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Effect of substrate concentration on the bioleaching of heavy metals from sewage sludge

CHEN Ying-xu*, HUA Yu-mei, ZHANG Shao-hui

(Department of Environmental Engineering, Zhejiang University, Hangzhou 310029, China. E-mail: yxchen@zju.edu.cn)

Abstract: The effect of elemental sulfur concentration on bioleaching of Cu, Zn and Pb and loss of fertilizer value from sewage sludge was investigated in flasks by batch experiments. The results showed that the ultimate pH of sludges with 3—5 g/L of sulfur added was about 1.3 and the production of SO_4^{2-} had good correlation with the elemental sulfur concentration. The sensitivity of removal efficiency of metals to sulfur concentration was: Pb > Cu > Zn. The sulfur concentration except for 3—5 g/L had significant effect on the solubilization of Cu, Pb and Zn. The highest solubilization efficiency for sludge with 3 g/L of sulfur was 87.86% for Cu, 32.72% for Pb and 92.14% for Zn, which could make the treated sludge easily meet the metal limitations for land application. The sulfur concentration of 3 g/L was enough for the solubilization of all three heavy metals. The influence of sulfur concentration on solubilization of total nitrogen and potassium from sludge was negligible, but that on solublization of total phosphorus was of great importance. The loss of nitrogen, phosphorus and potassium of sludge with 3 g/L of sulfur by bioleaching was 38.2%, 52.1% and 42.8% respectively, and the sludge still remained satisfactory fertilizer value after bioleaching.

Keywords: sewage sludge; heavy metals; bioleaching; substrate; fertilizer value

Introduction

The production of excess sewage sludge is increasing rapidly with the widespread of wastewater treatment, which causes serious problem to the disposal of sewage sludge. Land application of sewage sludge is the most cost-effective and feasible utilization approach at present. However, large amounts of sludge in China can not meet the metal limitations for land application and heavy metals become one of the most significant restraints. This is mainly because the industrial and domestic wastewater are collected and treated in municipal waste water treatment plant (WWTP). To make the sludge suitable for land application, the following two conditions must be met: heavy metal content of the sludge must be reduced to acceptable levels and the fertilizing and soil conditioning potential of the sludge must be preserved (Wong, 1984). Bioleaching of heavy metals from sludge is a process during which the metals combined with sludge are solved by the acid produced by *Thiobacillus*. Removal of heavy metals by bioleaching has advantages such as high efficiency, simple operation and low cost and so on, and it has been widely discussed in abroad. The relative research on the bioleaching of heavy metals from sludge in China is just at the beginning period. Zhou *et al*. (Zhou, 2001) discussed the bioleaching using *Thiobacillus ferrooxidans*, however there is no reports about mixed *Thiobacillus* as yet.

It need to add enough substrate to ensure that the *Thiobacillus* can produce acid successfully because there is too little substrate to meet the growth of *Thiobacillus*. Elemental sulfur, thiosulfate and some other sulphocompound can all serve as the substrate for *Thiobacillus*, but some research suggested that elemental sulfur is the most appropriate substrate as for bioleaching of sludge (Tygai, 1994). It is of great importance to determine proper concentration of elemental sulfur. On one hand, the remaining sulfur will lead to the reacidification of treated sludge during the final disposal if the elemental sulfur added is not completely oxidized to sulfuric acid (Ravishankar, 1994). On the other hand, the process with too much sulfur may also result in a significant loss of nutrient from the sludge through a variety of chemical reactions and biological mechanisms. The aim of this research was to investigate the effect of elemental sulfur concentration on the bioleaching of heavy metals and the loss of sludge fertilization using mixed *Thiobacillus* as inoculum.

1 Materials and methods

1.1 Sludge samples

Sewage sludge was obtained from the Sibao Urban Sewage Treatment Plant of Hangzhou, China, which receives both domestic and industrial wastewater. The sludge was concentrated raw sludge of the mixture of primary and second precipitated sludge, and its initial characteristics is presented in Table 1. The sludge was kept at 4℃ before use.

Table 1 Characteristics of sludge

Parameters	Values
Total solids, g/L	26.22
pH	6.8
SO_4^{2-} in supernatant, mg/L	521.8
Cu, mg/kg dry sludge	267.9
Pb, mg/kg dry sludge	391.4
Zn, mg/kg dry sludge	4031.5
Total nitrogen, g/kg dry sludge	38.4
Total phosphorus, g/kg dry sludge	23.1
Total potassium, g/kg dry sludge	14.2

* Corresponding author

1.2 Cultivation of inoculum of *Thiobacillus*

The cultures were incubated in 500 ml Erlenmeyer flasks with 10 g/L of elemental sulfur, agitated at 200 r/min and 28℃ for about 6 d until the sludge pH dropped to under 2. Took the cultivated bacterial mixture as inoculum and put it into the fresh raw sludge in concentration of 5%, and added 10 g/L sulfur at the same time. After the second cultivation, the pH decreased to 2.0 again. Continually cultivate the sludge for two other cultivation period. The whole cultivation cycle, including three consecutive transfers, was about 14 d. The enriched sludge was retained as the inoculum of bioleaching.

1.3 Batch experiments of bioleaching of heavy metals from sludge

The inoculum of all batch experiments was 2% (v/v), and the elemental sulfur concentration was 0.5, 1, 2, 3, 4, and 5 g/L respectively. The control was sludge with neither inoculum nor elemental sulfur. Each sample was performed triplicate. The bioleaching experiments were conducted in 500 ml Erlenmeyer flask in which 200 ml sludge was filled, and agitated at 100 r/min and 28℃. The loss of water in sludge due to evaporation during bioleaching was compensated by distilled water based on weighing everyday.

1.4 Analysis

About 15 ml of the sludge in the flask was taken everyday and centrifuged at 10000 r/min for 10 min. Then it was filtered by quantitative filter paper to obtain the supernatant which was considered as carrying the heavy metals in dissolved phase. Total heavy metals in sludge were determined after HF-$HClO_4$ digestion. The concentration of Cu, Zn and Pb were analyzed using the Atomic Absorption Spectrometer with Perkin-Elmer Analyzer-100. Sulfate was measured by Ion Chromatogram with Dionex-120. Potassium was analyzed by atomic emission spectrometer. Total nitrogen and total phosphorus were measured according to the standard methods of NEPA(NEPA, 1989).

2 Results and discussion

2.1 Variation of pH and SO_4^{2-}

Fig.1 shows the pH of sludges with elemental sulfur concentrations ranging from 0.5 to 5.0 g/L only had a slightly drop at the first two days. This was because that the microorganisms need to acclimate to the new substrate and environment initially. The samples with no less than 3 g/L sulfur concentration were somewhat unanimous, which all showed slight degree of drop since the fifth day. It indicated that 3 g/L sulfur concentration was probably enough for meeting the requirement of the growth of *Thiobacillus*. However, the pH of sludge with 2 g/L of sulfur was a little high than sludge with higher sulfur concentration, and it reached the same value until the 11 d. The pH of sludge with 0.5 g/L sulfur concentration only lightly decreased throughout the bioleaching process, which was almost the same as that of the control. Blais *et al*.(Blais, 1993) found bioacidification generally proceeds in two steps: an initial decrease in pH to below 4 by the less-acidophilic *Thiobacillus* followed by a further pH reduction to below 2 by acidophilic bacteria. The solubilization of heavy metals in sludge by activity of bacteria does not depend on the amount of acid produced, but on the pH value, for the alkalinity of the sludge resists the pH change and the difference of buffering ability of sludge will show different pH. pH decides the number proportion between less-acidophilic *Thiobacillus* and acidophilic *Thiobacillus*. The drop of pH couples with the increase of growth of acidophilic *Thiobacillus* and improves the solubilization of heavy metals from sludge.

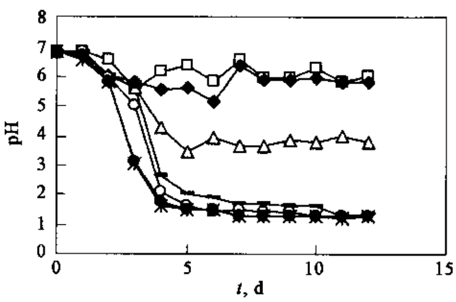

Fig.1 Variation of pH with different sulfur concentration during bioleaching
□ control; ◆ 0.5 g/L; △ 1 g/L; ■ 2 g/L; ○ 3 g/L; ● 4 g/L; * 5 g/L

The *Thiobacillus* in sludge oxidize added sulfur used as substrate (Fig. 2). This oxidation of sulfur leads to the production of sulfuric acid that, in turn, triggers the acidfication of sludge (Filali-Meknassi, 2000). Therefore, the sulfate concentration in the leachate could reflect the oxidation process of sulfur to certain extent. Initially, the production rate of sulfate was slow, i.e. there was a lag period at the beginning of bioleaching. The lag time was found to depend on: (1) the surface area of sulfur available for bacteria attachment and (2) the number of bacteria present in the sludge capable of initiating bacterial colonies on these surfaces (Sreekrishnan, 1996). The rate of sulfate production for the sludge with more than 3 g/L of elemental sulfur picked up rapidly after 2 d, and become slow until 8 d, then remained relatively stable. The sulfate concentration in the leachate increased drastically with the increase of sulfur concentration during bioleaching, and the correlation coefficient was high as $r^2 = 0.992$. The sulfate concentration at 12 d was higher than the theoretical amount once the elemental sulfur was all utilized and turned to sulfate by *Thiobacillus*, as Table 2 shown. It was mainly because that some metal sulfides were also used as the substrate of *Thiobacillus* and transformed to sulfate. The sulfate coming from the metal sulfides was up to more than 1000 mg/L. It was difficult to make clear the whole utilizing process of elemental sulfur or metal sulfides by *Thiobacillus*.

The *Thiobacillus* in sludge can not multiply a great deal if there is no enough substrate. As for the sludge with

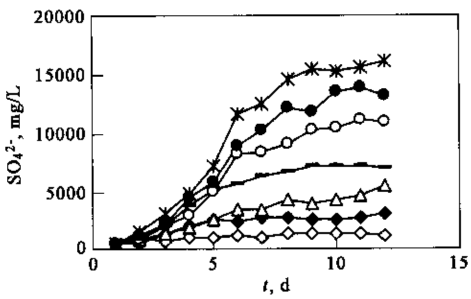

Fig. 2 Variation of SO_4^{2-} with different sulfur concentration during bioleaching
□ control; ◆ 0.5 g/L; △ 1 g/L; ▬ 2 g/L; ○ 3 g/L; ● 4 g/L; * 5 g/L

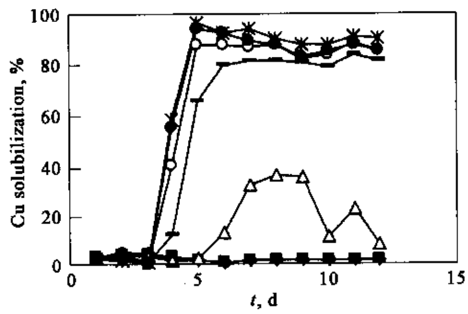

Fig. 3 Solubilization of Cu with different sulfur concentration during bioleaching
◇ control; ■ 0.5 g/L; △ 1 g/L; ▬ 2 g/L; ○ 3 g/L; ● 4 g/L; * 5 g/L

different concentration of sulfur added, the results showed that the bacteria were active in the sludge only in the presence of proper additional substrate. Adsorption of *Thiobacillus* onto sulfur particles was the primary and essential step for oxidation of elemental sulfur during bioleaching process. The oxidation of elemental sulfur was considered to take place with the adsorption of *Thiobacillus* onto the surface of solid substrate by Van der Waals attractive forces and then the metabolism of sulfur occurred (Shrihari, 1992). Under the condition of low sulfur concentration, the sulfur metabolized by *Thiobacillus* was little due to little amount of sulfur adsorbed on the sludge surface, and it need longer time to arrive the pH at which heavy metals can solve. The more was the surface of sulfur, the stronger was the absorbance between sulfur and *Thiobacillus* and the easier was the oxidation of sulfur.

Table 2 Comparison of theoretical SO_4^{2-} concentration with practical SO_4^{2-} concentration

Sulfur concentration, g/L	0.5	1	2	3	4	5
Theoretical SO_4^{2-}, mg/L	1500	3000	6000	9000	12000	15000
Practical SO_4^{2-}, mg/L	3041	5413	7046	10923	13119	16047

2.2 Bioleaching of heavy metals

Fig.3 shows that the bioleaching efficiency of Cu was relatively high as a whole, and the efficiency of sludges with 2—5 g/L sulfur concentration was between 78.9%—93.6% after stabilization. Little amount of Cu was dissolved during the first three days as the lag period, but the solubilization began to show significant increase after 3 d. The peak of removal rate was arrived at the fifth day and a slight decline was followed for the sludges with no less than 3 g/L of sulfur. As for the sludge with 1 g/L sulfur concentration, the solubilization of Cu was up to 35.8% at the eighth day, then decreased evidently after one day of maintenance and only 7.96% at the 12 d. It indicated that the acidification of sludge with 1 g/L of sulfur was not enough and the solubilized Cu was adsorbed by sludge again or complexed with some organic compound, resulting in the decrease of Cu concentration in leachate.

Pb could not attain high removal efficiency by bioleaching because the solubility of $PbSO_4$ produced with SO_4^{2-} under low pH was relatively low. Fig.4 shows that Pb began to solve after 4 d. The sludges with more than 3 g/L of sulfur achieved the top value at the sixth or seventh days, then appeared decreasing trend, and the ultimate removal efficiencies of all the three sludges were nearly the same. Obviously different from the removal of Cu, Pb concentration in the leachate of sludge with 2 g/L of sulfur was far lower than that of sludges with higher sulfur concentration though the pH value of the former was only 0.2—0.3 unit higher than that of the latter. It reflected that the solubilization of heavy metals from sludge did not only depend on pH.

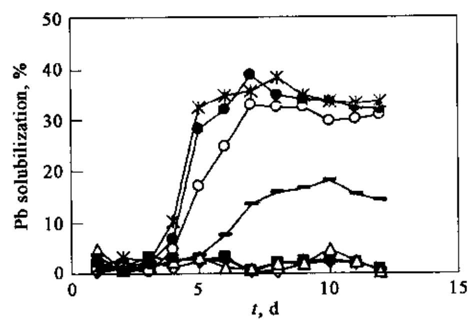

Fig. 4 Solubilization of Pb with different sulfur concentration during bioleaching
◇ control; ■ 0.5 g/L; △ 1 g/L; ▬ 2 g/L; ○ 3 g/L; ● 4 g/L; * 5 g/L

The solubilization efficiency of Zn was well correlated with pH (Table 3). It easily arrived to about 50% at the third day for sludges with 4 g/L and 5 g/L sulfur concentration, meanwhile pH was 3.0 (Fig. 5). From the forth day on, the solubilization of Zn of sludges with no less than 2 g/L of sulfur showed the similar trend. To the sludge with 1 g/L of sulfur, the solubilization of Zn can be up to 80% at the sixth day, but after 9 d the removal efficiency of Zn appeared continuous fall. It was because of the adsorption by sludge and complexation with organic compound just like the bioleaching of Cu.

For the sludges without sulfur added or with low sulfur concentration, very low metal solubilization was obtained, which meant appropriate substrate must be furnished if efficient metal bioleaching was desired. It showed that higher metal solubilization appeared with higher sulfur concentration for metals investigated. But for all metals 3 g/L sulfur concentration was enough. Further higher sulfur concentration could not significantly increase the solubilization efficiency. Metal solubilization from the sludge was shown to be primarily governed by pH. However, other important factors such as metal characteristics also affect the solubilization of metals.

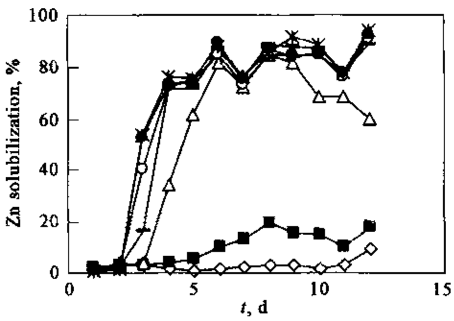

Fig. 5 Solubilizaion of Zn with different suolfur concentration during bioleaching
◇ control; ■ 0.5 g/L; △ 1 g/L; ■ 2 g/L; ○ 3 g/L; ● 4 g/L; * 5 g/L

Table 3 Correlation between different parameters and elemental sulfur concentration

Parameters	Correlation cofficients
pH	0.7394
SO_4^{2-}	0.992
Cu	0.7918
Pb	0.9045
Zn	0.7417
TN	0.769
TP	0.9247
TK	0.5367

2.3 Variation of fertilizer of sludge

Nitrogen is an essential nutrient for crop. Nitrogen in sludge maybe present as ammonia nitrogen, organic nitrogen (amino acids, hexoamines, and amides), and nitrite/nitrate nitrogen (Frost, 2001). Most of the organic nitrogen is associated with the sludge solids while inorganic forms of nitrogen (ammonia, nitrite and nitrate) are water soluble. The solubilization of heavy metals was deeply influenced by elemental sulfur as substrate, but total nitrogen in leachate was not. From Fig.6, we can see the total nitrogen in the leachate of all sludges showed a similar change during bioleaching, with a slow increase before 5 d and a quicker increase thereafter. The heavy metals after bioleaching can be removed from sludge by a solid-liquid process, so the nutrient amount in leachate reflects the loss of fertilizer value of sludge through bioleaching. The little difference of nitrogen in leachate between control and sludges with elemental sulfur added indicated that the loss of nitrogen in sludge could not be negatively influenced under acidic condition. Efficient destruction of indicator microorganisms and a substantial reduction in the concentration of sludge solids were observed during bioleaching, suggesting that the metal leaching and sludge digestion processes could be conducted simultaneously (Benmoussa, 1997). Filali-Meknassi *et al*. (Filali-Meknassi, 2000) found that the sludge achieved a reduction of near 40% of VSS following 14 d of bioleaching. This is mainly because bioacidification accumulates the growth of *Thiobacillus* and inhibits the growth of the microorganisms that can not tolerate the newly imposed acidic environment. Large amount of heterotrophic bacteria existing in initial sludge would die and hydrolyze, and release the nitrogen combined in biological mass. The amount of released nitrogen was enhanced with bioleaching time. The solubility of various nitrogen compounds has low relativity with pH. The change of total nitrogen in leachate during bioleaching was stable under various sulfur concentration conditions and the factor affecting the nitrogen in leachate was mainly the organic decomposition rate.

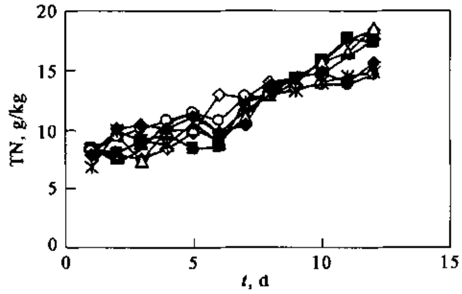

Fig.6 Vartiation of TN inleachate with different sulfur concentration during bioleaching
◇ control; ■ 0.5 g/L; △ 1 g/L; ■ 2 g/L; ○ 3 g/L; ◆ 4 g/L; * 5 g/L

Phosphorus in wastewater generally occurs as inorganic phosphorus compounds which may include calcium, ferric and aluminus phosphates and phosphorus sorbed on amorphous complexes of hydrous oxides. The primary form of phosphorus in biological sludge without the addition of chemicals is organic (Kyle, 1995). Phosphorus also exists as inorganic form and as stored polyphosphates in sludge. The relation of total phosphorus in leachate with elemental sulfur concentration was similar as that of solubilization of heavy metals, and more phosphorus in sludge released under higher sulfur concentration condition. The initial total phosphorus content in sludge was 23.1 g/kg dry sludge. For the sludges with 4 g/L and 5 g/L of sulfur the loss of phosphorus in the leachate can be up to about 14 g/kg dry sludge (Fig.7). The good correlation of releasing amount of phosphorus from sludge and elemental sulfur was because that high sulfur concentration caused environment of lower pH under which more phosphorus transform to HPO_4^{2-} and $H_2PO_4^-$, leading to stronger solubility of phosphorus to liquid phase of sludge.

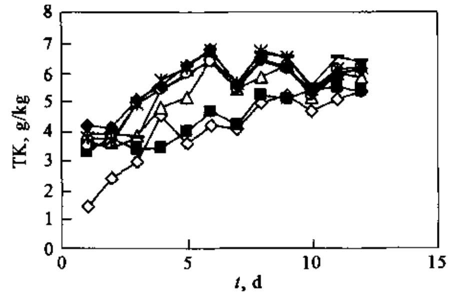

Fig.7 Variation of TP inleachate with different sulfur concentration during bioleaching
◇ control; ■ 0.5 g/L; △ 1 g/L; ■ 2 g/L; ○ 3 g/L; ◆ 4 g/L; * 5 g/L

The potassium did not show high proportion of solubilization in spite of its soluble nature, as Fig.8 shown. The potassium concentration in leachate of the sludge with elemental sulfur and the control was great different at the beginning, but the difference became less and less in the course of bioleaching. The potassium in leachate of control and sludge with 0.5 g/L of sulfur was 4.17 and 4.62 g/kg dry sludge respectively, and the loss of potassium of other sludges was 6.39—6.70 g/kg dry sludge at the sixth day. The potassium of control at last was 5.28 g/kg dry sludge and that of other sludges was 5.31—6.34 g/kg dry sludge.

Similar to the peak of metal leaching, the most loss of potassium was at the sixth day followed a subdued change. It indicated that the release of potassium from sludge was easily affected by the acidification of sludge. The death and cell hydrolysis due to the effective aerobic digestion of the control can also lead to the same level of loss of potassium, resulting in the similar potassium loss for all the samples at the end of bioleaching.

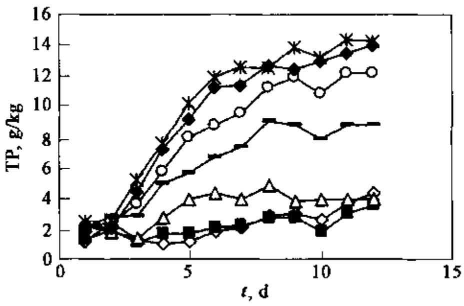

Fig.8 Variation of TK in leachate with different sulfur concentration during bioleaching
◇ control; ■ 0.5 g/L; △ 1 g/L; ▬ 2 g/L; ○ 3 g/L; ◆ 4 g/L; * 5 g/L

Land application following bioleaching means the great importance of fertilizer value in sludge. Therefore, one major concern about bioleaching is the potential leaching loss of nitrogen, phosphorus and potassium, which in turn will reduce its value as fertilizer. As for our experiments, the total nitrogen, phosphorus and potassium concentration in the sludge were 38.4, 23.1 and 14.2 g/kg dry sludge respectively, which reflects good fertilizer characteristic of the sludge. The loss of nitrogen and potassium after bioleaching was 38.8%—48.0% and 37.2%—44.6% under elemental sulfur concentrations ranging from 0 to 5.0 g/L. It indicated the fertilizer loss of these two nutrients was not affected by sulfur concentration because the similar phenomena would happen under other sludge treatment process such as anaerobic digestion and aerobic digestion which can cause the decomposition of bacteria in sludge. However, the loss of phosphorus was strongly correlated with elemental sulfur concentration, in the result of 15.1%—61.2% in leachate and the correlation coefficient was $r^2 = 0.9247$. The former results demonstrated that 3 g/L elemental sulfur could serve as the optimum substrate concentration, and the loss of nitrogen, phosphorus and potassium of sludge with 3 g/L sulfur by leaching was 38.2%, 52.1% and 42.8% respectively. Although there was certain loss of the fertilizer value from sludge after bioleaching, the nutrient level of nitrogen, phosphorus and potassium was still applicable for the land application. Analysis of sludge solids after bioleaching by Wong *et al*.(Wong, 2002) showed that 39% of nitrogen and 45% of phosphorus were lost at initial pH 3.0 of sludge. Another bioleaching experiment using indigenous sulphur-oxidizing microorganism running at pH 7 solubilized 76% of the total phosphorus and 38% of the TKN (Shanableh, 1999). The result of present experiments was in accordance with these values approximately.

3 Conclusions

The speed and degree of decrease of pH value increase with the increase of elemental sulfur concentration. The ultimate pH of sludges with 3—5 g/L of sulfur was about 1.3. The production of sulfate had good correlation with the elemental sulfur concentration.

The sulfur concentration except for 3—5 g/L has significant effect on the solubilization of Cu, Pb and Zn. The sensitivity of removal efficiency of metals to sulfur concentration was: Pb > Cu > Zn.

The sulfur concentration of 3 g/L could meet the requirement of solubilization of all three heavy metals, and the treated sludge with 3 g/L of sulfur could easily meet the metal limitations for land application. The peak value of solubilization of Cu, Pb and Zn was occurred at between 5 to 8 d.

The influence of sulfur concentration on solubilization of nitrogen and potassium from sludge was negligible, but that on solublization of total phosphorus was of great importance. The loss of nitrogen, phosphorus and potassium of sludge with 3 g/L of sulfur by leaching was 38.2%, 52.1% and 42.8% respectively, and the sludge still remained satisfactory fertilizer value after bioleaching.

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