



Column transport studies of 3-nitro-1,2,4-triazol-5-one (NTO) in soils



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HIGHLIGHTS

- This is the first study to examine transport behavior of dissolved NTO, a new IM compound.
- We measured adsorption, retardation and transformation of dissolved NTO in 8 soils.
- NTO adsorption and retardation were low ($K_d < 1 \text{ cm}^3 \text{ g}^{-1}$).
- Transformation rates increased with time for high OC soils.
- Organic carbon and microbial activity promoted NTO transformation.

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ABSTRACT

Development of the new, insensitive, energetic compound, NTO (3-nitro-1,2,4-triazol-5-one), creates need for the data on NTO's fate and transport to predict its behavior in the environment and potential for groundwater contamination. To measure the transport of NTO in soils, we conducted miscible-displacement experiments under steady state and interrupted flow conditions using eight soils having varying physical and geochemical properties. The breakthrough curve (BTC) data were analyzed using temporal moment analysis and simulated using HYDRUS-1D to determine transport parameters and better understand the mechanisms of sorption and transformation. Parameters determined from the miscible-displacement study were compared to results obtained from batch experiments conducted for the same soils, and examined in relation to soil properties. Column NTO linear adsorption coefficients (K_d) were low and correlated well ($P = 0.000049$) with measurements from the batch studies. NTO transformation rate constants increased and NTO recovery decreased with increase in soil organic carbon (OC) content. Autoclaved soils had slower transformation rates and greater NTO recoveries indicating that microorganisms play a role in NTO transformation. In addition, the transformation rate increased with time in soils with higher OC. Monod-type kinetics was implemented in HYDRUS-1D to simulate the observed increase in transformation rate with time. We think this phenomenon is due to bacterial growth. Results indicate very low adsorption of NTO in a range of soils, but natural attenuation through transformation that, depending on soil OC content and hydraulic residence time, could result in complete removal of NTO.

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1. Introduction

NTO (3-nitro-1,2,4-triazol-5-one) is a new energetic compound developed by the U.S. military for insensitive munitions (IMs). IMs retain the performance of traditional explosives but are better able to tolerate unplanned stimuli, such as mechanical shock and high temperatures (Beyard, 2007). As a result of incomplete detonation, solid IM explosives containing NTO may be deposited on range

Abbreviations and acronyms: ATO, 5-amino-1,2,4-triazol-3-one; DNAN, 2,4-dinitroanisole; IM, Insensitive Munition; NTO, 3-nitro-1,2,4-triazol-5-one.

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soils, and after dissolution (Taylor et al., 2013, 2015a, 2015b) can potentially reach groundwater. Complex interaction of the dissolved IM components with soil constituents is key to understanding the threat of groundwater contamination. NTO is highly soluble ($16,642.0 \text{ mg L}^{-1}$ at $25 \text{ }^\circ\text{C}$) (Spear et al., 1989) and its transport to groundwater used as potable water source could result in human exposure to the chemical. While NTO showed low toxicity to the freshwater organism *Ceriodaphnia dubia*, green algae *Selenastrum capricornutum* (Haley et al., 2009), and *Rana pipiens* tadpoles (Stanley et al., 2015), it is a male reproductive toxicant in rats (Wallace, 2011; Lent et al., 2015) and was reported to form transformation products that can be toxic if they behave similarly to metabolic intermediates of other nitro-compounds (Le Campion et al., 1999b). Based on these risks, accurate soil adsorption coefficients (K_d s) and transformation rate constants are necessary to simulate and predict NTO fate and transport to groundwater.

NTO's pKa is 3.7–3.76, and it is negatively charged at environmentally relevant pHs (Le Campion et al., 1997; Smith and Cliff, 1999). Low estimated octanol-water partition coefficients (K_{ow}) ($\log K_{ow}$ s 0.37–1.03) (Sokkalingam et al., 2008) suggest that NTO would not strongly adsorb to soil organic matter through hydrophobic interactions and, therefore, be more mobile than other components in IM mixtures, such as 2,4-dinitroanisole (DNAN) ($\log K_{ow}$ s 1.58–1.92) (Sokkalingam et al., 2008; Hawari et al., 2015). Microbial transformation studies have shown that NTO can undergo nitroreduction under anaerobic conditions (Krzmarzick et al., 2015) and form 5-amino-1,2,4-triazol-3-one (ATO). ATO can further transform under aerobic conditions to CO_2 , urea, and inorganic nitrogen species (Le Campion et al., 1999b; Krzmarzick et al., 2015).

Mark et al. (2016) conducted a series of kinetic and equilibrium batch experiments to determine first order transformation rate constants (k), K_d s and Freundlich adsorption coefficients (K_f s) for NTO using multiple soils with a range of properties. NTO was weakly adsorbed, with K_d s ranging between 0.02 and $0.51 \text{ cm}^3 \text{ g}^{-1}$. Soil affinity for NTO was not influenced by OC content of the soil, but was strongly affected by soil pH. Adsorption decreased as soil pH increased, probably due to the influence of protonation on the interaction between soil surfaces and NTO. This is supported by studies with pure minerals (Linker et al., 2015) that showed that NTO did not adsorb to negatively charged layer silicate clays, but had higher affinity for goethite that was positively charged. Estimates for NTO k s ranged between 0.0004 h^{-1} and 0.0221 h^{-1} . There was a significant positive relationship between OC in soils and measured k s (Mark et al., 2016) and sterilized soils experienced smaller mass loss than same soils without sterilization suggesting microbial transformation, possibly with OC as the energy source.

To further elucidate processes involved in the reactive transport of NTO in soils, we conducted miscible-displacement experiments using saturated repacked columns under steady state and transient flow conditions. NTO k and K_d were determined using temporal moment analysis (TMA) and HYDRUS-1D solute transport modeling software. Monod-type kinetics was implemented in HYDRUS to evaluate the occurrence of temporally dependent NTO transformation. To assess the impact of the experimental approach, transport parameters (k , transformation rate for Monod-type kinetics, and K_d) were compared to the results of the batch studies (Mark et al., 2016). This is the first study to examine transport behavior of dissolved NTO in soils.

2. Materials and methods

2.1. Soils

For the NTO transport experiments we selected eight uncontaminated surface soils (top 12 inches or 30 cm) with a wide range

of properties collected (except for Catlin) on military installations across the United States. They were a subset of eleven soils used for NTO batch adsorption and transformation studies by Mark et al. (2016). Some of these soils had been used previously in studies of explosive and propellant formulation constituents (Dontsova et al., 2006, 2007, 2009a, 2009b; Taylor et al., 2012). As the same soils were used in batch and saturated flow studies we were able to compare determined parameters to verify if the previously observed processes were responsible for NTO attenuation. The selected soils had mixed particle size, ranging from fine (clay loam) to coarse (loamy sand) and a wide range of pH (4.40–8.21) and OC concentrations (0.34–5.28%). Their characteristics are summarized in Table A1 and in Mark et al. (2016).

2.2. NTO solution preparation and quantification

Technical grade NTO used in the experiments was obtained from US Army Armament Research, Development and Engineering Center (ARDEC), Picatinny Arsenal. Calibration standards for NTO were prepared using the same NTO. One mg L^{-1} solutions were prepared and NTO concentration was quantified using Dionex Ultimate 3000 high performance liquid chromatograph (HPLC) equipped with a diode array detector (ThermoFisher, MA) as described in Mark et al. (2016). The HPLC quantification method was adapted from Le Campion et al. (1999a). Mobile phase, acetonitrile (ACN): deionized water (75:25) with 0.1% TFA, was run isocratically at 1 mL min^{-1} . Oven temperature was set at $32 \text{ }^\circ\text{C}$. NTO and its transformation products were separated using a Thermo Scientific Hypercarb Column. NTO was detected at approximately 2.5 min using a 315 nm wavelength. The UV detector was set at 220 nm to monitor for presence of ATO (Le Campion et al., 1999a). No ATO was detected in any analyzed samples.

2.3. Saturated flow experiments

To measure the transport of NTO in soils, we conducted saturated miscible-displacement experiments under steady state and transient conditions. Solutions of NTO were used as the source. Concentrations of NTO, as well as a conservative tracer (bromide), used to characterize water flow through the columns, were determined in the column effluent and used to construct breakthrough curves.

Small columns (7 cm length with 1.18 cm internal diameter glass tubes with PTFE caps, Supelco, Bellefonte, PA) allowed us to minimize the amount of source material needed and reduce hazardous waste produced in the study. Between 11 and 12 g of soil was packed homogeneously over a bottom layer of silanized glass wool that prevented migration of particles. Average packed bulk density (ρ) (determined from the mass of air-dried soil used to pack a column of known volume) ranged between $1.71 \pm 0.06 \text{ g cm}^{-3}$ (Camp Guernsey soil) and $1.31 \pm 0.02 \text{ g cm}^{-3}$ (Arnold AFB soil). Saturated flow parameters, water content (θ) and packed bulk density (ρ) for all studied soils are shown in Table B2.

A layer of glass wool overlaid the packed soil and helped to evenly distribute incoming flow. To avoid air entrapment, columns were saturated from the bottom up with the background solution (0.005M CaCl_2) for approximately 1–1.5 h. After saturation, pore volume (PV) was determined by measuring the volume of solution needed to saturate the packed column. Tygon microbore tubing connected to a Cole-Parmer (Vernon Hills, IL) Master flex peristaltic pump was attached to the top of each column to supply 1 mg L^{-1} NTO solution with 0.005M CaBr_2 tracer at a given flux. A 0.02 mL min^{-1} flow rate, or 1.1 cm h^{-1} Darcy flux, was used (measured average flow rate was $0.0197 \pm 0.0019 \text{ mL min}^{-1}$, or Darcy flux of $1.083 \pm 0.0017 \text{ cm h}^{-1}$). Effluent was collected

continuously into 4 mL amber vials using Teledyne ISCO (Lincoln, NE) Foxy 200 Fraction collector with a 200-vial capacity. Pump was calibrated for constant target flow prior to the start of the experiment. Volumetric flow rate was monitored based on collected effluent. After 4 to 17 PV, depending on the soil, inflow was switched to 0.005M CaCl₂ solution and the flow continued for several more pore volumes to evaluate NTO desorption and elution from the soil.

Flow interruption tests, wherein flow was stopped for 24 h, were performed for all soils to better characterize and quantify rate-limited mass-transfer and transformation processes (Brusseau et al., 1989, 1997). When flow is stopped during the arrival wave (NTO injection phase), mass-transfer and transformation processes continue, which will be reflected in a decrease in concentration if transformation or sorption is rate limited. These flow interruption experiments were repeated for sterilized Catlin and Sassafra soils (OC content of 5.28 and 1.30%, respectively) to determine whether solute concentration loss was caused by microbial transformation. Soils were sterilized by autoclaving three times over three days with 1 h exposure to 120 °C each time.

Bromide was analyzed using Ion Chromatography (Dionex ICS 5000 with conductivity detector). Effluent solution samples were filtered through 0.45 μm Millex-HV PVDF filter (EMD Millipore Darmstadt, Germany), and placed in labeled 4-mL amber vials prior to HPLC analysis for NTO. The tracer allowed us to distinguish between non-equilibrium processes attributed to physical versus chemical properties and to determine dispersion and diffusion in the columns.

After the end of each experiment, the columns were divided into thirds and weighed. Extractions were performed on each soil sample to determine the amount of NTO remaining in the soil. A mixture of soil and acetonitrile in 1:2 ratio was agitated for 24 h, centrifuged, filtered through 0.45 μm Millex-HV PVDF filter (EMD Millipore Darmstadt, Germany), and analyzed using HPLC (U. S. Environmental Protection Agency, 2006).

2.4. Numerical modeling

Temporal moment analysis (TMA) was used to analyze breakthrough curves. TMA described in Supplement appendix 1 allowed to quantify solute transport properties such as dispersivity, mass balance ratio, or a ratio between mass of effluent and inflow solute, and retardation factor R (equal to $1 + (\rho/\theta) K_d$, where ρ is the soil bulk density, K_d is the linear adsorption coefficient, and θ is the water content).

HYDRUS-1D model (Šimůnek and van Genuchten, 2008; Šimůnek et al., 2016) was also used to analyze breakthrough curves obtained from the column experiments. Constant pressure head boundary conditions were used to model continuous flow experiments, while variable pressure head boundary conditions were used to model flow interruption tests.

Solute transport was modeled using the advection-dispersion equation:

$$\theta \frac{\partial C}{\partial t} + \rho \frac{\partial S}{\partial t} = \theta D \frac{\partial^2 C}{\partial z^2} - q \frac{\partial C}{\partial z} - \phi \quad (1)$$

where C is the solution concentration [ML⁻³], S is the sorbed concentration [MM⁻¹] ($S = K_d C$), D is the dispersion coefficient which includes both molecular diffusion and hydrodynamic dispersion [L²T⁻¹], q is the volumetric fluid flux density (specific discharge) [LT⁻¹] evaluated using the Darcy-Buckingham law, and ϕ is a sink-source term which includes various zero and first order reactions [ML⁻³T⁻¹]. In this application, ϕ represents the transformation (mass loss) of NTO. In some cases, this process was represented as a

first order reaction occurring only in the liquid phase and is defined as follows:

$$\phi = k\theta C \quad (2)$$

Alternatively, we also used a modified version of HYDRUS-1D that employs Monod kinetics to describe BTCs where effluent concentration decreases with time (De Wilde et al., 2009). This model accounts for bacteria growth using the following kinetics:

$$\frac{dX}{dt} = \left(\mu_m \frac{C}{K_s + C} \right) X - k_{decay} X \quad (3)$$

where X is the biomass concentration [ML⁻³], C is the liquid NTO concentration [ML⁻³], μ_m is the specific biomass growth rate coefficient [T⁻¹], K_s is the half saturation constant [ML⁻³], and k_{decay} is the decay rate [T⁻¹]. The corresponding equation describing NTO concentration is as follows:

$$\frac{dC}{dt} = -\frac{1}{Y} \left(\mu_m \frac{C}{K_s + C} \right) X \quad (4)$$

where Y is the yield [MM⁻¹].

In this model, bacterial growth requires the presence of NTO. If the amount of bacteria remains constant, the NTO transformation rate ($\mu^* X$) stays the same and the BTC of NTO would attain steady state conditions wherein the effluent concentration of NTO remains constant during continued constant injection. However, depending on extant conditions, significant bacterial growth may cause an increase in the magnitude of NTO transformation, causing NTO effluent concentrations to decrease with time (e.g., Brusseau et al., 1999, 2006).

2.5. Parameter estimation

First, the TMA was used to analyze bromide breakthrough curves to determine physical parameters characterizing the column experiments, namely λ . Then, NTO BTC data were analyzed using TMA to provide estimates for NTO transport parameters, including λ (cm), R , K_d (cm³ g⁻¹), and k (h⁻¹). Mass balance calculations were performed for NTO by mass balance ratio, r . NTO mass balance estimates were compared to recovery of the conservative tracer.

In HYDRUS-1D simulations of bromide transport, λ for the tracer was optimized using the TMA value as the initial estimate. For the NTO simulations, the HYDRUS-1D-optimized λ obtained for the tracer and K_d calculated by TMA for NTO were used as initial estimates for optimization by HYDRUS-1D. The transformation rate coefficients (first order or Monod based) were also optimized.

R² values characterize the correspondence between simulated and measured solute concentrations and the confidence intervals for the optimized parameters show the behavior of the objective function around its minimum. Parameter estimates were considered statistically significant if confidence intervals did not intersect with zero. At least three experiments were conducted for each soil. Interrupted and continuous flow experiments were treated as replicates for the purposes of statistical analysis for TMA estimates and analyzed separately for HYDRUS-1D. Differences between treatments were considered significant if confidence intervals did not overlap at 95% probability. When multiple column experiments were performed for the same soil under either continuous or interrupted flow conditions, their HYDRUS-1D parameter estimates and confidence intervals were averaged. The reaction parameters determined using the inverse mode of HYDRUS-1D were compared to the same parameters independently determined in soil batch studies. Agreement between the two sets of values provides

confidence that the determined parameters accurately characterize the transport of NTO in soils.

3. Results and discussion

3.1. Conservative tracer

The mean λ determined from the tracer BTC for both interrupted and continuous flow experiments was larger for coarser soils such as Camp Butner and Camp Guernsey (0.28 ± 0.136 and 0.26 ± 0.198 cm, respectively) than for the finer Catlin and Camp Swift soils (0.11 ± 0.151 and 0.17 ± 0.194 cm, respectively) (Tables B1 and B2), but differences were not significant at a 95% confidence level. In general, the values were relatively small (many not significantly different from zero) as expected for short repacked

Table 1

Mass recovered (%) in effluent of column transport experiments involving NTO and conservative tracer, bromide. Soils are listed in order of decreasing OC content.

Soil	NTO		Bromide	
	Mean	SD	Mean	SD
Catlin	34.2	12.4	103.8	10.8
Arnold AFB	45.0	11.1	97.4	2.9
Butner	52.7	2.3	98.0	5.9
Limestone Hills	64.2	12.9	94.2	8.6
Sassafras	68.3	12.3	107.0	12.4
Camp Guernsey	85.9	8.3	101.5	5.6
Florence MR	88.6	1.6	99.9	5.8
Camp Swift	90.4	1.7	98.4	4.5

columns. Breakthrough of the conservative tracer occurred at one pore volume (~140 min), which indicates a lack of preferential flow. Air entrainment within the column was not observed. Tracer breakthrough curves were plotted on a pore volume basis to compare their behavior to that of NTO. After flow interruption, effluent concentrations of bromide resumed at similar values, indicating minimal impact of diffusive mass-transfer processes associated with physically heterogeneous domains (soil aggregates, dead-end porosity, etc). Mass balance calculations showed full recovery of the tracer (Table 1).

3.2. NTO

Fig. 1 presents breakthrough curves for Camp Guernsey and Arnold AFB soils and Fig. 2 presents breakthrough curves for Sassafras and Catlin soils. Solid triangles represent measured values for NTO concentration for experiments with and without flow interruption, while lines indicate BTCs simulated by HYDRUS-1D (Figs. 1 and 2). Values for λ determined by HYDRUS-1D for NTO were relatively similar to bromide (Table B2). 11 out of 16 treatments (flow interruption and continuous flow experiments for each soil type) showed that 95% confidence intervals for NTO and bromide dispersivity values overlapped. In the 5 instances where 95% CI did not overlap, in 4 soils dispersivity estimates for NTO were higher than that of bromide, and in Sassafras soil (continuous flow) they were lower.

NTO breakthrough was observed after breakthrough of the conservative tracer, suggesting NTO adsorption to the solid phase, but for all soils this delay (retardation) was small. Four out of eight

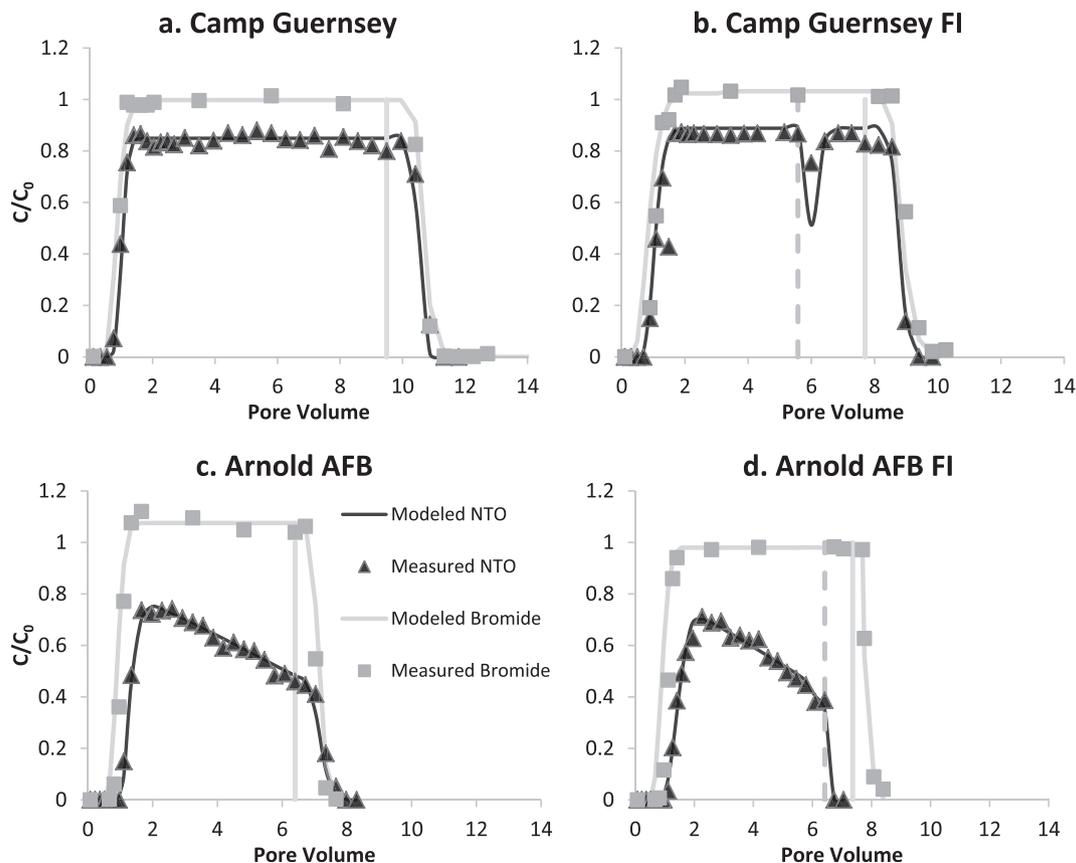


Fig. 1. Breakthrough curves for NTO in Camp Guernsey and Arnold AFB soils. Dashed grey vertical line indicates timing of 24-h flow interruption (FI). Grey solid vertical line indicates time when solution was switched back to saturating solution to observe desorption and elution of NTO.

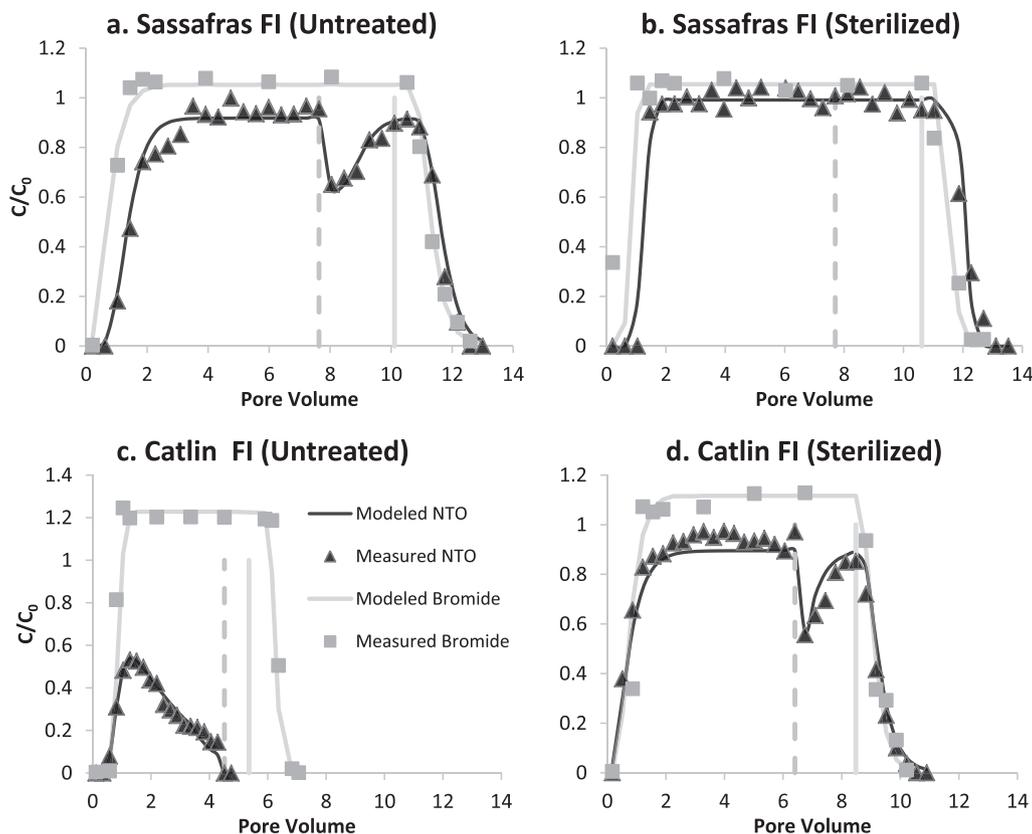


Fig. 2. Breakthrough curves for NTO in untreated and autoclaved Sassafra and Catlin soils. Dashed grey vertical line indicates timing of 24-h flow interruption. Grey solid vertical line indicates time when solution was changed back to saturating solution to observe desorption phase of the isotherm.

soils, Sassafra, Camp Guernsey, Florence MR, and Camp Swift, exhibited behavior consistent with adsorption and first order transformation observed in the batch studies (Mark et al., 2016). For example, for Camp Guernsey soil (Fig. 1a and b), there is very little retardation observed consistent with low K_d measured in batch studies ($0.02 \text{ cm}^3 \text{ g}^{-1}$). Steady state concentrations after breakthrough are consistent with first order transformation, as is the decrease in effluent NTO concentration after 24 h flow interruption and return to steady state observed for these soils (Figs. 1b and 2a).

However, in the other four soils, Catlin (Fig. 2a and b), Arnold AFB (Fig. 1c and d), Camp Butner, and Limestone Hills, effluent concentrations did not attain steady state, but rather decreased with time, indicating an increase in the transformation rate. They also exhibited sharper decreases in NTO concentration following flow interruption, such that NTO could no longer be detected, and after flow was restarted concentrations of NTO did not increase. Such changes in effluent concentrations with time can be explained by a change in conditions within the column. One possible mechanism is a change in redox state of the column as the experiment progresses. As has been shown by Krzmarzick et al. (2015), NTO reduction, a first step in its transformation, is favored under anaerobic conditions. Anaerobic conditions are more likely to develop as a result of microbial respiration when OC is present and this is consistent with the fact that the temporal increase in transformation rates was observed only for soils with high OC content (Table B1). However, we do not have a direct measure of change in oxygen concentration or redox potential of the effluent. It is also possible that microbial growth, stimulated by OC in the soil, could directly affect NTO concentration if microorganisms cometabolize NTO with the OC. NTO transformation linked to

bacterial growth is supported by our modeling results. Applying Monod-type kinetics for Catlin (Fig. 2c and d), Arnold AFB (Fig. 1c and d), Camp Butner, and Limestone Hills soil experiments in HYDRUS-1D generated R^2 values close to 1 (Table 2).

Mass balance calculations showed that NTO recovery was less than a 100% (Table 1) and similar between continuous and interrupted flow experiments (not shown) but decreased with increase in OC content of the soil (Table 1) consistent with positive correlation between k values and OC observed in batch studies (Mark et al., 2016). No NTO or transformation products were recovered from soil by acetonitrile extraction at the end of the column experiments. Since experiments were continued in desorption phase until no NTO was recovered in the effluent, no water extractable NTO remained in the soils.

Further evidence for a microbial contribution to NTO transformation comes from the results of experiments conducted with sterilized soils, which were compared to the non-sterilized soil data in Fig. 2 for Catlin (5.28% OC) and Sassafra (1.30% OC). For untreated Sassafra soil, the BTCs exhibited constant NTO concentrations and decreases in concentration upon flow interruption consistent with first order transformation. For autoclaved Sassafra soil, the effluent NTO concentration was similar to inflow concentration, and flow interruption had no effect, indicating no transformation. For Catlin soil, which had a Monod-type transformation behavior and no recovery after flow interruption in untreated columns, the effluent concentrations were constant and concentrations recovered after flow interruption for the sterilized treatments.

The k values (Table 3) were higher in untreated soils: k determined for untreated Sassafra soil was approximately 600 times higher than k determined for sterilized Sassafra soil (which was

Table 2
NTO fate and transport parameters from temporal moment analysis (TMA) and HYDRUS-1D, linear adsorption coefficients, K_d , first order transformation rates, k , and initial Monod transformation rate, with average rate shown in parentheses. In all cases, TMA estimates of k were determined for the portion of the BTC where steady state influent concentration was achieved. TMA results include data from both interrupted flow (FI) and continuous flow experiments. Interrupted and continuous flow experiments were treated as replicates for the purposes of statistical analysis. HYDRUS-1D results show interrupted flow (FI) and continuous flow experiments separately. NA indicates that results are not available. Soils are listed in order of decreasing OC content. CI = 95% confidence interval.

Soil	TMA						HYDRUS-1D						R^2
	$K_d, \text{cm}^3 \text{g}^{-1}$		R		k, h^{-1}		$K_d, \text{cm}^3 \text{g}^{-1}$		k, h^{-1}		Monod Trans. rate, h^{-1}		
	Est.	CI	Est.	CI	Est.	CI	Est.	CI	Est.	CI	Est.	CI	
Catlin	0.33	0.12	1.70	0.25	NA		0.15	0.04			0.058 (0.123)	0.015	0.97
Catlin FI							0.04	0.04			0.046 (0.133)	0.016	0.96
Arnold AFB	0.39	0.16	1.99	0.38	NA		0.17	0.01			0.072 (0.121)	0.008	0.99
Arnold AFB FI							0.13	0.02			0.067 (0.133)	0.010	0.98
Camp Butner	0.47	0.19	2.81	1.43	NA		0.12	0.01			0.207 (0.259)	0.024	0.99
Camp Butner FI							0.06	0.01			0.100 (0.183)	0.011	0.99
Limestone Hills	0.22	0.14	1.61	0.40	NA		0.0001	0.01			0.038 (0.154)	0.010	0.99
Limestone Hills FI							0.15	0.01			0.034 (0.186)	0.009	0.99
Sassafras	0.62	0.41	3.01	1.41	0.006	0.006	0.05	0.01	0.100	0.008			0.99
Sassafras FI							0.40	0.31	0.076	0.058			0.96
Camp Guernsey	0.12	0.07	1.58	0.27	0.020	0.005	0.03	0.01	0.009	0.009			0.98
Camp Guernsey FI							0.05	0.01	0.064	0.010			0.98
Florence MR	0.07	0.03	1.30	0.16	0.023	0.007	0.03	0.02	0.065	0.013			0.98
Florence MR FI							0.04	0.01	0.041	0.003			0.97
Camp Swift	0.02	0.05	1.07	0.19	0.049	0.008	2.55E-06	2.55E-06	0.040	0.008			0.98
Camp Swift FI							0.04	0.01	0.009	0.004			0.99

not significantly different from zero), and the average Monod transformation rate in untreated Catlin soil was approximately 3 times higher than the k value determined for columns using sterilized Catlin soil (Table 3). No transformation products were observed, and no NTO was recovered in soil by acetonitrile extraction. Between the soils, transformation rates were higher for the Catlin soil with or without sterilization.

Similarly to what was observed in batch studies (Mark et al., 2016), autoclaved and untreated soils also showed a significant difference in mass loss, particularly for soil with higher OC content, suggesting biotransformation (Fig. 2 and Table 3). Forty percent less NTO was recovered in the column effluent in untreated Catlin soil compared to recovery from columns using Catlin soil sterilized by autoclave (24 vs 68%) (Table 3). Similarly smaller mass balance ratios were determined for autoclaved compared to untreated Sassafras soils (81% and 91%, respectively). Combined evidence from impact of sterilization on DNAN transformation rates, mass loss, and shape of breakthrough curves, and from ability of Monod rates to describe change in NTO concentrations for high OC soils strongly supports importance of microbial activity for NTO transformation.

Good fits for BTC for Sassafras, Camp Guernsey, Florence MR, and Camp Swift were achieved using an equilibrium adsorption model suggesting that kinetic sorption was likely negligible (Table 2). With the exception of Florence MR and Camp Guernsey soils, the K_d values determined from HYDRUS-1D matched well with TMA derived values and fell within 95% confidence intervals

(Table 2). Irreversible attenuation and transformation, as indicated by k , was statistically significant in TMA and when modeled by HYDRUS-1D. The k values determined from HYDRUS-1D were generally in the same order of magnitude as TMA values but did not fall within 95% confidence intervals, with the exception of Florence MR FI and Camp Swift FI (Table 2). Soils that had higher OC content, Catlin, Arnold AFB, Camp Butner, and Limestone Hills, showed non-first order, irreversible attenuation as indicated by lower NTO recovery in effluent and changes in transformation rates with time, which was modeled by HYDRUS-1D using Monod-type kinetics. Obtained transformation rate constants for these soils could not be compared directly to the TMA estimates.

There was a general agreement between batch and column determined sorption parameters. Figures B1a and B1b show a comparison of NTO K_d s determined from batch and column experiments using TMA and HYDRUS-1D. Correlation between K_d s derived from TMA BTC data and measured in batch experiments was highly significant ($P = 0.00020$) (Figure B1a). The slope of the regression was higher than but not significantly different from 1. K_d s derived from HYDRUS-1D using BTC data were also significantly correlated to, but lower than, K_d estimates in batch experiments (Figure B1b). Correlating an average of TMA and HYDRUS-1D derived K_d versus batch K_d for the eight soils tested (Fig. 3a) yields a slope of regression equal to 0.97 (lower and upper 99% CI, 0.58 and 1.35, respectively, and $P = 0.000049$) indicating that the estimates are the same. This increases our confidence in the determined values, and as a result, in our ability to predict NTO

Table 3
Average NTO mass recovered (%) in the effluent and the HYDRUS-1D-calculated transformation rate constant for column transport experiments involving untreated and sterilized Catlin and Sassafras soils. Average Monod transformation rate constant (h^{-1}) (an average of the NTO transformation rate constants using the initial and final biomass concentration, X) is shown for Catlin FI Untreated, while first order transformation rate constant (h^{-1}) is shown for the remaining treatments. FI = flow interruption for 24 h.

Soil	Mass Recovery			Transformation rate constant, h^{-1}			R^2
	Estimate	95% CI		Estimate	95% CI		
Catlin FI Untreated	24	17	31	0.137 ^a	0.128	0.147	0.95
Catlin FI Sterilized	68	45	90	0.048	0.038	0.059	0.95
Sassafras FI Untreated	81	77	85	0.030	0.025	0.036	0.97
Sassafras FI Sterilized	91	90	92	4.99E-05	-1.76E-03	1.86E-03	0.96

^a Average Monod transformation rate (h^{-1}).

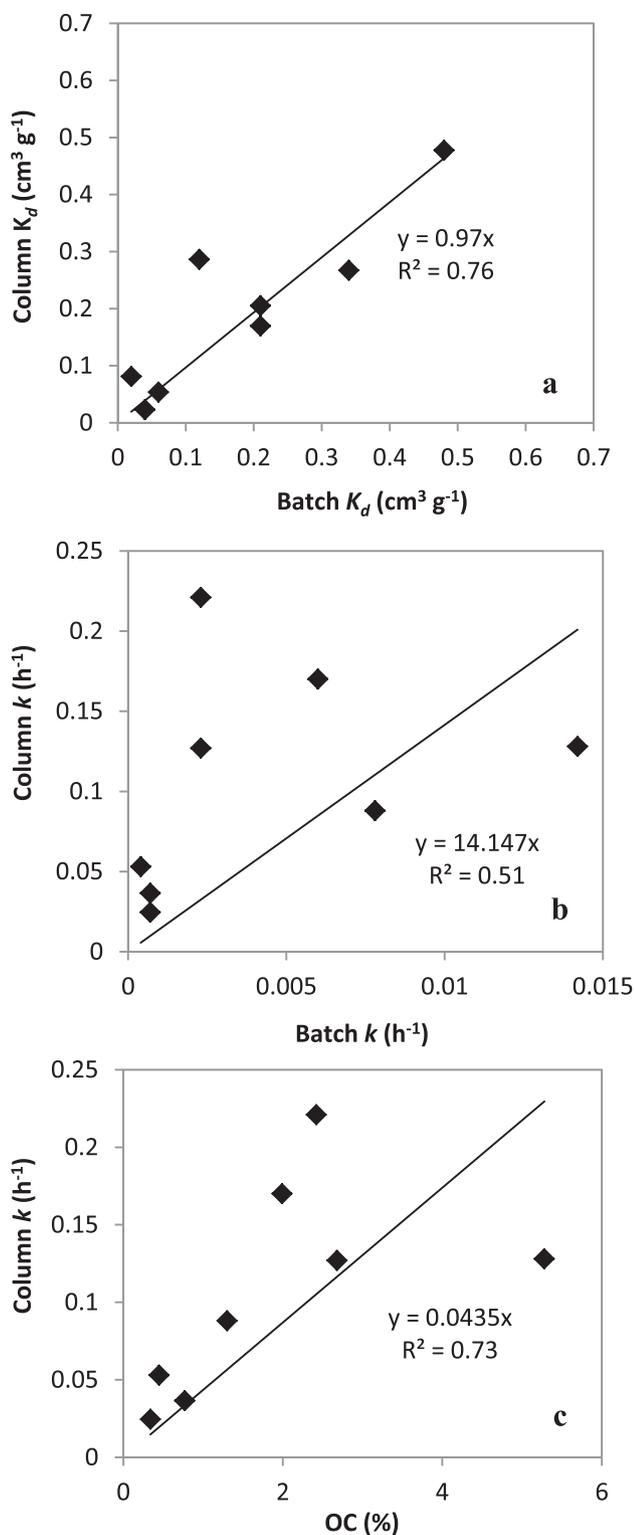


Fig. 3. Correlation of NTO linear adsorption coefficients (K_d) between batch measurements (Mark et al., 2016) and column measurements derived from the temporal moment analysis and HYDRUS-1D ($P = 0.000049$) (a). Correlation between batch (Mark et al., 2016) and column (derived from HYDRUS-1D (b, $P = 0.031698$)) NTO transformation rate constants; and between OC percent in the soil and column transformation rate constant (c, $P = 0.003306$). Values from continuous and flow interrupted experiments were averaged.

adsorption in other soils if we know their pH, a relationship developed by Mark et al. (2016) using batch study results.

The estimates of transformation rate constants, a combination of first order rates for soils with steady state conditions and mean ($0.1060 \pm 0.0339 \text{ h}^{-1}$) or initial ($0.0641 \pm 0.0228 \text{ h}^{-1}$) Monod rates for soils that did not, in column experiments were, on average, more than an order of magnitude higher than ones determined in the batch studies for the same soils ($0.0043 \pm 0.0033 \text{ h}^{-1}$). If we compared only soils that had steady state rates we saw similar trends: for batch studies rates equaled $0.0024 \pm 0.0035 \text{ h}^{-1}$ and for column studies $0.0505 \pm 0.0221 \text{ h}^{-1}$. Correlation between batch and HYDRUS-1D determined values was weak (Fig. 3b). It was not significant when the intercept was allowed to vary but significant ($P = 0.031698$) if the intercept was set to zero. Obtained transformation rate constants also correlated with percent OC ($P = 0.003306$) but also only with a zero intercept (Fig. 3c). A positive relationship between OC and k values was observed in batch studies (Mark et al., 2016). A slope of the correlation between k estimates using batch and column experiments was significantly different from one.

We need to understand the large difference between transformation rates observed in column and batch experiments to predict NTO behavior in the environment based on measurements done in the lab. One factor influencing the comparison of batch and column biodegradation rate coefficients is that the NTO supply is quite different. In a batch system the NTO is a fixed source that depletes with time. Conversely, for column experiments, there is a constant supply of NTO. This maintains a maximal rate of transformation, and also allows for greater microbial growth. We would expect that column experiments are closer to the field conditions in this respect and therefore provide a more realistic estimate of NTO transformation.

This work emphasized the importance of microbial transformation for NTO fate in soils. It showed that conditions that are favorable for microbial activity, such as high OC content, promote NTO transformation, main mechanism of natural attenuation for this compound that is highly soluble and negatively charged under conditions usually encountered in soils, and therefore weakly adsorbed. These findings also provide a potential mechanism for treatment of NTO-impacted soils.

4. Conclusion

We conducted a series of saturated flow experiments with and without flow interruption (for 24 h equilibration period) for eight soils collected on military ranges across the US and having a range of soil properties. Sorption K_d s obtained from the column experiments were consistent with those determined from prior batch studies. Conversely, larger transformation rate constants were obtained for the column study. NTO transformation rates increased with increase in soil OC, as was observed for the batch study. Soils with higher OC content also exhibited Monod kinetics of transformation characterized by increase in transformation rate with time caused by bacterial growth.

Overall, the results indicate that NTO exhibits generally low sorption. This would mean that NTO has a great potential for advective transport in the subsurface. However, the results also show that NTO is subject to significant mass loss via biological transformation. Depending upon extant site conditions, this transformation may cause significant attenuation during transport. The results of this study may be useful for evaluating the significance of attenuation and potential impacts on groundwater quality.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.chemosphere.2016.12.067>.

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