEP) characterizes the electrical properties of a mineral. Figure 1 shows that the IEP of *At.f6* cells is at pH 2.8 and there are two IEPs for djurleite and bornite: the IEPs of djurleite are at pH 4.0 and pH 7.9, and the IEPs of bornite are at pH 3.2 and pH 6.9. This may be because djurleite and bornite are easily dissolved in acidic environments and the locating ions are mainly Cu^{2+}, S^{2-} and HS$^-$. The zeta po-

Table 4 — Free energy of adhesion (mJ/m^2).

System	ΔG_{adh}^{LW}	ΔG_{adh}^{AB}	ΔG_{adh}^{TOT}
Djurleite-*At.f*$_6$	–0.53	–2.03	–2.56
Bornite-*At.f*$_6$	–2.62	28.06	25.44
Covellite-*At.f*$_6$	–1.47	35.3	33.83
Chalcopyrite-*At.f*$_6$	1.90	29.15	31.05

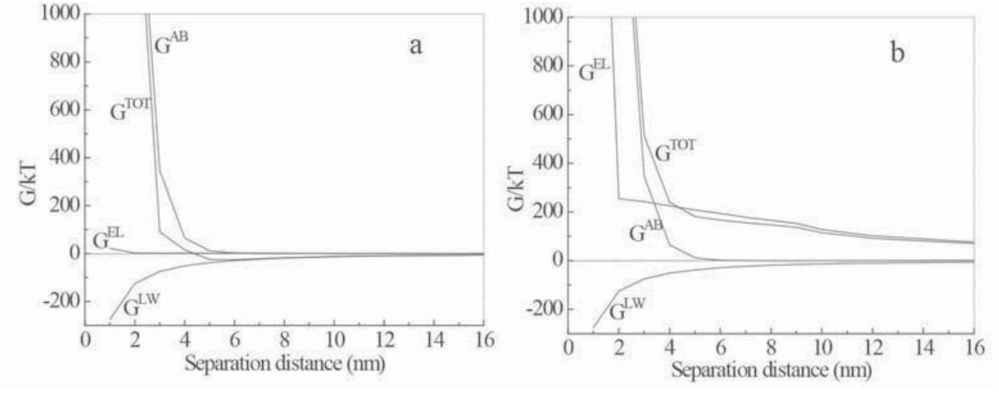

Figure 2 — Free energy versus separation distance curves for *At.f6-At.f6* system at (a) pH = 2 and (b) pH = 10, when I = 0.001 M.

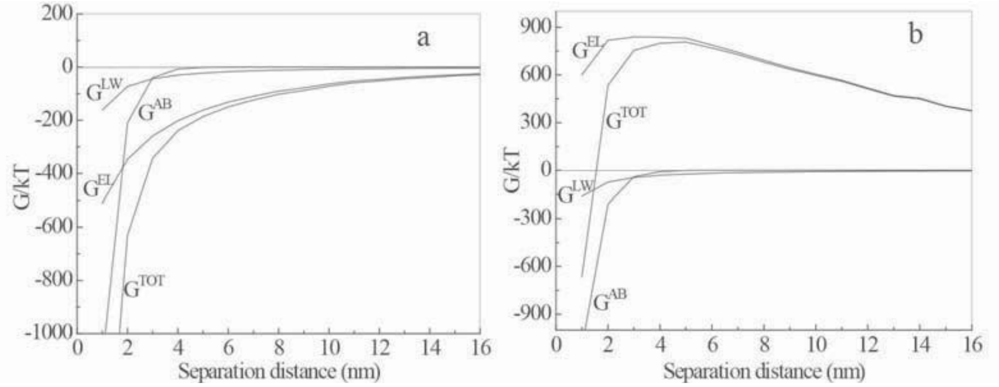

Figure 3 — Free energy versus separation distance curves for djurleite-*At.f6* system at (a) pH = 2 and (b) pH = 10, when I = 0.001 M.

tentials of djurleite and bornite in acidic environments can be calculated using the equation:

$$\varphi_0 = 0.059/Z(pM_{PZC} - pM),\ V$$

where φ_0 is the zeta potential, Z is the ion valence and M is locating cationic activity. The mineral dissolution gradually enhances with decreasing pH, resulting in the increase of pM and thus the decrease of φ_0. When $pM > pM_{PZC}$, $\varphi_0 < 0$. The locating ions of djurleite and bornite in the alkaline environment are mainly H^+ and OH^-, and the zeta potentials can be calculated using the equation:

$$\varphi_0 = 0.059/Z(pH_{PZC} - pH),\ V$$

where pH_{PZC} is the point of zero charge of the minerals. When $pH > pH_{PZC}$, $\varphi_0 < 0$.

Chalcopyrite has an IEP at pH 6.9, while the IEP of covellite is at pH 6.8. The zeta potential is negative for pH values smaller than the IEP value, and positive for pH values larger than the IEP value.

DLVO approach. *At.f6-At.f6 system.* The total interaction is the sum of van der Waals interactions, electrostatic interactions and acid-base interactions between the bacterial cells themselves. Van der Waals interactions, electrostatic interactions, acid-base interactions and the total interaction between bacterial cells themselves as a function of separation distance (H) at I = 0.001 M are shown in Fig. 2. The ΔG_{adh}^{LW}, ΔG_{adh}^{AB} and ΔG_{adh}^{TOT} of bacteria-bacteria are –0.92 mJ/m^2, 36.41 mJ/m^2 and 35.49 mJ/m^2, respectively.

Figure 2a shows that the total interaction energy, G_{TOT}, is positive when the separation distance H < 4.2 nm, indicating that the repulsive forces dominate. When H > 4.2 nm, G_{TOT} is negative, indicating that the adhesion between bacterial cells themselves is thermodynamically preferred. This means that aggregation of bacterial cells in the aqueous phase in the acidic pH region is impossible. Figure 2b shows G_{TOT} is positive in the alkaline pH region, indicating that the repulsive forces dominate interactions between bacterial cells. Although the van der Waals interactions G^{LW} is attractive, the acid-base interactions and electrostatic interactions are highly repulsive. The reason for this repulsion is the fact that bacterial cells are hydrophilic and hence they prefer to stay in the aqueous phase rather than form flocs (Sharma and Hanumantha Rao, 2003).

Mineral-At.f6 system. The total interaction, van der Waals interactions, electrostatic interactions and acid-base interactions versus separation distance curves of djurleite, bornite,

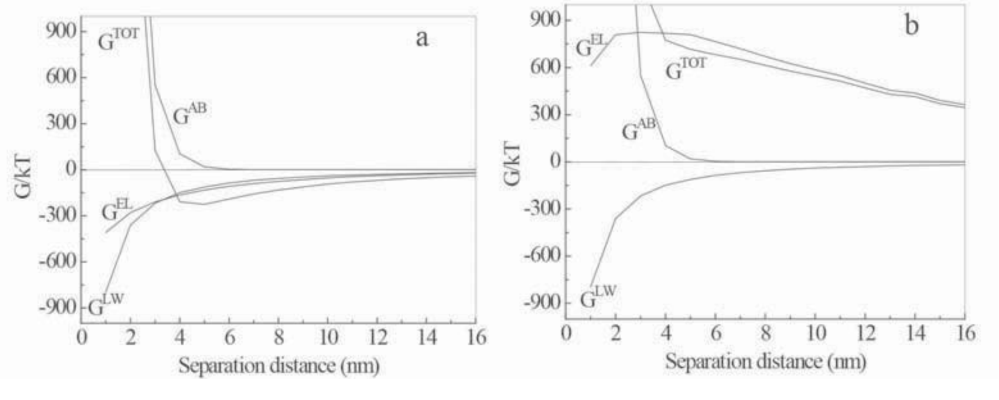

Figure 4 — Free energy versus separation distance curves for bornite-*At.f6* system at (a) pH = 2 and (b) pH = 10 when I = 0.001 M.

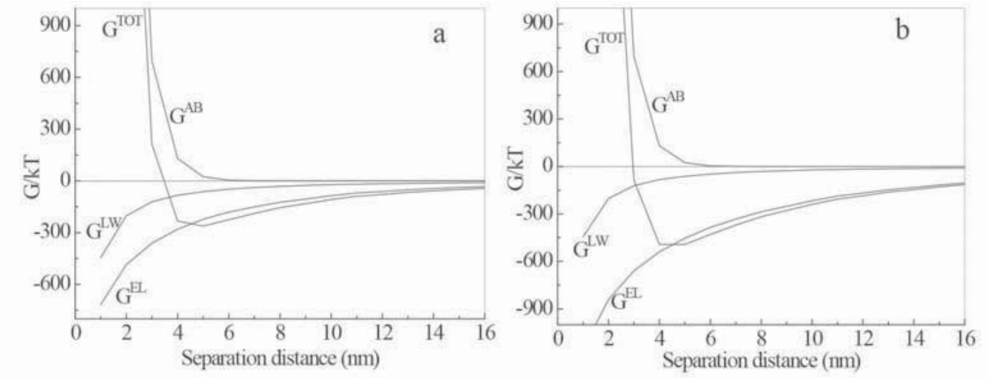

Figure 5 — Free energy versus separation distance curves for covellite-*At.f6* system at (a) pH = 2 and (b) pH = 10, when I = 0.001 M.

covellite and chalcopyrite interactions with *At.f6* are shown in Figs. 3, 4, 5 and 6, respectively.

Figure 3a shows that the total interaction energy, van der Waals interactions, electrostatic interactions and acid-base interactions between djurleite and *At.f6* at pH = 2 are attractive. The number of cells adsorbed on djurleite increases. The primary contribution to the total energy comes from the electrostatic energy G^{EL}, followed by the Lifshitz-van der Waals energy G^{LW} and the acid-base energy G^{AB}. The acid-base interaction is relatively short-range, and the interacting surfaces must approach closely (less than 5 nm) before these forces become operative (Hermansson, 1999). At pH = 2, the bacterial cells and mineral particles are oppositely charged, and the adhesion of bacterial cells to the mineral surfaces is possible. At pH = 10 (Fig. 3b), both djurleite and *At.f6* are negatively charged and hence electrostatic interaction is repulsive. Figure 3b shows the Lifshitz-van der Waals energy G^{LW} and the acid-base energy G^{AB} are attractive. In the basic pH range, when $H < 1.5$ nm, G^{TOT} is negative, indicating that the attractive force dominates interaction between djurleite and bacterial cells and the number of adsorbed cells increases. However, when $H > 1.5$ nm, G^{TOT} is positive. There is a maximum value $G^{TOT}{}_{max}$ at H = 5 nm, indicating that bacterial cells could adsorb onto the surface of djurleite when the kinetic energy of *At.f6* is larger than $G^{TOT}{}_{max}$.

The free energy versus separation distance (H) curves of bornite-*At.f6* and covellite-*At.f6* are shown in Figs. 4 and 5, respectively. Figures 4a and 5a show that the G^{LW} and G^{EL} of bornite-*At.f6* and covellite-*At.f6* are attractive, but the G^{AB} of both is repulsive. The total interaction energy G^{TOT} is negative at $H > 3.0$ nm and positive at $H < 3.0$ nm. This means that the *At.f6* cells can approach, then adsorb on the surfaces of bornite and covellite, but the adhesion strengths of the *At.f6* to the surfaces of the two minerals are weak. However, the free energy versus separation distance (H) curves of covellite interaction with *At.f6* at pH = 2 are similar to those at pH = 10, indicating that the interaction between covellite and cells in the acidic pH range agrees with that in the basic pH range. But the interaction between bornite and *At.f6* in the acidic pH range is different from that in the basic pH range. G^{TOT} is positive at pH = 10, showing that the repulsive forces dominate the interaction between bornite and the *At.f6*, and the adhesion of bacterial cells to bornite decreases.

Figure 6 shows that the G^{TOT} between chalcopyrite and bacterial cells is always positive at any given pH values, predicting that the repulsive forces dominate and hinder the adsorption process. The reason for this repulsion is the fact that both G^{LW} and G^{AB} are repulsive.

Adsorption experiment. The adsorption of the *At.f6* on the different copper sulfides as a function of the conditioning time at pH = 2.0 is shown in Fig.7.

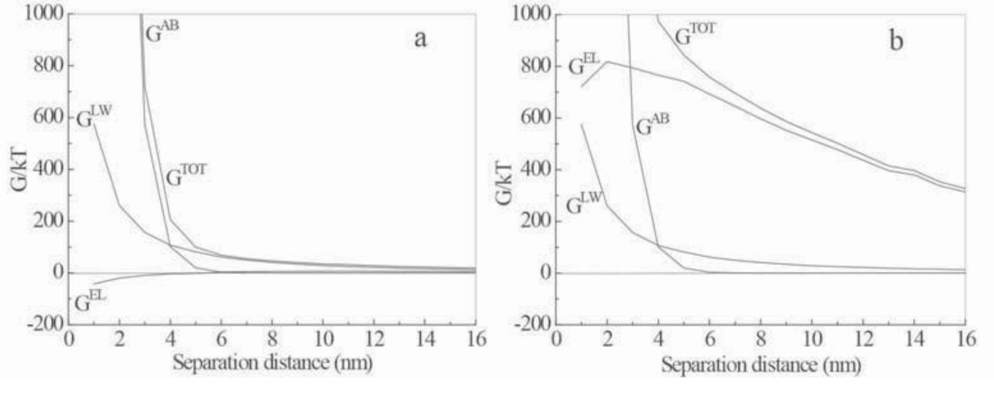

Figure 6 —Free energy versus separation distance curves for chalcopyrite-*At.f6* system at (a) pH = 2 and (b) pH = 10, when I = 0.001 M.

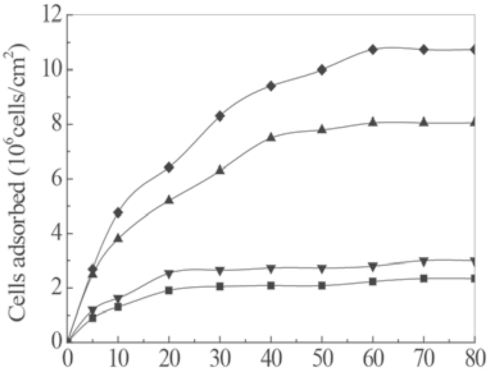

Figure 7 —Adsorption of *At.f6* on djurleite (□), bornite (▲), covellite (▼) and chalcopyrite (●).

Figure 7 shows the number of adsorbed cells increases for up to 60 minutes before saturation in the cases of djurleite and bornite, and 70 minutes before saturation for covellite and chalcopyrite. As for the four copper sulfides, the cell density on the surface of djurleite is higher than those on the surfaces of bornite, covellite and chalcopyrite. After conditioning for 80 minutes, the cell densities on the surfaces of djurleite, bornite, covellite and chalcopyrite are 10.74 × 10^6, 8.06 × 10^6, 3 × 10^6, 4.24 × 10^6 and 2.35 × 10^6 cells/cm^2, respectively. The order of adsorption of cells on copper sulfides is: djurleite > bornite > covellite > chalcopyrite.

Discussion

The thermodynamic approach predicts that there will be no attachment of bacterial cells to the mineral surfaces (Sharma and Hanumantha Rao, 2003). However, the total free energy of adhesion of the *At.f6* to djurleite is negative, indicating that the thermodynamic approach can predict the adhesion of the *At.f6* to djurleite. The reason behind this is the strong acid-base attraction and van der Waals attraction between djurleite and the *At.f6*. The thermodynamic approach predicts that there will be no attachment of *At.f6* to the surfaces of bornite, covellite, and chalcopyrite. The results are in good agreement with those reported by Sharma and Hanumantha Rao (2003). For bornite and covellite, the strong acid-base repulsion outweighs the van der Waals attraction. The other reason may be the fact that electrostatic interactions are not considered at all in the thermodynamic approach.

The DLVO approach predicts the possibility of flocs of the individual bacterial cells, and attachment of the *At.f6* to the surfaces of djurleite, bornite and covellite, and repulsion between chalcopyrite and the *At.f6*. However, the adsorption experiment shows the attachment of the *At.f6* bacterial cells to these minerals. The attachment of bacterial cells to chalcopyrite is not predicted by the DLVO approach or thermodynamic approach. However, the DLVO approach can explain the selective adhesion of bacterial cells to the surfaces of different copper sulfides.

Conclusions

Our adsorption experiment showed that, after conditioning for 80 minutes, the cell densities on the surfaces of djurleite, bornite, covellite and chalcopyrite were 10.74 × 10^6, 8.06 × 10^6, 3 × 10^6, 4.24 × 10^6 and 2.35 × 10^6 cells/cm^2, respectively. The order was: djurleite > bornite > covellite > chalcopyrite.

The thermodynamic approach predicts that there will be no attachment of the $At.f_6$ to the surfaces of bornite, covellite and chalcopyrite. For bornite and covellite, the strong acid-base repulsion outweighs the van der Waals attraction. The other reason may be the fact that electrostatic interactions are not considered at all in the thermodynamic approach. The DLVO approach predicts the possibility of flocs of the individual bacterial cells, and attachment of the $At.f_6$ to the surfaces of djurleite, bornite and covellite, and repulsion between chalcopyrite and the $At.f_6$. The attachment of bacterial cells to chalcopyrite is not predicted by the DLVO approach or thermodynamic approach. The DLVO approach can partially explain the selective adhesion of bacterial cells to the surfaces of different copper sulfides.

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References

Akcil, A. and Deveci, H., 2010, "Mineral biotechnology of sulphides," *Geomicrobiology*, S. Jain, A. Khan, and M.K. Rai, eds., Science Publishers, Enfield, NH, USA, pp. 101-137.

Behera, S.K., Panda, P.P., Singh, S., Pradhan, N., Sukla, L.B., and Mishra, B.K., 2011, "Study on reaction mechanism of bioleaching of nickel and cobalt from lateritic chromite overburdens," *International Biodeterioration & Biodegradation*, Vol. 65, No. 7, pp. 1035-1042.

Blessing, N.A.M., 1997, "Theory of hydration forces between surface," Langmuir, Vol. 13, No. 3, pp. 2113-2124.

Bosecker, K., 1997, "Bioleaching: metal solubilization by microorganisms," *FEMS Microbiology Reviews*, Vol. 20, No. 3-4, pp. 591-604.

Bos, R., Van, D.M.H.C., and Busscher, H.J., 1999, "Physico-chemistry of initial microbial adhesive interactions-its mechanisms and methods for study," *FEMS Microbiology Reviews*, Vol. 23, No. 2, pp. 179-230.

Brierley, J.A., and Brierley, C.L., 2001, "Present and future commercial applications of biohydrometallurgy," *Hydrometallurgy*, Vol. 59, No. 2-3, pp. 233-239.

Busscher, H.J., Norde, W., Sharma, P.K., and Van der Mei, H.C., 2010, "Interfacial re-arrangement in initial microbial adhesion to surfaces," *Current Opinion in Colloid & Interface Science*, Vol. 15, No. 6, pp. 510-517.

Chen, M.L., Zhang, L., Gu, G.H., Hu, Y.H., and Su, L.J., 2008, "Effects of microorganisms on the surface properties of chalcopyrite and bioleaching," *Transactions of Nonferrous Metals Society of China*, Vol. 18, No. 6, pp. 1421-1426.

Deryagin, B.V., and Landau, L.D., 1941, "Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes," *Acta Physicochim*, Vol. 14, pp. 633-662.

Dew, D.W., Van, B.C., Mcewan, K., and Bowker, C., 1999, "Bioleaching of base metal sulphide concentrates: A comparison of mesophile and thermopile bacterial cultures," *Biohydrometallurgy and environment toward the mining* *of 21st century, part A*, R. Amils and A. Ballester, eds., Amsterdam, Netherlands, Elsevier, pp. 229-238.

Ehrlich, H.L., 2004, "Beginnings of rational bioleaching and highlights in the development of biohydrometallurgy: A brief history," *The European Journal of Mineral Processing and Environmental Protection*, Vol. 4, No. 2, pp. 102-112.

Farahat, M., Hirajima, T., Sasaki, K., and Doi, K., 2009, "Adhesion of Escherichia coli onto quartz, hematite and corundum: Extended DLVO theory and flotation behavior," *Colloids and Surfaces B: Biointerfaces*, Vol. 74, No. 1, pp. 140-149.

Farahat, M., Hirajima, T., and Sasaki, K., 2010, "Adhesion of Ferroplasma acidiphilum onto pyrite calculated from the extended DLVO theory using the van Oss-Good-Chaudhury approach," *Journal of Colloid and Interface Science*, Vol. 349, No. 2, pp. 594-601.

Fu, K.B., 2012, "Study on Law and Mechanism of Copper Sulphides Bioleaching," Ph.D Thesis, University of Science and Technology Beijing, Beijing, China.

Fu, K.B., Lin, H., Cheng, H., Mo, X.L., and Dong, Y.B., 2013, "Bioleaching of djurleite using Acidithiobacillus ferrooxidans," *Minerals Engineering*, Vol. 40, pp. 38-41.

Fu, K.B., Lin, H., Mo, X.L., Dong, Y.B., and Zhou, L., 2011, "Study on bioleaching of different types of chalcopyrites," *Journal of University of Science and Technology Beijing*, Vol. 33, No. 7, pp. 806-811.

Harneit, K., Göksel, A., Kock, D., Klock, J.H., Gehrke, T., and Sand, W., 2006, "Adhesion to metal sulfide surfaces by cells of Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans and Leptospirillum ferrooxidans," *Hydrometallurgy*, Vol. 83, No. 1-4, pp. 245-254.

Hermansson, M., 1999, "The DLVO theory in microbial adhesion," *Colloids and Surfaces B: Biointerfaces*, Vol. 14, No. 1-4, pp. 105-119.

Jia, C.Y., Wei, D.Z., Liu, W.G., Han, C., Gao, S.L., and Wang, Y.J., 2008, "Selective adsorption of bacteria on sulfide minerals surface," *Trans. Nonferrous Met. Soc. China*, Vol. 18, No. 5, pp. 1247-1252.

Mills, A.L., Powelson, D.K., and Fletcher, M., 1996, "Bacterial adhesion: molecular and ecological diversity," *Surface Science Reports*, Vol. 37, No. 8, pp. 25-40.

Neumann, A.W., Good, R.J., Hope, and C.J., Sejpal, M., 1974, "An equation-of-state approach to determine surface tensions of low energy solids from contact angles," *Journal of Colloid and Interface Science*, Vol. 49, No. 2, pp. 291-304.

Neumann, A.W., Hum, O.S., Francis, D.W., Zingg, W., Van Oss, and C.J., 1980, "Kinetic and thermodynamic aspects of platelet adhesion from suspension to various substrates," *Journal of Biomedical Materials Research*, Vol. 14, No. 4, pp. 499-509.

Oliveria, R., 1997, "Understanding adhesion: a means for preventing fouling," *Therm Fluid Science*, Vol. 15, No. 5, pp. 16-322.

Owens, D.K. and Wendt, R.C., 1969, "Estimation of the surface free energy of polymers," *Journal of Applied Polymer Science*, Vol. 13, No. 8, pp. 1741-1747.

Poortinga, A.T., Bos, R., Norde, W., Busscher, and H.J., 2002, "Electric double layer interactions in bacterial adhesion to surfaces," *Surface Science Reports*, Vol. 38, No. 5, pp. 1-32.

Pradhan, N., Nathsarma, K.C., Srinivasa Rao, K., Sukla, L.B., and Mishra, B.K, 2008, "Heap bioleaching of chalcopyrite: A review," *Minerals Engineering*, Vol. 21, No. 5, pp. 355-365.

Sharma, P.K., 1999, "Physico-chemical Characterization of Microbial Cell Surface and Bioflotation of Sulfide Minerals," Licentiate Thesis, Luleå University of Technology.

Sharma, P.K., and Hanumantha Rao, K., 2003, "Adhesion of Paenibacillus polymyxa on chalcopyrite and pyrite: surface thermodynamics and extended DLVO theory," *Colloids and Surfaces B: Biointerfaces*, Vol. 29, No. 1, pp. 21-38.

Sharma, P.K. and Hanumantha Rao, K., 2002, "Analysis of different approaches for evaluation of surface energy of microbial cells by contact angle goniometry," *Advances in Colloid and Interface Science*, Vol. 98, No. 3, pp. 341-463.

Subramanian, S., Santhiya, D., and Natarajan, K.A., 2003, "Surface modification studies on sulphide minerals using bioreagents," *International Journal of Mineral Processing*, Vol. 72, No. 1-4, pp. 175-188.

Van Oss, C.J., 1993, "Acid-base interfacial interactions in aqueous media," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, Vol. 78, pp. 1-49.

Van Oss, C.J., 1994, *Interfacial Forces in Aqueous Media*, Marcel Dekker, New York.

Van Oss, C.J., and Good, R.J., 1984, "The equilibrium distance between two bodies immersed in a liquid," *Colloids and Surfaces*, Vol. 8, No. 4, pp. 373-381.

Verwey, E.J.W., and Overbeek, J.T.H.G., 1948, *Theory of the Stability of Lyophobic Colloids*, Leiden, Netherlands: Drukkerij, Luctor et Emergo.

Watling, H.R., 2006, "The bioleaching of sulphide minerals with emphasis on copper sulphides-A review," *Hydrometallurgy*, Vol. 84, No. 1-2, pp. 81-108.