Special Waste Treatment
EFFECTS OF ALUM SLUDGE CHARACTERISTICS ON THE EFFICIENCY OF COAGULANTS RECOVERY BY ACIDIFICATION

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ABSTRACT The purpose of this study is to evaluate the effects of alum sludge characteristics on the efficiency of coagulants recovery using the technique of acidification (H₂SO₄). Two alum sludge characteristics were studied: types of coagulant and textures of the suspended solid in raw water. The coagulant types are aluminum sulfate and polyaluminum chloride (PAC); the textures of the suspended solid are sand-based and clay-based. The sand-based and clay-based alum sludge were obtained from water treatment plants in southern and northern Taiwan, respectively. Efficiency of aluminum recovery at a pH of 2 was compared between different alum sludge.

The results showed that efficiency of aluminum recovery from alum sludge containing sand-based particles were higher than that from alum sludge containing clay-based particles. The recoverable aluminum from sand-based alum sludge ranged between 2.1 and 2.8 g/kg dry sludge, whereas it ranged between 0.7 and 0.9 g/kg dry sludge for clay-based alum sludge. As for the effect of coagulant types, the aluminum recovery rate for alum sludge using PAC ranged between 77 and 100 percent, whereas it ranged between 65 and 72 percent for alum sludge using aluminum sulfate as the coagulant. This means using PAC as the coagulant could result in higher recovery efficiency of coagulant and be beneficial when water treatment plants consider renewable materials and waste reduction as the factors for making decisions regarding plant operations. However, other metals, such as manganese, could be released with aluminum during the acidification process. This would limit the use of the recovered coagulants. It is suggested that the recovered coagulants be used in wastewater treatment processes.

Keywords: Alum sludge, Coagulant recovery, Acidification

Introduction

The cost of waste sludge including dewatering and final disposal is a major operation cost and has caused severe problems for water supply facilities [1]. Sludge acidification with aluminum recycle is one of the alternatives being considered for effectively reducing the sludge volume and treatment cost and has been applied in many countries [2-4]. The most significant advantage of this process is the cost reduction resulting from the recycling the coagulants and the decrease of sludge production. In addition, acidification of sludge also improves the dewaterability of sludges, thus reducing the volume of sludge cakes and following cost on sludge disposal [5-7]. Many reports also prove the coagulation performance and the treatment cost of recycled coagulants is comparable to new coagulant on the application on water and wastewater treatment [5, 6, 8, 9 ]. Significantly, the recycled alum coagulant has become in feasibly and readily available. In theoretical, the total alum recovery is released from the alum-coagulant and aluminum content in the sediment in alum sludge. Aluminum sulfate and poly aluminum chloride (PACl) are wildly used in Taiwan as coagulant. The characteristics of suspended solid at different parts of Taiwan are different. In general, the soil characteristics were sand-based in southern Taiwan, whereas it is clay-based in northern Taiwan. In the previous researches, the effects of coagulant and suspended solid type on alum recovery by acidification have not been studied. The purpose of this study is to evaluate the effects of alum sludge characteristics on the efficiency of coagulants recovery using the technique of acidification (H₂SO₄).

Materials and Methods

Alum sludge samples were taken from the Fang-Yuan (FY), Ping-Jhen (PJ) (northern Taiwan) and Ping-Ting (PT), Wu-San-To (WST) (southern Taiwan) water supply facility of the Taiwan Water Supply
Company in Taiwan. The types of coagulant used in the water treatment facilities were as the following: aluminum sulfate at WST and poly aluminum chloride (PACl) at the other 3 water supply facilities.

**Acidification and metal extraction of the sludge**

In this study, the sediments collected from the water resources and the alum sludge generated at the corresponding water treatment facility were subjected to acidification and metal extraction processes. The sediment samples were prepared by mixing 100 g of sediments with water obtained from the water source to a final volume of 500 mL, and stirred for 24 h at 200 rpm with a jar test equipment before being settled. The settled sludge was acidified with 98% sulfuric acid to a pH of 2.0 and stirred (200 rpm) for 2 h in a 1-L beaker placed in a water bath at 25 °C. In our preliminary study, the aluminum concentration in the supernatant of acidified sludge should reach a stable level after 2 h of acid extraction.

The metal extraction of sludge was conducted at the following pH values: 1.0, 2.0, 3.0, 4.0, and 5.0 by addition of 98% sulfuric acid. When the desired pH was reached, the solids were allowed to settle for 15 min. The supernatant was centrifuged at 35 rpm for 15 min. The total metal content in the solid and liquid phases was extracted by the aqua regia method and analyzed by ICP-OES after digestion. The metal released efficiency was calculated according to Eq. 1.

\[
\text{heavy metal released efficiency (\%) = } \frac{\text{metal content}_{\text{liquid}} \text{ after acidification} - \text{metal content}_{\text{solid}} \text{ after acidification}}{\text{metal content}_{\text{solid}} \text{ after acidification}} \times 100
\]  

(1)

**Aluminum recovery by acidification**

Acidification of alum sludge was conducted by adding 98% sulfuric acid to 500 mL of alum sludge to a pH value of 2.0 and stirred at 200 rpm for 2 h in a 1-L beaker placed in a water bath. Aluminum content of the acidified sludge and supernatant were analyzed. The efficiency of aluminum coagulants recovered from the alum sludge was determined according to Eq. 2-5. The method of determined aluminum recovery efficiency by acidification in reference was illustrated in Eq. 6 [9-12]. In this study, the recovered aluminum from the coagulant was considered in aluminum recovery efficiency estimation, and compared to the aluminum released from alum sludge that the recovery efficiency could be calculated according to Eq. 7.

\[
\text{Alum released efficiency(\%) = } \frac{\text{Released from field sludge (g/ Kg } \alpha_\theta) - \text{Released from the sediments (g/ Kg } \alpha_\theta)}{\text{Alum from coagulant (g/ Kg } \alpha_\theta)}
\]  

(2)

\[
\text{Alum released from field sludge by acidification (g/ Kg } \alpha_\theta) = \frac{\text{The conc. of alum in the supernatant (mg/L) - The volume of supernatant (L)}}{\text{Mass of the field sludge before acidification (g) \times [1 – moisture(\%)]}}
\]  

(3)

\[
\text{Alum released from the sediments by acidification (g/ Kg } \alpha_\theta) = \frac{\text{The conc. of alum in the supernatant (mg/L) - The volume of supernatant (L)}}{\text{Mass of the sediments before acidification (g) \times [1 – moisture(\%)]}}
\]  

(4)

\[
\text{Alum from the coagulant (g/ Kg } \alpha_\theta) = \text{Alum field sludge (g/ Kg } \alpha_\theta) - \text{Alum in the sediments (g/ Kg } \alpha_\theta)
\]  

(5)

\[
\text{Alum released (\%) = } \frac{\text{Released from acidified field sludge (g)}}{\text{Alum from field sludge (g)}}
\]  

(6)

\[
\text{Alum recovery (\%) = } \frac{\text{Released from acidified field sludge (g)}}{\text{Total Alum from coagulant added (g)}}
\]  

(7)
Results and Discussion

Effects of Coagulant Type on Aluminum Recovery

The percent release of aluminum ranged between 34.3 and 80.7 percent (Table 1), which is comparable to those found in the literature [9, 10]. Results in Table 1 also indicates that the percent release of aluminum was differs within the water treatment facility. Therefore, the percent release of aluminum is unable to distinguish the effect of coagulant type on the aluminum recovery efficiency. Table 2 shows the aluminum recovery calculated with Eq. 7. The aluminum recovery for WST was 65 and 71 % at different date. The aluminum recovery for the other water treatment facilities was between 77 and 101 %, which was higher than that of WST. As WST is the only facility that uses aluminum sulfate as coagulant, the results in Table 2 suggests that acidification of PACl-based sludge can achieve higher recovery rate than aluminum sulfate-based sludge. This can be contributed to the inter-particle bridging induced by PACl resulting in more aluminum ions being adsorbed on the solid phase of sludge. Results from Table 2 indicate that Eq. 7 is more appropriate than Eq. 1 for evaluating the aluminum recovery efficiency.

Table 1. Aluminum released from different field sludges by acidification

<table>
<thead>
<tr>
<th>Sample</th>
<th>WST21</th>
<th>WST12I</th>
<th>P131</th>
<th>P1319</th>
<th>P116</th>
<th>FY828</th>
<th>FY39</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS[1]</td>
<td>3240±597.7</td>
<td>2001±28.3</td>
<td>1453±40.3</td>
<td>2696±147.4</td>
<td>1395±591.3</td>
<td>2472±560.0</td>
<td>3365±21.3</td>
</tr>
<tr>
<td>AFS[2]</td>
<td>1052±312.4</td>
<td>9776±139.3</td>
<td>497.6±39.4</td>
<td>1287±9.4</td>
<td>631±32.5</td>
<td>680±85.0</td>
<td>2298±121.3</td>
</tr>
<tr>
<td>RFS[3]</td>
<td>1409±47.5</td>
<td>1231±10.9</td>
<td>1095±24.8</td>
<td>1405±19.7</td>
<td>659±247.7</td>
<td>1994±69.5</td>
<td>1153±273.4</td>
</tr>
<tr>
<td>Recovery(%)</td>
<td>46.13</td>
<td>59.76</td>
<td>75.36</td>
<td>52.14</td>
<td>47.24</td>
<td>80.66</td>
<td>34.26</td>
</tr>
</tbody>
</table>

Table 2. Aluminum recovery from coagulants added in different WTPs

<table>
<thead>
<tr>
<th>Coagulant type</th>
<th>WST21</th>
<th>WST12I</th>
<th>P131</th>
<th>P1319</th>
<th>P116</th>
<th>FY828</th>
<th>FY39</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA[1]</td>
<td>68.08</td>
<td>82.16</td>
<td>85.87</td>
<td>60.40</td>
<td>27.15</td>
<td>49.14</td>
<td>12.65</td>
</tr>
<tr>
<td>R.AFS[3]</td>
<td>44.51±0.45</td>
<td>59.00±0.04</td>
<td>74.13±0.18</td>
<td>46.80±2.26</td>
<td>21.33±0.16</td>
<td>50.02±0.12</td>
<td>9.87±0.38</td>
</tr>
<tr>
<td>Recovery(%)</td>
<td>65.38</td>
<td>71.81</td>
<td>86.33</td>
<td>77.48</td>
<td>78.56</td>
<td>101.85</td>
<td>78.02</td>
</tr>
</tbody>
</table>

Effects of Aluminum Content in Sediments

The release of aluminum from sediments during acidification process could affect the calculation of coagulant recovery efficiency as it adds to the amount of aluminum in the supernatant after acidification. Table 3 shows the results of aluminum released from sediments of different water treatment reservoirs when acidified to a pH value of 2.0. The sediments from northern Taiwan contain more clay, whereas the sediments from southern Taiwan contain more sand. The percent release of aluminum ranged between 11.8 and 18.5 percent and 4.6 and 5.0 percent for sandy and clayed sediments, respectively. The results confirm that the aluminum content of sediments could contribute to the total aluminum content in the supernatant after sludge acidification. Sandy sediment could contribute more aluminum than clayey sediments (Table 2).

The aluminum recovery efficiency shown in Table 2 was adjusted by considering the aluminum contribution from the sediments. After the adjustment, the aluminum recovery efficiency of sludges from the southern Taiwan and northern Taiwan was 61~70% and 72~100%, respectively (Table 4).
suggest the sludge from clay-based sediments can release more aluminum than sludge from sand-based sediments. This is probably due to the difference in the specific surface area of the sludge. Clay-based sludge has higher specific surface area than sand-based sludge. When clay-based sludge is subjected to acidification, its specific surface area tends to decrease [7]. On the other hand, sand-based sludge did not show significant reduction in specific surface area. The reduction of specific area means dissolution of solids from the sludge occurred during acidification. It is this dissolution of solid than could contribute to high aluminum recovery efficiency by acidification.

Table 3. Aluminum released from original sediment by acidification

<table>
<thead>
<tr>
<th>WTPs</th>
<th>South WST</th>
<th>South PT</th>
<th>North PT</th>
<th>North FY</th>
</tr>
</thead>
<tbody>
<tr>
<td>O.P.</td>
<td>15.15±0.86</td>
<td>18.03±0.58</td>
<td>19.81±2.55</td>
<td>13.13±0.93</td>
</tr>
<tr>
<td>A.O.P.</td>
<td>16.21±1.26</td>
<td>18.70±1.26</td>
<td>22.13±2.33</td>
<td>11.39±2.70</td>
</tr>
<tr>
<td>R.A.O.P.</td>
<td>2.80±0.49</td>
<td>2.13±0.27</td>
<td>0.92±0.64</td>
<td>0.66±0.04</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>18.48</td>
<td>11.81</td>
<td>4.64</td>
<td>5.03</td>
</tr>
</tbody>
</table>

Mean±SD: average of triple, unit: g/Kg dry

Table 4. Comparison of alum recovery from coagulant in south and north facilities

<table>
<thead>
<tr>
<th>Sample</th>
<th>South WST 2/1</th>
<th>South WST 1/21</th>
<th>South PT 1/16</th>
<th>North PJ 1/31</th>
<th>North PJ 3/19</th>
<th>North FY 8/28</th>
<th>North FY 3/9</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.</td>
<td>68.08</td>
<td>82.16</td>
<td>27.15</td>
<td>85.87</td>
<td>60.40</td>
<td>49.14</td>
<td>12.65</td>
</tr>
<tr>
<td>R.A.F.S.</td>
<td>44.51±0.45</td>
<td>59.00±0.04</td>
<td>21.33±0.16</td>
<td>74.13±0.18</td>
<td>46.80±2.26</td>
<td>50.02±0.12</td>
<td>9.87±0.38</td>
</tr>
<tr>
<td>R.A.O.P.</td>
<td>2.80±0.49</td>
<td>2.80±0.49</td>
<td>2.13±0.27</td>
<td>0.92±0.64</td>
<td>0.92±0.64</td>
<td>0.66±0.04</td>
<td>0.66±0.04</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>61.27</td>
<td>68.40</td>
<td>70.72</td>
<td>85.26</td>
<td>75.96</td>
<td>100.45</td>
<td>72.81</td>
</tr>
</tbody>
</table>

Mean±SD: Three repeats of each sample, unit: g/Kg dry

Heavy Metal Release by Acidification

The amount of heavy metal released by acidification decreased with pH (Fig. 1-4). Among the heavy metals quantified, lead, silver, and cadmium were undetected. Arsenic had the highest release efficiency (almost 100% when the sludge was acidified to pH = 2) followed by manganese. These patterns are similar to those found in the literature [13, 14]. The order of release efficiency of heavy metals follows the same pattern of the solubility of these metal hydroxides, i.e., Mn > Al > Cr > Zn > Cu > Pb > Fe > Cd. This means that manganese needs to be released before aluminum starts to dissolve when the sludge is acidified. Therefore, the manganese content in the sludge increases the consumption of acid and could decrease the aluminum recovery efficiency if insufficient acid was used in the acidification process.

The release of heavy metals from sludge acidification is undesirable as it could cause health concern and limit the use of the recovered coagulants. According to the Taiwan’s standards for impurities in coagulants, arsenic, zinc, and mercury all exceeded the limit, resulting in the covered coagulants being unable to be used in water treatment plants (Table 5). This problem can be addressed by applying the coagulants to wastewater treatment plants. Another concern is the pH of the sludge. Although the optimum condition for aluminum recovery efficiency is pH = 2, this low pH valued caused the residual sludge to be characterized as “hazardous” according to Taiwan’s regulation. This could significantly increase the cost for disposal of these sludge. Therefore, it is recommended that the final pH for acidification is set to 3.0 [6, 9, 10].
Table 5. Metal content in the supernatant after acidification vs Standard of reagent used for water treatment

<table>
<thead>
<tr>
<th>Metal</th>
<th>Standard of reagent for drinking water treatment</th>
<th>WST 2/1</th>
<th>( \text{pH}=1 )</th>
<th>( \text{pH}=2 )</th>
<th>( \text{pH}=3 )</th>
<th>( \text{pH}=4 )</th>
<th>( \text{pH}=5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>--</td>
<td></td>
<td>3658.97</td>
<td>3064.52</td>
<td>2731.11</td>
<td>774.48</td>
<td>0.00</td>
</tr>
<tr>
<td>Cu</td>
<td>3.0 ppm</td>
<td></td>
<td>1.32</td>
<td>0.33</td>
<td>0.12</td>
<td>0.02</td>
<td>N.D.</td>
</tr>
<tr>
<td>Ni</td>
<td>1.0 ppm</td>
<td></td>
<td>0.40</td>
<td>0.09</td>
<td>0.06</td>
<td>0.04</td>
<td>N.D.</td>
</tr>
<tr>
<td>Pb</td>
<td>1.0 ppm</td>
<td></td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Cr</td>
<td>2.0 ppm</td>
<td></td>
<td>0.35</td>
<td>0.08</td>
<td>0.06</td>
<td>0.02</td>
<td>N.D.</td>
</tr>
<tr>
<td>Cd</td>
<td>0.03 ppm</td>
<td></td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Ag</td>
<td>0.5 ppm</td>
<td></td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>As</td>
<td>0.5 ppm</td>
<td></td>
<td>1.44</td>
<td>1.38</td>
<td>1.15</td>
<td>0.24</td>
<td>0.03</td>
</tr>
<tr>
<td>Zn</td>
<td>5.0 ppm</td>
<td></td>
<td>10.98</td>
<td>5.32</td>
<td>1.15</td>
<td>1.34</td>
<td>0.28</td>
</tr>
<tr>
<td>Hg</td>
<td>0.005 ppm</td>
<td></td>
<td>5.08</td>
<td>1.39</td>
<td>1.62</td>
<td>0.46</td>
<td>N.D.</td>
</tr>
<tr>
<td>Mn</td>
<td>25 ppm</td>
<td></td>
<td>41.27</td>
<td>34.49</td>
<td>39.34</td>
<td>34.13</td>
<td>15.56</td>
</tr>
<tr>
<td>Fe</td>
<td>200 ppm</td>
<td></td>
<td>643.25</td>
<td>149.63</td>
<td>131.87</td>
<td>103.55</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Conclusions
The aluminum recovery efficiency by acidification for PACl-based sludge is higher than that of aluminum sulfate-based sludge. The aluminum recovery efficiency by acidification was affected by the type of sediments in the water sources. Sludge from clay-based sediments has higher aluminum recovery efficiency than sludge from sand-based sediments after acidification. Release of manganese, arsenic, zinc, and mercury during sludge acidification limits the use of the recovered coagulants.

Acknowledgments
The authors would like to acknowledge the financial support provided by the National Science Council of Taiwan.

References
MOLeCULAR SIEVES FROM WASTE CATHODERAY-TUBES (CRTS)

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ABSTRACT Due to Technological advancement cathode-ray tubes (CRTs) from computer monitors and TV sets are being replaced continuously by thinner and lightweight versions like Liquid Crystal Display (LCD) or Plasma screen monitors. As regular discarding e-waste from CRTs are continuously increasing day by day and in UK alone it is projected to reach around 100,000 tonnes/year. CRT composition consists of 50-72% SiO2, 10-15% PbO, 5-7% Al2O3 and 18-35% various oxides with 0-0.5% of bonded plastics and carbon. From the recycling point of view this waste is categorized into two parts; (i) Organic decomposable part and; (ii) Silicate with metal oxide part. Management of CRTs involves the following steps; (i) grinding and milling; (ii) Thermal decomposition of organic part and finally; (iii) silicates with metals oxides are then subjected to thermal and/or ionic treatment in the presence of fluoride to produce molecular sieves for various applications including separation chemistry. Thermo/ionic reactions were performed at temperature between 500-6250°C and in the presence of fluoride ion in such a way that 98% of the fluoride used can be recovered finally. Silica and metal oxide (mostly heavy metals) mixture from waste CRT: (i) pose serious environmental problem when landfilled due to heavy metal leaching and; (ii) the separation of heavy metals from silica is an expensive operation. It justifies the significance of Thermo/ionic reactions for the purpose. It was found that the product from thermionic reaction have more crystalline pattern as compared with thermo reaction, as confirmed by XRD and SEM analysis.

Keywords: E-waste, Cathode-ray tubes (CRTs), Recycling, Molecular sieves

Introduction

Due to continuous discarding, Cathode ray tubes (CRTs) have now become a global environmental problem [5]. This waste contains valuable materials as well as large quantities of potentially hazardous materials and therefore poses danger to the environment and human health, if inappropriately managed. CRT waste contains approximately 38-40% silicate material [4]. Silicate materials present in CRT glasses are chemically very different and their chemical compositions can differ from one glassmaker to another, it represents problems for recycling methods that require very tight material specifications [3]. The disposal of silicate materials from CRT by landfill should be avoided because the toxic heavy metals including lead, cadmium and mercury are environmental threats, due to the acidic leaching of high concentration of heavy metals in to the ground [4].

However silicate waste from glass can be reused for making concrete admixture [7], masonry blocks [8], and ceramic tile [2] but inconsistency of the waste glass streams, [1] makes the use of these materials in regular applications questionable. Chemical conversion of silicate waste to design material for specific application [6] appears to be a potential management technique to manage this glass waste. In this paper molecular sieve designing method via the chemical recycling of silicate from waste CRT was proposed as a potential management technique for the problem associated with silicates from the CRT waste.

Materials and Methods

Waste CRT glass having the 72% SiO2 composition (by XRF analysis) were purchased from local scrap dealer. This glass sample was mechanically pulverized by a grinder with added cyclone separator to specific mesh size range 200-300. Hydrogen fluoride (48%), ammonium fluoride and hydrochloric acid all are AR grade and used without further purifications.

Activation of Silicate from Waste CRT

Pulverized CRT powder (8.5 g, containing approximately 0.1 mole silicon oxide) was initially neutralized with dilute hydrochloric acid to remove all calcium oxide present on it and then washed several times with water. The resultant mass was then treated with ammonium fluoride reagent containing 16.7 mL of 48% HF (0.4 moles) and 7.4 g NH4F (0.2 moles) in 20 mL water. The resultant solutions were taken in a
polypropylene conical flask and placed over water bath for a period of 10 hours. Precipitate thus obtained was washed 2-3 times with 10 mL of water and then dried first at a temperature of 150°C for a period of 30 minutes and finally calcined at 600°C for another 45 minutes in air. Fluoride can be recovered from the resultant filtrate in the form of ammonium fluoride by evaporating it in a hot air oven at a temperature 50°C, white shining crystal of NH₄F are thus obtained; The yield was 19 g (85%).

Activation of silicate material was investigated by using X-ray diffraction pattern of the samples which was examined in Pan analytical Holland X Ray diffractometer, using Cu as an Anode material and results are analyzed by WinXPow Software. ZEISS SMT EVO scanning electron microscope (SEM) was used to obtain the micrographs. For this purpose 10–15 Å thickness films were prepared by pasting the material on carbon tape and gold coated to improve its conductivity. pH is measured by using Flucka Digital pH meter using the procedure as reported by Kalra and Maynard [9]. Before utilizing it, it was calibrated with standard acidic (pH 4) and neutral (pH 7) buffer.

Results and Discussion

Activation of Silicate Material

Activation of waste glass involved the following steps: (i) removal of calcium oxide by using hydrochloric acid leaching; (ii) fluorination of silicon oxide; (iii) silicon fluoride then reacted with ammonium fluoride to form ammonium fluorosilicate; and (iv) defluorination of ammonium fluorosilicate using ammonia solution resulted in activated silica, and this hypothesis is supported by the previous study by [10].

\[
Ca^{2+} + H^+ + H_2O \rightarrow Ca(OH)_2
\]  
\[
SiO_2 + 4HF \rightarrow SiF_4
\]  
\[
SiF_4 + 2NH_4F \rightarrow (NH_4)_2SiF_6
\]  
\[
(NH_4)_2SiF_6 + 2NH_4OH + x H_2O \rightarrow 6NH_4F + SiO_2 . x H_2O + H_2
\]

The suitable calcination temperature was decided with the help of pH study at various temperatures in between 400-600°C (Table 1), it was found that at 600°C obtained pH was 5.21. So this temperature was taken as suitable calcination temperature for the origination of Bronsted site on the silica surface.

**Table 1. Relation of pH with calcination Temperature (Time 45 minutes)**

<table>
<thead>
<tr>
<th>Calcination Temperature (in °C)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>9.95</td>
</tr>
<tr>
<td>500</td>
<td>9.92</td>
</tr>
<tr>
<td>600</td>
<td>5.21</td>
</tr>
</tbody>
</table>

Changes in Silicate Matrix and Molecular Sieve Formation

X-ray diffraction study was used to elucidate the information regarding the change in the initial silicate matrix and molecular sieve formation (Fig.1). Considerable improvement in crystalline pattern was obtained after the aforesaid activation experiment as compared with initial silicate matrix proved the formation of molecular sieve (Fig 1 a, b); it was further conformed by its SEM micrographs (Fig 2).
Proceedings of the International Conference on Solid Waste 2011- Moving Towards Sustainable Resource Management, Hong Kong SAR, P.R. China, 2 – 6 May 2011

Figure 1. Scanning Electron Microscopic (SEM) pictures of (a) Untreated and (b) treated silicate

Figure 2. XRD Curves of (a) initial; and (b) activated silicate material from waste CRT

References

THE BEHAVIOR OF TETRACYCLINES AND CU DURING THE ANAEROBIC DIGESTION OF SWINE MANURE

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ABSTRACT As the most commonly used veterinary antibiotics and heavy metal for promoting animal growth, the potential threat to environment and public health posed by tetracyclines (TCs) and Cu are recently paid more and more attention. In this study, the behaviors of TCs including tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC) and their degradation products, as well as Cu and its five speciation, were studied and compared in batch swine manure anaerobic digestion with and without inoculum at 37 ± 1°C. Obviously, the performance of anaerobic digestion with inoculum on both biogas production and TCs’ removal was much better than that without inoculum. The degradation products of epi-tetracycline (ETC) and epi-oxytetracycline (EOTC) were detected in both experiments with and without inoculum, and the removal rates of 80.0%, 42.1% and 47.8% for CTC, OTC and TC, respectively, during swine manure anaerobic digestion with inoculum demonstrated that TCs could be removed to some degree, and inoculum was useful to promote their removals. The behavior of Cu transferring from liquid phase to solid phase occurred and Cu bonding to Fe/Mn oxides was transformed to organically bound Cu during the anaerobic digestion.

Keywords: Tetracyclines, Cu, Anaerobic digestion, Swine manure

Introduction
Veterinary antibiotics and some micronutrients such as Cu, Zn, As are widely used as feed additives in animal husbandry not only for therapeutic and prophylactic treatment, but also to promote animal growth and stimulate feed efficiency [1-4]. However, most of the excessive administered antibiotics and heavy metals are excreted via feces or urines due to incomplete absorption in animal tissues, and thus result in serious problems of environmental pollution and pose a rising risk of environmental safety and public health along with a large amount of animal manure produced as the accelerated development of Concentrated Animal Feeding Operations (CAFOs) in China. Tetracyclines (TCs) such as tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC) are among the most common antibiotics used in animal husbandry [1]. High tetracyclines residues in animal manure due to the wide usage and incomplete absorption can be detected in the level of mg/kg or even up to several hundreds of mg/kg [5-6]. As one of the imperative micronutrients, Cu is also widely used as additives in animal feeds [3, 7-8]. However, previous studies have shown that excessive dosages of feed additives may not be absorbed completely and subsequently excreted [4], leading to a high concentration of Cu remained in animal manure. The residual TCs and Cu in animal manure bring in an increasing potential risk with the application of manure as fertilizers in agricultural lands. Thus, it is necessary and important to treat and dispose animal manure before its land application.

Anaerobic digestion is considered as an effective and environmental-friendly alternative in animal manure treatment to achieve energy recovery and pollution control simultaneously. Although a number of researchers have investigated the fate of antibiotics in anaerobic environmental compartments, such as soil interstitial water [9-10] or anaerobic lagoon [11], little information is given regarding the fate of TCs [12-13] or Cu during the anaerobic digestion of animal manure.

Therefore, the purpose of our study is to investigate the behavior of both TCs and Cu during batch swine manure anaerobic digestion in order to optimize the operation of anaerobic digestion.

Materials and Methods

Batch Assay Methodology
Swine manure was collected from a large-scale pig farm located in Beijing, while the inoculum was obtained from the upflow anaerobic sludge blanket (UASB) reactor for treating pig slurry. The batch assay was carried out in a series of 250 mL conical flasks capped with grey butyl rubber stoppers. 150 mL of pig slurry and 50 mL inoculum were transferred to each reactor in the experimental assay while 200 mL of pig slurry with no inoculum addition was placed to the reactors as controlled. After sealing with silica gel, the headspaces were flushed with nitrogen gas to remove traces of oxygen. Then the reactors were incubated in a rotary shaker at 150 rpm, 37 ± 1°C. Duplicate reactors were created and sacrificed on days 0, 5, 10, 17, 24, respectively. The biogas production was recorded each day by water displacement.

**Analytical Methods**

pH, alkalinity, total and volatile suspended solids (TSS and VSS) were analyzed according to Determination Methods for Examination of Water and Wastewater [14]. Total and soluble chemical oxygen demand (TCOD and sCOD) were determined by Hach methods 8000 with a DR 2800 spectrometer. Short-chain volatile fatty acids and methane concentration were determined by a gas chromatography with flame ion detection (GC-FID). The slurry sample was centrifuged and filtered by 0.45 μm glass fiber filters. TCs and Cu in the filtrate were determined by UPLC-MS/MS and ICP-MS, respectively. Total Cu concentration and its speciation in residual solid were analyzed according to Tessier [15] and determined by flame atomic absorption spectrophotometry or ICP-MS depending on the contained concentration of Cu inside.

**Batch Assay Performance**

Figure 1. Variation in pH, Alkalinity, SS, VSS, Total biogas, CH₄, sCOD, COD and VFA during the anaerobic digestion
For both treatments, the initial pH was set at about 7.80 (Fig.1A). During the 24 days’ incubation, pH fell down at the very beginning and then increased. By comparison swine manure with and without inoculum, 0.31 pH decrease was observed within 5 days before it began to increase in the inoculum group while the controlled one had reduced as much as 1.0 pH by 10 days before it increased again. Such phenomenon of decreasing pH value suggested that insoluble organic matters were converted through hydrolysis reactions by fermentative or acid-forming bacteria. The less alkalinity may account for the lower and quicker decreased pH in the controlled group. The SS and VSS concentrations were in the range of 20-29 g/L and 11-18 g/L, respectively, and declined gradually over the duration of both experiments (Fig.1B). As presented in Fig.1C, TCOD decreased from 45167 mg/L to 30100 mg/L, reaching a removal rate of 33.4% in the inoculum group. However, the TCOD removal rate was only 9.2% in the control. sCOD and VFA soon arrived the maximum value before they declined at the end of the anaerobic digestion, and 23.8% and 1.5% of sCOD were reduced in the inoculum and control group, respectively. It was found that acetate and propionate were predominated among the total VFA in both experiments. Fig.1D indicated the pronounced variance in total biogas and CH4 of the experiments with and without inoculum. The total biogas in the inoculum group increased quickly to almost 870 mL while no gas was produced after 5 days’ incubation in the control. CH4 concentration in the inoculum group increased sharply to 34% and then maintained almost constant, whereas that in the control did not increase until when it ended. The above results suggested less methanogenic activity during swine manure anaerobic digestion without inoculum.

The Behavior of TCs During Swine Manure Anaerobic Digestion

The concentrations of TCs were in the level of μg/L, much lower than the previously reported level of mg/L in pig slurry [16-17], due to the fact that the determined concentrations in our study were in the slurry filtrate rather than the slurry itself. Except for OTC, all the parent TCs in the inoculum group were observed much lower than that in the control. The initial concentrations of CTC, OTC and TC in the filtrate (Fig.2) were 16.5 μg/L, 21.4 μg/L and 4.5 μg/L, and reduced by 80.0%, 42.1% and 47.8% respectively during the anaerobic digestion with inoculum, comparable with those obtained by Arikan [12-13], who reported approximately 59% and 75% removal of OTC and CTC during the anaerobic digestion of manure from medicated calves. A 57% reduction of CTC was also reported over the course of 216-day swine manure anaerobic digestion by Stone [17]. The removal rates of CTC, OTC and TC in the control reached 89.0%, 34.9% and 36.7% respectively, suggesting that inoculum was slightly useful to promote TCs’ removal during the anaerobic digestion. The degradation products of ETC and EOTC were detected in both experiments. The initial concentrations of ETC and EOTC were almost half of their parents and fluctuated slightly in the inoculum group, ranging between 1.6-2.8 μg/L and 6.3-16.7 μg/L, respectively, but limited OTC and EOTC were observed and ETC was in the range of 3.2-5.8 μg/L in the control.
The Behavior of Cu During Swine Manure Anaerobic Digestion

The total Cu concentrations (Fig.3a) in the residual solid calculated by dry weight (DW) increased from 1356 mg/kg and 952 mg/kg to 1920 mg/kg and 1403 mg/kg, respectively in the experiments with and without inoculum. Meanwhile, total Cu concentrations in the liquid phase, meaning that in the slurry filtrate, declined from 92.3 µg/L and 200.0 µg/L to 49.5 µg/L and 88.0 µg/L, respectively. Hence, it was inferred that the behavior of Cu transferring from liquid phase to the solid phase occurred in the course of anaerobic digestion.

![Figure 3. Total Cu and its speciation in the residual solid during anaerobic digestion with and without inoculum](image)

It was noticed that Cu speciation was predominantly existed in the form of combining with organics, in accordance with the reported findings in swine manure [18]. Over 83% of Cu was in the organically bound fraction, followed by around 10% of Cu combining with Fe-Mn oxides in swine manure anaerobic digestion with inoculum while Cu fractions binding to organics and Fe/Mn oxides in swine manure anaerobic digestion without inoculum were in the range of 70-89% and 7-25%, respectively. The other three fractions involving exchangeable, carbonates-bound and residual Cu constituted less than 10% and showed no obvious variance for all treatments during anaerobic digestion. However, an increase of organically bound Cu and a decrease of Fe/Mn oxides bounded Cu in concentrations were observed in Fig.3b and 3c, indicating that Cu binding to Fe/Mn oxides was transformed to organically bound Cu during anaerobic digestion.

**Conclusions**

The detected degradation products of both ETC and EOTC and a removal rate of 80.0%, 42.1% and 47.8% for CTC, OTC and TC respectively during swine manure anaerobic digestion with inoculum demonstrated that TCs could be removed and inoculum was useful to promote their removal. The behavior of Cu transferring from liquid phase to the solid phase occurred and Cu binding to Fe/Mn oxides was transformed to organically bound Cu during anaerobic digestion.

**Acknowledgements**

This work is financially supported by the National 863 Program of China (No. 2007AA06Z344) and National Natural Science Fund of China (No. 50578156 and No. 21077122).

**References**


DISPOSAL OF OBSOLETE PESTICIDES INCLUDING DDT IN CHINESE CEMENT PLANT AS A BLUEPRINT FOR FUTURE ENVIRONMENTALLY SOUND CO-PROCESSING OF HAZARDOUS WASTE INCLUDING POPS IN CEMENT INDUSTRY

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ABSTRACT In September 2009, 350 tons of Obsolete Pesticides (OP) including 160 tons of DDT (dichlorodiphenyltrichloroethane, a Persistent Organic Pollutant, POP) have been successfully disposed as a business case at the Huaxin Jinmao Cement Company in Suzhou, China. The cement production is in a New Suspension Pre-calciner (NSP) kiln with clinker capacity of 3'200 tons per day. Followed up a comprehensive trial burn the disposal was carried out to ensure reliable Destruction Removal Efficiency (DRE) of DDT and the emission level of Dioxins/Furans during the processing. In the trial, the actual DDT feeding rates ranged from 150kg/h, 500kg/h, and 1'000 kg/h to 2'000 kg/h at maximum. The results showed that a DRE of 99.99999% can be reached with a maximum 2 tons feeding rate of DDT of the highest concentration of 30%; the emission of Dioxins/Furans was well below 0.1 ng TEQ/Nm3.

Keywords: DDT, Cement kiln, Co-processing, DRE, PCDD/F

Introduction

Two main objectives of Sino-Germany Obsolete Pesticide Management Project were 1) to conduct the inventory mapping of obsolete pesticides in three pilot provinces, Jiangsu, Hubei and Jilin and 2) consequently to introduce BAT to dispose the wastes found in an environmental sound manner. In Jiangsu a total of 350 tons of OP had been found including 160 tons of DDT, which is one of the most difficult substances to be decomposed in nature. Based on technical assessment by the expertise and panel, and also global experience [1], Huaxin Jinmao Cement Company had been chosen to conduct the task for disposal in new suspension pre-heater and pre-calciner cement kiln with clinker production capacity 3,200t/d. For ensuring the DRE for DDT, a comprehensive trial burn plan had been prepared. The execution of the trial was done by the Company under supervision of Chinese Research Academy of Environmental Sciences.

A trial burn planned is consisting of a base line and trial burn tests. In this trial, DDT was fed into pre-calciner where gas temperature is about 1000°C and DDT feeding rate was adjusted from 0 kg/h as base line test, to 150kg/h, 500kg/h, 1'000kg/h and 2'000kg/h at maximum. The trial burn was carried out on September 11, 12, 13, 14, 15 of 2009 respectively. To get the parameters for DDT DRE, a sampling plan was scheduled and shown in Table.1.

To evaluate the environmental impact from cement kiln exhaust gas and abide by the national regulation, the exhausting gas had been sampled and the following items analyzed: PCDD/F, Dust, CO, CO2, SO2, NOX, Benzene, DDT, Cl, HCl, HF, NH3 and TVOC.

Materials and Methods

Clinker production process is shown in Figure.1.
Results and discussion:

For having a DRE, the results can be calculated according to the formula [2] shown as below

\[
\text{DRE} = \left(\frac{W_{\text{in}} - W_{\text{out}}}{W_{\text{in}}}\right) \times 100\%
\]

\[
W_{\text{in}} = M_{\text{i}} \times C_{\text{DDT,I}}
\]

\[
W_{\text{out}} = V_{\text{G}} \times C_{\text{DDT,G}}
\]

- \(W_{\text{in}}\): DDT input
- \(W_{\text{out}}\): DDT output
- \(M_{\text{i}}\): DDT input volume to cement kiln, t/h,
- \(C_{\text{DDT,I}}\): DDT concentration in DDT input,
- \(V_{\text{G}}\): Exhausting gas from cement kiln system, m³/h,
- \(C_{\text{DDT,G}}\): DDT concentration in exhausting gas,

All the data for DDT DRE calculation and results are shown in Table.2.
Table 2: DDT DRE parameters

<table>
<thead>
<tr>
<th>Sample number</th>
<th>DDT feeding rate (kg/h)</th>
<th>DDT conc. (%)</th>
<th>Gas DDT conc. (mg/m³)</th>
<th>Gas volume (m³/h)</th>
<th>Bag filter dust DDT (mg/kg)</th>
<th>DDT DRE (%)</th>
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<tbody>
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<td>-</td>
<td>-</td>
<td>UD</td>
<td>3.19×10⁵</td>
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<td>-</td>
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<td>-</td>
<td>UD</td>
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<td>UD</td>
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<td>6.9×10⁵</td>
<td>3.3×10⁵</td>
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<td>99.999996</td>
</tr>
<tr>
<td>091503</td>
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<td>6.3×10⁵</td>
<td>3.25×10³</td>
<td></td>
<td>99.999996</td>
</tr>
</tbody>
</table>

UD: Under Detection Limit; Detection Limit for DDT: 0.03μg/M³

Table 3: PCDD/F from exhaust gas

<table>
<thead>
<tr>
<th>Sample number</th>
<th>PCDD/F (ng I-TEQ/Nm³)</th>
</tr>
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<td>091101</td>
<td>0.012</td>
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<tr>
<td>091501</td>
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<tr>
<td>091502</td>
<td>0.013</td>
</tr>
<tr>
<td>091503</td>
<td>0.087</td>
</tr>
</tbody>
</table>

Conclusion:
From this DDT trial burn, the following conclusions can be summarized:

1) DDT can be highly decomposed with high feeding rate (up to 2000kg/h) in such a new technology cement kiln.
2) All the stack gas emissions can meet the national standards and are far below the limits
3) DDT can not be found in the product (clinker)
4) DDT can be detected in bag filter dust, but only if the feeding rate is as high as 2000kg/h
5) The bag filter dust is fed to the kiln again; hence, there is no environmental impact from this source
6) Chinese environmental authorities will be encouraged to adopt and to spread co-processing as a successful method to dispose Persistent Organic Pollutants in cement factories for an efficient implementation of the Stockholm Convention.

Acknowledgements:
This work was supported by the Sino-Germany Obsolete Pesticide Management Project (Project number: 09.2277.3-001.00).

Reference:
POTENTIAL APPLICATIONS OF THE SOLID WASTES GENERATED DURING JATROPHA BIODIESEL PRODUCTION

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ABSTRACT The oil extracted from the seeds of a non-food crop, Jatropha curcus, has been processed to convert into high quality biodiesel globally for over ten years. Today, this oilseed plant, originated in Central America, is widely cultivated in the tropical and subtropical regions because of its potential to become an important alternative energy crop for the production of biofuels to replace the non-renewable fossil fuels. Some of the apparent advantages of J. curcus over other oilseed plants are (1) its high oil content (up to 40%), (2) its broad adaptability and the presence of toxicity (no competition among other food crops for arable farmlands), as well as (3) the negative net carbon dioxide emissions and other promising environmental issues.

The common solid wastes generated during the production of biodiesel from J. curcus are the hull of the fruits, the shell of the seeds and the residual meal (seedcake) left after oil extraction of the seed kernels. Moreover, the crude glycerine, a co-product (about 10% by volume) separated from the refined Jatropha biodiesel is of limited value if not undergoing further purification.

In this study, the seedcake waste was converted into organic fertiliser in compliance with the British compost standard (PAS 100:2005) and China organic fertiliser standard (NY525-2002). In addition, the seedcake was also able to be processed into high quality powdered activated carbon. Besides, the co-product, crude glycerine, could be purified to over 95% with relatively simple procedures mainly involving ion exchange chromatography.

At present, the production cost of Jatropha biodiesel is still higher than that of petroleum diesel. Further incorporating the potential applications of the wastes in the production will certainly increase the competitiveness of Jatropha biodiesel in the fuel markets.

Keywords: Jatropha curcas, Biofuels, Jatropha biodiesel, Solid wastes
THE ROLE OF G*ALACTOMYCES* SP. Z3 IN IMPROVING PIG SLURRY BIOLEACHING


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* Corresponding author: E-mail address: lxzhou@njau.edu.cn Tel: +86 25 84395160

ABSTRACT
In this paper, we aim to isolate a slurry dissolved organic matter-degrader and investigate its role in improving the activities of A. ferrooxidans LX5 and A. thiooxidans TS6 in successive multi-batch bioleaching experiments. Results showed that the isolated heterotrophic microorganism Z3 was identified as Galactomyces sp., which could grow well from pH 3 to pH 7 and degrade pig slurry DOM from 1,973 mg/L to 942 mg/L within 48 hours. The removal efficiencies of Cu (>85%) and Zn (>94%) in the cooperative systems were higher than in the single systems. In addition, the bioleaching process could eliminate the pathogens in pig slurry with more than 99% of the elimination efficiencies for total coliforms (TC) and fecal coliforms (FC) and also completely remove the offensive odors released from pig slurry.

Keywords: Bioleaching, Pig slurry, Acidithiobacillus species, Heterotrophic microorganism, Heavy metals

Introduction
It is well known that manures were rich in N, P and organic matter. However, for economical purposes, some metal salts are added into the feed stuffs to stimulate the growth of pigs and control the pork quality in China and other countries, such as Cu, Zn, As, and Mn could be found in pig feeds [1]. After these manures are applied in agricultural soil, the accumulation of trace metals in plant may pose a health risk when humans or livestock consume them [2]. On the other hand, there are the offensive odors and the risk of disease spreading for land application of livestock slurry.

In the last decade, bioleaching has been developed as an attractive method to remove heavy metals from sludge, sediments and soils, not only as a scientific novelty but also for its efficiency, simplicity and cost effectiveness [3]. The bioleaching process is affected by the concentration of organic acids or dissolved organic matter (DOM) in sludge strongly inhibits bioleaching process. Furthermore, it was found that the introduction of acid-tolerant heterotrophic microorganism into sludge bioleaching system could readily degrade DOM or organic acid and shorten bioleaching time [4]. Therefore, the purposes of this study are to (1) isolate a new heterotrophic microorganism from bioleached pig slurry and investigate its role in degrading pig slurry DOM, (2) investigate the effect of the co-inoculation of the heterotrophic microorganism with two acidophilic thiobacillus on improving pig slurry bioleaching efficiency.

Materials and Methods
Pig Slurry Sample
The pig slurry used in the study was collected from the pig farm of Jiangpu, Nanjing, China. The selected physiochemical properties of the pig slurry were measured according to standard methods recommended by APHA [5], and which were given in Table1.

Isolation Slurry DOM-Degradar
Heterotrophic microorganisms capable of degrading pig slurry DOM were isolated from the bioleached pig slurry (pH=2.0) by plating method described by Wang et al. [6]. A strain with white and powdery to cottony colonies on PDA medium exhibited a strong ability of degrading slurry DOM and was considered as DOM-degrader (hereinafter named by Z3).

Acidithiobacillus spp and the Inoculum Preparation
A. ferrooxidans LX5 and A. thiooxidans TS6 obtained from China General Microbiological Culture Collection Center were cultivated in modified 9K and SM liquid medium, respectively. The inoculum was
prepared by mixing 15 mL of *A. ferrooxidans* and 15 mL of *A. thiooxidans* in 500 mL Erlenmeyer flasks containing 270 mL of pig slurry supplemented with 0.4% Fe$^{2+}$ and 0.2% of S$^{0}$. The flasks for inoculum preparation were agitated on a horizontal shaker at 28 °C and 180 rpm for 13 days until pH dropped to 2.0.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value (dry weight)</th>
<th>Parameters</th>
<th>Value (dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.11</td>
<td>Zn (mg kg$^{-1}$)</td>
<td>2710</td>
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<tr>
<td>Total solid (%)</td>
<td>3.0</td>
<td>Cu (mg kg$^{-1}$)</td>
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<td>Organic matter (%)</td>
<td>65.4</td>
<td>Cr (mg kg$^{-1}$)</td>
<td>10.4</td>
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<tr>
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<td>2.57</td>
<td>Mn (mg kg$^{-1}$)</td>
<td>510</td>
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<tr>
<td>Total P (%)</td>
<td>1.82</td>
<td>Ni (mg kg$^{-1}$)</td>
<td>18.7</td>
</tr>
<tr>
<td>Total K (%)</td>
<td>1.5</td>
<td>Pb (mg kg$^{-1}$)</td>
<td>10.7</td>
</tr>
</tbody>
</table>

**Table 1. Physicochemical characteristics of pig slurry**

Co-inoculation of Sludge DOM-degrader Z3 with *A. ferrooxidans* and *A. thiooxidans* on Pig Slurry Bioleaching

The bioleaching experiment was conducted in 500 mL Erlenmeyer flasks, each containing 150 mL of fresh pig slurry and 150 mL pre-acidified pig slurry as inoculum mentioned in 2.3. 0.4% of Fe$^{2+}$, 0.2% of S$^{0}$, and 10 loops of viable strain Z3 scraped from the PDA plates were added for the above flasks. The control was also performed but without the addition of strain Z3. The flasks were incubated in a gyratory shaker at 28 °C and 180 rpm. During the incubation, 10 mL of slurry samples were withdrawn from the flasks at 12 h intervals and determined for pH, subsequently, these samples were centrifuged at 12 000 rpm for 15 min, filtered through 0.45 μm membrane filter, determined for Zn, Cu solubilization efficiency using ICP-AES. When the pH in slurry dropped to 2.0, which is regarded as the termination of each batch bioleaching, 150 mL acidified bioleached slurry was withdrawn and added to new flasks containing 150 mL of fresh pig slurry, 0.6 g Fe$^{2+}$ (0.4%) and 0.3 g S$^{0}$ (0.2%). Then these new flasks were incubated again in a gyratory shaker at 28 °C and 180 rpm. The recycle of acidified bioleached slurry were performed for successive four batches of bioleaching experiments. The total coliforms (TC) and fecal coliforms (FC) were measured before and after bioleaching in the first batch reaction systems by the methods of multiple tube fermentation method [7].

**Results and Discussion**

**Identification of Strain Z3 and its Optimum pH for Growth and the Ability of Degrading Pig Slurry DOM**

The feature of the strain Z3 was cultured on the PDA plate for 2 days, and unicell morphology was shown in Fig. 1a. It was identified as *Galactomyces* species (hereinafter called as *Galactomyces* sp. Z3) based on morphology feature. As shown in Fig. 1b, *Galactomyces* sp. Z3 could grow well between 2.5 and 7.0. When *Galactomyces* sp. Z3 was inoculated in the medium containing DOC derived from pig slurry and incubated for 48 h, DOC in the medium declined from 1 973 mg L$^{-1}$ to 942 mg L$^{-1}$.

**Solubilization of Cu and Zn during the Consecutive Multi-batch Bioleaching Processes**

Remove efficiencies of pig slurry-borne Cu and Zn by microbial leaching during the consecutive multi-batch bioleaching processes were shown in Fig. 2. Zn solubilization was 51% for the uninoculated treatment in the first batch trial, however, Cu solubilization was hardly observed. The Zn solubilization efficiency was also higher than Cu in the inoculated treatments. The solubilization efficiency of Zn exceeded 94% at the end of the bioleaching process, while the solubilization efficiency of Cu was just about 85%. The Cu and Zn solubilization in inoculated treatments was different in the first and the third batch trial. For instance, 84% of Cu and 98% of Zn solubilization efficiencies for the cooperative system (the treatment simultaneously inoculated with *Acidithiobacillus* species and *Galactomyces* sp. Z3) were achieved in day 4 while only 68% and 84% for the single system (The treatment only inoculated *Acidithiobacillus* species)
was observed at the same time in the first batch trial. Likewise, 90% of Cu and 97% of Zn in pig slurry were dissolved in day 14 for the cooperative system while 84% of Cu and 90% of Zn for the single system in the third batch trial.

Figure 1. Colonies and unicell morphology of *Galactomyces* sp. Z3 (10×100), and its growth in different pH

The Variation of Total Coliforms (TC) and Fecal Coliforms (FC) Before and After Bioleaching

After bioleaching, both TC and FC in pig slurry greatly decreased with compared to those in fresh pig slurry. The amounts of TC declined from the $3.0 \times 10^7$ cells g$^{-1}$ before bioleaching to the $1.6 \times 10^3$ cells g$^{-1}$ after bioleaching, and the amounts of FC also decreased from the $1.7 \times 10^7$ cells g$^{-1}$ to the $2.0 \times 10^2$ cells g$^{-1}$ after bioleaching, which was below the limited value of safe land application of livestock manure proposed by China (GB-18596-2001).

Conclusions

The co-inoculation of *Galactomyces* sp. Z3 and two acidophilic thiobacillus in the successive multi-batch bioleaching systems could improve pig slurry bioleaching efficiency, as exhibiting that the removal efficiency of Cu and Zn and oxidation rate of Fe$^{2+}$ and S$^0$ were higher than those in the single system. The removal efficiencies of pig slurry-borne Zn and Cu were more than 94% and 85%, respectively. Moreover, the elimination efficiencies of TC and FC exceeded 99%. The offensive odor could also be removed. Obviously, bioleaching was a feasible technology for the removal of heavy metals and elimination of pathogens from pig slurry.

Figure 2. Cu and Zn solubilization during the successive multi-batch bioleaching process. Pig slurry of un inoculated treatment (■); pig slurry with co-inoculation of *Galactomyces* sp. Z3 and *Acidithiobacillus* species (△); pig slurry with inoculation of only *Acidithiobacillus* species (control) (○)
Acknowledgments

This work was Supported by the National High-Tech Research and Development Program of China (863 Program) (2009AA06Z317).

References

REMEDIATION OF LEAD FROM CONTAMINATED SEDIMENTS USING ELECTROCOAGULATION PROCESSES

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ABSTRACT The electrocoagulation (EC) was introduced to eliminate lead (Pb) from contaminated sediment slurry. The ionisation of coagulant and Pb involving the electro-flocculation are key factors that can stimulate the kinetic rate of EC. The aluminium ions (Al) could be more quickly generated than ferrous ions (Fe). Al ions could rapidly stabilise Pb and form a dense white floc that could sink down. After treating, the effluent contained low concentration of Pb but the Al ions were remained with very high concentration. The high concentration of Al coagulant might perform a charge neutralisation and sweep coagulation with Pb ions. On the other hand, EC with Fe electrode was limited with lack of Fe and Pb ions. Ferrous ion could be immediately oxidised to ferric ions, the rust floculants was presented in the system. The EC process using aluminum electrode with 20 mA of DC current applying is optimum condition for removing Pb from contaminated sediment and wastewater.

Keywords: Aluminium electrode, Electrocoagulation, Flocculation, Lead, Sediments

Introduction

The EC can be effectively applied to eliminate many refractory constituents in high strength wastewater 0. The electrochemical reaction can precipitate heavy metals and generate the highly stable oxide sludge [2]. So, EC is an effective tool for remediating contaminants in both of suspended, colloidal and dissolved particles. Lead can pose a serious health problem in the western region of Thailand [3]. Treating of Pb by chemical precipitation can generate chemical sludge, heat and toxic gases [4].

Materials and Methods

Lead contaminated soil and sediments were collected from agricultural area around the mine. The physical and chemical properties of samples were examined in accordance with the ASTM standards. Sediment slurry was prepared by mixing 3% of sediments into deionised water and it was stirred by magnetic stirrer until achieving the equilibrium of Pb hydrolysis. The metal concentrations including lead, aluminium and iron were analysed according to ICP-OES (Method 200.7) and US EPA guidelines. For particulate samples, they were digested by microwave acid digestion in accordance with the method for extraction of the metals from soil (EPA method 3052). The borosilicate beaker with a working capacity of 1.0 L was used as the EC reactor as shown in Figure 1. The portion of lead presented in sediments was determined using sequential extraction according to Tessier’s method.
Results and Discussions

Sediment Properties

The sediment was composted of sand. It contained high concentration of lead, which was bounded with organic matter. The organic matter could be a source of minerals that could react with lead.

Optimum Condition of Electrocoagulation for the Remediation

The equilibrium time of Pb hydrolysis is 50 minutes as presented in Figure 2. The SS was enough for the mechanism of adsorption-destabilisation. The EC with aluminium electrodes was undertaken under a various voltage level, as presented in Figure 3. The electrodes could generate Al$^{3+}$ and electrons. Electrons could enhance Pb ionisation in a very short period. The higher electrical current could much encourage the ionisation of coagulant and Pb. The ionic Al and Pb could immediately form the white-floc that could sink down. The Pb concentration was lower than the maximum allowable concentration of Pb in drinking water (0.01 mg/L). The aluminium ion remained in effluent was higher than 0.1 mg/L, which could induce the risk on Alzheimer’s disease [6]. Figure 4 presents the EC with mild steel electrodes.

![Equilibrium Time = 50 min](attachment:equilibrium_time.png)

**Figure 2. Equilibrium time of Pb hydrolysis**

![Al and Pb concentration in effluent grabbed from EC with Al electrode](attachment:al_pb_concentration.png)

**Figure 3. (a) Al and (b) Pb concentration in effluent grabbed from EC with Al electrode**
Figure 4. (a) Al and (b) Pb concentration in effluent grabbed from EC with Fe electrode

The interaction between Fe$^{2+}$ and Pb might be sweep coagulation, as small portions of Fe and Pb ions were governed. The rust coloured effluent was observed due to the oxidation of Fe$^{2+}$ to Fe$^{3+}$. The Fe$^{2+}$ remained in effluent was at very low concentration of 0.02 mg/L, which was less than the maximum allowable concentration (0.3 mg/L). After 90 minutes of reaction, Pb could be released from the sludge. This suggested that Pb might not be stabilised. The kinetic reaction rate constants were calculated to describe the influence of electrical current on coagulant generating and EC reactions. The reaction between Al and Pb ions was fast enough to be well fitted with 2nd order kinetic reaction equation. The kinetic rate constants for system with 10, 20 and 30 mA applying were 0.37, 0.99 and 1.26 L/mg-min, respectively. The high current density could stimulate the cationic hydrolysis products of aluminium to further react with OH$^{-}$ ions that was Al(OH)$_{3(0)}$ [7]. The system with Fe electrodes was fitted with 1st order kinetic reaction equation. The kinetic rates of Pb ionisation were 0.04, 0.05 and 0.06 min$^{-1}$ for systems with 10, 20 and 30 mA current applied, respectively. The kinetic rates of Fe ionisation were 0.06 and 0.07 and 0.08 min$^{-1}$ for systems with 10, 20 and 30 mA current applied, respectively. This revealed that the ionisation of Fe and Pb were in competitive condition. The EC was inhibited as lack of Fe and Pb ions.

Stability of Pb in Sludge

The sequential extraction result is given in Table 1. Lead in natural condition was predominantly bounded on carbonate. The EC could activate the ionic Pb to be bounded with ionic coagulant as residue.

Table 8. Chemical forms of Pb in treated and untreated samples

<table>
<thead>
<tr>
<th>Chemical form (%)</th>
<th>Untreated</th>
<th>30mA$^{-}$</th>
<th>20mA$^{-}$</th>
<th>10mA$^{-}$</th>
<th>30mA$^{-}$</th>
<th>20mA$^{-}$</th>
<th>10mA$^{-}$</th>
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<td>0.62</td>
<td>0.36</td>
<td>0.40</td>
<td>0.43</td>
<td>0.09</td>
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<td>0.14</td>
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<td>26.38</td>
<td>24.92</td>
<td>34.44</td>
<td>19.79</td>
<td>26.75</td>
<td>36.64</td>
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<tr>
<td>Bound-to-Fe &amp; Mn oxides</td>
<td>17.86</td>
<td>8.61</td>
<td>10.63</td>
<td>10.90</td>
<td>12.09</td>
<td>10.72</td>
<td>8.61</td>
</tr>
<tr>
<td>Bound-to-organic matter</td>
<td>3.31</td>
<td>1.95</td>
<td>2.37</td>
<td>2.26</td>
<td>2.18</td>
<td>2.39</td>
<td>2.18</td>
</tr>
<tr>
<td>Residual</td>
<td>8.22</td>
<td>62.70</td>
<td>61.68</td>
<td>51.97</td>
<td>65.85</td>
<td>59.93</td>
<td>52.43</td>
</tr>
</tbody>
</table>
Conclusions

The EC with Al electrodes can more effectively treat Pb than Fe, since; the reaction between Al and Pb ions was faster than the ionisation of Fe and Pb. The treated water from using aluminum electrodes is very clear but the treated water from system with steel electrodes is yellow-green that result from insoluble rust.

Acknowledgements

Laboratory facilities are provided by Department of Environmental Engineering, Faculty of Engineering King Mongkut's University of Technology Thonburi. Funding are sponsored by NCE-EHWM and NRU project-Computational Modeling for Environmental systems and Application, KMUTT.

References

INFLUENCE OF PH AND EC ON SETTLING VELOCITY OF LEAD CONTAMINATED SEDIMENTS

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ABSTRACT The release of heavy metals from sediments has relied upon the environment factors, mainly pH, specific electrical conductivity (EC). The lead contaminated sediments are classified as discrete river sand particles with 20,250 mg/kg lead content. The settling velocity of the sand particles is calculated by using Stroke’s Law. EC and pH factors can influence the stability of particle by increasing roughness and charge gain/loss on surface of particles. The settling columns are used to provide data of the vertically settling velocity of particles. Sediments can sink very quickly when pH of system is at neutral, but the settling velocity is increased in acidic condition and decreased when pH of systems was alkaline. Besides, the settling velocity was high when no NaCl present in the solution and decreased with 1 and 2% NaCl added.

Keywords: Modified Strokes equation, Lead, Movement, Sediments, Settling velocity

Introduction

Under natural conditions, heavy metals tended to be accumulated onto sediments [1]. The environmental factors, particularly pH and specific EC could influence on releasing of heavy metals after settling of sediment [2]. The settling velocity of particles could be estimated using general law for settling as given in Equation 1 [3].

\[ V_c = \left( \frac{2g V}{\rho_s - \rho} \right)^{1/2} \]  

where \( V_c \) is settling velocity [m/s], \( g \) is acceleration due to gravity [kg-m/s²], \( \rho_s \) and \( \rho \) are density of particle and fluid, respectively [kg/m³]. \( V \) is volume of particle [m³], \( A \) is cross sectional area of particle [m²] and \( C_D \) is drag coefficient [\(-\)]. When \( Re \ll 1 \), \( C_D \) is yielded as \( C_D \approx 24 / \sqrt{Re} \). Where \( u \), \( D \) and \( \nu \) are velocity [m/s], spherical diameter [m] and kinematic viscosity [m²/s]. The Stoke’s law is derived as \( V_c = (\rho_s - \rho)gD^2 / 18\mu \).

However, the particles are naturally coarse and not spheres. The Dietrich’s settling curve can be applied to the case of particles with a rough surface. The equation for estimating the drag coefficient (\( C_D \)) is provided as follows.

\[ C_D = \frac{1}{3} \left( \alpha + \sqrt{\alpha^2 + 16\beta / Re} \right) \]  

where \( V_c^{*} \), \( S \), are dimensionless settling velocity [m/s] and fluid sediment parameter [4]. \( \alpha \) and \( \beta \) are constants and \( S \) is specific gravity [\(-\)]. \( \mu \) is fluid viscosity [kg/s-m] and \( \nu = \mu / \rho \). Variables are defined as \( Re = Vc^* \nu = 4Vc^*S \), \( 1 = \alpha + \beta / S \), \( S = \frac{D}{4\nu} \left( \frac{S - 1}{S + 1} \right) gD \) and \( Vc^* = \begin{cases} S / 4.5 & S < 1 \\ 1.83 & 150 < S < 4 \times 10^4 \end{cases} \).

For non sphere particles, the Corey shape factor (csf) is applied. The equations are given as [5]:

\[ V_c = \frac{MV}{ND} \left[ \frac{1}{4} \left( \frac{4N}{3M^2} \right) \right]^{1/n} \]  

For non sphere particles, the Corey shape factor (csf) is applied. The equations are given as [5]:
where $M$, $N$ and $n$ are the statistical curve fitting values of a various types of sediments; $M = 53.5 \exp(-0.65csf)$, $N = 5.65 \exp(-2.5csf)$, $n = 0.7 + 0.9csf$. $D_*$ is dimensionless function of particle diameter [$\left[\frac{D_*/D_w - 1}{\sqrt{v'}}\right]^{1/3}$]. The Cory shape function is defined as $c/\sqrt{ab}$. $a$, $b$ and $c$ are the lengths of longest, intermediate and shortest side of particles.

The reaction occurred between sediments and solvent are estimated using the complicating factor. The settling velocity is determined as follows [6].

$$V'_C = V_C (1 - C_S)^{m}$$

(4)

where $V'_C$ is actual settling velocity, $C_S$ is the sediments content (%w/w) and $m$ is empirical function of particle diameter and container diameter $(= 4.65 + 19.5D/D_w)$. $D_w$ is diameter of container.

Materials and Methods

The settling column was fabricated from acrylic tube with an inner diameter of 4.3 cm and height of 35 cm. The sediment slurry was prepared with a concentration of 3% (w/w). The solvents used are including of deionised water, 3.6% HCl (pH=4) or 1.2% NaCl (pH=9) and 1 and 2% (w/w) NaCl solution. The Suspended Solids (SS) and Pb content in sediments and effluent were analysed in accordance with standard methods given by ASTM (1997) and APHA (2005), respectively.

Results and Discussion

Sediment sample utilised in this study was collected from mining area, it contained 10% of organic matter, 4.2% silt and 6.73% of clay. The pH (1:5) of sediment was 7.31 and the specific electrical conductivity (EC) was 33.58 µS/cm. This contaminated sediment contained 20,250 mg Pb/kg. The settling velocity data is presented in Figure 1. The sediments soaked with deionised water, the settling velocity can be well match with Storkes Law and Dietrich settling curve with csf at 0.7 indicating that the particles were relatively round and the surface of particle was slightly rough. In case of sediment slurry with 3.6% HCl, the acid could digest some organic matter contained in silt and clay particles. The size of particles became smaller than the original ones. The actual settling velocity was lower than the estimated using Stokes Law. The csf was 0.34 that was relatively reduced as a result of fragile particles. In case of sediments settled into 1.2% NaOH, the csf was 0.58 and the size of particles became bigger than the original ones. The settling velocity was also increased from the control test with sediments-deionised water slurry. The influence of electrical conductivity on settling velocity was proved with the test of sediments soaked with 1 and 2% NaCl solution, the csf values were 0.57 and 0.59, respectively. The higher settling velocity was observed in the system with lower NaCl concentration. The sediments could disperse due to electromotive forces of NaCl ions.

The Pb could highly leach from sediment when the acid solution was applied. On the other hand the Pb releasing was reduced in alkaline solution. The loss of Pb in saline solution was not much different from the one observed in system with deionised water. The loss of Pb could affect to sediment properties, resulting in fluctuation of settling velocity. These findings based on the fact that the release and dispersal of Pb contaminated sediments in a various pH and EC solution, these could affect the settling of sediments [7].
Figure 1 Simulations and observations of settling velocities of sediments soaked with (a) deionised water (b) 3.6% HCl (c) 1.2% of NaOH (d) 1% NaCl and (e) 2% NaCl solutions

Conclusions

pH and EC could affect the settling velocity of sediment and solubility of Pb. The sediments soaked with deionised water, Storkes and Dietrich settling curve could generate the high accuracy on predicting of
sediment settling velocity as there was no reaction between sediments and deionised water. The change of pH and EC of solution could make the different chemical properties of sediments. The Pb loss and settling velocity of sediments were decreased in alkaline solution. The Pb releasing and settling velocities of sediments were increased in acid solution. The Pb does not directly react with NaCl, but the ions of Na⁺ could be adhered on surface of sediments, resulting in suspension due to electromotive force.

Acknowledgements

Laboratory facilities are provided by the Department of Environmental Engineering, Faculty of Engineering, King Mongkut’s University of Technology Thonburi. Funding is partially supported by Nation Research University (NRU) project-Computational Modelling for Environmental Systems and Applications.

References


LEACHABILITY OF ANTIMONY AND ARSENIC FROM MINE TAILINGS AROUND AN ANTIMONY MINE, CHINA

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ABSTRACT Laboratory experiments of the mine residues from Xikuangshan antimony mine of Hunan Province, China showed that with increasing amounts of antimony (Sb) and arsenic (As) in the materials, increasing amounts of Sb and As were leached and went into solution. And the amounts released into solution in anaerobic conditions were comparable with that in aerobic water. Residue weathering by rain of Xikuangshan area is the cause of mine seepage or drainage, which would deteriorate ground water quality. Underground water and waste water from the mine showed high levels of antimony and arsenic. Both Sb(III) and As(III) were found in the waters.

Keywords: Antimony, Mine tailing, Waste water, Ground water, Leachate

Introduction

Mine tailings produced from the mining activities are the major solid wastes in China and are of the environmental concern due to potential hazards of surface or groundwater pollution. The Xikuangshan antimony (Sb) mine, which is located at the west of Hunan province, China, is one of the world largest Sb mines. Exploitation during several periods from 1897 to present has resulted in huge amount of solid wastes. Stibnite (Sb₂S₃) is the only ore mineral here [1]. Trace amounts of pyrite and pyrrhotite occur in the ore along with the mesothermal stibinite, which contains arsenic in solid solution from tens to thousands of mg/kg [2]. Sb and As would released into the environment when the stibnite, and commonly associated arsenopyrite and arsenian pyrite dissolves.

This paper investigated the distribution and speciation of Sb and As in underground water and waste water produced in the mining activities. Environmental mobilities of Sb and As in the mine residues were also examined.

Materials and Methods

Three surface tailings sands (T1, T2 and T3) were collected randomly at 0-20 cm of tailing reservoirs and one arsenic-alkali residue samples (T4) was collected from antimony smelter; six ground water (U1-U6) and five waste water samples (W1-W5) from the pit near the tailings reservoirs were also collected in 2008. The total organic carbon (TOC) content was determined using an elemental analysis (Vario EL, Elementar, Germany) and NO₃⁻, SO₄²⁻ and Cl⁻ were determined by Ion Chromatography (DX-600). Laboratory experiments were designed to investigate the amount of antimony likely to dissolve from the four solid samples. Coarse solids (sieved to >1mm) were placed into two sets of low density polyethylene (LDPE) plastic jars (50 ml capacity) with screw lids. One set of the coarse stibnite-containing tubes (with five grams of solids) were used for aerobic leaching (1, 4, 7, 10, 14, 20 and 28 days, 25°C). The other set of tubes (with two grams of solids) were used for anaerobic leaching (1, 7, 14 and 28 days, 25°C). Sb species were then analyzed by Hydride Generation-Atomic Fluorescence Spectrometer (AFS-230, Beijing Haiguang Company).
Results and Discussion

Leaching of Sb and As from the Mine Tailings

The three surface tailing samples and one arsenic-alkali residue samples contain 3226, 1778, 1117, 9630 mg/kg Sb, and 349, 263, 283 and 18298 mg/kg As in solid solution, respectively. Leachates from the tailings showed a little variation in pH during the course of leaching, e.g., pH in leachates from the arsenic-alkali residue ranged from 11.1-11.8, with only minor fluctuations occurring. With increasing amounts of Sb and As in the mine tailings, increasing amount of Sb and As were leached and went into solution.

Aerobic experiments released up to 680 mg/kg and 193 mg/kg Sb from the tailing sand 2 and 3 in the first 10 days, and then the rate released subsequently decreased for the two tailing sands. The arsenic-alkali-residue released an almost identical amount of Sb under the aerobic conditions, up to 129.7 mg/kg in the first 14 days. Tailing sand 1 (T1) initially released similar amount of Sb to the tailing sand 3 (T3), but the increase in amount released over time was less for T1 than for T3. Tailing sand 2 from the old mine tailing sand dam would dissolve higher levels of Sb than those from other dams and the one arsenic-alkali-residue. There is no clear relationship between pH and dissolved Sb. In the waste water from the pit near the tailing reservoirs, Sb was present in the form of Sb(III) and Sb(V) species. The release of Sb(III) in the two redox conditions was also observed during laboratory experiments. Sb (III) increased with time both in aerobic and anaerobic experiments (Figure 2). The release of Sb(V) always exceeded that of Sb(III) after days of leaching period.

The three tailing sands released less As than Sb under aerobic conditions, with less than 74.3 mg/kg As going into solution. The amount of dissolved As in the four materials increased with time both in aerobic and anaerobic conditions. The arsenic-alkali-residue released over 23.5 mg/kg As initially under aerobic conditions, and the amount increased with time to almost 440 mg/kg. Similar as Sb(III), As(III) also take a large portion in the total dissolved As concentrations (Figure 3). (III) released from the three tailing sands in the aerobic experiments was much lower than in the anaerobic conditions. However, As(III) released from the arsenic-alkali-residue in the aerobic experiments was much higher than in the anaerobic conditions.

The liberated Sb and As were environmentally significant on a time scale of days. Our experiments suggested that Sb and As were readily leached by water from the tailing sands and arsenic-alkali-residue on the short time scale. The elevated amount of Sb released in the short time experiments is in accord with observations of Wilson et al. [3] who examined environmental dissolution of smelter residues from a historic antimony smelter site in New Zealand, and found Sb and As to be highly mobile locally.

Antimony and Arsenic in Waste Water and Underground Water

The leaching experiments suggest that Sb and As are readily released into water on this short time scale. The mine residues were impounded in dams or piles in Xikuangshan area. Residue weathering by rain here is the cause of mine seepage or drainage, which would deteriorate ground water quality. The waste and underground water samples differed in their pH values. It varied from 6.5 to 8.8 in underground water; W1 and W2 were two leaching adits of arsenic-alkali-residue heaps from the South mine, in which the pH values were as high as 11.1 and 12.4, respectively. Sb solubility in underground waters would not alter severely by the almost neutral pH values since only low or high pH values will increase the solubility of the element [4]. Sulphate concentrations in underground water and waste water ranged from 62.5 to 376.4 mg/L and 395.3 to 4093 mg/L, respectively. The higher sulphate contents at the underground water and waste water reflected the lithology of the mine area, which might be linked to the oxidation of stibnite and arsenopyrite from the stratum or tailing dams. Probable reactions are [2, 5]:

\[ \text{Sb}_2\text{S}_3 (\text{stibnitt e}) + 3\text{H}_2\text{O} + 6\text{O}_2 = 3\text{SO}_4^{-2} + 2\text{Sb}_2\text{O}_3 (\text{senarmontite}) + 6\text{H}^+ \] (1)

\[ \text{FeAsS} (\text{arsenopyrite}) + 7\text{H}_2\text{O} = \text{Fe}^{2+} + \text{H}_3\text{AsO}_4 (aq) + 11\text{H}^+ + 11\text{e}^{-} + 2\text{SO}_4^{-2} \] (2)
Table 1. Results of experimental mobilization of Sb and As from tailing sands and arsenic-alkali-residue (mg/kg)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Time (days)</th>
<th>Tailing 1</th>
<th></th>
<th></th>
<th>Tailing 2</th>
<th></th>
<th></th>
<th>Tailing 3</th>
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<tr>
<td></td>
<td>Sb</td>
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<td>153.3</td>
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</table>

Figure 2. Experimental dissolution of Sb(III) in tailing sands and arsenic-alkali-residue in aerobic and anaerobic conditions

Figure 3. Experimental dissolution of As(III) in tailing sands and arsenic-alkali-residue in aerobic and anaerobic conditions
If carbonate is present in the system, an alkaline carbonate solution may neutralize the hydrogen ion [6]. Xikuangshan antimony mine is carbonate type deposit, which occurs in mineralised veins with carbonate sequence. The acid generated in equation (1) and (2) is balanced by acid consumption and the pH in waste water and underground water remains at near neutral. The overall oxidation could be presented as:

\[
Sb_2S_3(s) + 3H_2O + 6O_2 + 6CO_2^- = 3SO_4^{2-} + 2Sb_2O_3(senarmontite) + 6HCO_3^- \tag{3}
\]

\[
FeAsS(arsenopyrite) + 7H_2O + 11CO_2^- = Fe^{2+} + H_3AsO_4(aq) + 11HCO_3^- + 11e^- + SO_4^{2-} \tag{4}
\]

Underground water U2 near the North mine showed high levels of arsenic. As(III) and total As in the sample were as high as 3.60 and 10.6 mg/L, the other underground water samples had lower As levels. Sb concentrations in U1 to U5 exceeded China drinking water limits (5 µg/L). The minimum dissolved Sb value (4.60 µg/L) in underground waters was found in U6, which was located at the Lengshuijiang City and was a little far from the mine area compared with the other sites. The two seepage adits (W2 and W3) from arsenic-alkali-residue heaps in the South mine contained up to 31.9 mg/L Sb and 690 mg/L As. The other waste waters contained much lower Sb and As concentrations. Waste water from the tailing sand dam (W1) contained 5.22 mg/L Sb and 1.54 mg/L As, beneficiation waste water (W1) contained 1.79 mg/L Sb and 2.07 mg/L As, and mine water (W5) from the South mine contained 5.51 mg/L Sb and 3.32 mg/L As.

| Table 2. pH, concentrations of SO_4^{2-} (mg/l), Cl^(-) (mg/l), NO_3^(-) (mg/l), Sb(µg/l) and As(µg/l) in underground water and waste water |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Samples | Sb(III) | Sb_{tot} | As(III) | As_{tot} | pH | Cl^(-) | NO_3^(-) | SO_4^{2-} | TOC |
| U1    | 33.3   | 60.2   | n.d.    | 3.12    | 8.3 | 15.5  | 18.3  | 152.7  | 14.5 |
| U2    | 85.4   | 342.8  | 3606    | 10565   | 8.8 | 3.22  | 12.7  | 146.2  | 13.4 |
| U3    | 592.1  | 2975   | 4.69    | 10.69   | 6.5 | 11.8  | 13.9  | 376.4  | 11.9 |
| U4    | 1.42   | 5.87   | n.d.    | 2.62    | 7.1 | 1.52  | 5.10  | 62.5   | 7.81 |
| U5    | 0.00   | 48.9   | 2.36    | 4.32    | 7.8 | 8.56  | 19.9  | 63.8   | 12.9 |
| U6    | 0.77   | 4.60   | 1.67    | 2.80    | 6.7 | 64.5  | 31.3  | 142.8  | 15.5 |
| W1    | 2215   | 5215   | 572     | 1545    | 6.5 | 10.5  | 15.5  | 455.1  | 28.0 |
| W2    | 7467   | 13561  | 156643  | 450675  | 11.1 | 531.0 | 46.9  | 409.3  | 518 |
| W3    | 11616  | 31885  | 321461  | 690979  | 12.4 | 657.5 | 104.4 | 2607   | 1306 |
| W4    | 502.7  | 1785   | 280     | 2074    | 6.8 | 17.1  | 8.77  | 446.3  | 43.9 |
| W5    | 478.9  | 5511   | 1483    | 3317    | 5.7 | 11.9  | 14.7  | 395.3  | 23.9 |

Conclusions
Mobilization of Sb and As is readily to occur in mine residues in Xikuangshan area. Historical disposal of Sb-As-mineralised mine waste has released Sb and As into water body. Antimony and arsenic concentrations were up to 31.9 and 690 mg/L in seepage water of arsenic-alkali-residue. Dissolved antimony and arsenic in underground water exceeded 2.98 and 105 mg/L, respectively.

Acknowledgements
This work was supported by the Nonprofit Environment Protection Specific Project (201009037-06) and National Natural Science Foundation of China (40873077, 20777009).

References


THE BIO-OXIDATION OF ENERGY SUBSTANCES ACCELERATED BY INTERACTION OF A.THI OOXIDANS AND A.FERROXIDANS DURING SEWAGE SLUDGE BIOLEACHING AND OPTIMUM PH VALUE FOR DEHYDRATION OF BIOLEACHED SLUDGE


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ABSTRACT This study was conducted to evaluate the behaviors of the bio-oxidation of energy substances during the bioleaching process through flask-shaking experiments with co-inoculation of two Acidophilic thiobacillus (A.thiooxidansTS6 and A.ferrooxidans LX5) and the addition of energy substrates (Fe2+ and S0) at a different ratio. In the experiments with the addition of a fixed 2g/L of S0 but various amount of Fe2+, the sulfur oxidation rate were calculated as 8.3%, 51.8%, 49.1%, 46.1%, and 35.9% for the treatments with the addition of 0g/L, 1g/L, 2g/L, 3g/L and 4g/L Fe2+, respectively. In another experiment with the addition of various S0 but a fixed 2g/L of Fe2+, the Fe2+ oxidation percentage in 66h are 64.9% for the treatment with addition of 1g/L S0 and 100% for these with addition of ≥2g/L S0. Moreover, it is found that there is an optimum pH ~2.4 for highest dewater ability for bioleached sludge.

Keywords: Sewage sludge, Bioleaching; Fe2+; S0, Bio-oxidation, Dewaterability

Introduction
At present, there are more than 2,000 municipal wastewater treatment plants working in China. The amount of treated wastewater exceeds 100,000,000 M3/d, which generates about 7,000,000 dry tons of sewage sludge each year. Moreover, large amounts of municipal sewage sludge are expected to be generated in the next decade due to the increasing number of wastewater treatment facilities being constructed. The treatment of municipal sewage sludge using bioleaching approach has been extensively studied in laboratory scale [1-5]. Acidithiobacillus ferrooxidans (A.ferrooxidans) and Acidithiobacillus thiooxidans (A. thiooxidans) have been employed as the most significant microorganisms involved in bioleaching processes. We found in previous study that the co-addition of Fe2+ and S0 in municipal sewage sludge bioleaching involving in A. ferrooxidans could accelerate pH decrease and enhance metal removal efficiency. However, the bio-oxidation rates of ferrous ion and element sulfur during sludge bioleaching with co-inoculation of A. thiooxidansTS6 and A.ferrooxidans LX5 and the addition of Fe2+ and S0 at different ratio have never been studied. The previous finding showed that bioleaching will improve sludge dewater ability more than 4–10 times than the control sludge without any pretreatment [6-7]. Therefore, the objectives of the present study were 1): to investigate the oxidation rates of ferrous ion and element sulfur during sludge bioleaching with co-inoculation of A. thiooxidansTS6 and A.ferrooxidans LX5 and the addition of Fe2+ and S0 at different ratio, and 2): to explore optimal pH value for sludge dewatering during the bioleaching process.

Materials and Methods

Municipal sewage sludge sampling
The municipal sewage sludge used in this work was obtained from the first wastewater treatment plant in high-tech industrial park, Suzhou city, Jiangsu province, China.

Bioleaching Experiments

Changes of sulfur or ferrous oxidation rate with the addition of different concentration of Fe2+ or S0: A series of 15 mL of the newly prepared inoculum was added to 250-ml Erlenmeyer flasks containing 135ml fresh municipal sewage sludge followed by addition of (1) 2 g/L S0 and 0 g/L Fe2+; (2) 2 g/L S0 and 1 g/L Fe2+; (3) 2 g/L S0 and 2 g/L Fe2+; (4) 2 g/L S0 and 3 g/L Fe2+; (5) 2 g/L S0 and 4 g/L Fe2+ in the experiment.
series 1; (6) 2 g/L Fe^{2+} and 0 g/L S_0; (7) 2 g/L Fe^{2+} and 1 g/L S_0; (8) 2 g/L Fe^{2+} and 2 g/L S_0; (9) 2 g/L Fe^{2+} and 3 g/L S_0; (10) 2 g/L Fe^{2+} and 4 g/L S_0 in the experiment series 2. All treatments were done in triplicate. The flasks were shaken at 28°C and 180 r/min for bioleaching. During bioleaching process, the pH of sludge was periodically monitored and 8 ml sludge samples were withdrawn from each of the Erlenmeyer flasks and centrifuged at 12000 r/min for 15 min to separate solid from liquid fraction. The liquid fraction was filtered through 0.45 µm membrane filter, acidified with HCl to about pH 2.0 and then stored at 4°C prior to determination of Fe^{2+}, Fe^{3+}, total Fe and SO_4^{2-} concentration.

Optimum pH value for municipal sludge dewatering during bioleaching:

Batch experiments were conducted through shaking 250-ml Erlenmeyer flasks containing 150 ml of fresh municipal sewage sludge inoculated with bio-acidified bioleached sludge at 28°C and 180 r/min. 30 ml of acidified bioleached sludge (pH=2.0, collected from the above experiments with the addition of 2 g/L Fe^{2+} and 2 g/L S_0) was added into the flask containing 120 ml of the fresh municipal sewage sludge followed by the addition of 2 g/L Fe^{2+} and 2 g/L S_0. As described above, pH of sludge was periodically determined and 10 ml sludge samples were withdrawn from each flask for the determination of sludge CST.

Results and Discussion

Bio-oxidation of Fe^{2+} during Bioleaching

It is noted in Fig. 1a that bio-oxidation of Fe^{2+} in all treatments is finished in 96 hours and the oxidation of Fe^{2+} coupled with Fe^{3+} generation.

Fe^{3+} concentration in liquid fraction for different treatments with the addition of 0 g/L, 1 g/L, 2 g/L, 3 g/L, and 4 g/L Fe^{2+} gradually increased from initial 18 mg/L, 110 mg/L, 138 mg/L, 193 mg/L and 230 mg/L to 63 mg/L, 147 mg/L, 628 mg/L, 1130 mg/L and 1593 mg/L after 144 h for bioleaching, respectively. In experiment series 2, the variation of Fe^{2+}, Fe^{3+} and total Fe concentrations in liquid fraction of municipal sludge during bioleaching with the addition of 2 g/L Fe^{2+} and 0–4 g/L S_0 are given in Fig. 1b. It is found that Fe^{2+} concentration in the treatment with addition of 2 g/L Fe^{2+} and 0 g/L S_0 declines from 553 mg/L to 278 mg/L in the first 18 hours and the ferrous oxidation rate just increases to 67% after 96h. Moreover, ferrous...
oxidation rate could be accelerated by added S\(^0\) as energy substance in the bioleaching systems, as indicating in Fig. 1b that ferrous oxidation rates in 66 h are 64.9% and 100% with addition of 1 g/L S\(^0\) and \(\geq 2\) g/L S\(^0\), respectively.

**Bio-oxidation of S\(^0\) during Bioleaching**

It is found in Fig. 2a. After 24 h for reaction, the sulfate concentrations gradually increase until the end of bioleaching for all but the treatment with 2 g/L S\(^0\) and 0 g/L Fe\(^{2+}\). For example, sulfate concentration in bioleaching systems increases from initial 1124 mg/L, 2823 mg/L, 4375 mg/L, 6099 mg/L and 7641 mg/L to final 1624 mg/L, 5933 mg/L, 7321 mg/L, 8864 mg/L, and 9794 mg/L for the treatments with 2 g/L S\(^0\) + 0 g/L Fe\(^{2+}\), 2 g/L S\(^0\) + 1 g/L Fe\(^{2+}\), 2 g/L S\(^0\) + 2 g/L Fe\(^{2+}\), 2 g/L S\(^0\) + 3 g/L Fe\(^{2+}\), and 2 g/L S\(^0\) + 4 g/L Fe\(^{2+}\), respectively. Correspondingly, sulfur oxidation percentages are calculated as 8.3%, 51.8%, 49.1%, 46.1%, and 35.9% in the above-mentioned treatments. Obviously, the elemental sulfur oxidation can be increased from 8.3% for the treatment only with 2 g/L S\(^0\) to about 50% when 1–2 g/L Fe\(^{2+}\) as well as 2 g/L S\(^0\) is added into fresh sludge as energy substance before bioleaching.

**Figure 2. Variation of SO\(_4^{2-}\) during bioleaching with different ratio of Fe\(^{2+}\) to S\(^0\)**

![Figure 2](image.png)

As shown in Fig. 3, in the first batch, with the decline of pH from 5.3 to 1.9, sludge CST is decreased from initial 28.4 s to 14.6 s at 40 h, and then unexpectedly increased to 30.0 s at 84 h. The lowest point of CST is 14.6 s, in which corresponding pH value is around 2.7. Likewise, in the second batch, the lowest point of CST is 11.0 s, and corresponding pH value is around 2.4 (see Fig. 3). This indicates that there is an optimum pH (2.4–2.7) for highest dewater ability for bioleached sludge.

**Optimum pH Value for Dewatering of Municipal Sewage Sludge during Bioleaching**

As shown in Fig. 3, in the first batch, with the decline of pH from 5.3 to 1.9, sludge CST is decreased from initial 28.4 s to 14.6 s at 40 h, and then unexpectedly increased to 30.0 s at 84 h. The lowest point of CST is 14.6 s, in which corresponding pH value is around 2.7. Likewise, in the second batch, the lowest point of CST is 11.0 s, and corresponding pH value is around 2.4 (see Fig. 3). This indicates that there is an optimum pH (2.4–2.7) for highest dewater ability for bioleached sludge.
Conclusions

The ferrous bio-oxidation rate could be accelerated by added $S^0$ as energy substance in bioleaching system, and the elemental sulfur oxidation can be increased by added $Fe^{2+}$ as energy substance in sludge bioleaching system. The bio-oxidation rates of $Fe^{2+}$ reaches 100% simultaneously with the addition of $\geq 2$ g/L $S^0$. Sulfur oxidation rate can be reached to 50%. pH ~2.4 was the optimum end point for bioleaching.

Acknowledgements

This work described in this paper was supported by the National High-Tech Research and Development Program of China (863 Program) (2009AA06Z317) and the National Natural Science Foundation of China (20977098).

References

BIODEGRADATION OF OPnEO: POTENTIAL ENDOCRINE DISRUPTORS FROM TANNERY SEWAGE

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ABSTRACT Octylphenol polyethoxylate (OPnEO) is a potential endocrine-disrupting chemical and biodegradation provides a cheap and effective means to its removal. Therefore the aim of the present study was to investigate the use of the bacterium Aeromonas sp. TXBc10 isolated from activated sludge of a tannery waste treatment plant of Hunan Province, China for the biodegradation of OPnEO. The microorganism was capable of utilizing OPnEO as the sole source of carbon and energy while OPnEO was degraded under aerobic batch culture conditions. Microbial degradation of OPnEO was studied in batch experiments for several environmental factors. The effect of initial OPnEO concentrations on the degradation was investigated between 100 and 1000 mg·L⁻¹, and the results showed that the biodegradation process conformed to the first-order kinetic model. The optimum pH, temperature, and salinity for OPnEO degradation by Aeromonas sp. TXBc10 were found to be 8.0, 30 degrees C, and 25 per thousand (w/w), respectively. The process of OPnEO biodegradation was monitored by reversed-phase high-performance liquid chromatography with ultra-violet detection after extraction. The major metabolites of OPnEO degradation were identified as short-chain octylphenol polyethoxylate without octylphenol by liquid chromatography-mass spectrometry.

Keywords: Octylphenol polyethoxylate, Degrading strain, Isolation, Degradation characteristics

Introduction

Octylphenol polyethoxylate (OPnEO) surfactants are used in numerous commercial and industrial (e.g. leather industry) products [1]. Large amounts of such surfactants and their various residual biodegradation by-products are ultimately released into the environment. Thus, there is much interest in the environmental fate of the OPnEO surfactant metabolites, because they can mimic natural hormones and thus have the potential to act as endocrine disrupters in aquatic organisms, wildlife and even humans [2-4]. Evidence suggests that a lifetime of exposure to ovarian estrogens may be a principal risk factor in the development of breast cancer. Similarly, excessive exposure during in utero development results in irreversible alterations in the structure and function of the female genital tract [5-6].

As a result, concerns over the toxicity of alkylphenol ethoxylates (APEOs) and their biodegradation products led to a voluntary ban in Europe on the use of APEOs in household cleaning products, however, industrial use continues in Europe [7]. Also, some political bodies have begun to implement risk management strategies for EDCs. For example, the European Union and some individual European nations have banned or are phasing out the use of some endocrine disruptor-related chemicals such as nonylphenol polyethoxylate surfactants and certain phthalate esters [8]. In Switzerland, NP and A9PEO products have been banned since 2006, and since August 2008 OP and OPEO have been banned as well [9].

These compounds are ubiquitous and persistent in various environmental media and biota. Thus, they remain a cause for concern. Recently, many studies on OPnEO mainly focused on the isolation and identification of OPnEO degrading strains [10-11] and determination of OPnEO [12-14]. All the isolated bacteria accumulate octylphenol triethoxylate (OP3EO), octylphenol diethoxylate (OP2EO) and octylphenol (OP) in culture medium [10, 15-17]. However, complete mineralization of surfactants is accomplished by mixed cultures of microorganisms suggested Ginkel [18].

The objectives of this investigation were to isolate microorganisms capable of degrading OPnEO, characterize the biochemical degradation pathway, and elucidate the environmental factors influencing the degradation process.
Materials and methods

Chemicals

Reagents. 4-octylphenol polyethoxylates glycol (OPnEO, purity >99%) with an average ethoxylation degree of 9.5 was purchased from Sigma-Aldrich (Missouri, USA). HPLC-grade methanol from TEDIA Co (USA). All other reagents were of analytical-reagent grade.

Medium. Mineral salt medium (MSM, mg·L⁻¹): NaCl (2.5), (NH₄)₂SO₄ (2.0), MgSO₄·7H₂O (0.3), CaCl₂ (0.01), Na₃HPO₄ (3.7), KH₂PO₄ (0.9), Vitamin [19] (2 mL/L), Trace element [19] (1 mL/L).

Enrichment Culture and Culture Medium

The initial enrichment culture was established by inoculating a 250 mL Erlenmeyer flask containing 100 mL mineral salts medium (MSM) supplemented with OPnEO (100 mg·L⁻¹) as the sole carbon and energy source with 5 g fresh sewage from tannery waste treatment plant of Hunan Province, China. The pH of the culture medium was adjusted with HCl or NaOH to 7.0 ± 0.1. The flasks were incubated in a ZHWY-2102 Incubator Shaker operating at 150 rpm and 30.0 ± 0.5 °C. The OPnEO-degrading cultures were obtained through enrichment transfer at approximately 5-days intervals on the basis of depletion of OPnEO, by transferring 2.0 mL of the active culture to a new Erlenmeyer flask containing 100 mL of freshly made MSM with gradually increasing concentrations of OPnEO (100-1000 mg·L⁻¹). The OPnEO-degrading enrichment cultures were transferred more than 10 times prior to the isolation of bacteria from the enrichment cultures.

Isolation of Microorganism

Bacteria in the enrichment culture showing ability in degrading OPnEO were diluted in MSM before plating on the nutrient agar (NA) plates. After 48 h of incubation at 30 °C, a number of well-separated individual colonies of different morphological types appeared and were further streaked onto fresh NA plates to purify the cultured organisms. Pure cultures were used subsequently for identification using DNA Sequencing method as described by Mergaert et al. [20].

Biodegradation of OPnEO

All flasks were incubated at 30 °C on a rotary shaker operated at 150 rpm. Experiments on OPnEO degradation were conducted in 250 mL Erlenmeyer flasks with 50 mL MSM. All tests were conducted in triplicate. Sterile controls were prepared by autoclaving before addition of OPnEO, which passed through a membrane filter 0.45 μm pore size. After 5 d of incubation, each of the culture medium (0.5 mL) was extracted with an equal volume of methanol and then analyzed by HPLC with a ultra-violet detector or liquid chromatography-mass spectrometry (LC-MS).

Analysis of OPnEO and Its Metabolites

The Agilent 1100/1200 series HPLC and an Agilent ZORBAX Eclipse XDB-C18 Column (150 × 4.6 mm, particle size 5 μm) set at 280 nm, was used for the quantification of OPnEO concentration. The mobile phase was a methanol: water mixture (90:10, v/v) and the flow rate was 1 mL·min⁻¹. Under these chromatographic conditions, baseline separation could be obtained within 12 min for OPnEO and its metabolites. All compounds studied were quantified using external standards.

OPnEO degradation metabolites were identified using an Agilent 1100 Series LC/MSD (Agilent, USA). The column used was a VP-ODS C18 (4.6 mm × 15 cm 5 μm). Gradient elution consisted of a solution of 75:25 (v/v) methanol: water for 3.5 min, followed by an increase in methanol to 95% over 10 min. The injection volume was 10 μL and the flow-rate was 0.5 mL·min⁻¹ for 10 min. Precursor ion was [M+NH₄]⁺ for OPnEOs in positive ion mode. Ionization in the ESI source was achieved using nitrogen as a nebulizer and drying gas. The nebulizing gas pressures were fixed 40 psi. The collision induced dissociation (CID) voltage was set to 100 eV.
Results and discussion

**Biodegradation of OPnEO by Aeromonas sp. TXBc10**

One bacterium capable of utilizing OPnEO as sole source of carbon and energy was isolated from the culture medium and was identified as *Aeromonas* sp. with 99% similarity. OPnEO was degraded quickly during the enrichment process without an apparent lag period at the beginning of degradation.

In order to determine the effect of initial concentrations of OPnEO on degrading efficiency, *Aeromonas* sp. was inoculated and cultured at a range of OPnEO concentrations. During incubation, OPnEO was utilized for microbial growth and cell maintenance. The biodegradation of OPnEO by *Aeromonas* sp. at initial concentrations of 100-1000 mg·L⁻¹ is shown in Fig. 1. The OPnEO biodegradation by *Aeromonas* sp. is assumed to fit to the Monod first-order kinetic equation, which has the following forms: lnC = -Kt + C₀ (1); t₁/₂ = ln2/k (2)

![Figure 1](image1.png)

![Figure 2](image2.png)

![Figure 3](image3.png)

Where C is the concentration of OPnEO at time t; t is time; K is the first-order rate constant; C₀ is a constant and t₁/₂ is the half-life period of the OPnEO biodegradation by *Aeromonas* sp.

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The kinetic equations for the OPnEO biodegradation by *Aeromonas* sp. are listed in Table 1. The relationship between the different initial substrate concentrations and the degradation rates was linear (R² > 0.95). As substrate concentrations increased from 100 to 1000 mg·L⁻¹ in the culture, the half-life value also showed an increase from approx. 4.60 to 6.27 h, respectively.

**Effects of pH**

The hydrogen ion concentration in the culture medium greatly influences the bacterial growth since pH value limits activity of enzymes. Degradation of OPnEO was particularly sensitive to low pH [21-22]. Accumulation of degradative intermediates may inhibit the further bacterial degradation of intermediates. The relationship between the degradation rate and pH for *Aeromonas* sp. is shown in Fig. 2. The rate of OPnEO degradation increased quickly when pH value of the culture was increased from 5.0 to 8.0. A high degradation rate was achieved for *A*. sp. TXBc10 at pH 8.0. The pH selected for degradation by *Aeromonas* sp. for subsequent experiments was pH 8.0.
Effects of Temperature

When the effect of temperature on OPnEO degradation by *Aeromonas* sp. TXBc10 was assessed (Fig. 3), the degradation rate increased with the increase of temperature between 15 °C and 30 °C. Further increases in temperature resulted in the lowering of degradation rate; hence the optimum was 30 °C, at which the degradative enzyme reached the highest activity for the temperature tested in this study.

Effects of Salinity

The effect of salinity gradients on the degradation of OPnEO by *Aeromonas* sp. TXBc10 was shown in Fig. 4. Salinity studies showed that *Aeromonas* sp. TXBc10 adapted a wide range (2.5-10%) of salinity. This meant the identified strain *Aeromonas* sp. TXBc10 was salt tolerant. Salinity concentrations were changed from 2.5-10% (v/v) for strain TXBc10, keeping other growth parameters constant. Samples were withdrawn at regular time intervals and analyzed for growth by recording absorbance value at 550 nm. Maximum biomass yield was taken as criterion to find the optimum value. A salinity concentration of 2.5% (w/w) was found to be optimum for strain TXBc10 and the activity of *Aeromonas* sp. TXBc10 decreased as the salinity of the medium increased (2.5-10 %, Fig. 4).

Identification of OPnEO Degradation Metabolites of by Aeromonas sp. TXBc10

OPnEO was rapidly transformed in the culture medium inoculated with *Aeromonas* sp. TXBc10 when OPnEO served as the sole source of carbon and energy. A representative LC-MS chromatogram of the culture filtrate from OPnEO degradation experiment is presented in Figs. 5 and 6. In addition to the parent compound peak of OPnEO, short chains OPnEO were observed. Main compounds in OPnEO degradation culture by *Aeromonas* sp. TXBc10 were identified as OP5EO, OP6EO, OP7EO and OP8EO. The result obtained by LC-MS was consistent with that by HPLC, confirming the identity of these degradation intermediates.

Metabolic Pathway of OPnEO Degradation by Aeromonas sp. TXBc10

Decreasing concentrations of OPnEO, transient appearance of short chain OPnEO (OP3EO, OP4EO, OP5EO, OP6EO and OP7EO) intermediates and decrease of initial OP9EO and OP10EO were observed over 120 h. However, unlike previous reports [1, 23], OP and OP2EO that possess intrinsic estrogenic activity were not observed. Meanwhile, acetaldehyde and acetic acid were not among the intermediates. The results indicated that the strain TXBc10 is of most importance towards OPnEO degradation because no OP was produced. Based on the above results, a combination of TXBc10 and other effective degrading bacteria may lead the complete mineralization of OPnEO [18].

Conclusions

The results obtained in the present study showed that OPnEO could be rapidly degraded by *Aeromonas* sp.TXBc10 isolated from tannery sewage. The optimum pH for the degradation was 8.0 and *Aeromonas* sp.TXBc10 grew vigorously under the temperature of 30 °C with a salinity of 2.5% (w/w). The biodegradation kinetics could be described using a first-order kinetic model. Degradation of OPnEO by
Aeromonas sp. TXBe10 proceeded short chains OPnEO without OP and OP2EO. The results suggest that OPnEO can be degraded by natural bacteria indigenous to the environment.

Acknowledgments

We gratefully acknowledge Dr Huang Lu, Hunan Chemical Industry Research Institute, for supervision of LC-MS analysis. This research was supported by Graduate Innovation Fund of Hunan Province (CX2010B299) and Natural Science Foundation of Hunan Province, China. NO. 09JJ4014.

References


FATE OF ANTIBIOTIC RESISTANCE GENES AND THE CHANGES IN BACTERIAL DIVERSITY DURING COMPOSTING OF SWINE MANURE

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ABSTRACT This study investigated the copy numbers of the antibiotic resistance genes (ARGs) as well as the distribution of antibiotic-resistant microorganisms during swine manure composting with the aim to assess the persistence of these ARGs. Non-antibiotic treated swine manure was composted as a control; while two other treatments were prepared by spiking swine manure with high or low concentrations of tetracycline, sulfadiazine and ciprofloxacin. The real-time PCR (RT-PCR) was applied to quantify the bacterial 16S rDNA gene to determine the total bacteria (cultivable and non-cultivable), and the ARGs for tetracyclines and sulfonamides. The ratio of the copy numbers of ARG to total 16S rDNA was calculated to reveal the distribution profile of the corresponding ARG carrying bacteria. PCR-DGGE (denaturing gradient gel electrophoresis) was performed to reveal the bacterial diversity during composting. The bacterial population was apparently affected by the addition of antibiotics in the beginning stage (day 1 and 3); while such pronounced effect was not observed afterwards.

Keywords: Composting, Swine manure, Antibiotic resistance, Real-time PCR, DGGE

Introduction

Recently, the potential adverse effects of the extensive use and disposal of veterinary antibiotics on the environmental health have been continually reported resulting in the increasing public awareness during the past decade (Kümmerer et al, 2003). Since only 50-60% veterinary antibiotics can be degraded by animals, the non-metabolized antibiotics are excreted into environment, causing serious environmental problems and jeopardizing human health.

As a promising bioremediation technology, composting has been applied to remove antibiotics from livestock and poultry manure. It has been reported that the efficiency of composting on the removal of antibiotics was inconsistent and sensitive to the types and concentrations of antibiotics, since the antibiotics present may cause effects on both microbial quantity and diversity in a composting process, thus result in distinct removal consequence.

Antibiotic resistant genes existed in highly concentrated microbial community may be transmitted among bacteria, and could indicate the spreading of the antibiotic-resistant bacteria. We initiated this study with the aim to answer the questions of whether and how antibiotics affect the microbial distribution, and what is the fate of antibiotic resistance genes during swine manure composting process. Real-time PCR was used to quantify the copy number of tetracycline, sulfadiazine as well as ciprofloxacin resistant genes in the swine manure compost samples with or without antibiotics treatment, and PCR-DGGE was performed to elucidate the microbial community changes upon antibiotics addition.

Materials and methods

Reactor and Operation

Swine manure was collected from a local swine farm, spiked with sulfadiazine (SDZ), chlortetracycline (CTC) and ciprofloxacin (CIP), mixed with saw dust (1:1 on dry weight basis) to adjust the C/N ratio and moisture content to about 29 and 60%, respectively, and then composted in a bench-scale 20 L computer controlled composter for 56 days. The aeration was provided at a flow rate of 1 L kg⁻¹ dry weight of swine manure min⁻¹. All treatments received 7 kg of the mixture of swine manure and saw dust. Non-antibiotic treated swine manure was composted as a control; while two other treatments were prepared by spiking swine manure with high (100 mg kg⁻¹ CTC +, 20 mg kg⁻¹ SDZ + 20 mg kg⁻¹ CIP on dry weight basis) or low (10 mg kg⁻¹ CTC + 2 mg kg⁻¹ SDZ + 2 mg kg⁻¹ CIP) concentrations of antibiotics.

Total Genomic DNA Isolation
Swine manure compost samples were collected directly from the control and antibiotic-treated composters at days 0, 1, 3, 7, 14, 21, 28, 42 and 56. 200mg of each collected sample was subjected to total DNA extraction by QIAamp DNA stool Mini kit (Cat.# 51504, Qiagen) according to the manufacturer’s protocol. The purified total genomic DNA was stored at -20 °C, and used as templates for the subsequent polymerase chain reactions.

**PCR Amplification**

Total bacterial 16S rDNA, tetracycline resistant genes (tetW, tetQ, tetC, tetG, tetY and tetZ) and sulfadiazine resistance genes (sul1, sul1, drfA1 and drfA7) were amplified from the total genomic DNA isolated from compost samples by PCR, using the primer pairs listed in Table 1. The 2×PCR reaction kit was used and the reactions were performed on a Thermo Cycler with the following reaction conditions: 95 °C for 5 min, followed by 34 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The PCR products were analyzed by electrophoresis on 2 % (w/v) agarose gel with 100 bp DNA ladder (Promega) to confirm the size and the approximate quantity of the amplicons.

**Plasmid Construction**

The PCR products of the V3 region of the total bacterial 16S rDNA and the individual antibiotic resistant genes were purified by Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281, Promega), and then applied for the subsequent cloning to the pGEM-T vector (Promega). The ligation reactions were performed according to the manufacturer’s instructions, and 2 μl of each ligation product was transformed into E. coli DH5α competent cells. Plasmid DNA of selected transformants was purified by PureYield Plasmid Miniprep System (Cat. # A1222, Promega). The purified plasmids were subjected to Nanodrop for concentration identification before applied to the subsequent RT-PCR.

**Real-time PCR**

The initial plasmid DNA for 16S rDNA as well as ARGs was serially 10 fold diluted for making standard curve. A master mix for each primer set was prepared such that each well contained the following: 2× SYBR Green Master Mix (Applied Biosystems) that contained all the nucleotides, polymerase, reaction buffer and SYBR green dye; forward and reverse primers; and nuclease-free water to a total of 49μl. 1μl of each sample or standard was added to the master mix, followed by gentle pipetting. The resultant mixture was then applied to thermal cycler. Real time quantitative PCR was performed using the Applied Biosystem, with fluorescence detection of SYBR green dye. Amplification consisted of an initial hold for 10 min at 95 °C, followed by 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min. A melting curve temperature profile was obtained by programming the Light cycler at 95°C for 0 s, 55°C for 60 s, and 95°C for 0 s.

**PCR-DGGE**

The total DNA isolated at different time points of the compost samples was normalized to identical concentration and was subsequently used as template to amplify the total bacterial 16S rDNA as described by Muyzer et al., 1993, using primer pair GC341F and V3-RV (Table 1). The PCR products, 50μl, were separated in a vertical denaturing gradient gel at 60 °C. The polyacrylamide gel (6%) with gradients of 40%~75% denaturants (where 100% denaturants contains 7M urea and 40% formamide) and a running time of 14h at 75V were selected, as these conditions optimally separated a maximal number of bands. After electrophoresis, gels were stained with SYBR gold, and photographed under ultraviolet (UV) light. The distinct bands were excised from the gel, and crushed into sterilized water. The DNA liberated into the water was used as template to perform PCR for the amplification of the DGGE band, using primer pair: 357F-GC-M13R and 518R-AT-M13F (Table 1). The PCR amplicons were sequenced with standard M13 sequencing primers.
Table 1. Synthetic oligonucleotides used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>5’→3’ sequence</th>
<th>Purpose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>V3-FW</td>
<td>CCTACGGAGGCGACG</td>
<td>Amplification of bacterial 16S rDNA V3 region</td>
<td>[2]</td>
</tr>
<tr>
<td>V3-RV</td>
<td>ATTACGGCGGCTGCTGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TetW-FW</td>
<td>GAGAGCTGTATATGCCAGC</td>
<td>Amplification of tetracycline resistant gene (tetW)</td>
<td>[3]</td>
</tr>
<tr>
<td>TetW-RV</td>
<td>GGGCGCTGCCGCCATTATGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TetQ-FW</td>
<td>AGAATCTGCTGTTTGCCAGTG</td>
<td>Amplification of tetracycline resistant gene (tetQ)</td>
<td>[3]</td>
</tr>
<tr>
<td>TetQ-RV</td>
<td>CCAGGTGCTAATGATATTGCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tet-C-FW</td>
<td>GCCGCTATGCCTCCATTCCG</td>
<td>Amplification of tetracycline resistant gene (tetC)</td>
<td>[3]</td>
</tr>
<tr>
<td>Tet-C-RV</td>
<td>GCGTATAGGATCCACAGGACG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TetG-FW</td>
<td>GCAAGACAGTGTGCTGCT</td>
<td>Amplification of tetracycline resistant gene (tetG)</td>
<td>[3]</td>
</tr>
<tr>
<td>TetG-RV</td>
<td>CCGGACTGCGGAGCGAAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TetY-FW</td>
<td>ATTTGTACCGGCAGGACAAAC</td>
<td>Amplification of tetracycline resistant gene (tetY)</td>
<td>[3]</td>
</tr>
<tr>
<td>TetY-RV</td>
<td>GCAGAGTGTGCTGCTGG</td>
<td></td>
<td></td>
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<tr>
<td>TetZ-FW</td>
<td>CCTTCTGGACAGCTTGG</td>
<td>Amplification of tetracycline resistant gene (tetZ)</td>
<td>[3]</td>
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<tr>
<td>TetZ-RV</td>
<td>ACCACAGCGTCTCCGTTC</td>
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<td></td>
</tr>
<tr>
<td>Sul1-FW</td>
<td>CGGCGTGGCGCTACCTGAACG</td>
<td>Amplification of sulfadiazine resistant gene (sul1)</td>
<td>[3]</td>
</tr>
<tr>
<td>Sul1-RV</td>
<td>GCCGCTAAGGCTATCCTGAAACG</td>
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</tr>
<tr>
<td>Sul2-FW</td>
<td>GGCCTCAAGGCGAGATGGCAT</td>
<td>Amplification of sulfadiazine resistant gene (sul2)</td>
<td>[4]</td>
</tr>
<tr>
<td>Sul2-RV</td>
<td>GCCGATCGCTCCTATGGCGGCACC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dfrA1-RV</td>
<td>TTTCGACAAAGATACCGACGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dfrA7-FW</td>
<td>AAAATGGCGTAATCGGTAATG</td>
<td>Amplification of sulfadiazine resistant gene (dfrA7)</td>
<td>[5]</td>
</tr>
<tr>
<td>dfrA7-RV</td>
<td>GTGAACAGTGAACAAATGAAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC341F</td>
<td>GGGCAGCGGGGGGTACCGGAGGCGCAG</td>
<td>GC-clamp addition for 16S rDNA V3 region</td>
<td></td>
</tr>
<tr>
<td>35F-GC-M1</td>
<td>CAGGAAAAACAGCTATGACGGGGCGGGGCGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3R</td>
<td>GCACGGGGGGCTTACCGGAGGCGCAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>518R-AT-M1</td>
<td>GTAAAAAACAGGCCCACTAATAAATAAAATAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3F</td>
<td>ATGTAATGAAAATTACCACGCGGCTGCTGG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results and discussion

Quantification of the Total Bacteria

Standard curve obtained by plotting copy number of pGEM-16S rDNA versus threshold cycles, allowed the absolute quantification of total bacterial 16S rDNA, which was used to estimate the overall bacterial abundance. With an initial high in copy number of 16S rDNA in all treatments, the copy number of 16S rDNA decreased sharply until day 3, and then rose slightly to a peak at day 7, and declined gradually thereafter (Figure 1). It has been well characterized that, at the initial phase of the composting process, pH decreases due to the accumulation of VFAs (volatile fatty acids) accompanied by a temperature increase, known as the “thermoacidophilic stage”. The sudden change of the living environment might prohibit the growth of a variety of the sensitive microbes, while selectively enrich the tolerant ones that could adapt fast and survive along the composting conditions, thus resulted in the sharp decrease followed by a reviving in terms of the total population profile. The gradual decline since day 14 could be due to a decrease of the available nutrient contents owing to microbial consumption.

Figure 1 Total bacterial population represented by 16S rDNA copy number. PM+SD: pig manure mixed with saw dust at time zero; C: control; L: treated with low concentration of antibiotics; H: treated with high concentration of antibiotics. Error bars indicate the standard deviation.
Quantification of the Tetracycline and Sulfadiazine Resistance Genes

Normal PCR was performed to preliminarily screen the existence of 13 of the tetracycline as well as 6 of the sulfadiazine resistance genes (tetW, tetT, tetS, tetQ, tetO, tetM, tetC, tetE, tetG, tetH, tetY, tetZ and tetB/P; sul1, sul2, sul3, drfA1, drfA2 and drfA7) for all the genomic DNA samples isolated during the composting process. Tetracycline resistance genes of tetW, tetQ, tetC, tetG, tetY and tetZ, as well as all of the tested sulfadiazine resistance genes except sul3 and drfA7 were positively selected (data not shown) and subjected to real-time PCR.

The absolute copy number of each ARG was quantified by referring to the individual standard curve fabricated by plotting copy number of the constructed ARG-carrying pGEM-T plasmid versus threshold cycles. The ratio of the copy numbers of ARG to total 16S rDNA was then calculated to normalize the resistant genes to the bacterial population, thus revealing the distribution profile of the corresponding ARG carrying bacteria. As shown in Figure 2, the individual ARG carrying bacteria accumulated during composting in a time-point dependent manner, and none of the tested ARG was detected after 28 days. The

Figure 2. Real-time PCR for quantification of tetracycline as well as sulfadiazine resistance genes. The symbols were presented as in Fig.1

The absolute copy number of each ARG was quantified by referring to the individual standard curve fabricated by plotting copy number of the constructed ARG-carrying pGEM-T plasmid versus threshold cycles. The ratio of the copy numbers of ARG to total 16S rDNA was then calculated to normalize the resistant genes to the bacterial population, thus revealing the distribution profile of the corresponding ARG carrying bacteria. As shown in Figure 2, the individual ARG carrying bacteria accumulated during composting in a time-point dependent manner, and none of the tested ARG was detected after 28 days. The
tetW, tetY and tetZ carrying bacteria reached to a peak accumulation at the 1st, 3rd and 21st day, respectively, and declined afterwards. While the bacteria carrying tetQ, tetC and tetG distributed in a more persistent way, covering relatively even in the detected time points. In general, the bacteria carrying sulfadiazine resistance gene occupied in a larger concentration in the total bacteria community compared to the presence of tetracycline determinants carrying bacteria, while the time-point dependent trend for their distribution was observed as well. In most cases, the addition of antibiotics increased the copy number of ARGs, indicating that the antibiotic selection pressure promoted the prosperity of the corresponding antibiotic resistance gene carrying bacteria.

DGGE Profiles of the Swine Manure Composting Microbial Community

DGGE was performed using the bacterial 16S rDNA V3 region to discover the distribution profile of microbial communities along the composting process. As shown in Figure 3, compare to day 0, the number of the visible bands was clearly decreased from day 1 onwards, suggesting that the alteration of the surroundings by the initiation of the composting process might reduce the microbial diversity in the composting piles, favouring the growth of tolerant microbes. The microbial communities were very dynamic at the initial stage (day 1 and 3), responding to both time points and the antibiotic treatments, while such effects disappeared in the followed sampling days.

Sequencing of the dominant DGGE bands showed the predominance of Ruminofilibacter (01), Microbulbifera (04), Verrucomicrobium (09) as well as Lachnospiraceae (010) in day 0, and Bacillus (1 and 2), Psedomonas (3), Psychrobacter (6, 9 and 10) as well as Sporosarcina (7 and 8) in day 1, and Alicyclobacillus (12), Lachnospiraceae (14), Rhizobiaceae (19) as well as Clostridium (20) in day 3, and Proteobacteria (32 and 33), Bacteroidetes (44), Sphingobacterium (51), as well as Erythrobacter (50) from day 7 onwards. On day 1, the major two bands resulted from Bacillus in lane C disappeared in lane L and H, and four bands representing Psedomonas, Psychrobacter as well as Sporosarcina were selectively enriched in lane L and H, indicating the sensitivity of Bacillus to added antibiotics, and the potential capability to degrade antibiotics by the selectively enriched microbes.

Conclusions

Overall, this study demonstrates differential distribution profiles for antibiotic resistance determinants during swine manure composting process, and the temporal changes of microbial community structure upon antimicrobials treatments were reflected in the initial stage of composting. During the initial stages of composting, the bacterial population was affected by the antibiotic pressure; however, when the...
composting progresses, the population increased might be due to the degradation of antibiotics or the adaptation of the bacterial population to the pressure. Besides, persistency of ARGs specifically, the sul genes indicate that the bacteria tends to retain the ARG for few weeks in the composting system even in the absence of antibiotics. This situation implies that the treatment was improper or inadequate, the ARGs could persist in the manure and transported to the land upon application.

References
MICROEMULSIONS ENHANCE THE BIOREMEDIATION OF LINDANE (Γ-HEXACHLOROCYCLOHEXANE) CONTAMINATED SOILS VIA THE INOCULATION OF SPHINGOBIOUM INDICUM

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ABSTRACT Bioremediation is becoming a more appropriate technology for the decontamination of soils contaminated with hydrophobic organic compounds (HOCs) due to its environmental and economic advantages; however, the low bioavailability of HOCs seriously restricts the bioremediation efficacy. The present study addressed this issue through the application of microemulsions, which is a novel surface active agent consisting of surfactants, cosurfactant and oil phase, into the bioremediation of lindane (γ-hexachlorocyclohexane, γ-HCH) contaminated soils with the inoculation of Sphingobium indicum B90A. In aqueous phase, the biodegradation of γ-HCH could be effectively accelerated from 0.0393 mg/(L·h) for the control without any surface active agent in the system to 0.0799 mg/(L·h) when 2000 mg/L of microemulsion formed with Tween 80 was present, indicating that microemulsion formed with Tween 80 could effectively enhance the bioavailability of γ-HCH for the degradative S. indicum B90A. Further experiments in soil slurry system also revealed that the removal rate of γ-HCH from contaminated soil could be enhanced to 0.2539 mg/(kg·d) which is 2.5 times of that of bacterial inoculation only, and all 50 mg/kg of γ-HCH in soil was effectively removed within 21 days of incubation. Addition of microemulsions effectively enhances the bioremediation of γ-HCH contaminated soils through enhancing both the bioavailability of γ-HCH and the growth of the degradative bacteria.

Keywords: Bioremedation, Lindane, Soil, Sphingobium indicum

Introduction

Bioremediation is becoming a more appropriate technology for the decontamination of soils contaminated with hydrophobic organic compounds (HOCs) due to its environmental and economic advantages over other physical-chemical remediation alternatives, such as low temperature thermal desorption [1], incineration, photodegradation [2] and phytoremediation [3, 4]. However, a major concern of bioremediation is to enhance the bioavailability of HOCs for microbial degradation to make the bioremediation effective. Many surfactants including synthetic surfactants, natural surfactants and biosurfactants have been used with the aim to increase the bioavailability of HOCs through partitioning HOCs into the hydrophobic cores of surfactant micelles above their critical micelle concentration (CMC) and thus enhancing the contact between degradative microorganisms and pollutants [5, 6].

Microemulsion (μE), as a combination of surfactants, co-surfactants and oil phase, may be another potential candidate for remediation of soils due to its high solubilization capacity relative to the surfactant micelle solutions. Microemulsions are thermodynamically stable, isotropic, and macroscopically homogeneous dispersions of two immiscible fluids, generally oil and water, stabilized by surfactant molecules either alone or mixed with a cosurfactant [7]. Our previous study revealed that microemulsions formed with Tween 80, 1-pentanol and linseed oil possessed much higher solubilizing capacity for γ-HCH than the counterpart Tween 80 solution [6]. For instance, about 22.1 mg/L of lindane (γ-hexachlorocyclohexane, γ-HCH) can be solubilized by 1000 mg/L of Tween 80 solution; while its intrinsic solubility in water is only 7.3 mg/L. However, more than 37.7 mg/L of γ-HCH can be successfully solubilized by 1000 mg/L of microemulsion formed with Tween 80, 5 times higher than its intrinsic water solubility.

To date, the effect of microemulsions, i.e. oil-in-water microemulsions, on the biodegradation of HOCs it still unclear, and the aim of this study was therefore to study the possibility of applying microemulsions to enhance the bioremediation of lindane contaminated soils with the inoculation of Sphingobium indicum B90A.
Materials and Methods

Preparation of Microemulsions

The oil-in-water microemulsions formed with Tween 80, 1-pentanol and linseed oil, with the cosurfactant to surfactant ratio (C/S ratio, w/w) of 1:3 and oil to surfactant ratio (O/S ratio, w/w) of 1:10, were prepared as described in our previous study [6]. Unless otherwise indicated specifically, the microemulsions employed in this study used Tween 80 as the surfactant phase.

Effect of Microemulsions on the Biodegradation of γ-HCH by Sphingobium Indicum B90A in Aqueous System

50 μg of γ-HCH dissolved in 0.5 mL of acetone was added to each 20 mL autoclaved glass vial and allowed to evaporate completely, which produced γ-HCH coating on the bottom of each vial. After that, 8.8 mL autoclaved Bushnell-Haas Broth, 0.2 mL of S. indicum B90A inoculum prepared as described in our previous study [8], and 1 mL of surface active agents including 20,000 mg/L of Tween 80 solution or microemulsions were then added to vials to make the final concentrations of surfactant or microemulsions at 2000 mg/L and S. indicum B90A density at 7×10⁶ cells/mL. Control without any surface active agent was prepared with only 9.8 mL of Bushnell-Haas Broth and 0.2 mL of S. indicum B90A inoculum in vials. Then all the vials were incubated on a rotary shaker at 180 rpm and 30°C, during which the vials were scarific periodically for the measurement of γ-HCH loss following the procedures described in previous study [8].

Table 1. Details of treatments employed in γ-HCH bioremediation experiments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H₂O (g/g soil)</th>
<th>Sawdust immobilized with S. indicum B90A (g/g soil)</th>
<th>Surface active agents (mg/kg soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.3</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>B¹</td>
<td>0.3</td>
<td>0.17</td>
<td>×</td>
</tr>
<tr>
<td>B1S²</td>
<td>0.3</td>
<td>0.17</td>
<td>1000 (Tween 80)</td>
</tr>
<tr>
<td>B1M³</td>
<td>0.3</td>
<td>0.17</td>
<td>1000 (Microemulsion)</td>
</tr>
</tbody>
</table>

¹ B = Bacterial inoculum; ² S = Surfactant; ³ M = Microemulsion; 1 = 1000 mg/kg.

Effect of Microemulsions on the Removal of γ-HCH by S. indicum B90A in Soil Slurry

Table 1 shows the treatments employed in γ-HCH bioremediation experiment, respectively. 300 g of γ-HCH spiked soil (50 mg/kg) was mixed with 51 g (dry weight) of sawdust which had been immobilized with S. indicum B90A, then predetermined amounts of Tween 80 solution or microemulsions prepared with H₂O were added and mixed with soil and sawdust thoroughly. The moisture content of this mixture was about 65% of soil’s water holding capacity which was 47.8%. All soil or soil-sawdust mixtures were placed in 1 L flasks which were then incubated at 30°C with regular mixing on alternate days. 10 g of soil samples were collected from each flask, subjected to soxhlet extraction and analyzed for γ-HCH using GC/ECD [8].

Results and discussion

As shown in Figure 1, Sphingobium indicum B90A can degrade γ-HCH from 50 mg/L to about 4.9 mg/L in 42 h without microemulsion or surfactant addition, indicating that the hydrophobicity or γ-HCH did not seriously restrict its biodegradation by S. indicum B90A probably due to the relative higher dissolution rate of γ-HCH in water. However, the degradation processes are usually much slower in soil environment than in aqueous phase because of the strong sorption ability of soil particles, which indicates that shortening biodegradation period might be important and useful for real remediation processes of contaminated soils. The addition of 2000 mg/L of Tween 80 or 2000 mg/L of microemulsion accelerated the biodegradation of γ-HCH by S. indicum B90A. After 18 h incubation, only 24.8% of γ-HCH added was degraded in the control system without any surfactant or microemulsion; whereas, over 52.4% and 86.1% of γ-HCH were degraded in the system supplemented with 2000 mg/L of Tween 80 and 2000 mg/L of microemulsion, respectively.
To compare the effect of different surface actives agents on the biodegradation processes, \( \gamma \)-HCH biodegradation by \textit{S. indicum} B90A was fitted to the first-order kinetic equation. The degradation rate constant \((k)\) was determined using the algorithm:

\[
\frac{C_t}{C_0} = e^{-kt}
\]

where \(C_0\) is the amount of \( \gamma \)-HCH present in the liquid medium at time 0, \( C_t \) is the amount of \( \gamma \)-HCH in liquid medium at time \( t \) and, \( k \) and \( t \) are the rate constant \( (h^{-1}) \) and degradation period in hours, respectively.

The fitting of \( \gamma \)-HCH biodegradation to experimental data using the first-order kinetic model is shown in Table 2. Obviously, the biodegradation of \( \gamma \)-HCH was effectively accelerated from 0.0393 mg/(L·h) for the control without any surface active agent to 0.0799 mg/(L·h) when 2000 mg/L of microemulsion was added, indicating that microemulsion could effectively enhance the bioavailability of \( \gamma \)-HCH for the degradative \textit{S. indicum} B90A. In contrast, the addition of 2000 mg/L of Tween 80 did not enhance the biodegradation process and also lowered the biodegradation rate to 0.0319 mg/(L·h).

During the biodegradation of \( \gamma \)-HCH in soil slurry system, the residual \( \gamma \)-HCH in the control was about 50 mg/kg soil throughout the incubation period, indicating the persistency of \( \gamma \)-HCH in soil environment and lack of degradative microbes in soil [9]. In contrast, residual \( \gamma \)-HCH concentration of all three treatments decreased from 50 mg/kg soil to less than 1 mg/kg soil in just 21 days, but with different degradation rates.

As presented in Table 2, the degradation data of all treatments fit well with the first-order equation \((R^2 > 0.99)\), and the addition of Tween 80 or microemulsions effectively increased the degradation rate constant. Without surface active agent, the degradation rate of \( \gamma \)-HCH by \textit{S. indicum} B90A in soil slurry was only 0.1155 mg/(kg·d); while, the addition of Tween 80 and microemulsion effectively increased the degradation rate of \( \gamma \)-HCH to 0.1629 by the addition of 1000 mg/kg Tween 80, and to 0.2539 with 1000 mg/kg of microemulsion. Undoubtedly, the biodegradation rate of \( \gamma \)-HCH by \textit{S. indicum} B90A was enhanced by about 2.5 times by the addition of microemulsion compared to the control without any surface active agent. On the other hand, microemulsion was much more effective than the same concentration of Tween 80 in accelerating the removal of \( \gamma \)-HCH from contaminated soil.

![Figure 1](image-url)

Figure 1. Effect of microemulsions on the biodegradation of \( \gamma \)-HCH by \textit{S. indicum} B90A at 30°C in both aqueous system (a) and soil slurry system (b). Control - without any surfactant or microemulsion; T80 - Tween 80; T80-M - microemulsion formed with Tween 80
Table 2. Kinetic data of γ-HCH biodegradation in aqueous and soil slurry system

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Regression equation</th>
<th>$R^2$</th>
<th>$k$ (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$\ln(C_t/C_0) = -0.0393t + 0.0045$</td>
<td>0.9785</td>
<td>0.0393±0.0051</td>
</tr>
<tr>
<td>2000 mg/L T80-M</td>
<td>$\ln(C_t/C_0) = -0.0799t + 0.0076$</td>
<td>0.9650</td>
<td>0.0799±0.0171</td>
</tr>
<tr>
<td>2000 mg/L T80</td>
<td>$\ln(C_t/C_0) = -0.0319t + 0.0115$</td>
<td>0.9488</td>
<td>0.0319±0.0061</td>
</tr>
<tr>
<td>Soil slurry system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$\ln(C_t/C_0) = -0.0045t + 0.0014$</td>
<td>0.9478</td>
<td>0.0045±0.0010</td>
</tr>
<tr>
<td>B</td>
<td>$\ln(C_t/C_0) = -0.1155t + 0.0016$</td>
<td>0.9901</td>
<td>0.1155±0.0086</td>
</tr>
<tr>
<td>B1S</td>
<td>$\ln(C_t/C_0) = -0.1629t + 0.0006$</td>
<td>0.9965</td>
<td>0.1629±0.0087</td>
</tr>
<tr>
<td>B1M</td>
<td>$\ln(C_t/C_0) = -0.2539t + 0.0004$</td>
<td>0.9980</td>
<td>0.2539±0.0151</td>
</tr>
</tbody>
</table>

Conclusions

The biodegradation of γ-HCH by *S. indicum* B90A in both aqueous and soil slurry system was effectively accelerated by microemulsion formed with Tween 80, and microemulsion is much more effective than the same concentration of Tween 80 to enhance the biodegradation process. Microemulsion coupled with the inoculation of *S. indicum* B90A effectively removed all 50 mg/kg of γ-HCH in soils within 21 days through enhancing the bioavailability of γ-HCH.

Acknowledgements

The authors would like to thank the financial support from the Research Grant Council of the Hong Kong Special Administrative Region, People’s Republic of China (Grant HKBU261307) and the Faculty Research Grant of the Hong Kong Baptist University (FRG/06-07/II-44).

References

THE RECYCLING TECHNIQUES FOR WASTEWATER FROM DISCARD DISC RECOVERY

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ABSTRACT The purpose of this study is to establish a processing program of the wastewater from discard compact disc recovery. Chemical oxygen demand (COD) reduction of wastewater by filtration, electrolysis and photolysis were investigated in batch study. Effects of operating parameters such as type of adsorbent, current intensity, time of electrolysis/photolysis, and photocatalyst (TiO₂) addition were studied to optimize conditions for treatment. The results indicated that the optimal removal efficiency of COD by filtration, electrolysis and photolysis were up to 51%, 56% and 44%, respectively. After filtration (activated carbon of bone black with 2 mL/min) and electrolysis (2.0 A for 4 h) treatment processes, the removal efficiency of COD was up to 98.4% (from 4640 ppm to 72 ppm). Moreover, the high pH value of the solvent was retained. Our results provide an alternative for factories to reuse the wastewater in future recycling process, and fulfill the goal of zero-waste discharge.

Keywords: Discard compact disc, Recycling technique, Filtration, Electrolysis, Photolysis

Introduction

The purpose of this study is to establish a processing program of the wastewater from discard compact disc recovery. In Taiwan, most techniques used in discard disc recovery were wet alkali treatment. Therefore, wastewater from discard disc recovery was alkali and contains high organic content and heavy metals. Chemical precipitation and electrolysis were used in some studies to retreat valuable metals from wastewater [1]. But few studies in wastewater treatment of discard compact disc recovery were found. The residual high alkali and organic content without proper disposal was considered a threat to environment. However, filtration, electrolysis and photolysis were employed to remove organic content in the wastewater treatment elsewhere [2, 3]. In our study, the operation parameters for filtration, electrolysis and photolysis were studied to optimize the treatment conditions. Chemical oxygen demand (COD) was used as indicator for removal efficiency evaluation. As a result of the optimal condition evaluation, filtration and electrolysis were selected for use in treatment. After processing, the organic content in the wastewater was minimal. Furthermore, the treated alkali solvent can be reused for further recycling process.

Materials and Methods

Wastewater from discard disc recovery process was pretreated with Whatman No.1 filter paper to remove large particulates of printing material.

Filtration

Activated carbon of bamboo and bone black were used to evaluate the removal efficiency of mechanical and chemical filtration for chemical oxygen demand (COD). The filtration rate was set at 2 mL/min.

Electrolysis

An experimental reactor cell 10 cm x 7 cm x 10 cm, (L x W x H) was prepared and used for electrolysis. Removal of COD from wastewater was investigated at various current intensities (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 A) by using Pt/Ti and stainless steel electrodes and at various electrolysis times. The distance between electrode plates was fixed at 4 cm. In each batch study, 400 mL sample was used and a magnetic stirrer was employed to ensure complete mixing.

Photolysis

A photolysis cell made of acrylic with diameter of 10 cm and height of 17 cm was prepared. A UV lamp (9 W, 254 nm wavelength) was employed as light source for photolysis. TiO₂ was used as the photocatalyst. In
each batch study, 100 mL of sample was used and the addition of photo catalyst was none, 0.1 g, 0.2 g and 0.3 g, respectively. The photolysis time lasted for 8 h.

**Analytical Assay**

Chemical oxygen demand was analyzed by using closed potassium dichromate reflux method (NIEA W517.52B). Aluminum concentration was measured by Graphite Furnace Atomic Absorption Spectrometer (Hitachi Z8200, Japan). The measurement of pH was done using pH meter (Suntex SP-2200, Taiwan).

**Results and Discussion**

The characteristic of wastewater after pretreatment was analyzed. The COD, pH and Al concentration in the wastewater from discard disc recovery process were 4640 mg/L, 13.85, 66.2 mg/L, respectively. Thus, the wastewater could be categorized as an alkali solvent with high organic content.

**Filtration**

The concentration of COD was reduced from 4640 mg/L to 2160 mg/L and 1630 mg/L on passing through activated carbon of bamboo and bone black, respectively, with a corresponding COD removal efficiency of 51.3% and 64.9% respectively, at a filtration rate of 2 mL/min. The reduction in metal concentration upon filtration with activated carbon of bamboo was minimal. However, filtering with activated carbon of bone black did have some effect on the same. In the later case, Al concentration was reduced from 66.2 mg/L to 44.7 mg/L. The reduction rate of Al concentration was 32.5%.

**Electrolysis**

The reduction in COD after electrolysis process is shown in Figure 1. The reduction of COD was increased with increasing current intensity application as well as electrolysis time. After 4 h of electrolysis, the COD removal efficiencies were 45.7%, 50% and 56 for current intensity of 2.0 A, 2.5 A and 3.0 A, respectively. However, at lower current intensity (> 1.5 A ), the energy supplied were not enough to fully oxidize the organic content. In such cases, the COD removal efficiency was all less than 30%.

**Photolysis**

The results of photolysis are shown in Figure 2. The COD removal efficiency was 17.2%, 20.7%, 35.3% and 44% the addition of none, 0.1 g, 0.2 g and 0.3 g of photocatalyst, respectively. The addition of photocatalyst (TiO₂) did enhance the reduction of COD. The addition of 0.3 g TiO₂ increased the COD reduction rate as the photolysis time increased. Most of the COD were reduced in the first hour of photolysis. The reduction rate was doubled from 2 to 8 h of photolysis for 0.3 g TiO₂ addition.
Figure 2. Reduction in chemical oxygen demand (COD) after photolysis process for 8 h of addition of photocatalyst addition at varying amounts

Optimal Condition Evaluation

Removal efficiency and cost effectiveness were taken into account for decision making. Filtration and electrolysis were selected as our optimal treatment process. Both kinds of activated carbon were employed for evaluation. The optimal conditions of electrolysis were 2.0 A current intensity and 4 h of electrolysis time. The results of optimal condition evaluation are shown in Figure 3. The COD was reduced from 4064 mg/L to 124 mg/L and 72 mg/L for activated carbon of bamboo and bone black respectively. The reduction rate of COD was 97.3% and 98.4% for activated carbon of bamboo and activated carbon of bone black, respectively.

Figure 3. The reduction in chemical oxygen demand (COD) and reduction rate for optimal treatment process

Discussion

The optimal removal efficiency of COD by filtration, electrolysis and photolysis were up to 51%, 56% and 44%, respectively. As a result of the optimal condition evaluation, filtration and electrolysis were selected. After filtration and electrolysis treatment processes, the removal efficiency of COD was up to 98.4%. The COD was reduced from 4640 mg/L to 72 mg/L. Aluminum concentration was also reduced to acceptable level. Furthermore, the high pH value of the solvent was retained and therefore, could be reused for further recycling processes. Few studies in wastewater treatment of discard compact disc recovery were found, but when compared to two related studies [2, 3], the removal efficiency of COD was higher in our study.
However, the filtration rate in our study has to be improved for full scale application. Further study of higher filtration rate with effective COD removal rate should be addressed.

**Conclusions**

Processing program of the wastewater from recycling process of discard compact disc recovery was established in our study. Electrolysis after filtration processing, reduced the organic content in the wastewater to a minimum. The removal efficiency of COD was up to 98.4%. Furthermore, the treated alkali solvent can be reused for further recycling process. Our results provide an alternative for factories to reuse the wastewater in future recycling process, and fulfill the goal of zero-waste discharge.

**References**


CO-DEPOSITION OF ENVIRONMENTALLY HAZARDOUS OIL SHALE ASH AND SEMICOKE WASTE MATERIALS

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ABSTRACT Growing energy demand in fast-growing economies forces the use of low quality fossil fuels such as oil shale. The total world resources of oil shale type fuels, mainly located in China, Brazil and U.S.A., are at least 500 billion tonnes. Effective and sustainable usage of oil shale, however, is complicated. The mineral matter content of the commercial-grade oil shale can be as high as 85%. As a result large amount of solid waste is produced. Large-scale exploitation of oil shale type fuel is operational only in Estonia, where kerogenous oil shale (8-12 MJ kg-1) is burned in electric power plants as well as retorted to produce shale oil. An inevitable issue in Estonian oil shale industry is the formation of environmentally hazardous solid waste materials – oil shale ash and semicoke, which amounts 45-48% of the raw oil shale after combustion and about 4 tonnes per every tonne of shale-oil. The re-use of those highly alkaline waste materials is limited to only few applications and most of the waste is landfilled. The purpose of the current study was to investigate possible co-deposition of two main residues – oil shale ash and semicoke. Due to high content of free CaO in burnt oil shale the water draining off the landfills is highly alkaline. Those landfills are considered as major pollution source in Estonia. Therefore, during the landfill construction low hydraulic conductivity of the material is required to reduce infiltration through the waste deposits and thus protect underlying soil and groundwater. Intensive delayed secondary mineralization causes significant expansion of co-deposited ash and semicoke mixtures resulting in highly permeable layers. This expansion effect can be controlled by choosing suitable ratios of ash and semicoke in landfill mixture. Five different mixtures with ash and semicoke ratios 2:1, 1:1, 1:2, 1:3 and 1:4 were prepared in semicoke landfill. Changes in dry density, hydraulic conductivity and composition/structure of the mixtures were periodically recorded in 12 months period under the field conditions. The results reveal that dry density shows a slight decrease in all the mixtures during the test period with the reduction being more apparent in mixtures with higher ash content. Hydraulic conductivity shows apparent decrease in few weeks after the deposition, which is related to the development of cementation in the pore space. After the first few weeks hydraulic conductivity starts to increase due to delayed ettringite mineralization, achieving its maximum 4-5 months after deposition due to the start of heavy rainfall period. In general, with increasing ash content higher hydraulic conductivity values were measured, which was caused by expansion of material due to intensive secondary mineralisation process in ash material. As a result microscopic cracks are formed which lead to more intensive erosion of the material due to precipitation. The co-deposition of ash and semicoke is possible, but the limiting factor is the ash content of the mixture. As ash and semicoke ratio exceeds 1:4, hydraulic conductivity and dry density become critical.

Keywords: Oil shale ash, Oil shale semicoke, Landfilling
DESIGN OF SAFE DISPOSAL SYSTEM FOR MERCURY BEARING BRINE SLUDGE FROM CHLOR-ALKALI INDUSTRY


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ABSTRACT Studies were undertaken to design a secured landfill facility for safe disposal of brine sludge generated from chlor-alkali plant of Jayshree Chemicals Ltd. The sludge contains 50 to 100 mg/kg of mercury. As per Hazardous Waste (Management, Handling and Transboundary Movement) Rules, 2008, the waste comes under hazardous category. Secured landfill is a disposal system where special provisions are made for isolation of waste from air and water environment. Design considerations of secured landfill included landfill area, lining and leachate collection system, and covering system. Operational guidelines and closure and post-closure methodologies were also delineated. Ranking exercise of the site indicates that it comes under ‘moderate’ category as per the potential level of hazard.

Keywords: Secured landfill, Chlor-alkali plant, Brine sludge, Mercury

Introduction

Sodium hydroxide and chlorine are the major products of chlor-alkali industries. The manufacturing process involves electrolysis of brine solution in mercury cell. In a mercury cell, high quality titanium is used as anode and mercury as cathode. Brine sludge is generated during the process of purification of brine. In brine purification system, the recycled brine which remains unutilized in cell is used. The recycled brine is a major source of mercury in the brine sludge, especially during process upset when leakage of excess mercury occurs. Jayshree Chemicals Ltd. is a well known industry located at Ganjam in Orissa state of India. The industry produces sodium hydroxide, chlorine and hydrogen chloride in the tune of 1890 ton per month, 1241 ton per month and 1351 ton per month respectively. About 1729 ton of brine sludge is generated per year in this industry. For safe disposal of sludge, a secured landfill system has been designed which was successfully implemented in collaboration with the industry.

Materials and Methods

Characteristics of Brine Sludge

In India, management of hazardous waste is governed by the Hazardous Waste (Management, Handling and Transboundary Movement) Rules, 2008. As per the provision of these rules, brine sludge is considered as hazardous waste as it contains mercury in the range of 50 to 100 mg/kg. As such, the industry needed a secured disposal system for the management of brine sludge within the framework of regulations. Accordingly a secured landfill system was designed, constructed and put in operation.

Design Considerations for Secured Landfill

Major design features/parameters of secured landfill system are as follows

- Landfill area
- Lining system
- Leachate collection system
- Covering system/closure process of landfill

Area estimation is needed to assess the land requirement. Area for landfilling was estimated using the data related to quantity of sludge, operating period, density of sludge and depth of fill. Land requirement for accessories was also considered in estimation.
Results and Discussion

Landfill Area

The filling area is computed from the equation

\[
A = \frac{Q \times P}{\rho \times d} \quad (\text{sq. m})
\]

where

- \( Q \) = quantity, ton per year
- \( P \) = operation period, year
- \( \rho \) = density of sludge, ton per cum
- \( d \) = depth of landfill, m

The industry required a secured landfill system for a short term operating period. Accordingly, the estimated area was 4788 sq.m. including accessories.

Lining and Leachate Collection System

The liner acts as an impervious barrier and prevents the leachate from entering the subsoil body and around water. A combination of clay of permeability 1 x 10^-7 cm/sec. and High Density Polyethylene (HDPE) was used in two layers, which were termed as primary and secondary layer. The function of secondary layer is to prevent the entry of leachate to the subsoil and ground water if any leakage occurs in the primary layer. Geotextile sheet was provided to protect the HDPE liner. Sand acted as a filter media. Gravels were used as drainage media. Figure 1 shows the arrangement of liners in the secured landfill of Jayshree Chemical Ltd. Design specifications of thickness are also indicated in Figure 1. Perforated HDPE pipes of 15-20 cm diameter were provided and 2% slope was maintained at the base to facilitate leachate collection. Leachate is collected at leachate collectors and stored in storage sumps.

![Figure 1. Arrangement of Liners in Secured Landfill of Jayshree Chemicals Ltd](image)

Construction of Secured Landfill

The following measures were adopted during construction of secured landfill:

- Excavation of soil was conducted in keeping with design requirements
- Soil stability test was conducted
The HDPE sheets were properly joined and anchored
The mechanical properties of the liner were tested

Operation of Secured Landfill
Various measures adopted during operation are as follows:

- Cell wise filling
- Application of soil cover over the waste
- Regular monitoring of leachate and ground water quality around the site
- Leak detection measures

Closure and Post Closure Measures for Secured Landfill
Final cover is applied after a phase of filling is completed and level of the waste reaches the designed height. The final cover comprises of the components in the following order from the top to the waste layer.

- Vegetation cover at the topmost layer
- Top soil
- Filter layer
- Drainage layer
- Geotextile
- HDPE
- Compacted clay/clayey soil
- Waste

The monitoring of leachate and ground water is needed to be continued even after closure of the secured landfill. The final cover should be properly maintained. Secured landfill of Jayshree Chemicals Ltd. is shown in Exhibit 1 and Exhibit 2.

Exhibit 1. Construction of Secured Landfill of Jayshree Chemicals Ltd


**Site Assessment**

The types of rocks available at the site are granite, gneiss and weathered Khondalites. The aquifers, which are water bearing zones, occur in the unconfined condition above the base rock. Soil characteristics indicate that clay with core sand is available at the top followed by fine sand with silty clay and fine to medium sand with traces of clay. Ranking of the site has been conducted following the guidelines of the Ministry of Environment & Forests, India. Location, geological and hydrogeological features of the site, distance of the water body, climatic condition, soil quality, population and many other factors were taken into consideration. In ranking exercise, weightage was assigned to various attributes in accordance with the relative magnitude of the impact using ranked pairwise technique. For each of the attribute, sensitivity scale was assigned. The value of the sensitivity index multiplied by the corresponding weightage was score of each attribute. The least is the score; the least is the impact on environmental quality. The score of the site was 441 out of maximum score 895. As per the level of potential hazards, the site comes under ‘moderate’ category.

**Conclusions**

Mercury is highly toxic and poses potential risk for environment and human health. Brine sludge from chlor-alkali plant of Jayshree Chemicals Ltd. contains mercury in the range of 50 to 100 mg/kg. Secured landfill system is a viable and highly professional tool in hazardous waste management where special attention is taken to ensure that the waste does not come in contact air and water. A secured landfill system was designed for Jayshree Chemicals Ltd. for safe disposal of brine sludge. The system has been successfully implemented and is in operation.

**References**

[1] Central Pollution Control Board, Govt. of India. 2001. Criteria for Hazardous Waste Landfills