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Removal of Cr from tannery sludge by bioleaching method

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Abstract: Bioleaching of Cr(III) from tannery sludge using the mixture of ingenious iron- and sulfur-oxidizing bacteria was investigated in batch flasks. Experiments involved the adaptation of indigenous iron- and sulfur-oxidizing bacteria, the pre-acidification the sludge to pH 6.0 and the addition of energy substrates. Results showed the inoculation of mixture of ingenious iron- and sulfur-oxidizing bacteria and co-addition of Fe²⁺ and elemental sulfur accelerated acid production and increase of oxidation-reduction potential originating from the bio-oxidation of Fe²⁺ and elemental sulfur. Dissolved Cr concentration increased concomitant with pH decreased in the sludge and reached its maximum removal of 95.6%. The amelioration of the odor of bioleached sludge could be noted. However, 20.4% of N, 24.5% of P and 14.3% of organic matter were lost in the bioleaching process. The residual Cr content in the leached tannery sludge was acceptable for use in agriculture. This study had shown the feasibility of applying the bioleaching process, developed for sewage sludge, to tannery sludge with high Cr.

Keywords: tannery sludge; Cr; removal; bioleaching

Introduction

Chromium(III) salts are the most widely used tanning agent in the leather industry, and is an important source of contamination due to the large volume of exhausted liquid discharges and solid sludge produced (Macchi *et al.*, 1992; Calvanese *et al.*, 2002). About 300000 dry t of Cr(III)-containing (1%—4%) tannery sludge are annually produced by tanneries in China (Yu *et al.*, 2000). Illegal dumping and uncontrolled landfills are currently the common practice, which had caused serious environmental pollution. Land application provides an economical and attractive alternative for the final disposal of tannery sludge because it consists largely of organic substances and also contains nitrogen and phosphorus (Zhou *et al.*, 1999, 2003). However, high Cr content in the sludge is always an issue restricting its general use (Lopez *et al.*, 1991).

Although Cr is an essential nutrient for human, there is no doubt that Cr(VI) compounds are both acutely and chronically toxic (Anderson, 1989; Iyengar, 1989; Rinehart, 1989). In contrast to Cr(VI), Cr(III) is less toxic to mammalian, aquatic organism and plants due to its low solubility, mobility and bioavailability (Adriano, 1986; Rinehart, 1989). However, the possibility that organic ligands and/or acid conditions increase Cr(III) mobility in soils and that MnO₂ allow its oxidation to the more toxic and mobile Cr(VI) forms, cannot be excluded (Bartlett and James, 1979; Dreiss, 1986; Early and Rai, 1987). Therefore, removal of Cr from tannery sludge is indispensable for safe land application of sludge. There are two ways for reducing Cr in tannery sludge: source control and Cr removal. Cleaner technologies used to reduce and recover Cr in wastewater such as

high exhaustion process, direct or indirect Cr recycling, cannot eliminate completely Cr from effluent coming from post tanning section. In addition, these processes are relatively expensive. Thus, the Cr concentration in tannery sludge always exceeds the recommended limit for agricultural use.

Accordingly, several efforts aimed at the removal of Cr from tannery sludge using mineral acid solubilization have been made (Chuan and Liu, 1996; Shen *et al.*, 2000a, b). The effect of various factors including the extraction time, the type of mineral acid, consumption of acid, pH, sludge solids concentration, temperature on leaching yield of metals was systematically investigated. However, high cost, operational difficulties and large acid consumption have made a practical application of chemical method unattractive.

In the last decade, bioleaching have been developed as a potential method to remove heavy metals from sewage sludge using *Acidithiobacillus ferrooxidans* (an iron and sulfur oxidizing bacterium) or *Acidithiobacillus thiooxidans* (an sulfur oxidizing bacterium) (Couillard and Zhu, 1992; Blais *et al.*, 1993; Tyagi and Tran, 1993; Tyagi *et al.*, 1996; Wong *et al.*, 2002). This process used biological reduction of sludge pH and phase separation to remove toxic metals from sewage sludge. It was demonstrated that sludge pH was decreased to 2.0 and more than 80% of Cu, Zn, Cd and Ni was leached from the sludge. However, removal efficiency of Cr was usually less than 30%—40% (Zhou *et al.*, 2002). Moreover, little information on bioleaching of tannery sludge with extremely high Cr content was available (Zhou *et al.*, 2005). Compared with sewage sludge in the same solid concentration, tannery sludge possesses greater acid buffering capacity and higher concentration of Cr

(III) and dissolved organic matter, which will drastically inhibit the growth of autotrophic *Acidithiobacilli* (Tyagi and Tran, 1993, Zhou *et al.*, 2005). Therefore, the aims of the present study were: (1) to investigate the presence of indigenous iron-oxidizing bacteria and sulfur-oxidizing bacteria in tannery sludge and (2) to ascertain if the biolcaching technology developed for sewage sludge could be applied into tannery sludge.

1 Materials and methods

1.1 Sampling and characterization of tannery sludge

The tannery sludge used in this work was obtained from a tannery factory in Haining City, Zhejiang Province, China. Being representative of tannery plant in China, the factory is equipped with a coagulation-precipitation primary treatment unit and a secondary biological treatment unit (activated sludge process) that handles wastewater of 2500 t/d. A mixture of primary (90% v/v) and secondary (10% v/v) sludges was taken in February, 2002. The pH and oxidation-reduction potential (ORP) of the tannery sludge were determined immediately after collection while solid contents were measured by oven-drying at 105°C. Sub-samples were digested with di-acid digestion (conc. HNO₃ + conc. HClO₄) and heavy metals in the digestates were determined by atomic absorption spectrometry. Dissolved organic matter (DOM) in tannery sludge was determined by using a TOC analyzer (TOC-5000A, Shimaduz, Japan).

The physicochemical characteristics of the sludge used are presented in Table 1.

Table 1 Physicochemical characteristics of tannery sludge

| Parameter | Value | Parameter | Value |
|----------------------------------|--------|--------------------------|-------|
| pH | 8.05 | Total Fe, % | 3.15 |
| ORP, mV | -156.9 | Total S, % | 1.90 |
| Total solid, % | 11.84 | Cr ³⁺ , mg/kg | 15970 |
| Organic matter, % | 44.6 | Cr ⁶⁺ , mg/kg | ND |
| Total N, % | 3.03 | Zn, mg/kg | 110 |
| Total P, % | 1.02 | Cu, mg/kg | 31 |
| Total K, % | 0.49 | Pb, mg/kg | 105 |
| Dissolved organic matter, mgC/kg | 26008 | | |

It should be noted that the total solid contents (TS) of 11.84% in this study was greater than the 2.0%—4.5% that was performed in the decontamination of sewage sludge (Couillard and Zhu, 1992; Blais *et al.*, 1993; Tyagi and Tran, 1993; Wong *et al.*, 2002). Increasing TS could contribute to decreasing the volume of the reactor, which, in turn decreased the treatment cost.

1.2 Microorganisms and inoculum

The acclimation of indigenous iron-oxidizing bacteria in tannery sludge was performed according to

the technique of Tyagi and Tran (1993) with some modifications. Samples (150 ml) were transferred to a 500-ml Erlenmeyer flasks. The tannery sludge was adjusted to pH 2.0 with sulfuric acid and 3.0 g FeSO₄·7H₂O was added to each flask. The flask was then incubated at 28°C in a horizontal shaker at 200 r/min. After 15—18 d of initial adaptation when more than 95% of the Fe²⁺ in the medium was oxidized to Fe³⁺, 10% of the acid sludge was mixed with 150 ml of pre-acidified (pH 2.0) tannery sludge containing 3.0 g FeSO₄·7H₂O followed by re-incubation. The procedure was repeated until the rate of Fe²⁺ oxidation was constant in two consecutive transfers. At this time, iron-oxidizing bacteria were assumed to be fully adapted. The control with sterilized sludge containing 4.0 g/L of Fe²⁺ at pH 2.0 was also run to distinguish between chemical oxidation and biological oxidation of ferrous iron. The presence of indigenous iron-oxidizing bacteria in tannery sludge was examined by mixing 1 ml adapted sludge in 100 ml improved 9 K synthetic liquid medium (pH 2.0) containing 44 g of FeSO₄·7H₂O according to Yates and Holmes (1987). After incubation at 28°C and being shaken 200 r/min for 3—5 d, the solution turned to dark reddish-brown color, which was an indicator of the existence of iron-oxidizing bacteria.

The acclimation of indigenous sulfur-oxidizing bacteria in tannery sludge was carried out according to the technique of Blais *et al.* (1992). 150 ml of sludge sample was pre-acidified with sulphuric acid to pH 6.0 and then transferred to a 500-ml Erlenmeyer flask containing 1% of tyndalized powdered sulfur. The control without the addition of sulfur was also done. The flask was incubated at 28°C in a horizontal shaker at 200 r/min for a period of 15—20 d for initial acidification (pH less than 2.0). Samples of 5 ml were withdrawn every 48 h for pH measurement. After the initial acidification, the 150 ml pre-acidified sludge (pH 6.0) combined with 1% of tyndalized powdered sulfur was mixed with 5% of the above-mentioned sludge and reincubated in a horizontal shaker at 28°C and 200 r/min. This step was repeated successively until the rate of pH reduction (initial pH 6.0) to 2.0 was maximized over two consecutive transfers. At this stage, indigenous sulfur-oxidizing bacteria were assumed to be adapted.

The inoculum was prepared by mixing 15 ml of indigenous iron-oxidizing bacteria and 15 ml of sulfur-oxidizing bacteria in 500 ml Erlenmeyer flasks containing 120 ml of pre-acidified tannery sludge supplemented with 0.4% Fe²⁺ and 0.3% of tyndalized sulfur. The flasks for inoculum preparation were agitated on a horizontal shaker at 200 r/min and 28°C for 4 d, which was determined in a preliminary experiment to delineate the time period for the highest biological activity.

1.3 Bioleaching experiments

Batch experiments were carried out at 28°C and 200 r/min in a 500-ml Erlenmeyer flask containing 250 ml tannery sludge. Tannery sludge was pre-acidified to pH 6.0 with sulphuric acid and then agitated at 28°C and 200 r/min for 12 h to adapt the sludge to bioleaching temperature. This pH adjust did not solubilize Cr in sludge. An inoculum of 15% volume was added to the pre-acidified sludge followed by addition of (1) 6.0 g/L Fe²⁺ and 2.0 g/L elemental S; (2) 0 g/L Fe²⁺ and 2 g/L elemental S; (3) 0 g/L Fe²⁺ and 0 g/L elemental S. Control experiments without inoculum were always performed for comparison. The water loss due to evaporation will be replenished with distilled water. All treatments were done in triplicate.

1.4 Analytical methods

Samples of 15 ml were withdrawn at 3 d intervals for pH and ORP measurement and subsequent centrifugation at 12000 ×g for 15 min to separate solids and liquid. The solid fraction was freeze-dried and then used to measure total N, P and total organic matter (Blais *et al.*, 1992). The supernatant were then filtered with 0.45 μm membrane filters prior to determination of Fe²⁺ and Fe³⁺ by the 1, 10-phenanthroline method (Wong *et al.*, 2002), and Cr⁶⁺ by the *s*-dephenycarbazide method (Bartlett and James, 1979), total Cr and SO₄²⁻ by ICP-AES (Optival, USA). Cr removal efficiency was calculated as the ratio between the solubilized Cr in the supernatant and total Cr present in the sludge as defined by Couillard and Zhu (1992).

2 Results and discussion

2.1 Adaptation of the iron- and sulfur-oxidizing bacteria

Results in Fig.1 show that Fe²⁺ oxidation in sterilized sludge at pH 2.0 was negligible throughout the incubation period. In nonsterilized sludge, Fe²⁺ oxidation was initiated by 9 d of incubation and drastically enhanced and then reached maximum value at the day 17. Successive adaptation process greatly accelerated the rate of Fe²⁺ oxidation until complete oxidation could be achieved in 2–3 d. This is in good agreement with results obtained by Pesic *et al.* (1989) that the abiotic oxidation of Fe²⁺ with O₂ was extremely slow at low pH (< 4.5), but iron-oxidizing bacteria, especially *Acidithiobacillus ferrooxidans*, can accelerate the rate of Fe²⁺ oxidation by a factor of 10⁶. The microscopic examination of culture medium in third transfer revealed the presence of rod-shaped bacteria with 1.0–1.5 μm similar to *Acidithiobacillus ferrooxidans*.

As shown in Fig.2, the pH decreased in the test runs combined with the tyndalized sulfur from 6.0 to about 2.0 during 24 d, while the pH in control without sulfur increase from 6.0 to about 6.9, it is possibly due

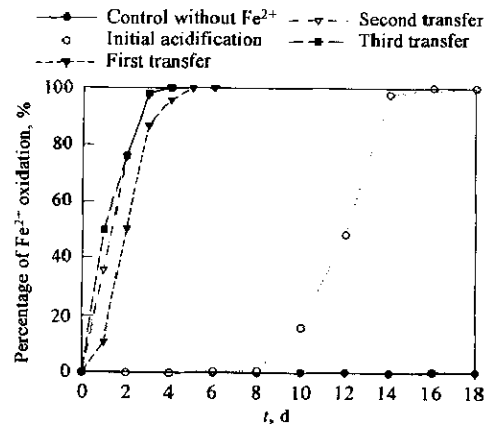


Fig.1 Presence of iron-oxidation bacteria evaluated by ferrous oxidation efficiency as function of time

to ammonification liberating ammonia from decomposition of organic matter. This indicated that the pH decrease was due to the oxidation of sulfur into sulfuric acid by indigenous sulfur-oxidizing bacteria. Successive transfers of acidified sludge to corresponding fresh sludge accelerated the decrease of pH and reduced the lag phase.

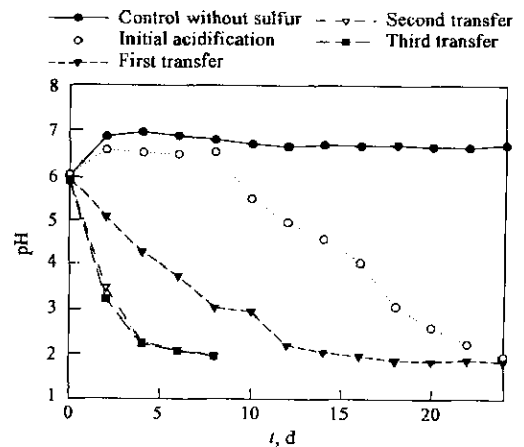
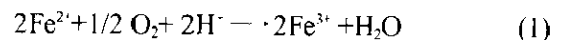


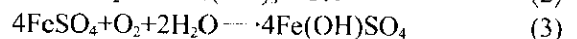
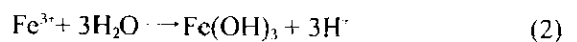
Fig.2 Adaptation of sulfur-oxidation bacteria evaluated by pH as function of time

2.2 Oxidation of ferrous iron and/or elemental sulfur

The oxidation of ferrous ions coupled with the Fe³⁺ generation originating from the added ferrous sulfate or native ferrous sulfide in tannery sludge may naturally occur or biological catalyzed (Fig.3a and b).



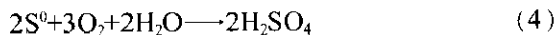
As above-mentioned, Fe²⁺ at neutral pH is chemically unstable and will be oxidized to Fe³⁺ that precipitates as iron hydroxides or ferric hydroxy-sulfates lowering the pH.



It was the reason that the Fe²⁺ concentration in the uninoculated treatment with addition of Fe²⁺

rapidly decreased from 6292 to 2524 mg/L in 3 d.

The oxidation of added elemental sulfur with concomitant sulfate production is shown in Fig.3c.



The initial sulfate concentration in tannery sludge was approximately 1600 mg/kg. It was virtually stable for the control and other uninoculated treatments during bioleaching, which indicated that elemental sulfur was chemically stable. Inoculum alone caused a slight increase in sulfate from 1620 to 1820 mg/L indicating the possible utilization of sulfide in tannery sludge as energy source for growth of iron- and sulfur-oxidizing bacteria. Due to biooxidation of added S^0 , there was a net increase in sulfate for the inoculated treatments with co-addition of Fe^{2+} and S^0 and addition of S^0 alone.

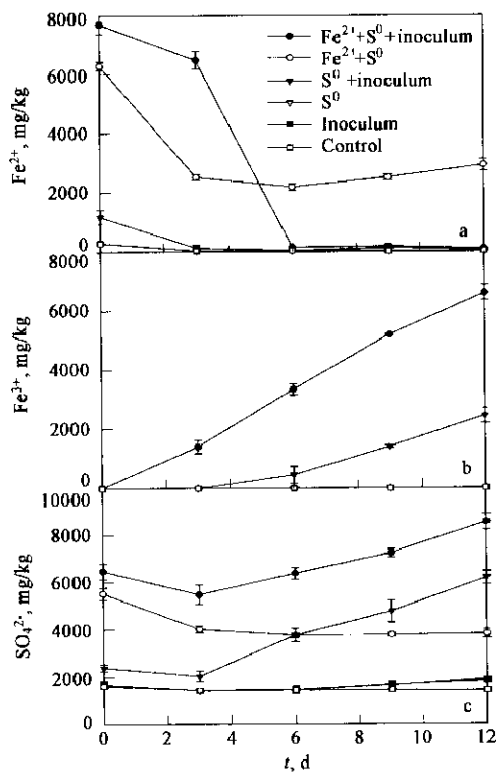


Fig.3 Changes of Fe^{2+} , Fe^3+ and SO_4^{2-} during the bioleaching of tannery sludge

2.3 Changes in pH and ORP with time

Oxidation of ferrous iron and/or elemental sulfur directly led to sludge acidification and increase of ORP. The sludge acidification pattern and changes in ORP during the bioleaching are presented in Fig.4a and b, respectively. In control and uninoculated treatments with addition of S^0 alone, there was an increase from 6.0 to about 7.3 in pH. In the uninoculated treatment with addition of ferrous sulfate, the pH decreased from 6.0 to approximately 3.8 and maintained this level till the end of bioleaching. This pH decrease was attributed hydrolysis of Fe^{2+} and Fe^{3+} generated by chemical oxidation of Fe^{2+} at $pH > 4.5$.

The pH in inoculated treatments decreased at a different rate. The relative rates of acid production were $Fe^{2+}+S^0+inoculum > S^0+inoculum > inoculum$ alone. Their final pH were 1.85, 1.98 and 5.51, respectively. The reason why the pH in $Fe^{2+}+S^0+inoculum$ was lower than that in $S^0+inoculum$ was that the rapid decrease of pH initiated by the chemical oxidation of Fe^{2+} favored the growth of acidophilic iron- and sulfur-oxidizing bacteria, which in turn accelerated further fall of pH.

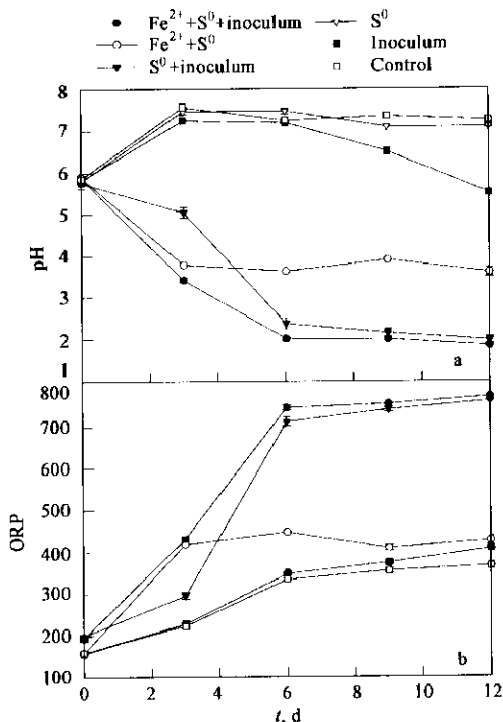


Fig.4 Changes of pH and ORP during the bioleaching of tannery sludge

High ORP coupled with low pH value have been considered as indicators of the presence of substantial population of iron- and sulfur-oxidizing bacteria (Wong *et al.*, 2002). Opposite to the trends of changes in pH, the ORP of different treatments increased at a different rate: $Fe^{2+}+S^0+inoculum > S^0+inoculum > Fe^{2+}+S^0 > inoculum > S^0 > control$.

2.4 Removal of Cr from tannery sludge

As shown in Fig.5, solubilization of Cr seemed to be closely dependent on the presence of bacteria and energy substrates as indicated by the low Cr solubilization efficiencies (< 2.2%) for the all uninoculated treatments and inoculated treatment without adding energy substrate, throughout the bioleaching period. Cr solubilization also was governed by pH because it occurred only when the pH in sludge was lower than about 3.0. Therefore, there was a lag period of 3–4 d prior to leaching of Cr from the sludge for the inoculated treatments receiving energy substrates. From the day 3, the dissolved Cr concentration increased drastically as pH decreased. The maximum removal efficiency observed after 12 d

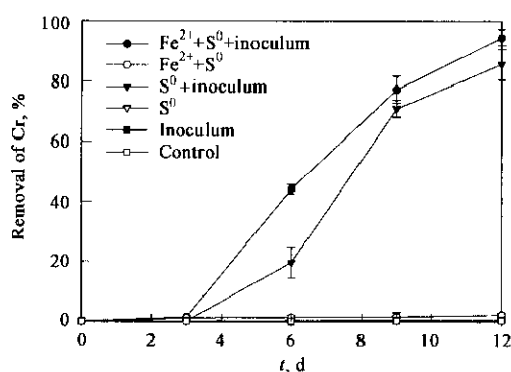


Fig.5 Removal of Cr from tannery sludge by bioleaching

of bioleaching in co-addition of Fe²⁺ and S⁰ was 95.6%. Having achieved 95.6% removal of Cr in tannery sludge brings its concentration below the required limit (GB 4284-84).

Cr is notoriously problematical metal whose removal efficiency is usually less than 30%—40% in sewage sludge bioleaching (Blais *et al.*, 1993; Chuan and Liu, 1996; Tyagi *et al.*, 1996; Shen *et al.*, 2001a). Studies carried out on sewage sludge demonstrated that the metal solubilization efficiency was strongly affected by metal speciation (Tyagi *et al.*, 1988; Lombardi and Garcia, 1999). It has been reported that 10%—20% Cr(III) was bounded to organic matter and 80%—90% existed as mixed oxides of Cr-Fe coprecipitates in tannery sludge (Chuan and Liu, 1996). It is widely accepted that Cr associated with organic fraction is less available for solubilization than that in inorganic form (Tyagi *et al.*, 1988; Zhou *et al.*, 2002). Macchi *et al.* (1999) reported that only 90% of Cr could be extracted from tannery sludge at pH 1.0 using sulfuric acid. Data presented here showed bioleaching for solubilizing Cr seemed to be more effective than chemical acid solubilization at the same pH. Plausible interpretation may be that microbiological assimilation and the highly oxidizing condition facilitated breakage of organic fraction and thus Cr release from tannery sludge during the bioleaching.

The yield of Cr solubilization was about 10% lower in the uninoculated treatment with addition of S⁰ alone than that with co-addition of Fe²⁺ and S⁰. It could be ascribed to its higher pH, lower ORP and Fe³⁺ concentration. Further research is required for enhancing Cr removal using the less expensive substrate such as S⁰ alone.

Analyses also showed that Cr(III) was predominant species and Cr(VI) was not present in leachates during bioleaching. This indicated that *Acidithiobacilli* and the chemical oxidant (Fe³⁺) can not transform less toxic Cr(III) into higher toxic Cr(VI), which excluded the concern that the bioleaching process might modify Cr speciation and increase its bioavailability (Lombardi and Garcia, 1999).

2.5 Nutrient loss in bioleaching

One major concern about bioleaching was the potential loss of nutrients including N, P and organic matter, which in turn reduced its value as soil fertilizer or conditioner. The loss of N, P and total organic matter after 12 d of bioleaching is shown in Table 2.

Table 2 Loss of N, P and organic matter in bioleaching in tannery sludge

| Treatment | N loss, % | P loss, % | Organic loss, % |
|--|--------------|-------------|-----------------|
| Fe ²⁺ +S ⁰ +inoculum | 20.4 ± 0.35* | 24.5 ± 0.65 | 14.3 ± 0.24 |
| Fe ²⁺ +S ⁰ | 2.6 ± 0.04 | 2.9 ± 0.03 | 5.8 ± 0.05 |
| S ⁰ +inoculum | 17.8 ± 0.47 | 19.6 ± 0.41 | 10.1 ± 0.04 |
| S ⁰ | 1.7 ± 0.02 | 3.9 ± 0.02 | 1.8 ± 0.00 |
| Inoculum | 2.6 ± 0.01 | 2.0 ± 0.01 | 1.6 ± 0.01 |
| Control | 1.2 ± 0.01 | 2.0 ± 0.00 | 1.3 ± 0.00 |

* Standard deviation

The patterns of nutrient loss were similar to that of Cr solubilization, the greater the amount of Cr solubilized, the greater the amount of N, P, and organic matter lost for all the treatments. There was less than 6% change in N, P, and organic matter in uninoculated treatments, indicating the loss of nutrients was not essential. In inoculated treatment with co-addition of Fe²⁺ and S⁰, 20.4% of N, 24.5% of P and 14.3% of organic matter were lost in bioleached sludge, respectively. This loss can be caused by the highly oxidizing condition and the low pH during bioleaching resulting in an environment suitable for dissolving the metals and also enhancing the decomposition of organic matter in the sludge. It should be noted that the amelioration of the odor of bioleached sludge compared to the control sludge could be observed. Similar observations also were obtained by Couillard *et al.* (1992), whose interpretation was that the reduction in ammonia content following bacterial assimilation and the oxidation of H₂S to sulfate by sulfur-oxidizing bacteria.

3 Conclusions

The iron-oxidizing bacteria and sulfur-oxidizing bacteria are naturally present in tannery sludge and can acclimated readily by mending with ferrous sulfate or elemental sulfur, in spite of extremely high concentrations of Cr and dissolved organic matter.

The utilization of mixture of the indigenous iron- and sulfur-oxidizing bacteria and co-addition of Fe²⁺ and elemental sulfur is particularly efficient for the removal of Cr, which renders tannery sludge suitable for use in agriculture. 20.4% of N, 24.5% of P and 14.3% of organic matter were lost in the bioleaching process.

This study has shown the feasibility of applying the bioleaching process, developed for sewage sludge,

to tannery sludge.

References:

- Adriano D C, 1986. Trace elements in the terrestrial environment[M]. New York: Springer. 58—76.
- Anderson R A, 1989. Essentiality of chromium in humans [J]. *Sci Total Environ*, 86: 75—81.
- Bartlett R J, James B R, 1979. Behaviour of chromium in soils: oxidation[J]. *J Environ Qual*, 8: 31—35.
- Blais J F, Tyagi R D, Auclair J C, 1992. Bioleaching of trace metals from sewage sludge by indigenous sulfur-oxidizing bacteria[J]. *J Environ Engin*, 118: 690—707.
- Blais J F, Tyagi R D, Auclair J C, 1993. Biocaching of metals from sewage sludge: microorganisms and growth kinetics [J]. *Water Res*, 27: 101—110.
- Calvanese G, Cioffi R, Santoro L, 2002. Cement stabilization of tannery sludge using quaternary ammonium salts exchanged Bentonite as pre-solidification adsorbent [J]. *Environ Technol*, 23: 1051—1062.
- Chuan M C, Liu L C, 1996. Release behaviour of chromium from tannery sludge[J]. *Water Res*, 30(4): 932—938.
- Couillard D, Zhu S, 1992. Bacterial leaching of heavy metals from sewage sludge for agricultural application [J]. *Water Air Soil Pollut*, 63: 67—80.
- Dreiss S J, 1986. Chromium migration through sludge treated soils[J]. *Ground Water*, 24: 312—321.
- Early L E, Rai D, 1987. Kinetics of chromium(III) oxidation to chromium (VI) by reaction with manganese dioxide [J]. *Envir Sci Technol*, 21: 1187—1193.
- Iyengar G V, 1989. Nutritional chemistry of chromium [J]. *Sci Total Environ*, 86: 69—74.
- Lombardi A T, Garcia O Jr, 1999. An evaluation into the potential of biological processing for the removal of metals sewage sludge[J]. *Crit Rev in Microbiol*, 25(4): 275—288.
- Lopez A, Rotunno T, Palmisano F *et al.*, 1991. Simultaneous determination of chromium, aluminum, and iron in tannery sludge acid extracts by reversed-phase high-performance liquid chromatography [J]. *Environ Sci Technol*, 25: 1262—1266.
- Macchi G, Pagano M, Pettine M *et al.*, 1991. A bench study on chromium recovery from tannery sludge [J]. *Water Res*, 25(8): 1019—1026.
- Pesic B D, Oliver J, Wichlacz P, 1989. An electrochemical method of measuring rate of ferrous to ferric iron with oxygen in the presence of *Acidithiobacillus ferrooxidans*[J]. *Biotech Bioeng*, 33: 428—439.
- Renoux A Y, Tyagi R D, Samson R, 2001. Assessment of toxicity reduction after metal removal in bioleached sewage sludge [J]. *Water Res*, 35(6): 1415—1424.
- Rinehart W E, 1989. Recapitulation [J]. *Sci Total Environ*, 86: 191—193.
- Shen S B, Tyagi R D, Blais J F, 2001a. Extraction of Cr(III) and other metals from tannery sludge by mineral acids[J]. *Environ Technol*, 22(9): 1007—1014.
- Shen S B, Tyagi R D, Blais J F, 2001b. Prediction of metal precipitates in tannery sludge leachate based on thermodynamic calculations [J]. *Environ Technol*, 22(9): 961—970.
- Tyagi R D, Couillard D, Tran F, 1988. Heavy metals removal from digested sludge by chemical and microbiological method [J]. *Environ Pollu*, 50: 295—316.
- Tyagi R D, Tran F T, 1993. Bacterial leaching of metal from digested sewage sludge by indigenous iron-oxidizing bacteria [J]. *Environ Pollut*, 82: 9—12.
- Tyagi R D, Meunier J, Blais J F, 1996. Simultaneous sewage sludge and metal leaching: effect of temperature [J]. *Appl Microbiol Biotechnol*, 46: 422—431.
- Wong J W C, Xiang L, Chan L C, 2002. pH requirement for the bioleaching of heavy metals from anaerobically digested sewage sludge[J]. *Water Air Soil Pollut*, 138: 25—35.
- Yates J R, Holmes D S, 1987. Two families of repeated DNA sequences in *Acidithiobacillus ferrooxidans* [J]. *J Bacteriol*, 169(5): 1861—1870.
- Yu C Z, Zhang C B, Ding S L, 2000. The present conditions of tannery sludge and prospect of composting with sludge[J]. *China Leather*, 29(23): 4—7.
- Zhou L X, Hu A T, Ge N F, 1999. Study on utilization of municipal sewage sludge in farmland and forest land [J]. *Acta Ecologica Sinica*, 9(2): 185—193.
- Zhou L X, Fang D, Wang S M *et al.*, 2005. Bioleaching of Cr from tannery sludge: The effects of initial acid addition and recycling of acidified bioleached sludge [J]. *Environ Technol*, 26: 277—284.
- Zhou S G, Zhou L X, Wong J W C, 2002. Removal of heavy metals from sewage sludge by bioleaching[J]. *Acta Ecologica Sinica*, 22(1): 125—133.
- Zhou S G, Wang S M, Yu S P *et al.*, 2003. Isolation of *Acidithiobacillus ferrooxidans* and its application on heavy metal bioleaching from sewage sludge[J]. *Environ Sci*, 24(3): 55—60.

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