Leaching of Copper from Furnace Dust by Pure and Mixed Culture of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*

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The relationship between bacterial adsorption and leaching of copper from copper-containing furnace dust has been investigated in the culture systems of *T. ferrooxidans* (*Thiobacillus ferrooxidans*), *T. thiooxidans* (*Thiobacillus thiooxidans*) and their mixture. Maximum adsorption amounts of *T. ferrooxidans* and *T. thiooxidans* were 1.39×10^{11} cells/g and 1.08×10^{11}, respectively while maximum adsorption amount of mixed culture was 2.06×10^{11} cells/g which is close to the sum of that of each bacterial component. These indicate that the two bacterial species have almost independent preferential adsorption sites on furnace dust except ca. 17% of competitive sites. However, the efficiency of leaching was not linearly related to the ratio of adsorption in three different systems even though it was increased in parallel with the bacterial adsorption within the same bacterial system.

Key Words : Adsorption, Copper, Leaching, *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*

Introduction

Microbial leaching processes are useful to recover precious metals such as copper, gold, and uranium from their ores or to leach out some environmentally hazardous heavy metals. *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* are frequently utilized to solubilize Cu^{2+} from copper-containing sulfide ores.[1-4] *T. ferrooxidans* is able to oxidize metallic sulfides to sulfate directly via direct attack of substrates or indirectly via oxidation of ferrous ion to ferric ion in solution.[5-7] *T. thiooxidans* attacks sulfur phase of some of the sulfide ores and oxidizes to sulfate but it can not oxidize ferrous ion to ferric ion. However, it is able to leach insoluble covellite in the presence of iron.[8]

Even though *T. ferrooxidans* and *T. thiooxidans* differ in their capabilities of adsorptions and in their bioleaching actions, they often naturally occur together in acid mines. Dugan & Apel reported that naturally occurring mixed cultures containing both of *T. ferrooxidans* and *T. thiooxidans* had shown to be highly effective in the catalysis of pyrite dissolution.[9] However, Donati *et al.* reported that the mixed cultures never leached better than the most efficient pure culture in covellite dissolution.[10] Shrihari *et al.* reported that the adsorptions of *T. ferrooxidans* cells on substrate played a significant role in leaching of chalcopyrite mineral.[11] Based on the direct mechanism, bacterial attachment on substrate plays an important role in bioleaching.

In this study we investigated the leaching of copper from a copper-containing furnace dust by pure and mixed cultures of *T. ferrooxidans* and *T. thiooxidans* in order to examine the possibility to recover copper. We compared the leaching efficiency of copper by pure cultures of *T. ferrooxidans* and *T. thiooxidans* with that of their mixed cultures in relation to the bacterial adsorption onto the substrates.

Experimental Section

Substrates. A furnace metallic waste produced from an industrial Cu-scrap melting process was used as a substrate for bacterial adsorption. The main elements in the furnace dust were analyzed with using atomic absorption spectrophotometer (Varian spectra AA-800, USA) and elemental analysis (Fisions EA 1110 CHNS-O, Italy). It contains C (24.4%), Cu (9.57%), Pb (8.24%), Sn (6.17%), S (2.55%), Cd (0.37%) and Fe (0.18%). Other components could be oxide and hydrate forms of compounds and minor elements.

Microorganism. *T. ferrooxidans* (KCTC 2677) and *T. thiooxidans* (KCTC 2505) were obtained from the Korean Collection for Type Cultures. The culture of *T. ferrooxidans* was inoculated in an aqueous solution containing 3.0 g of $(NH_4)_2SO_4$, 0.1 g of KCl, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 10.0 mg of $Ca(NO_3)_2$, 1.0 mL of 5 M H_2SO_4 and 44 g of $FeSO_4 \cdot 7H_2O$ per liter. An aqueous solution containing 0.1 g of NH_4Cl, 3.0 g of K_2HPO_4, 0.1 g of $MgCl_2 \cdot 6H_2O$, 0.14 g of $CaCl_2 \cdot 2H_2O$ and 10 g of sulfur powder per liter was used for *T. thiooxidans*.

After bacterial growth had reached the exponential phase, the cultures were centrifuged at 1700G for 10 min to remove insoluble ferric compounds from the culture solutions. The *T. ferrooxidans* and *T. thiooxidans* supernatant were passed through a membrane filter (pore size 0.45 μm) to collect the cells, respectively. Then the cells were washed twice with an aqueous sulfuric acid solution (pH 2.0) and twice with a fresh medium solution same as growth medium but only devoid of iron for *T. ferrooxidans* and only devoid of sulfur for *T. thiooxidans*, respectively. Then, the *T. ferrooxidans* and *T. thiooxidans* cells were suspended in an iron-free growth medium and a sulfur-free growth medium as described above, respectively.

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Bacterial adsorption. 0.10 g of substrate, a furnace dust, was incubated with 10 mL of bacterial suspension (7×10^7 - 2×10^9 cells·mL^{-1}) in a glass flask under controlled agitation at 200 rpm for 10 min to reach equilibrium. The number of cells remained free in the supernatant was then determined by direct counting of 0.1 mm depth and 1/400 mm^2 area with using a hemocytometer.

Bioleaching. Bioleaching experiments of a copper-containing furnace dust were carried out in agitated flasks. The experiments were performed in 250 mL volumetric flasks with 100 mL of bacteria suspension (1×10^7 cells·mL^{-1}) and 10% (wt/v) furnace dust. Base on our previous work, bacterial adsorptions of *T. ferrooxidans* and *T. thiooxidans* on the furnace dust were highest at 35 °C.[13] Thus, the samples were incubated at 35 °C and pH 2.0 in a shaker at 200 rpm. Small portions of the samples were withdrawn at every 24 hrs from the flasks for analysis. Sterilized blank solutions were employed to determine the metal extraction from furnace dust by acid attack.

Results and Discussion

Adsorption equilibrium. The adsorption behavior of pure and mixed culture of *T. ferrooxidans* and *T. thiooxidans* were examined since bacterial attachment to substrates has been considered as an important step in bioleaching processes. In order to obtain a quantitative analysis of bacterial adsorption, the adsorption parameter values were determined using the Langmuir adsorption isotherm equation.[12] The adsorption of bacteria can be described as follows:

$$X_A = K_A X_{Am} X_L / (1 + K_A X_L) \qquad (1)$$

where X_A and X_{Am} are the adsorption amount and maximum adsorption amount per unit mass of furnace dust, respec-

tively. X_L is the concentration of free cells in liquid phase at adsorption equilibrium and K_A is the adsorption equilibrium constant. Figure 1 shows the adsorption of *T. ferrooxidans*, *T. thiooxidans* and the mixed culture of them on furnace dust at 35 °C. We reported in our previous paper that the degree of adsorption of *T. ferrooxidans*, *T. thiooxidans* and their mixed culture increased with increasing temperature from 5 °C up to 35 °C which was the optimum temperature for growth of these bacteria.[13] Equation (1) can be rearranged as follows:

$$X_L / X_A = X_L / X_{Am} + 1 / X_{Am} K_A \qquad (2)$$

K_A and X_{Am} were determined from the plots of X_L / X_A against X_L using a least-square analysis. The plot of X_L / X_A versus X_L resulted in fairly good straight line as shown in Figure 2, which indicates that the adsorptions of *T. ferrooxidans*, *T. thiooxidans* and their mixture on the furnace dust well obey the Langmuir isotherm, as a similar result observed by Konishi *et al.*[14] The K_A values, an intensive property as a measure of adsorption affinity, were 4.45×10^{-9} for *T. ferrooxidans*, 3.30×10^{-9} for *T. thiooxidans* and 14.91×10^{-9} mL/cell for the mixture. X_{Am} is proportional to the number of adsorption sites per unit surface and the bacteria accessible specific surface area of adsorbent. The X_{Am} values were 1.39×10^{11} for *T. ferrooxidans*, 1.08×10^{11} for *T. thiooxidans* and 2.06×10^{11} cells/g for the mixture. The X_{Am} value of the mixed culture is much larger than that of each pure bacteria, but it is slightly smaller than the sum of that of each bacterial component. These results indicate that the adsorption sites of *T. thiooxidans* and *T. ferrooxidans* in furnace dust particles are mostly composed of independent selective sites even though some competitive sites may exist. Berry *et al.* studied the adsorption of *T. ferrooxidans* to low-grade copper waste ore and reported that *T. ferrooxidans* had preferential
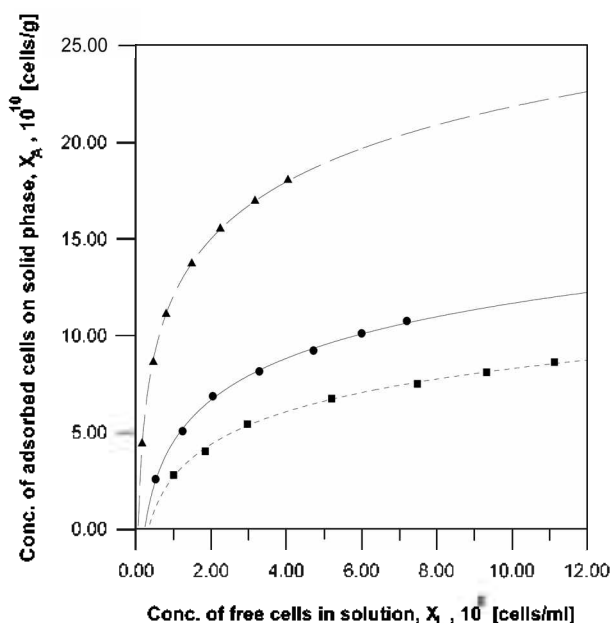

Figure 1. Equilibrium adsorption isotherm of *T. thiooxidans* (■), *T. ferrooxidans* (●) and their mixed culture (▲) on furnace dust at 35 °C.
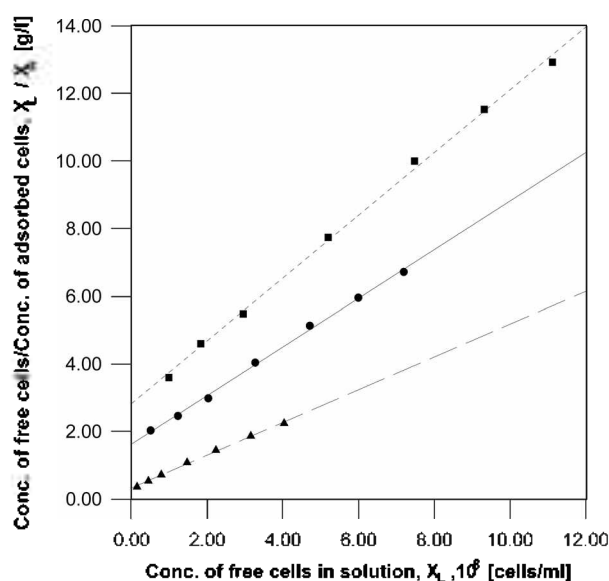

Figure 2. Langmuir plots for the adsorption isotherm of *T. thiooxidans* (■), *T. ferrooxidans* (●) and their mixture (▲) on furnace dust at 35 °C.
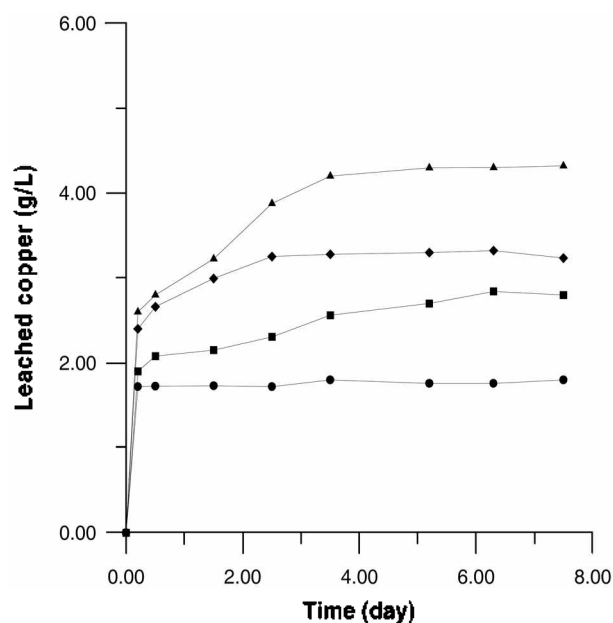
Figure 3. Copper extraction from furnace dust by *T. thiooxidans* (■), *T. ferrooxidans* (●), mixed culture (◆) and sterile system (▲). The concentration of copper in solution was analyzed with using atomic absorption spectrophotometer.

adsorption sites.[15]

Microbial leaching. Figure 3 shows the concentration changes of copper released from a furnace dust by pure culture of *T. ferrooxidans* or *T. thiooxidans* and mixed culture of them. The highest copper extraction was obtained by *T. ferrooxidans* and the lowest by *T. thiooxidans* but somewhat higher than that of sterile system. Curutchet *et al.* reported that *T. thiooxidans* is able to oxidize soluble sulfides, but it is not able to oxidize insoluble sulfides such as CuS without iron.[8] It is consistent with our result that shows small amount of copper released from the relatively insoluble furnace dust by *T. thiooxidans*. However, the copper extraction by *T. thiooxidans* is much larger than that expected from the results of Curutchet *et al.*, which may caused by small amount of iron (Fe/Cu =1.9%) contained in the furnace dust that can catalyze the copper leaching by *T. thiooxidans*.[8] The highest copper extraction by *T. ferro-oxidans* may be involved in both of direct copper dissolution by adsorbed *T. ferrooxidans* and of indirect copper oxidation by ferric ions produced by *T. ferrooxidans* oxidation of ferrous ions. Even though the mixed culture showed the highest adsorption, the amount of copper extraction was lower than that by *T. ferrooxidans* but somewhat higher than that by *T. thiooxidans*. It might be caused by the *T. thiooxidans* occupation at some competitive sites that *T. ferrooxidans* can also be adsorbed. Donati *et al.* reported similar results that a higher level of covellite dissolution was observed in a system containing *T. ferrooxidans* than that in a system containing mixed culture of *T. ferrooxidans* and *T. thiooxidans* without iron and sulfur.[10] However, Qiu *et al.* reported that the bioleaching efficiency of chalcopyrite with Fe^{2+} and sulfur was higher in mixed culture of *T. ferro-*
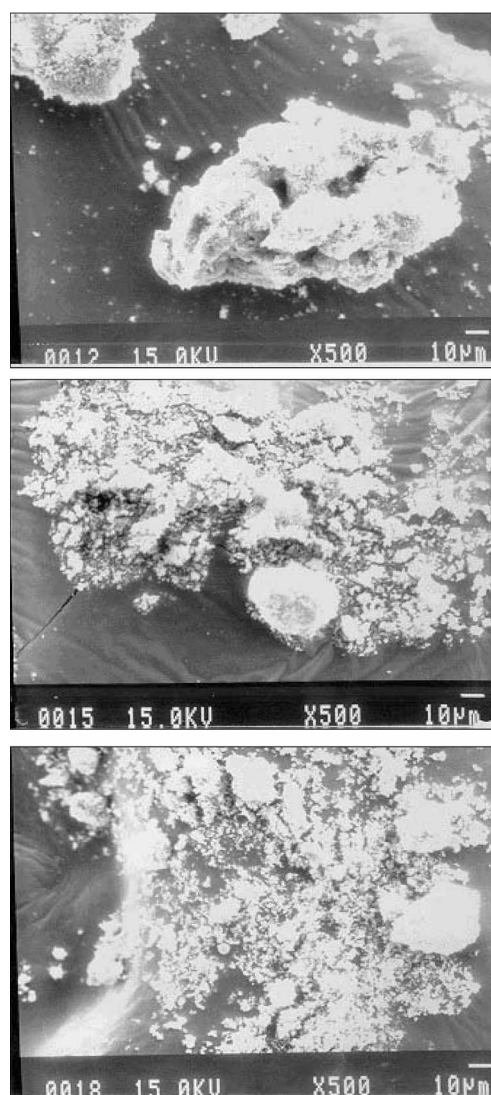

Figure 4. Scanning electron micrographs of furnace dusts at various degrees of bioleaching by mixed culture after 10 min (top), 3 days (middle) and 7 days (bottom).

oxidans and *T. thiooxidans* than in each of their pure culture.[16] This discrepancy between researchers can be ascribed to the difference of components of their systems such as substrate, pulp density, relative amount of Fe^{2+} and sulfur, *etc.*

Figure 4 shows scanning electron micrographs of furnace dust at various degrees of bioleaching by mixed culture after 10 min (top), 3 days (middle) and 7 days (bottom). As bio-leaching is progressed the substrate particles are disinte-grated. However, leaching is not much proceeded after 4 days. The relatively fast leaching rate might be partly attributed to the brittleness of the furnace dust. In order to get information of size effect on bacterial adsorption and copper extraction, the substrate particles were sieved into three fractions according to particle size as follows: A ($D_p <$ 45 μm), B (45 μm $< D_p <$ 74 μm), C (75 μm $< D_p <$149 mm), where D_p is the diameter of particle. The X_{Am} values mixed cultures were 2.06×10^{11}, 1.78×10^{11} and 1.58×10^{11}

cells/g for A, B and C, respectively. And the K_A values were 14.91 × 10^{-9}, 8.61 × 10^{-9} and 5.82 × 10^{-9} mL/cell for A, B and C, respectively. As the particle size of furnace dust decreased, the values of X_{Am} and K_A increased because of increase in available specific surface area for the bacterial adsorption, and copper extraction also increased parallel with bacterial adsorption.

Conclusions

A linear relationship between the ratio of adsorption and the efficiency of bioleaching was not observed in three different bacterial culture systems of *T. ferrooxidans*, *T. thiooxidans* and their mixed culture. Maximum adsorption amounts of mixed culture of *T. ferrooxidans* and *T. thiooxidans* was close to the sum of that of each bacterial component. These indicate that the two bacterial species have almost independent preferential adsorption sites on furnace dust except *ca.* 17% of competitive sites. However, the efficiency of leaching was not linearly related to the ratio of adsorption in three different systems even though it was increased in parallel with the bacterial adsorption within the same bacterial system. As decreased in substrate particle size, the amount of bacterial adsorption and the apparent rate of copper extraction increased linearly. The amount of copper extraction from furnace dust increased as the adsorption amount increased in the same bacterial system. We concluded that *T. ferrooxidans* was the most efficient in copper leaching from copper-containing furnace dust with no or relatively small amount of iron and sulfur even though the maximum amount of bacterial adsorption was the highest in mixed culture of *T. ferrooxidans* and *T. thio-*

oxidans. However, the bioleaching efficiency of copper appears to depend on many components of the systems such as substrate property, pulp density, relative amount of Fe^{2+} and sulfur, *etc*. Our future work will focus on the influence of these factors on our system.

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