

Bioleaching of chromium from tannery sludge by indigenous *Acidithiobacillus thiooxidans*

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Abstract

Chromium in tannery sludge will cause serious environmental problems and is toxic to organisms. The acidophilic sulfur-oxidizing *Acidithiobacillus thiooxidans* can leach heavy metals from urban and industrial wastes. This study examined the ability of an indigenous sulfur-oxidizing *A. thiooxidans* to leach chromium from tannery sludge. The results showed that the pH of sludge mixture inoculated with the indigenous *A. thiooxidans* decreased to around 2.0 after 4 days. After 6 days incubation in shaking flasks at 30 °C and 160 rpm, up to 99% of chromium was solubilized from tannery sludge. When treated in a 2-l bubble column bioreactor for 5 days at 30 °C and aeration of 0.5 vvm, 99.7% of chromium was leached from tannery sludge. The results demonstrated that chromium in tannery sludge can be efficiently leached by the indigenous *A. thiooxidans*.

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Keywords: Chromium; Tannery sludge; *Acidithiobacillus thiooxidans*; Bioleaching

1. Introduction

In leather industry, tanning is the main process that protects leather against some environmental effects such as microbial degradation, heat, sweat or moisture [1]. About 90% of tanneries in the world use chromium salts as tanning materials because of the excellent properties of the chromium compounds in the tanning [2]. During the tanning process, chromium forms crosslinks between the collagen fibers and the resulting hides have a good mechanical resistance, an extraordinary dyeing suitability and a better hydrothermic resistance in comparison with hides treated with vegetable substances [3]. However, only 60% of the total chromium reacts with the hides. The rest of the chromium remains in the tanning effluent and are subsequently sent to a tannery wastewater management plant where the chromium salts end up in the sludge [1,4,5].

Chromium is not biodegradable and tends to accumulate in living organisms, causing serious diseases and disorders [6,7]. Chromium is found in either +3 or +6 oxidation states [Cr(III) or Cr(VI)]. Cr(III) is toxic only at high concentrations, whereas Cr(VI) is toxic to mammals even at low concentrations, with

a potential carcinogenic effect [8,9]. Chromium in tannery sludge can be released to the environment [10]. Researches also revealed the released Cr(III) in aqueous phase could be oxidized through interaction with manganese dioxide (MnO₂) surface to Cr(VI) [11,12]. Therefore tannery sludge, with high level of chromium (1.0–4.0%) compounds and other pollutants, was considered as toxic and hazardous waste by many nations. Thus the disposal of tannery sludge has becoming one of the major emerging environmental problems of the tanning industry [12].

Generally, tannery sludge is disposed of in landfills, ocean dumping and incineration or solidification [5,13,14]. Landfills and ocean dumping of tannery sludge are under increasing pressure in many countries because of environmental consideration. When the sludge was incinerated, the gases and soluble toxic chemicals were generated which can cause serious environmental problems such as air, soil and water pollution plus the high cost. Cr(III) can be converted to its more soluble hexavalent form in the air atmosphere. While tannery sludge was solidified, chromium can be immobilized in the ceramic materials and suitable for building materials such as bricks production. However, sulfur compounds, zinc and chlorine are released to the air [5].

One promising long-term solution appears to be recycling and using tannery sludge for beneficial purposes by removing

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hazardous components such as chromium from it. Land application of detoxified sludge on agricultural lands, forest and disturbed lands represents one of the better options for utilization of this material by recycling plant nutrients and organic matter to soil for crop production [15].

Chemical and microbiological methods have been investigated to leach chromium and other metals from tannery sludge and other wastes. Compared to the mineral acids consuming chemical approach, the sulfur-oxidizing bacteria using microbiological method have the distinct advantages [16–22]. In the microbiological method, metals can be readily solubilized by oxidation of metal sulfide to soluble sulfate or via acid dissolution with sulfuric acid formed by oxidation of elemental sulfur [23]. The released chromium can be efficiently removed or recovered for reuse with many ways [1–4,6,8–10]. Thus treatment of tannery sludge with sulfur-oxidizing bacteria will be advantage for the tanning industry for environmental and economical considerations.

Since sulfur-oxidizing bacteria play an important role in the removal of chromium and other metals from tannery sludge and other wastes, interests in bacterial treatment of tannery sludge aroused. Besides time-consuming (from 8 to 25 days) [18–22], bacterial leaching of chromium from tannery sludge using *Acidithiobacillus thiooxidans* also required high amounts of elemental sulfur (up to 30 g l⁻¹) [18,19] or preacidification with sulfuric acid plus addition of elemental sulfur [22]. This will increase the operational cost. Therefore, it is necessary to reduce the treatment time and amount of elemental sulfur used in bioleaching of chromium and other metals using *A. thiooxidans*. Furthermore, bioleaching of chromium using *A. thiooxidans* were mainly performed with shake flasks and results from bumble column bioreactor had not been reported. Thus it is needed to understand the performance of *A. thiooxidans* in chromium leaching from tannery sludge in bubble column reactor. In this report, we examined the possibility of bacterial treatment of tannery sludge using an indigenous *A. thiooxidans* with shake flask and bubble column reactor. The results suggested that chromium in tannery sludge can be efficiently leached in less time and lower elemental sulfur amount without preacidification.

2. Materials and methods

2.1. Chemicals

All reagents used in the study are all of analytical grade.

2.2. Collection of tannery sludge sample

Tannery sludge used in this study was sampled from a tannery plant in Haining (China). Samples were stored at 4 °C. The water content of the sludge was 80.7%. Soluble metals were measured with IRIS Intrepid ICP-AES and total carbon, total phosphorus as well as total nitrogen were measured according to APHA [24]. Selected physicochemical properties including the metal and nutrient (nitrogen and phosphorous) concentrations of the sludge are shown in Table 1.

Table 1
Selected physiochemical properties of tannery sludge sample

Total C (g kg ⁻¹ dry sludge)	277.80 ± 13.25
Total P (g kg ⁻¹ dry sludge)	3.40 ± 0.14
Total N (g kg ⁻¹ dry sludge)	75.60 ± 3.54
S (g kg ⁻¹ dry sludge)	5.81 ± 0.27
Cr (g kg ⁻¹ dry sludge)	35.00 ± 1.36
Fe (g kg ⁻¹ dry sludge)	6.87 ± 0.27
Ca (g kg ⁻¹ dry sludge)	5.35 ± 0.31

2.3. Microorganism

The indigenous sulfur-oxidizing *A. thiooxidans* was isolated from tannery sludge sample. Enrichment of the purified *A. thiooxidans* was performed by incubating the bacterium in 2.0 g l⁻¹ tannery sludge containing improved SM medium (g l⁻¹): KH₂PO₄ 3.0, (NH₄)₂SO₄ 0.4, MgSO₄·7H₂O 0.5, FeSO₄·7H₂O 0.01, CaCl₂·2H₂O 0.25, pH 4.0. Tyndallized elemental sulfur powder (S⁰) of 10.0 g l⁻¹ was amended to each flask. The enriched *A. thiooxidans* was grown in improved SM medium for 5 days at 30 °C, 160 rpm then the culture was maintained at 4 °C. The active *A. thiooxidans* inoculum used in this study was prepared by two consecutive transfers in improved SM medium for 2 days. All flask incubations were performed on a rotary shaker at 30 °C, 160 rpm.

2.4. Experimental procedure

The investigations of chromium solubilization by the indigenous *A. thiooxidans* were conducted in 250-ml Erlenmeyer flask containing 100 ml mixture of tannery sludge supplemented with 2.0 g l⁻¹ S⁰. In all bioleaching experiments, treatment only inoculated with *A. thiooxidans* was set as biological control and treatment only added with S⁰ was set as chemical control. Unless otherwise noted, inoculation volume of *A. thiooxidans* and concentration of tannery sludge were 10% (v/v) and 100 g l⁻¹, respectively. In investigation of the effect of initial pH on chromium leaching and pH changes, sludge pH was adjusted to the tested levels with 1 mol l⁻¹ H₂SO₄ or 2 mol l⁻¹ NaOH. Laboratory level scale-up study was carried out with a 2-l glass bubble column bioreactor (Fig. 1) containing 1 l 100 g l⁻¹ tannery sludge supplemented with 2.0 g l⁻¹ S⁰ at 30 °C and 0.5 vvm of aeration. The height and inner diameter of the bubble column bioreactor were 50 and 10 cm, respectively. The changes of sludge pH were monitored everyday and samples were withdrawn daily to determine the concentration of chromium released from the sludge. The water loss in flasks due to evaporation during bioleaching was compensated with distilled water by weight difference method. All experiments were performed with triplicates.

2.5. Analytical methods

Sludge pH was determined with a Metler Toledo 320 pH meter. Samples were first centrifuged at 10,000 × g for 10 min, and the supernatants were collected for chromium assay. Chromium concentration in supernatants was determined colorimetrically with 1,5-diphenylcarbazide method [24] by Unicob 2000 Spectrophotometer at 540 nm.

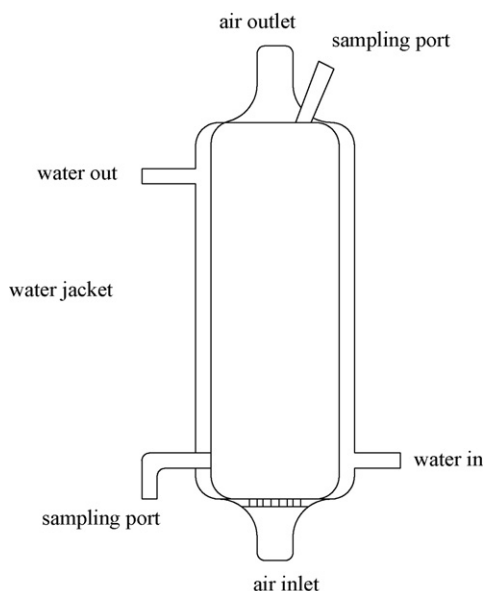


Fig. 1. Design of the used 2-l glass bubble column bioreactor.

3. Results and discussion

3.1. Solubilization of chromium from tannery sludge by the indigenous *A. thiooxidans* in shaking flasks

3.1.1. Effects of initial pH on chromium solubilization

As can be seen in Fig. 2(a), the final pH of treatment at initial pH 3.0, 4.0, 5.0, 6.0, 7.0 and natural pH was 1.61, 1.84, 1.85, 1.89, 1.98 and 1.84, respectively. Final pH of biological control also decreased to 2.34 whereas final pH of chemical control only reached 6.25. Sludge pH of treatments with initial pH of 3.0 and 4.0 first reached 4.01 and 4.58, respectively at day 1 then dropped whereas sludge pH of other treatments decreased throughout the experiment.

As shown in Fig. 2(b), chromium release of all inoculated treatments increased throughout the incubation whereas almost no increase in solubilized chromium was determined in the chemical control. Final chromium solubilization at initial pH 3.0, 4.0, 5.0, 6.0, 7.0 and natural pH reached 93.2, 87.4, 87.3, 86.0, 84.0 and 87.5%, respectively.

The data showed sludge pH of treatments at tested initial pH can decrease to below 2.0. According to the results, except treatment at initial pH of 3.0, there was no appreciable difference in final pH and chromium solubilization for other initial sludge pH. The results suggested that increase of chromium solubilization was closely related to reduction of sludge pH. The profiles of sludge pH and chromium solubilization were similar to the earlier reports on removal of metals from tannery sludge and other wastes by sulfur-oxidizing bacteria using S^0 [19–22,25].

3.1.2. Effect of inoculation volume of active indigenous *A. thiooxidans* on chromium solubilization

As shown in Fig. 3(a), sludge pH with tested inoculation volumes had a similar decrease trend but with different rates throughout. While pH of treatment with 5% (v/v) inoculation

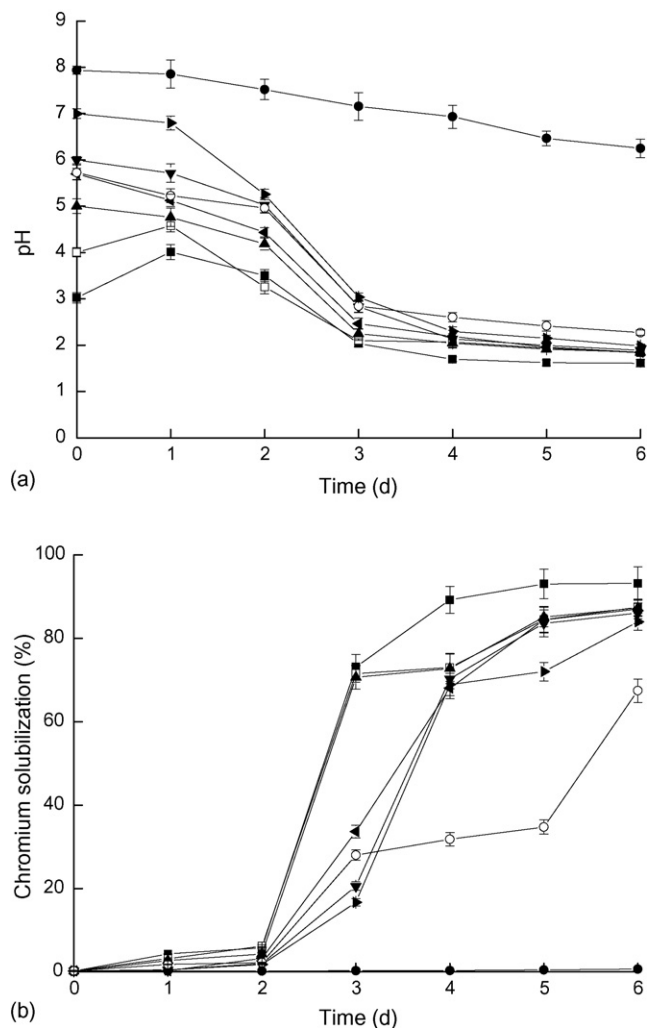
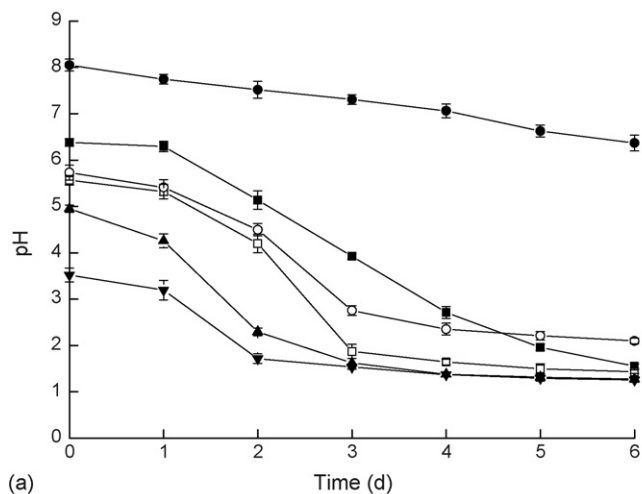


Fig. 2. Effect of initial pH on chromium solubilization from tannery sludge by the indigenous *A. thiooxidans* in shake flask (30 °C, rotation rate 160 rpm, inoculation volume 10% (v/v), sludge concentration 100 g l⁻¹ and S⁰ 2.0 g l⁻¹): (a) pH of sludge mixture; (b) chromium solubilization. Symbol: pH 3.0 (■), pH 4.0 (□), pH 5.0 (▲), pH 6.0 (▼), pH 7.0 (►), natural pH (◄), chemical control (●), and biological control (○).

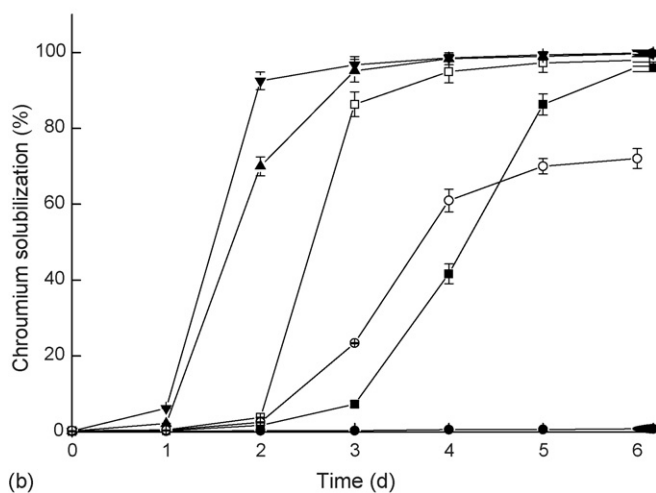
volume reached 2.72 at day 4, treatment with 10, 15 and 20% (v/v) inoculation volume reduced to 1.65, 1.38 and 1.37, respectively. Final pH with inoculation volume of 5, 10, 15 and 20% (v/v) was 1.56, 1.43, 1.28 and 1.26, respectively.

As can be seen in Fig. 3(b), chromium release with tested inoculation volume increased at various rates throughout the incubation. The increase of treatments with higher inoculation volume was faster than that of treatments with lower inoculation volume. While chromium solubilization of treatment with 5% (v/v) inoculation volume reached 7.3% at day 3, chromium release of treatments with 10, 15 and 20% (v/v) inoculation volume already reached 86.3, 95.0 and 96.8%. Final chromium solubilization with inoculation volume of 5, 10, 15 and 20% (v/v) reached 96.2, 98.0, 99.7 and 99.9%, respectively.

The results of pH changes demonstrated that there was no significant difference in final pH and chromium release with tested inoculation volumes. The results also suggested that high inoculation volume of *A. thiooxidans* can accelerate sludge pH



(a)



(b)

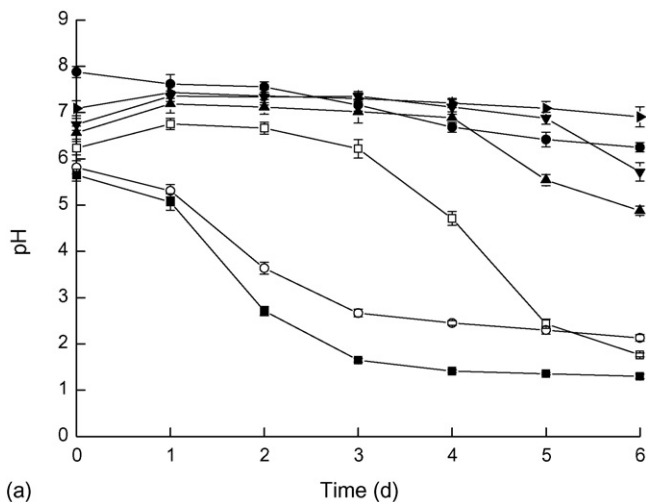
Fig. 3. Effect of inoculation volume of indigenous *A. thiooxidans* on chromium solubilization from tannery sludge in shake flask (30 °C, 160 rpm, sludge concentration 100 g l⁻¹ and S⁰ 2.0 g l⁻¹): (a) pH of sludge mixture; (b) chromium solubilization. Symbol: 5% (■), 10% (□), 15% (▲), 20% (▼), chemical control (●), and biological control (○).

reduction and chromium release but cannot affect final pH reduction and chromium release.

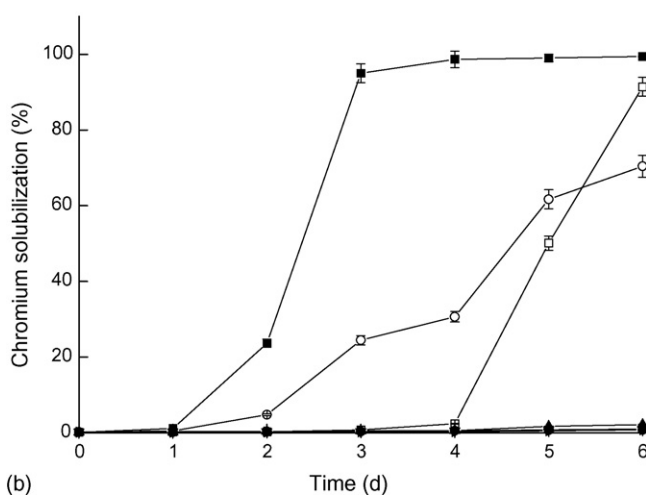
3.1.3. Effect of sludge concentrations on chromium solubilization by the indigenous *A. thiooxidans*

As can be seen in Fig. 4(a), pH changes of treatment with various sludge concentrations were different. The pH of treatment with 100 g l⁻¹ sludge decreased throughout the incubation. It reduced to 5.07 at day 1 and reached 1.65 at day 3. The pH of treatment with 200 g l⁻¹ sludge increased to 6.76 at day 1 then decreased at the rest of the incubation. Final pH of treatment with sludge concentration of 100, 200, 300, 400 and 500 g l⁻¹ was 1.30, 1.76, 4.90, 5.72 and 6.91, respectively.

As depicted in Fig. 4(b), chromium solubilization of treatments with various sludge concentrations was different. Chromium release of treatment with 100 g l⁻¹ sludge increased throughout the incubation. It increased to 1.2% at day 1 then reached 95% at day 3. Chromium release of treatment with 200 g l⁻¹ sludge only reached 2.4% at day 4. However, almost



(a)



(b)

Fig. 4. Effects of sludge concentrations on chromium solubilization from tannery sludge by the indigenous *A. thiooxidans* in shake flask (30 °C, 160 rpm, inoculation volume 10% (v/v) and S⁰ 2.0 g l⁻¹): (a) pH of sludge mixture; (b) chromium solubilization. Symbol: 100 g l⁻¹ (■), 200 g l⁻¹ (□), 300 g l⁻¹ (▲), 400 g l⁻¹ (▼), 500 g l⁻¹ (▶), chemical control (●), and biological control (○).

no chromium was leached from treatments with 300, 400 and 500 g l⁻¹ sludge. Final chromium solubilization of treatment with 100 and 200 g l⁻¹ sludge was 99.4 and 91.4%, respectively.

The results indicated that pH reduction and chromium solubilization increase during bioleaching can be hindered by increased tannery sludge concentrations.

Data of above experiments strongly demonstrated that chromium solubilization was negatively related to sludge pH. According to the results, pH 2.0 seemed to be a threshold for considerable chromium solubilization during the bioleaching experiment. This was similar to earlier reports on microbial leaching of chromium from tannery sludge [19,21,22]. The results also indicated that final pH of treatments under low sludge concentration was lower than that of the biological control. Therefore, sulfur addition might be necessary for higher chromium solubilization. Additionally, the data also indicated that there was no significant difference in final pH and chromium solubilization at the tested initial pH under low tannery sludge

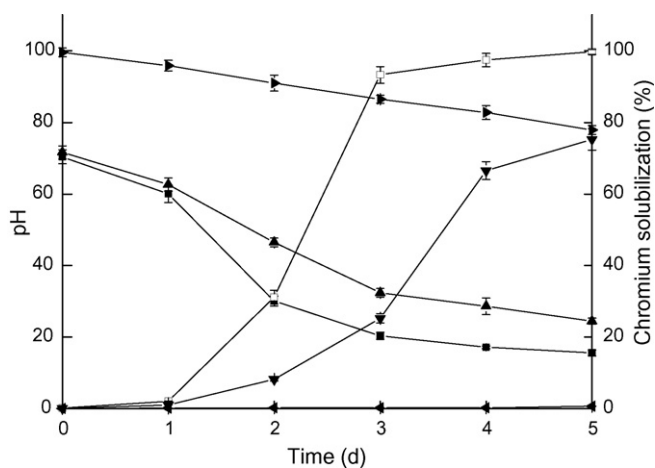


Fig. 5. Chromium solubilization by the indigenous *A. thiooxidans* using a 2-l bubble column bioreactor (30 °C, aeration rate 0.5 vvm, sludge concentration 100 g l⁻¹, inoculation volume of 10% (v/v) and S⁰ 2.0 g l⁻¹). Symbol: pH of *A. thiooxidans* inoculated and elemental sulfur added treatment (■), chromium solubilization of *A. thiooxidans* inoculated and elemental sulfur added treatment, % (◀), pH of biological control (▲), chromium solubilization of biological control, % (▼), pH of chemical control (▶), chromium solubilization of chemical control, % (▶).

concentration. Therefore, chromium solubilization using the indigenous *A. thiooxidans* at low sludge concentration might not require the preacidification process. Moreover, with a shorter time and lower amount of sulfur, chromium release efficiency of this experiment was almost the same as demonstrated [19,21,22]. However, efforts should be made to improve the efficiency of chromium bioleaching at high sludge concentration.

3.2. Solubilization of chromium from tannery sludge by *A. thiooxidans* with a 2-l bubble column bioreactor

As shown in Fig. 5, pH of all treatments decreased at various rates during the incubation. Final pH of the chemical control, biological control and *A. thiooxidans* inoculated treatment added with S⁰ dropped to 6.37, 2.0 and 1.28, respectively. Chromium solubilization increased throughout the experiments. Chromium solubilization of the *A. thiooxidans* inoculated treatment added with S⁰ increased to 1.2% at day 1 then reached 93.5% at day 3. Then chromium removal reached 99.7% at day 5. Chromium solubilization of the biological control also increased during the incubation whereas almost no increase in solubilized chromium was examined in the chemical control. Finally, 75.3% of chromium was solubilized from biological control.

According to the results, chromium in tannery sludge can be efficiently bioleached using the 2-l bubble column bioreactor. It was in accordance with that obtained from the shaking flask experiments. Thus this method might be carried out at a larger scale. The data further stressed that pH 2.0 was a threshold for significant chromium release.

4. Conclusions

This study investigated the possibility of removing chromium from tannery sludge using an indigenous acidophilic *A. thioox-*

idans. Chromium solubilization conditions such as sludge concentrations, initial pH and inoculation volume were examined in shake flasks. Laboratory scale-up test was carried out with a 2-l bubble column bioreactor. The results showed that sludge pH can decrease to 2.0 or below. Chromium solubilization can be deeply influenced by the sludge pH. The pH and chromium removal can be accelerated by increasing inoculation volume. They can be hindered by increasing sludge concentrations. After 6 days incubation in shake flask, over 99% of chromium was solubilized from tannery sludge. It was similar to the result in 2-l bubble column bioreactor after 5 days incubation. The profiles of sludge pH and chromium release were in agreement with several experimental studies on microbial removal of chromium using sulfur-oxidizing bacteria [19,21,22,25].

This work showed the possibility of using the indigenous *A. thiooxidans* as a potential candidate for microbial removal of chromium from tannery sludge because of its high chromium solubilization efficiency.

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References

- [1] M. Erdem, Chromium recovery from chrome shaving generated in tanning process, *J. Hazard. Mater.* B129 (2006) 143–146.
- [2] L.M. Ortega, R. Lebrun, I.M. Noël, R. Hausler, Application of nanofiltration in the recovery of chromium(III) from tannery effluents, *Sep. Purif. Technol.* 44 (2005) 45–52.
- [3] A.A. Dantas Neto, T.N.C. Dantas, M.C.P.A. Moura, Evaluation and optimization of chromium removal from tannery effluent by microemulsion in the Morris extractor, *J. Hazard. Mater.* B114 (2004) 115–122.
- [4] A. Cassano, E. Drioli, R. Molinari, C. Bertolutti, Quality improvement of recycled chromium in the tanning operation by membrane processes, *Desalination* 108 (1997) 193–203.
- [5] T. Basegio, F. Berutti, A. Bernardes, C.P. Bergmann, Environmental and technical aspects of the utilisation of tannery sludge as a raw material for clay products, *J. Eur. Ceram. Soc.* 22 (2002) 2251–2259.
- [6] M. Ajmal, R.A.K. Rao, B.A. Siddiqui, Studies on removal and recovery of Cr(VI) from electroplating wastes, *Water Res.* 30 (1996) 1478–1482.
- [7] S.E. Bailey, T.J. Olin, R.M. Bricka, D.D. Adrian, A review of potentially low-cost sorbents for heavy metals, *Water Res.* 33 (1999) 2469–2479.
- [8] C.K. Lee, K.S. Low, K.L. Kek, Removal of chromium from aqueous solution, *Bioresour. Technol.* 54 (1995) 183–189.
- [9] K.S. Low, C.K. Lee, A.Y. Ng, Column study on the sorption of Cr(VI) using quaternized rice hulls, *Bioresour. Technol.* 68 (1999) 205–208.
- [10] M.C. Chuan, J.C. Liu, Release behavior of chromium from tannery sludge, *Water Res.* 30 (1996) 932–938.
- [11] A.D. Apte, S. Verma, V. Tare, P. Bose, Oxidation of Cr(III) in tannery sludge to Cr(VI): field observations and theoretical assessment, *J. Hazard. Mater.* B121 (2005) 215–222.
- [12] L.E. Eary, D. Rai, Kinetics of Cr(III) oxidation by manganese dioxide, *Environ. Sci. Technol.* 21 (1987) 1187–1193.
- [13] J.R. Rao, P. Thanikaivelan, K.J. Sreeram, B.U. Nair, Green route for the utilization of chrome shavings (chromium-containing solid waste) in tanning industry, *Environ. Sci. Technol.* 36 (2002) 1372–1376.
- [14] M. Giugliano, A. Paggi, Use of tannery sludge in brick production, *Waste Manage. Res.* 3 (1985) 361–368.
- [15] J.W.C. Wong, L. Xiang, X.Y. Gu, L.X. Zhou, Bioleaching of heavy metals from anaerobically digested sewage sludge using FeS₂ as an energy source, *Chemosphere* 55 (2004) 101–107.

- [16] S. Skrypski-Mantele, T.R. Bridle, Environmentally sound disposal of tannery sludge, *Water Res.* 29 (1995) 1033–1039.
- [17] S.B. Shen, R.D. Tyagi, J.F. Blais, Extraction of Cr(III) and other metals from tannery sludge by mineral acids, *Environ. Technol.* 22 (2001) 1007–1014.
- [18] S.B. Shen, A.S. Vidyarthi, R.D. Tyagi, J.F. Blais, R.Y. Surampalli, Effect of sulphur concentration on bioleaching of Cr(III) and other metals from tannery sludge by indigenous sulphur-oxidizing bacteria, *Prac. Period. Hazard., Toxic, Radioact. Waste Manage.* 6 (2002) 244–249.
- [19] S.B. Shen, R.D. Tyagi, J.F. Blais, R.Y. Surampalli, Bacterial leaching of metals from tannery sludge by indigenous sulphur-oxidizing bacteria—effect of sludge solids concentration, *J. Environ. Eng.* 129 (2003) 513–519.
- [20] L.X. Zhou, S.G. Zhou, S.M. Wang, D. Fang, D.Z. Wang, Cr removal and improving the settling and dehydrating capability from tannery sludge simultaneously through bioleaching approach, *J. Environ. Sci.* 24 (2004) 1014–1020.
- [21] L.X. Zhou, D. Fang, S.G. Zhou, D.Z. Wang, S.M. Wang, Removal of Cr from tannery sludge by acidophilic *Thiobacilli*, *Environ. Sci.* 25 (2004) 62–66.
- [22] L.X. Zhou, D. Fang, S.M. Wang, J.W.C. Wong, D.Z. Wang, Bioleaching of Cr from tannery sludge: the effects of initial acid addition and recycling of acidified bioleached sludge, *Environ. Technol.* 26 (2005) 277–284.
- [23] L.C. Chan, X.Y. Gu, J.W.C. Wong, Comparison of bioleaching of heavy metals from sewage sludge using iron- and sulfur-oxidizing bacteria, *Adv. Environ. Res.* 7 (2003) 603–607.
- [24] APHA, WEF and AWWA, *Standard Methods for Examination of Water and Wastewater*, 19th ed., APHA, Washington, DC, USA, 1995.
- [25] 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.