Degradation of TNT under Different Metabolic Regimes of Slurry Phase Bioreactor

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In this study, degradation of TNT under different metabolic regimes of slurry phase bioreactor for TNT-contaminated soil treatment was evaluated. The most rapid degradation of TNT was observed in aerobic treatment at the early stage of operation and its degradation efficiency was 71.5% until 60 days. After 200 days of operation, however, degradation efficiency was 93.2%. This was relatively low compared to anaerobic and anaerobic/aerobic treatments which showed degradation efficiencies of 97.0% and 95.0%, respectively. In anaerobic/aerobic treatment, the degradation of TNT was not significantly enhanced by subsequent aerobic stage compared to anaerobic treatment alone.

1. Introduction

The worldwide annual production of TNT (2,4,6-trinitrotoluene), the primary explosive used in the manufacture of munitions, is estimated at two million tons1. It is widely used in commercial explosives as a good sensitizer and is much safer in production and handling than nitroglycerine2. TNT has a melting point of 80.65°C and a boiling point of 240°C at which point it explodes. The water solubility is 120 mg/L. TNT is introduced into soil and water ecosystems mainly by military and manufacturing activities. TNT is toxic to a number of organisms including humans and may be carcinogenic. Because of its toxic and recalcitrant properties, the contamination of soil and groundwater by TNT represents a significant international environmental problem3.

Many researchers have studied the biological degradation of TNT and other hazardous energetic nitroaromatic compounds. However, most of the work done during the last few decades have been directed toward aerobic degradation and showed very little mineralization of TNT, mainly because the nitroso-substituents on the aromatic ring are resistant to electrophilic attack by oxygenases4. TNT has been found in particulate form (chunk) and heterogeneously dispersed in explosive contaminated sites5. Unfortunately, chunk TNT isn't broken down easily by the microbes and remains in the soil at the end of the composting period. Therefore, alternative method for increasing the biodegradation of TNT needs to be developed. Slurry phase treatment may offer an advantage in this case, since dissolution rates can be increased by agitation of the slurry. Slurry phase bioremediation has been demonstrated to be an effective process for contaminated soils, sediments, and sludges6. However, effectiveness of aerobic or anaerobic slurry phase bioremediation has rarely evaluated between the two.

This study was performed to evaluate the degradation of TNT under different metabolic regimes of slurry phase bioreactor for TNT-contaminated soil treatment.

2. Materials and Methods

2.1 Materials and Analysis

The soil used for this research was collected from the top 15 cm of the surface soil and sieved through a 2-mm sieve to remove large fraction such as stone and gravel. The soil was ground with mortar and pestle to obtain finer fraction. The texture of the soil was
classified as a typical loam (the portions of sand, silt, and clay of soil were 36.1%, 37.3%, and 26.6%, respectively) according to the USDA definition. The soil had a slight alkaline pH of 8.1 and contained organic matter of 4.5% by dry weight. Target contaminant of this research was TNT, which was spiked at 1,000 mg TNT/kg soil. Military-grade TNT was used for this research and its purity was about 76% by wet basis. Also, it contained ADNT (aminodinitrotoluene) and DNT (dinitrotoluene) as the minor purities.

TNT and their metabolites have properties such as thermal lability and polarity. Many of these compounds thermally degrade or explode at temperature below 300°C. Thus, the analytic methods based on gas chromatography are not recommended for routine use. Therefore, the TNT-extracted solution was analyzed using RP-HPLC (reversed-phase high performance liquid chromatography) method (SW-846 Method 8330A).

2.2 Experimental Condition

Different metabolic regime experiments were performed with anaerobic, anaerobic/aerobic, and aerobic reactors. To prevent photolysis of the added TNT, the reactors were incubated in the dark and operated at 30°C. In anaerobic/aerobic reactor, anaerobic stage was maintained for 60 days and then transferred to aerobic reactor that was operated until 200 days.

Molasses of 0.3% (w/v) as co-substrate was added to the slurry based on total solid in all reactors. Soil loading was 30% (w/v) slurry of TNT-contaminated soil in water. The phosphate buffer adjusted to neutral pH with KH₂PO₄ and K₂HPO₄ was added to the slurry in 50mM. The experimental condition for this research is shown in Table 1. Fig. 1 shows the bioreactor sets for this research.

Table 1 Experimental condition for this research

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Operational Condition</th>
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<tr>
<td>Anaerobic</td>
<td>Anaerobic stage during 200 days</td>
</tr>
<tr>
<td>Anaerobic/aerobic</td>
<td>Anaerobic stage for initial 60 days and aerobic for the last 140 days</td>
</tr>
<tr>
<td>Aerobic</td>
<td>Aerobic stage during 200 days</td>
</tr>
</tbody>
</table>

3. Results and Discussion

The performance of slurry phase bioreactors under different metabolic regimes is shown in Fig. 2. TNT concentration of anaerobic/aerobic treatment was decreased after subsequent aerobic stage. After 200 days of operation, however, degradation efficiency was above 95% in both anaerobic and anaerobic/aerobic treatment (Fig. 3). Among the different metabolic regimes studied, the most rapid degradation of TNT was observed in aerobic treatment at the early stage of operation and its degradation efficiency was 71.5% until 60 days. This was about three times larger than that of anaerobic treatment (Fig. 3). This result was consistent with the investigation by Craig et al. (1995), who evaluated slurry phase bioremediation technologies of TNT-contaminated soil and summarized based on DRE (destruction and removal efficiency) and treatment time². DRE of anaerobic treatment was 97.1% after operation period of 150 days, while aerobic treatment showed 99.1% of DRE within 35-70 days. Harvey et al. (1997) also evaluated performance of aerobic and anaerobic slurry phase treatment using bench scale slurry reactors³. Results indicated that aerobic treatment was more rapid than anaerobic approaches.

Fig. 2 Variation of TNT concentration in different metabolic regimes experiments
Breitung et al. (1996) investigated the issue of TNT-contaminated soils bioremediation using two different composting regimes (e.g. aerobic and anaerobic/anaerobic systems)\(^{10}\). In the anaerobic/anaerobic system, TNT was almost completely transformed to ADNT in the initial anaerobic stage. In the subsequent aerobic stage, TNT was almost entirely disappeared. In addition, the compost produced by anaerobic/anaerobic system was less toxic than that produced by aerobic system. Lenke et al. (1998) performed anaerobic/aerobic treatment of originally contaminated soil for a former ammunition plant in a laboratory slurry reactor \(^{11}\). The experiment showed that the anaerobic bacteria completely reduced the nitro groups of TNT and ADNTs. To mineralize the fermentation products after the anaerobic treatment, a subsequent aerobic treatment was necessary to complete the bioremediation process. The two primary benefits of the anaerobic/aerobic treatment were decreased foaming due to aeration and decreased formation of intermediates during the aerobic stage. In particular, the cycling between anaerobic and aerobic conditions is believed to select for a wider diversity of bacteria capable of rapidly reducing TNT and subsequently oxidizing the ring backbone of the parent TNT molecule.

**Fig. 4** shows variation of redox potential in anaerobic and anaerobic/aerobic reactors, and dissolved oxygen in aerobic reactor. The degree of oxidation or reduction of any system can be defined by the redox potential. It can be a more precise index of anaerobiology\(^{12}\). As shown in **Fig. 4**, Redox potential in anaerobic/aerobic reactor dropped to -220 mV by 60 days of anaerobic stage. After aerobic conversion, it rapidly increased up to 200 mV. Dissolved Oxygen in the aerobic reactors was monitored in order to confirm aerobic condition. DO concentration of initial sample was approximately 3 mg/L, and then dropped to 2 mg/L and remained throughout the experiment.

Degradation kinetic parameters based on the first order kinetic model in different metabolic regimes are presented in **Table 2**. The degradation of TNT in anaerobic and anaerobic/aerobic treatment was described with high correlation coefficients both zero order kinetic model (\(r = 0.98\) for anaerobic treatment and \(r = 0.97\) for anaerobic/aerobic treatment; data for zero order kinetics are not presented) and first order kinetic model (\(r = 0.96\) for anaerobic and anaerobic/aerobic treatment). In case of aerobic treatment, the correlation coefficients for the kinetic models indicated that the first order model (\(r = 0.96\)) described the degradation of TNT with high correlation compared to the zero order model (\(r = 0.89\)). Therefore, in this study the first order kinetics was used in comparing the degradation rate. The overall first order degradation rate (\(k\)) indicated that more active degradation of TNT occurred in anaerobic/aerobic treatment (\(k = 0.0175/\text{day}\)) than anaerobic or aerobic treatment. However, significant difference of the first order degradation rate constant (\(k\)) was not observed in them.

**Table 2** Degradation kinetics based on the first order kinetic models

<table>
<thead>
<tr>
<th>Reactor Condition</th>
<th>First order</th>
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<tr>
<td></td>
<td>(k_1)^(a)</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>0.0161</td>
</tr>
<tr>
<td>Anaerobic/aerobic</td>
<td>0.0056</td>
</tr>
<tr>
<td>Aerobic</td>
<td>0.0223</td>
</tr>
</tbody>
</table>

\(a\) First order kinetic constant and correlation coefficient (anaerobic stage from 0 to 60 days)

\(b\) First order kinetic constant and correlation coefficient (aerobic stage from 0 to 60 days)

\(c\) Overall first order kinetic constant (from 0 to 200 days)
Fig. 5 presents kinetic model parameters that were divided into two parts according to the conversion of metabolic regime in anaerobic/aerobic treatment. The first order degradation rate ($k_1 = 0.0056$/day) for anaerobic stage was slower than that ($k_2 = 0.0223$/day) of aerobic stage. The degradation rate of aerobic stage for the last 140 days was approximately 4 times higher than that of anaerobic stage for the initial 60 days.

![Graph showing kinetic model parameters for anaerobic/aerobic treatment.]

**Fig. 5** Linearization for evaluation of first order kinetic model in anaerobic/aerobic treatment.

Fig. 6 shows variation of ADNT metabolites concentration in different metabolic regimes. The major metabolites observed during TNT metabolism under different metabolic regimes were 4-ADNT, 2-ADNT and unknown metabolites. Bradley et al. (1995) investigated the ability of aquifer microorganisms to degrade TNT using aerobic microcosms [12]. The most intermediate products of TNT transformation were 4-ADNT and 2-ADNT. The concentration of 4-ADNT was at least twice that of 2-ADNT. They suggested that para nitro group was reduced most readily in aerobic condition. This pattern is consistent with this study. The 2-ADNT was predominately observed in the early stage of anaerobic stage, whereas the 4-ADNT was major metabolite in aerobic treatment.

**4. Conclusions**

The most rapid degradation of TNT was observed in aerobic treatment at the early stage of operation and its degradation efficiency was 71.5% until 60 days. After 200 days of operation, however, degradation efficiency was 93.2%. This was relatively low compared to anaerobic and anaerobic/aerobic treatment which showed degradation efficiencies of 97.0% and 95.0%, respectively. In anaerobic/aerobic treatment, the degradation of TNT was not significantly enhanced by subsequent aerobic stage compared to anaerobic treatment alone. The major metabolites observed during TNT metabolism under different metabolic regimes were 4-ADNT and 2-ADNT.

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