Anaerobic degradation of 2,4,6-trinitrotoluene in granular activated carbon fluidized bed and batch reactors

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Abstract: In this study, an anaerobic fluidized bed reactor (AFBR) was used to treat a synthetically produced pink water waste stream containing trinitrotoluene (TNT). The synthesized waste consisted of 95 mg/l-TNT, the main contaminant in pink water, which was to be co-metabolized with 560 mg/l ethanol. Granular activated carbon was used as the attachment medium for biological growth. TNT was reduced to a variety of compounds, mainly 2,4,6-triaminotoluene (2,4,6-TAT), 2,4-diamino-6-nitrotoluene (2,4-DA-6-NT), 2,6-diamino-4-nitrotoluene (2,6-DA-4-NT), 2-amino-4,6-dinitrotoluene (2-A-4,6-DNT), and 4-amino-2,6-dinitrotoluene (4-A-2,6-DNT). These conversions resulted through the oxidation of ethanol to carbon dioxide under anoxic conditions, or reduction to methane under methanogenic conditions. The anaerobic reactor was charged with 1.0 kg of 16×20 U.S. Mesh Granular Activated Carbon (GAC) and was pre-loaded with 200 g of TNT prior to the addition of the mixed seed culture. During the first three weeks of operation, ethanol was completely degraded and no methane was produced. Effluent inorganic carbon revealed stoichiometric conversion of the feed ethanol to dissolved inorganic carbon with accumulation of carbon dioxide in the headspace of the reactor. GAC extraction showed incremental reduction of the nitro groups to amino groups, with 2,4,6-TAT as the final product. After three weeks, the oxygen from the nitro groups was depleted and methane production commenced. The reproducibility of this phenomenon was confirmed by repeating the experiment in the same manner using an identical AFBR. Furthermore, serum bottle tests were conducted using TNT loading ratios of 0.2, 0.4, 0.8, 1.0 g-TNT/g-GAC as well as experiments in the absence of GAC. Similar behavior to that of the columns was observed, with degradation rates varying according to the particular condition. GAC greatly enhanced the degradation rates and the higher TNT loading resulted in slower degradation rates of ethanol.

Keywords: Anaerobic; biodegradation; fluidized-bed; methanogenic bacteria; TNT

Introduction

2,4,6-Trinitrotoluene (TNT) is classified by the USEPA as a priority pollutant and, based on animal studies, as a possible human carcinogen. It is frequently found in the contaminated areas of munitions manufacturing plants. Long exposure to TNT causes serious health problems including anemia, abnormal liver function, cataract development and skin irritation. The increasing number of contaminated sites due to demilitarization and disposal of obsolete weapons increases the need for more innovative clean-up techniques. Current munitions waste disposal practices include discharge to deep-sea disposal sites, landfilling, incineration when the quantities are small, and adsorption followed by incineration of the spent activated carbon.

Biological systems such as the AFBR offer promising alternatives to the currently employed disposal strategies. The AFBR has been proven effective in treating various inhibitory wastes such as coal gasification wastewater, and wastewater containing chlorinated hydrocarbons and phenols, dinitrotoluene, and formaldehyde (Suidan et al., 1983, 1991, 1997, 1998). The adsorptive capacity of the GAC serves both as a buffer against perturbation in waste loads and an excellent attachment medium for biological growth. A previous study showed that it is possible to stoichiometrically reduce 2,4-dinitrotoluene to...
2,4-diaminotoluene (2,4-DAT) under anaerobic conditions, followed by aerobic mineralization of 2,4-DAT (Berchtold et al., 1995).

TNT is almost refractile under aerobic conditions and therefore requires an anaerobic step before it can be metabolised. Furthermore, although denitrification is often a major reaction in the biodegradation of compounds containing nitro groups, it is rarely the case with TNT. Martin et al. (1997) reported that P. savastanoi can reduce TNT to 2,4-DNT. Reduction of TNT nitro groups to amino groups, resulting in the formation of amino-nitro compounds, is often reported. For example, Boopathy et al. (1998) reported 2-A-4,6-DNT, 4-A-2,6-DNT, 2,4-DA-6-NT and toluene as the byproducts under different electron donor and acceptor conditions. Preuss et al. (1993) observed the formation of TAT from TNT by sulfate reducing bacteria isolated from sewage sludge. They also reported that the nitro groups in positions 2 and 4 are easily reduced, with position 6 being the rate-limiting step in the reduction process of the intermediate 2,4-diamino-6-nitrotoluene. Funk et al. (1995) reported the remediation of TNT contaminated soil in an anaerobic soil-slurry reactor, using potato starch as a co-substrate. p-Cresol as well as the compounds listed above was identified among the end products.

The objective of this research project is to study the effectiveness of the AFBR in the treatment of pink water containing TNT. Special emphasis was placed on the identification of the TNT degradation intermediates and products.

Materials and methods

Anaerobic bioreactor

The 9.1 l bioreactor consisted of a jacketed main column, an influent header, and an effluent header (Figure 1). The inner-jacketed tube (96.5 cm long, 10.2 inner diameter) was constructed of Plexiglas and was enclosed in an outer Plexiglas tube. Water was circulated through the annular space from a constant temperature bath (model MW1120A Magna Whirl, Blue M Electric Co., Blue Island, IL) to maintain a constant temperature of 35°C within the column. The recycle lines were constructed from polyvinyl chloride tubing and the feed and effluent lines were Tygon and neoprene tubing. The column was charged with 1.0 kg of 16×20 U.S. Mesh Filtrasorb 400 GAC (Calgon Corporation, Pittsburgh, PA) and pre-loaded with 200 grams of TNT. Effluent recycle was used to maintain a bed-expansion of 50%. The influent header was filled with marbles to distribute the flow evenly across the column cross section. The effluent header served to separate and convey the liquid effluent and off gas to respective effluent ports.

The column was equipped with a side arm. The entrance to this side arm was above the effluent header and the exit was below the recycle withdrawal port inside the main column. The purpose of this side-arm was to provide a port for the introduction of preloaded GAC or solid TNT, if necessary, without being caught in the recycle stream. The column was also equipped with a GAC withdrawal port located at the top of the effluent header. This allowed the GAC to be withdrawn from the column using a constant volume cup that could be lowered to the desired level in the bed to ensure representative sampling of the GAC medium. The buffer and nutrient solutions were fed into the recycle lines using pump drives (model 7015-20 pump head for the buffer and model 7016-20 pump head for the nutrients, Cole-Palmer Instruments Co., Chicago, IL). Bacterial growth in the feed reservoirs and feed lines was minimized by separating the growth nutrients and buffer solutions into different feed reservoirs and by pumping the nutrient and buffer solutions into the recycle line at different points in the recycle loop.

Chemicals. TNT and all its byproducts were provided to the University of Cincinnati by Construction Engineering Research Laboratory (CERL), Urbana, IL. Salts, nutrients, and
carbonate were purchased from Fisher Scientific, Pittsburgh, PA, and were 95% purity or greater.

**Seed culture.** The seed culture was obtained from an anaerobic digester from a municipal wastewater treatment plant in Urbana, IL.

**Methods**

The reactor was monitored daily for feed flow rates, temperature, and effluent pH. Daily anaerobic gas production was measured with a wet tip gas meter (Environmental and Water Research Engineering, Nashville, TN). The off-gas composition was determined daily. Aqueous effluent samples were withdrawn daily from the reactor to analyze for DOC, IC, volatile fatty acids, TNT, and TNT byproducts. Aqueous effluent samples were withdrawn weekly from the anaerobic bioreactor to analyze for alcohols, nitrate, and ammonia. GAC and effluent samples were withdrawn three times during the study for PLFA analyses of the attached and suspended biomass. GAC samples were also withdrawn monthly for extraction and analyzed for TNT and byproducts. Aqueous samples for the various analysis were filtered through 0.22 mm Magna Nylon supported plain filters (Micron Separations Inc., Westboro, MA) and were kept at a pH of approximately 7.20.

**Gas composition.** Effluent gas samples from the anaerobic reactor were analyzed for nitrogen, oxygen, carbon dioxide and methane with a Hewlett Packard 5890 Series II GC with a thermal conductivity detector (TCD). Argon was used as the carrier gas through a 3.2-mm ID, 3-m steel column packed with 45/60 molecular sieve (Hewlett-Packard Company, San Fernando, CA).

**Volatile fatty acids (VFAs).** Volatile fatty acids (acetic, propionic, butyric, iso-butyric, valeric, and formic acids) were analyzed by 1050 series Hewlett Packard High Performance Liquid Chromatograph (HPLC) equipped with a diode array detector. The column used for the analysis is a resin packed type, 300 mm × 7.8 mm, Aminex HPX-87H (Bio-Rad, Hercules, CA). The mobile phase was sulfuric acid 0.01 N at a flow rate of 0.75 ml/minutes.

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![Diagram of AFBGAC Reactor](https://iwaponline.com/wst/article-pdf/43/1/67/428676/67.pdf)

Figure 1 Anaerobic fluidized bed bioreactor
Dissolved organic carbon and inorganic carbon (DOC and IC). The pH of the aqueous sample was first reduced below 2 with hydrochloric acid. Subsequent purging was with ultra pure air, with less than 0.1 ppm hydrocarbons, to strip inorganic carbon as CO₂. The DOC was then determined by injection into a Shimadzu DOC analyzer, model 5000 (Shimadzu Corp., Tokyo, Japan). Inorganic carbon of non-acidified samples was measured using the same instrument.

Ammonia. The ammonia concentration was measured with model 720A Orion pH meter (Orion Research Co., Boston, MA) using a model 13-620-505 ammonia ion selective electrode (Fisher Scientific, Pittsburgh, PA) and an Orion model 215284-A01 ATC probe.

Nitrate/nitrite. Standard Method 418A (16th ed.) includes UV absorption at 220 nm with subtraction of interference by dissolved organic matter estimated by absorption at 275 nm. Samples were acidified with sulfuric acid to convert any nitrite to nitrate prior to sample storage. A minimum of 24 hours was allowed for complete conversion of the nitrite.

Alcohols. Ethanol was analyzed by aqueous injection into a Hewlett Packard 5890 gas chromatograph equipped with flame ionization detectors.

2,4,6-Trinitrotoluene and byproducts. TNT, 2-A-4,6-DNT, 4-A-2,6-DNT, 2,4-DA-6-NT, and 2,6-DA-4-NT were analyzed using a Hewlett Packard High Pressure Liquid Chromatograph HPLC 1050 series equipped with a diode array detector. The column used was an Accubond C-18 (150×4.6 mm, J & W Scientific, Inc., Folsom, CA). An isocratic mobile phase of 50% water and 50% acetonitrile was used to elute the amino-nitro compounds. Flow rate was kept at 0.7 ml/min and detection limits of TNT were at 17 ppb. The identification of TNT byproducts, with the exception of TAT, was confirmed using a Hewlett Packard GC/MS.

2,4,6-Triaminotoluene (TAT). TAT was analyzed separately on the HPLC, HP 1050 series using a Supelcosil LC-CN column (250×4.6 mm, Supelco, Bellefonte, PA). The mobile phase consisted initially of 1% acetonitrile and 99% of 10 mM dibasic sodium phosphate buffer for a period of 5 minutes. The gradient increased to 100% acetonitrile over 1.5 minutes and was maintained for 2 minutes; then reduced to 1% again over 4.5 minutes. Flow rate of the mobile phase was kept at 1.0 ml/min.

Results and discussion
The AFBR was operated on a synthesized pink water waste stream for a continuous operating period of 240 days. The wastewater, which was introduced into the reactor at a flow rate 6.1 litres per day, contained 95 mg/l of TNT and 560 mg/l of ethanol, which served as the primary substrate. The AFBR was charged with 1.0 kg of 16×20 U.S. Mesh Granular Activated Carbon (GAC), which serves as an attachment medium for biological growth. The GAC was pre-loaded with 200 g of TNT prior to the addition of the seed culture. The reactor was seeded with anaerobic digester sludge from the municipal wastewater plant in Urbana, Illinois. The combined volume of the anaerobic bioreactor and the recycle loop was 10 litres, resulting in a system Hydraulic Retention Time (HRT) of approximately 1.5 days. Based on the volume of the GAC and the attached biomass, the Empty Bed Contact Time (EBCT) of the reactor was approximately 9 hours.

The AFBR was monitored daily for pH, flow rates, temperature, and gas production. During the first 20 days of operation, the effluent remained void of ethanol, VFAs, TNT, or any of its byproducts. However, no methane production was observed. On day 18, the efflu-
ent was analyzed for inorganic carbon and the results showed an almost equivalent conversion of the feed ethanol into dissolved inorganic carbon. (Figure 2). This observation led us to conclude that ethanol was oxidized to carbon dioxide through the reduction of TNT to TAT according to the following equation:

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1.5 \text{C}_2\text{H}_5\text{O} + \text{C}_7\text{H}_5\text{N}_3\text{O}_6 \xrightarrow{\text{microorganisms}} \text{C}_7\text{H}_11\text{N}_3 + 3\text{CO}_2 + 1.5\text{H}_2\text{O}
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Figure 3 shows the time-varying influent and effluent loading of TNT and its byproducts for the operating period between days 140 and 240. The data prior to day 140 are not shown since TAT was not measured during that period. It is important to note that between days 140 and 240 TNT was introduced into the reactor in solid form at the rate of one gram per day in addition to its normal concentration in the feed in order to stress the system and accelerate reactor response. The data in Figure 3 reveal that the reduction of TNT was essentially complete with TAT as the main TNT byproduct in the effluent. Between days 140 and 240 of operation, TNT appeared in the reactor effluent as TAT, while the remaining TNT and its byproducts were retained on the GAC. Attempts to quantify the mass of TNT and its byproducts that were adsorbed on the GAC were unsuccessful since the extraction efficiency of these compounds was relatively very low. Vanderloop et al. (1994) reported similar low extraction efficiencies for TNT and TAT.

Since the initial phase of the first experiment was not well characterized, this experiment was repeated using the same initial and operating conditions. The data in Figure 4 show a
carbon balance on the new experiment. These data confirm that during the first 22 days of the experiment, ethanol was oxidized to CO₂ through the reduction of the nitro groups of TNT. The data from the second experiment present a good replicate of those obtained from the first experiment, confirming the reproducibility of the results. Between days 2 and 10, the effluent DOC was primarily due to ethanol, while between days 22 and 49, the effluent DOC was primarily due to ethanol, while between days 22 and 49, the effluent DOC was primarily due to ethanol, while between days 22 and 49, the effluent DOC was primarily due to ethanol, while between days 22 and 49, the effluent DOC was primarily due to ethanol, while between days 22 and 49, the effluent DOC was primarily due to ethanol, while between days 22 and 49, the effluent DOC was primarily due to ethanol, while between days 22 and 49, the effluent DOC was primarily due to ethanol, while between days 22 and 49, the effluent DOC was primarily due to ethanol, while between days 22 and 49, the effluent DOC was primarily due to ethanol, while between days 22 and 49, the effluent DOC was primarily due to ethanol, while between days 22 and 49, the effluent DOC was primarily due to ethanol, while between days 22 and 49, the effluent DOC was primarily due to ethanol, while 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DOC was primarily due to VFA. During other periods, the effluent DOC was consistently below 7 mg/l and was of an unidentified nature. GAC samples withdrawn from the second AFBR run were frequently collected and extracted. While the extraction efficiency was very low, no compounds with residual nitro groups were observed after day 25 suggesting that TNT was completely reduced to TAT in the reactor. Figure 5 shows the GAC extraction results.

**Figure 7** Batch reactors with GAC (0.2 g-TNT/g-GAC)

**Figure 8** Ethanol degradation in batch reactors with various TNT loadings

**Figure 9** Inorganic carbon accumulation in batch reactors with various TNT loadings

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The biodegradation of TNT was evaluated in serum bottles (160 ml total volume) under conditions similar to those maintained in the AFBR. The role of GAC was examined, as well as any possible abiotic transformation of TNT. The serum bottles were divided into six groups in order to evaluate under all permutations the role of GAC, inorganic nutrients, and the seed culture in the biodegradation of TNT. No transformation of the TNT was observed under abiotic conditions, while GAC was found to greatly enhance the rate of biodegradation of ethanol and TNT (Figures 6 and 7). Another set of batch reactors was prepared to examine the effect of the mass of TNT present in the serum bottle on the degradation rates of ethanol. The reactors were divided into two groups, with one group having the TNT preadsorbed on one gram of GAC while the same mass of TNT was added to the second set of serum bottles without any addition of GAC. The mass of TNT tested in the serum bottles was 0.2, 0.4, 0.8, and 1.0 g. The data in Figure 8 suggest that higher loading of TNT on the GAC tended to decrease the rate of ethanol oxidation. This may be due to the reduced availability of GAC active sites with increased TNT loading on the GAC. This observation also suggests that the free GAC surface may serve as a catalyst for the oxidation reduction reactions involved.

The data in Figure 9 show the accumulation of inorganic carbon in the batch reactors. Similar to what was observed in Figure 8, inorganic carbon appeared to accumulate at a faster rate when the TNT adsorbed per gram of GAC was lower. All the other serum bottles that contained no GAC showed much lower rates of ethanol reduction with a maximum transformation of 47.2% occurring in one set after 40 days. No methane or VFA formation was observed during the 40 days incubation of the serum bottles suggesting that coupled ethanol oxidation and TNT reduction was responsible for the observed transformations.

Conclusions

The data reported in this paper provide new insights into the anaerobic degradation of 2,4,6-trinitrotoluene. The oxidation potential of all the nitro groups was utilized to mineralize ethanol to carbon dioxide with a concomitant reduction of TNT to TAT. Depending upon the availability of the oxidizing equivalents of TNT, the remaining ethanol was fermented to methane and carbon dioxide. The AFBR was shown to be an effective process for the complete transformation of TNT to TAT. Preliminary data suggest that the GAC surface plays an important role in catalyzing the coupled ethanol oxidation and TNT reduction reactions.

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References


