

# Bioleaching of heavy metals from contaminated sediment by indigenous sulfur-oxidizing bacteria in an air-lift bioreactor: effects of sulfur concentration

Shen-Yi Chen, Jih-Gaw Lin\*

*Institute of Environmental Engineering, National Chiao Tung University, 75 Po-Ai Street, Hsinchu, Taiwan*

Received 18 July 2003; received in revised form 16 April 2004; accepted 30 April 2004

## Abstract

The effects of sulfur concentration on the bioleaching of heavy metals from the sediment by indigenous sulfur-oxidizing bacteria were investigated in an air-lift reactor. Increasing the sulfur concentration from 0.5 to 5 g/l enhanced the rates of pH reduction, sulfate production and metal solubilization. A Michaelis–Menten type equation was used to explain the relationships between sulfur concentration, sulfate production and metal solubilization in the bioleaching process. After 8 days of bioleaching, 97–99% of Cu, 96–98% of Zn, 62–68% of Mn, 73–87% of Ni and 31–50% of Pb were solubilized from the sediment, respectively. The efficiency of metal solubilization was found to be related to the speciation of metal in the sediment. From economical consideration, the recommended sulfur dosage for the bioleaching of metals from the sediment is 3 g/l.

© 2004 Elsevier Ltd. All rights reserved.

*Keywords:* Bioleaching; Indigenous; Sediment; Heavy metal; Sulfur concentration; *Thiobacilli* species

## 1. Introduction

Sediment is the ultimate fate for natural and anthropogenic pollutants in the aquatic environment. Sediment is generally highly contaminated with various types of pollutants in the countries of dense industrialization. For a successful remediation, dredging of contaminated sediment is one of important alternatives besides controlling the pollutants at sources and building sewer systems. Most of the sediment dredged from contaminated aquatic bodies such as river and lake contains high concentration of toxic pollutants such as heavy metals (Förstner and Calmano, 1998). Because of stringent disposal standards, this metal-contaminated sediment cannot be disposed off on lands without proper

treatment. Environmental engineers and managers are facing new challenges regarding the management and treatment of a large quantity of contaminated sediment. From this perspective, development of a suitable and a cost-effective technology for removal of heavy metals from the contaminated sediment is needed.

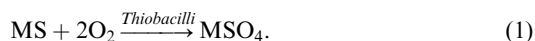
Conventionally, physical or chemical technologies are used for treatment of metal-contaminated sediments. However, these technologies often have some limitations in practical application (Rulkens et al., 1995). The microbial method is an efficient and cost-effective alternative to chemical and physical methods of remediating many environmental pollution problems because of its low demand for energy, material and less generation of waste byproduct (Hsu and Harrison, 1995). Bioleaching is one of the microbial methods lately considered highly promising for the treatment of metal-containing or metal-contaminated solids, e.g. ores, sludges, soils, swine manure and sediments (Blais et al.,

\*Corresponding author. Tel.: +886-3-5725958; fax: +886-3-5722681.

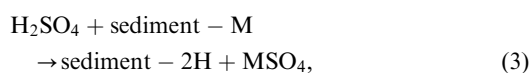
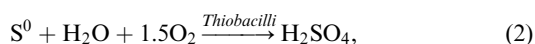
E-mail address: jglin@mail.nctu.edu.tw (J.-G. Lin).

1992; Ahonen and Tuovinen, 1995; Tichy et al., 1998; Chen and Lin, 2000, 2001). The most important microorganisms that are known to facilitate metal bioleaching reactions belong to the autotrophic *Thiobacillus* species (Boseker, 1997). The two acidophilic bacteria, namely *T. ferrooxidans* and *T. thiooxidans*, are frequently used in the bioleaching process (Donati et al., 1996). However, the bioleaching process inoculated with only acidophilic *Thiobacillus* species needs pre-addition of acid to lower the pH to the acidic range ( $\text{pH} \leq 4$ ) suitable for the growth of these bacteria and it increases the operational cost. A more economical and effective bioleaching process inoculated with a mixed culture of *T. thioparus* (less-acidophilic) and *T. thiooxidans* (acidophilic) has been recently investigated (Blais et al., 1992; Chen and Lin, 2000). These mixed microbial cultures could grow and solubilize the metals at neutral pH thereby eliminating the addition of acid to initiate bioleaching process. Theoretically, the bioleaching process is primarily achieved by two mechanisms: (1) direct (Eq. (1))—the bacteria involved in bioleaching process can catalyze the oxidation of insoluble metal sulfides to soluble metal sulfates; and (2) indirect (Eqs. (2) and (3))—elemental sulfur or reduced sulfur compounds are oxidized to sulfuric acid by these bacteria, thereby lowering the pH and subsequently enhancing the metal solubilization (Rulkens et al., 1995).

(1) Direct mechanism



(2) Indirect mechanism



where M is a bivalent metal.

The bioleaching process is governed by the activity of microorganisms involved. The maximum efficiency of metal solubilization can be achieved only when the conditions of the bioleaching process correspond to the optimum growth conditions of the bacteria (Boseker, 1997). However, the activity of bacteria is affected by various physical, chemical and biological factors. It is therefore crucial to understand the pragmatic operational conditions for optimum growth of the microorganisms involved in bioleaching process. Because elemental sulfur ( $\text{S}^0$ ) is less expensive than other substrates (Tyagi et al., 1994), it is extensively used as the substrate for the growth of *Thiobacilli* species (sulfur-oxidizing bacteria) in the bioleaching process (Tichy et al., 1998; Chen and Lin, 2000, 2001). When using elemental sulfur as the substrate, the production of

acid is a rate limiting step in the overall bioleaching process (Tichy et al., 1993). Generally, the acid production and pH changes are affected by the amount of sulfur added. However, if excess sulfur is added than required for metal solubilization, the residual sulfur will cause reacidification of treated sediments or soils during land application (Ravishankar et al., 1994). Thus, sulfur concentration is one of the important parameters and has to be optimized in bioleaching process. Typically, most of the bioleaching processes are operated in shaking flasks or conventional stirred tank reactors. However, these kinds of reactors have some inherent drawbacks such as high energy consumption, large area requirement and poor mixing property (Zhou et al., 2003; Chisti and Jauregui-Haza, 2002). In comparison, air-lift reactors have outstanding advantages over stirred tank reactors: a simple construction, an excellent heat transfer capacity, a reasonable interphase mass transfer rate and good mixing properties at low energy consumption, as the gas phase serves the dual function of aeration and agitation (Vial et al., 2002). The objectives of this study were to investigate the feasibility of bioleaching process for metal-contaminated sediments in an air-lift reactor and to examine the effects of sulfur concentration on the performance of bioleaching process.

## 2. Materials and methods

### 2.1. Sediment source and its characterization

The sediment sample used throughout this study was taken from the lower reaches (near Nan Ding Bridge) of the Ell Ren River in southern Taiwan. The Ell Ren River, which has a catchment area of about 350 km<sup>2</sup> and a total length of 65 km, was chosen because it receives many untreated and/or partially treated municipal, industrial and agricultural wastewaters from uncontrolled effluents and the sediment has been highly contaminated by heavy metals. The sediment samples were collected in sealed plastic bags and stored at 4°C prior to their use. Every batch of sediment sample was characterized before its use in the bioleaching experiment. The characterization includes determination of total solids (TS), volatile solids (VS), pH, and important metals and their speciation. The measured characteristics and metal speciation of the contaminated sediment are listed in Tables 1 and 2.

### 2.2. Seed microorganisms and activation

The indigenous sulfur-oxidizing bacteria were acclimated in a 3 l batch reactor containing sediment slurry of 20 g (dry weight)/l and a tyndallized elemental sulfur (200–300 µm in diameter) of 5 g/l. This acclimation

reactor was stirred with a mixer running at 200 rpm and aerated at a rate of 1.21 air/min at  $30 \pm 0.5^\circ\text{C}$  constant temperature. The pH of sediment slurry was measured daily. A 150 ml of acclimated sediment slurry was then transferred to 31 freshly prepared sediment slurry with 5 g/l elemental sulfur under the same conditions when the pH dropped to less than 2.0. The above procedure was repeated until the indigenous sulfur-oxidizing bacteria in the sediment slurry had the maximum activity of acidification as evident from the rate of pH drop. The inocula thus obtained were believed to be well acclimated and rich in indigenous sulfur-oxidizing bacteria.

### 2.3. Bioleaching in air-lift reactor

The batch air-lift reactor consisted of three parts: top part, main column with water jacket and bottom part with an air diffuser. Fig. 1 shows a schematic diagram of the experimental setup. The main column consisted of an internal tube of 5 cm internal diameter and 150 cm height, an external tube of 15 cm diameter and 150 cm height and a total working volume of 25 l. Compressed air was sparged at a rate of 6 l/min from the bottom of the column into the internal tube, which produced a recirculation flow pattern of the sediment slurry in the column. The top part of the reactor had a 25 cm diameter and 30 cm height and was employed to prevent the overflow of sediment slurry from the reactor. The

reactor was maintained at  $30 \pm 0.5^\circ\text{C}$  using a water jacket. In each bioleaching experiment, the air-lift reactor was fed with 25 l sediment slurry of 1% TS content, 2.5 l well acclimatized inoculum and a pre-determined amount of sterilized (tyndallized) elemental sulfur. The elemental sulfur concentrations in the reactor were varied from 0.5, 1, 3 to 5 g/l. During each

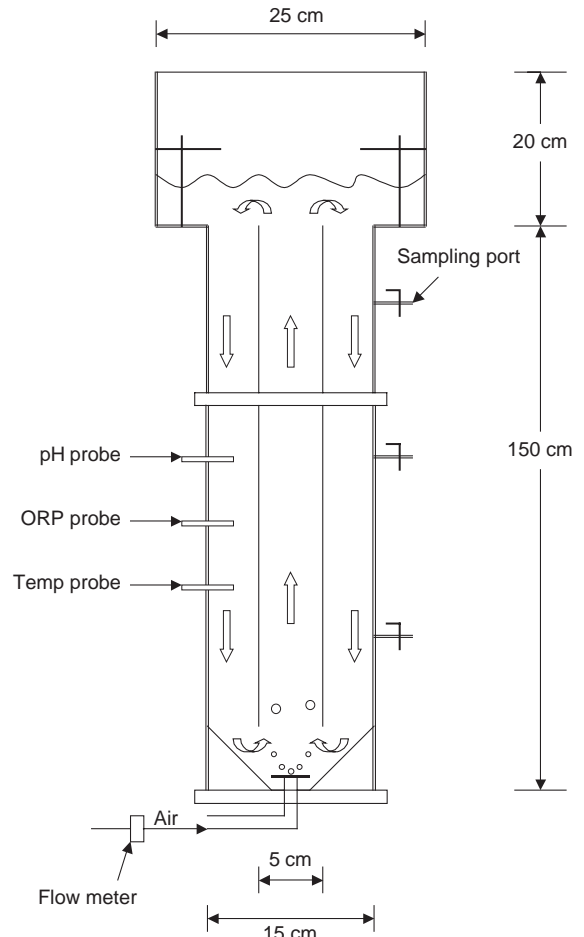


Fig. 1. Schematic diagram for air-lift reactor of bioleaching experiment.

Table 1  
Measured characteristics of the contaminated sediment

Parameter	Value
Total solids (%)	$44.8 \pm 0.2^a$
Volatile solids (%)	$5.0 \pm 0.2$
pH	$7.9 \pm 0.1$
Cu (mg/kg dry weight)	$145.3 \pm 1.8$
Zn (mg/kg dry weight)	$519.7 \pm 22.5$
Mn (mg/kg dry weight)	$519.7 \pm 9.7$
Pb (mg/kg dry weight)	$43.2 \pm 3.0$
Ni (mg/kg dry weight)	$87.3 \pm 3.5$

<sup>a</sup>Mean  $\pm$  standard deviation ( $n = 12$ ).

Table 2  
Metal speciation in the sediment used in this study

Metal	Exchangeable	Carbonates	Fe/Mn oxides	Sulfide/organic	Residual
Cu	0.2 (0.2) <sup>a</sup>	0.3 (0.2)	1.2 (1.0)	106.2 (84.5)	17.8 (14.2)
Zn	0.5 (0.1)	68.3 (14.4)	192.9 (40.7)	164.0 (34.6)	48.1 (10.2)
Pb	0.6 (1.3)	3.9 (8.2)	21.1 (44.3)	13.7 (28.8)	8.3 (17.4)
Ni	1.2 (1.6)	10.5 (14.0)	18.3 (24.4)	30.5 (40.7)	14.5 (19.3)

<sup>a</sup>Numbers in parentheses represent the percentage (%) of metal speciation in sediment.

bioleaching experiment, samples were collected from the reactor routinely and analyzed, for sulfate and soluble heavy metals such as Cu, Zn, Mn, Pb and Ni. Additionally, pH and oxidation–reduction potential (ORP) were also monitored online.

#### 2.4. Analytical methods

The pH and ORP values were measured by pH and ORP electrodes installed in an online digital recorder (Model RD-500, Tank). The sediment slurry taken from the reactor was centrifuged at a speed of 10,000 rpm for 20 min, and filtered through a 0.45 µm membrane filter. The filtrate was analyzed for sulfate concentrations according to the Standard Methods (APHA et al., 1995). The sediment TS and VS were also determined following the procedure outline in Standard Methods (APHA et al., 1995). The total heavy metals in the sediment were determined after HF-HNO<sub>3</sub>-HCl digestion method (USEPA, 1995). The speciation of metals in the sediment was determined by a sequential extraction procedure, which was based on the method reported by Tessier et al. (1979) with some modification (Lin et al., 1999). This sequential extraction procedure divided heavy metals in sediments into five binding forms, namely, exchangeable, carbonate-bound, Fe/Mn oxide-bound, sulfide/organic matter-bound and residual. The heavy metal concentrations were measured by a flame/graphite atomic absorption spectrophotometer (Model Z-8100, Hitachi).

#### 2.5. Identification and enumeration of microorganisms

Since the traditional culture-based techniques for identification and enumeration of sulfur-oxidizing bacteria were of biases and time-consuming (4–6 weeks) (Blais et al., 1992), a molecular technique called fluorescent in situ hybridization (FISH) was used to confirm the presence and quantification of sulfur-oxidizing bacteria in the acclimated sediment slurry. This technique targets the small sub-unit (SSU) ribosomal RNA (rRNA) in bacteria based on their genetic evolution. For FISH experiments, samples collected from the acclimation reactor on day 2 and day 20 were fixed on ice in 1:3 (v/v) dilutions of 4% ethanol for 2 h. The fixed samples were then spotted onto gelatin-coated multi-well slides, dried and dehydrated. The dehydrated fixed samples were hybridized with oligonucleotide probes and then stained with 4',6'-diamidino-2-phenylindole (DAPI) under the conditions as described in the study of Bond and Banfield (2001). Two oligonucleotide probes specific for *T. thioparus* (THIOPA511, 5'-CACCCCTCCTGACGCA-3') and *T. thiooxidans* and *T. ferrooxidans*, (THIO820, 5'-ACCAAACATC-TAGTATTCATCG-3') (Peccia et al., 2000) used in this study were synthesized and labeled with Cy3. The

fluorescent signal of FISH was analyzed with an epifluorescence microscope (Axioscope2 plus, Zeiss). Images were captured with a digital charge coupled device camera (Axio Cam HR, Zeiss) using AxioVision 3.0. To enumerate, the DAPI-stained cells detected with probes were counted individually for each probe on each of the samples.

### 3. Results and discussion

#### 3.1. Sulfur-oxidizing bacteria in acclimated sediment

The sediment samples taken from the acclimation reactor on day 2 and day 20, were subjected to DAPI staining and hybridization with two oligonucleotide probes: one for the species *T. thioparus* (THIOPA511) and the other for species *T. thiooxidans* and *T. ferrooxidans* (THIO820). Results of FISH confirmed that less-acidophilic (*T. thioparus*) and acidophilic *Thiobacillus* (*T. thiooxidans* and *T. ferrooxidans*) indeed occurred in the acclimated sediment (Fig. 2). Additionally, the enumeration results indicated that 7% and 2% of DAPI-stained cells were detected with THIOPA511 and THIO820, respectively, on the 2nd day of acclimation. However, after 20 days of acclimation, *T. thiooxidans* and *T. ferrooxidans* were estimated to make up 60% of DAPI-stained cells in the acclimated sediment, but *T. thioparus* only made up less than 1% of DAPI-stained cells. It was found that the acidification of sediment slurry and the solubilization of heavy metals were accomplished by the combined activities of less-acidophilic and acidophilic *Thiobacillus* (Chen and Lin, 2001).

#### 3.2. Sediment acidification

To evaluate the rate of sediment acidification during bioleaching experiment, the reactor was operated without pH controlled. During bioleaching, the oxidation of elemental sulfur produced sulfuric acid that caused a decrease in pH and solubilization of metals. Fig. 3 shows the effect of sulfur concentration on acidification of sediment slurry. From the figure, it was apparent that the pH decreased from the neutral value of about 7.5 to the acidic value of 2.0 within 8 days of bioleaching experiment at all sulfur concentrations, except at 0.5 g/l. The rate of pH reduction increased with an increase in feed sulfur concentration. The microbial oxidation of elemental sulfur is known to proceed through the initial adsorption of bacteria on the insoluble elemental sulfur and followed by its oxidation by sulfur-oxidizing bacteria (Shrihari et al., 1992). It is well known that adsorption of *Thiobacilli* to sulfur particles is necessary and plays an important role in the microbial oxidation rate of sulfur (Porro et al., 1997). Therefore, the rate of

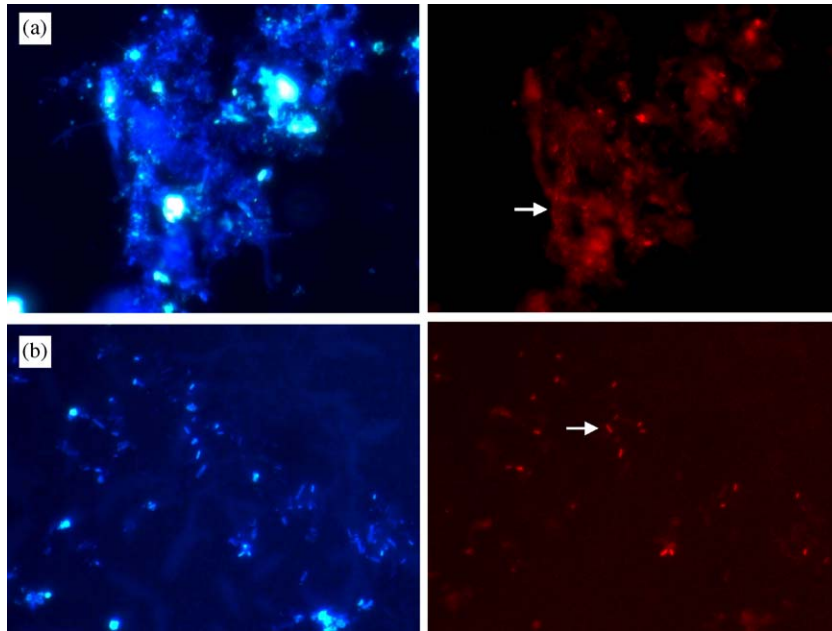


Fig. 2. Epifluorescent micrographs of FISH with different oligonucleotides probes. Horizontally paired images represent the same field of view. On the left are cells stained by DAPI. On the right is the corresponding view of cells hybridized with (a) probe THIOPA511 for acclimated sediment on the day 2 and (b) probe THIO820 for acclimated sediment on day 20.

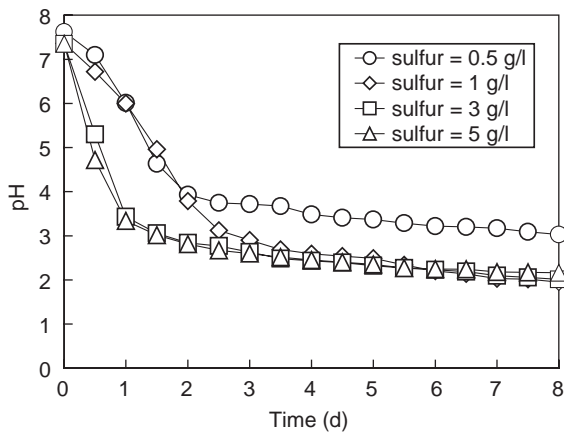


Fig. 3. Changes of pH in the bioleaching process for different sulfur concentrations.

oxidation of elemental sulfur also depends on the availability of total surface of sulfur particles. An increase in the sulfur concentration means an increase in the availability of total surface area of sulfur particles, which enhances the acidification rate. However, adding 5 g/l of elemental sulfur into the reactor did not improve the acidification rate appreciably compared to that of 3 g/l of elemental sulfur. In the bioleaching process, the microbial oxidation of sulfur led not only to sediment acidification but also to an increase in the ORP (Fig. 4).

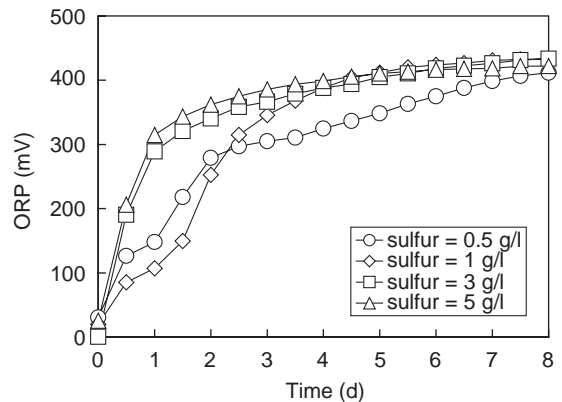


Fig. 4. Changes of ORP in the bioleaching process for different sulfur concentrations.

Fig. 4 shows the effects of sulfur concentration on the variations of ORP in the bioleaching process. From the figure it was evident that the oxidation of sulfur increased the ORP rapidly and then gradually approached a limiting value (+410 to +430 mV) within 8 days. The increase of ORP had a similar trend with decrease of pH. That is the higher the sulfur concentration was, the greater the rate of ORP increase was. Based on the pH-pe diagram established from the chemical conditions of the sediment slurry in the reactor,

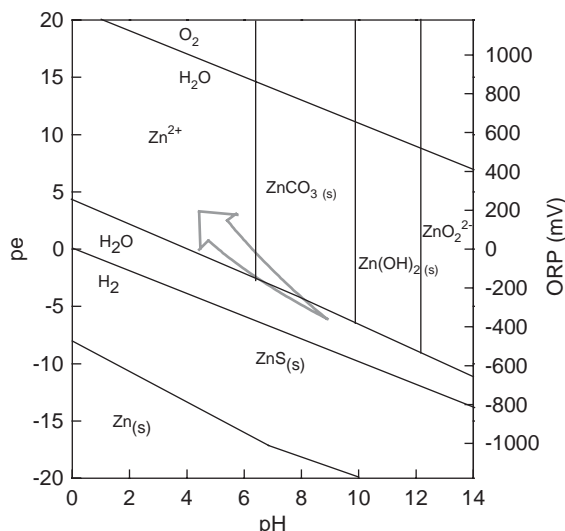


Fig. 5. Predominance area diagram in terms of pe and pH for Zn (the arrow indicates changes of pH and ORP caused by the bioleaching process:  $C_T = S_T = 10^{-2}$  M,  $Zn_T = 10^{-4}$  M) (modified from Theis and Hayes, 1980; Bourg, 1994).

low pH and high ORP conditions were found to favor the transformation of insoluble metal salts to soluble metal forms (taking Zn for example, Fig. 5). Therefore, the efficient solubilization of heavy metal needs an optimum condition of pH and ORP in the reactor during the bioleaching process.

### 3.3. Sulfur oxidation

It is believed that elemental sulfur is completely oxidized to sulfuric acid by *Thiobacilli* when elemental sulfur is used as a substrate (Tyagi et al., 1994). As a result sulfate ions start to accumulate in bioleaching process. Therefore, the sulfur oxidation can be directly correlated to sulfate formation. Fig. 6 shows the trend of sulfate production at different elemental sulfur concentrations. It is apparent from the figure that the concentration of sulfate ( $[SO_4^{2-}]$ ) increased linearly with time ( $t$ ), supporting the observations of Laishley et al. (1986) and Konishi et al. (1995). Therefore, the rate of sulfate ( $V_{\text{sulfate}}$ ) production in the bioleaching process can be calculated by the following equation:

$$V_{\text{sulfate}} = \frac{d[SO_4^{2-}]}{dt} = k_s, \quad (4)$$

where  $k_s$  is the rate constant of sulfate production (mg/l/d). Good correlation coefficients ( $R^2 > 0.97$ ) were obtained by Eq. (4), indicating that the variations of sulfate in bioleaching process conformed to the linear equation (Table 3). The adsorption of *Thiobacilli* on sulfur particles was a significant step for oxidation of elemental sulfur in this bioleaching process. The rate of

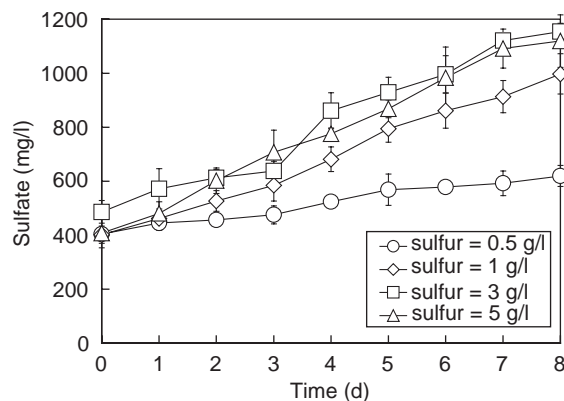


Fig. 6. Sulfate production in the bioleaching process for different sulfur concentrations.

Table 3

Predicted rate of sulfate production in the bioleaching process for different sulfur concentration

Sulfur (g/l)	$V_{\text{sulfate}}$ (mg/l/d)	$R^2$
0.5	27.3	0.98
1	71.0	0.99
3	90.0	0.97
5	93.4	0.99

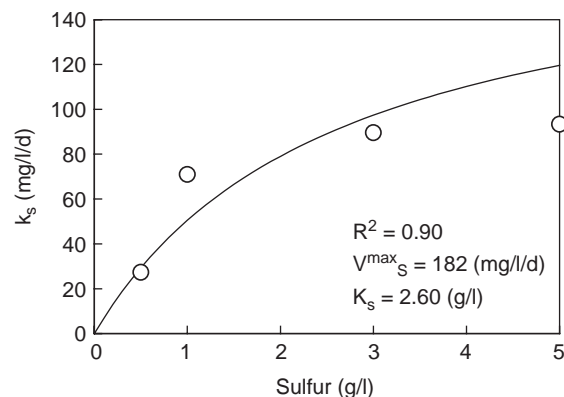


Fig. 7. Rate of sulfate production rate for different sulfur concentrations in the bioleaching process (the solid line is the fit to Michaelis–Menten type of equation).

sulfate production increased with increasing total surface area of sulfur (i.e. sulfur concentration) (Fig. 7). The results shown in Fig. 7 indicate that a Michaelis–Menten type of equation can best describe the effects of sulfur concentration on rate of sulfate production:

$$V_{\text{sulfate}} = \frac{V_S^{\max} S}{K_s + S}, \quad (5)$$

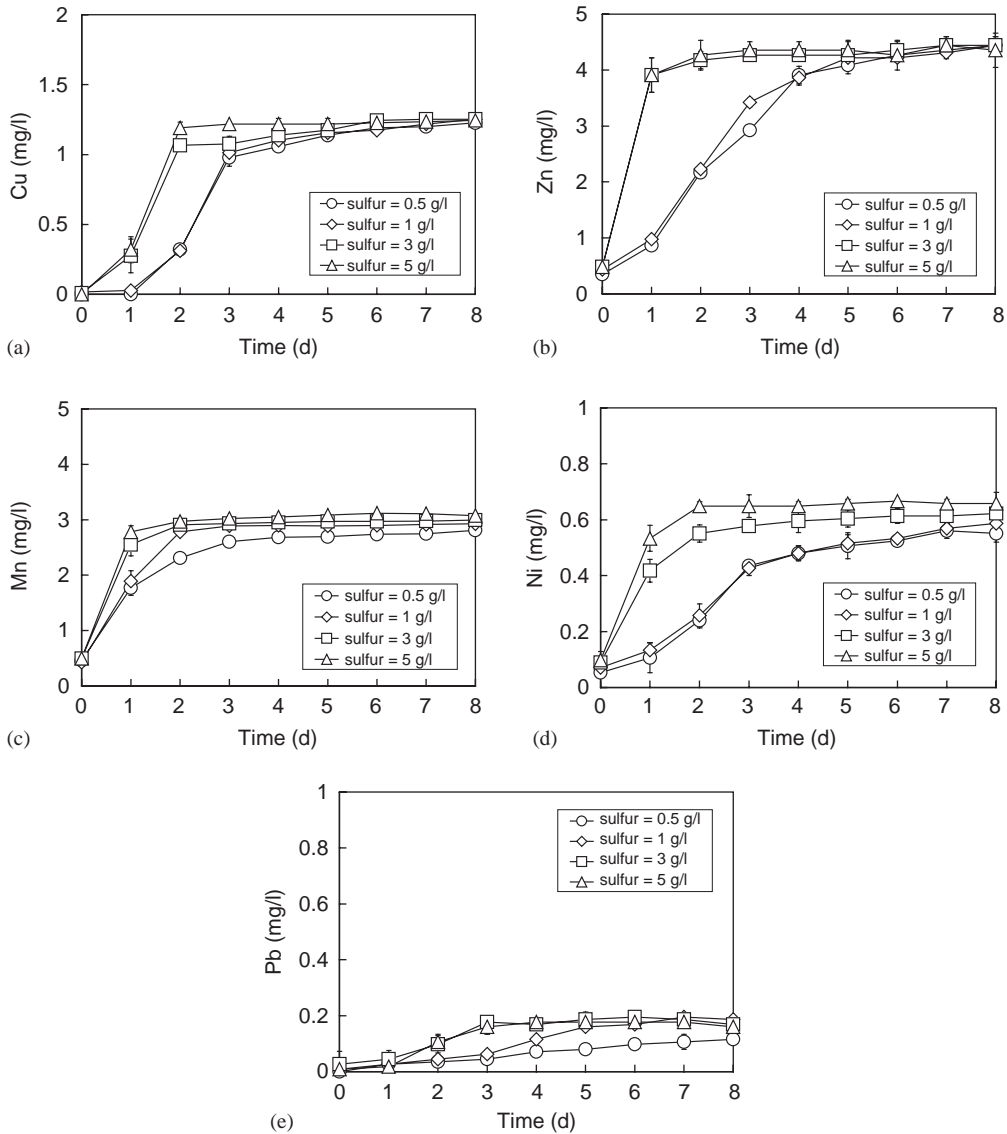


Fig. 8. Metal solubilization in the bioleaching process for different sulfur concentrations (a) Cu, (b) Zn, (c) Mn, (d) Ni and (e) Pb.

where  $K_s$  is the saturation constant (g/l),  $V_S^{\max}$  the maximum rate of sulfate production (mg/l/d) and  $S$  the sulfur concentration (g/l). The values of  $V_S^{\max}$  and  $K_s$  in Eq. (5) were determined to be 182 mg/l/d and 2.60 g/l, respectively. The Michaelis–Menten type of equation predicted from the estimated parameters values of  $V_S^{\max}$  and  $K_s$  was consistent with the experimental data (Fig. 7).

Based on the results in Fig. 6, it was found that about 48–88% of the sulfur powder added in the bioreactor was not oxidized (utilized) during the bioleaching process. The residual sulfur powder was necessary to be removed and recycled from the treated sediments

before its further disposal. However, the sulfur powder was not easy to be removed and recycled. Actually, the elemental sulfur in the form of large particle size is preferable for recycling in the bioleaching process, but its small specific surface area deters the performance of the bioleaching process. Some recoverable forms of sulfur for the bioleaching process were prepared in our previous study (Chen et al., 2003) and were proven to be reusable and helpful to the performance and cost effectiveness of the bioleaching process in the batch tests. These recoverable forms of sulfur can also be applied to the air-lift bioreactor for bioleaching in our future research.

### 3.4. Metal solubilization

For investigating the effect of sulfur concentration on metal solubilization in the bioleaching process, rates of metal solubilization were determined from the slopes of the initial linear section of the curves in Fig. 8. When the sulfur concentration was between 0.5 and 3 g/l, the rate of metal solubilization significantly increased as the sulfur concentration increased (Fig. 9). Then the rate of metal solubilization increased only slightly with the sulfur concentration of 5 g/l. It was observed that the trends of rate of metal solubilization in the bioleaching process also followed Michaelis–Menten type model:

$$V_{\text{metal}} = \frac{V_m^{\text{max}} S}{K_m + S} \quad (6)$$

where  $K_m$  is the saturation constant (g/l),  $V_m^{\text{max}}$  the maximum rate of metal solubilization (mg/l/d) and  $S$  the sulfur concentration (g/l). The estimated values of parameters ( $V_m^{\text{max}}$  and  $K_m$ ) for different metals were presented in Table 4. It was found that the maximum rate of metal solubilization was highly related to the initial metal contents in the sediment (Table 1). The rate of metal solubilization from contaminated sediment was

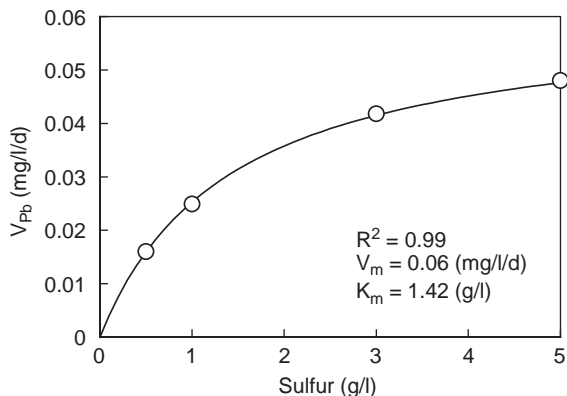


Fig. 9. Rate of Pb solubilization rate in the bioleaching process for different sulfur concentrations (the solid line is the fit to Michaelis–Menten type of equation).

Table 4

Parameters of Michaelis–Menten type of equation for metal solubilization in the bioleaching process

Metal	$V_m^{\text{max}}$ (mg/l/d)	$K_m$ (g/l)	$R^2$
Cu	0.43	0.19	0.81
Zn	1.96	0.66	0.81
Mn	1.31	0.19	0.91
Pb	0.06	1.42	0.99
Ni	0.17	0.26	0.90

Table 5

Percentage (%) of metal solubilization after 8 days of bioleaching

Metal	Sulfur concentration (g/l)			
	0.5	1	3	5
Cu	97	99	99	98
Zn	98	98	98	96
Mn	62	64	66	68
Pb	31	50	45	43
Ni	73	77	82	87

in the decreasing order of  $Zn > Mn > Cu > Ni > Pb$ . As the production of acid is considered as a rate limiting step in overall bioleaching process (Tichy et al., 1993), the rate of metal solubilization is affected by the rate of acid production. Therefore, the observed dependence of sulfur concentration on rates of sulfate production and metal solubilization followed the Michaelis–Menten type of equation. However, because the relationship between metal solubilization and pH is non-linear and not the same for each metal (Chen and Lin, 2001), the parameters listed in Table 4 and Fig. 7 were different.

The efficiency of solubilization of heavy metals after 8 days of bioleaching for different sulfur concentrations is listed in Table 5. From the table it was clear that the highest solubilization was measured for Cu and Zn, with about 96–99% of these two metals being solubilized for different sulfur concentrations after 8 days of bioleaching. Mn and Ni were solubilized by about 62–68% and 73–87%, respectively. Pb was the least solubilized (31–50%) among all metals after a period of 8 days. Metals in exchangeable, carbonate-bound and Fe/Mn oxide-bound speciation, which are contributed by anthropogenic pollution, are considered to be more mobile, dangerous and bioavailable. The organic matter/sulfide-bound, and residual metals are stable and non-bioavailable (Perin et al., 1997). According to the partition of metals in sediment (Table 2), Cu (85%) is mainly associated with sulfides/organic matter and this form of Cu can be efficiently oxidized (solubilized) by *Thiobacilli* (direct mechanism of bioleaching). On the other hand, Zn (55%) was predominantly found in more available and mobile fractions (i.e. exchangeable, carbonate and Fe/Mn oxides fractions). The above reasons explained why the efficiency of Cu and Zn solubilization was often high in the bioleaching of metal-contaminated sediment (Chen and Lin, 2000). Moreover, because of the formation of insoluble  $PbSO_4$  ( $K_{sp} = 1.62 \times 10^{-8}$ ), the efficiency of Pb solubilization was generally lower than other metals in the bioleaching process (Tyagi, 1992; Chen and Lin, 2001).



#### 4. Conclusions

The bioleaching process appears to be a potentially efficient technology for removing heavy metals from the sediment. It is very important to obtain the optimum operating parameters for the field application of this process. Among the factors affecting bioleaching process, the substrate concentration, i.e. sulfur concentration, is the most basic and significant parameter. The increasing of sulfur concentration resulted in the increased rates of acidification, sulfate production and metal solubilization. The Michaelis–Menten type of equation was able to describe the effects of sulfur concentration on both sulfate production and metal solubilization. In addition, the binding forms of each metal in sediment had a profound effect on metal solubilization efficiency in bioleaching process. Metals in loose binding forms (exchangeable, carbonates and Fe/Mn oxides) were more easily solubilized from the sediment than the organic matter/sulfide-bound, and residual metals. Though a high sulfur concentration generated an efficient acidification and metal solubilization in the bioleaching process, it also increased the operational costs and hindered the disposal of treated sediment. Based on the results obtained from this study, the preferable concentration of sulfur added was considered to be 3 g/l for the bioleaching process with indigenous sulfur-oxidizing bacteria in the air-lift reactor.

#### References

- Ahonen, L., Tuovinen, O.H., 1995. Bacterial leaching of complex sulfide ore samples in bench-scale column reactors. *Hydrometallurgy* 37, 1–21.
- APHA, AWWA and WEF, 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.
- Blais, J.F., Auclair, J.C., Tyagi, R.D., 1992. Cooperation between two *Thiobacillus* strains for heavy metal removal from municipal sludge. *Can. J. Microbiol.* 38, 181–187.
- Bond, P.L., Banfield, J.F., 2001. Design and performance of rRNA targeted oligonucleotide probes for in situ detection and phylogenetic identification of microorganisms inhabiting acid mine drainage environments. *Microb. Ecol.* 41, 149–161.
- Boseker, K., 1997. Bioleaching: metal solubilization by microorganisms. *FEMS Microbiol. Rev.* 20, 591–604.
- Bourg, A.C.M., 1994. Speciation of heavy metals in soils and groundwater and implications for their natural and provoked mobility. In: Salomons, W., Förstner, U., Mader, P. (Eds.), *Heavy Metals*. Springer, Berlin, pp. 19–31.
- Chen, S.Y., Lin, J.G., 2000. Influence of solid content on bioleaching of heavy metals from contaminated sediment by *Thiobacillus* spp. *J. Chem. Technol. Biotechnol.* 75, 649–656.
- Chen, S.Y., Lin, J.G., 2001. Bioleaching of heavy metals from sediment: significance of pH. *Chemosphere* 44, 1093–1102.
- Chen, S.Y., Chiu, Y.C., Chang, P.L., Lin, J.G., 2003. Assessment of recoverable forms of sulfur particles used in bioleaching of contaminated sediments. *Water Res.* 37, 450–458.
- Chisti, Y., Jauregui-Haza, U.J., 2002. Oxygen transfer and mixing in mechanically agitated airlift bioreactors. *Biochem. Eng. J.* 10, 143–153.
- Donati, E., Curutchet, G., Pogliani, C., Tedesco, P., 1996. Bioleaching of covellite using pure and mixed cultures of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. *Proc. Biochem.* 31, 129–134.
- Förstner, U., Calmano, W., 1998. Characterization of dredged materials. *Water Sci. Technol.* 38, 149–157.
- Hsu, C.H., Harrison, R.G., 1995. Bacterial leaching of zinc and copper from mining wastes. *Hydrometallurgy* 37, 169–179.
- Konishi, Y., Asai, S., Yoshida, N., 1995. Growth kinetics of *Thiobacillus ferrooxidans* on the surface of elemental sulfur. *Appl. Environ. Microbiol.* 61, 3617–3622.
- Laishley, E.J., Bryant, R.D., Kobryn, B.W., Hyne, J.B., 1986. Microcrystalline structure and surface area of elemental sulphur as factors influencing its oxidation by *Thiobacillus albertis*. *Can. J. Microbiol.* 32, 237–242.
- Lin, J.G., Chen, C.Y., Chen, S.Y., 1999. Effects of pH on metals speciation in a contaminated sediment. *J. Chin. Inst. Environ. Eng.* 9, 49–56.
- Peccia, J., Marchand, E.A., Silverstein, J., Hernandez, M., 2000. Development and application of small-subunit rRNA probes for assessment of selected *Thiobacillus* species and members of the genus *Acidiphilium*. *Appl. Environ. Microbiol.* 66, 3065–3072.
- Perin, G., Fabris, R., Manente, S., Wagener, A.R., Hamacher, C., Scotto, S., 1997. A five-year study on the heavy metal pollution of Guanabara bay sediments (Rio De Janeiro, Brazil) and evaluation of the metal bioavailability by means of geochemical speciation. *Water Res.* 31, 3017–3028.
- Porro, S., Ramirez, S., Reche, C., Curutchet, G., Alonso-Romanowski, S., Donati, E., 1997. Bacterial attachment: its role in bioleaching processes. *Proc. Biochem.* 32, 573–578.
- Ravishankar, B.R., Blais, J.F., Benmoussa, H., Tyagi, R.D., 1994. Bioleaching of metals from sewage sludge: elemental sulfur recovery. *J. Environ. Eng.* 120, 462–470.
- Rulkens, W.H., Grotenhuis, J.T.C., Tichy, R., 1995. Methods of cleaning contaminated soils and sediments. In: Salomons, W., Förstner, U., Mader, P. (Eds.), *Heavy Metals*. Springer, Berlin, pp. 151–191.
- Shrihari, Bhavaraju, S.R., Modak, J.M., Kumar, R., Gandhi, K.S., 1992. Dissolution of sulphur particles by *Thiobacillus ferrooxidans*: substrate for unattached cells. *Biotechnol. Bioeng.* 41, 612–616.
- Tessier, A.P., Campbell, G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.* 51, 844–851.
- Theis, T.L., Hayes, T.D., 1980. Chemistry of heavy metals in anaerobic digestion. In: Rubin, A.J. (Ed.), *Chemistry of Wastewater Technology*. Ann Arbor Science, Michigan, pp. 403–440.

- Tichy, R., Grotenhuis, J.T.C., Rulkens, W.H., 1993. Bioleaching of zinc-contaminated soils with *Thiobacilli*. In: Eijsackers, H.J.P., Hamers, T. (Eds.), *Integrated Soil and Sediment Research: a Basis for Proper Protection*. Kluwer Academic Publishers, Dordrecht, pp. 686–687.
- Tichy, R., Rulkens, W.H., Grotenhuis, J.T.C., Nydl, V., Cuypers, C., Fajtl, J., 1998. Bioleaching of metals from soils or sediments. *Water Sci. Technol.* 37, 119–127.
- Tyagi, R.D., 1992. Microbial leaching of metals from municipal sludge: effects of sludge solid concentration. *Proc. Biochem.* 27, 89–96.
- Tyagi, R.D., Blais, J.F., Deschenes, L., Lafrance, P., Ville-neuve, J.P., 1994. Comparison of microbial sulfuric acid production in sewage sludge from added sulfur and thiosulfate. *J. Environ. Qual.* 23, 1065–1070.
- USEPA, 1995. Microwave assisted acid digestion of sediments, sludge, and oils. Method 3052, US Environmental Protection Agency, Washington, DC.
- Vial, C., Poncin, S., Wild, G., Midoux, N., 2002. Experimental and theoretical analysis of the hydrodynamics in the riser of an external loop airlift reactor. *Chem. Eng. Sci.* 57, 4745–4762.
- Zhou, P., He, J., Qian, Y., 2003. Biofilm airlift suspension reactor treatment of domestic wastewater. *Water Air Soil Pollut.* 144, 81–100.