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# Bioleaching of heavy metals from livestock sludge by indigenous sulfur-oxidizing bacteria: effects of sludge solids concentration

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## Abstract

A technologically and economically feasible process called bioleaching was used for the removal of heavy metals from livestock sludge with indigenous sulfur-oxidizing bacteria in this study. The effects of sludge solids concentration on the bioleaching process were examined in a batch bioreactor. Due to the buffering capacity of sludge solids, the rates of pH reduction, ORP rise and metal solubilization were reduced with the increase of the solids concentration. No apparent influence of solids concentration on sulfate produced by sulfur-oxidizing bacteria was observed when the solids concentration was less than 4% (w/v). A Michaelis–Menten type of equation was able to well describe the relationship between solids concentration and rate of metal solubilization. Besides, high efficiencies of metal solubilization were achieved after 16 d of bioleaching. Therefore, the bioleaching process used in this study could be applied to remove heavy metals effectively from the livestock sludge.


*Keywords:* Bioleaching; Heavy metal; Livestock sludge; Solids concentration; Sulfur-oxidizing bacteria

## 1. Introduction

Because of land limitation in Taiwan, the livestock farming tends toward the indoor system instead of pasture-based system. The uncontrolled wastewater discharged from livestock farms was one of the main pollution sources of rivers in Taiwan. Recently, because of the promotion of high-tech livestock farming, most wastewater treatment plants of livestock farms are guided by the government for protection of water quality of rivers. Therefore, the management of waste sludge produced from the wastewater treatment plants becomes the most important issue of environmental protection for the livestock farming. The livestock sludge often contains high concentrations of organic matter and nutrients. The application of these livestock sludges on the agricultural lands as soil conditioners or fertilizers has the benefits of resource recycling and waste minimization from livestock farms. However, for economical purposes, some metal salts are added into the feedstuffs to stimulate the growth of pigs and control the pork quality in Taiwan. Consequently, high contents of heavy metals are found in sludges produced from livestock farms (Hsu and Lo, 1999). The presence of elevated heavy metals in livestock sludge will limit the land application of livestock sludges. In order to resolve the above problems, it is important to develop a suitable and economical technology for treatment of the large quantity of livestock sludge for recycling these sludges in land application as soil fertilizers.

The physical and chemical technologies for removal of heavy metals from sludge have been well documented.

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Although these physical and chemical treatment techniques have been extensively applied in practice, they show some limitations such as low efficiency and high cost (Rulkens et al., 1995). Generally, biotechnology is a powerful and versatile alternative to chemical and physical methods for resolving many problems of environmental pollution because of low demand for energy and materials, and low generation of waste and emissions. A bioleaching process has been recently developed to remove heavy metals from sludges, sediments and soils (Sreekrishnan and Tyagi, 1996; Tichy et al., 1998; Chen and Lin, 2000, 2001). 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 (Rulkens et al., 1995). The microorganisms intensively used in bioleaching belong to the genus *Thiobacillus*. The oxidation and acid producing activity of sulfur-oxidizing bacteria are the primary mechanisms of solubilization of heavy metals in the bioleaching process. These sulfur-oxidizing bacteria can obtain energy from the oxidation of elemental sulfur or reduced inorganic sulfur, and cause bioacidification and solubilization of heavy metals. This process is a novel technology for treatment of heavy metals from sludges by application of the sulfur biocycle.

The performance of bioleaching process is affected by various physical, chemical and biological parameters (Jensen and Webb, 1995). Among these affecting parameters, solids concentration plays an important role in optimization of the bioleaching process. Because of buffering capacity, the variation of pH during the bioleaching process was found to be governed by solids concentration in the system (Chen and Lin, 2000). However, pH has been considered to be a key factor in determining the solubilization of heavy metals in the bioleaching process (Chen and Lin, 2001). Moreover, it was found that the costs of sludge treatment and disposal increased with increasing solids concentration treated in the bioleaching process (Sreekrishnan and Tyagi, 1996). Therefore, a thorough understanding of this affecting parameter is useful to optimize the bioleaching process. The purpose of this study is to develop a bioleaching process for removal of heavy metals from livestock sludges. At the same time, the effects of sludge solids concentration on this bioleaching process were investigated in this study.

## 2. Materials and methods

### 2.1. Livestock sludge collection

In Taiwan, a three-step treating system, including solid–liquid separation, anaerobic treatment process and activated sludge process, is the most typical and suitable wastewater treatment system for pig farms. The livestock sludge used in this study was collected from the anaerobic baffled reactor of the wastewater treatment plant of the pig farm of Hsinchu Branch of the Taiwan Livestock Research Institute in Hsinchu, Taiwan. The sludge was well mixed and stored at 4 °C before use. The general properties, total solids, volatile solids (APHA, 1995), pH (LaBauve et al., 1988) and total metal contents (USEPA, 1995) of the livestock sludge were determined and are listed in Table 1.

Table 1
The characteristics of the livestock sludge

| Parameter | Value[a] |
|---|---|
| Total solids ($mg\,l^{-1}$) | 29 200 ± 1200 |
| Volatile solids ($mg\,l^{-1}$) | 10 400 ± 300 |
| pH | 7.90 ± 0.10 |
| *Metal ($mg\,kg^{-1}$ dry weight)* | |
| Cu | 382 ± 28 |
| Zn | 3283 ± 425 |
| Mn | 1400 ± 15 |
| Pb | 40 ± 5 |
| Ni | 33 ± 5 |
| Cr | 152 ± 8 |

[a] Mean ± standard deviation ($n = 6$).

### 2.2. Microorganisms and inoculum

In the activation process of the indigenous sulfur-oxidizing bacteria, 3 l livestock sludge (solids concentration: 2% (w/v)) and 0.5% (w/v) tyndallized elemental sulfur were placed in a completely mixed batch reactor and mixed at 200 rpm and 30 °C. The pH of the sludge was monitored. When pH dropped to 2.0, 150 ml acidified sludge was then transferred to 3 l fresh sludge with 0.5% elemental sulfur under the same conditions. The inoculum for the bioleaching experiment was obtained by repeating the above procedures until the indigenous sulfur-oxidizing bacteria in the sludge had the highest rate of acidification.

### 2.3. Bioleaching experiments

The bioleaching experiments were performed in 12 l completely mixed batch reactors agitated by stirring at 200 rpm and aerated at a flow of 1.2 $l\,min^{-1}$. Ten liter of sludge with various solids concentrations (0.5%, 1%, 2%, and 4% (w/v)) was added to the reactors, respectively. These reactors were inoculated with 5% (v/v) of previously activated indigenous sulfur-oxidizing bacteria and maintained at 30 °C. 0.3% (w/v) of tyndallized elemental sulfur was then fed into the reactors. During the bioleaching experiment, pH and oxidation–reduction potential (ORP) in the reactor were measured with an on-line monitor (Tank, model RD-500). A portion of sludge taken from the reactor 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 sulfate (APHA, 1995) and heavy metal (Cu, Zn, Mn, Pb, Ni, and Cr). In this study, heavy metals were determined using a flame/graphite atomic absorption spectrophotometer (Model Z-8100, Hitachi).

Besides, for determination of binding forms of metal in the livestock sludge before and after the bioleaching process, a sequential extraction procedure was used in this study. This sequential extraction was based on Tessier et al. (1979) and modified by Lin et al. (1999). This procedure defines the following five binding forms of metals: exchangeable, carbonate-bound, Fe/Mn oxide-bound, organic matter/sulfide-bound, and residual. All extracts obtained from the sequential extraction procedure were analyzed for heavy metals.

## 3. Results and discussion

### 3.1. Variations of pH and ORP in bioleaching

The variations of pH during the bioleaching process with different sludge solids concentrations are shown in Fig. 1(a). The activated indigenous sulfur-oxidizing bacteria in the sludge were able to use reduced sulfur for their growth, then sulfuric acid was produced and pH decreased in the bioleaching process. After 16 d of reaction time, the pH decreased from neutral pH to an acidic pH of 1.2, 1.3, 1.4, and 2.7 for solids concentrations of 0.5%, 1%, 2%, and 4% (w/v), respectively. The sludge solids concentration significantly affected the rate of pH decrease. Generally the acidification rates of sludge decreased as the solids concentration increased. Because sludge of higher solids concentration had higher buffering capacity, it required more time and acid for pH decrease (Chen and Lin, 2000). Therefore, sludge solids concentration plays a key role in the variation of pH during the bioleaching process.

Besides the acidification of sludge, the oxidation of sulfur and aeration resulted in an increase in the ORP during the bioleaching process. The variations of ORP during the bioleaching process are given in Fig. 1(b). Compared to the variation of pH in bioleaching, the ORP increased to the highest values (425–561 mV) after 16 d of bioleaching. The variations of ORP were much related to those of pH. It was observed that the increase of ORP followed the same trend as decrease of pH. The higher solids concentration of sludge induced a drop in the rate of ORP increase. It is known that lower pH and higher ORP values favor the solubilization of heavy metals from sludge in the bioleaching process.

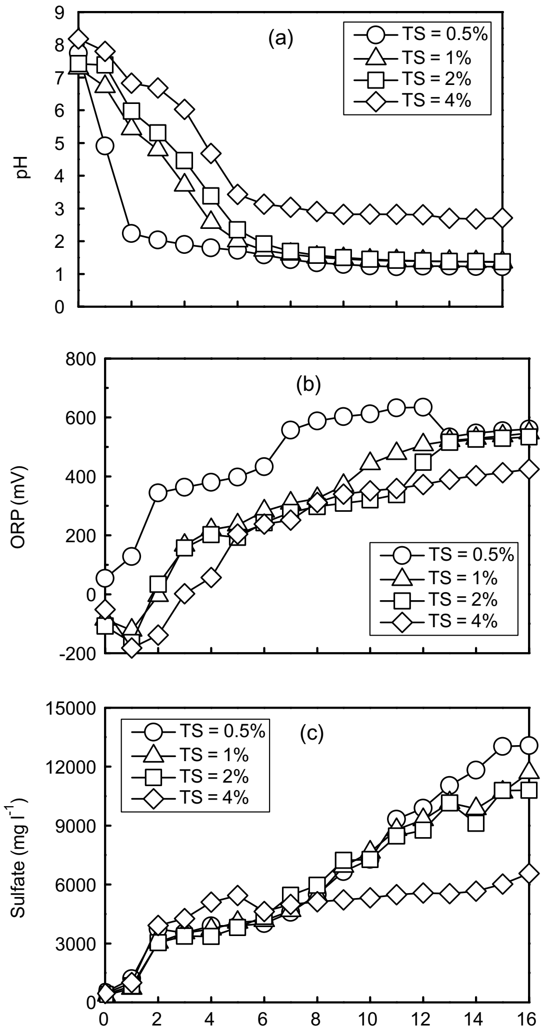


Fig. 1. Variations of pH, ORP and sulfate during the bioleaching process with different solids concentrations (a) pH, (b) ORP and (c) sulfate.

### 3.2. Sulfate production in bioleaching

The observed sulfate production in the bioleaching process can be explained by the bio-oxidation of elemental sulfur to sulfate. Fig. 1(c) shows the effects of sludge solids concentrations on production of sulfate during the bioleaching process. Sulfate production seems not to be affected by the solids concentration except for the sludge solids concentration of 4% (w/v). In this study, because some nutrients (e.g. N, P, and K) essential for bacterial growth can be supplied by the sludge itself, except elemental sulfur, no other nutrients were added into the reactor of the bioleaching experiment. Livestock sludges generally contain relatively high levels of nutrients (e.g. N, P, and K) (Hooda et al., 2000). Therefore, it was found that these nutrients were enough for bacterial growth and solids concentration did not affect the sulfate production in the bioleaching process. However, the rate of sulfate production decreased after 8 d of bioleaching for the solids concentration of 4% (w/v). Due to the highest buffering capacity and the lowest rate of pH decrease, the pH values were the highest in the bioleaching process with 4% (w/v) of solids concentration (Fig. 1(a)). In this study, the bioleaching process was carried out by successive growth of less acidophilic and acidophilic sulfur-oxidizing bacteria (Blais et al., 1993; Chen and Lin, 2000). The increase of pH prolonged the lag period of acidophilic sulfur-oxidizing bacteria (Tyagi et al., 1993), so the rate of sulfate production slowed down in the latter half of the bioleaching process.

### 3.3. Metal solubilization in bioleaching

The effects of solids concentration on the solubilization of heavy metals from sludge during the bioleaching process are shown in Fig. 2. The results indicated that a longer lag period of metal solubilization was found in the bioleaching process with higher sludge solids concentration. This is because the higher the sludge solids concentration, the higher the buffering capacity. Thus,

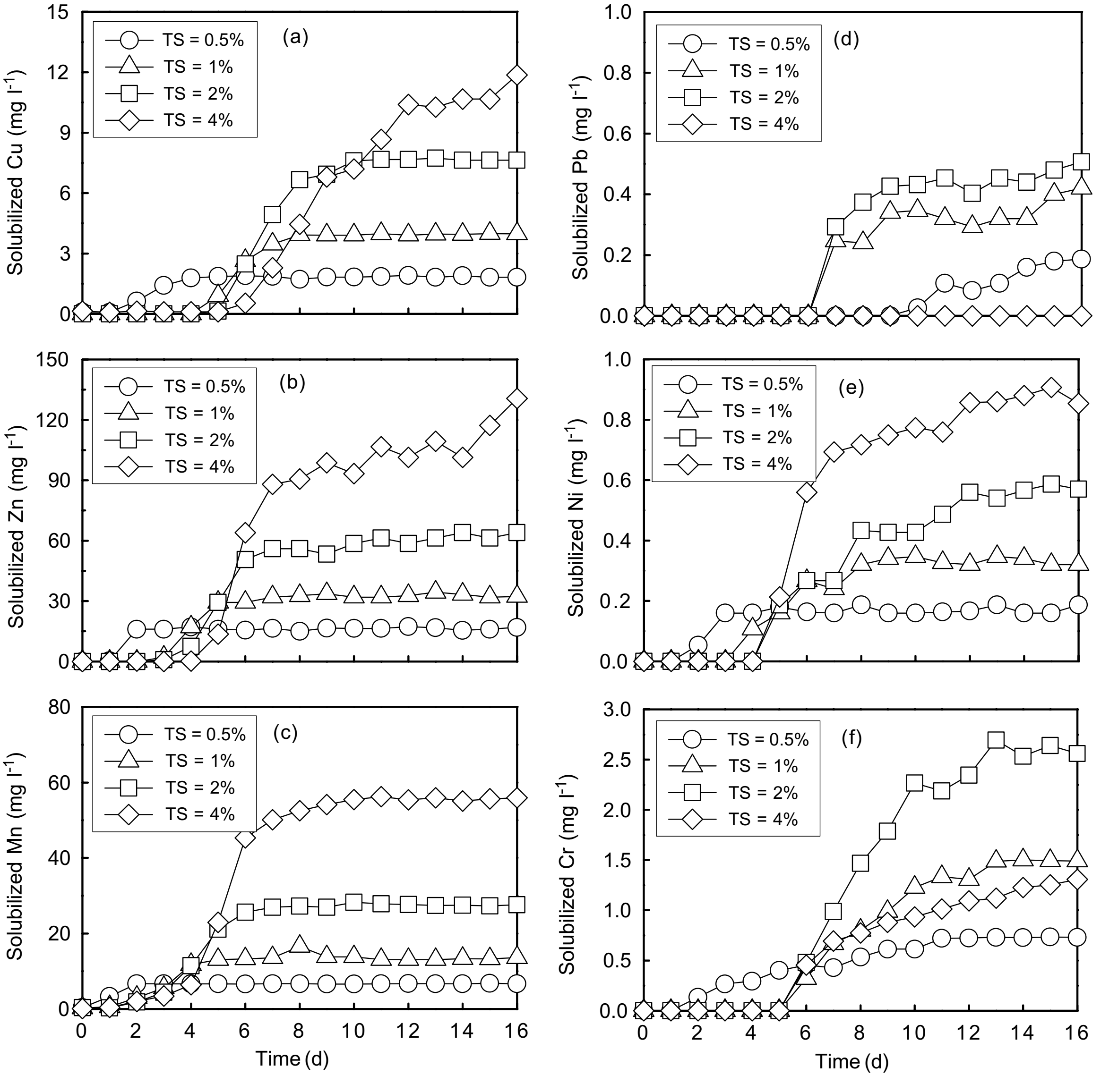


Fig. 2. Metal solubilization during the bioleaching process with different solids concentrations (a) Cu, (b) Zn, (c) Mn, (d) Pb, (e) Ni and (f) Cr.

Table 2
The percentage of metal solubilization in the bioleaching process with different sludge solids concentrations

| Metal | Solids concentration (%, w/v) | | | |
|---|---|---|---|---|
| | 0.5 | 1 | 2 | 4 |
| Cu | 99[a] | 98 | 98 | 78 |
| Zn | 99 | 98 | 98 | 97 |
| Mn | 99 | 98 | 97 | 97 |
| Pb | 95 | 95 | 65 | 0 |
| Ni | 96 | 96 | 94 | 65 |
| Cr | 95 | 94 | 87 | 22 |

[a] The final percentage of metal solubilization after 16 d.

the rate of pH decrease was slower in the bioleaching process with higher solids concentration. It takes more time to attain the pH at which heavy metals can be leached from sludge. However, the pH value was not low enough for Pb to solubilize from sludge during the bioleaching process with the solids concentration of 4% (w/v) (Fig. 2(d)). In general, after the lag periods, the amount of the solubilized metals increased significantly and then became stable or continued to increase slightly. Table 2 shows the percentage of metal solubilization in the bioleaching process. Cu, Zn, and Mn had high solubilization percentages (97–99%) in the bioleaching process. For Pb, Ni, and Cr, the solubilization percentage was low in the bioleaching process with high solids concentration. It can be seen that the percentage of metal solubilization was affected by the solids concentration. This is attributed to the fact that the final pH value was higher for the system with higher sludge solids concentration (Fig. 1(a)). The percentage of metal solubilization was found to highly depend on the pH value during the bioleaching process (Chen and Lin, 2001). Overall, the percentage of metal solubilization from sludge was in the decreasing order: Zn > Mn > Cu > Ni > Cr > Pb.

The binding forms of metal in the sludge were responsible for the difference in efficiency of metal solubilization. Metals in exchangeable, carbonate-bound, and Fe/Mn oxide-bound fractions 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). It was found that Mn (93%), Zn (89%), and Ni (89%) in the sludge predominantly existed in the mobile forms (i.e. exchangeable, carbonate, and Fe/Mn oxides fractions) (Fig. 3(a)). Most of Cu (86%) existed as sulfides and this form of Cu was efficiently oxidized (solubilized) by sulfur-oxidizing bacteria in the bioleaching process. Pb (91%) in the livestock sludge was found to exist in residual (crystal lattice-held) fraction. Therefore, Mn, Zn, Cu, and Ni had high solubilization efficiency in this study. The efficiency of Pb solubilization was the lowest. After the bioleaching process, metals remaining in the livestock sludge had

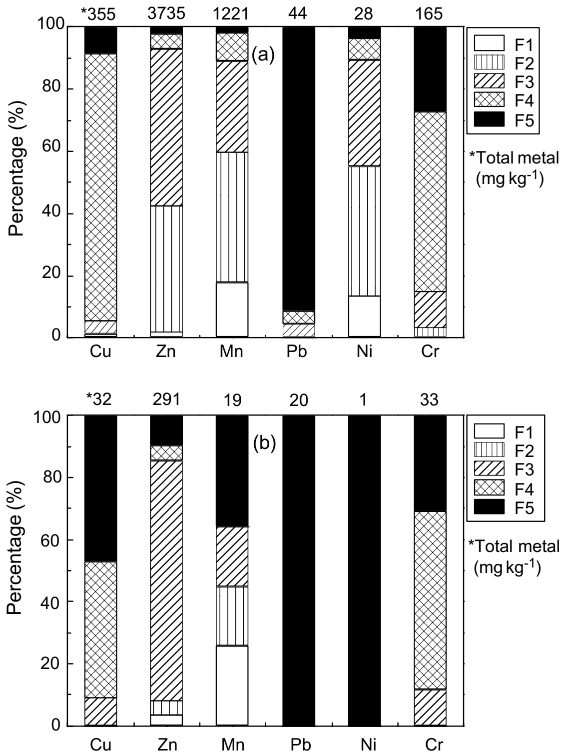


Fig. 3. Binding forms of metal in the sludge (a) before and (b) after the bioleaching process (F1: exchangeable; F2: bound to carbonates; F3: bound to Fe–Mn oxides; F4: bound to sulfides and organic matter; F5: residual).

relatively low contents and were mainly found in the stable fractions (Fig. 3(b)). These results show the applicability of the treated sludge to agricultural lands.

The effect of solids concentration on the initial rate of metal solubilization during the bioleaching process was also studied and representative results obtained are shown in Fig. 4. These results indicate that the dependence of rate of metal solubilization on solids concentration followed a Michaelis–Menten type of equation (Swamy et al., 1995):

$$V = \frac{V_m S}{K + S} \qquad (1)$$

where $V$ is rate of metal solubilization, $K$ is the saturation constant and is the solids concentration which yields $V_m/2$, $V_m$ is the maximum rate of metal solubilization, and $S$ is the solids concentration, respectively. Taking the inverse of Eq. (1), a linear expression between the term $1/V$ and $1/S$ is obtained (Eq. (2)) and the values of $V_m$ and $K$ can be evaluated from the slope ($K/V_m$) and intercept ($1/V_m$) of the straight line.

$$\frac{1}{V} = \frac{1}{V_m} + \frac{K}{V_m S} \qquad (2)$$

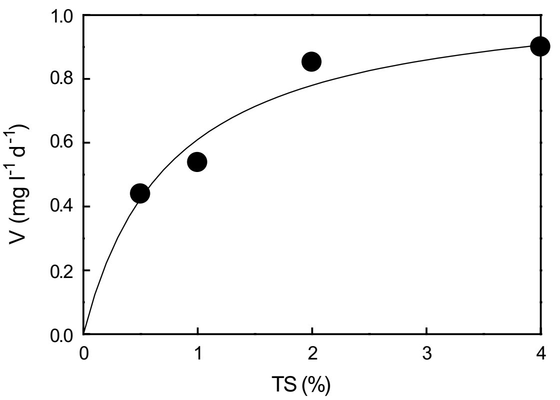


Fig. 4. Effect of solids concentration on the rate of Cu solubilization during the bioleaching process.

Table 3
Parameters of Michaelis–Menten equation for bioleaching of heavy metals from livestock sludge

| Metal | $V_m$ ($mg\,l^{-1}\,d^{-1}$) | $K$ (%) | $R^2$ |
|---|---|---|---|
| Cu | 1.08 | 0.77 | 0.92 |
| Zn | 17.48 | 1.94 | 0.99 |
| Mn | 13.55 | 3.76 | 0.99 |
| Pb | –[a] | – | – |
| Ni | 0.22 | 8.24 | 0.99 |
| Cr | 0.87 | 6.38 | 0.99 |

[a] Not available.

The data of $V_m$ and $K$ obtained from Eq. (2) are given in Table 3. The correlation coefficients ($R^2$) obtained in the regression analysis of Eq. (2) were greater than 0.92 for all metals. The maximum rates of metal solubilization decreased in the order: Zn > Mn > Cu > Cr > Ni. It was found that the rate of metal solubilization (Fig. 2 and Table 3) was highly related to the initial contents of metal in the sludge. Moreover, the binding forms of metal in the sludge also affected the rate and extent of metal solubilization in the bioleaching process (Chen and Lin, 2000; Chartier et al., 2001).

## 4. Conclusions

For the application of livestock sludge on agricultural land, the high levels of heavy metals in the sludge should be removed. The bioleaching process was recognized as a possible alternative to traditional chemical and physical techniques for treatment of sludge containing heavy metals. The solids concentration, one of the important factors affecting the bioleaching process, was investigated in this study. The solids concentration of sludge predominately affected the buffering capacity in the system. Accordingly, the variations of pH were governed by the solids concentration. Because the pH value was related to the metal solubilization during the bioleaching process, the percentage of metal solubilization was also affected by the solids concentration. The effects of solids concentration on the rate of metal solubilization were found to follow a Michaelis–Menten equation. However, most of the heavy metals were efficiently solubilized from the sludge after the bioleaching process. For Cu, Zn, and Mn, the percentages of metal solubilization even exceeded 90%. The bioleaching process with indigenous sulfur-oxidizing bacteria used in the present work was shown to be a very feasible technology for the removal of heavy metals from livestock sludge.

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