Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2013.817374

52 (2014) 6185–6193
September

Taylor & Francis
Taylor & Francis Group

Tolerance levels of sulfur-oxidizing micro-organisms to Methylene blue and Remazol black B dyes during sewage sludge bioleaching

Kapil Kumar[a,*], M.G. Dastidar[a], T.R. Sreekrishnan[b]

[a]*Centre for Energy Studies, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India*
Tel. +91 8475805221; email: Kapil.iitd05@gmail.com
[b]*Department of Biochemical Engineering & Biotechnology, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India*

Received 28 April 2012; Accepted 30 May 2013

ABSTRACT

The present study was undertaken to investigate the tolerance levels of indigenous sulfur-oxidizing micro-organisms to Methylene blue (MB), Remazol black B (RBB), and mixture of both the dyes (DM) during bioleaching of heavy metals from sewage sludge. The experiments were performed with anaerobically digested sewage sludge at initial neutral pH of the sludge containing 0–35,000 mg/l of the MB, RBB, and DM. The results show that the bioleaching process was affected by the increase in concentration of MB, which decreased the growth of indigenous sulfur-oxidizing micro-organisms leading to the slow rate of decrease in pH and hence decreased solubilization of metals. Similar effects were observed on the activity of indigenous sulfur-oxidizing micro-organisms using RBB and a mixture (DM) of MB and RBB. The presence of RBB and DM were found to be more toxic as compared to MB at the same initial concentration of the dyes. At initial concentration of 30,000 mg/l, a drastic reduction in pH drop was observed with decreased solubilization of metals, irrespective of any dye. The results of the present study shall be useful to develop a suitable bioleaching process for the sludges contaminated with a variety of dyes.

Keywords: Sewage sludge; Heavy metal; Leaching; Acidithiobacillus; Dyes

1. Introduction

Dyes are the coloring agents used in textile, pharmaceutical, pulp and paper, food, tanneries, paint, plastics, electroplating, and cosmetics industries. The wastewater generated in these industries are highly colored and it is estimated that around 15% of the used dyestuff is released into the process water during manufacturing of dye [1,2]. Many dyes and pigments are toxic and are known to have carcinogenic, mutagenic, and tetratogenic effects [3]. Besides dyes, the dye contaminated wastewater is also known to contain a variety of inorganic salts and heavy metals such as Cu, Cr, Zn, etc., which are present as an integral part of various dyes [4]. Further, various chemicals and compounds used in the dyeing process also add to the total metal load in the wastewater coming out from the textile mills [5].

During the treatment of wastewater contaminated with dye, the heavy metals present originally in the wastewater find their way into the sludges, the disposal of which is a serious environmental problem.

*Corresponding author.

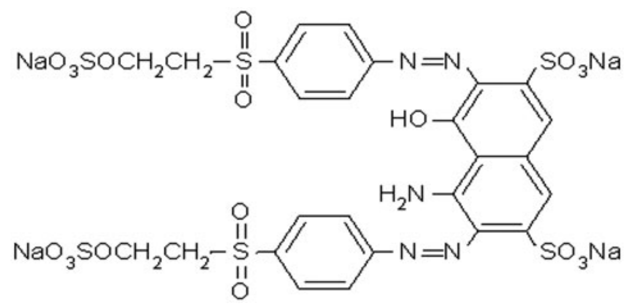
It was reported that the concentration of metals in the sludge contaminated with dye is typical of that of the sewage sludge. The average concentrations of Cu, Ni, Zn, Cr, Mn, and Pb in the sludge from dye industries were found to be in the range of 25–740 mg/kg of dry sludge [5]. Further, sewage sludges are often contaminated with dye, as in the absence of adequate treatment facilities in the small and medium scale dye industries and hence, wastewater contaminated with dyes are often discharged in the sewer line without giving any treatment. The heavy metals can leach out and contaminate the soils as well as the surface and groundwater sources when metal-laden sludges are disposed of on land or in landfills [6]. There is ample information available in the literature on various chemical methods employed for the elution of heavy metals from soil, sludges, and solid wastes [7]. However, the practical application of the chemical processes is still limited due to the requirement of large amount of chemicals, high operating cost, operational difficulties, and secondary pollution problems associated with them [8].

Over the years, the bioleaching process using sulfur-oxidizing (*At. thiooxidans*) micro-organisms has been reported to be an efficient and economical method for the removal of heavy metals from sewage sludges [9]. The applications of these micro-organisms along with other micro-organisms in metal leaching from industrial sludges have also been reported [10–14]. The micro-organisms involved in bioleaching can survive at low pH and high oxidizing conditions developed during bioleaching [15]. Further, being resistant to a variety of toxic metals, the micro-organisms have a great potential in metal bioleaching. There is sufficient information in the literature on the metal tolerance of *Acidihiobacilli* [16]. However, there is still a lack of information about the tolerance level of *Acidithiobacilli* indigenous to sludge contaminated with other toxicants such as dyes. It is worth mentioning that the most of the dyes are considered as toxic and for the development of a successful bioleaching process for sludges contaminated with metals and dyes, there is a need to examine the metal leaching in the presence of different dyes. The present study is probably a first attempt to examine the tolerance levels of indigenous sulfur-oxidizing micro-organisms to different dyes such as Methylene blue (MB), Remazol black B (RBB), and a mixture of both the dyes (DM) during metal bioleaching. The batch bioleaching experiments were carried out using anaerobically digested sewage sludge inoculated with indigenous sulfur-oxidizing micro-organisms. The effects of different concentrations (5,000, 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l) of MB, RBB, and DM on bioleaching of metals were investigated.

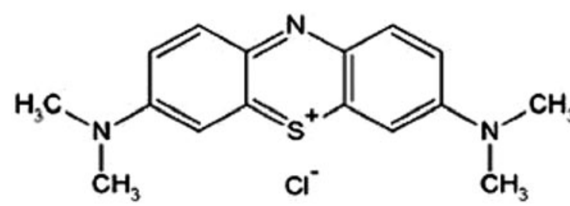
2. Materials and methods

2.1. Synthetic solutions of dyes

In the present study, RRB (molecular formula $C_{26}H_{21}N_5Na_4O_{19}S_6$, molecular weight: 991.82 and pKa 6.9) and MB (molecular formula: $C_{16}H_{18}N_3SCl$, molecular weight: 319.85 and pKa 3.8) were used. Stock solutions of RBB and MB dyes of 1,00000 mg/l concentration were prepared by dissolving the appropriate quantities of powdered dyes in tap water and the solutions of the desired concentrations for various experiments were obtained by successive dilution. The dyes were procured from the textile engineering department of IIT Delhi and were of analytical grade with 95% purity. The chemical structure of the dyes are shown in Scheme 1(a) and (b).



Scheme 1(a). Structure of Remazol black B.



Scheme 1(b). Structure of Methylene blue.

2.2. Characterization of the sludge

The anaerobically digested sewage sludge was procured from a sewage treatment plant (treatment capacity > 100 million gallon/d) located in Delhi, the capital city of India. The characteristic properties of the sludge before bioleaching are shown in Table 1. The pH, total solids content, organic and inorganic matter contents, total Kjeldahl nitrogen, and total phosphorus content of the sludge were determined according to the standard methods [17]. For the determination of the total heavy metal content, the sludge samples were subjected to di-acid digestion ($HNO_3 + HClO_4$) and the heavy metals in the digested liquid were determined with the help of atomic

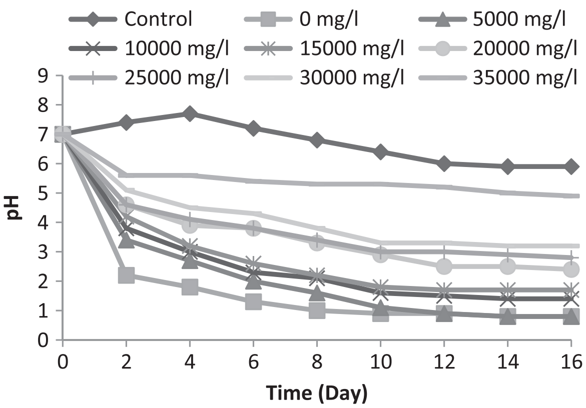


Fig. 1. Change in pH with time in sewage sludge during bioleaching at different concentrations of MB using sulfur-oxidizing micro-organisms.

Table 1
The characteristic properties of sewage sludge

Parameters	Value
pH	7.1
Total solids (g/l)	20.2
Organic matter (mg/kg dry sludge)	43
Inorganic matter (mg/kg dry sludge)	57
Nitrogen (mg/kg dry sludge)	2.7
Phosphorus (mg/kg dry sludge)	1.2
Cu (mg/kg dry sludge)	472
Ni (mg/kg dry sludge)	294
Zn (mg/kg dry sludge)	1,310
Cr (mg/kg dry sludge)	332

absorption spectrophotometer (Perkin Elmer AAnalyst 200).

2.3. Preparation of inoculum

For inoculum preparation, the secondary activated sludge was fortified with 0.5% (w/v) elemental sulfur for sulfur-oxidizing micro-organisms at 28℃ and 180 rpm. When pH of the sludge decreased from an initial value of 7 to 2, the culture was transferred to fresh sludge for further enrichment. This procedure was repeated three times so as to get an active inoculum (enriched sludge) for using it in the subsequent bioleaching experiments.

2.4. Batch bioleaching experiments

The batch bioleaching experiments were conducted in 500 ml Erlenmeyer flask with 250 ml of sewage sludge (20 g/l of the solids). The sludge was inoculated with 10% (v/v) of the inoculum and 0.5% (w/v) elemental sulfur at 28℃ and 180 rpm. The control experiment was also conducted using the same sludge under similar conditions without adding any inoculum and elemental sulfur. To examine the effect of dyes on sulfur-oxidizing micro-organisms, MB, RBB, and DM dyes, each in the concentration range of 0–35,000 mg/l were added to the sludge in separate flasks. For example, to achieve a dye concentration of 10,000 mg/l, 10,000 mg dye was directly added to 1 L of sludge and 250 ml of the sludge aliquot was used for the bioleaching experiments. All the bioleaching experiments were performed in duplicate for 16 days. The change in pH, ORP, and solubilization of heavy metals in the absence and presence of dyes was monitored with time.

3. Results and discussion

3.1. Change in pH and ORP

The change in pH with time, in sludge having MB (0–35,000 mg/l) along with the control (without dye and sulfur) is shown in Fig. 1. In the control, the pH initially increased from an initial value of 7–7.4 on the fourth day and then decreased to 5.9 on the sixteenth day of bioleaching studies. No significant decrease could be observed in pH in the control. On the other hand, in the sludge having elemental sulfur and inoculum, the indigenous sulfur-oxidizing micro-organisms were able to oxidize elemental sulfur even in the presence of high concentration of dyes resulting in rapid decrease in pH.

However, the decrease in pH was slower at higher initial concentration of dye. This is because at higher concentrations, the inhibitory effect of the dye was more pronounced on the growth of sulfur-oxidizing

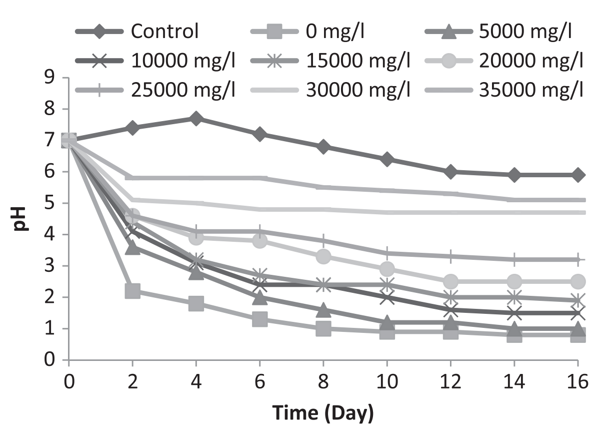


Fig. 2. Change in pH with time in sewage sludge during bioleaching at different concentrations of RBB using sulfur-oxidizing micro-organisms.

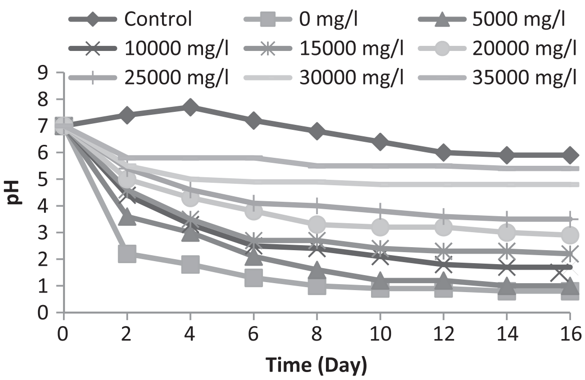


Fig. 3. Change in pH with time in sewage sludge during bioleaching at different concentrations of DM using sulfur-oxidizing micro-organisms.

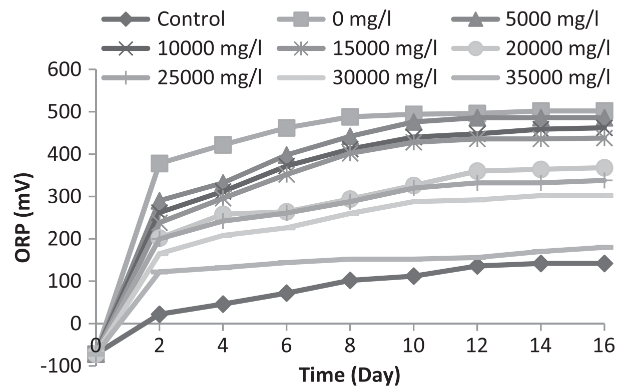


Fig. 4. Change in ORP with time in sewage sludge during bioleaching at different concentrations of MB using sulfur-oxidizing micro-organisms.

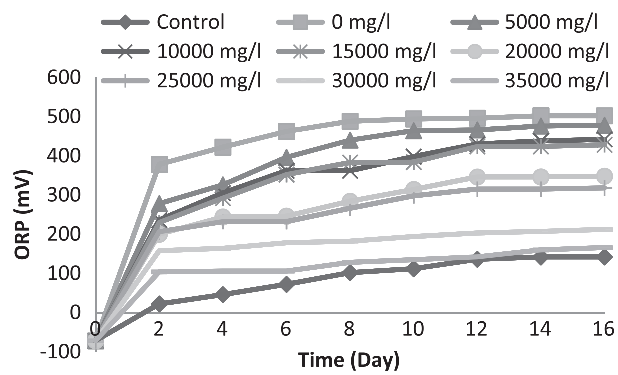


Fig. 5. Change in ORP with time in sewage sludge during bioleaching at different concentrations of RBB using sulfur-oxidizing micro-organisms.

micro-organisms. This led to less oxidation of elemental sulfur and hence, less production of acid resulting in slow decrease of pH. In the sludge having only elemental sulfur and inoculum (0 mg/l dye), the pH decreased sharply to less than 2 in 4 days and reached 1 on the eighth day. In the sludge having 5,000 mg/l MB, it took 6 days to reach the pH 2 and 10 days to reach pH less than 1. However, the final pH (0.8) was identical in both the sludges and remained constant till the sixteenth day. However, MB concentration beyond 5,000 mg/l seems to reduce the growth of indigenous sulfur-oxidizing microbes as indicated by the final pH values 1.4, 1.7, 2.4, 2.8, and 3.2 achieved in the sludges having 10,000, 15,000, 20,000, 25,000, and 30,000 mg/l MB, respectively, after 16 days of bioleaching. With a further increase in MB concentration up to 35,000 mg/l, the final pH remained at 4.9 after 16 days of bioleaching. The results show that the increasing concentration of MB dye proved more toxic to sulfur-oxidizing micro-organisms. A similar trend in decrease in pH with time during bioleaching was observed in the presence of RBB (Fig. 2). In the sludge having 5,000 mg/l RBB, the pH decreased sharply to less than 2 in 6 days and reached finally 1 in 16 days of bioleaching, as compared to pH 0.8 in the sludge containing MB. The final pH achieved in the sludges containing 10,000, 15,000, and 20,000 mg/l RBB was 1.5, 1.9, and 2.5, respectively, in 16 days of bioleaching. At RBB concentration of 35,000 mg/l, the final pH remained constant at 5.1 till the sixteenth day of bioleaching. The above results show that the presence of RBB in sludge had more toxic effect on the sulfur-oxidizing microbes as compared to MB.

Fig. 3 shows that in the sludge having 5,000 mg/l DM (combination of RBB and MB in equal proportions), the pH decreased to less than 2 in 8 days and reached finally 1 in 16 days of bioleaching. This indicates that presence of DM had more toxic effect on the micro-organisms as compared to the individual dyes. Further increase in DM concentration indicated reduced growth of sulfur-oxidizing micro-organisms as higher final pH values, i.e., 1.7, 2.2, 2.9, and 3.5 were achieved in the sludges containing 10,000, 15,000, 20,000, and 25,000 mg/l DM, respectively, in 16 days. These pH values were higher than the final pH achieved in the sludges having MB and RBB individually. At DM concentration of 35,000 mg/l, the final pH achieved was 5.4.

3.2. Change in ORP

The change in ORP with time during bioleaching in the presence of MB is shown in Fig. 4. In the sludges having different concentrations of MB, the ORP decreased with increase in dye concentration. In the sludge having 5,000 mg/l MB, the ORP increased to 378 mV on the second day and finally to 486 mV on the sixteenth day. Increase in MB concentration beyond 5,000 mg/l resulted in lower rate of increase

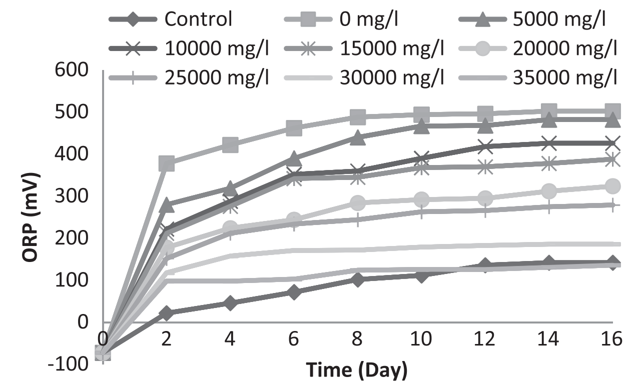


Fig. 6. Change in ORP with time in sewage sludge during bioleaching at different concentrations of DM using sulfur-oxidizing micro-organisms.

in ORP. The final ORP achieved in the sludges containing 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l MB was 462, 438, 368, 338, 302, and 180 mV, respectively, in 16 days of bioleaching.

A similar trend in change in ORP with time was observed in the sludges having RBB (Fig. 5). However, the increase in ORP was slower in the sludges having RBB compared to the sludges containing MB. In the sludges having 5,000, 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l RBB, the ORP value was 478, 442, 428, 348, 318, 212, and 166 mV, respectively, on the sixteenth day of bioleaching.

The ORP values achieved in the sludges having DM were observed to be lower compared to the sludges containing MB and RRB, separately (Fig. 6). The final ORP values obtained in the sludges having 5,000, 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l DM were found to be 482, 426, 388, 324, 279, 186, and 136 mV, respectively, in 16 days of bioleaching. The above results confirm that the increasing concentration of DM had more inhibitory effect on the growth of bioleaching micro-organisms leading to reduced oxidation of elemental sulfur.

The bio-oxidation of elemental sulfur by indigenous sulfur-oxidizing micro-organisms is associated with a change in ORP of the system. The utility of elemental sulfur by the sulfur-oxidizing bacteria led to the increase of sludge ORP. A rise in ORP and lowering of pH are indicators of substantial growth of bacteria and hence higher solubilization of metals. Bioleaching process involves metal solubilization due to in situ production of the acid by the action of iron and sulfur-oxidizing bacteria.

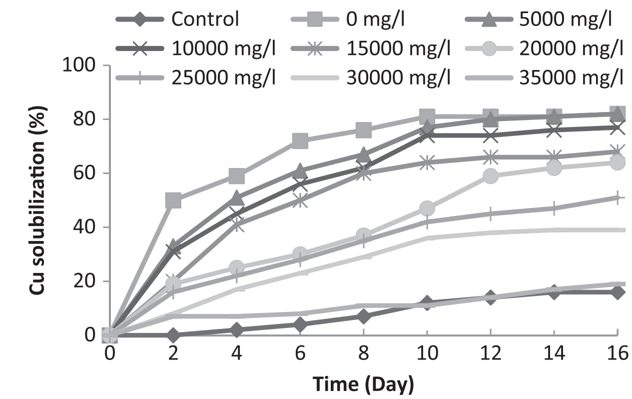


Fig. 7. Solubilization of Cu with time from sewage sludge during bioleaching at different concentrations of MB using sulfur-oxidizing micro-organisms.

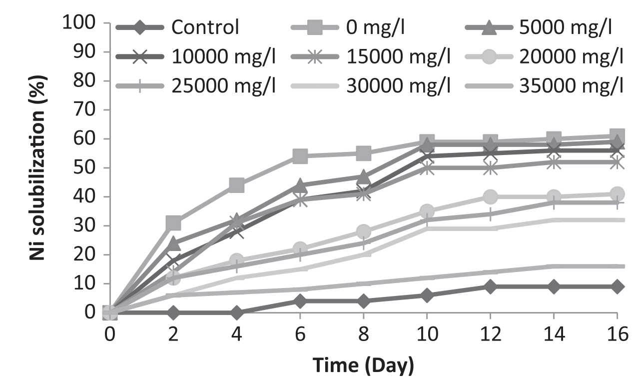


Fig. 8. Solubilization of Ni with time from sewage sludge during bioleaching at different concentrations of MB using sulfur-oxidizing micro-organisms.

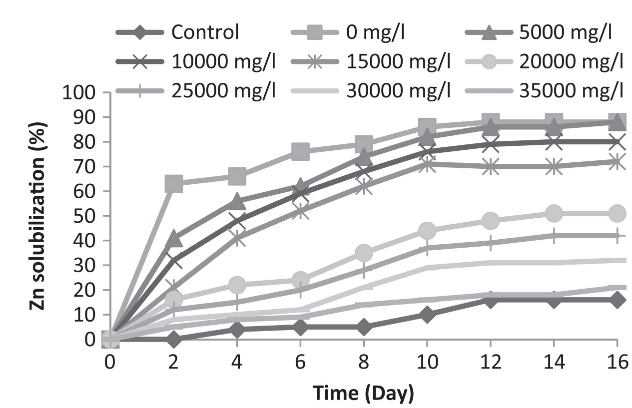


Fig. 9. Solubilization of Zn with time from sewage sludge during bioleaching at different concentrations of MB using sulfur-oxidizing micro-organisms.

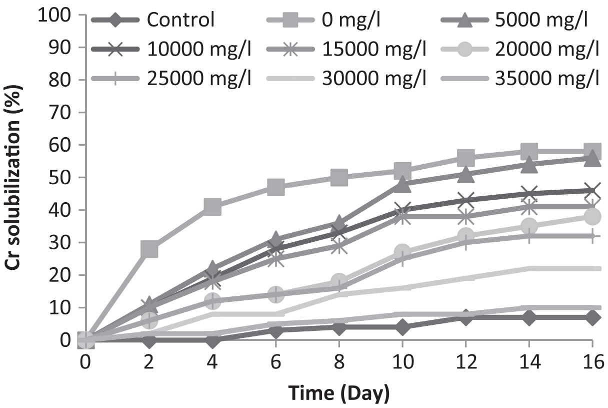


Fig. 10. Solubilization of Cr with time from sewage sludge during bioleaching at different concentrations of MB using sulfur-oxidizing micro-organisms.

3.3. Solubilization of heavy metals with time during bioleaching using sulfur-oxidizing micro-organisms

The solubilization of Cu, Ni, Zn, and Cr with time from the sludges having MB (0–35,000 mg/l) along with control is shown in Figs. 7–10. In the control, without the addition of inoculum and sulfur, only 16% Cu was solubilized in 16 days. In the sludge having only inoculum and sulfur as an energy source (0 mg/l dye), a maximum of 82% Cu was solubilized at a sludge final pH of 0.8 on the sixteenth day. The previous study carried out in the absence of dye has shown that bioleaching was effective in metal removal from the sludge. Using only 2 g/l sulfur, about 79% Cu was solubilized within 12 days of bioleaching operation [18], which is almost comparable to what has been achieved in the present study in the absence of any dye in the sludge. On the other hand, in the sludge having 5,000 mg/l MB, only 33% Cu was solubilized in the first 2 days. However, after 16 days, the final solubilization of Cu was similar (82%) to that of the sludge containing 0 mg/l MB. Further increase in the MB concentration (beyond 5,000 mg/l) led to decrease in Cu solubilization, i.e. 77, 68, 64, 51, 39, and 19 for sludges containing 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l of MB, respectively. The results show that the presence of MB proved toxic to the bioleaching micro-organisms ultimately leading to reduced solubilization of metals. Further, lower solubilization of Ni (Fig. 8) as compared to Cu was achieved in the presence of MB. In the sludge having only inoculum and sulfur as energy source (0 mg/l dye), a maximum of 61% Ni was solubilized on the sixteenth day. On the other hand, in the sludge having 5,000 mg/l MB, reduced Ni solubilization (59%) was observed after 16 days of bioleaching. Further increase in MB concentration led to a significant reduction in Ni solubilization which was 56, 52, 41, 38, 32, and 16% for sludges containing 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l of MB, respectively.

Compared to Cu and Ni, higher solubilization of Zn was observed in the presence of MB (Fig. 9). In the control, although 16% Zn was solubilized in 16 days, about 88% Zn solubilization was achieved in the sludges having 0 mg/l and 5,000 mg/l MB. However, reduced solubilization of Zn was seen in the presence of higher concentrations of MB, which were 80, 72, 51, 42, 32, and 21% for sludges containing 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l of MB, respectively. The bioleaching experiments reported using sewage sludge in the absence of dyes also showed that about 86% Zn was solubilized using only 2 g/l of sulfur [19]. This is quite similar to the results obtained in the present study in the absence of dyes (0 mg/l). The solubilization pattern of Cr was similar to that of

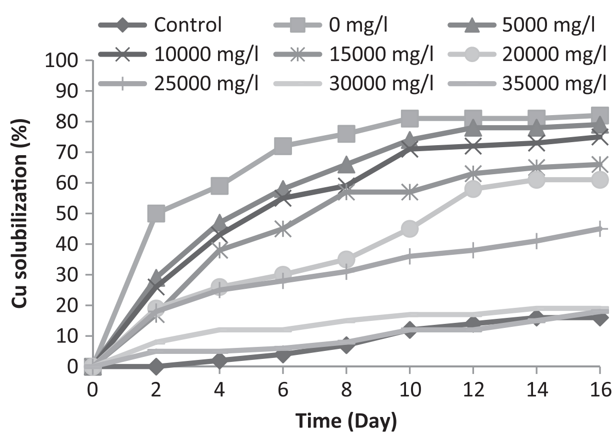


Fig. 11. Solubilization of Cu with time from sewage sludge during bioleaching at different concentrations of RBB using sulfur-oxidizing micro-organisms.

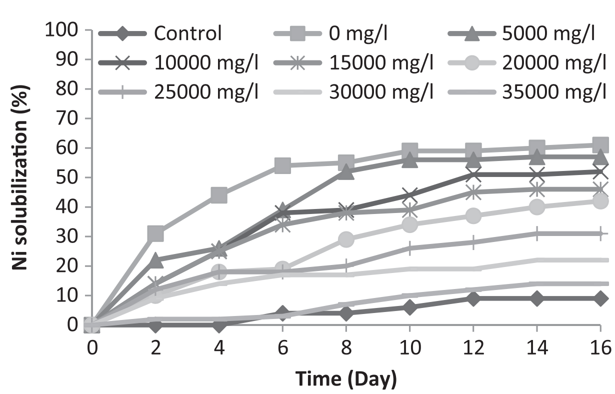


Fig. 12. Solubilization of Ni with time from sewage sludge during bioleaching at different concentrations of RBB using sulfur-oxidizing micro-organisms.

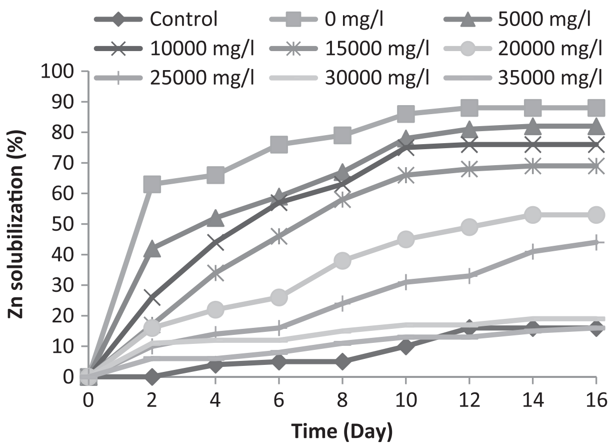


Fig. 13. Solubilization of Zn with time from sewage sludge during bioleaching at different concentrations of RBB using sulfur-oxidizing micro-organisms.

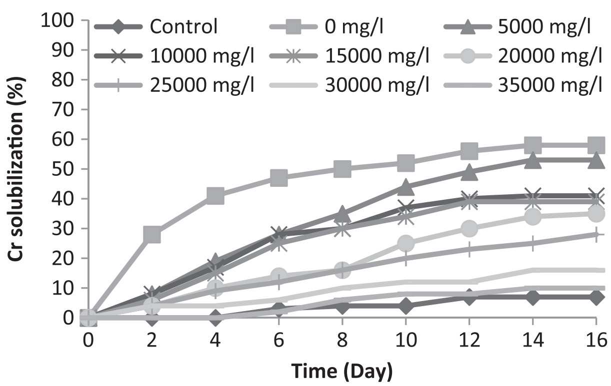


Fig. 14. Solubilization of Cr with time from sewage sludge during bioleaching at different concentrations of RBB using sulfur-oxidizing micro-organisms.

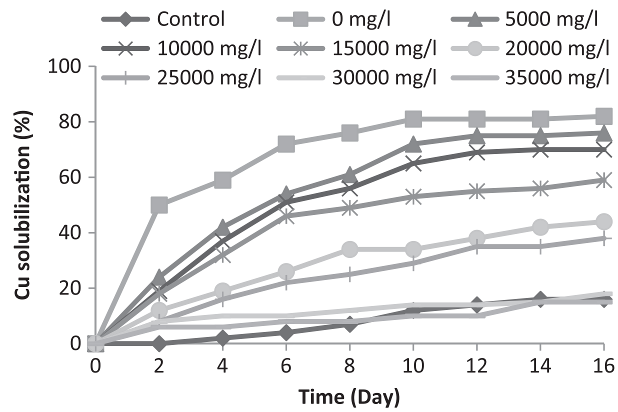


Fig. 15. Solubilization of Cu with time from sewage sludge during bioleaching at different concentrations of DM using sulfur-oxidizing micro-organisms.

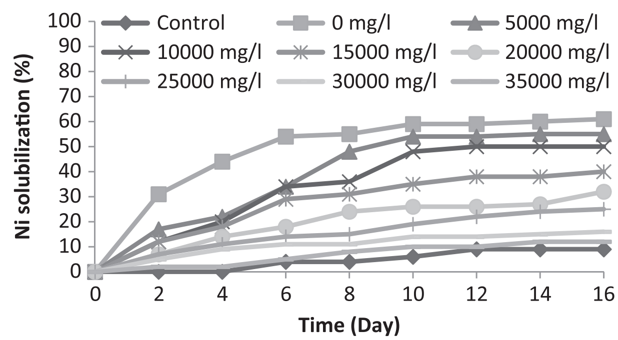


Fig. 16. Solubilization of Ni with time from sewage sludge during bioleaching at different concentrations of DM using sulfur-oxidizing micro-organisms.

Ni (Fig. 10). In the control, only 7% Cr was solubilized, compared to 58% in the sludge having only inoculum and sulfur (0 mg/l MB). In the sludge having 5,000 mg/l MB, a reduced Cr solubilization (56%) was achieved after 16 days of bioleaching. At higher MB concentrations, beyond 5,000 mg/l, a more pronounced reduction in Cr solubilization was observed, which was 46, 41, 38, 32, 22, and 10% for sludges containing 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l of MB, respectively.

The solubilization pattern of different heavy metals in the sludge-containing RBB (0–35,000 mg/l) along with control is shown in Figs. 11–14, respectively. The rate of Cu solubilization was found to be comparatively slower in the sludge containing 5,000 mg/l RBB and about 79% Cu was solubilized after 16 days of bioleaching (Fig. 11). An increase in the RBB concentration beyond 5,000 mg/l caused decrease in Cu solubilization and final Cu solubilization was observed to be 75, 66, 61, 45, 19, and 18% for sludges containing 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l of RBB, respectively.

Similarly, about 57% Ni was solubilized after 16 days of bioleaching in the presence of 5,000 mg/l RBB. In the absence of the RBB (0 mg/l), about 61% Ni was solubilized after 16 days. A significant reduction in Ni solubilization was observed at higher RBB concentrations, which was 52, 46, 42, 31, 22, and 14% for the sludges containing 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l of RBB, respectively (Fig. 12). In the case of Zn, comparatively higher solubilization was observed. However, percentage solubilization was less compared to the sludge containing MB. In the presence of 5,000 mg/l RBB, only 82% Zn was solubilized which reduced further to 76, 69, 53, 44, 19, and 19% in the presence of 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l RBB, respectively (Fig. 13). Compared to the other metals, solubilization of Cr was least and about 58% Cr was solubilized in the absence of RBB (0 mg/l). The solubi-

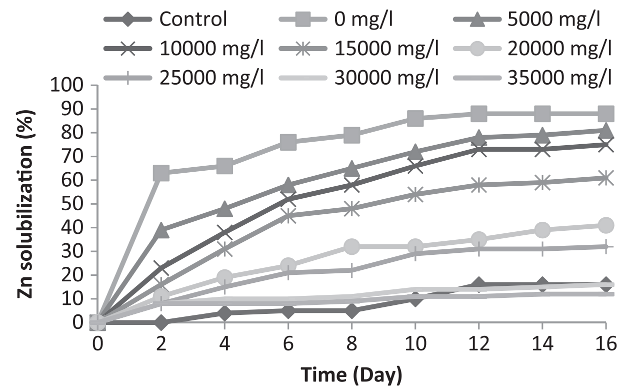


Fig. 17. Solubilization of Zn with time from sewage sludge during bioleaching at different concentrations of DM using sulfur-oxidizing micro-organisms.

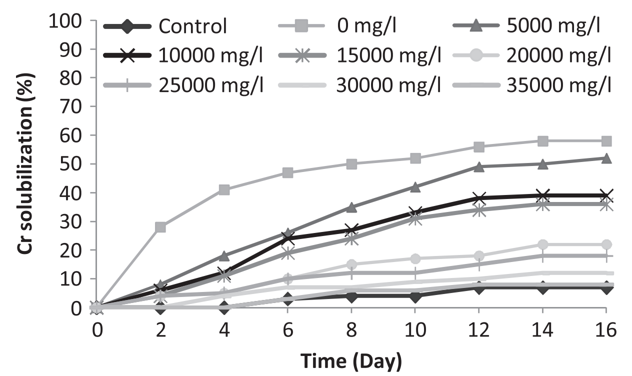


Fig. 18. Solubilization of Cr with time from sewage sludge during bioleaching at different concentrations of DM using sulfur-oxidizing micro-organisms.

lization of Cr reduced to 53% in the presence of 5,000 mg/l RBB. A further increase in RBB concentrations beyond 5,000 mg/l caused reduction in Cr solubilization which was 41, 39, 35, 28, 16, and 10% Cr in the sludges containing 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l of RBB, respectively (Fig. 14).

The lower solubilization of Cr during bioleaching is well documented [20]. The results of the above study have shown that during sewage sludge bioleaching, only 65% Cr was solubilized compared to 99% Zn and 84% Ni after 16 days of bioleaching. The lower solubilization of Cr can be explained on the basis of its predominant presence as Cr (III) in the sludge.

The lowest solubilization of all the heavy metals (Figs. 15–18) was achieved in the sludges containing DM (0–35,000 mg/l). Only 76% Cu was solubilized after 16 days in the presence of 5,000 mg/l DM compared to 82 and 79% Cu solubilization in the sludges having 5,000 mg/l MB and RBB, respectively. The solubilization of Cu was found to decrease further at higher concentrations of DM and about 70, 59, 44, 38, 18, and 15% Cu was solubilized in the presence of 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l of DM, respectively (Fig. 15). The lowest solubilization of Cu in the sludge containing DM shows that the combined effect of mixed dyes was inhibitorier to the sulfur-oxidizing micro-organisms compared to the sludge containing MB and RBB, separately.

Similar results were also observed for Ni where about 55, 50, 40, 32, 25, 16, and 12% Ni were solubilized in the presence of 5,000, 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l of DM, respectively (Fig. 16). The highest solubilization in the presence of DM was also observed for Zn, among all the metals. The solubilization efficiencies of Zn were found to be 81, 75, 61, 41, 32, 16, and 12% in the sludges containing 5,000, 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l of DM, respectively (Fig. 17).

Chromium was solubilized the least, among all the metals and only 52% Cr was solubilized after 16 days in the presence of 5,000 mg/l DM (Fig. 18). A significant reduction in Cr solubilization was observed beyond 5000 mg/l DM concentration and only 39, 36, 22, 18, 12, and 8% Cr was solubilized in the sludge containing 10,000, 15,000, 20,000, 25,000, 30,000, and 35,000 mg/l of DM.

4. Conclusions

The results of the above study clearly show that bioleaching process is an efficient method for decontamination of sludge. The leaching micro-organisms can grow in the presence of different dyes, though higher concentration of dyes proved inhibitory for the growth of *Acidithiobacillus*. However, the rate of change of pH and ultimate solubilization of heavy metals are affected by the presence of dyes, which being highly toxic caused suppression of the activity of leaching micro-organisms. The presence of MB proved less toxic compared to RBB and DM. In general, the concentration of dyes in industrial effluent is found in range between 200 and 500 mg/l. In the present study, the minimum concentration of RBB, MB, and DM was 5,000 mg/l and which is very much higher in concentration, in comparison to that found in industrial effluent. On the basis of the results of the present study, suitable treatment strategy can be developed for the bioleaching of heavy metals from sludges contaminated with a variety of dyes.

References

[1] Q. Husain, Potential applications of the oxidoreductive enzymes in the decolorization and detoxification of textile and other synthetic dyes from polluted water: A review, Crit. Rev. Biotech. 60 (2006) 201–221.

[2] F.I. Hai, K. Yamamoto, K. Fukushi, Hybrid treatment system for dye wastewater, Crit. Rev. Env. Sci. Technol. 37 (2007) 315–377.
[3] G. McKay, M.S. Otterburn, D.A. Aga, Fullers earth and fired clay as adsorbent for dye stuffs, equilibrium and rate constants, Wat. Air Soil Pollut., 24 (1985) 307–322.
[4] A. Netzer, H.K. Miyamato, P. Wilkinson, Heavy metals concentration of exhausted dye bath effluents, Bull. Env. Cont. Toxic. 14 (1975) 301–303.
[5] S. Celebi, S. Kendir, Toxicity assessment of a dye industry treatment sludge, Waste Manage. Res. 20 (2002) 541–545.
[6] J. Balasubramanian, P.C. Sabumon, J.U. Lazar, R. Langovan, Reuse of textile effluent treatment plant sludge in building materials, Waste Manage. 26 (2006) 22–28.
[7] A.Z. Antonis, M. Loizidou, The applications of inorganic and organic acids for the treatment of heavy polluted sewage sludge and the evaluation of the remaining metal with sequential chemical extraction, Desalin. Wat. Treat. 12 (2009) 229–237.
[8] J.F Blais, N. Meuneir, J.L. Sasseville, R.D. Tyagi, G. Mercier, F. Hammy, Hybrid chemical and biological process for decontaminating sludge from municipal sewage, U.S. Patent No. 6 (2005) 855, 256 B2.
[9] L.D. Villar, O. Garcia, Jr., Effect of anaerobic digestion and initial pH on metal bioleaching from sewage sludge, J. Env. Sci. Health Part A. 41 (2006) 211.
[10] S. Wang, G. Zheng, L. Zhou, Heterotrophic microorganism *Rhodotorula mucilaginosa* R30 improves tannery sludge bioleaching through elevating dissolved CO_2 and extracellular polymeric substances levels in bioleach solution as well as scavenging toxic DOM to *Acidithiobacillus* species, Wat. Res. 44 (2010) 5423–5431.
[11] G. Zheng, L. Zhou, Supplementation of inorganic phosphate enhancing the removal efficiency of tannery sludge-borne Cr through bioleaching, Wat. Res. 45 (2011) 5295–5301.
[12] D. Fang, L. Zhou, Enhanced Cr bioleaching efficiency from tannery sludge with coinoculation of *Acidithiobacillus thiooxidans* TS6 and Brettanomyces B65 in an air-lift reactor, Chemosphere 69 (2007) 303–310.
[13] F. Liu, L. Zhou, J. Zhou, X. Song, D. Wang, Improvement of sludge dewaterability and removal of sludge-borne metals by bioleaching at optimum pH, J. Hazard. Mater. 222 (2012) 170–177.
[14] A. Kim, K.Y. Lee, B.T. Lee, S. Kim, K.W. Kim, Comparative study of simultaneous removal of As, Cu, Pb using different combinations of electrokinetics with bioleaching by Acidithiobacillus ferrooxidans, Wat. Res. 46 (2012) 5591–5599.
[15] C. Solisio, A. Lodi, F. Veglio, Bioleaching of zinc and aluminium from industrial waste sludges by means of *Thiobacillus ferrooxidans*, Waste Manage. 22 (2002) 667–675.
[16] K. Bosceker, Bioleaching: Metal solubilization by microorganism, FEMS Microbiol. Rev. 20 (1997) 591–604.
[17] R.P.R. Barreira, L.D. Villar, O. Garcia, Jr., Tolerance to copper and zinc of *Acidithiobacillus thiooxidans* isolated from sewage sludge, World J. Microbiol. Biotech. 21 (2005) 89–91.
[18] APHA (American Public Health Association) In Standards methods for examination of water and wastewater, Washington DC, USA, 1989.
[19] A. Pathak, M.G. Dastidar, T.R. Sreekrishnan, Bioleaching of heavy metals from anaerobically digested sewage sludge, J. Env. Sci. Health Part A 43 (2008) 402–411.
[20] J.W.C. Wong, L. Xiang, X.Y. Gu, L.X. Zhou, Bioleaching of heavy metals from anaerobically digested sewage sludge using FeS_2 as an energy source, Chem. 43 (2004) 101–107.