Influence of solid content on bioleaching of heavy metals from contaminated sediment by *Thiobacillus* spp

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Abstract: The bioleaching process is one of the promising methods for removing heavy metals from contaminated sediments. In this study the effects of sediment solid content on the performance of the bioleaching process using a mixed culture of two sulfur-oxidizing bacteria were investigated. The results showed that the rate of pH reduction decreased with increasing sediment solid content because of the buffering capacity of sediment solids. It was found that there was a linear relationship between buffering capacity and sediment solid content. For different solid contents (10–100 g dm^{-3}), 82–95% (w/w) of Cu; 58–70% (w/w) of Zn; 55–73% (w/w) of Mn; 33–72% (w/w) of Pb; 35–65% (w/w) of Ni and 9–20% (w/w) of Cr were leached from sediments in this bioleaching process. The rate of metal leaching was found to decrease with an increase in sediment solid content. The solubilization of heavy metal from sediments was well described by a solid content-related kinetic equation.

Keywords: bioleaching; contaminated sediment; heavy metals; *Thiobacillus* spp

NOTATION

β	Buffering capacity (mmol H^{+} dm^{-3})
k	Rate constant of kinetic equation (d^{-1})
k_s	Coefficient of rate, related to solid content (g dm^{-3} d^{-1})
M	Weight of metal in the aqueous phase (mg)
M_s	Initial weight of metal in the sediment (mg)
S	Sediment solid content (g dm^{-3})

1 INTRODUCTION

In natural water systems, bottom sediments play an important role both as a sink where contaminants can concentrate and as a source of these contaminants to the overlying water and to biota.[1] The management of contaminated sediments has become an issue of critical importance to government agencies responsible for managing waterways and for the people living adjacent to such waterways.[2] Sediments dredged from contaminated rivers often contain substantial amounts of heavy metals and hence cannot be disposed of on the land and in waterbodies without treatment.

Bosecker[3] applied a microbial leaching process to recover metals and detoxify industrial wastes. This biotechnological method is able to effectively extract heavy metals from some non-sulfide industrial wastes. Blais *et al*[4] demonstrated that microbial leaching processes can solubilize heavy metals from sewage sludges very rapidly. In addition, Tyagi *et al*[5] developed a conceptual model for predicting metal solubilization from sewage sludge in the bioleaching process. The primary benefits of bioleaching process are less labor and energy requirements, lower capital investment, and ease of application. Moreover, the adverse effects on the environment are considerably reduced.[6,7] Therefore, it is possible that microbial leaching with sulfur-oxidizing bacteria may be one of the promising methods for removing heavy metals from contaminated aquatic sediments.

Thiobacillus spp that are known to play a role in microbial leaching include *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans* and *Thiobacillus thioparus*.[4] Recently, a more economical and efficient process for metal leaching using a mixed culture of *T thioparus* and *T thiooxidans* at neutral pH (compared with the process using *T ferroxidans* or *T thiooxidans* at acidic pH) has been developed. The advantage of using the mixed culture is that the initial sulfur oxidation and acidification carried out by *T thioparus* results in a decrease in pH to approximately 4, at which *T thiooxidans* can grow without adding any acid. The microbial leaching process may be defined as the solubilization of metals from solid substrates either directly by the metabolism of leaching bacteria or indirectly by the products of metabolism.[8] The main

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Item	Ell Ren river	SRM[b] 2704 (certified values)	SRM 2704 (analytical values)
Total solids ($mg\,g^{-1}$)	724.3 ± 0.1[a]	–	–
Organic matter ($mg\,g^{-1}$)	35.1 ± 0.1	–	–
pH	7.8 ± 0.1	–	–
Metal ($\mu g\,g^{-1}$)			
Cu	190.8 ± 6.7	98.6 ± 5.0	91.3 ± 2.3
Mn	424.1 ± 14.1	555.0 ± 19.0	545.2 ± 15.1
Zn	400.9 ± 24.7	438.0 ± 12.0	425.0 ± 10.3
Pb	142.9 ± 10.5	161.1 ± 17.2	145.0 ± 10.0
Ni	49.7 ± 2.3	44.1 ± 3.0	43.2 ± 2.4
Cr	74.1 ± 2.8	135.1 ± 5.0	120.5 ± 5.3

Table 1. The properties of sediment of the Ell Ren River

[a] Mean ± standard deviation ($n = 12$).
[b] Standard Reference Material (sediment).

mechanisms involved in microbial leaching of heavy metals by *Thiobacillus thiooxidans* and *Thiobacillus thioparus* include direct and indirect mechanisms, which can be described by the following equations:[9]

(i) Direct mechanism

$$MS + 2O_2 \xrightarrow{\text{thiobacilli}} MSO_4 \quad (1)$$

(ii) Indirect mechanism

$$S^0 + H_2O + \frac{3}{2}O_2 \xrightarrow{\text{thiobacilli}} H_2SO_4 \quad (2)$$

$$H_2SO_4 + \text{sediment} - M \rightarrow \text{sediment} - 2H + MSO_4 \quad (3)$$

where M is a bivalent metal. The performance of the microbial leaching process is affected by various parameters such as bacterial strain, quantity and type of substrate, solid content, particle size, carbon dioxide and oxygen concentrations in system, pH and redox potential.[10,11] In the microbial leaching process, pH has been considered to be a key factor in determining the solubilization of heavy metals.[12,13] In bioleaching of heavy metals from sewage sludges, solubilization of Cu and Pb is affected by the organic matter content of sludges because Cu and Pb tend to form complexes with the organic matter in sludges. In addition, increasing sulfate concentration can have unfavorable effects on the solubilization of Pb due to the formation of less soluble $PbSO_4$ during the bioleaching process.[14] Good understanding of these parameters is important for optimizing microbial leaching processes. The objectives of this work were to develop a microbial leaching process to solubilize metals from dredged sediments using a mixed culture of thiobacilli and to investigate the effects of sediment solid content on microbial leaching of heavy metals from contaminated sediments. In addition, a kinetic equation related to sediment solid content was used to describe the solubilization of heavy metals from sediments.

2 MATERIALS AND METHODS

2.1 Sediment

Sediment samples were taken from a highly polluted river (Ell Ren River), located in southern Taiwan. The total solids and content of organic matter (ie volatile solids) of the sediment were determined using the Standard Method 2540G[15] and the pH values were analyzed as per the procedures of LaBauve *et al.* [16] The heavy metals in the sediment were extracted using the HF–HNO_3–HCl acid microwave digestion method.[17] In addition, the lattice-held fraction (lithogenic bonding form) of Cr in sediment was analyzed by the sequential extraction procedure.[18] Then the concentration of metals was determined using a flame atomic absorption spectrophotometer (AAS) equipped with a graphite burner (Model Z-8100, Hitachi). The characteristics of the sediment are presented in Table 1. On a dry weight basis, the organic content equaled $35.1\,mg\,g^{-1}$ measured as volatile solids. The pH value was about 7.8. The content of heavy metals, Cu, Mn, Zn, Pb, Ni, and Cr, was equal to 190.8, 424.1, 400.9, 142.9, 49.7 and $74.1\,\mu g\,g^{-1}$, respectively. These results indicate the contamination of sediment by heavy metals of industrial origin.

2.2 Culture strains and media

Two sulfur-oxidizing bacteria, *T thiooxidans* (CCRC 15612) and *T thioparus* (CCRC 15623), were obtained from Culture Collection and Research Center (CCRC) of the Food Industry Research and Development Institute (FIRDI), Hsinchu, Taiwan. Growth media for both bacterial strains were chosen as recommended by the CCRC. Medium 317 was used for *T thiooxidans*, this contained 0.3 g $(NH_4)_2SO_4$, 3.5 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.25 g $CaCl_2$, and 5.0 g tyndallized sulfur powder per dm^3 of deionized water. The pH value was adjusted to 4.5 using $0.5\,mol\,dm^{-3}$ H_2SO_4. Medium 318 used for *T thioparus* contained 0.3 g $(NH_4)_2SO_4$, 4.0 g K_2HPO_4, 1.5 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 10.0 g $Na_2S_2O_3.5H_2O$, and $10.0\,cm^3$ trace salts solution per dm^3 of deionized water. The trace salts solution was prepared

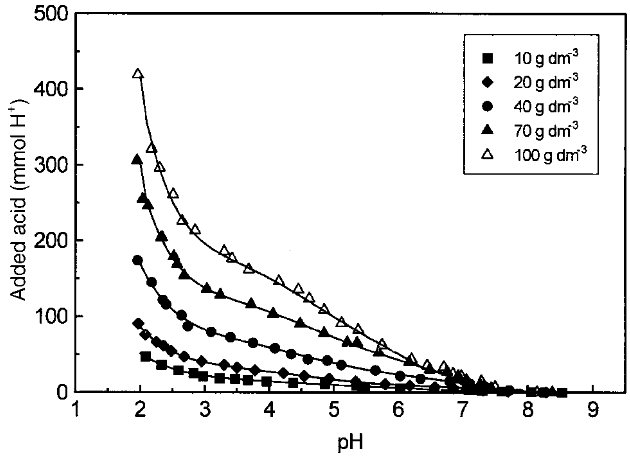

Figure 1. The relationship between added acid and pH in bioleaching for different sediment solid contents.

by dissolving 50.0 g Na_2EDTA, 2.2 g $ZnSO_4.7H_2O$, 7.3 g $CaCl_2.2H_2O$, 2.5 g $MnCl_2.6H_2O$, 0.5 g $CoCl_2.6H_2O$, 0.5 g $(NH_4)_6Mo_7O_{24}.4H_2O$, 5.0 g $FeSO_4.7H_2O$, 0.2 g $CuSO_4.5H_2O$, 11.0 g NaOH in deionized water and diluting to 1 dm^3. The pH value was adjusted to 7.0 using 0.5 mol dm^{-3} H_2SO_4.[19] The liquid cultures were incubated in 500 cm^3 flasks and shaken in a 3.3 Hz rotary incubator shaker at 30 °C.

2.3 Bioleaching process

A mixed inoculum of 5 cm^3 of the 5-day-old subculture of *T thiooxidans* and *T thioparus* was transferred to 500 cm^3 of autoclaved sediment (solids content: 20 g dm^{-3}) with 2.5 g of tyndallized sulfur powder. The culture was incubated in a 500 cm^3 flask and shaken in a 3.3 Hz rotary incubator shaker at 30 °C. The process of acclimation of the bacteria in sediments was monitored by measuring pH, ie montoring acid production, during sulfur oxidation.[4] Then 150 cm^3 of this acclimated growing mixed culture was added to 3 dm^3 of sediments containing selective amounts of solids (10, 20, 40, 70 and 100 g dm^{-3}) with 15 g of tyndallized sulfur powder in a completely mixed batch (CMB) reactor. The sediment was stirred using a 3.3 Hz Teflon agitator and aerated with an air diffuser at a rate of 20 $cm^3 s^{-1}$. The reactor temperature was maintained at 30 °C using a water bath. The duration of the experiments with various solid contents was determined by the pH of the system. The experiments were stopped when the pH of the system was about 2.4. The pH and oxidation–reduction potential (ORP) in the reactor were measured using an on-line monitor (Tank, model RD-500). A 15 cm^3 sample was withdrawn from the CMB reactor at regular intervals. The samples were then centrifuged at 10 000 rpm for 20 min and filtered through a 0.45 μm filter membrane. The filtrate was collected for chemical analyses (see Section 2.4).

2.4 Analyses

Sulfate concentrations in the filtrate were determined by Standard Method 4500E[15] and the concentrations of heavy metals in the filtrate (Zn, Mn, Cu, Pb, Ni, and Cr) were determined using a flame and graphite atomic absorption spectrometer (Hitachi, model Z-8100). The efficiency of solubilization of heavy metals from sediment in the bioleaching process was calculated as the ratio of heavy metal content in the liquid phase to that in the sediment. At the same time, an identical CMB reactor under the same experimental conditions was used to determine the volumetric oxygen transfer coefficient (k_La) following the procedures recommended by Benefield and Randall.[20] The QA/QC programs of analyses followed the principles presented in Ref 15. As a reference for the analytical quality, a standard reference material (SRM 2704, National Institute of Standards and Technology (NIST)) was analyzed with each batch of sample sediments from the reactor. The reproducibility and repeatability of the analyses were good as there were no significant differences between duplicates (Table 1).

3 RESULTS AND DISCUSSION

3.1 Quantification of buffering capacity of sediment

Sediment with different solid contents (10, 20, 40, 70, and 100 g dm^{-3}) was abiotically acidified by stepwise addition of 0.1 mol dm^{-3} sulfuric acid in a CMB reactor equipped with a 3.3 Hz Teflon agitator. The pH after each step of acid addition was noted at equilibrium and the pH versus volume of acid added curve was plotted (Fig 1). Since acid is a by-product of the metabolic activity of sulfur-oxidizing bacteria in this study, it would lead to a decrease of pH and solubilization of heavy metals from the sediment. However, the variation in pH is dependent on the buffering capacity of sediment solids in the bioleaching process. The slope of the acid curve in Fig 1 depends on the pH in the reactor. It requires a large quantity of acid to change the pH at a lower pH and a small

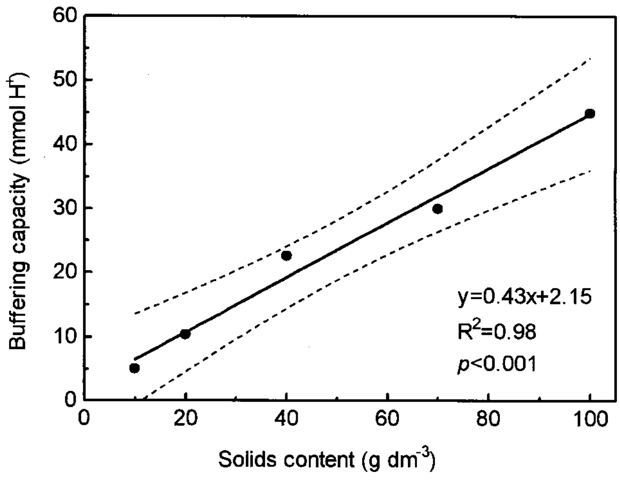

Figure 2. Buffering capacity in bioleaching for different sediment solid contents (dotted lines are the prediction interval at the 95% confidence level).

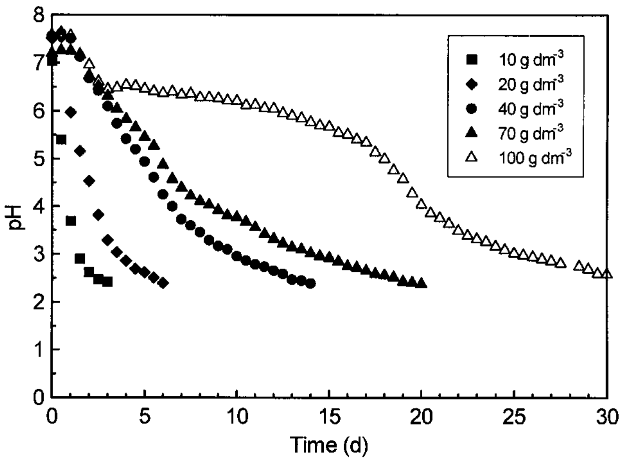

Figure 3. Variations in pH during bioleaching for different sediment solid contents.

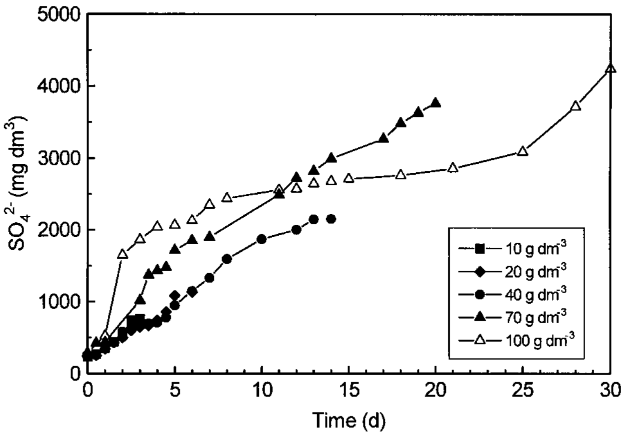

Figure 5. Sulfate production during bioleaching for different sediment solid contents.

quantity will affect a pH change at a higher pH. Therefore, the buffering capacity (β) of sediment is defined as the quantity of hydrogen ion required to change the pH by one unit at a pH of 4.0.

$$\beta = \left.\frac{d(H^+)}{d(pH)}\right|_{pH=4.0} \qquad (4)$$

The calculated buffering capacities for different sediment solid contents are displayed in Fig 2. It is observed that there is a linear relationship between buffering capacity and sediment solid content, ie the buffering capacity increases as the sediment solid content increases. Sreekrishnan *et al*[12] defined a parameter called 'buffering capacity index (BCI)' as the quantity of sulfate required to change the pH by one unit at a pH of 4.0.

$$BCI = \left.\frac{d(SO_4^{2-})}{d(pH)}\right|_{pH=4.0} \qquad (5)$$

During bioleaching, sulfate can be produced by oxidation of sulfides (eqn (1)) and elemental sulfur (eqn (2)). But sulfate does not account for the entire acid production during the process. Therefore, eqn (4) using hydrogen ion concentration will give the better result for the buffering capacity of sediment solids.

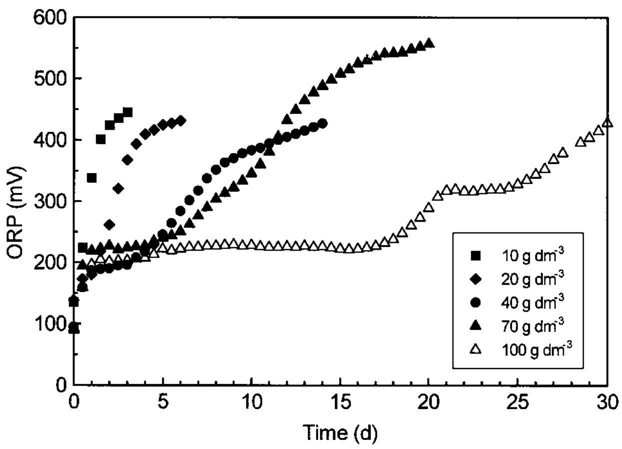

Figure 4. Variations in ORP during bioleaching for different sediment solid contents.

3.2 Variations in pH and oxidation–reduction potential (ORP)

Figure 3 illustrates variations of pH during microbial leaching of different sediment solids contents. It is found that the sediment pH value dropped to about 2.4 from 7.5 at all solid contents studied, but the rate of decline in pH decreases as the sediment solid content increases. It took 3, 6, 14, 20 and 30 days to reach the required pH value of about 2.4 for solids contents of 10, 20, 40, 70 and 100 g dm^{-3}, respectively. These results indicate that higher the sediment solids content, the longer the experimental period to reach the required pH value. This is attributed to the higher sediment solids content with higher buffering capacity (Fig 2). Thus, sediment solid content plays an important role in the variation of pH during the microbial leaching process. Aeration and oxidation of sulfur compounds to sulfate through microbial leaching result in an increase of sediment ORP. The changes in ORP during microbial leaching of different sediment solids content are shown in Fig 4. The rate of ORP rise decreases with increases in the sediment solids content and the maximum ORP values attained during the microbial leaching process are 450–550 mV.

3.3 Sulfate production

Figure 5 shows the formation of sulfate during the oxidation of sulfur by sulfur-oxidizing bacteria for different sediment solids contents. It is seen that the rate of formation of sulfate and its concentration in the system are greater for the sediment with higher solid contents. In the bioleaching experiment of this study, no other nutrients, except elemental sulfur powder, were added into the CMB reactor. This may be due to the higher concentration of nutrients in sediments with higher solid content.[12] Only 10–30% of elemental sulfur fed was utilized during growth, and so it is reasonable to assume that the growth-limiting substrate for the bacteria is not the elemental sulfur, but

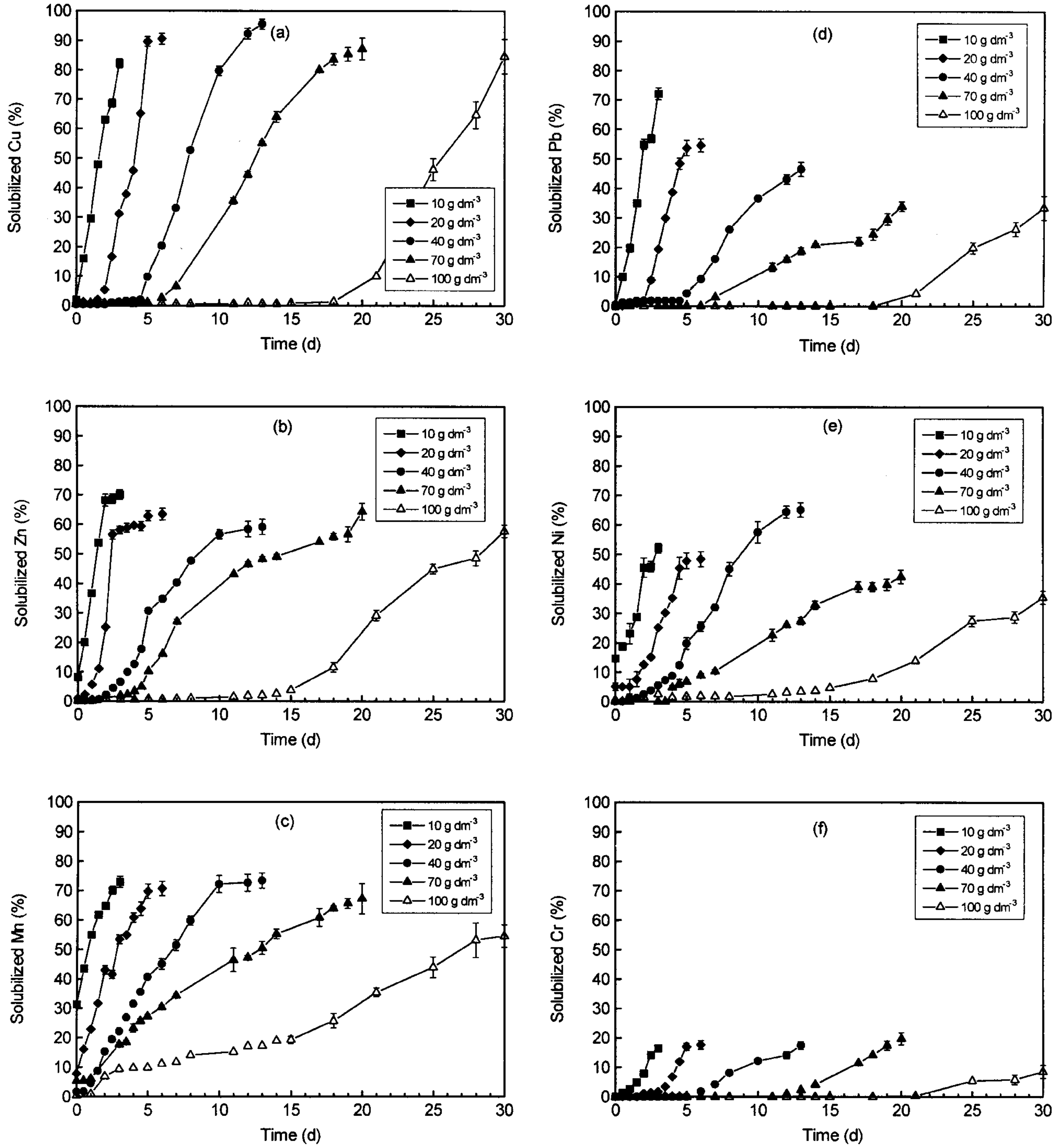

Figure 6. Metal solubilization of sediment during bioleaching for different sediment solid contents: (a) Cu, (b) Zn, (c) Mn, (d) Pb, (e) Ni and (f) Cr.

the other micronutrients (eg ammonium, sulfate, phosphate, etc) or trace metals. These growth-limiting substrates which are essential for bacterial growth can be provided by the sediment itself.

3.4 Metal leaching

In this study, the efficiencies of solubilization of different metals were compared when the pH reached the value of 2.4. Figure 6 shows the solubilization of heavy metals from the sediment and Table 2 summarizes the final solubilization efficiencies (%) of heavy metals in the bioleaching process. The solubilization efficiencies of Cu, Zn and Mn for different solids contents are 82–95% (87.8 ± 5.0%), 58–70% (63.0 ± 4.6%) and 55–73% (67.6 ± 7.4%), which are not significantly influenced by solids content. From these results, it is found that the efficiencies of solubilization of Pb (48.0 ± 16.1%), Ni (48.4 ± 11.3%) and Cr (16.0 ± 4.2%) are affected by the solid contents of sediments. Table 2 shows that the solubilization efficiency of Cu ranged between 82 and 95% for the solid contents of 10–100 g dm^{-3}, which is greater than those of sewage sludges shown in another study.[21] The reason for this result is that Cu has a strong affinity with organic matter,[22] and the sediment used in

Table 2. The efficiency of solubilization of heavy metals from the sediments in bioleaching with different solid contents

Solid content ($g\,dm^{-3}$)	Efficiency[a] (%) Cu	Zn	Mn	Pb	Ni	Cr
10	82±1.5	70±1.6	73±1.9	72±2.0	52±1.7	16±0.8
20	90±1.8	63±2.0	70±2.4	54±2.3	48±2.6	17±1.4
40	95±1.7	60±2.6	73±2.5	47±2.4	65±2.5	18±1.3
70	87±3.8	64±2.8	67±5.1	34±1.6	42±2.3	20±2.0
100	85±4.8	58±2.2	55±3.8	33±4.0	35±2.3	9±2.1

[a] Efficiency (mean±standard deviation) of solubilization was calculated as the ratio of heavy metal content in liquid phase to that in sediment at the pH value of 2.4.

Table 3. Rate constants (*k*) of the kinetic equation in bioleaching of heavy metals from the sediment with different solid contents

	Cu		Zn		Mn		Pb		Ni		Cr	
Solid content ($g\,dm^{-3}$)	k (d^{-1})	R^2	k (d^{-1})	R^2	k (d^{-1})	R^2	k (d^{-1})	R^2	k (d^{-1})	R^2	k (d^{-1})	R^2
10	0.890	0.93	0.261	0.76	0.242	0.91	0.398	0.95	0.208	0.93	0.064	0.93
20	0.771	0.76	0.155	0.78	0.171	0.98	0.151	0.95	0.116	0.97	0.040	0.89
40	0.218	0.88	0.069	0.98	0.037	0.99	0.045	0.89	0.086	0.95	0.013	0.86
70	0.111	0.91	0.053	0.97	0.054	0.99	0.018	0.85	0.031	0.98	0.010	0.75
100	0.040	0.75	0.025	0.76	0.024	0.91	0.010	0.75	0.013	0.78	0.002	0.70

this study had a lower organic content (3.5% (w/w) of total solids) than that of sewage sludge (30–88% (w/w) of total solids).[23] The higher concentration of sulfate in sediments with higher solid contents results in a lower solubilization efficiency of Pb, due to the formation of $PbSO_4$.[14] Most of the Cr ($57.3 \pm 2.6\,\mu g\,g^{-1}$, ie 77% (w/w) of total Cr) exists in the lattice-held fraction of the sediments, therefore, it requires an extremely acidic condition for Cr to be solubilized from the sediments.[18] Thus, the solubilization efficiency of Cr in this study is very low. Figure 6 shows that the heavy metals (Zn, Cu, Pb and Cr) show a lag phase before solubilizing from sediments with higher solid contents. Because of the buffering capacity of the sediment with higher solid content (Fig 2), the sulfur-oxidizing bacteria may take more time to produce acid in order to lower pH to the level at which solubilization of each metal can take place efficiently.

According to the results obtained from this study, the metal solubilization in microbial leaching follows a kinetic equation. The rate of solubilization of metals in this process can be described by the equation:

$$\frac{dM}{dt} = k(M_s - M) \qquad (6)$$

where k is the rate constant (d^{-1}), M_s and M are the initial weight of metal in the sediment (mg) and weight of metal in the aqueous phase (mg), respectively. The rate constants of the kinetic equation in microbial leaching and the coefficient of determination of linear regression, R^2, are summarized in Table 3. The rates of metal leaching decrease in the order: Cu> Zn> Mn> Pb> Ni> Cr. The rate constants of the kinetic equation decrease with increasing sediment solid contents. It is found that the rate constants at solid content below $100\,g\,dm^{-3}$ can be determined by using

Table 4. Coefficient k_s of the modified kinetic equation in bioleaching of heavy metals from the sediment

Metal	k_s ($g\,dm^{-3}\,d^{-1}$)	R^2
Cu	10.048	0.86 ($p<0.05$)
Zn	2.719	0.98 ($p<0.001$)
Mn	2.579	0.92 ($p<0.01$)
Pb	3.638	0.94 ($p<0.001$)
Ni	2.181	0.95 ($p<0.005$)
Cr	0.661	0.97 ($p<0.005$)

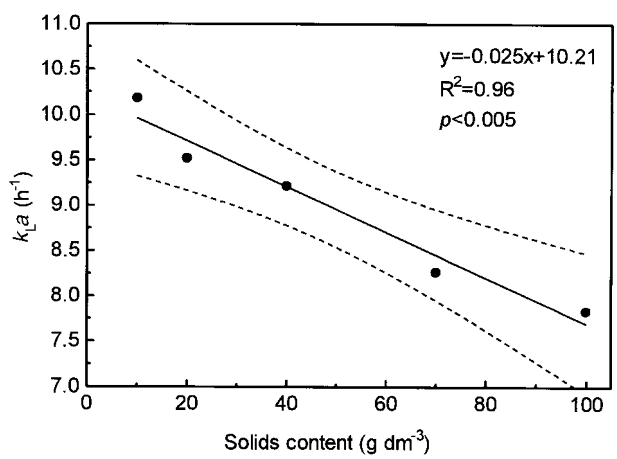

Figure 7. The relationship between volumetric oxygen transfer coefficients (k_La) and solid content of sediment in bioleaching (dotted lines are the prediction interval at the 95% confidence level).

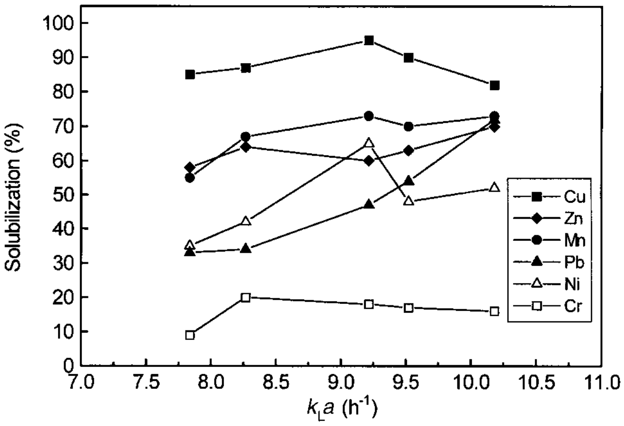

Figure 8. The relationship between volumetric oxygen transfer coefficient (k_La) and efficiency of solubilization of heavy metals in bioleaching.

the following expression:

$$k = \frac{k_s}{S} \tag{7}$$

where k_s is a coefficient ($g\,dm^{-3}\,d^{-1}$) of rate, which depends on solid content and S is the solid content of the sediment ($g\,dm^{-3}$). From eqn (7), k_s is calculated and the results are listed in Table 4. Table 4 shows that the coefficient of determination of linear regression, R^2, was high for all the heavy metals. The coefficient k_s decreases in the order: Cu> Zn> Mn> Pb> Ni> Cr. Therefore, there is an inverse relationship between the rate of metal solubilization and sediment solid content in the microbial leaching process. When the rate constant k from eqn (7) is substituted in eqn (6), a modified kinetic equation (eqn (8)) is obtained.

$$\frac{dM}{dt} = \frac{k_s}{S}(M_s - M) \tag{8}$$

In addition, the sediment solid content influences the solubility and transfer of gaseous nutrients, which may be the factors responsible for the metal solubilization in the microbial leaching process.[10] The purpose of aeration and agitation in the microbial leaching process is to achieve oxygen transfer and to mix the solid substrate and nutrient solution thoroughly so that a uniform suspension of sediment particles and bacteria can be maintained. Figure 7 is a plot of volumetric oxygen transfer coefficient (k_La) determined in microbial leaching process with different sediment solid contents. It is observed that the k_La value decreases with increasing sediment solid content. The bacteria (*T thiooxidans* and *T thioparus*) inoculated in this study are strictly aerobic,[24] thus availability of oxygen in microbial leaching is an important factor which affects the leaching process. The combined effects of aeration and agitation are expressed as k_La which is the volumetric oxygen transfer coefficient under the microbial leaching process conditions. Generally, a higher k_La value favors metal leaching because the bacterial activity increases with increasing oxygen uptake efficiency (oxygen transfer coefficient).[10] Since the variations (from 7.84 to 10.18 h^{-1}) of k_La and concentrations of dissolved oxygen (6.8–7.2 $mg\,dm^{-3}$) are very small in this study, the solubilization efficiencies of Cu, Zn and Mn were not significantly influenced by the k_La values (Fig 8). Tyagi and Couillard[24] found that k_La values were between 7.3 and 7.9 h^{-1} in the leaching of metals from digested sludge in a 3.0 dm^3 bioreactor at agitation rate of 425 rpm and aeration rate of 0.1 vvm (volume of air flowing/dm^3 of working volume/min), and it required a minimum oxygen concentration of 35% of the saturation using sludge as a substrate. The present study shows that the dissolved oxygen level (89–94% of saturation) was sufficient for the bacteria to solubilize heavy metals from the sediment, even though the k_La is known to be affected by the sediment solid content.

4 CONCLUSIONS

A microbial leaching process with a mixed culture of two sulfur-oxidizing bacteria can be used to remove heavy metals from contaminated sediments. The performance of the bioleaching process is affected by various parameters. Sediment solid content is important in determining the size of the bioreactor and the operational time of the bioleaching process. Other parameters, such as buffering capacity and oxygen transfer coefficient, are themselves influenced by the solid content. Changes in the pH value of the bioleaching process are influenced by the buffering capacity of sediment solids. Thus, with high buffering capacity, the pH of sediment with higher solid content declines slowly to the required value. Under similar pH conditions, the efficiency of solubilization of heavy metal decreases in the order Cu> Zn> Mn> Pb> Ni> Cr. Concurrently, there are apparent influences of sediment solid contents on the final efficiencies of Pb, Ni and Cr (except Cu, Zn and Mn). The rates of metal leaching are well described by a kinetic equation related to solids content. In addition, higher oxygen transfer coefficient is often favorable for bacterial activity and solubilization of heavy metals in the bioleaching process. In this study, though the volumetric oxygen transfer coefficient was affected by the solid content, the dissolved oxygen level was sufficient for bacteria to solubilize heavy metals from the sediment.

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REFERENCES

1 Baudo R, Giesy JP and Muntau H, *Sediments: Chemistry and Toxicity of In-place Pollutants*, Lewis Publishers, Ann Arbor, MI (1990).

2 Timberlake DL and Garbaciak S, Bench-scale testing of

selected remediation alternatives for contaminated sediments. *J Air Waste Manage Assoc* **45**:52–56 (1995).

3 Bosecker K, Bacterial metal recovery and detoxification of industrial waste. *Biotechnol Bioeng* **16**:105–120 (1986).

4 Blais JF, Auclair JC and Tyagi RD, Cooperation between two *Thiobacillus* strains for heavy metal removal from municipal sludge. *Can J Microbiol* **38**:181–187 (1992).

5 Tyagi RD, Sreekrishnan TR, Campbell PGC and Blais JF, Kinetics of heavy metal bioleaching from sewage sludge-II. Mathematical model. *Water Res* **27**:1653–1661 (1993).

6 Hsu CH and Harrison RG, Bacterial leaching of zinc and copper from mining wastes. *Hydrometallurgy* **37**:169–179 (1995).

7 Mercier G, Chartier M and Couillard D, Strategies to maximize the microbial leaching lead from metal-contaminated aquatic sediments. *Water Res* **30**:2452–2464 (1996).

8 Wong LTK and Henry JG, Bacterial leaching of heavy metals from anaerobically digested sludge, in *Biotreatment systems*, Ed by Wise DL, CRC Press, Boca Raton, FL. pp 125–169 (1988).

9 Rulkens WH, Grotenhuis JTC and Tichy R, Methods of cleaning contaminated soils and sediments, in *Heavy Metals*, Ed by Salomons W, Förstner U and Mader P, Springer-Verlag, Berlin. pp 191–191 (1995).

10 Couillard D and Zhu S, Bacterial leaching of heavy metals from sewage sludge for agricultural application. *Water Air Soil Pollut* **63**:67–80 (1992).

11 Haddadin J, Dagot C and Fick M, Models of bacterial leaching. *Enzyme Microb Technol* **17**:290–305 (1995).

12 Sreekrishnan TR, Tyagi RD, Blais JF and Campbell PGC, Kinetics of heavy metal bioleaching from sewage sludge –I. Effects of process parameters. *Water Res* **27**:1641–1651 (1993).

13 Tyagi RD, Couillard D and Tran F, Heavy metals removal from anaerobically digested sludge by chemical and microbiological methods. *Environ Pollut* **50**:295–316 (1988).

14 Tyagi RD, Microbial leaching of metals from municipal sludge: effects of sludge solid concentration. *Proc Biochem* **27**:89–96 (1992).

15 APHA, *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington (1995).

16 LaBauve JM, Kotuby-Amacher J and Gambrell RP, The effect of soil properties and a synthetic municipal land-fill leachate on the retention of Cd, Ni, Pb and Zn in soil and sediment materials. *J Water Pollut Control Fed* **60**:379–385 (1988).

17 USEPA, *Microwave assisted acid digestion of sediments, sludge, and oils*. Method 3052, US Environmental Protection Agency, Washington (1995).

18 Lin JG, Chen CY and Chen SY, Effects of pH on metals specification in a contaminated sediment. *J Chinese Inst Environ Eng* **9**:49–56 (1999).

19 FIRDI, *Catalogue of Strains: Supplement II*, Food Industry Research and Development Institute, Hsinchu, Taiwan (1994).

20 Benefield LD and Randall CW, *Biological Process Design for Wastewater Treatment*, Prentice-Hall, Englewood Cliffs, NJ (1980).

21 Blais JF, Tyagi RD and Auclair JC, Bioleaching of metals from sewage sludge by sulfur-oxidizing bacteria. *J Environ Eng* **118**:690–707 (1992).

22 Lin JG and Chen SY, The relationship between adsorption of heavy metals and organic matter in river sediments. *Environ Int* **24**:345–352 (1998).

23 Metcalf and Eddy Inc, *Wastewater Engineering*, 3rd edn, McGraw-Hill, New York, NY (1991).

24 Tyagi RD and Couillard D, Bacterial leaching of metals from sludge, in Encyclopedia of Environmental Control Technology, Vol 3: *Wastewater Treatment Technology*, Ed by Cheremisinoff PN, Gulf Publishing Co, Houston. pp 557–591 (1989).