Heavy metal removal from wastewater and leachate co-treatment sludge by sulfur oxidizing bacteria

L.C. Aralp, A. Erdincler and T.T. Onay

Institute of Environmental Sciences, Bogazici University, 80815 Bebek, Istanbul, Turkey

Abstract Heavy metal concentration in sludge is one of the major obstacles for the application of sludge on land. There are various methods for the removal of heavy metals in sludge. Using sulfur oxidizing bacteria for microbiological removal of heavy metals from sludges is an outstanding option because of high metal solubilization rates and the low cost. In this study, bioleaching by indigenous sulfur oxidizing bacteria was applied to sludges generated from the co-treatment of municipal wastewater and leachate for the removal of selected heavy metals. Sulfur oxidizing bacteria were acclimated to activated sludge. The effect of the high organic content of leachate on the bioleaching process was investigated in four sets of sludges having different concentrations of leachate. Sludges in Sets A, B, C and D were obtained from co-treatment of wastewater and 3%, 5%, 7% and 10% (v/v) leachate respectively. The highest Cr, Ni and Fe solubilization was obtained from Set A. Sulfur oxidizing bacteria were totally inhibited in Set D that received the highest volume of leachate.

Keywords Bioleaching; heavy metals; leachate; sludge; sulfur oxidizing bacteria

Water Science and Technology **Vol 44 No 10 pp 53–58**

Introduction

In the last fifteen years, ultimate disposal of wastewater sludges has gained considerable attention due to gradually increasing problems. Treating the wastewater sludge and then using it for agricultural purposes is a very popular option but there are quite a lot of parameters that must be taken into account before the ultimate disposal of sludge. Heavy metal content of sludge is probably the most important parameter in reuse of the sludge.

Heavy metal content of sludge is about 0.5 to 2% on a dry weight basis and may even reach 4% w/w of chromium, copper, lead and zinc (Tyagi and Couilard, 1987). Furthermore great importance is given to the topic and many countries have heavy metal limits set for the ultimate disposal of sludge.

The two methods of heavy metal removal from sludge are chemical leaching and biological leaching. Biological leaching is more feasible than chemical leaching since enormous amounts of sludge have to be handled and the cost of chemicals wasted for the chemical leaching is much higher than the operational costs for the biological leaching process (Sreekrishnan and Tyagi, 1994). Bioleaching is also a very efficient metal removal method.

Bioleaching process is demonstrated under two headings. First one is iron oxidation where iron oxidizing bacteria (*Thiobacillus ferrooxidans*) are used to obtain heavy metal solubilization. *Thiobacillus ferrooxidans* can also be used together with *Thiobacillus thiooxidans*. Tyagi *et al*. (1988) showed that the efficiency of bioleaching can be increased by the use of mixed culture of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans. Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* are active below pH 4.0. The disadvantage of the iron oxidation method is that the initial pH of the system must be brought to 4.0 by external acid addition in order to start the bacterial activity. In the sulfur oxidation method, the mixed culture of *Thiobacillus thioparus* and *Thiobacillus thiooxidans* is used for metal solubilization. *Thiobacillus thioparus* reduces initial pH from 7.0 to around 4.0 without any need for external acid addition. The sulfur oxidation method is more efficient in metal solubilization when compared to iron oxidation (Blais *et al.*, 1993; Sreekrishnan and Tyagi, 1994).

Leachate generated from municipal solid waste (MSW) landfill sites requires a careful collection and treatment due to its high organic and heavy metal contents. Various biological and physico-chemical unit processes have been used for landfill leachate treatment. Due to its high organic and heavy metal content, leachate requires pretreatment and/or dilution with a municipal wastewater prior to biological treatment proceses. Biological treatment of leachate is effective for heavy metal and organic matter removal (Cameron and Koch, 1980; Ray and Chan, 1986; Avezu *et al.*, 1995) leading to high concentrations of heavy metals and potentially toxic trace organics in the produced sludges.

This study investigates the applicability of bioleaching process by sulfur oxidation to the sludges produced from co-treatment of municipal wastewater and leachate. It also investigates the ratio of leachate to total wastewater tolerable by sulfur oxidizing bacteria sensitive to the organic content of the environment.

Materials and methods

Sludges used in the experiments were produced in four 4-L plexiglass tanks by applying activated sludge process to wastewater and leachate mixture. The ratios of leachate in the mixture were adjusted as 3%, 5%, 7% and 10% (v/v) in the tanks A, B, C and D respectively. Mixed liquor volatile suspended solids concentration was maintained as 5000 mg/L in all of the tanks. Sludge age was adjusted to 10 days.

Leachate was obtained from Kemerburgaz MSW landfill site in İstanbul and maintained in a cold room at 4°C. Wastewater was prepared synthetically by using whey powder. The initial COD concentrations in the leachate and synthetic wastewater were 9000 mg/l and 500 mg/l respectively. The sludge produced from the co-treatment of leachate and synthetic wastewater mixture was used in the bioleaching experiments to observe the metal solubilization capacity of sulfur oxidizing microflora.

Acclimation of sulfur oxidizing microflora

The acclimation of sulfur oxidizing microflora to undigested sewage sludge was carried out according to a technique modified from Blais *et al.* (1993). A 600 ml sludge sample was transferred to a 2 L plexiglass tank. 1% (w/v) powdered elemental sulfur was added to the sludge. The sludge sample was aerated continuously and the tank was kept in a water bath at constant temperature of 25°C. The process was carried out until the initial acidification (pH $\cong$ 2.0) was reached in 50 days. After the initial acidification, 600 ml of fresh sludge was transferred to a 2 L plexiglass tank and 1% (w/v) powdered elemental sulfur was added to the sludge. This sludge was then mixed with 5% acidified sludge and reincubated. This process was repeated for 2 more times to reach the acidification (pH $\cong$ 2.0) in a shorter time of about 5 days.

Metal solubilization by sulfur oxidizing bacteria

Sludges obtained from co-treatment of wastewater and four different ratios of leachate were transferred to four identical 2 L plexiglass tanks. They were mixed with 1% (w/v) powdered elemental sulfur and 5% acidified sludge. The tanks were continuously aerated and kept at 25°C in a water bath.

The pH and temperature of the sludges were measured at 24-hour intervals. The 50 ml sludge samples were drawn from the tanks at different pH values. The samples were centrifuged for 10 minutes at 2,800g for the solids–liquid separation. The liquid portion (supernatant) was used to determine the solubilized trace metal and sulfate concentrations in sludge samples.

Cu, Cr, Ni and Fe concentrations in the supernatants of the sludge samples were determined by digesting the 25 ml samples according to EPA Method 3010. A Perkin Elmer Atomic Absorption Spectrophotometer (Analyst 300) was used to measure heavy metal concentrations in the digested samples. Sulfate concentration in the supernatants of sludge samples were measured by using a Hach spectrophotometer.

Results and discussions

The effect of leachate, having high organic content, on the bioleaching process was investigated in four sets of sludges having different ratios of leachate. Sludges in Sets A, B, C and D were obtained from the co-treatment of wastewater and 3%, 5%, 7% and 10% (v/v) leachate respectively. Solubilization of Cu, Cr, Ni and Fe was investigated in all three sets and reported in Table 1. Data on the initial pH, temperature, total solids content and heavy metal concentrations of the sludges in Sets A,B,C and D are provided in Table 1.

The general trend in the experiments was that the solubilization of Cr increased when the sludge pH dropped below 2.5. On the other hand, the solubilization of Ni did not show a sharp increase below pH 3.0. It started to increase below pH 4.0 and continued until the end of the experiments. The solubilization of Fe increased below pH 2.5.

The pH of sludge in Set A decreased to 2.9 on the 5th day of introduction of elemental sulfur. After the 5th day the pH of the sludge remained almost constant in a range of pH 2.5–2.9 until the end of 21st day. (Figure 1). The small increases in sludge pH can be attributed to the reducing effect of leachate on the activity of acidophilic bacteria being active in the pH range of 4.0–2.0. The effect of leachate was lower on the less acidophilic bacteria being active at pH 7.0–4.0. The less acidophilic bacteria decreased the pH of sludge from 5.8 to 4.0 in 4 days. Generally it takes only 24–36 hours for these bacteria to drop the pH of sewage sludge itself to the same level (Blais *et al*., 1993; Sreekrishnan and Tyagi, 1994).

The sulfate concentration at the end of 21st day at pH 2.9 was 2,400 mg/L verifying the bacterial activity in sludge. Several researchers recorded sulfate concentrations of 1,500–2,200 mg/L in sewage sludges at pH around 3.0 (Blais *et al*., 1993; Sreekrishnan *et al*., 1993; Tyagi *et al*., 1993; Ravishankar *et al*.,1994).

In Set A, the solubilization of metals was 80%, 17%, 77% and 4% for Cu, Cr, Ni and Fe respectively. The solubilization of Cu, Cr and Ni remained almost constant after the pH of sludge reached 2.88 on the 5th day (Figure 1). Fe has the lowest solubilization among the selected heavy metals. Due to the high initial concentration of Fe (123 mg/L) in the sludge, solubilization of Fe was not complete at the end of the 21st day.

In Set B a similar pH trend as in Set A was observed. The pH of the sludge in Set B dropped to 3.05 on the 5th day and raised to 3.70 on 18th day. This was also a result of reducing effect of leachate on the activity of acidophilic bacteria. However, the final sulfate concentration of 5,400 mg/L showed that the bacteria functions properly at this pH. The increase in pH on the 2nd day was due to the continuing activity of hetotrophic bacteria.

A pH increase was observed in Set B. The pH started to increase slowly after the 5th day from 3.06 until 3.7 because of the inhibiting effect of leachate. The heavy metal solubilization percentages in Set B were found to be 15% for Cu, 16% for Cr, 41% for Ni and 1.4% for Fe. Copper is known as a difficult element to be solved because of its strong affinity to organic matter (Couillard and Chartier, 1991). The critical pH value for Cu solubilization from sewage sludge is 2.0 (Wozniak and Huang, 1982). Although pH of the Set B was the lowest among the three sets, the solubilization of Cu was also the lowest. This may be a result of the respectively higher TS concentration of Set B (Table 1). The solubility of Cu decreases with higher total solids concentration (Tyagi *et al*., 1988). High total solids content does not have a direct effect on metal solubilization but has an indirect effect since it has buffering capacity on pH where more acid is needed to decrease pH (Sreekrishnan *et* *al.*, 1993). Solubilization of heavy metals increased when the pH droped below 3.0. The increase in the solubility of Cr and Fe was higher at the pH values below 3.0 (Figure 2).

In Set B, different from other sets, another 13-day period was added on to 21-day process time. At the end of 33 days solubilization of Cu, Cr, Ni and Fe increased dramatically to 71%, 78%, 74% and 48% respectively. It seems that although it would not be feasible to

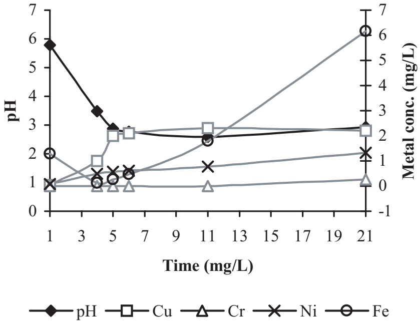

Figure 1 pH and heavy metal concentrations in supernatant of sludge in set A

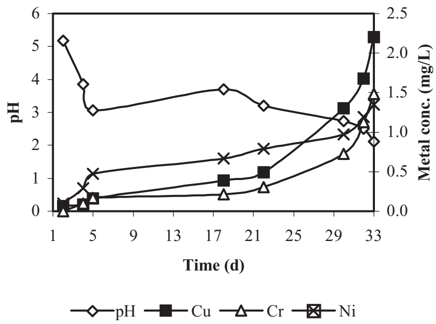

Figure 2 pH and heavy metal concentrations in supernatant of sludge in set B

Table 1 Initial trace metal concentrations and solubilization rates for Set A, Set B and Set C

		SET A	SET B	SET C
	Initial pH	5.78	4.84	5.52
	Final pH	2.92	2.12	3.30
	Initial COD of sludge supernatant (mg/L)	422	444	612
	Final COD of sludge supernatant (mg/L)	283	273	290
	COD removal (%)	33	39	53
	Initial total solids (g/L)	5.65	8.00	6.10
	Final total solids (g/L)	4.52	5.4	4.99
	TS removal (%)	20	35	18
Cu	Recommended* trace metal conc. in sludge to be used in agricultural land (mg/kg)	1,200	1,200	1,200
	Initial trace metal conc. in sludge (mg/kg)	478	375	541
	Final trace metal conc. in sludge supernatant (mg/kg)	382	56	498
	Required removal (%)	0	0	0
	Achieved solubilization rate (%)	80	15	92
Cr	Recommended* trace metal conc. in sludge to be used in agricultural land (mg/kg)	1,200	1,200	1,200
	Initial trace metal conc. in sludge (mg/kg)	273	238	395
	Final trace metal conc. in sludge supernatant (mg/kg)	46	38	0
	Required removal (%)	0	0	0
	Achieved solubilization rate (%)	17	16	0
Ni	Recommended* trace metal conc. in sludge to be used in agricultural land (mg/kg)	200	200	200
	Initial trace metal conc. in sludge (mg/kg)	283	213	344
	Final trace metal conc. in sludge supernatant (mg/kg)	218	87	230
	Required removal (%)	29	6.5	72
	Achieved metal solubilization (%)	77	41	67
Fe	Recommended* trace metal conc. in sludge to be used in agricultural land (mg/kg)	–**	–**	–**
	Initial trace metal conc. in sludge (mg/kg)	21,770	26,116	22,660
	Final trace metal conc. in sludge supernatant (mg/kg)	871	366	0
	Required removal (%)	–	–	–
	Achieved solubilization rate (%)	4	1.4	0

* Adapted from Turkish Environmental Legislation
** No regulation found

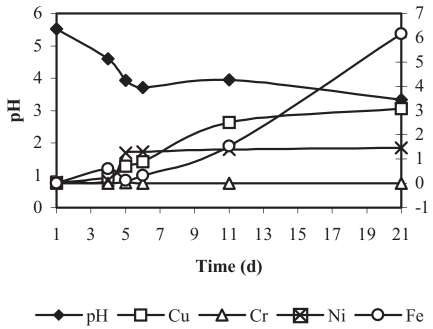

Figure 3 pH and heavy metal concentrations in sludge in set C

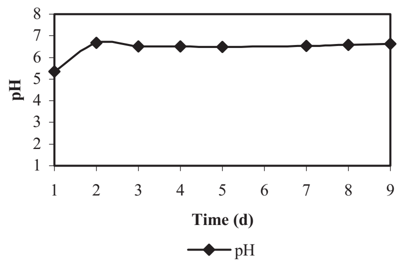

Figure 4 pH Profile of sludge in set D

apply such a long process time, the solubilization of heavy metals in sludges carrying higher leachate concentrations may be enhanced by increasing the process time.

In Set C, a small pH increase was observed between the 6th and 11th day similar to the increase in Set A and Set B (Figure 3). This period can be interpreted as the adaptation period of acidophilic bacteria to the environment. Among the three sets the highest pH was achieved in Set C. At the same time, the highest sulfate production of 5,600 mg/L was also obtained in this set. This can be attributed to the buffering capacity of leachate on pH. It should also be noted that higher total solids concentration for the bioleaching process is known to give higher sulfate concentrations due to the gradual drop in pH (Ravishankar *et al.*, 1994, Sreekrishnan *et al.*, 1993).

The solubilization of Fe and Cr was 0% for both at the end of 21st day (Table 1). On the other hand, the solubilization of Cu was unexpectedly high at 92%. The reason for high Cu solubilization could not be understood clearly.

Set D received sludge from co-treatment of wastewater and 10% leachate. At this high ratio of leachate the activity of *Thiobacillus* species, sensitive to high organic loads, was totally inhibited due to the high organic content of applied leachate. The system pH for Set D was constant until the end of the experiment (Figure 4).

Except Ni, the initial metal concentrations in the three sets were below the recommended concentrations given in the Turkish Environmental Regulations. The criteria for sludge to be used in agriculture were not fulfilled in Set C. The results then showed that the bioleaching process was not completely satisfactory for the sludge from co-treatment of wastewater and 7% leachate.

The solubilization of selected heavy metals in the three sets were compared on the basis of 21-day process time, Set B achieved the lowest solubilization for Cu, and Ni. In this case higher solubilization rates were achieved in Set A, which received the lowest leachate concentration of 3%. Although the initial heavy metal concentrations of sludge in Set A were higher than those of Set B, the solubilization of metals was also higher in this sludge. The higher heavy metal concentrations of sludge in Set A did not have an adverse effect on their solubilization.

The acidophilic and less acidophilic bacteria were affected by the increasing leachate concentration in the wastewater–leachate mixture. The hydraulic retention times needed for acidification of the subject sludge were very long. The pH 2.12 is reached on the 34th day in Set B. Generally, 2–4 days would be enough for sulfur oxidizing bacteria to reduce pH to 2.0 in sewage sludge (Blais *et al.*, 1993; Ravisahnkar *et al.*, 1994; Sreekrishnan *et al.*, 1994; Tyagi *et al.*, 1993). However, it should be noted that in this study, experiments were conducted with bacteria acclimated to undigested sewage sludge. In future studies, the higher heavy metal solubilizations can be achieved if the acclimation of bacteria is done to sludge generated from the co-treatment of wastewater and leachate.

Conclusions

The purpose of this study was to investigate the applicability of bioleaching process by sulphur oxidation to the sludges produced from co-treatment of municipal wastewater and leachate. Based on the results of this research the following conclusions were drawn.

The bioleaching process functions properly for sludges, sourced from co-treatment of domestic wastewater and leachate, depending on the ratio of leachate volume to total wastewater volume.

The efficiency of the bioleaching process was the highest for the sludges from co-treatment of wastewater and 3% (v/v) leachate. When the leachate ratio was increased, the efficiency of the bioleaching process decreased. Increase of leachate ratio to 10% caused total inhibition of the sulfur oxidizing bacteria in the system.

The bioleaching process time for sludge from co-treatment of wastewater and leachate was very long compared to the process time required for sewage sludge itself. The process time increased with increasing ratio of leachate. It may be possible to shorten the process time through the enrichment of sulfur oxidizing bacteria in wastewater–leachate sludge.

Leachate reduced the activity of acidophilic bacteria due to its high organic content and buffering capacity on sludge pH. *Thiobacillus thiooxidans* are more sensitive to leachate concentration compared to *Thiobacillus thioparus*.

Further studies should be performed to investigate the effect of temperature, total solids content and higher heavy metal concentrations of sludge on the efficiency of the bioleaching process.

Acknowledgement

This study has been supported by TUBITAK under project number YDABCAG-199Y109, and Bogazici University Research Fund under project number 01HY101.

References

Avezzu, F., Bissolotti, G., Collivignarelli, C. and Ghirardini, A. Volpi (1993). Behaviour of Heavy Metals in Activated Sludge Biological Treatment of Landfill Leachate. *Waste Manage. Res.*, **13**, 103–121.

Blais, J.F., Tyagi, R.D. and Auclair, J.C. (1993). Bioleaching of Metals from Sewage Sludge: Microorganisms and Growth Kinetics. *Wat. Res.*, **27**(1), 101–110.

Cameron, R.D. and Koch, F.A. (1980). Trace Metals and Anaerobic Digestion of Leachate. *J. WPCF*, **52**(2), 282–292.

Couillard, D. and Chartier, M. (1991). Removal of Metals from Aerobic Sludges by Biological Solubilization in Batch Reactors. *J. Biotechnol.*, **20**, 163–180.

Ravishankar, B.R., Blais, J.F., Benmoussa, H. and Tyagi, R.D. (1994). Bioleaching of Metals from Sewage Sludge: Elemental Sulfur Recovery. *J. Environ. Eng.*, **120**(2), 462–470.

Ray, C. and Chan, P.C. (1986). Heavy Metals in Landfill Leachate. *Int. J. Environ. Studies*, **27**, 225–237.

Sreekrishnan, T.R., Tyagi, R.D., Blais, J.F. and Campbell, P.G.C. (1993). Kinetics of Heavy Metal Bioleaching from Sewage Sludge-I. Effects of Process Parameters. *Wat. Res.*, **27**(11), 1641–1651.

Sreekrishnan, T.R. and Tyagi, R.D. (1994). Heavy Metal Leaching from Sewage Sludges: A Techno-Economic Evaluation of the Process Options. *Environ. Technol.*, **15**, 531–543.

Tchobanoglous, G., Theisen, H. and Vigil, S. (1993). *Integrated Solid Waste Management: Engineering Principles and Management Issues*. McGraw-Hill, Inc., Singapore.

Tyagi, R.D. and Couilard, D. (1987). Bacterial Leaching of Metals from Digested Sewage Sludge. *Process Biochem.*, 114–117.

Tyagi, R.D., Couilard, D. and Tran, F. (1988). Heavy Metals Removal from Anaerobically Digested Sludge by Chemical and Microbiological Methods. *Environ. Pollut.*, **50**, 295–316.

Wozniak, D.J. and Huang, J.Y.C. (1982). Variables Affecting Metal Removal from Sludge. *J. WPCF*, **54**(12), 1574–1580.