Anaerobic bioventing of unsaturated zone contaminated with DDT and DNT


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Abstract Initial degradation of highly chlorinated compounds and nitroaromatic compounds found in munition waste streams is accelerated under anaerobic conditions followed by aerobic treatment of the degradation products. The establishment of anaerobic environment in a vadose zone can be accomplished by feeding appropriate anaerobic gas mixture, i.e., “anaerobic bioventing”. The gas mixture contains an electron donor for the reduction of these compounds. Lab scale study was conducted to evaluate potential of anaerobic bioventing for the treatment of an unsaturated zone contaminated with 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) and 2,4-dinitrotoluene (DNT). Hydrogen was used as the electron donor. Using the soil columns innoculate with anaerobic microorganisms, it was observed that by feeding a gas mixture of 1% hydrogen, 1% carbon dioxide and nitrogen, methanogenic conditions were established and DDT was reductively dechlorinated. 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD) accumulated as the intermediate product. The half life of DDT was calculated to be 8.5 months. DNT completely disappeared after six months of operation and no intermediates could be detected.

Keywords Anaerobic bioventing; DDT; DNT; munition wastes; pesticides; vadose zone

Introduction

Introduction and large scale production of synthetic halogenated organic chemicals over the last few decades has resulted in a widespread contamination of these compounds. These compounds exhibit very low solubility, and high toxicity coupled with a tendency to accumulate in food chains, make them particularly relevant targets for remediation activities. Halogenated compounds are relatively oxidized by the presence of halogen substituents, which are highly electronegative and thus more susceptible to reduction. Similarly, due to the electron withdrawing nature of nitro, nitroaromatic compounds are electron deficient impeding electrophilic attack by aerobic bacteria (Rieger and Knackmuss, 1995). The degradation of these compounds thus requires the presence of strong anaerobic conditions for degradation.

Bioventing is an in situ process appropriate for bioremediation in the unsaturated zone. It is the use of induced gas movement through unsaturated soils, with or without the addition of nutrients, to stimulate the growth of indigenous microbes that are capable of converting organic contaminants to less hazardous substances. It is an innovative technology for clean up of hazardous compounds in the vadose zone. Existing soil vapor extraction facilities can be easily converted to establish aerobic conditions promoting biodegradation. Bioventing has been successfully applied to remove a wide range of contaminants, especially petroleum hydrocarbons and BTEX compounds (Du Pont, 1993). It offers a cost-effective remediation technology as the required flow rates are much lower and the system rarely requires emission permitting or expensive off gas treatment (Ratz et al., 1997). Using the same gas delivery system as employed in aerobic bioventing, anaerobic gas mixture can be introduced to the unsaturated zone to establish anaerobic conditions and provide...
required amendments such as nutrient and electron donors. A gas mixture with low levels of \( \text{H}_2 \) and \( \text{CO}_2 \) in \( \text{N}_2 \) can be delivered to displace soil oxygen and drive the reduction reaction with \( \text{H}_2 \) as the reducing agent (Mihopoulos et al., 2000).

The objective of the work presented here was to demonstrate, on a laboratory scale, the feasibility of anaerobic bioventing. Bench scale reactors simulating columns of unsaturated soil, were operated to establish proper anaerobic conditions and promote the reduction (dechlorination and nitro reduction) reaction. DDT was chosen as a chlorinated pesticide while 2,4-DNT was selected to represent the class of nitroaromatic compounds. Though the use of DDT was banned in 1972 by the U.S. Environmental Protection Agency (USEPA) in 1972, DDT and its natural degradation products are still present in surface sediments at levels harmful to the benthic organisms, indicating a long half life for these compounds (Renner, 1998). Currently DDT is listed as a priority pollutant by the USEPA and many DDT-contaminated sites appear on the Superfund National Priority List (Sayles et al., 1997).

Microbial degradation of DDT and its residues is one of the mechanisms for loss of DDT from soil. The reduction in the levels of DDT in the field has also been observed with flooding and frequent tillage (Boul et al., 1994). Fertilizer application has also shown to be effective in the remediation of the DDT contaminated sites (Pritchard and Costa, 1991). Volatilization, erosion and uptake by plants and animals are other mechanisms by which DDT is lost from soil (Aislabie et al., 1997). Sayles et al., 1997, investigated the capability of powdered zero-valent iron to dechlorinate DDT and related compounds.

Nitroaromatic compounds like 2,4-dinitrotoluene (DNT) are major components in the production of propellants and widely used in industry (Freedman et al., 1996). Some of the possible products from DNT biodegradation are 2-amino-4-nitrotoluene (2-A-4-NT), 4-amino-2-nitrotoluene (4-A-2-NT) and 2,4-diaminotoluene (DAT) (Freedman et al., 1996). Some of the current treatment technologies for DNT in aqueous streams comprise fluidized bed anaerobic granular activated carbon reactors (Berchtold et al., 1995), photoxidation with hydrogen peroxide (Ho, 1986), aerobic degradation by Phanerochaete chrysosporium (Valli et al., 1992).

Materials and methods

Chemicals

DDT (99% purity) was purchased from Sigma Chemicals (St. Louis, MO, USA), and DNT (97% purity) was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). A calibration standard for methane (\( \text{CH}_4 \)), carbon dioxide (\( \text{CO}_2 \)), hydrogen (\( \text{H}_2 \)) and helium (\( \text{He} \)) balanced in \( \text{N}_2 \) was obtained from Scott Specialty Gases, Chicago, IL, USA. The sand used (ASTM # D-1556) was purchased from ELE International Soiltest, Lake Bluff, IL. CaCO\(_3\) was obtained from Fisher Scientific. The top soil was supplied by a local garden supply vendor (Cincinnati, OH, USA).

Column Operation

The unsaturated zone was simulated in the laboratory with Plexiglas columns (3” dia. × 6” long) filled with soil. The columns had three (1/4”) sampling ports, 6.5 cms apart, placed along the length of the columns. The columns were pressurized for leak detection before the experiments commenced. The operating conditions and parameters are given in Table 1. 1 kg of soil mixture consisting of 83% spiked sand, 5% sea-shells, 2% top soil and 10% water, all percentages on a weight basis, was charged to the columns. Sea-shells provided necessary buffering capacity to the soil. Total Kjeldahl nitrogen (TKN) was 100 mg/kg. The growth of photosynthetic organisms was prevented by keeping the columns foil wrapped. Inoculation of the columns was carried out by injecting 5 ml of anaerobic culture (Wilson et al., 1997) through each of the mid and effluent sampling ports.
Table 1 Operational Parameters for the Anaerobic Bioventing Columns

<table>
<thead>
<tr>
<th>Unit</th>
<th>Value</th>
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<tbody>
<tr>
<td>Column Height cm</td>
<td>15.0</td>
</tr>
<tr>
<td>Column Inside Diameter cm</td>
<td>7.62</td>
</tr>
<tr>
<td>Empty bed residence time min</td>
<td>480</td>
</tr>
<tr>
<td>Sand Porosity % v/v</td>
<td>0.4</td>
</tr>
<tr>
<td>DDT &amp; DNT initial Conc. mg/kg</td>
<td>100</td>
</tr>
</tbody>
</table>

Four columns (two each for DDT and DNT) were operated as up-flow anaerobic continuous reactors (Fig. 1). Gas delivery system consisted of a mass flow controller assembly and the gas composition was maintained at 1% hydrogen, 1% carbon dioxide, 5% helium balanced in nitrogen. Helium was detected as a passive tracer while hydrogen and carbon dioxide were fed as energy sources for the microorganisms. The anaerobic mixture was bubbled through a humidifier containing 100 mg/l sodium sulfite and 25 mg/l cobalt chloride ensuring that the gas would not dry out the soil to a point which would be detrimental to microbial growth.

The performance of columns was monitored weekly by withdrawing gas samples from the sampling ports with Pressure-Lok gas tight syringes (Dynatech Corp. Baton Rouge, LA) and analyzing immediately. The final concentrations of contaminants and intermediates were determined after operating one column for two months and the other for six months. The columns were also sectionally analyzed for pH and moisture content.

Analytical Methods

Gas Analysis: CO₂ and CH₄ were analyzed by GC (HP 5890 Series II) equipped with thermal conductivity detector using a 1 mL gas sample. At a carrier gas (He) flow of 30 ml/min, retention times for CO₂ and CH₄ were 2.402 and 5.29 min on a 6 ft × 1/8” od 80/100 HP Hayesep Q and 10 ft × 1/8” od 45/60 HP Molecular Sieve columns in series (50°C for 3.2 minutes, ramped to 80°C at 10°C/min). He and H₂ were routinely determined by GC (Hewlett Packard 5890 Series II) a thermal conductivity detector using a 0.5 ml gas sample. At a carrier gas (argon) flow of 30 ml/min, retention times for He and H₂ were 0.61 and 0.72 min on a 10 ft × 1/8” od 45/60 HP Molecular Sieve column (50°C isothermal).

![Diagram of the experimental setup](https://iwaponline.com/wst/article-pdf/43/2/35/429149/35.pdf)
DDT and degradation products: The compounds were extracted from soil by ultrasonication with methanol and methylene chloride being the extracting solvents. The extracts were analyzed by Hewlett-Packard 5890 Series II gas chromatograph (GC) fitted with DB-5 capillary column on ECD. The temperature was ramped from 100°C to 250°C at 20 degrees/min and was held at 250°C for 7 minutes. The detector (ECD) and the injection port were held at 300°C and 250°C, respectively. The carrier gas (helium) flow was set at 10 mL/min and the make-up gas (5% methane and 95% argon) at 50 mL/min.

DNT, 2-A-4-NT and 4-A-2-NT: Soxhlet extraction was carried out to remove the compounds from soil. Methanol and methylene chloride were used as the extracting solvents. These extracts were quantified on a Hewlett Packard High Performance Liquid Chromatograph HPLC 1050 Series provided with a diode array detector. The analysis was performed on an accubond C-18 (150 × 4.6 mm, J & W Scientific Inc., Folsom, California, USA) column with an isocratic mobile phase of 50% water and 50% acetonitrile at 0.7 ml/min flow rate.

DAT: Analysis of DAT was also accomplished on the same HPLC. However, the mobile phase composition was varied as follows:
0–5 min 99% water, 1% acetonitrile at 1 ml/min;
5–8.5 min 100% acetonitrile at 1 ml/min;
8.5–20 min 99% water, 1% acetonitrile at 1 ml/min.

The Standard Operation Procedure described in SW-846 9045B was followed to measure soil pH. Soil moisture was determined according to ASTM Method D 2216.

Results and discussions

DDT Columns

The gas analyses for DDT columns are shown in Figures 2 and 3. DDT column 1 represents the one operated for two months while DDT column 2 was run for a longer period of time (6 months).

Hydrogen was consumed to a significant extent in both the DDT columns. After 53 days of operation, no hydrogen was detected in the effluent for the DDT column 1 while no detectable level of hydrogen was measured in the second DDT column after 67 days. The difference in hydrogen uptake could be due to the different rate at which methanogens grow in the columns. The hydrogen was converted to methane and stoichiometric reduction in carbon dioxide concentration was measured. The methanogenic activity was sustained for the entire length of the experiment. Methanogenic activity in both the columns indicated a very low redox potential in the system and maintenance of strict anaerobic conditions. An oxygen contamination on the 78th day for the DDT column 2 resulted in increase in both hydrogen and carbon dioxide concentrations and drop in methane concentration in the effluent indicating that methanogenic activity required strict anaerobic conditions. However, the system did recover from the accident within two weeks and the methane concentration returned to its original value and remained steady till the end of the operation. The sectional analysis for the DDT columns is shown in Tables 2 and 3, where the initial DDT loading was 81.5 ± 2.2 mg/kg (0.23 mmol/kg).

The results indicate that DDT was reductively dechlorinated to DDD under methanogenic conditions. At the end of two months, the DDT levels in all three sections dropped by 10%, and stoichiometric conversion of DDT to DDD was observed. This is supported by the DDT + DDD mole balance (~0.23 mmol/kg). No other intermediates were detected. Further degradation of DDT occurred, upon operating the other DDT column for
a longer period of time. The three sections of the column showed 30%, 34% and 42% reduction in initial DDT concentration. DDD was the only intermediate detected. DDD levels also increased corresponding to decrease in DDT concentrations. The mole balance, however, indicates presence of other intermediates that were either not identified or were strongly adsorbed to the soil. The first order rate constant for DDT degradation based on the experiment is 0.0814 (month)^{-1}, giving a half life of DDT of 8.5 months. This half-life is much shorter than the natural degradation rate where a half life of DDT has been reported to be close to 20 years.

**DNT columns**

The gas analyses for the DNT columns are shown in Figs 4 and 5. The hydrogen concentration in the effluent was measured at about 4500 ppmv for the first two months of operation. It dropped below the detection limit for the DNT column 2 after four months and methane production began resulting in stoichiometric reduction in carbon dioxide influent levels. This could be attributed to the acclimation of the anaerobic culture to DNT sorbed onto

<table>
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<tr>
<th>Table 2</th>
<th>Sectional Analysis for DDT and degradation products</th>
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<tr>
<td></td>
<td>DDT (mg/kg)</td>
</tr>
<tr>
<td></td>
<td>2 Months</td>
</tr>
<tr>
<td>Top</td>
<td>74.1±3.5</td>
</tr>
<tr>
<td>Middle</td>
<td>72.1±1.4</td>
</tr>
<tr>
<td>Bottom</td>
<td>74.9±2.9</td>
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</tbody>
</table>

<table>
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<tr>
<th>Table 3</th>
<th>Sectional pH and Moisture Analysis for DDT columns.</th>
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<tbody>
<tr>
<td></td>
<td>Soil pH</td>
</tr>
<tr>
<td></td>
<td>2 Months</td>
</tr>
<tr>
<td>Top</td>
<td>8.7±0.04</td>
</tr>
<tr>
<td>Middle</td>
<td>8.7±0.07</td>
</tr>
<tr>
<td>Bottom</td>
<td>8.4±0.07</td>
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</table>
sand particles. Methanogenic activity was sustained thereafter for two more months before the column was sacrificed for analysis.

The results of sectional analyses for moisture, pH and parent compound analyses are shown in Table 4, where the initial DNT loading is 77.2±2.4 mg/kg.

The analysis performed after two months of operation showed no significant decrease in DNT concentration. However, for the second DNT column, the analysis indicated that DNT was below the detection limit and no intermediates could be detected. The complete disappearance of DNT could be attributed to the methanogenic activity in the column after four months of operation. Initially, DNT could have inhibited the growth of methanogens, following the acclimation period, methane formation commenced. The observation that DNT completely disappeared without any metabolites is consistent with what was shown by Noguera and Freedman, 1997. Their study indicated complete removal of 2,4-DNT without accumulation of any of the intermediates 2-A-4-NT, 4-A-2-NT and 2,4-DAT after 80 days of operation. In our case, venting was carried out after a period of 180 days under strong anaerobic conditions. Also, the exposure of soil samples to oxygen might have triggered a slow transformation of DAT to a dark brown polymer rendering DAT difficult to detect.

The soil pH for all the columns and in all sections did not vary much and remained close to 8.7. This shows that methanogenic activity could be sustained at higher pH values. The moisture content expressed as weight of water/weight of dry sand increased from top to bottom. This is consistent with the drainage of pore volume and accumulation of pore water in the deeper sections of the column.

Conclusion
The feasibility of anaerobic bioventing for the treatment of contaminants such as DDT and DNT loaded onto soil in the vadose zone was demonstrated. Under methanogenic conditions DDT was reductively dechlorinated to DDD. However, accumulation of DDD is also
of concern as it is also listed as a priority pollutant by the USEPA. In the present case, DDT is reductively dechlorinated to DDD, the first degradation product. However, further degradation of DDD may occur via the same metabolic pathway, yielding 1-chloro-2,2-bis(p-chlorophenyl)ethylene (DDMU) and further less chlorinated intermediates. However, these intermediates were not observed.

Similarly, reduction of DNT with hydrogen leads to intermediate products such as 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene. Complete reduction of nitro-groups results in the formation of 2,4-diaminotoluene. In this study complete removal of DNT was observed but no metabolites could be detected. From a toxicological point of view, the identification of transformation products from DNT is essential, as they may pose a similar or greater threat than the parent compound (Noguera and Freedman, 1997).

Hydrogen plays an important role as an electron donor in anaerobic bioventing systems. It can be easily delivered in the gaseous phase. Besides acting as a reducing agent, it promotes methane formation establishing a low redox potential in the soil environment and can be utilized by the $H_2$ (Knallgas) oxidizing bacteria to remove trace levels of oxygen (Aragno, 1998). These three reactions are described as follows:

**Electron Donor**

$$H_2 + R-\text{Cl} \rightarrow R-H + HCl$$

$$3H_2 + R-\text{NO}_2 \rightarrow R-\text{NH}_2 + 2H_2O$$

where $R$ can be any alkyl or aryl group.

**Methanogenesis**

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

$$HCO_3^- + H^+ + 4H_2 \rightarrow CH_4 + 3H_2O$$

**Hydrogen Oxidation**

$$2H_2 + O_2 \rightarrow 2H_2O$$

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**References**


