ANAEROBIC BIODEGRADATION OF SULFOLANE IN SOIL ENVIRONMENT

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(received June 1999, accepted August 1999)

Abstract: Batch degradation studies were conducted with soil inoculum, collected from the contaminated site, spiked with aqueous sulfolane and stirred under anaerobic conditions. The results of the study show that sulfolane was degraded up to 64% after 68 d of incubation time for initial concentration of 6.6 mmol l⁻¹ in the absence of nutrients. Headspace analyses indicate that approximately 1 mmol l⁻¹ carbon for the sulfolane (2.6 mmol l⁻¹ carbon) fully degrades to C11₄, CO₂ as the ultimate products. The rest of the degraded sulfolane presents as unidentified intermediate aqueous products. Sulfur balance indicates that approximately 94% of sulfur still remained in the reactor while hydrogen sulfide was observed at 2.5 mg l⁻¹ in the headspace.

Key Words: bioremediation, soil contamination, sulfolane

INTRODUCTION

The site subject to this investigation is formerly Sulfinol waste sludge disposal site located in Brisbane, Australia. Approximately 6 ha of the 240 ha site have been impacted by an annual quantity of approximately 20,000 l of a Sulfinol sludge over the last 20 y with careless disposal of sulfolane used in Sulfinol process to remove carbon dioxide from the air stream in an ammonia plant. Further chemical analysis of groundwater in the site indicated that sulfolane was the major contaminant in the study area and the concentration was ranged from 3153 to 4344 mg l⁻¹.

Sulfolane (CAS: 126-33-0) was regulated as toxic organic compound in US EPA report (Federal Register, 1991) and can be readily migrated through aquifer due to ion-dipole interaction with water molecules showing higher dielectric constant of 43.3 (Morrison and Boyd, 1987). The first largest commercial use of sulfolane is employed for various industrial purposes such as the industrial extraction of organic hydrocarbons, fatty acids and fatty acid esters. The second largest commercial use for sulfolane is the removal of acidic components, e.g., H₂S, CO₂, COS, CS₂, and mercaptans, from sour gas streams from the process known as the Sulfinol process. Toxic property of sulfolane would be an environmentally harmful, which was on the U.S. Environmental Protection Agency’s list of priority pollutants (Federal Register, 1991). Toxicity of sulfolane reveals that LD₅₀ for rats is 1941 mg/kg (Chapman and Hall, 1996). The sulfolane chronic toxicity test with guinea pigs has shown that the threshold dose of sulfolane was 2.5 mg/kg, whereas the non-effective dose was 0.25 mg/kg. By extrapolation this experimental result to humans, the
maximum allowable concentration of sulfolane in surface water has been recommended as 5 mg/l (Zhu et al., 1987).

MITI (1987) reported that sulfolane was not biodegradable by microorganisms. Boettcher et al. (1994) accomplished a feasibility test on biodegradability for 1000 mg l\(^{-1}\) of sulfolane by introducing aerobic bacteria, but aerobic heterotrophic bacteria could not use sulfolane as a carbon source. However, Chou and Swatloski (1982) and Cowan and Stover (1984) investigated the biodegradability of sulfolane in petroleum refinery wastewater stream by performing bench scale experiment. Sulfolane was biodegradable up to 200 mg l\(^{-1}\) in aerobic condition. Bagnall et al. (1984) investigated biological decomposition of sulfolane using rotating biological contactor (RBC) at ranged from 11°C to 25°C and pH 6.5 to 8.5. Sulfolane (2365 to 2750 mg l\(^{-1}\)) was introduced into influent at steady state, which could be degraded by 86 to 100% after 8 to 22 d of operation. Ying et al. (1994) has had three steps of biological degradation of sulfolane using biological activated carbon (BAC) in aerobic condition. The sulfolane has been degraded in a few days since BAC column was inoculated with activated sludge from petrochemical plant. In pilot scale test, they introduced groundwater contaminated with 3000 \(\mu g\) l\(^{-1}\) of sulfolane into 1.8 m (L) \(\times\) 10.2 cm (D) of PVC column packed with activated carbon (Calgon F-400). Sulfolane ranged from 600 to 1500 \(\mu g\) l\(^{-1}\) was significantly removed by as low as 100 \(\mu g\) l\(^{-1}\). McLeod et al. (1991) and Fedorak and Coy (1996) have also studied aerobic biodegradation of sulfolane.

Schrementi and Meganathan (1986, 1987) report that tetrahydrothiophene-1-oxide was anaerobically reduced as an electron acceptor into thiolane. Zinder and Brock (1978a, b) also reports that sulfolane would be reduced into thiolane by facultatively anaerobic culture.

Although most of works mentioned above have been devoted to biodegradability of sulfolane in aerobic condition, relatively little attention has been paid to the anaerobic degrada-

dation of sulfolane even though sulfolane would be presented at anoxic environment beneath the ground.

This paper reports the importance of potential natural biodegradation process, which can lead the removal of sulfolane from the aquifer at the waste disposal site. It also provides evidence that sulfolane can be gradually degraded under anaerobic condition without supplying any nutrients. In addition, the role of sulfolane as electron acceptor was examined employing 4-methylphenol which is one of phenolic compounds found in 1982 at up to 18 mg l\(^{-1}\), but it has not been detected through the current study for groundwater samples taken from the site even though sulfolane has still been present at higher concentration in the site.

**MATERIALS AND METHODS**

**Chemicals**

Sulfolane and 4-methylphenol were purchased from Aldrich Chemical Co., Inc. (Ex.\(\geq\)99%).

**Soil and Groundwater Samples**

Subsurface soil samples were obtained at depths ranging from 2 to 3 m, near to the water table. In order to obtain soil samples in the saturated zone, a Selby tube (45 cm \(\times\) 4.8 cm I.D.) was pushed through the soil at the bottom of a freshly dug 3 m pit. The Selby tube was steam cleaned (Gerni 660 Turbo Laser) and rinsed with 70% ethanol prior to sampling to prevent cross contamination. The tube was then capped with butyl rubber stoppers at both ends, brought to the laboratory, and purged for 1 h with oxygen-free nitrogen. The soil samples were used immediately to reduce the possibility of changes in the soil biota.

In addition, groundwater was collected from a reference well, beyond the extent of the contamination plume. The groundwater was collected from a reference well located approximately 2 km apart from the site and then filtered with a 0.45 \(\mu m\) membrane filter (Davis et al., 1994) and then sterilized with an autoclave.
Reactor Design and Procedures

The reactor used in this experiment is illustrated in Figure 1. The reactor was sealed with a butyl rubber stopper, equipped with two teflon tubes (5 mm I.D.) to obtain slurry and headspace samples. The soil slurry was heated and stirred using a magnetic stirrer (Ika Labortechnik, Germany). The temperature of the water bath was controlled at 35 ± 2°C.

To start the experiment, the reactor was filled with 200 g of soil and 1800 ml of sterilized groundwater in anaerobic condition. No nutrients or any carbon source were added to the reactor. The reactor was purged with oxygen-free nitrogen for 1 h. The reactor was then connected to a manometer, pressurised with nitrogen and monitored for 24 h to check for any gas leaks.

The degradation experiments was initiated in duplicate by adding 1 ml of a sterilized aqueous sulfolane solution such that the sulfolane concentration in the reactor was about 6.6 mmol l⁻¹ (790 mg l⁻¹). In addition, a set of test employing 50 mg l⁻¹ of 4-methylphenol in the presence of 1500 mg l⁻¹ of sulfolane was conducted to evaluate a role of sulfolane as an electron acceptor to degrade a phenolic compound found in the site. For a while, MLVSS (Mixed Liquor Volatile Suspended Solid) and pH were observed to monitor microbial growth. MLVSS was measured based on the Standard Methods (1994).

Control Experiment

The slurry sample was autoclaved at 121°C and 15 psi for 15 min on the three consecutive days (Tomy, High Pressure Steam Sterilizer, ES-315) and then amended with 1000 mg l⁻¹ of HgCl₂ to be final concentration in the reactor.

The purpose of this experiment was to assess volatile loss of sulfolane or any conversion of sulfolane into thiolane in abiotic condition by measuring volatile sulfolane proportion using Tenax tube connected into the control reactor as shown in Figure 2. The flow rate was calibrated with foam blowing into the injection point as 40 ml min⁻¹ using Aircheck Sampler (Model 224-PCXR7, SKC Inc.). The volume of gas passed through Tenax tube was adjusted with the head space volume in the reactor so that sulfolane may not avoid coming from the soil slurry inocula. Since the suction rate was determined, a 5 mm (I.D.) of teflon tube was directly connected into the reactor and then gas sampling was initiated. Perkin Elmer Automatic Thermal Desorption System (ATD 400) was used to run ATD tube automatically (oven temperature: 290 °C, 15 min, valve temperature: 220 °C) and subsequently the sample was analyzed by Perkin-Elmer Gas Chromatograph equipped with flame ionization detector (FID).

Analytical Methods

The concentration of sulfolane, 4-methylphenol
and any detectable degradation products were monitored throughout the experiment. Aqueous phase species, including sulfolane and 4-methylphenol, were monitored by collecting 5 ml slurry samples from the reactor. The slurry samples were centrifuged at 1500 rev min\(^{-1}\) for 15 min and filtered with a 0.2 \(\mu\)m membrane filter. Subsequently, it was extracted with 2 ml of methylene chloride in a small glass vial by hand-shaking for 1 min (Ying et al., 1994). The separated aqueous layer was discarded. Any moisture retained in the methylene chloride was removed by Na\(_2\)SO\(_4\).

The methylene chloride extracts were analysed using gas chromatography (GC). A Perkin Elmer AutoSystem gas chromatograph equipped with a flame ionization detector (FID) was employed. A 30 m long (0.25 mm) and 0.25 \(\mu\)m thick DB5 (5% phenyl methyl poly siloxane) column was used for sulfolane analysis. High purity helium was used as the carrier gas at a flow rate of 1.7 ml min\(^{-1}\). The injector and detector temperatures were 300 and 350 °C, respectively. FID response was monitored with a Perkin-Elmer LCI-100 integrator. Sulfolane and 4-methylphenol concentrations were determined by comparing peak areas against calibration curves.

Headspace gaseous samples were collected using a Hamilton gas-lock syringe to be 7 ml and then analysed by gas chromatography (Perkin Elmer, AutoSystem) equipped with a thermal conductivity detector (TCD). In addition, H\(_2\)S concentration from the headspace was quantified employing 10-S Plus Photovac Digital Gas Chromatograph equipped with Photo Ionization Detector (PID). Prior to obtain gas sample from the reactor, the sampling bag (SKC Inc., Eighty Four, PA, USA) was flushed with high purity air to remove any residual gas in the bag. A 100 ml of Millilitres SGE (Australia) gas tight syringe was used to take headspace gas sample from the reactor and then introduced into the sampling bag. Subsequently, a 0.5 ml of gas was introduced from the sampling bag into the column (CP-CILS, 10 m long) at 48 °C by high purity compressed air as carrier gas.

pH, COD, redox potential and sulphate concentrations in the aqueous phase were also observed.

RESULTS AND DISCUSSION

Soil and Groundwater Characterisation

The soil sample was characterized as presented in Table 1. Each parameter was determined after 200 g of soil was mixed with 1.8 l of sterilized groundwater except for temperature, redox potential (E\(_h\)).

Soils at the location were surrounded in anaerobic condition as showing -276 mv of E\(_h\) and 22 mg l\(^{-1}\) of SO\(_4^{2-}\). The concentration of TKN and T-P indicates that potential degradation of organic contaminants can be occurred by soil microorganisms. Table 2 lists the results of chemical analysis of groundwater used in inoculum preparation. In this analysis, potential toxic metal compounds to limit microbial activity were also measured for the ensued biological degradation tests.

Especially, Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICPAES) was used for quantitative analysis of inorganic compounds presented in filtered reference groundwater sample. Total 42 elements were scanned based on the standard solutions for each of ions prepared for a quantitative com-

<table>
<thead>
<tr>
<th>pH</th>
<th>Temp. (°C)</th>
<th>E(_h) (mv)</th>
<th>COD (mg l(^{-1}))</th>
<th>SO(_4^{2-}) (mg l(^{-1}))</th>
<th>TKN(^+) (mg l(^{-1}))</th>
<th>T-P(^*) (mg l(^{-1}))</th>
<th>Alkalinity (mg l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.9</td>
<td>18</td>
<td>-276</td>
<td>50</td>
<td>22</td>
<td>53</td>
<td>0.34</td>
<td>20</td>
</tr>
</tbody>
</table>

* Total Kjeldahl Nitrogen, * Total Phosphorus
Table 2. Characteristics of reference groundwater

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (mg L⁻¹)</th>
<th>Analytical instruments</th>
</tr>
</thead>
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<tr>
<td>Sulfolane</td>
<td>0</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>SO₄</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td>DOC*</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>T-P</td>
<td>0</td>
<td></td>
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<tr>
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<td>pH</td>
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<td>TPS 90-FLMV</td>
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</tr>
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<td>P</td>
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<td></td>
</tr>
<tr>
<td>Si</td>
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<td></td>
</tr>
<tr>
<td>Sr</td>
<td>0.14</td>
<td></td>
</tr>
</tbody>
</table>

Note: As, B, Cd, Cr, Cu, Li, Mo, Se, Ti, V, Zn, Hg, Sc, Sn, Pb, Co, Ni, Sb, Bi, Be, Nb, Ag, Ti, Zr, U, La, Ce, Sm (Not detected)

* Dissolved Organic Carbon

Comparison against those ions in the samples. As shown in Table 2, pH is mostly suitable for microorganisms to degrade substrate. Furthermore, no toxic compounds were found in the ICPAES analyses. The composition of DOC exposed humic substances by GC analysis.

Degradation Study

During period of test, the control was concurrently tested. The initial concentration of sulfolane was 7.4 (± 0.18) mmol in the slurry and then reduced into 7.15 (± 0.16) mmol after the experiment was completed. Any volatile compounds were not detected from the Tenax tube analysis, so the loss of approximately 0.25 mmol in the slurry was supposed to be occurred by adsorption on either soil sample or potential adsorption on the surface of reactor.

At first, the results of sulfolane degradation test was shown in Figure 3 as based on carbon balance. The amount of sulfolane was converted into the corresponding mole of carbon and then multiplied by the reactor volume as shown in y-axis. Sulfolane is shown to decrease from over 50 mmol to less than 20 mmol in 70 d.

The degradation products detected during the experiment are methane and CO₂. In addition, trace amount of H₂S was also observed. Figure 3 shows that the total amount of detectable car-
Figure 5. CH$_4$ variations with sulfolane degradation as COD. □ Theoretical COD of sulfolane; △ Observed COD of sulfolane; ◇ CH$_4$.

Figure 6. SO$_4^{2-}$ formation on degradation of sulfolane. ◇ SO$_4^{2-}$; □ Sulfolane; △ SO$_4^{2-}$+Sulfolane.

Carbon diminishes as the experiment progresses, indicating that not all products are accounted for. It indicates that intermediate compounds accumulate in the aqueous phase. Furthermore, COD is another parameter that can be used to indicate the presence of nonidentified compounds as shown in Figure 6. Prior to interpretation of Figure 5 further, Figure 4 was obtained to show that the measured COD of a pure solution of sulfolane corresponds closely with the theoretical COD of the same sulfolane solution.

\[ \text{C}_4\text{H}_8\text{SO}_2+7\text{O}_2 \rightarrow 4\text{CO}_2+4\text{H}_2\text{O}+\text{SO}_4 \]

where theoretical oxygen demand is 224 g O$_2$/120 g sulfolane=1.86 g O$_2$/g sulfolane

Therefore, in the absence of any other intermediate compounds, the COD of the aqueous phase in the reactor should follow the theoretical COD of the sulfolane in solution. However, Figure 5 shows that the measured COD of the aqueous phase climbs steady above the COD that is theoretically attributable to sulfolane. This indicates an accumulation of aqueous intermediate products. A small anomaly in the experiment is that the measured COD is initially less than the theoretical COD attributable to sulfolane in the first 10 d of the experiment. The difference between them observed at 790 mg l$^{-1}$ of sulfolane (Figure 4) was correspondingly consistent with the difference made at time zero (Figure 5). As the time progress on Figure 5, observed amount of COD has rather been increased compared to theoretical COD for sulfolane. It strongly demonstrated that amount of intermediate still present in the reactor as 380 mg l$^{-1}$ of corresponded COD at 70 d of incubation period.

A further check on the fate of sulfolane is to monitor sulphur. No sulphides were detected in the aqueous solution except for only trace amount of H$_2$S observed in the headspace. However, small concentrations of sulphate persisted and accumulated slightly in solution. Figure 6 shows that total accountable sulphur decreases with time, again indicating the presence of intermediate compounds.

An attempt was made to monitor microbial growth by measuring Mixed Liquor Volatile Suspended Solids (MLVSS) concentrations as present Figure 7. Figure 7 shows that MLVSS fluctuates during the first month of the experiment. This is not surprising as microbial populations will fluctuate while stable conditions are established. On the other hand, the pH of the reactor contents decreased from 7.4 to 3.3 over 130 d. This alone indicates the environment is suitable for microbial action. Degra-
Figure 7. Variations of MLVSS on decreasing of pH during sulfolane degradation. [ ] MLVSS; ◀ pH.

Table 3. Sulphur observed from the soil and aqueous inoculum in the reactor (Sulfur concentration: mmol/L).

<table>
<thead>
<tr>
<th>Soil inocula</th>
<th>Initial sulfolane</th>
<th>Final sulphur</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.09</td>
<td>6.34</td>
<td>6.25</td>
</tr>
</tbody>
</table>

Dissolution of sulfolane, a saturated organic compound, may well release protons into solution as reported by Chou and Swatloski (1982). They have found that biodegradation of sulfolane needs pH control to meet with stoichiometric release of sulfur molecules as sulfite.

After completion of this test, the sulphur balance was established by measuring total sulphur concentration using ICP-AES after the samples were oxidized by 0.1 N HCl as present in Table 3.

In considering of 6.59 mmol l⁻¹ of sulfolane initially accommodated into the reactor, the total amount of sulphur was still correspondingly left in the reactor even if sulfolane would be transformed into daughter compounds. The differences of approximately 0.43 mmol l⁻¹ in the balance would partly attribute to amount of H₂S produced during test of period, which was observed at 2.5 mg l⁻¹ after test was completed. Even if considering possible analysis error and a presence of H₂S, there are still observed the differences in the balance. It may be explained that an uncertainty would be occurred from loop of whole experimental procedures and sample preparation prior to analysis.

To assess potential of sulfolane as electron acceptor, one of phenol compounds was chosen as electron donor in the anaerobic experiment. In 1982, several phenolic compounds such as 2-chlorophenol, 4-chlorophenol and 4-methylphenol were observed in the study area at levels ranging from 7 to 18 mg l⁻¹, but those phenolics were not detected in the current chemical analysis conducted in 1996 and 1997.

The anaerobic experiment employing 50 mg l⁻¹ of 4-methylphenol was conducted in the presence of 1500 mg l⁻¹ of sulfolane. The result was compared with that obtained from the test including only 790 mg l⁻¹ of sulfolane as carbon source. Figure 8 reports that the degradation of sulfolane in the presence of 4-methylphenol was stimulated by 4 times greater degradation rate than that of sulfolane only applied for the reactor. It is well consistent with the result obtained by Zinder and Broek (1978a, 1978b). They used dimethyl sulfoxide (DMSO) and lactate as electron acceptor and electron donor. When DMSO was supplied with lactate,
the degradation rate of those compounds were significantly increased. It is expected that DMSO can play a role as electron acceptor when lactate was degraded in anaerobic condition.

Therefore, the degradation of sulfolane was accelerated when it was consumed as electron acceptor in the presence of 4-methylphenol. From the test, it was assumed that chlorophenol derivatives might be disappeared from the site as they could be used as an electron donor.

CONCLUSIONS

The anaerobic degradation experiment shows that sulfolane would be readily degraded under the natural conditions in the subsurface at the site. Although degradation has been demonstrated, the fate of sulfolane has not been fully described. The experiment shows a steady accumulation sulphur in solution, in the form of unidentified intermediate compounds.

The presence of electron donor can significantly accelerate the degradation of sulfolane since it behaves as electron acceptor in anaerobic condition.

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