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Transformations of TNT and related aminotoluenes in groundwater aquifer slurries under different electron-accepting conditions

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The transport and fate of pollutants is often governed by both their tendency to sorb as well as their susceptibility to biodegradation. We have evaluated these parameters for 2,4,6-trinitrotoluene (TNT) and several biodegradation products. Slurries of aquifer sediment and groundwater depleted TNT at rates of 27, 7.7 and 5.9 μM day^{-1} under methanogenic, sulfate-reducing and nitrate-reducing conditions, respectively. Abiotic losses of TNT were determined in autoclaved controls. Abiotic TNT loss and subsequent transformation of the products was also observed. These transformations were especially important during the first step in the reduction of TNT. Subsequent abiotic reactions could account for all of the transformations observed in bottles which were initially nitrate-reducing. Other controls removed TNT reduction products at much slower rates than slurries containing live organisms. 2-Amino-4,6-dinitrotoluene was produced in all slurries but disappeared in methanogenic and in sulfate-reducing slurries within several weeks. This compound was converted to 2,4-diamino-6-nitrotoluene in all slurries with subsequent removal of the latter from methanogenic and sulfate-reducing slurries, while it persisted in autoclaved controls and in the nitrate-reducing slurries. Aquifer slurries incubated with either 2,4- or 2,6-diaminotoluene showed losses of these compounds relative to autoclaved controls under nitrate-reducing conditions but not under sulfate-reducing or methanogenic conditions. These latter compounds are important as reduced intermediates in the biodegradation of dinitrotoluenes and as industrial chemicals. In experiments to examine sorption, exposure to landfill sediment resulted in losses of approximately 15% of diaminotoluene isomers and 25% of aminodinitrotoluene isomers from initial solution concentrations within 24 h. Isotherms confirmed that the diaminotoluenes were least strongly sorbed and the amino-dinitrotoluenes most strongly sorbed to this sediment, while TNT sorption capacity was intermediate. In our studies, 2,4,6-triaminotoluene sorption capacity was indeterminate due to its chemical instability. Coupled with biodegradation information, isotherms help describe the likelihood of contaminant removal, persistence, and movement at impacted sites.

Keywords: TNT; groundwater; anaerobic biodegradation; sorption; nitroaromatics

Introduction

2,4,6-Trinitrotoluene (TNT) and related dinitrotoluenes are often found as contaminants in soils and waters. These compounds are toxic to humans [14], aquatic organisms [7,28] and microorganisms [eg 6], and therefore their destruction is of concern. Rapid degradation of these compounds under aerobic conditions is unlikely, due to their highly oxidized nature. In recent years, researchers have focused mainly on anaerobic processes for the treatment and degradation of polynitroaromatic compounds.

As it is often difficult to treat large tracts of contaminated land due to technical and economic constraints, intrinsic bioremediation of pollutants is often favored. However, in order to evaluate this option reliably, basic information on the abilities of the indigenous microflora to degrade the contaminants of interest must be available. In particular, kinetic parameters associated with the intrinsic biodegradation of contaminants must be established so that professionals can predict the fate and transport characteristics of pollutant materials. Since the initial anaerobic transformation of nitroaromatic compounds is reductive [4,21,22], the initial degradation rate may depend on the redox potential of the anaerobic environment. The latter is, in turn, a function of the terminal electron acceptor and the presence of either sulfate, nitrate or iron could have a major influence on the rate of TNT reduction. However, there is little information on the impact of the electron-accepting process on the transformation of TNT.

Multiple pathways have been proposed for the anaerobic biodegradation of TNT. In several cases 2,4,6-triaminotoluene (TAT) has been postulated as a key intermediate [3,17,21]. The related compounds 2,4-diaminotoluene (2,4-DAT) and 2,6-diaminotoluene (2,6-DAT) are produced during the degradation of dinitrotoluenes and are important industrial chemicals in their own right. They are used in the manufacture of foams and resins and have an annual US production of the order of 3×10^8 kg [13]. It is therefore important to understand the fate of TAT and diaminotoluenes in the environment.

In this study, we examined the fate of TNT under methanogenic, sulfate-reducing and nitrate-reducing conditions in sediment slurries prepared from landfill leachate

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and delineated the influence of abiotic reactions on the processes. Slurries were monitored for nearly one year. The fates of TAT, 2,4-DAT and 2,6-DAT were also studied in separate incubations. Experiments were designed to measure depletion of the parent compounds as well as the production of methane or the consumption of sulfate or nitrate by the resident microflora. Short-term experiments to determine sorption kinetics and isotherms were also conducted to determine the relative importance of sorption on the fate of these compounds. Sorption of amino-nitrotoluenes, the partially reduced products of TNT, has not been addressed [20]. Sorption has long been recognized as an important factor relating to contaminant immobilization and transport in soil and groundwater systems. It is also recognized as an important consideration for understanding the performance of composting, which is cited as the most well-developed technology for remediation of soils contaminated with explosives [18]. Sorptive effects have been considered in modeling of biofilm [11,24] and groundwater [25] systems. Investigators have implicitly considered the effects of soil adsorption, desorption and other mechanisms on bioavailability of organic compounds when studying the effects of sample aging on biodegradation. Compounds found to exhibit such effects have included the triazine herbicide simazine [23], phenanthrene and 4-nitrophenol [12].

Recently, Nielsen *et al* [19] evaluated the importance of sorption on the aerobic fate of contaminants using *in situ* microcosms. They determined sorption constants based on 'instantaneous' and 'rate-limited' conditions and used the values to modify calculated first-order degradation rate constants for each of their test compounds. They concluded that accounting for sorption effects made it possible to reconcile differences between *in situ* microcosms with those of parallel laboratory incubations. Our results from experiments to examine sorption comprise an initial attempt at better understanding and interpretation of the availability of TNT and related products for biodegradation in the presence of sediments and groundwater.

Materials and methods

Sampling and incubation of aquifer slurries

The environmental samples used were collected from a methanogenic site located within the anoxic aquifer that underlies the municipal landfill in Norman, OK. The characteristics of the site were described previously [1]. Aquifer solids and groundwater were collected in late summer by digging to the top of the water table (4 m depth) followed by collection of the solids and water independently into sterile vessels. The water temperature was 22°C and contained a relatively high level of endogenous sulfate (700 μM). Samples were transferred to an anaerobic glovebox within 4 h of sample collection where the headspace was exchanged with $N_2 : H_2$ (95 : 5). Samples were then stored at 4°C for no more than 2 days.

Additions to the water and slurries were from sterile stock solutions. Groundwater was bubbled with $N_2 : CO_2$ (80 : 20) and amended with either sodium sulfate or sodium nitrate to an initial concentration of 1.2 and 1.8 mM, respectively. Some groundwater was left unamended with electron acceptor, for use in methanogenic incubations. All aquifer slurries received sterile Na_2S and resazurin to initial concentrations of 1 mM and 1 mg L^{-1}, respectively. Slurries were prepared in an anaerobic glovebox by filling sterile 160-ml serum bottles with 50 g wet weight of aquifer sediment and 75 ml of anaerobic groundwater as previously described [16]. Bottles were sealed with 1-cm thick serum stoppers held in place by aluminum crimp seals. The headspace was then flushed with $N_2 : CO_2$ (80 : 20). The final pH was 7.0.

All bottles were incubated at room temperature (22°C) for 11 days prior to the start of the experiments. Methanogenic aquifer slurries were then pressurized to 200 kPa with $H_2 : CO_2$ (80 : 20) to stimulate the removal of endogenous sulfate via sulfate reduction. These bottles were reflushed 2 days later with $N_2 : CO_2$ (80 : 20). During this time, the sulfate levels were reduced to 120 μM. After 2 weeks incubation, control bottles were autoclaved, and the nitroaromatic contaminant was added from a sterile stock solution. TNT (50 μl) was added from a methanolic stock solution, and the aminotoluenes TAT, 2,4-DAT and 2,6-DAT were added from aqueous filter-sterilized stock solutions (originally at 45 mM). The initial concentration in the serum bottles was approximately 100 μM for each substrate of interest. Each treatment included triplicate incubations and duplicate autoclaved controls. Substrate-unamended controls were prepared in the same way. Samples were periodically obtained by syringe and stored at –20°C prior to analysis. Samples from slurries containing triaminotoluene were sampled and analyzed within a 2-h period.

After 12 weeks incubation, the TNT-containing bottles were reamended with electron acceptor, either 15 mM sodium sulfate or 20 mM sodium nitrate. The aminotoluene-containing slurries were reamended with either 1 mM sodium sulfate or 2 mM sodium nitrate at 14 weeks for the sulfate and nitrate-reducing slurries respectively. An additional 2 mM sodium nitrate was added to these latter slurries at 39 weeks.

Sorption kinetics and isotherm studies

Adsorption experiments were carried out in two phases, 24-h kinetic tests and 4-h isotherm studies. Kinetic tests were conducted on samples containing 75 ml reverse osmosis/deionized (RO/DI) water along with aquifer material in 250-ml flasks on a shaker table. Initial concentrations were: 2,4-DAT, 246 μM; 2,6-DAT, 246 μM; 2-amino-4,6-dinitrotoluene (2-ADNT), 152 μM; 4-amino-2,6-dinitrotoluene (4-ADNT), 152 μM; and TAT, 146 μM, with 15 g of aquifer material (1 g per 5 ml sediment-to-solution ratio) for TAT and the ADNT isomers and 30 g (2 g per 5 ml) for the diaminotoluene isomers. To minimize other biotic or abiotic effects, flasks were sealed with parafilm and covered with aluminum foil. Sodium azide (4.6 mM) was added to some duplicate flasks. TAT was dissolved in boiled, degassed water at pH 8.5 and handled under an argon atmosphere to avoid instability when in contact with oxygen and other chemicals. The initial pH of all flasks was adjusted to 7.5 after mixing of reactants, and samples consisting of 1.5 to 2.0 ml of mixed suspension were taken at intervals over the 24-h test period.

Isotherm studies were conducted on 15 ml of RO/DI

water and sediment in 20-ml glass vials. Sediment to water ratios were the same as for the kinetic tests. TNT isotherms were run earlier at a ratio of 1 g sediment to 5 ml RO/DI water, over a concentration range of 22–440 μM. Aminodinitrotoluene isomers were tested at concentrations in the range of 25–152 μM. TAT and diaminotoluene isomers were tested at concentrations from 15–164 μM. Other aspects of sample handling were as described previously for the kinetic tests. Vials were equilibrated on a shaker for 4 h, shown from kinetic tests to be adequate to establish steady-state conditions, and greater than the 2-h equilibration time reported by Pennington and Patrick [20] in their studies of TNT adsorption.

Following completion of sorption isotherms, the liquid phase was removed from each vial, and the solid phase was extracted in 5–7 ml methanol with approximately 1-min manual shaking followed by centrifugation and extract collection. Three sequential extractions were combined for each sample and analyzed for desorbed test compounds. Results are presented as percent recoveries of compounds presumed lost from solution by sorption.

Analytical methods

The concentrations of TNT, its transformation products and the aminotoluenes were measured by HPLC analysis. This was done using a C-18 column with a buffer : acetonitrile (60 : 40) system containing 20 mM potassium phosphate (pH 4.0). Diaminotoluenes were determined using the same system at pH 6.5. TAT was similarly measured except that the acetonitrile concentration was 8%. Sulfate and nitrate concentrations were determined with an ion chromatography system (Dionex Corp, Sunnyvale, CA, USA) using an AS4A column. Methane was determined by flame ionization gas chromatography as previously described [16]. The theoretical methane yield was calculated using the Buswell equation [26].

The sorption experiments were also monitored by HPLC using a reversed phase C-18 column with a mobile phase of 10 mM phosphate buffer : methanol. The buffer : methanol ratio was 50 : 50 at pH 5.0 for TNT and the ADNT isomers, 75 : 25 at pH 6.5 for the DAT isomers, and 92 : 8 at pH 6.5 for TAT. These samples were filtered through 0.45-mm pore size membrane filters and kept frozen prior to analysis. Blank tests showed that sorption on glass vials and filter assemblies was insignificant.

Sterility testing

Autoclaved slurries were tested for the presence of viable microorganisms after the experiments were completed. Media were prepared anaerobically [15] and contained a mineral solution [15], vitamins, trace metals [27], 2 g L^{-1} TES, 1 g L^{-1} $NaHCO_3$, 15 g L^{-1} Bacto agar and 10 g L^{-1} Bacto Plate Count Broth (Difco). The medium was adjusted to pH 7.2, then boiled under $N_2 : CO_2$ and autoclaved in a sealed flask. Petri plates were poured and subsequently incubated in a glovebox under $N_2 : H_2$ (~95 : 5) headspace. Medium for aerobic cultivation of cells was prepared as above except without the $NaHCO_3$. These were incubated in air. Both autoclaved and live aquifer slurries (0.1 ml) were spread over each plate. Plates were incubated at room temperature for 6 days and examined for the presence of colonies.

Chemicals

2,4-Diamino-6-nitrotoluene (2,4-DANT) was a gift of Dr Ronald Spanggord. Aldrich Chemical Co (Milwaukee, WI, USA) supplied 2,6-DAT and 2,4-DAT. TNT and TAT were obtained from Chem Services (West Chester, PA, USA). The analytical standards 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT) were obtained from Accustandard Inc (New Haven, CT, USA). All other chemicals were reagent grade or higher purity.

Results

Degradation of TNT

TNT was removed from all of the aquifer slurries (Figure 1a, d, g). Methanogenic slurries transformed TNT more rapidly than autoclaved controls. We estimate initial rates of TNT removal for aquifer slurries as: 27, 7.7 and 5.9 μM day^{-1} under methanogenic, sulfate-reducing and nitrate-reducing conditions respectively. When sterile and live slurries were compared, the former exhibited similar rates of TNT loss with sulfate or nitrate as electron acceptors. However, the methanogenic controls lost TNT at a slower rate (6.1 μM day^{-1}; Figure 1a).

HPLC analysis allowed us to discern three major chromatographic peaks which absorbed at 230 nm. All three peaks were identified based on their retention time and UV absorbance spectra as TNT (12.3 min), 2-ADNT (9.6 min) and 2,4-DANT (4.6 min).

The greatest differences among the various incubations were observed when the fates of the partially reduced compounds were compared as a function of electron acceptor availability (Figure 1). For example, under methanogenic conditions 2-ADNT and subsequently 2,4-DANT were rapidly produced and removed. Sulfate-reducing slurries (Figure 1d, e) underwent a similar transformation, but these compounds accumulated more slowly and were more slowly removed than in comparable methanogenic slurries. The controls accumulated one or more of these metabolites.

The nitrate-reducing slurries underwent a series of reactions which appeared to be carried out by abiotic processes. Plots of each of the three major peaks in the live slurries were mirrored by the autoclaved controls (Figure 1g, h, i). The 2-ADNT was again produced rapidly but was removed to a limited extent with persistence at a level of approximately 20 μM (Figure 1h). This seems to reflect a dynamic equilibrium concentration, because 2,4-DANT was produced and accumulated over the course of the incubation as what appeared to be a major product of 2-ADNT conversion (Figure 1i).

Sterility tests conducted after more than a year of incubation revealed no microbial contaminants in the sterile control bottles. Bottles containing live organisms sampled at the same time revealed abundant microbial growth. Slurries amended with benzoate (500 μM) were methanogenic, sulfate- or nitrate-reducing during the incubation by demonstrating production of methane or the consumption of sulfate or nitrate during the early stages of the incubation. TNT-amended methanogenic bottles began producing

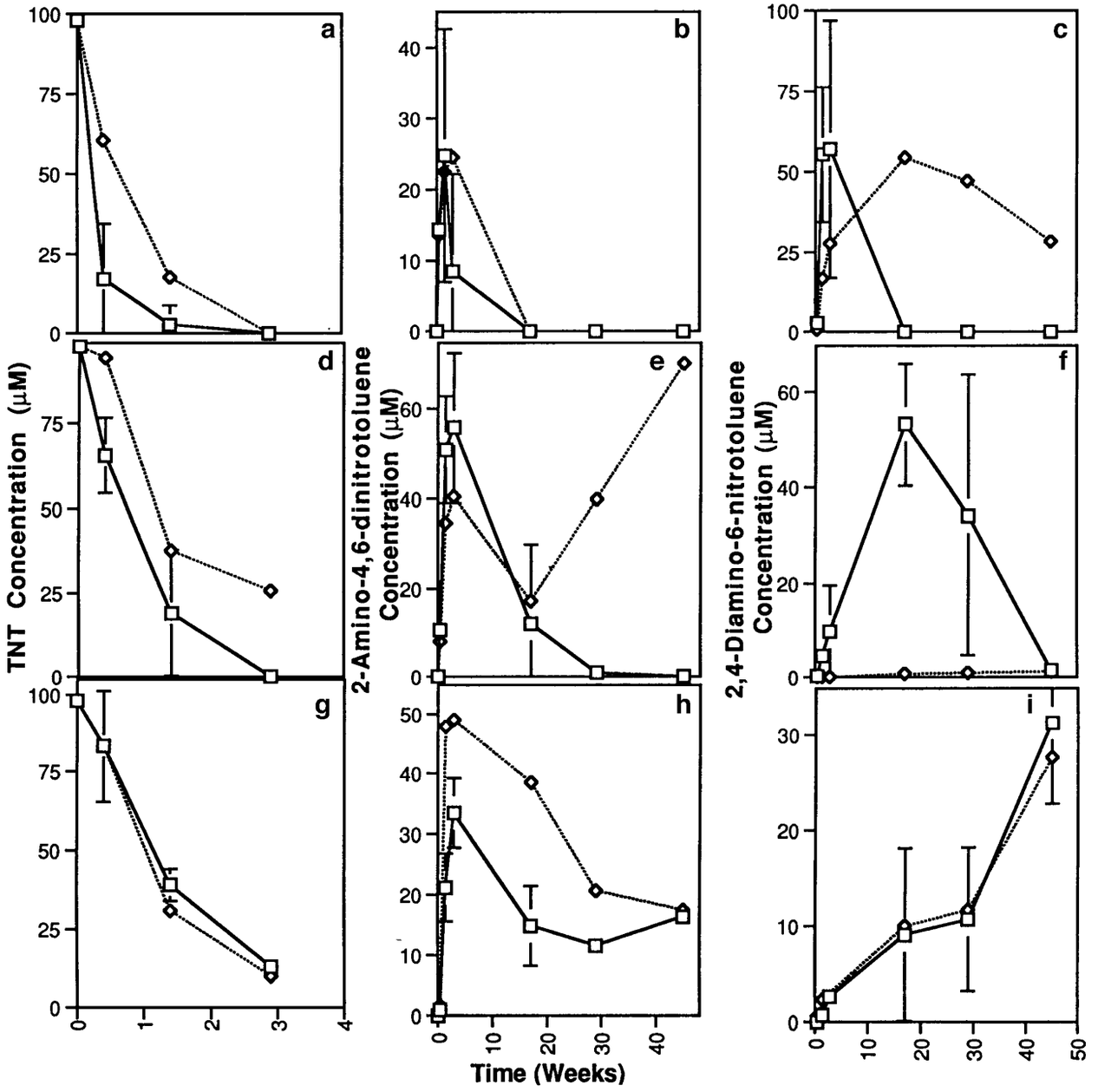

Figure 1 Levels of TNT (a, d, g), 2-ADNT (b, e, h) and 2,4-DANT (c, f, i) in cultures. Experiments were carried out under methanogenic (a, b, c), sulfate-reducing (d, e, f) or nitrate-reducing conditions (g, h, i). Slurries with live organisms (squares) are compared to autoclaved controls (diamonds) in each plot. Standard deviations are indicated for slurries with live organisms. Because of the rapid initial transformation of TNT, the zero time point has been calculated from the amount added and corrected for dilution. By the 17-week time point, TNT had dropped below the detection limit in all of the slurries.

methane at 74 days of incubation, which roughly corresponds with the date that the last detectable intermediate disappeared. Sulfate concentrations did not decrease in the TNT-containing slurries or the unamended slurries after the start of the experiment. A blackening of the sediments (most likely due to sulfate reduction) did occur after several months of incubation. A significant depletion of nitrate was observed in TNT-amended bottles at samplings over the course of the incubation with a total of 17.5 mM nitrate consumed over the 45-week incubation period.

Fate of aminotoluenes

Diaminotoluene levels were monitored over a period of 54 weeks. No apparent change in the parent substrate concentration was observed for either 2,4-DAT or 2,6-DAT incubated under methanogenic or sulfate-reducing conditions (data not shown). Furthermore, no differences were observed in the amount of sulfate consumed in the substrate-amended and unamended slurries. While we observed methane produced in the substrate-amended cultures at levels above the unamended controls, we could not

correlate this gas production with consumption of the diaminotoluenes. On the other hand, both of these compounds were shown to be removed biologically under nitrate-reducing conditions (Figure 2). This was apparent as the diaminotoluene-containing incubations diverged from the autoclaved controls after 22 weeks. Because of the relatively high background level of nitrate consumption observed in substrate-unamended controls (data not shown), it was difficult to assess whether nitrate consumption was linked to removal of this electron acceptor.

TAT concentrations were reliably determined only at the final time point taken at 69 weeks. In these samples, no TAT was detected in the autoclaved controls. TAT levels in the methanogenic, sulfate- and nitrate-reducing slurries were 6.3, 20.2 and 0 μM respectively.

Sorption of aminotoluenes and TNT to aquifer solids

The DAT and ADNT isomers reached near-long-term equilibrium solution concentrations within 4 h (Figure 3). Equilibrium was not reached in vials containing TAT (Figure 3e). Rather, this compound disappeared steadily from solution and reached undetectable concentrations within 24 h. Variability between duplicates ranged from 6 to 22%.

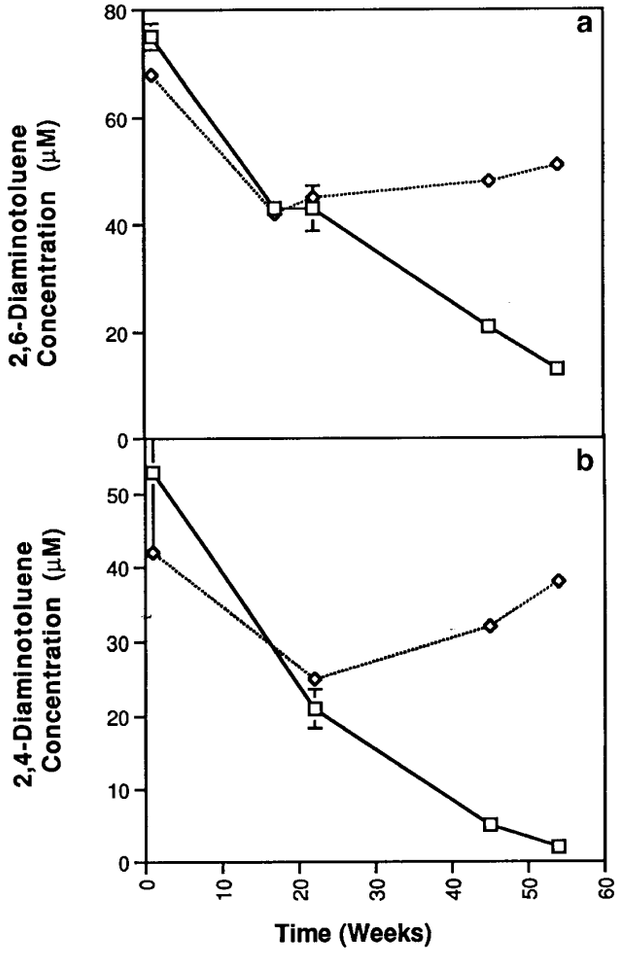

Figure 2 Disappearance of the two diaminotoluene isomers incubated under nitrate-reducing conditions with groundwater and aquifer material in live (squares) and autoclaved controls (diamonds). (a) 2,6-diaminotoluene-containing slurries, (b) 2,4-diaminotoluene-containing slurries. Standard deviations are indicated for the slurries containing live organisms where they are greater than 2 μM.

In calculating isotherms, amounts of adsorbed mass were calculated from known solution volumes, measured initial concentrations, and decreases in solution concentrations. In these experiments, variability between duplicates also ranged from 6 to 22%. Results were fit to linearized Langmuir and Freundlich isotherms, given as follows by Pennington and Patrick [20]:

$$\text{Langmuir:} \quad 1/q = (1/Q) + (1/bQ) \cdot (1/C)$$
$$\text{Freundlich:} \quad \ln(q) = \ln(K) + (1/n) \cdot \ln(C)$$

where q is the solid phase concentration of the test compound (mg g^{-1}), Q is the monolayer sorption capacity (mg g^{-1}), b is the Langmuir constant related to entropy, C is the equilibrium solution concentration (mg L^{-1}), K is the Freundlich adsorption coefficient, and n is the Freundlich characteristic constant. Mass concentration units are standard for isotherm studies.

Both models accurately described the experimental data, as indicated by regression coefficients (Table 1). Both models also demonstrated comparable trends among the different compounds with respect to their sorption characteristics. The pattern is suggested most clearly by comparison of Langmuir Q values, which indicate that the monolayer sorption capacity of this sediment for ADNT isomers is at least twice that for TNT and an order of magnitude greater than the capacity for DAT isomers. Isotherm results for TAT are questionable due to the lack of steady state kinetic data for this compound, and the fact that isotherm coefficients appear to be inconsistent with other results. The linearized Langmuir isotherm plots for each compound are shown in Figure 4.

Desorption recovery was calculated as follows:

$$\%\ \text{Recovery} = [(\text{Desorbed Mass})/(\text{Adsorbed Mass})] \cdot 100$$
$$\%\ \text{Recovery} = \{[(\text{Extract Conc}) \cdot (\text{Extract Vol})]/[(\text{Solution Conc Decrease}) \cdot (\text{Solution Vol})]\} \cdot 100$$

Percent recovery values for each compound were: 2,4-DAT, 94; 2,6-DAT, 91; 2-ADNT, 99; 4-ADNT, 75; TAT, 0; and TNT, 87.

Discussion

Our results show the differences in degradation rates for TNT that can occur under various electron-accepting conditions. The initial transformation reaction of TNT was by far the most rapid under methanogenic conditions. Subsequent transformations of both 2-ADNT and 2,4-DANT followed a trend with the relative rates a function of the incubation condition: methanogenic > sulfate-reducing > nitrate-reducing. Abiotic reactions in slurries initially incubated under the three conditions and subsequently autoclaved demonstrated that many transformations can occur in the absence of microbial activity, the most rapid of which is the initial reduction of TNT to 2-ADNT. These results

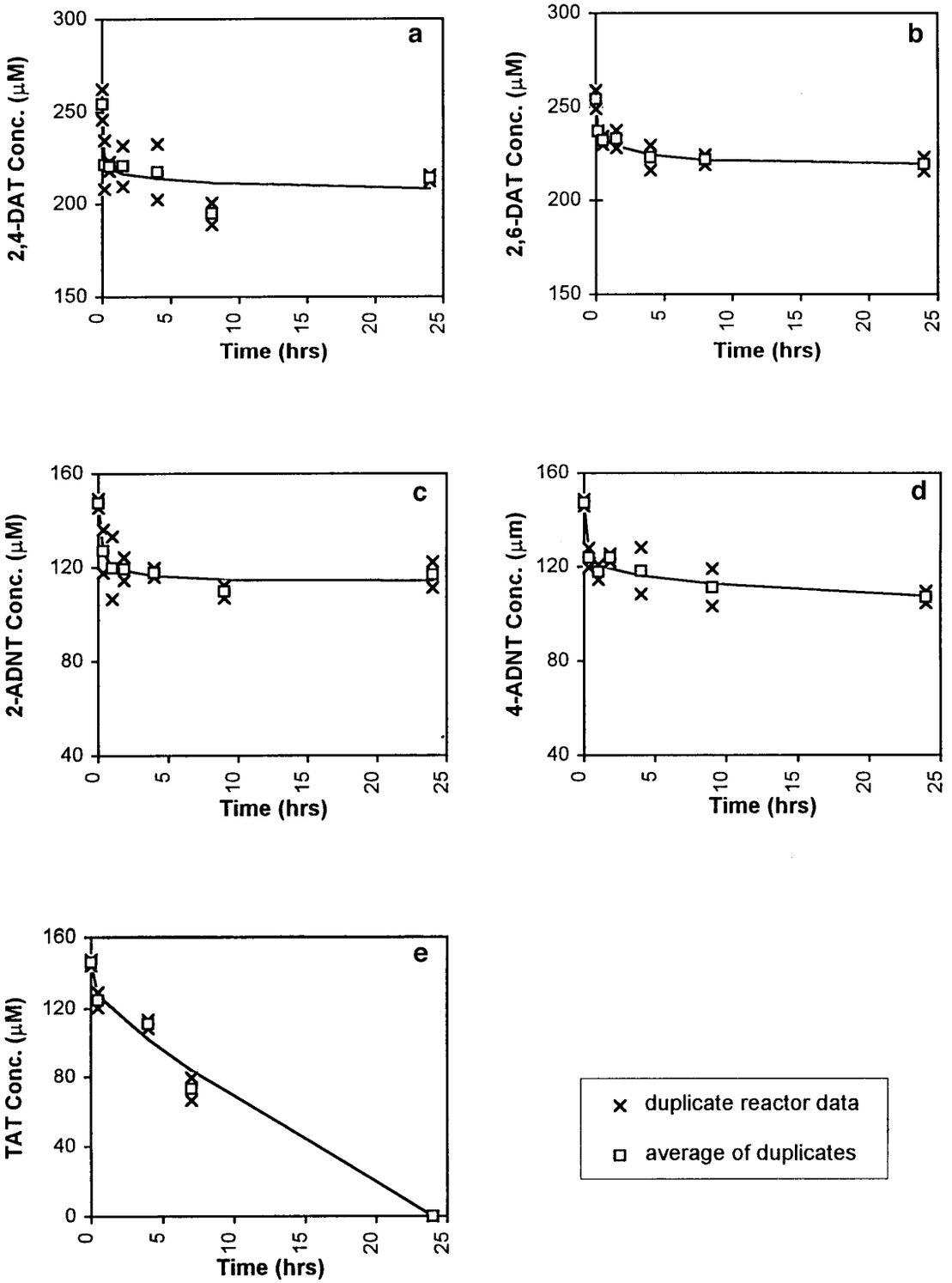

Figure 3 Adsorption kinetics curves for the amino- and nitrotoluene compounds. Data for individual replicates are shown (x) with means plotted as squares.

follow the predictions of Dunnivant *et al* [8] which state that rate constants for the reduction of nitroaromatic compounds are proportional to decreasing redox potential. We conclude that initial transformation of the nitro groups beyond 2,4-DANT occurs most rapidly under methanogenic conditions but will still occur to a significant extent under sulfate-reducing conditions. Furthermore, in the presence of the excess nitrate, the accumulation of partially reduced products may be predicted.

We are familiar with only one other published study in

Table 1 Values determined from isotherm data

Compound	Langmuir			Freundlich		
	r^2	Q (μg g^{-1})	b	r^2	K	n
2,4-DAT	0.981	8.7	0.14	0.983	1.2	1.6
2,6-DAT	0.990	6.6	0.23	0.987	1.4	1.9
2-ADNT	0.941	84	0.020	0.943	1.8	1.1
4-ADNT	0.987	112	0.013	0.975	1.5	1.1
TAT	0.999	27	0.93	0.902	12	3.1
TNT	0.996	41	0.026	0.993	1.5	1.4

which the degradation of TNT has been characterized under different electron-accepting conditions. Boopathy *et al* [4] grew enrichment cultures under methanogenic, sulfate- and nitrate-reducing conditions from a soil inoculum with either H_2, lactate or acetate, respectively, as electron donors. These cultures were tested for their abilities to reduce TNT. In these experiments, a relatively homogeneous microbial community had been selected based on its ability to use the specific inorganic electron acceptor. In contrast, our experiments represent a model system which contains both a wide variety of potential electron donors as in landfill leachate and a heterogeneous microbial community. We were therefore not surprised to see further and more rapid transformation of TNT than had been observed previously. In their experiments [4], neither sulfate-reducing nor methanogenic enrichment cultures transformed TNT to a significant extent. Under nitrate-reducing conditions, 80% of 440 μM TNT was removed over a 40-day period. Of the transformation products, only the monoamino-dinitrotoluenes were observed. In our study, we have observed complete disappearance of 100 μM TNT after as little as 1 week.

The abiotic reduction of TNT and aminonitrotoluenes is a very important process. Previous efforts have recognized the ability of natural organic matter, sulfide, or minerals containing sulfide to catalyze the reduction of nitroaromatic compounds [8,29]. Our slurries contain all of the above and these results point out the relative importance of abiotic reductive reactions in ecosystems in which sulfide is present. We were familiar with the ability of sulfide to catalyze the reduction of nitro groups, but felt it necessary to add a small amount of sulfide to slurries in order to scavenge oxygen that may have been introduced during sampling. Shortly after the addition of sulfide, a heavy black ferrous sulfide precipitate was observed in the groundwater. The aquifer material used in this study typically contains iron sulfide due to the cycling of iron and sulfur in the system (unpublished observation).

Although abiotic reactions are capable of reducing TNT, we have presented evidence that even under the most highly reduced (methanogenic) conditions, 2,6-DANT accumulated over the 45-week incubation period. Although the trend was different, this same intermediate accumulated under nitrate-reducing conditions. An interesting phenomenon occurred in which only a low level of 2,6-DANT accumulated in the sterile sulfate-reducing controls. A possible explanation is that the high sulfide levels that may have accumulated in these incubations prior to autoclaving reacted in some way during the sterilization process to inhibit the conversion of 2-ADNT to 2,4-DANT.

Our TNT transformation profiles have demonstrated the conversion of TNT to 2-ADNT and subsequently to 2,4-DANT. In slurries containing live methanogenic and sulfate-reducing organisms, the latter compound is further transformed. We assume that the next step in the transformation is the conversion of 2,4-DANT to TAT, as has been demonstrated in previous studies [17,21]. Although we did not measure TAT throughout the experiment in the TNT-containing slurries and could not because of its inherent instability in air (during sample storage), we did determine the fate of aminotoluenes added directly to slurries under different redox conditions. At the end of a 69-week experiment, TAT levels were assayed in all slurries amended with this compound. Because no TAT was detected in any of the autoclaved controls, it is impossible to prove whether TAT was biologically transformed. However, since the diaminotoluenes were transformed in the presence of nitrate, other aminotoluenes including TAT may be transformed under these conditions. The fact that the two diaminotoluene isomers are transformed in the live slurries under nitrate-reducing conditions, and not with the other electron acceptors, indicates that more-oxidized conditions may facilitate transformation of the reduced products. The idea of a sequential anaerobic/aerobic process has been previously implemented for the treatment of TNT [10,22]. Another report has documented the effectiveness of a sequential anaerobic–aerobic process for the degradation of nitrobenzene [5]. Beunink and Rehm [2] investigated a similar system for the degradation of 4-chloro-2-nitrophenol which involves a coupled anaerobic–aerobic process carried out by bacteria immobilized in calcium alginate beads. This latter process is particularly interesting in that anaerobic bacteria living in the center of the beads carry out the anaerobic reaction while aerobes on the surface carry out the aerobic step. The use of nitrate as an electron acceptor for the treatment of diaminotoluenes produced by the chemical industry may also avoid the potential problem of oxidation of the amino groups to nitro groups, resulting in a more toxic waste.

Short term experiments to evaluate sorption indicate that interaction with sediment particles affects solute availability, and there were significant differences among the compounds tested. Exposure to landfill sediment resulted in losses of approximately 15% of DAT isomers and 25% of ADNT isomers within 24 h (Figure 3). These values may be compared with apparent TNT losses of approximately 30–75% from adsorption kinetics curves presented by Pennington and Patrick [20] for 14 Army Ammunition Plant (AAP) soil samples with differing characteristics. This is the only previous study we know of which addresses directly the question of TNT sorption to soil particles.

Isotherms confirm that diaminotoluenes were least strongly sorbed and the amino-dinitrotoluenes most strongly sorbed to this sediment. TNT sorption capacity was intermediate. The Langmuir coefficient (Q) describing TNT sorption was less than those values previously observed for the AAP soils [20]. In our experiments, TAT sorption was not determined because it was unstable.

These observations may help explain results of radiolab-

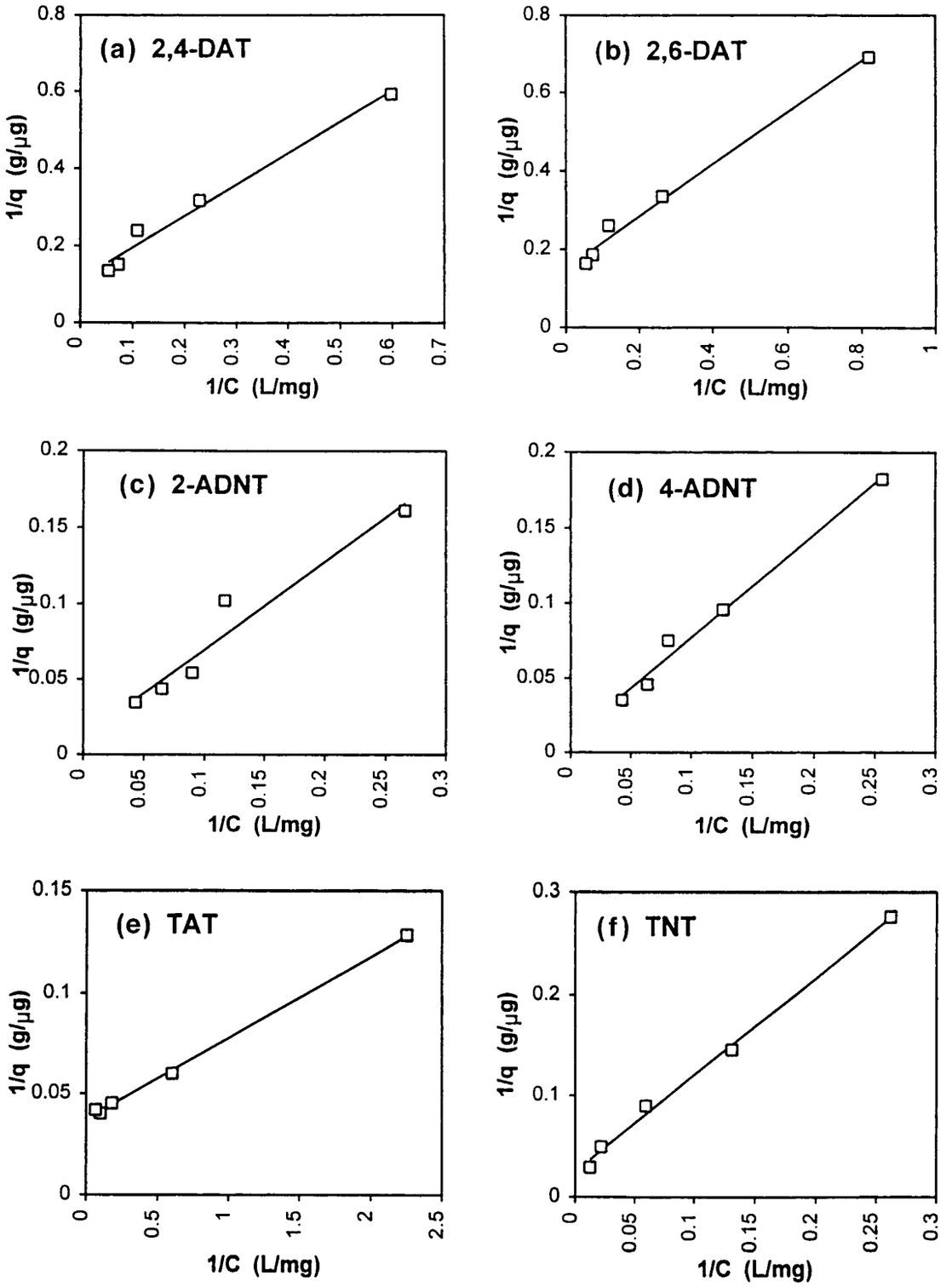

Figure 4 Langmuir isotherm plots for the amino- and nitrotoluene compounds. Isotherms are plotted in mass concentration units to conform with standard practice and facilitate comparison of results with other studies.

eled TNT mineralization studies with the white-rot fungus *Phanerochaete chrysosporium* reported by Fernando *et al* [9]. At a low initial TNT concentration of 1.3 mg L^{-1}, about 35% of labeled compound was mineralized to CO_2 in liquid culture within 18 days, whereas only about 6% was mineralized in soil cultures within 30 days. Almost complete loss of TNT was indicated, as less than 5% of the original ^{14}C-labeled TNT remained in either system. In longer-term

studies at high initial concentrations (100 mg L^{-1} and 10 000 mg kg^{-1} respectively), both aqueous and solid-phase cultures degraded approximately 85% and mineralized 18–20% of the TNT over a 90-day incubation period. However, mineralization occurred at a much slower rate in the soil-containing cultures. These differences were possibly due to the relative unavailability of the sorbed fraction of TNT and metabolites.

Our results suggest some interesting refinements in efforts to understand and model the behavior of nitro- and amino-aromatic compounds. For example, previous studies [20] concluded that groundwater movement of TNT should not be greatly retarded by soil adsorption, because sorbed TNT was readily desorbed in water (87.5–93.75% recoveries, depending on soil type, within 10 h). We also obtained high desorption efficiencies, but ADNT isomers produced by TNT biodegradation were more highly sorbed than TNT (Table 1), and the 4-ADNT isomer was the least readily desorbed compound for which results were obtained in this study. Whether this trend would continue for 2,4-DANT, known to be formed as a further biodegradation product, is unclear. However, the fully reduced aminotoluenes adsorbed less strongly and once formed, are likely to be transported most rapidly of the compounds tested.

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