ELSEVIER

Journal of Hazardous Materials 49 (1996) 1–14

JOURNAL OF HAZARDOUS MATERIALS

Aerobic biodegradation of 2,4-dinitrotoluene, aminonitrotoluene isomers, and 2,4-diaminotoluene

D.L. Freedman [a,*], R.S. Shanley [b], R.J. Scholze [b]

[a] *Department of Civil Engineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801 USA*
[b] *U.S. Army Construction Engineering Research Laboratories, Champaign, IL 61821 USA*

Received 1 September 1995; accepted 5 December 1995

Abstract

2,4-Dinitrotoluene (DNT) is widely used in industry, including the manufacture of propellants. 2,4-Diaminotoluene (DAT), 2-amino-4-nitrotoluene (2A4NT), and 4-amino-2-nitrotoluene (4A2NT) are among the products that can result from biological treatment of DNT. The objective of this study was to examine the biodegradability of these hazardous compounds. Using an inoculum from a munitions wastewater treatment plant, enrichment cultures were developed that readily consumed DAT, 4A2NT, 2A4NT, and DNT when they were provided as sole organic substrates (84–100 mg l^{-1}) or when ethanol (600 mg l^{-1}) was added as a cosubstrate. Low effluent chemical oxygen demand (COD) levels suggested that the aromatics were mineralized, rather than simply transformed to other possibly hazardous compounds. The addition of ether (142 mg l^{-1}) as a cosubstrate had no significant effect on the biodegradation of DAT and 4A2NT, but did inhibit the consumption of DNT and 2A4NT. The presence of ether in munitions wastewater may therefore present a challenge to effective biological treatment. When DNT was added to enrichment cultures as sole substrate, nitrite was released stoichiometrically. However, when ethanol was also added, nitrite release was only 59% of the maximum, indicating that most of the DNT was reduced prior to ring cleavage. When provided to enrichment cultures as the sole substrates, the observed yield coefficients for the aromatic compounds ranged from 0.10 to 0.28 mg of volatile suspended solids per mg COD.

Keywords: Aerobic biodegradation; Diaminotoluene; Dinitrotoluene

1. Introduction

Nearly one billion pounds of 2,4-dinitrotoluene (DNT) are produced annually in the United States, most of which is used as a chemical intermediate during the manufacture of polyurethanes, pesticides, and rubber chemicals [1]. DNT is also an important

* Corresponding author. Fax: 217-333-9464; e-mail: freedman@ux1.cso.uiuc.edu.

PII S0304-3894(96)01756-6

component of propellants used by the U.S. military. Release of DNT to the environment is a concern because, like most nitrated aromatic compounds, DNT poses a potential health risk. It is on the Environmental Protection Agency's Drinking Water Priority List, which includes substances known or anticipated to occur in public water systems that might require regulation under the Safe Drinking Water Act Amendments. In Virginia, a maximum permissible discharge limit for DNT in wastewater from munitions manufacturing has recently been set at 113 $\mu g\ l^{-1}$ [2].

Biological treatment of DNT-contaminated water is a potentially low cost alternative to physical and chemical methods such as activated carbon adsorption. The most widely recognized pathway for DNT biotransformation is reductive, resulting in the formation of metabolites such as 2-amino-4-nitrotoluene (2A4NT), 4-amino-2-nitrotoluene (4A2NT), 2,4-diaminotoluene (DAT), azoxytoluene isomers, and 4-acetamido-2-nitrotoluene [3,4]. Reduction of the nitro groups to amines proceeds through nitroso and hydroxylamino intermediates, both of which are very unstable and therefore difficult to identify [5]. The distribution of reduction products appears to depend, at least in part, on redox conditions. For example, reduction of DNT to the aminonitrotoluene isomers has been demonstrated under aerobic, anoxic and anaerobic conditions. Formation of azoxy compounds is restricted primarily to aerobic conditions, and significant reduction of DNT to DAT appears to occur only in the absence of oxygen. Stoichiometric reduction of DNT to DAT has been demonstrated in an ethanol-grown methanogenic enrichment culture [6,7].

Only one report thus far has demonstrated a substantial degree of DNT oxidation under aerobic conditions. Spanggord et al. [8] isolated a *Pseudomonas* species that uses DNT as a sole organic substrate. When ammonia was provided as a nitrogen source, oxidation of DNT led to the stoichiometric release of nitrite. Oxidation of DNT by the lignin-degrading fungus *Phanerochaete chrysosporium* has also been reported, although less than one third of the DNT carbon was recovered as CO_2 [9]. Glucose was required as a cosubstrate for the fungus. This is of interest because, in many environments where DNT is a contaminant, other organics are likely to be present. In munitions manufacturing wastewater, for example, high levels of ethanol and ether are typically found along with DNT [2].

Reductive biotransformation of DNT is a concern because the aromatic metabolites also pose a health hazard. DAT is a known animal carcinogen, and 2A4NT is an experimental carcinogen and mutagen [10,11]. Comparatively few health studies have been undertaken with 4A2NT, perhaps because it has not been produced in commercially significant quantities.

Compared with DNT, relatively little is known about the microbial biodegradability of the aminonitrotoluene isomers and DAT. Matsui et al. [12] tested the treatability of 35 organic compounds from the petrochemical industry and concluded that DAT was more readily biodegradable in activated sludge than DNT. Successful treatment of DAT in activated sludge has also recently been demonstrated by Suidan and coworkers [2,7,13]. However, no studies were found that specifically examined the biodegradability of 2A4NT or 4A2NT.

The objective of this study was to evaluate the relative biodegradability of 2A4NT, 4A2NT, DAT, and DNT under aerobic conditions, using inoculum from a munitions

manufacturing wastewater treatment plant. Treatment of each compound was examined in the presence and absence of other organic substrates.

2. Materials and methods

2.1. Chemicals and analytical methods

DNT, 2A4NT, 4A2NT, and DAT were obtained from Aldrich Chemical Company ($\geq$ 98% purity). They were added to cultures as high-concentration solutions prepared in basal medium (BM; described below). The average concentrations were: 160 mg l^{-1} for DNT, 200 mg l^{-1} for 2A4NT and 4A2NT, and 500 mg l^{-1} for DAT.

Aromatic compounds were measured by reversed phase high performance liquid chromatography (HPLC) using a 25 cm C-18 column (Vydac) in conjunction with a Waters Module 1 system. Detection was by UV at 254 nm. Isocratic separation of DNT, 2A4NT, and 4A2NT was achieved with a mobile phase consisting of 57.7% methanol, 41.6% water, and 0.7% triethylamine (v/v/v) at a flow rate of 1.5 ml min^{-1}. Isocratic analysis of DAT was performed with 39.5% methanol, 59.5% water, and 1.0% triethylamine at a flow rate of 1.3 ml min^{-1}. The lower percentage of methanol in the mobile phase for DAT analysis was necessary to increase its retention time on the C-18 column. Addition of triethylamine to the mobile phase greatly improved peak resolution for all compounds, especially DAT.

Samples were prepared for analysis of aromatics by mixing a 1.0 ml aliquot with 1.0 ml of methanol, mixing vigorously, and then filtering (0.45 μm PTFE). Adding methanol desorbed any of the aromatics and their metabolites that were partitioned to the biomass, and also minimized adsorption of the aromatics onto the filter [14]. Standards were prepared by dissolving the aromatics in methanol, mixing with an equal volume of deionized water, and filtering. The average recovery after filtration through PTFE filters ranged from 93% for 2A4NT to 98% for DAT. As the standards and samples were treated identically, no correction was made for these minor filtration losses.

Nitrate and nitrite were analyzed by HPLC (same system as above), using a Waters IC-Pack Anion HR column and a glucose borate/gluconate buffered mobile phase (1.0 ml min^{-1}), with UV detection at 214 nm. Samples were prepared by diluting the filtered methanol/sample mixture 50-fold with deionized water. Chemical oxygen demand (COD) and volatile suspended solids (VSS) were determined according to Standard Methods [15].

2.2. Cultures and experimental design

The biodegradability of each aromatic compound was examined using inoculum from the wastewater treatment system at the Radford Army Ammunition Plant (Radford, VA). DNT is manufactured at this facility and is a component of their wastewater. Secondary treatment at Radford consists of rotating biological contactors followed by secondary clarifiers. A sample of active biomass was obtained from the clarifier underflow.

Treatability studies were started by combining 1 ml of the inoculum with 99 ml of BM, described by Spanggord et al. [8]. The main constituents of the medium include a phosphate buffer (0.7 g l^{-1} of K_2HPO_4 and 0.3 g l^{-1} of KH_2PO_4), ammonium sulfate (0.5 g l^{-1}), and trace elements. The cultures were incubated at room temperature, on a shaker table, in 250 ml amber serum bottles, and in the dark (to minimize the possibility of photodegradation of the aromatic compounds). Whenever ether was added as a cosubstrate (see below), the serum bottles were sealed with gray butyl rubber septa and screw caps to prevent volatilization losses of the ether. Prior to sealing the ether-amended bottles, the headspaces were purged with pure oxygen, to ensure at least a two-fold excess of the terminal electron acceptor.

After setting up the bottles, approximately 0.55 mM of each aromatic compound was added. This represents a typical DNT concentration in one of Radford's process waste streams. Following several days of incubation, the concentration of aromatic compound remaining in the mixed liquor was measured by HPLC. Addition of methanol to the sample (see above) ensured recovery of aromatics that had adsorbed to the biomass. The culture was then centrifuged to retain the biomass, some of the clarified liquid was withdrawn, and an equivalent volume of BM plus aromatic-containing BM was added so that the aromatic concentration was returned to approximately 0.55 mM. COD, NO_3^- and NO_2^- were measured in aliquots of the clarified liquid.

Duplicate serum bottles were set up for each aromatic compound and tested under three substrate conditions: only the aromatic compound (0.55 mM) was added, the aromatic compound and ethanol (600 mg l^{-1}) were added, and the aromatic compound plus ether (142 mg l^{-1}) were added. The ethanol and ether concentrations are typical of one of Radford's process waste streams that contains DNT.

3. Results

3.1. Biodegradability of DAT, 4A2NT, 2A4NT and DNT

The biodegradability of DAT was first examined with ethanol added as a cosubstrate. As shown in Fig. 1(a), disappearance of DAT was somewhat erratic over the first 17 days, but thereafter each 0.55 mM addition was consumed within 2–4 days. Although ethanol levels were not measured, the minor amount of COD in the clarified effluent (< 30 mg l^{-1}) suggested that essentially all of the added ethanol and DAT were mineralized (Fig. 1(b)). When only DAT was added between days 39 and 56, there was no apparent impact on DAT consumption, and effluent COD actually decreased slightly (91% COD removal). Between days 56 and 77, addition of ether along with DAT did not significantly alter DAT consumption. However, the effluent COD rose noticeably in this interval, due to incomplete oxidation of the DAT or ether. During the final phase of operation (days 77–99), only DAT was added. With one exception (day 86), DAT was again readily consumed as a sole substrate every 2–4 days, and very low COD in the effluent suggested that oxidation was occurring.

The pattern with 4A2NT was similar to that with DAT. Following an acclimation period, 4A2NT was repeatedly biodegraded when added together with ethanol (Fig.

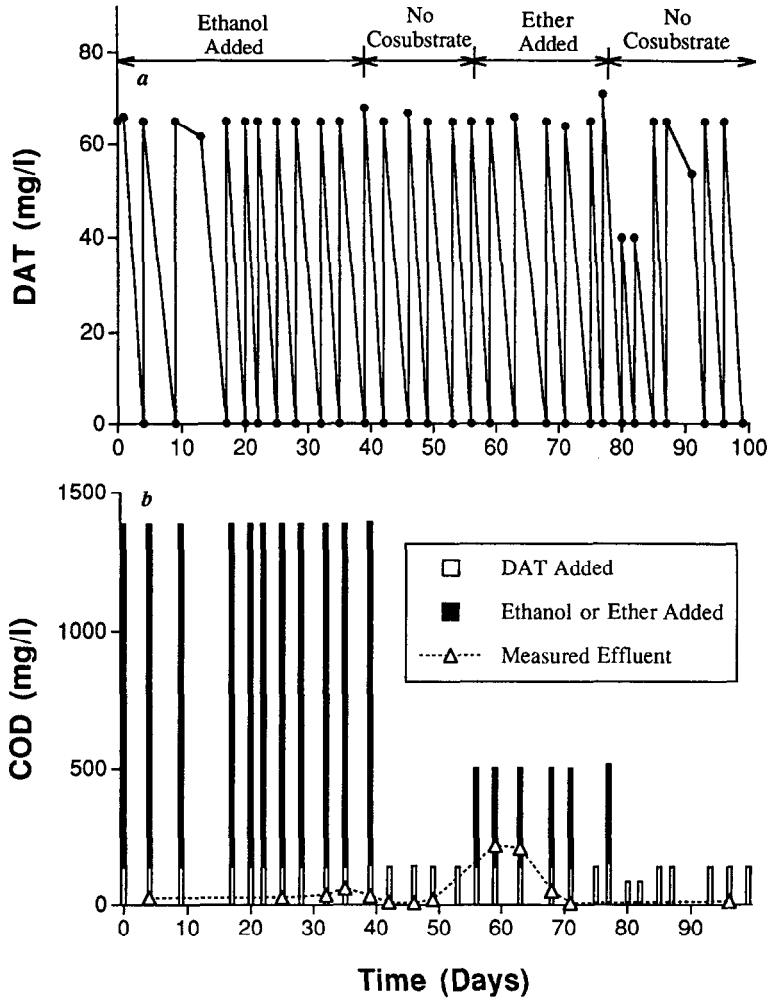

Fig. 1. Biodegradation of DAT, showing (a) repetative additions, with and without ethanol and ether added; and (b) COD contributed by DAT plus ethanol or ether, and soluble COD in effluent samples. Results shown are for one bottle of a pair; the behavior of the duplicate was very similar.

2(a)). Two effluent COD measurements in this interval demonstrated nearly complete mineralization of both compounds (Fig. 2(b)). When only 4A2NT was added, continued good removal was observed, and low effluent COD levels were maintained (82% COD removal). The addition of ether had no apparent effect on 4A2NT removal, and COD levels continued to stay below 35 mg l^{-1}. During the last phase of operation, when only 4A2NT was added, the cultures continued their efficient removal of the compound.

The initial pattern with 2A4NT was similar to that for 4A2NT. Repeated biodegradation in the presence of ethanol was observed after an acclimation period of approximately 20 days (Fig. 3(a)). Effluent COD measurements made near the end of this

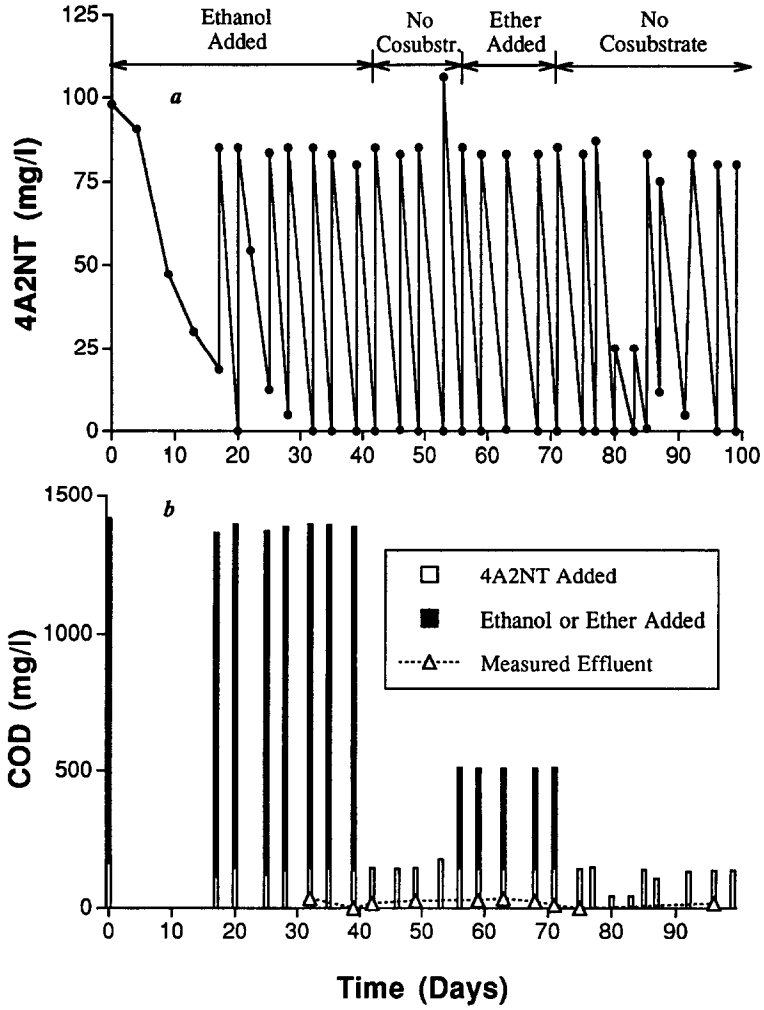

Fig. 2. Biodegradation of 4A2NT, showing (a) repetitive additions, with and without ethanol and ether added; (b) COD contributed by 4A2NT plus ethanol or ether, and soluble COD in effluent samples. Results shown are for one bottle of a pair; the behavior of the duplicate was very similar.

interval demonstrated essentially complete mineralization of both compounds (Fig. 3(b)). When only 2A4NT was added, continued good removal was observed (82% COD removal). However, unlike the behavior of DAT and 4A2NT, addition of ether on days 56 and 59 was extremely inhibitory to 2A4NT biodegradation, and resulted in a significant rise in the COD of the clarified effluent. The rate of 2A4NT biodegradation did improve when no more ether was added, but not quite to the pre-ether rate.

It was more difficult to establish consistent biodegradation of DNT in the presence of ethanol than for any of the other compounds. As shown in Fig. 4(a), DNT consumption within each 2–4 day monitoring interval was not predictable until day 39. Effluent COD

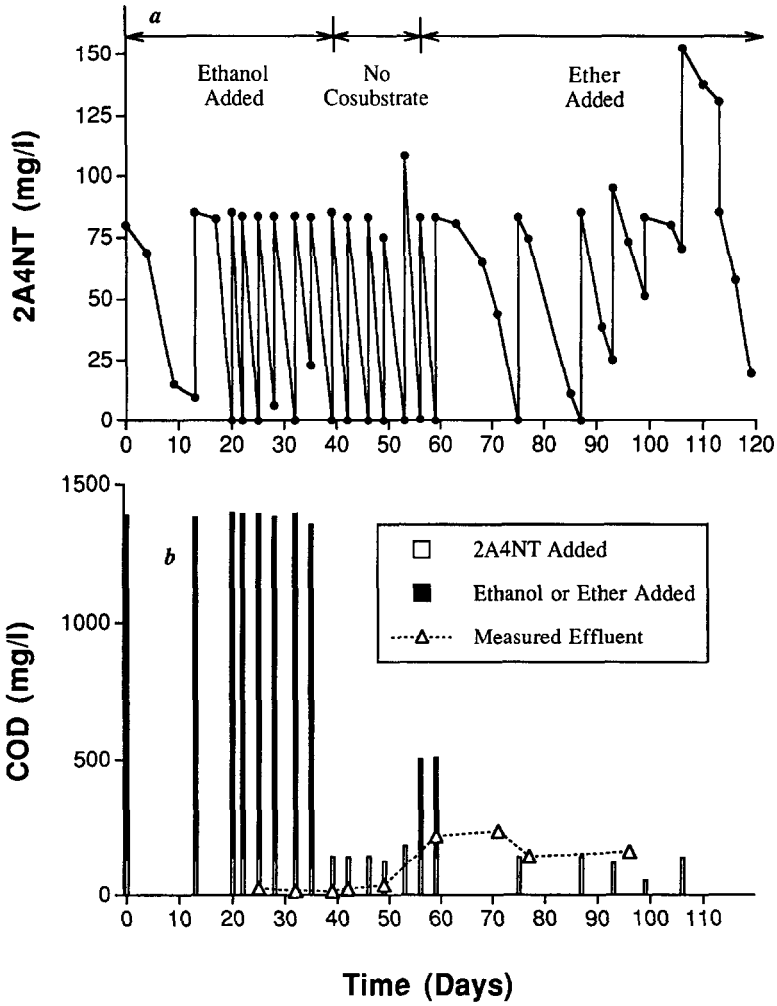

Fig. 3. Biodegradation of 2A4NT, showing (a) repetitive additions, with and without ethanol and ether added; and (b) COD contributed by 2A4NT plus ethanol or ether, and soluble COD in effluent samples. Results shown are for one bottle of a pair; the behavior of the duplicate was very similar.

levels were also higher than with the other compounds. In addition, the culture turned yellow for brief periods while DNT was being consumed. This color is characteristic of the aminotoluene isomers and of possible catechol intermediates. HPLC analysis of the effluent confirmed the appearance of aminonitrotoluene isomers, but since 2A4NT and 4A2NT were coeluted with the HPLC method used, it was not possible to quantify the amounts formed. The color disappeared, as did their detection by HPLC, whenever the DNT level fell close to zero.

Because of the lag in establishing DNT biodegradation, the "no-cosubstrate" phase was skipped with this pair of bottles (Fig. 4(a)). Ether was added in place of ethanol

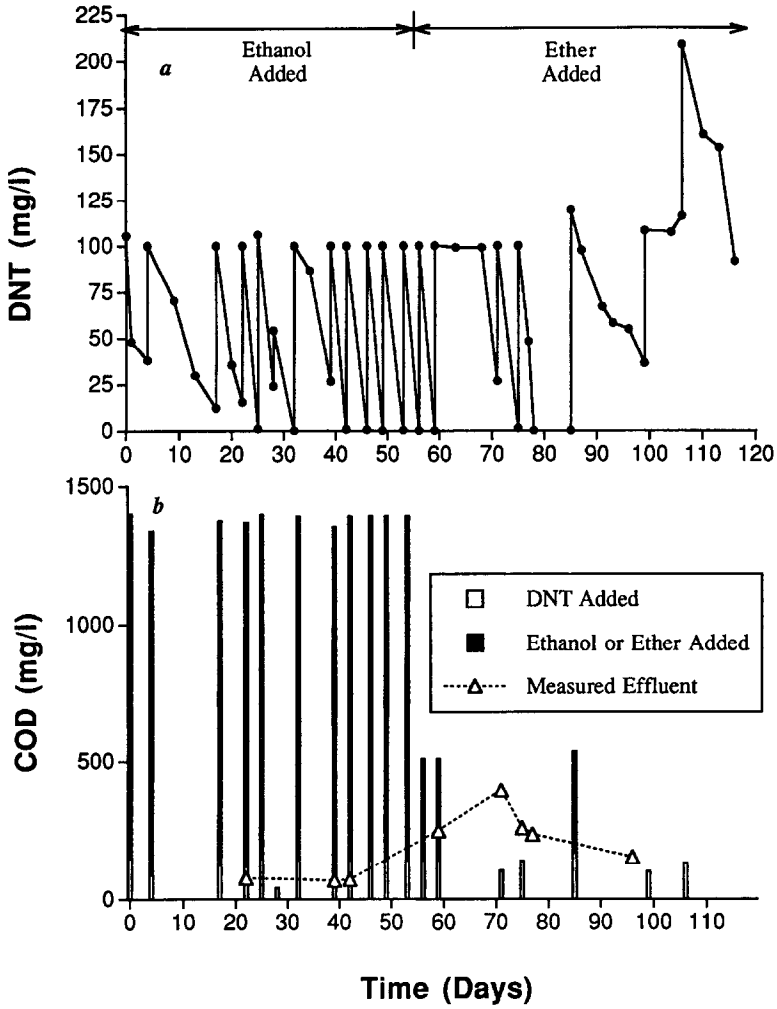

Fig. 4. Biodegradation of DNT, showing (a) repetitive additions, with ethanol or ether added; and (b) COD contributed by DNT plus ethanol or ether, and soluble COD in effluent samples. Results shown are for one bottle of a pair; the behavior of the duplicate was very similar.

starting on day 56 and it immediately inhibited DNT biodegradation. As with 2A4NT, DNT biodegradation over the next 60 days was very erratic, coinciding with a significant rise in effluent COD. A separate experiment was undertaken with DNT provided as the sole substrate, and repetitive biodegradation was observed in duplicate bottles (see Section 3.2).

In addition to the inoculated bottles described above, controls were also tested. Approximately the same initial concentrations as shown in Figs. 1–4 were added to duplicate serum bottles containing one of the aromatic compounds plus deionized water. Following 37 days of incubation, the loss of compounds from these controls was

minimal, ranging from 0.74% for DNT to 2.9% for DAT. This demonstrated that disappearance of the aromatics shown in Figs. 1–4 was not a result of volatilization or similar non-biotic processes.

3.2. Estimate of yield coefficients

A separate experiment was conducted to evaluate the use of each aromatic compound as a growth substrate. Eight bottles were set up with 99 ml of BM plus 1 ml of sample from one of the bottles described in Section 3.1, when they were being operated without the addition of ethanol or ether. Approximately 0.55 mM of one of the aromatic compounds was added, and its concentration was followed by HPLC. Whenever the compound was depleted, the culture was centrifuged, clarified liquid was decanted, and more BM containing the dissolved aromatic was added. The amount of aromatic compound consumed, the VSS formed, and the observed yield coefficients Y_{obs} are summarized in Table 1.

3.3. Stoichiometry of nitrite release

As mentioned above, biodegradation of DNT in the presence of ethanol resulted in transient accumulation of aminonitrotoluene isomers, whereas none appeared when DNT served as a sole substrate. This suggested that biodegradation of ethanol was driving at least a partial reduction of DNT prior to its oxidation. In order to evaluate this, the stoichiometry of nitrite release was measured. The extent of reduction was determined based on the percent recovery of nitrite, with a maximum of two moles NO_2^- expected per mole of DNT, and one mole of NO_2^- per mole of 2A4NT and 4A2NT. Little or no consumption of the released nitrite was expected for biosynthesis, because ammonium was always available as a nitrogen source in the BM in excess of the total COD demand.

Two experiments were conducted, as indicated in Table 2. In the first, nitrite release was measured in the same bottles used for estimation of the yield coefficients. Consequently, no ethanol or other organic substrates were added with the aromatics. Essentially stoichiometric release of nitrite was observed from DNT, 2A4NT and 4A2NT. In the second experiment, inoculum from the growth experiment was used to set up four bottles with DNT as a substrate (0.55 mM); two of these also received ethanol (17.8 mM). As in the first experiment, nitrite recovery was essentially stoichio-

Table 1
Measurement of yield coefficients [a]

Compound	Amount		Increase in VSS (mg)	Y_{obs} (mg VSS/mg COD)
	(μmol) [a]	COD (mg)		
DAT	161 (±19)	41.1	5.49(±0.13)	0.13
DNT	75.9 (±3.7)	19.4	2.00(±0.00)	0.10
2A4NT	61.9 (±0.7)	15.8	4.40(±1.70)	0.28
4A2NT	149 (±11)	38.0	3.60(±0.57)	0.10

[a] Average of duplicate bottles; numbers in parentheses are one standard deviation.

Table 2
Stoichiometry of nitrite release [a]

Compound and Experiment	Ethanol Added (mM)	Amount of Aromatic Consumed (mM)	Maximum Possible NO_2^- Release (mM)	Observed NO_2^- release [b] (mM)	NO_2^- Recovery (%)
2A4NT, Exp. 1	0	0.55	0.55	0.57	104
4A2NT, Exp. 1	0	0.55	0.55	0.54 (±0.006)	98
DNT, Exp. 1	0	0.18	0.36	0.37 (±0.057)	103
DNT, Exp. 2	0	0.55	1.10	1.10 (±0.017)	100
DNT, Exp. 2	17.8	0.55	1.10	0.65 (±0.035)	59

[a] Nitrate accumulation was also measured, but was insignificant in all bottles expect with one of the 4A2NT duplicates; in this case, the overall increase in NO_2^- + NO_3^- was used to calculate the average.
[b] Average of duplicate bottles (only one bottle reported for 2A4NT); numbers in parentheses are one standard deviation.

metric in the bottles that received only DNT. However, when ethanol was added as a cosubstrate, the percent recovery of nitrite dropped to 59%. This confirmed that a significant amount of the DNT was reduced in the presence of ethanol prior to being oxidized. As NH_4^+ was present in the medium in excess of the COD added, the lower than stoichiometric recovery of nitrite could not be attributed simply to the uptake of nitrite derived from DNT.

4. Discussion

Under the conditions of this study, the aerobic biodegradability of DNT, DAT, 2A4NT, and 4A2NT was demonstrated, both as sole substrates and in the presence of ethanol as a cosubstrate. Repetitive consumption of the aromatics coupled with low effluent soluble COD concentrations suggested that the compounds were mineralized to a significant degree, rather than simply transformed. It is also possible that some of the compound removal was attributable to formation of insoluble azoxy-type polymers, resulting from reactions among the aromatics and/or their metabolites. However, use of the compounds as growth substrates suggests that biological processes were most likely responsible for their disappearance from the cultures, and hence the decrease in effluent COD. Additional work with ^{14}C-labeled compounds is still needed to demonstrate more definitively mineralization and incorporation into cell material.

The effect of adding ether was mixed. In the case of DAT and 4A2NT, little or no impact was observed except for a transient rise in effluent COD. With DNT and 2A4NT, however, ether was quite inhibitory at the concentration added (142 mg l^{-1}). This is a potential concern for treatment of wastewaters containing DNT, since ether is often present, although volatilization during aeration will tend to mitigate this effect. Why ether had a bigger effect on the biodegradation of DNT and 2A4NT is not yet known, but the effect may be related to the nitro group at the *para* position. Previous studies have indicated that this is the site where transformation of many nitroaromatic compounds is initiated, typically by reduction and/or acetylation [3,16].

Surprisingly little is known about the pathway for aerobic oxidation of DNT, 2A4NT, 4A2NT, and DAT. The *Pseudomonas* strain isolated by Spanggord et al. [8] initiates its attack on DNT with a dioxygenase, converting it to 4-methyl-5-nitrocatechol. Subsequent ring cleavage was accompanied by stoichiometric release of nitrite when DNT was provided as a sole organic carbon source and NH_4^+ was added as a nitrogen source for biosynthesis. We also observed stoichiometric release of nitrite from DNT (and also from 2A4NT and 4A2NT) as long as it was provided as the sole organic carbon source. However, this condition is not typical of munitions wastewater, nor of many other environments in which nitroaromatic contamination is found.

Based on the lack of nitrite recovery during DNT biodegradation with ethanol (Table 2), and of the detection of aminonitrotoluenes as transient intermediates, our results indicated that reduction of DNT is a predominant pathway prior to ring cleavage when a cosubstrate is present. A number of other reports have also demonstrated reduction of DNT and trinitrotoluene under aerobic conditions [3,4,17,18], although significant subsequent mineralization did not occur. The maximum involvement of the reductive pathway during DNT biodegradation can be estimated by assuming that reduction did not proceed past 2A4NT or 4A2NT, since anoxic or anaerobic conditions are needed for significant reduction to DAT. To get 0.65 mM of NO_2^- from 0.55 mM DNT, 0.45 mM of DNT would have to be reduced to aminonitrotoluenes prior to ring cleavage (releasing 0.45 mM NO_2^-) and 0.10 mM would have to be directly oxidized (releasing 0.20 mM NO_2^-). This calculation suggests that as much as 82% of the DNT was reduced to 2A4NT and/or 4A2NT prior to ring cleavage. Formation of 2A4NT and 4A2NT is a concern because these aromatics also present a significant toxicological hazard, yet their concentration in water and wastewater is not currently slated for monitoring or regulation.

As these studies were undertaken with mixed aerobic enrichment cultures, the possibility existed for nitrification to occur. However, the concentration of aromatics used (84–100 mg l^{-1}) was apparently high enough to completely to inhibit the nitrifiers. In all but one bottle tested, the increase in nitrate during biodegradation of the aromatics was consistently minor (Table 2). Blum and Speece [19] observed a 50% inhibition of *Nitrosomonas* with only 0.92 mg l^{-1} of nitrobenzene, although a higher level was required with 2,6-DNT; they did not test 2,4-DNT, 2A4NT, or 4A2NT.

Much as in the work of Spanggord et al. [8], the results of this study demonstrated the use of DNT as a growth substrate. However, they did not report a yield coefficient for DNT, and no coefficients for DAT, 2A4NT, or 4A2NT were found in the literature. In order to provide a basis for evaluating the Y_{obs} values we measured (Table 1), the bioenergetic procedure developed by McCarty [20] was used to estimate yield coefficients. The following electron donor reactions were used, in combination with the appropriate cell synthesis (NH_4^+ as the N source, as it was always available in excess of the COD added) and oxygen (terminal e^- acceptor) half reactions:

$$C_7H_6(NH_2)_2 + 14H_2O \rightarrow 7CO_2 + 2NH_4^+ + 30H^+ + 32e^- \quad \Delta G^{o'} = -1028\,kJ$$

$$C_7H_6NO_2NH_2 + 14H_2O \rightarrow 7CO_2 + NH_4^+ + NO_2^- + 32H^+ + 32e^-$$

$$\Delta G^{o'} = -1062\,kJ$$

$$C_7H_6(NO_2)_2 + 14H_2O \rightarrow 7CO_2 + 2NO_2^- + 34H^+ + 32e^- \quad \Delta G^{o'} = -1042\,kJ$$

where ΔG^{α} represents the free energy change under standard conditions at pH 7. The free energy of formation ΔG_f° for DAT (234.1 kJ mol^{-1}), the aminonitrotoluene isomers (203.2 kJ mol^{-1}), and DNT (173.2 kJ mol^{-1}) were estimated using the method of Joback, as described in Reid et al. [21]. All other ΔG_f° values were from Brock et al. [22].

Assuming no endogenous decay, the calculated yield for all four compounds based on bioenergetics is 0.44 mg VSS per mg COD. This is several times higher than the Y_{obs} values reported in Table 1. A portion of this discrepancy is very likely attributable to endogenous decay. Given the length of incubation (11–20 days) relative to the amount of COD consumed, some decay of the biomass formed was expected. Another explanation for the difference in calculated and measured yield coefficients may involve transformation of the substrates to hydroxylated aromatic intermediates. Several hydroxylated nitroaromatic compounds are known to act as uncouplers, i.e. they disrupt the link between catabolism and synthesis of adenosine triphosphate, which is needed for biosynthesis. This has been demonstrated most extensively with nitrophenols, which are generally regarded as poor growth substrates [23]. When DNT is provided to a *Pseudomonas* isolate as the sole substrate, it is initially transformed to a catechol [8], which might then act as an uncoupler. The extent to which other nitroaromatic metabolites may serve as uncouplers must still be determined.

5. Summary and conclusions

Aerobic enrichment cultures were developed that repetitively biodegraded DAT, 4A2NT, 2A4NT, and DNT when they were provided as sole organic substrates (84–100 mg l^{-1}) or when ethanol (600 mg l^{-1}) was added as a cosubstrate. Consistently low effluent COD levels suggested that the aromatics were mineralized to a significant degree, rather than simply transformed to other possibly hazardous compounds.

The response of the enrichment cultures to the addition of ether (142 mg l^{-1}) as a cosubstrate was mixed. There was essentially no effect on 4A2NT biodegradation or overall COD removal. Biodegradation of DAT was also unaffected, although a transient rise in effluent COD did occur. With 2A4NT and DNT, addition of ether was very inhibitory and corresponded to a rise in effluent COD. Thus, the presence of ether may present a challenge to achieving efficient aerobic biological treatment of munitions wastewater.

When DNT, 2A4NT, and 4A2NT were added to enrichment cultures as sole organic substrates, and ammonium was provided in excess as a nitrogen source, there was a stoichiometric release of nitrite from each compound. This was in agreement with a previous report of nitrite release from DNT when it served as a sole substrate for a *Pseudomonas* strain [8]. However, when DNT was biodegraded in the presence of ethanol, only 59% of the maximum possible nitrite was recovered, indicating that as much as 82% of the DNT was reduced to 2A4NT and/or 4A2NT prior to ring cleavage. This result demonstrates the importance of studying biodegradative pathways under representative conditions, including the presence of cosubstrates.

Observed yield coefficients for the aromatic compounds ranged from 0.10–0.28 mg VSS per mg COD. As no other studies involving DNT, 2A4NT, 4A2NT, and DAT have reported yield coefficients, the only basis for comparison was to values calculated using bioenergetic principles. The experimentally determined values were significantly lower than the calculated yields. At least a portion of this discrepancy was attributable to endogenous decay. It is also possible that hydroxylated metabolites of these aromatic compounds acted as uncouplers, thereby lowering the percentage of electron donor used for biosynthesis.

Acknowledgements

This research was a joint effort between the Department of Civil Engineering at the University of Illinois at Urbana–Champaign and the U.S. Army Construction Engineering Research Laboratory.

References

[1] Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 9, 4th edn., John Wiley & Sons, 1979.
[2] S.W. Maloney, S. VanderLoop, M.T. Suidan and M.A. Moteleb, Two Phase Biodegradation of Ordnance Waste: Bench-Scale and Pilot Studies, Proc. 67th Annual Water Environment Federation Conference, Chicago, IL, 1994.
[3] N.G. McCormick, F.E. Feeherry and H.S. Levinson, Appl. Environ. Microbiol., 31 (1976) 949.
[4] N.G. McCormick, J.H. Cornell and A.M. Kaplan, Appl. Environ. Microbiol., 35 (1978) 945.
[5] D. Liu, K. Thomson and A.C. Anderson, Appl. Environ. Microbiol., 47 (1984) 1295.
[6] S. Maloney, E. May, M. Suidan and S. Berchtold, DNT abatement in propellant production wastewater on-site using expanded bed granular activated carbon anaerobic reactors, 19th Environmental Symposium and Exhibition, American Defense Preparedness Association, Albuquerque, NM, 1993.
[7] S.R. Berchtold, S.L. VanderLoop, M.T. Suidan and S.W. Maloney, Water Environ. Res., 67 (1995) 1081.
[8] R.J. Spanggord, J.C. Spain, S.F. Nishino and K.E. Mortelmans, Appl. Environ. Microbiol., 57 (1991) 3200.
[9] K. Valli, B.J. Brock, D.K. Joshi, and M.H. Gold, Appl. Environ. Microbiol., 58 (1992) 221.
[10] H.C. Crabtree, D. Hart, M. Thomas, B. Witham, I. McKenzie and C. Smith, Mutat. Res., 264 (1991) 155.
[11] National Cancer Institute, Bioassay of 5-Nitro-*o*-toluidine for Possible Carcinogenicity, National Cancer Institute, Bethesda, MD, National Cancer Institute of Health Carcinogenesis Technical Report Series No. 107, DHEW Publ. N (NIH) 78-1357, 1978.
[12] S. Matsui, T. Murakami, T. Sasaki, T. Hirose, and Y. Iguma, Water Technol., 3 (1975) 645.
[13] S.L. VanderLoop, M.T. Suidan, M.A. Moteleb and S.W. Maloney, Biodegradation of 2,4-dinitrotoluene in a two stage system, Proc. National Conference on Environmental Engineering, Boulder, CO, 1994.
[14] T.F. Jenkins, C.F. Bauer, D.C. Leggett and C.L. Grant, Reverse phase HPLC method for analysis of TNT, RDX, HMX, and 2,4-DNT in munitions wastewater, U.S. Army Corps of Engineers, Cold Regions Research and Engineering Laboratory, CRREL Rep. 84-29, 1984.
[15] Standard Methods for the Examination of Water and Wastewater, 16th edn., American Public Health Association, 1985.
[16] T. Glinsukon, T. Benjamin, H. Grantham, E. Weisburger and P. Roller, Xenobiotica, 5 (1975) 475.
[17] L.E. Hallas and M. Alexander, Appl. Environ. Microbiol., 45 (1983) 1234.
[18] A. Schackmann and R. Mueller, Appl. Microbiol. Biotechnol., 34 (1991) 809.
[19] D.J.W. Blum and R.E. Speece, Res. J. WPCF, 63 (1991) 198.
[20] P.L. McCarty, Prog. Water Technol., 7 (1975) 157.

[21] R.C. Reid, J.M. Prausnitz and T.K. Sherwood, The Properties of Gases and Liquids, McGraw–Hill Book Co., New York, 1977.
[22] T.D. Brock, M.T. Madigan, J.M. Martinko and J. Parker, Biology of Microorganisms, Prentice Hall, Englewood Cliffs, NJ, 1994.
[23] R.W. Okey, and H.D. Stensel, Toxicol. Environ. Chem., 40 (1993) 235.