

Transformation of heavy metal forms during sewage sludge bioleaching

Ying-Xu Chen^{a,*}, Yu-Mei Hua^a, Shao-Hui Zhang^b, Guang-Ming Tian^a

^a Department of Environmental Engineering, Zhejiang University, Hangzhou 310029, PR China

^b Department of Municipal Engineering, Wuhan University of Technology, Wuhan 430070, PR China

Received 12 June 2004; received in revised form 29 March 2005; accepted 31 March 2005

Available online 17 May 2005

Abstract

The transformation of heavy metal forms during sewage sludge bioleaching with elemental sulfur as substrate was investigated. The sequential selective extraction procedure was an effective way to reflect the variation in heavy metal forms. The exchangeable form of Cu, Pb and Zn after bioleaching accounted for 81.6, 40.2 and 75.8%, respectively. Cu at initial sludge mainly existed as sulfide precipitate form, most of which turned to exchangeable form during bioleaching, and was solubilized mostly by direct mechanism. The original forms of Pb and Zn were mainly carbonate precipitate and organically bound. Most of the carbonate precipitate Pb transformed into exchangeable form but a little of organically bound Pb. Pb was dissolved from the sludge due to transformation of carbonate precipitated and sulfide precipitated to exchangeable form by cooperation of both direct and indirect mechanisms. A majority of exchangeable Zn was transformed from organically bound and carbonate precipitated forms, and the bioleaching mechanism of Zn was mainly indirect. After bioleaching, the sewage sludge could be applied to land more safely because the heavy metals mainly existed in stable forms. The exchangeable Cu and Pb achieved an obvious increase at pH about 2, while exchangeable Zn showed higher percentage at pH about 3. The transformation of chemical forms for Cu had good relationship with ORP during bioleaching, but Zn was not influenced by ORP of sludge, which appeared to have high bioleaching efficiency at ORP less than 200 mV.

© 2005 Elsevier B.V. All rights reserved.

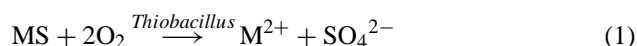
Keywords: Sewage sludge; Heavy metals; Bioleaching; Chemical form; Transformation

1. Introduction

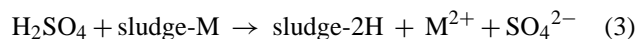
The production of excess sewage sludge increases substantially with the development of wastewater treatment facilities, which makes the proper disposal of sewage sludge become more and more urgent. The high content of organic matter and nutrient concentrations suggest sludge use preferably as fertilizer in agriculture or as regenerator for soil [1]. However, high concentration of heavy metals in sludge is one of the most important environmental impacts. An effective approach to reduce the toxicity of heavy metals in sewage sludge is bioleaching, during which the heavy metals can be dissolved under the acidic condition produced by *Thiobacillus* and then be removed through centrifugation or

other methods to separate solid and liquid. The main mechanisms involved in bioleaching of heavy metals have been categorized into two ways: direct and indirect, as shown below [2]:

Direct mechanism:



Indirect mechanism:



The toxic effects of heavy metals depend not only on concentrations but also on bioavailability [3]. During wastewater treatment, the heavy metals encounter solubilization, cation exchange, precipitation, adsorption, complexation and other reactions, resulting in various chemical forms in sludge. The

* Corresponding author. Tel.: +86 571 86971159; fax: +86 571 86971898.
E-mail address: yxchen@zju.edu.cn (Y.-X. Chen).

different forms of heavy metals stand different energy states, and affect not only the efficiency of bioleaching but also the bioavailability of heavy metals after bioleaching [4]. Concern was begun to be impressed over partitioning of heavy metals during sludge bioleaching in recent years because partitioning information of metals would allow for an investigation of which metal and its form are solubilized during bioleaching [5,6], and having immense significance to the exploitation and utilization of sludge as well.

It is essential to find out the dynamic transformation of chemical forms of heavy metals during bioleaching, which is of great help to clarify the reaction process and forecast the environmental chemical behavior of heavy metals (i.e. mobility, bioavailability and toxicity) after land application. However, there were no reports on the daily change and transformation of heavy metal forms during bioleaching. In present work, the transformation of chemical forms of heavy metals during bioleaching was investigated and bioleaching mechanisms were presumed.

2. Materials and methods

2.1. Sewage sludge sampling

Settled primary and secondary sewage sludge collected in a common pond was taken for the experiment from Sibao sewage treatment plant, Hangzhou, China, which receives both domestic and industrial wastewater. Table 1 gives the characteristics of the initial sludge used for the experiments.

2.2. Batch experiment of bioleaching

The cultivation of mixed *Thiobacillus* inoculums lasted for 12 days [7]. The experiments were carried out in twenty four 500 mL Erlenmeyer flask with 200 mL sewage sludge in, and agitated at 120 rpm and 28 °C. The sludge in 12 flasks was mixed with 2% (v/v) inoculums and 3 g/L (w/v) elemental sulfur powder as substrate and the rest were with neither inoculums nor elemental sulfur, set as control. A set of three flasks was sampled for 3 consecutive days and started another set on the 4th day. The total sampling period was 12 days. The loss of water in sludge due to evaporation during bioleaching was compensated by adding distilled water based on weight loss everyday.

Table 1
Characteristics of initial sludge

	Value
Total solids (g/l)	28.53
pH	6.8
ORP (mV)	−47
SO ₄ ^{2−} in supernatant (mg/l)	435.7
Cu (mg/kg dry sludge)	296.4
Pb (mg/kg dry sludge)	351.3
Zn (mg/kg dry sludge)	3756.2

Table 2
Sequential selective extraction procedure

Reagent	Volume/weight (ml/g SS)	Extraction time (h)	Extracted metal form
1 M KNO ₃	50:1	16	Exchangeable
0.5 M KF	80:1	16	Adsorbed
0.1 M Na ₄ P ₂ O ₇	80:1	16	Organically bound
0.1 M EDTA	80:1	16	Carbonate precipitated
1 M HNO ₃	50:1	16	Sulfide precipitated
Residual	—	—	Residual

2.3. Analysis

With the help of a plastic tube, 40 mL of sludge was taken everyday from flasks and centrifuged at 10,000 rpm for 10 min, followed by filtration by quantitative filter paper. Then the pH and ORP of supernatant were measured with pH meter. The concentration of Cu, Pb and Zn was determined by atomic adsorption spectrometry with Perkin-Elmer analyzer-100. Another sludge sample containing solid of 0.5 g (17.5 mL, which was calculated from the SS of the sludge) was taken and the chemical forms of heavy metals were analyzed everyday. The sequential selective extraction procedure of Stover et al. [8] was modified, in which liquid sludge instead of dry sludge was used for forms determination. The solid KNO₃, which finally amount to concentration of 1 M in the sample sludge, was used in the first extraction step, and other four steps were as same as the method of Stover et al. [8], as shown in Table 2.

The KNO₃ was chosen as an initial extractant for metals bound by exchange sites in sludge. If the sludge sample is saturated with K⁺, exchangeable metals will be displaced from exchange sites, which are located in the organic and inorganic component of the sludge. The second step in sludge fractionation involves extraction of adsorbed metals with KF. The pH and concentration of the KF permit the removal of adsorbed metals through the formation of soluble metal–fluoride complexes but minimize solubilization of organically bound metals. The following step involves organic matter extraction with Na₄P₂O₇, which will remove metals chelated or complexed by the organic fraction of sludge. EDTA is commonly used to release of elements bound by organic matter. However, if Na₄P₂O₇ is used previously, the metals recovered by the EDTA should be primarily in the carbonate form. Based on pH stability properties, HNO₃ should dissolve nearly all metal sulfides and other metal species not extracted by Na₄P₂O₇ or EDTA.

3. Results and discussion

3.1. Variation in pH and OPR

The change of sludge pH and OPR with bioleaching time is shown in Fig. 1. The pH showed an obvious increase followed a drastic fall during the first 5 days. The pH variation

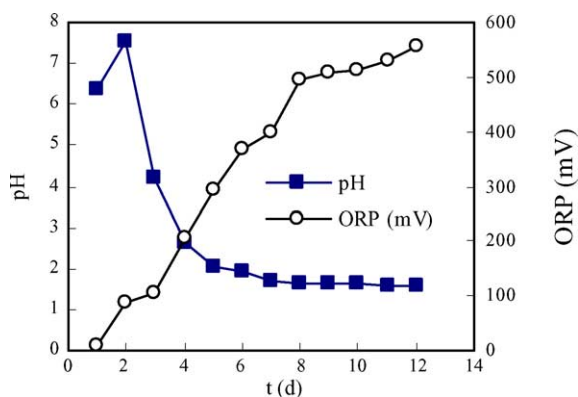


Fig. 1. Variation in pH and ORP during bioleaching.

could demonstrate the activity of sulfur-oxidizing bacteria. Blais et al. [9] 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 below 2 by acidophilic bacteria. The ORP kept rapid and continuous increase during first 8 days and slower thereafter. The utility of elemental sulfur by the sulfur-oxidizing bacteria led to the increase of sludge ORP, while the relatively slow increase during last few days of bioleaching was due to the lack of substrate, i.e. sulfur, for the growth of bacteria.

3.2. Variation in chemical form of heavy metals

3.2.1. Control

The partitioning of heavy metals with time in control is shown in Fig. 2. The carbonate precipitated form of Cu increased substantially during the experimental period, however, that of Pb and Zn kept relatively stable. As for the organically bound form, all of the metals showed an increasing trend. For all the three metals of control, the exchangeable, adsorbed and residual forms had slight change during the running, while the sulfide precipitate Cu, Pb and Zn showed an obvious increase due to the oxidizing condition caused by aeration with agitation.

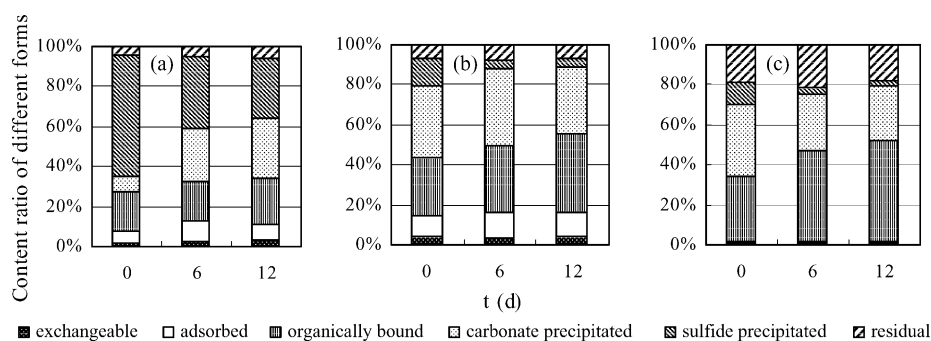


Fig. 2. Variation in partitioning of chemical forms for metals of control (a) Cu solubilization (b) Pb solubilization (c) Zn solubilization.

Table 3

Correlation between soluble metals and exchangeable metals

Metal	Linear equation	R^2
Cu	$y = 0.98x - 2.99$	0.9418
Pb	$y = 1.20x + 1.95$	0.9339
Zn	$y = 0.89x + 0.64$	0.9491

y: Percentage content of exchangeable metals.

x: Percentage content of soluble metals.

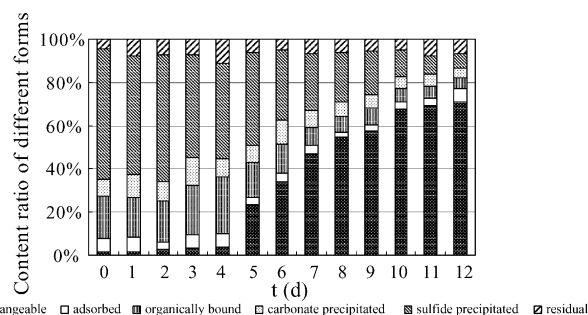


Fig. 3. Variation in partitioning of chemical forms for Cu during bioleaching.

3.2.2. Bioleaching sludge

It was difficult to clarify from which forms dissolved metals come. Some alternative should be exploited to investigate the change of the dissolved metals indirectly. The KNO_3 extraction mechanism based on ion-exchange reactions was used to remove a labile soluble/exchangeable form [6]. Table 3 shows the relation between soluble and exchangeable metals based on the percentage content during the 12 days of bioleaching process. It indicated that soluble heavy metals had good correlation with exchangeable ones. The transformation of exchangeable metal was easily determined by the sequential selective extraction procedure. Therefore, the bioleaching mechanism of soluble metals could be reflected by the transformation of exchangeable metals to a certain extent.

The Cu in the initial sludge existed mainly in sulfide precipitate form up to 60.4%, and followed by in organically bound form (about 20%) (Fig. 3). The sulfide precipitate Cu was gradually transformed to exchangeable Cu. The content of sulfide precipitate Cu, decreased up to 7.6% at the end of bioleaching showing an approximate linear trend of drop.

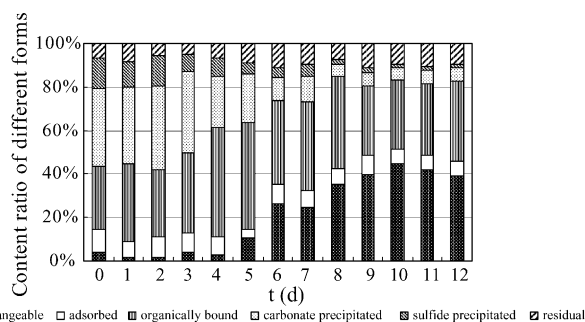


Fig. 4. Variation in partitioning of chemical forms for Pb during bioleaching.

The carbonate precipitated Cu was decreased from 11.0 to 5.0%, during which no obvious regulation was taken place. In the organically bound form, slight increase during the initial several days and sudden decrease between the 4th day and the 7th day was observed. The content of adsorbed Cu was low in original sludge and slightly decreased, while the residual Cu remained steady during the bioleaching process.

The drastic drop of organically bound Cu could be attributed to the release of Cu combined with biomass in sludge. The biomass containing heterotrophic bacteria was decomposed by the bioacidification of *Thiobacillus*. It was reported that Cu existed as an organic complex in sludge and was difficult to be dissolved because of its strong affinity to organic matter [10,11]. Moreover organically complex Cu^{2+} was bound more tightly than any other divalent transition metals [12]. A prolonged exposure under more extreme acidic conditions would be required to remove the organically bound metals [13]. In fact, the decreased extent of organically bound Cu was lower than that of sulfide precipitate during present experiment.

As a whole, more than 75% of exchangeable Cu was transformed from sulfide precipitated, and the second main transformation was organically bound (about 13%). However, the adsorbed and carbonate precipitated Cu played less role because of their low original proportion. Therefore, the bioleaching of Cu was mostly carried out in term of formula (1), i.e. the bioleaching mechanism of Cu was mainly direct.

As for Pb, the bioleaching efficiency was not so high as that of Cu (81.6%), which could be demonstrated by lower exchangeable Pb (40.2%) at the end of bioleaching (Fig. 4). The adsorbed Pb changed negligibly, while the residual form showed a little increase. The carbonate precipitate experienced acute fall at 3–6 days and remained 6% after 8 days.

The partitioning of chemical forms was affected by sludge characteristics, and the chemistry of metals was also an important parameter in determining the forms [6]. There was 36.0% carbonate precipitated Pb in the experimental sludge. The carbonate precipitated form was mostly referred to the heavy metals combined with the carbonate in sludge or carbonate precipitation itself. This form of heavy metals was most sensitive to the variation in pH and easy to be released under acid condition, which could explain the drastic transformation of carbonate precipitate during 3–6 days. The sol-

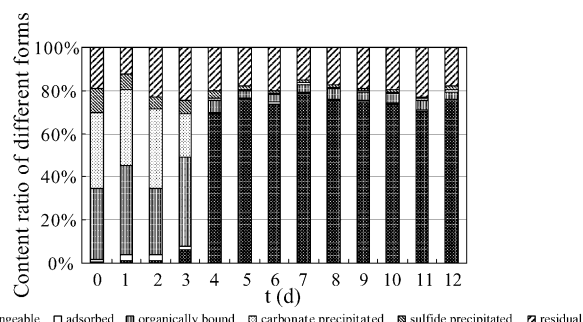


Fig. 5. Variation in partitioning of chemical forms for Zn during bioleaching.

ubility constant (K_s) of PbCO_3 was 3.3×10^{-14} , lower than that of PbSO_4 ($K_s = 1.06 \times 10^{-8}$). The PbCO_3 could solubilize due to the sulfuric acid produced and free Pb^{2+} was released. Theoretically the organically bound Pb with initial content of about 36% could gradually dissociate as the sludge cell died and broke up, but little variation in organically bound form was observed at present work. It could be explained by the lower ability of SO_4^{2-} bound Pb than that of organic biomass.

In conclusion, the exchangeable form of Pb was mainly transformed from sulfide precipitate and carbonate precipitate after the run. It implied that bioleaching of Pb was affected by the cooperation of direct mechanism and indirect mechanism and the function of the latter was more important.

The variation in chemical forms of Zn is showed in Fig. 5. Zn mainly existed in organically bound (33.1%) and carbonate precipitated forms (35.3%) before the bioleaching, which was similar to Pb. However, the transformation of these two chemical forms was far different from that of Pb. The two forms changed on the 4th day (pH 2.63) to a large degree. Almost all the carbonate precipitated Zn was turned to exchangeable, and most of the organically bound Zn also experienced a transformation to exchangeable. The transformation among different chemical forms was approximately constant after 4 days. The partitioning of Zn was more sensitive to the change of pH than that of Cu and Pb. At the end of bioleaching, only 5.5% of the exchangeable Zn came from sulfide precipitate, while the proportion of exchangeable Zn transformed from organically bound and carbonate precipitated forms could be up to 72.4% in all. It indicated that the bioleaching of Zn was mostly according to indirect mechanism. Because the initial percent of residual Zn was close to 20%, the bioleaching efficiency of Zn could not be improved further, although most of sulfide precipitate, carbonate precipitate and organically bound of Zn were transformed to exchangeable form.

The variation in forms of heavy metals in sewage sludge demonstrated that the residual form of the above three metals kept steady during the whole bioleaching process. It indicated that bioleaching had slight effect on the residual metals. It was because that the metals in residual form were mainly bound into resistant lattice structures, and the produced sulfuric acid could not have enough opportunity to react with

them. The dominant form and the specific characteristics of heavy metals in sewage sludge were of great diversity. When subjected to different treatment processes, the dominant inorganic form of heavy metals in sewage sludge was also different. The heavy metals associated with organic fraction of sludge were less available to solubilization than that with inorganic precipitates. The harm of heavy metals was mainly caused by exchanged, adsorbed and carbonate precipitated forms, and the other three forms were relatively stable after land application of sewage sludge. The removal of dissolved heavy metals was realized by solid–liquid separation, which meant most of the exchangeable metals could be removed because the exchangeable form determined by sequential selective extraction was greatly similar to the dissolved metals. As a result, a majority of the remaining heavy metals in sewage sludge could exist in stable forms which made a higher security for land application.

3.3. Relation between heavy metal forms and pH, ORP during bioleaching

Fig. 6 shows the relationship of metals' chemical forms and pH. For Cu, the exchangeable form achieved an obvious increase at pH about 2. The transformation of other forms to exchangeable was not achieved instantly in spite of pH as low as 2, and the quantity of exchangeable form experienced a course of gradual augment, from 23.5 to 81.6%. The correlation between pH and exchangeable metals, as shown in Table 4, indicated that Cu solubilization had slight relativity with the pH, which was in accordance with the results of Couillard et al. [14] using the aerobic sludge ($R^2 = 0.50$). The change of exchangeable Pb was similar to that of Cu. The exchangeable Zn showed a different pattern, which demonstrated higher percentage when pH was less than 3. It indicated that the solubilization of Zn was more sensitive to variation in pH and it showed close relationship with the pH.

Most of the colloidal materials adsorbed Cu^{2+} strongly and its ability of adsorption increased as the pH was raised. The change of pH affected the content of inorganic carbon, and the formation and solubilization of carbonate. The carbonate precipitate metals had direct correlation with the pH and content of carbonate, and were released due to the solubilization of carbonate when pH was low enough. The mechanism of pH affecting the transformation of heavy metals form had relationship with the chemical form of metals. With the decreasing pH, the solubility of organic matter in sludge and the ability of complex went down, resulting in decrease of organically bound metals. The organic matter was decom-

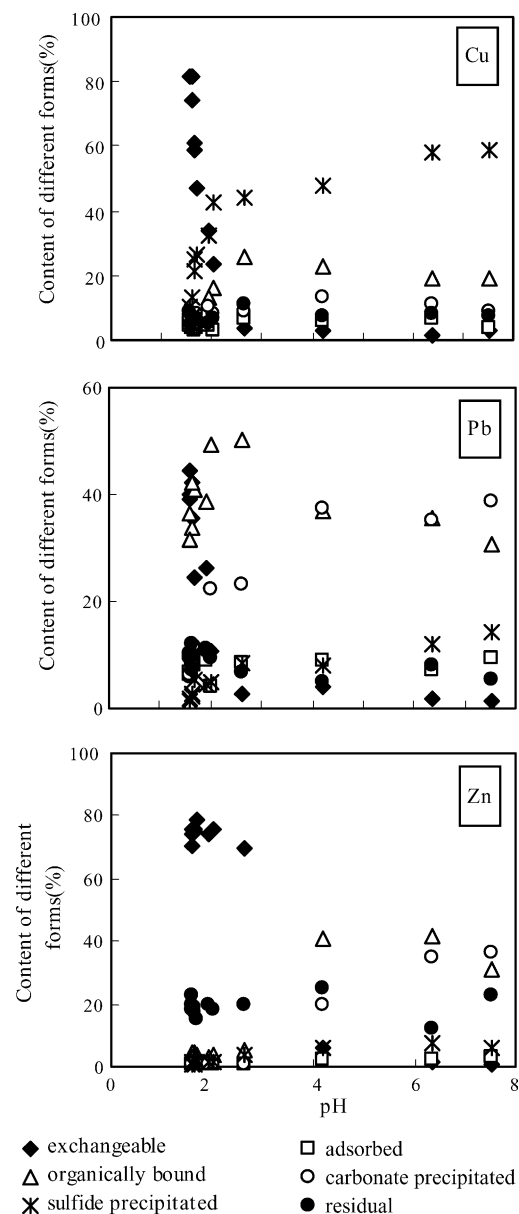


Fig. 6. Relation between heavy metal forms and sludge pH during bioleaching.

posed to materials having low molecular weight under acidic condition. The particles having low molecular weight could combine with metals to build up complex compound with high solubility. Given the low pH, it was less likely that mono or bivalent metals were adsorbed to the biomass. However, the adsorbed Cu did not completely but slightly transform to exchangeable form. The exchangeable form of heavy metals varied with the pH presumably because: (1) The negative charge of the sludge surface decreased with the increase of pH in the system, resulting in the weakening of adsorption to heavy metals and the increase of metals ion concentration; (2) The stability of complex action between sludge organic matter and metals also decreased with the increase of pH, which led to the solubilization of metals to liquid phase; (3) The K_s

Table 4
Correlation between exchangeable metals and pH

Metal	Linear equation	R^2
Cu	$y = -11.48 \text{ pH} + 72.37$	0.5422
Pb	$y = -6.38 \text{ pH} + 41.06$	0.5597
Zn	$y = -14.87 \text{ pH} + 99.06$	0.8848

y: Percentage content of exchangeable metals.

of multivalent cation and the hydroxyl ion decreased under low pH, and the production of metal hydroxide decreased, which reduced the adsorbance of metals to sludge.

The solubility of metals was governed primarily by pH but other factors were also important. For anaerobic sludge, Tyagi et al. [13] found that mere lowering of pH did not result in a shift of metal sulfides to the soluble ionic forms unless acidification was preceded by a rise in the sludge ORP. However, instant solubilization of a few metals such as Zn has been observed by merely lowering the pH and without raising ORP. As the raw sludge with higher initial ORP was used in this research, the function of ORP was not as important as that in anaerobic sludge. It was probable that the dependence of Cu solubilization toward oxidizing conditions explained the

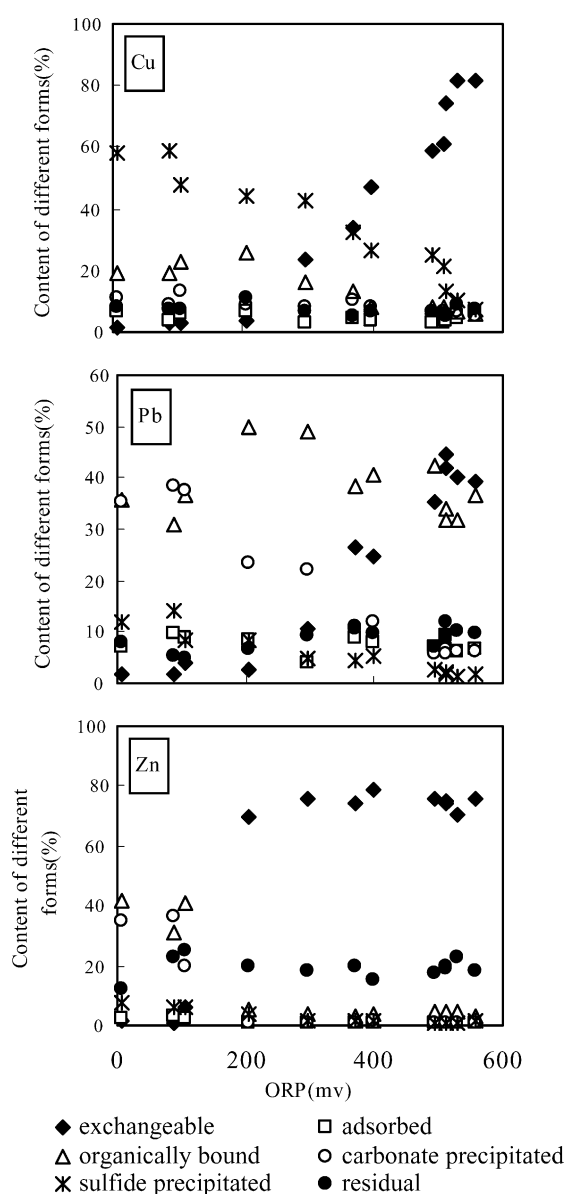


Fig. 7. Relation between heavy metal forms and sludge ORP during bioleaching.

Table 5

Correlation between exchangeable metals and ORP

Metal	Linear equation	R^2
Cu	$y = 0.16 \text{ ORP} - 13.90$	0.9170
Pb	$y = 0.08 \text{ ORP} - 6.38$	0.9139
Zn	$y = 0.14 \text{ ORP} + 8.32$	0.7247

y: Percentage content of exchangeable metals.

high percentage removal of organically bound and sulfide precipitated metals [15]. The obvious role of ORP could be demonstrated in the bioleaching of Cu (Fig. 7).

The chemical forms of metals could influence the function of ORP. It could be observed that the sulfide precipitated Cu showed an approximate linear fall when ORP varied from -47 to 500 mV. But the exchangeable Cu began to rise at ORP about 200 mV. It indicated that the solubilization of Cu, especially sulfide precipitated Cu, dependant not only on the pH, but also on the oxidation activity of *Thiobacillus*. The change in pattern of exchangeable Pb was similar to that of exchangeable Cu. The required ORP for solubilization of Zn was far lower than that of Cu. The exchangeable Zn had obtained a drastic increase in spite of the ORP less than 200 mV. It indicated that the ORP played fewer roles on the bioleaching of Zn than on that of Cu and Pb. The correlation of metals and ORP could be reflected from Table 5. The exchangeable Cu, and Pb had good relationship with ORP, and the correlation coefficient of both were more than 0.9 , while R^2 between exchangeable Zn and ORP was only 0.7247 . In comparison to the results of Couillard et al. [14] (correlation coefficient between Cu, Zn and ORP was 0.74 and 0.27 , respectively), the relationship between exchangeable metals and ORP at present work was more significant. This maybe caused by the different forms of metals between the present sludge and the experimental sludge used by Couillard et al., however, they did not refer the partitioning of metals in their research report. Therefore, it could not be compared each other further.

4. Conclusions

- (1) The transformation of heavy metal forms could be described well by the sequential selective extraction procedure during sewage sludge bioleaching.
- (2) The exchangeable Cu, Pb and Zn in control varied slightly after the running. The Cu in sludge mainly existed as sulfide precipitate, most of which turned in to exchangeable form during bioleaching, and Cu was solubilized mostly by direct mechanism. Most of carbonate precipitated Pb transformed to exchangeable form, and the bioleaching of Pb was realized by cooperation of both direct and indirect mechanism. A majority of exchangeable Zn was transformed from organically bound and carbonate precipitated forms, and the bioleaching mechanism of Zn was mainly indirect.

- (3) After bioleaching and solid–liquid separation, the exchangeable form of heavy metals in sewage sludge was removed and the remaining heavy metals were mostly stable, resulting in a higher security for land application of sludge.
- (4) The exchangeable Cu and Pb achieved an obvious increase at pH about 2, while higher percentage of exchangeable Zn showed at pH less than 3. The transformation of chemical forms of Cu had good relationship with ORP during bioleaching, but not for Zn.

Acknowledgements

The authors wish to acknowledge Dr. Kan Wang and Mr. Anton Perera for their assistance. The research was supported by the fund from Zhejiang Province (Project 2004C23024).

References

- [1] J. Ščančar, R. Milačič, M. Stražar, O. Burica, Total metal concentrations and partitioning of Cd, Cr, Cu, Fe, Ni and Zn in sewage sludge, *Sci. Total Environ.* 25 (2000) 9–19.
- [2] S.Y. Chen, J.G. Lin, Effect of substrate concentration on bioleaching of metal-contaminated sediment, *J. Hazard. Mater.* B82 (2001) 77–89.
- [3] A.Y. Renoux, R.D. Tyagi, R. Samson, Assessment of toxicity reducing after metal removal in bioleaching sewage sludge, *Water Res.* 35 (2001) 1415–1424.
- [4] H. Morita, H. Tsubol, Basic investigation on the chemical forms of heavy metals in a sewage treatment plant, *Water Sci. Technol.* 42 (2000) 159–165.
- [5] L.J. Tsai, K.C. Yu, S.F. Chen, P.Y. Kung, Effect of temperature on removal of heavy metals from contaminated river sediments via bioleaching, *Water Res.* 37 (2003) 2449–2457.
- [6] A.T. Lombardi Jr., O. Garcia, Biological leaching of Mn, Al, Zn, Cu and Ti in an anaerobic sewage sludge effectuated by *Thiobacillus ferrooxidans* and its effect on metal partitioning, *Water Res.* 36 (2002) 3193–3202.
- [7] T.R. Sreekrishnan, R.D. Tyagi, J.F. Blais, N. Meunier, P.G.C. Campbell, Effect of sulfur concentration on sludge acidification during the SSDML process, *Water Res.* 30 (1996) 2728–2738.
- [8] R.C. Stover, L.E. Sommers, D.J. Silveira, Evaluation of metals in wastewater sludge, *J. Water Pollut. Control Fed.* 47 (1976) 2165–2175.
- [9] J.F. Blais, R.D. Tyagi, J.C. Auclair, Bioleaching of metals from sewage sludge: microorganisms and growth kinetics, *Water Res.* 27 (1993) 101–110.
- [10] J.W. Olver, W.C. Kreye, P.H. King, Heavy metal release by chlorine oxidation of sludge, *J. Water Pollut. Control Fed.* 47 (1975) 2490–2497.
- [11] D. Couillard, M. Chartier, Removal of metals from anaerobic sludges by biological solubilization in batch reactors, *J. Biotechnol.* 20 (1991) 163–180.
- [12] L. Lazzari, L. Sperti, P. Bertin, B. Pavoni, Correlation between inorganic (heavy metals) and organic (PCBs and PAHs) micropollutant concentrations during sewage sludge composting process, *Chemosphere* 41 (2000) 427–435.
- [13] R.D. Tyagi, D. Couillard, F. Tran, Heavy metals removal from anaerobically digested sludge by chemical and microbiological methods, *Environ. Pollut.* 50 (1988) 295–316.
- [14] D. Couillard, M. Chartier, G. Mercier, Effect of pH and oxidation-reduction potential on biological solubilization of metals from aerobically digested sewage sludge, *Environ. Technol.* 12 (1991) 1095–1105.
- [15] M. Chartier, G. Mercier, J.F. Blais, Partitioning of trace metals before and after biological removal of metals from sediments, *Water Res.* 35 (2001) 1435–1444.