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Effect of substrate concentration on bioleaching of metal-contaminated sediment

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Abstract

The remediation of metal-contaminated sediment was studied using the bioleaching process with a mixed culture of sulfur-oxidizing bacteria. The effects of substrate concentration (elemental sulfur) on sediment acidification, sulfur oxidation and metal solubilization from contaminated sediment during the bioleaching process were investigated with free-cell suspensions. Sulfur concentration greater than 0.5% (w/v) was found to be inhibitory to bacterial activity and metal solubilization from sediment. The sulfate production was well described by a substrate inhibition expression and Haldane's equation. In addition, an empirical equation related to sulfur concentration was also used to describe the metal solubilization in the bioleaching process. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Bioleaching; Contaminated sediment; Heavy metal; Sulfur concentration; Thiobacilli

1. Introduction

Recently, the two serious problems related to sediments are huge volumes of dredged materials and high concentrations of toxic substances [1]. Contaminated sediments containing high concentrations of toxic pollutants may pose risks to both human health and environmental quality. Such sediments have been frequently found in the streams, rivers and lakes of Taiwan because of high industrialization. The contaminated sediments usually have to be dredged for water quality and waterways management reasons. The management of contaminated sediment has become one of the most important issues faced by environmental scientists and managers. For resolving the problems caused by contaminated sediments, suitable techniques for detoxification and decontamination of large quantities of contaminated sediments during the remediation of contaminated rivers have to be developed.

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Nomenclature

A_0, A_1	Constants
k_m	rate constant of metal solubilization (per day)
k_s	rate constant of sulfate production (mg/l per day)
K_i	inhibition constant (%, w/v)
K_s	Michaelis constant (%, w/v)
M	weight of metal in the aqueous phase (mg)
M_{s0}	initial weight of metal in the sediment (mg)
S	sulfur concentration (%, w/v)
SO_4^{2-}	sulfate concentration (mg/l)
t	time (days)
$V_{SO_4^{2-}}$	sulfate production rate (mg/l per day)
$V_{SO_4^{2-}\text{-max}}$	maximum sulfate production rate (mg/l per day)

In general, contaminated sediments are very similar to contaminated soils with respect to the physical and chemical characteristics. It shows that technologies potentially applicable for treatment of contaminated soils can be adapted to treat the contaminated sediments. The remediation of metal-contaminated sediments can be accomplished either by physical or chemical methods such as chemical extraction, thermal treatment and solidification/stabilization. The chemical extraction of contaminated sediment has been extensively applied in practice, but it has some limitations [2]. Bioremediation of heavy metals has recently gained increased attention since it is innovative, environmentally friendly and economical. Bioleaching is a simple, economical and effective process for metal solubilization from industrial wastes or biosolids [3–7]. In particular, this process is performed by the application of the sulfur cycle mediated by bacteria. Metal solubilization from solid wastes or other solids is achieved through the activity of some chemolithotrophic bacteria, including *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans* and *Thiobacillus thioparus*, which can catalyze the oxidation of reduced sulfur compounds to sulfuric acid causing pH lowering. The bioleaching process also has some potential for removal of heavy metals from contaminated sediments. Recently, a bioleaching process with a mixed culture of two thiobacilli, *Thiobacillus thioparus* and *Thiobacillus thiooxidans*, has been developed for remediation of metal-contaminated sediments [8]. The bioleaching 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 [9]. Consequently the main mechanisms involved in bioleaching of heavy metals by *Thiobacillus thiooxidans* and *Thiobacillus thioparus* are direct (Eq. (1)) and indirect (Eqs. (2) and (3)) mechanism, which can be described by the following equations [2]:

$$MS + 2O_2 \xrightarrow{\text{thiobacilli}} MSO_4 \tag{1}$$

$$S^0 + H_2O + 1.5O_2 \xrightarrow{\text{thiobacilli}} H_2SO_4 \tag{2}$$

$$H_2SO_4 + \text{sediment-M} \rightarrow \text{sediment-2H} + MSO_4 \tag{3}$$

where M is a bivalent metal.

Most of the physical, chemical and biological parameters have an influence on the bioleaching process. A complete understanding of the parameters that control the bioleaching process is important to optimize the process. It is well known that certain bacteria participating in the removal of heavy metals from contaminated sediments, can utilize reduced sulfur compounds as energy sources and have the ability to tolerate the high concentrations of heavy metals and hydrogen ions in which other bacteria cannot exist. In this bioleaching process, elemental sulfur is generally added as the substrate for thiobacilli. The acid production and pH variation in the bioleaching process are influenced by the concentration of added sulfur. However, the added elemental sulfur is not completely oxidized to sulfuric acid and the remaining sulfur will lead to the reacidification of treated sediments or soils during the final disposal [10]. It is therefore, necessary to optimize sulfur concentration added in the bioleaching process. In the present work, effects of sulfur concentration on the acid production, pH lowering and metal solubilization in the bioleaching process while remediating the metal-contaminated sediments were investigated.

2. Experimental

2.1. Microorganisms

Two strains of thiobacilli (*Thiobacillus thiooxidans* (CCRC 15612) and *Thiobacillus thioparus* (CCRC 15623)), from the culture collection and research center (CCRC) of the food industry research and development institute (FIRDI) in Hsinchu, Taiwan, were used throughout this study. The medium 317 and medium 318 according to FIRDI [11] were employed for growth and maintenance of *Thiobacillus thiooxidans* and *Thiobacillus thioparus*, respectively. The cultures were incubated for subculture in a shake flasks at 200 rpm and at 30°C before use in leaching experiments.

2.2. Bioreactor operation

The bioleaching experiments were carried out with a metal-contaminated sediment obtained from the downstream (Nan Ding Bridge) of the Ell Ren river in Taiwan. The Ell Ren river, located in southern Taiwan, has a catchment area of about 350 km^2 and total length of 65 km. Since receiving many municipal, industrial and agricultural wastewater from uncontrolled effluents, its downstream part has been highly polluted by heavy metals. Initially, the subculture of thiobacilli was acclimated to the environment with contaminated sediment and elemental sulfur. In this acclimation process, a mixed inoculum of 1% (v/v) of 5-day-old subculture of *Thiobacillus thiooxidans* and *Thiobacillus thioparus* was transferred to 150 ml of 2% (w/v) total solids of autoclaved sediment suspension with 0.5% (w/v) tyndallized elemental sulfur. Cultures were incubated in a 500 ml shake flask at 30°C and 200 rpm. The acclimation of thiobacilli in contaminated sediment was accomplished until the sediment pH dropped to 2.0. The bioleaching experiments were conducted in a 3 l completely mixed batch (CMB) reactor (Fig. 1) agitated by stirring at 200 rpm to maintain solids suspension. The reactor was also aerated with an air diffuser at a flow of 1.2 l/min. For all experiments the sediment solids concentration was 2% (w/w). The inoculum was 5% (v/v) of growing mixed culture of thiobacilli obtained from the acclimation process.

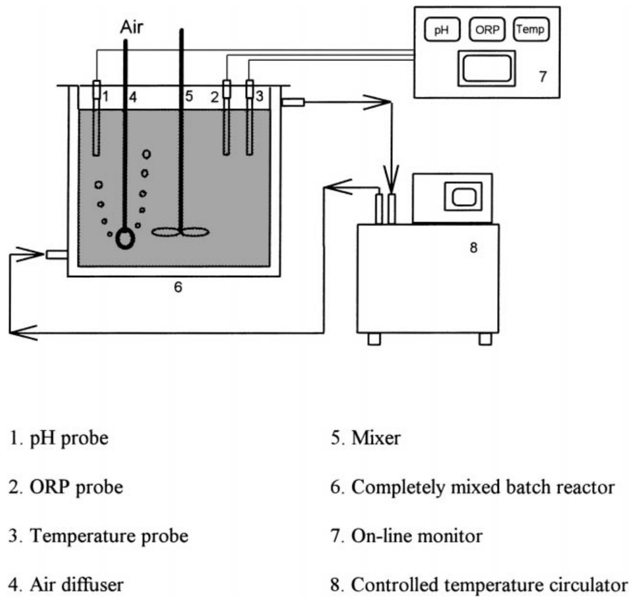

Fig. 1. Schematic diagram of the bioleaching experiment.

Various contents (0.1, 0.25, 0.375, 0.5, 0.75 and 1% (w/v)) of tyndallized elemental sulfur were fed into the CMB reactor, which was maintained at 30°C. The changes during each bioleaching experiment were monitored by periodic sampling and analyzing the sediment suspension for pH, ORP, sulfate and soluble heavy metals (Cu, Zn, Mn, Pb, Ni and Cr).

2.3. Analyses

The total and volatile solids of sediment were determined according to Standard Methods [12]. Sediment pH was determined using 25 ml of 1.0 M KCl added into 10 g samples. The samples were well shaken and the pH was immediately determined [13]. The concentrations of heavy metals in the sediment were determined by a HF–HNO_3–HCl microwave-digestion method [14]. In the bioleaching experiment, pH and ORP in the bioreactor were measured with an on-line monitor (Tank, model RD-500). The sediment suspension taken from the bioreactor was centrifuged at a speed of 10,000 rpm for 20 min, and then filtered through a 0.45 μm membrane. The filtrate was analyzed for its sulfate concentrations according to Standard Methods [12] and heavy metal concentrations. In this study, all heavy metals were determined using a flame/graphite atomic absorption spectrophotometer (model Z-8100, Hitachi).

3. Results and discussion

The characteristics of sediment used in this study are shown in Table 1. The contaminated sediment had the following characteristics. Total solids: 72.43% (w/w); volatile solids

Table 1
The characteristics of the contaminated sediment

Parameter	Value[a]
Total solids	72.43 ± 0.08%
Volatile solids	3.51 ± 0.09%
pH	7.85 ± 0.10
Metal	
Cu	190.83 ± 6.69 μg/g
Zn	400.91 ± 24.68 μg/g
Mn	424.10 ± 14.11 μg/g
Pb	142.92 ± 10.54 μg/g
Ni	49.67 ± 2.27 μg/g
Cr	74.08 ± 0.01 μg/g

[a] Mean ± S.D. ($n = 12$).

(organic matter): 3.51% (w/w); pH: 7.85; Cu: 190.83 μg/g; Mn: 424.10 μg/g; Zn: 400.91 μg/g; Pb: 142.92 μg/g; Ni: 49.67 μg/g and Cr: 74.08 μg/g.

3.1. Sediment acidification during bioleaching process

Fig. 2 shows the variation of pH in the bioreactor during the bioleaching process. As thiobacilli have grown on elemental sulfur, sulfuric acid accumulated in the bioreactor and the sediment pH decreased from the initial pH of 8.0 to a final value of 2.5 in 35, 20, 20, 13, 28 and 30 days for sulfur concentrations of 0.1, 0.25, 0.375, 0.5, 0.75 and 1% (w/v), respectively. The rate of pH reduction increased with the increasing sulfur concentrations in the bioreactor until it was above 0.5% (w/v). Due to the insolubility of sulfur, the microbial oxidation of elemental sulfur is considered to take place with the adsorption of bacteria onto

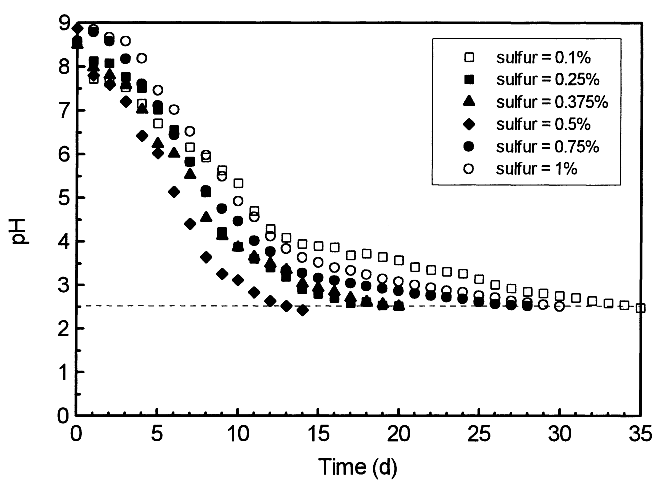

Fig. 2. Variation in pH during the bioleaching process with different sulfur concentrations.

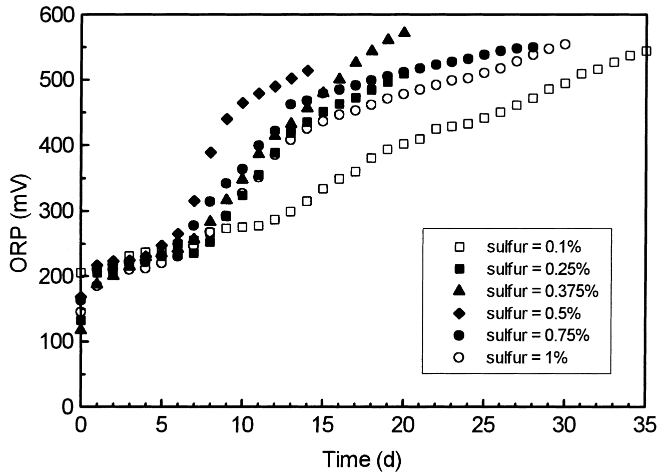

Fig. 3. Variation in ORP during the bioleaching process with different sulfur concentrations.

the solid substrate by Van der Waals attractive forces and then metabolism of sulfur [15]. Greater surface area of the sulfur particles leads to an increase in the adsorption of bacteria onto sulfur particles and sulfur is oxidized. However, the oxidation of elemental sulfur was inhibited by a higher sulfur concentration (greater than 0.5% (w/v)).

In addition to the acidification of sediment, the oxidation of sulfur caused an increase in the oxidation–reduction potential (ORP) of sediment during the bioleaching process. It was found that mere lowering the pH of anaerobic sludge by acidification did not result in a shift of metal sulfides to the soluble ionic forms unless it was preceded by a rise in the sludge ORP [16]. Therefore, solubilization of heavy metal requires an optimum adjustment of pH and ORP of the sediment so that the chemical equilibrium will be shifted in favor of soluble metallic ion formation. The variations in ORP during the bioleaching process are presented in Fig. 3. Acidification of the sediment and aeration increased the ORP significantly in the bioreactor. The ORP values increased to 510–570 mV when the final pH of 2.5 was reached. In this study, the ORP values depended on the changes in the pH. Similarly, the increase of ORP is faster when the sulfur concentration is higher. It became slower when sulfur concentration is greater than 0.5% (w/v), however.

3.2. Sulfur oxidation in bioleaching

Adsorption of bacteria onto sulfur particles is the primary and essential step for oxidation of elemental sulfur during this bioleaching process. Greater surface area of sulfur favors the oxidation of sulfur. Fig. 4 shows the oxidation of sulfur in the bioleaching process. It was found that the metabolic final product, sulfate was produced linearly with time [17,18] and the rate of sulfate production was calculated using Eq. (4).

$$\frac{\mathrm{d}\,SO_4{}^{2-}}{\mathrm{d}t} = k_\mathrm{s} \tag{4}$$

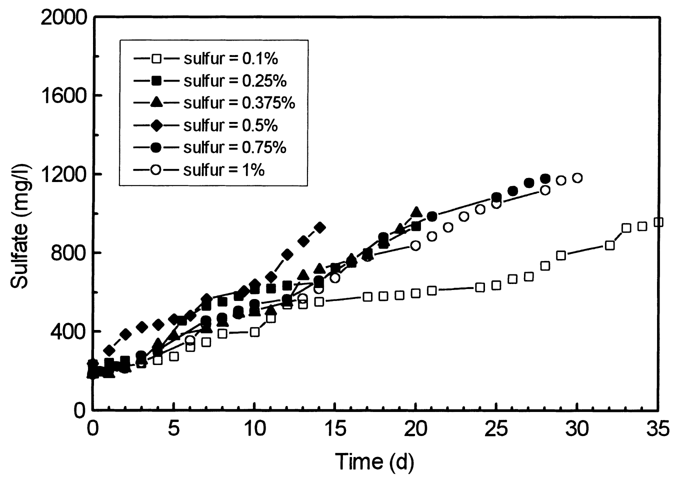

Fig. 4. Sulfate production during the bioleaching process with different sulfur concentrations.

where k_s is the rate constant of sulfate production (mg/l per day). The rate constants of sulfate production are shown in Table 2. The correlation coefficients (R^2) obtained in the regression analysis of Eq. (4) were greater than 0.95 for different feeding sulfur concentrations. The maximum sulfate production rate was recorded for a sulfur concentration of 0.5% (w/v) and a substantial decline in sulfate production rate was observed when the sulfur concentration increased above 0.5% (w/v). It was found that the oxidation of elemental sulfur is inhibited by an excess substrate. The observed dependence of sulfate production rates on sulfur concentration, as shown in Fig. 5, is characteristic of substrate inhibition. The solid lines shown on the figure represent the best fit of Haldane's equation:

$$\frac{\mathrm{d}\,SO_4{}^{2-}}{\mathrm{d}t} = V_{SO_4{}^{2-}} = \frac{V_{SO_4{}^{2-}\text{-max}}}{1 + (K_s/S) + (S/K_i)} \qquad (5)$$

where $V_{SO_4{}^{2-}\text{-max}}$ is the maximum sulfate production rate (mg/l per day), K_s is the Michaelis constant (%, w/v), S is the sulfur concentration (%, w/v) and K_i is the inhibition constant (%, w/v). The rate equation parameters, evaluated by fitting Eq. (5) to the data of different sulfur concentration, are $V_{SO_4{}^{2-}\text{-max}} = 113.64$ mg/l per day, $K_s = 0.43\%$ (w/v), and

Table 2
Rate constants of sulfate production in the bioleaching process with various concentrations of elemental sulfur

Sulfur (%, w/v)	k_s (mg/l per day)	R^2
0.1	20.14	0.96
0.25	35.60	0.97
0.375	39.63	0.98
0.5	43.72	0.95
0.75	36.76	0.97
1.0	35.10	0.99

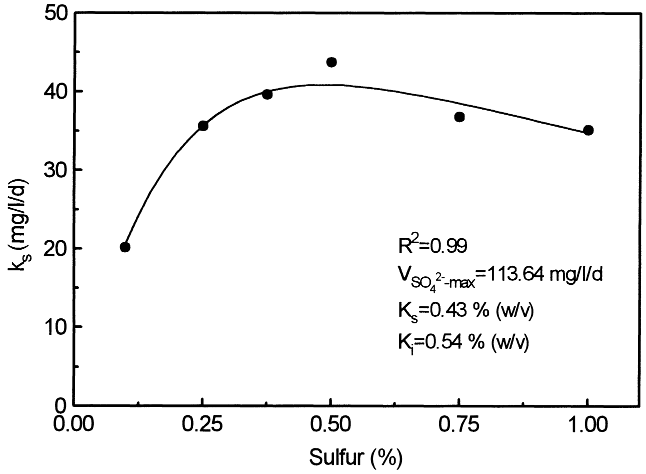

Fig. 5. Variation in sulfate production rate with sulfur concentration during the bioleaching process.

$K_i = 0.54\%$ (w/v), respectively. The observed inhibitory effect of sulfur concentration on bacterial growth, as indicated by the sulfate production rates, is in agreement with the results of Jain and Tyagi [19] and Sreekrishnan et al. [20]. There was an increase in sulfate production rate as the sulfur concentration increased from 0 to 0.5% (w/v), but the inhibitory effect of sulfur concentration on sulfate production rate was not found in both investigations.

3.3. Metal solubilization in bioleaching

Fig. 6 shows the solubilization of heavy metals from contaminated sediment during the bioleaching process. It was found that there were lag phases for heavy metals to solubilize from sediment with lower sulfur concentrations. This is because surface of sulfur was lower for bacterial adsorption and the rate of sulfate production was slower in the bioreactor with lower sulfur concentration, it requires more reaction time to reach the pH at which heavy metals can be leached from the sediment. It was shown (Fig. 6(a)) that Cu had good solubilization (85–95%) efficiency during the bioleaching process. The efficiency of Cu solubilization from the contaminated sediment in the present study was much better than when compared with the Cu solubilization from the sludge with the same bioleaching process. [8]. In general, the efficiency of Cu solubilization is not significantly affected by sulfur concentration when the sulfur concentration increases upto 1% (w/v). The feeding sulfur concentration can only affect the rate of metal solubilization in the bioleaching process. The results of other heavy metals (Zn, Mn, Pb, Ni and Cr) are similar to that of Cu (Fig. 6(b)–(f)). The efficiency of metal solubilization from contaminated sediment is in the decreasing order: Cu>Zn>Mn>Pb>Ni>Cr. The efficiency of Pb solubilization is not high (39–45%) in the bioleaching process fed with elemental sulfur because the solubilized Pb can form into low solubility of $PbSO_4$ ($K_{sp} = 1.62 \times 10^{-8}$) with sulfate. A large part (77%) of Cr mainly exists in the crystal lattices of sediment and it is just solubilized from the sediment in an extremely acidic condition [8,21], so the efficiency of Cr solubilization is the lowest (12–20%). The remaining concentrations of heavy metals in sediment after the treatment by the bioleaching process are shown in Table 3. Overall, most of the heavy metals (such as Cu, Zn and Cr) in the contaminated sediment are cleaned satisfactorily and the remaining concentrations of these three metals are unlikely to be toxic. The remaining concentrations of Pb and Ni in sediment seem to be somewhat unsatisfactory, however.

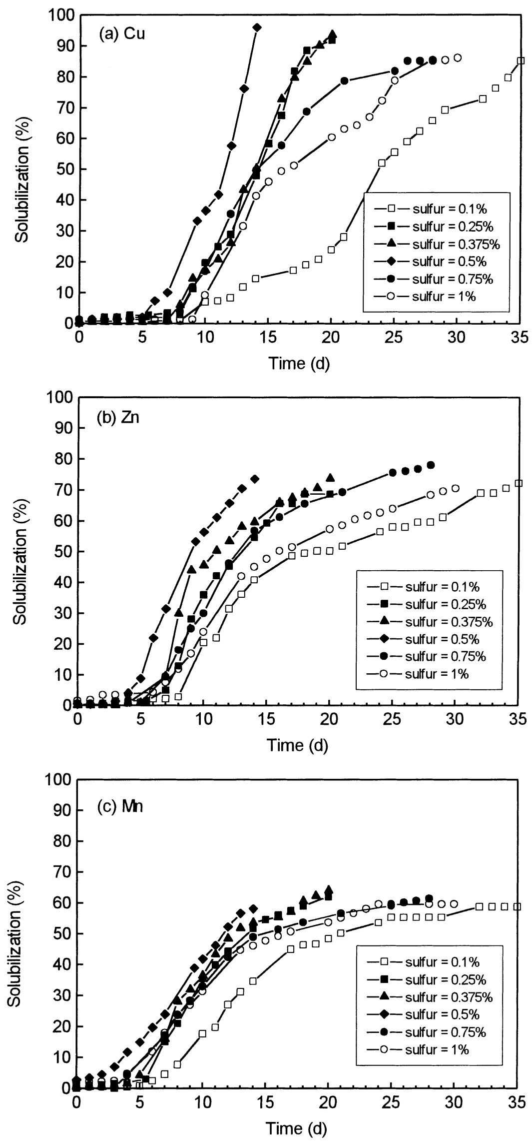

Fig. 6. Metal solubilization from sediment during the bioleaching process with different sulfur concentrations: (a) Cu; (b) Zn; (c) Mn; (d) Pb; (e) Ni; (f) Cr.

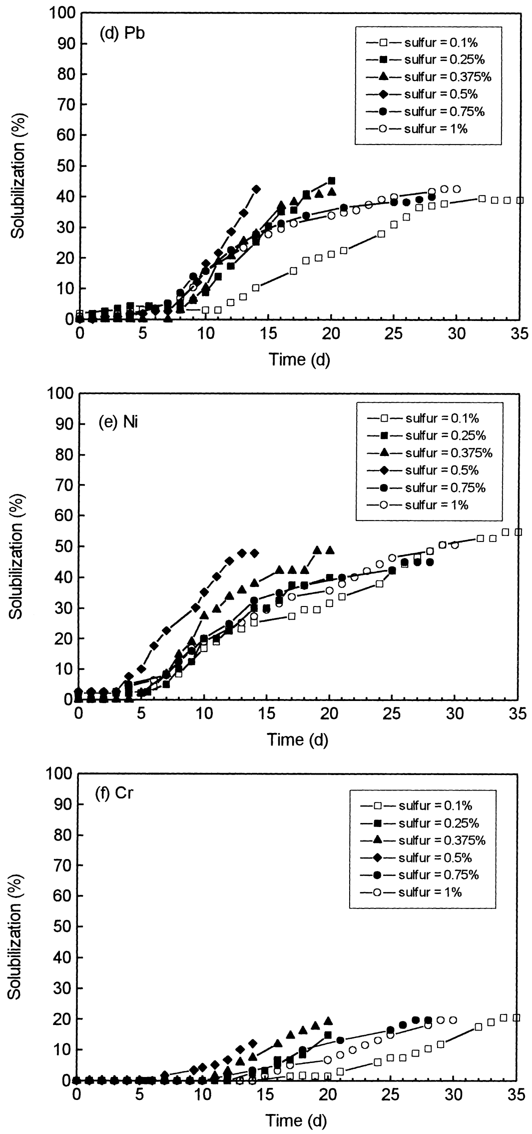

Fig. 6. (*Continued*).

The solubilization of heavy metal can be described by the following empirical equation in the bioleaching process:

$$\frac{dM}{dt} = k_m(M_{s0} - M) \tag{6}$$

where k_m is the rate constant of metal solubilization (per day), M_{s0} and M are the initial weight of metal in the sediment (mg) and weight of metal in the aqueous phase (mg),

Table 3
The concentrations (μg/g) of heavy metal in sediment after the treatment by the bioleaching process[a]

Metal	Sulfur (%, w/v)						Effects range	
	0.1	0.25	0.375	0.5	0.75	1	Low[b]	Medium[b]
Cu	28.35 (85)[c]	15.76 (92)	12.34 (93)	7.77 (96)	28.26 (85)	26.63 (86)	34	270
Zn	111.65 (72)	125.60 (69)	105.36 (74)	106.04 (74)	88.07 (78)	117.94 (71)	150	410
Mn	175.07 (59)	161.45 (62)	153.06 (64)	177.82 (58)	163.96 (61)	171.51 (60)	–	–
Pb	87.35 (39)	78.32 (45)	83.88 (41)	82.24 (42)	85.78 (40)	82.05 (43)	47	220
Ni	23.50 (53)	28.34 (43)	25.60 (48)	25.95 (48)	27.35 (45)	24.55 (51)	21	52
Cr	58.90 (20)	63.10 (15)	59.99 (19)	65.43 (12)	59.43 (20)	59.43 (20)	81	370

[a] Concentrations are obtained by subtracting the amount of solubilized metal from initial concentration of metal in sediment.

[b] Effects range-low are bulk sediment concentrations (dry-weight) below which sediments are unlikely to be toxic. Effects range-medium are concentrations above which toxicity is probable [23].

[c] Numbers in parentheses represent the efficiency (%) of metal solubilized from sediment in the bioleaching process.

respectively. The rate constants of metal solubilization in the bioleaching process are presented in Table 4. The correlation coefficients (R^2) obtained in the regression analysis of Eq. (6) were greater than 0.91 for different sulfur concentrations. The rate of metal solubilization increased up to 0.5% (w/v) sulfur concentration above which the rate of metal solubilization decreased. It is believed that the production of acid is a limiting step for the bioleaching process, using sulfur as a substrate [22]. The rate of metal solubilization from sediment will be affected by the rate of acid production in the bioleaching process fed with elemental sulfur. It is understood that there are some similarities between the rate of metal solubilization (Fig. 7) and sulfate production (Fig. 5). The rate of metal solubilization from sediment was inhibited by a higher sulfur concentration in the bioleaching process. Unfortunately, the effect of sulfur concentration on the rate of metal solubilization cannot be expressed by the same Haldane's equation (Eq. (5)). However, the rate of metal solubilization increased linearly with sulfur concentration in the sulfur concentration range from 0.1% (w/v) to 0.5% (w/v), as shown in Fig. 7. Therefore, when the feed sulfur concentration

Table 4
Rate constants of metal solubilization in the bioleaching process with various concentrations of elemental sulfur

Metal	Sulfur (%, w/v)											
	0.1		0.25		0.375		0.5		0.75		1.0	
	k_m (per day)	R^2	k_m (per day)	R^2	k_m (per day)	R^2	k_m (per day)	R^2	k_m (per day)	R^2	k_m (per day)	R^2
Cu	0.067	0.92	0.209	0.91	0.227	0.92	0.257	0.75	0.108	0.98	0.093	0.98
Zn	0.041	0.97	0.083	0.97	0.091	0.97	0.122	0.98	0.066	0.97	0.049	0.97
Mn	0.032	0.93	0.060	0.97	0.061	0.97	0.066	0.96	0.038	0.93	0.035	0.92
Pb	0.017	0.94	0.029	0.86	0.043	0.96	0.052	0.90	0.030	0.94	0.022	0.96
Ni	0.025	0.98	0.031	0.96	0.042	0.97	0.062	0.99	0.034	0.96	0.025	0.97
Cr	0.012	0.93	0.019	0.93	0.022	0.99	0.026	0.91	0.014	0.99	0.013	0.99

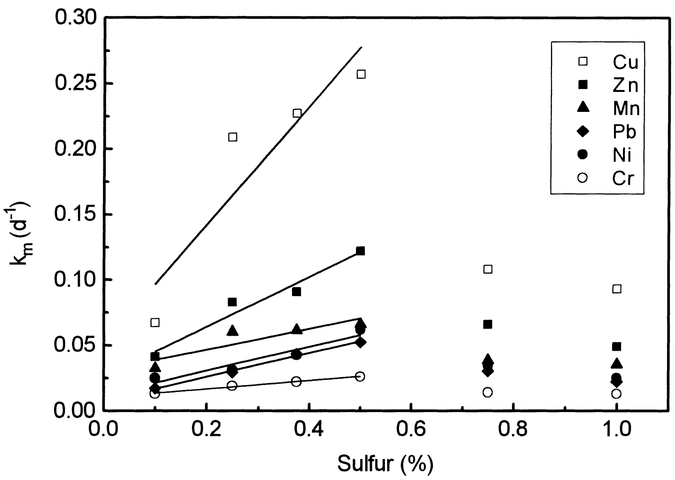

Fig. 7. Variation in metal solubilization rate with sulfur concentration during the bioleaching process.

Table 5
The linear relationship between rate constant of metal solubilization and sulfur concentration

Metal	Linear equation	R^2
Cu	$k_m = 0.0514 + 0.4527S$	0.86
Zn	$k_m = 0.0259 + 0.1906S$	0.96
Mn	$k_m = 0.0304 + 0.0796S$	0.81
Pb	$k_m = 0.0078 + 0.0897S$	0.99
Ni	$k_m = 0.0121 + 0.0909S$	0.92
Cr	$k_m = 0.0103 + 0.0318S$	0.99

is less or equal to 0.5% (w/v), the kinetic equation (Eq. (6)) describing the solubilization of heavy metal in the bioleaching process can be modified into the following equation:

$$\frac{dM}{dt} = (A_0 + A_1 S)(M_{s0} - M) \quad (7)$$

where A_0 and A_1 are the constants. The linear relationship between the rate of metal solubilization and sulfur concentration in the bioleaching process is shown in Table 5. As shown in Table 5, the constant, A_1, reflects the extent of effect of sulfur concentration on the rate of metal solubilization in the bioleaching process. It was found that the rate of Cu solubilization in the bioleaching process is most easily affected by the feeding sulfur concentration. Conversely, the sulfur concentration has considerably less effect on the rate of Cr solubilization in the bioleaching process.

4. Conclusions

The bioleaching process is a promising alternative for the treatment of metal-contaminated sediments. Elemental sulfur is an essential substrate for the growth of thiobacilli and bacterially catalyzed metal solubilization in the bioleaching process. The effects of sulfur concentration on the performance of a bioleaching process with a mixed culture of sulfur-oxidizing bacteria were evaluated in this study. Bacteria attachment on sulfur particles is an important step for oxidation of elemental sulfur in the bioleaching process. Greater surface area of sulfur accelerates the oxidation of sulfur and, consequently, metal solubilization. When the sulfur concentration is greater than 0.5% (w/v) the rates of sulfur oxidation and metal solubilization decrease. Almost all the concentrations of heavy metals are decontaminated to the nontoxic levels except Pb. The metal solubilization from sediment in the bioleaching process can be expressed in terms of a sulfur concentration related kinetic equation. The results obtained in this study show that the optimal sulfur concentration for sediment acidification and metal solubiliztion is found to be 0.5% (w/v) in the bioleaching process.

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