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Complete Reduction of TNT and Other (Poly)nitroaromatic Compounds under Iron-Reducing Subsurface Conditions

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Contamination of soils and aquifers with (poly)nitroaromatic compounds ((P)NACs) is a widespread problem. This work demonstrates that (P)NACs such as the explosive 2,4,6-trinitrotoluene (TNT) can be completely reduced to the corresponding aromatic polyamines by Fe(II) present at the surface of Fe(III)(hydr)oxides or, less efficiently, by hydroquinone moieties of (natural) organic matter in the presence of H₂S. The reduction kinetics of (P)NACs were investigated in sterile batch systems as well as in columns containing either FeOOH-coated sand and a pure culture of the iron-reducing bacterium *Geobacter metallireducens* or ferrogenic consortia in aquifer sediments. The relative reactivities as well as the competition behavior of (P)NACs in batch and column systems, respectively, correlated well with their one-electron reduction potentials, $E_h^{1'}$, which we determined for TNT and its aminonitrotoluene transformation products. A similar reactivity pattern of (P)NACs was found irrespective of the processes that (re)generated the surface-bound Fe(II), i.e., adsorption of Fe(II) from aqueous solution or microbial reduction of Fe(III)(hydr)oxides. The apparent stability of the toxic arylamine products under ferrogenic conditions may compromise intrinsic attenuation as an acceptable remediation option for (P)NAC contaminated anoxic aquifers. Iron-reducing conditions would, however, be favorable as a first step in a two-stage anaerobic/aerobic treatment of PNAC contaminated sediments since aromatic polyamines are biodegradable and/or bind irreversibly to the solid matrix under oxic conditions.

Introduction

Mono- and polynitroaromatic compounds ((P)NACs) are widely used as agrochemicals, explosives, textile dyes, and chemical intermediates, and they have been found to be ubiquitous pollutants, particularly in soil and subsurface

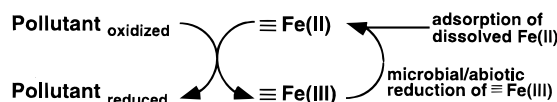
environments (1, 2). Among these chemicals the explosive 2,4,6-trinitrotoluene (TNT) and related compounds including (poly)nitroated toluenes and benzenes as well as their reduction products (e.g., aminodinitrotoluenes (ADNTs) and diaminonitrotoluenes (DANTs)) are presently of particular interest because of numerous subsurface contaminations (3–7). Obviously, a proper risk assessment and evaluation of appropriate remediation strategies of such contaminations require a basic understanding of the processes that govern the transport and transformation of (P)NACs in the subsurface.

In earlier and parallel work, we have shown that the transport of (P)NACs at conditions typical for most aquifers is governed by electron donor–acceptor (EDA) complexes with phyllosilicate minerals such as clays (8–10). This specific adsorption, which may also affect significantly the (bio)-availability of (P)NACs in the subsurface, is strongest for planar aromatic compounds with several nitro groups and/or other electron-withdrawing substituents (for more details see refs 8, 11, and 12).

With respect to transformation reactions of (P)NACs, it has been found that microbial as well as abiotic processes may be important in subsurface environments. Reduction of nitro groups is the predominant transformation pathway of (P)NACs under anaerobic as well as aerobic conditions (13–19). Since reduced (P)NACs will inevitably occur as intermediates or products of (P)NAC transformation in the subsurface, it has been proposed to remediate ammunition-contaminated soil matrix by a two-stage anaerobic/aerobic treatment, i.e., by a complete reduction of the nitroaromatic explosives and the subsequent irreversible binding of the aromatic (poly)amines to natural organic matter (20–27). Such a procedure requires detailed knowledge of possible transformation pathways of TNT to 2,4,6-triaminotoluene (TAT) in reducing environments. However, since different types of biological and chemical transformation processes may take place concurrently in the subsurface, a clear identification of the microorganisms or chemical reductants involved is, in most cases, rather difficult. Considering abiotic transformation reactions of (P)NACs and other reducible pollutants, it is likely that under anoxic conditions in the subsurface, reduced sulfur and iron species are the most important reductants (28). As has become evident from various recent investigations, ferrous iron species play an important role in the reduction of a variety of organic and inorganic pollutants in natural and engineered systems (for a review see ref 29).

Our current understanding is that very reactive Fe(II) surface species may be formed either through adsorption of aqueous Fe(II) to iron(III)(hydr)oxides or other mineral surfaces (30–32) or through microbial/abiotic reduction of ferric iron containing minerals (Scheme 1).

SCHEME 1



Using mononitroaromatic compounds (NACs), we have shown that surface-bound Fe(II) species are efficient reductants of a range of NACs exhibiting very different reactivities. Consequently, one may expect (P)NACs such as TNT to be reduced in soils and aquifers by surface-bound Fe(II) species, which may open new perspectives for the remediation of contaminated subsurface matrixes.

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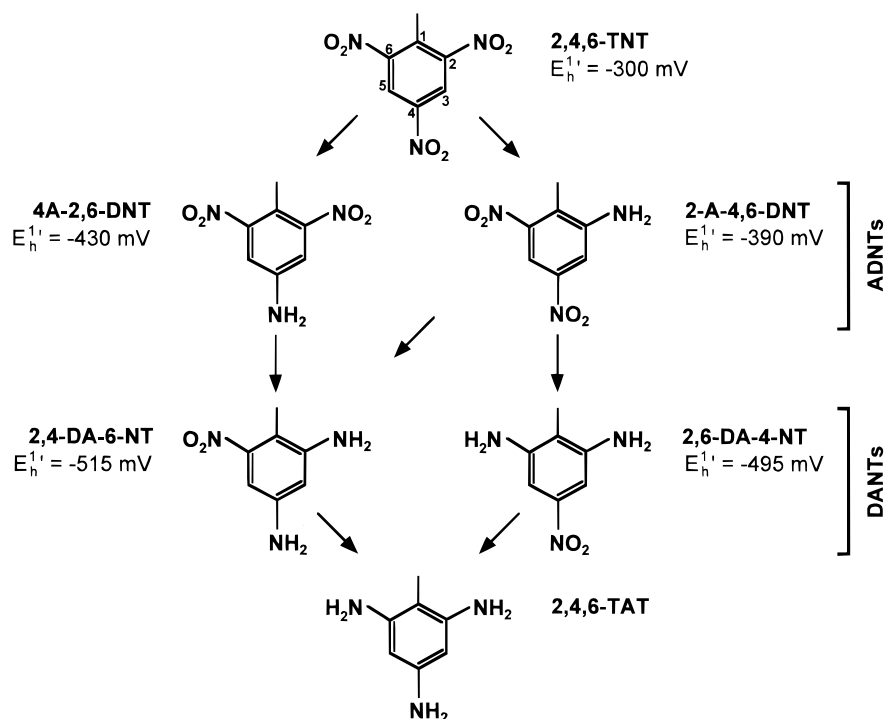


FIGURE 1. Reduction pathway of TNT to aminodinitrotoluenes (ADNTs), diaminonitrotoluene (DANTs), and TAT. $E_h^{1'}$ stands for the one-electron reduction potentials of the compounds at pH 7 (see eq 3).

The major goals of the work presented in this paper were (1) to investigate the complete reduction of (P)NACs by reactive Fe(II) surface species (exemplified by TNT and related aminonitrotoluenes (ANTs)), (2) to establish structure–activity relationships to evaluate the kinetics and the product distribution of (P)NAC reduction (Figure 1), and (3) to provide further evidence for the biogeochemical mechanisms involved in the formation and regeneration of reactive Fe(II) surface sites in the subsurface (Scheme 1). To this end, we chose TNT and its major metabolites as model PNACs. We investigated the reactivity of these compounds as single solutes as well as in binary mixtures in model systems mimicking iron-reducing conditions in the subsurface. Experiments were performed in sterile batch suspensions containing goethite FeCl_2 -electrolytes as well as in columns containing either pure cultures of a dissimilatory iron-reducing bacterium or sediment material with consortia of active iron-reducing microorganisms. To evaluate the reactivity of (P)NACs quantitatively in these systems, the one-electron reduction potentials, $E_h^{1'}$, of the compounds were determined from independent experiments.

Experimental Section

Chemicals. The suppliers of the (P)NACs as well as compound abbreviations used in this study are listed in Table 1. Additional compounds used and their producers follow: 2,4,6-triaminotoluene trihydrochloride (Promochem, Wesel, Germany); 3-(*N*-morpholino)propanesulfonic acid (MOPS), 8-hydroxy-1,4-naphthoquinone (juglone), 2-propanol, KH_2PO_4 , Na_2S , NaHCO_3 , FeCl_3 , FeCl_2 , CaCl_2 , KCl , NH_4Cl , and sodium acetate (Fluka AG, Buchs, Switzerland); methanol and acetonitrile (Scharlau, Barcelona, Spain); ethyl acetate (Burdick & Jackson); HCl , NaOH , NaCl , and HClO_4 (Merck AG, Dietikon, Switzerland); N_2 , N_2/H_2 , and N_2/CO_2 ($\geq 99.999\%$; Carbagas, Rümlang, Switzerland); and goethite (α - FeOOH , Bayferrox 910, BET-surface area 17.5 m^2 ; Bayer, Germany). All chemicals were of analytical grade or higher purity and were used without further purification.

Chemical Analyses. All samples containing (P)NACs and 2,4,6-triaminotoluene (TAT) were analyzed by reversed-phase

HPLC (Gynkoteck; pump M480, diode array UV detector 340S, GINA 50 autosampler, data system V5–30). TNT and aminonitrotoluenes were analyzed on a Supelcosil C-18 column ($250 \times 4.6 \text{ mm}$, $5 \mu\text{M}$ spheres, 20 mm Supelcoguard C-18 guard columns; Supelco, Gland, Switzerland). The eluent consisted of a mixture of 2-propanol/acetonitrile/ H_2O (20%/5%/75%; v:v:v) buffered with 5 mM phosphate at pH 7.0. TAT was quantified separately on a reversed-phase C-8 column ($125 \times 4 \text{ mm}$, $5 \mu\text{M}$ spheres; Merck, Darmstadt, Germany) with 1% of acetonitrile and 99% of phosphate buffer (pH 7.0) as mobile phase. Analyses of substituted mononitroaromatic compounds were performed on either reversed-phase C-18 or C-8 columns (Merck, Darmstadt, Germany) with various $\text{MeOH}/\text{H}_2\text{O}$ mixtures, buffered with 5 mM phosphate at pH 7.0. The flow rate was 1.0 mL/min for all measurements, UV–vis detection was performed simultaneously at four wavelengths, namely at 220 nm, 254 nm, and two wavelengths at the absorption maxima of the compounds analyzed (Table 1). Acetate was measured by ion chromatography (Metrohm, Model 690, Herisau Switzerland) as described in Heijman et al. (31).

Preparation of Oxygen-Free Solutions for Batch and Column Experiments. Autoclaved buffer solutions and methanolic spike solutions containing the (P)NACs for batch experiments were made oxygen free by purging with N_2 for at least 3 h. All batch assays (57 mL) were prepared in serum flasks in an anaerobic glovebox (ALK 421, Coy Laboratory Prod. Inc., Grass Lake, MI). Feeding solutions for column experiments were prepared with an autoclaved solution of 10 mM NaHCO_3 and purged with N_2/CO_2 (90/10) gas for at least 3 h. The $\text{HCO}_3^-/\text{CO}_2$ system chosen buffered the reservoirs and the columns at pH 7.2.

Batch Experiments for Derivation of $E_h^{1'}$ Values of (P)-NACs. Experiments in homogeneous solution containing 5 mM hydrogen sulfide and variable concentrations of juglone (8-hydroxy-1,4-naphthoquinone) as electron-transfer mediator were performed as described by Schwarzenbach et al. (33) and Perlinger et al. (34), using autoclaved glassware, phosphate buffer (50 mM, pH 6.60), 1 M HCl , and aliquots of 0.6 M Na_2S . The juglone stock solution (0.02 M) was

TABLE 1. Names, Abbreviations, One-Electron Reduction Potentials (E_h^1), Reaction Rate Constants, and Competition Coefficients (Q_c) of TNT, Aminonitrotoluenes, and NACs in Various Experimental Systems

compound	abbreviation	λ_{\max} (nm)	E_h^1 (mV)	batch systems		column systems			
				juglone/H ₂ S	Fe(II)/goethite	consortium of iron-reducing bacteria/aquifer matrix		<i>G. metallireducens</i> / FeOOH-coated sand	
				k_{HJUG^-} (M ⁻¹ s ⁻¹)	$k_{\text{obs, Fe(II)/goethite}}$ (s ⁻¹)	$k_{\text{obs, aquifer col}}$ ^{a,b} (mM h ⁻¹)	Q_c , aquifer col. ^c (-)	$k_{\text{obs, pure culture}}$ ^{a,d} (μ M h ⁻¹)	Q_c , pure culture ^c (-)
2,4,6-trinitrotoluene	TNT ^e	225	-300 ^f	$(7.4 \pm 0.52) \times 10^2$ ^g	$(4.6 \pm 1.2) \times 10^{-3}$ ^g	2.3	50		
2-amino-4,6-dinitrotoluene	2-A-4,6-DNT ^h	211	-390 ^f	$(9.7 \pm 0.37) \times 10^0$	$(4.6 \pm 0.3) \times 10^{-4}$	1.4	2.7		
4-amino-2,6-dinitrotoluene	4-A-2,6-DNT ^h	215	-430 ^f	$(1.2 \pm 0.36) \times 10^0$	$(3.6 \pm 9.2) \times 10^{-4}$	1.9	3.2		
2,4-diamino-6-nitrotoluene	2,4-DA-6-NT ^h	211	-515 ^f	$(2.1 \pm 0.13) \times 10^{-2}$	$(5.3 \pm 0.67) \times 10^{-5}$	1.7	0.35		
2,6-diamino-4-nitrotoluene	2,6-DA-4-NT ^h	210	-495 ^f	$(5.6 \pm 0.16) \times 10^{-2}$	$(6.1 \pm 0.57) \times 10^{-5}$	1.7	0.14		
nitrobenzene	NB ⁱ	267	-486 ^{j,k}			0.82 ^l	0.33 ^l	2.4	0.34
2-nitrotoluene	2-CH ₃ -NB ⁱ	265	-590 ^m			0.82 ^l	0.75 ^l	2.6	0.60
3-nitrotoluene	3-CH ₃ -NB ⁱ	273	-475 ^m			0.66 ^l	0.55 ^l	2.8	0.40
4-nitrotoluene	4-CH ₃ -NB ⁱ	285	-500 ^m	$(4.7 \pm 0.40) \times 10^{-2}$		0.66 ^l	0.40 ^l	2.7	0.30
2-chloronitrobenzene	2-Cl-NB ⁱ	258	-485 ⁿ			0.82 ^l	4.4 ^l	2.9	4.2
3-chloronitrobenzene	3-Cl-NB ⁱ	263	-405 ^m			0.66 ^l	3.0 ^l	2.5	3.0
4-chloronitrobenzene	4-Cl-NB ⁱ	276	-450 ^m	$(4.8 \pm 0.080) \times 10^{-1}$	$(2.2 \pm 0.73) \times 10^{-4}$	0.82 ^l	1.0 ^l	2.7	1.0
2-acetylnitrobenzene	2-COCH ₃ -NB ⁱ	242	-470 ⁿ			0.71 ^l	1.5 ^l	2.5	1.2
3-acetylnitrobenzene	3-COCH ₃ -NB ⁱ	265	-405 ⁿ			0.90 ^l	2.5 ^l	2.8	2.1
4-acetylnitrobenzene	4-COCH ₃ -NB ⁱ	266	-358 ^l	$(5.1 \pm 0.16) \times 10^1$		0.90 ^l	6.0 ^l	2.9	5.2
1,3-dinitrobenzene	1,3-DNB ⁱ	242	-345 ^j	$(6.1 \pm 0.12) \times 10^1$			3.0		
1,4-dinitrobenzene	1,4-DNB ⁱ	264	-257 ^j	$(6.9 \pm 3.7) \times 10^3$					
2-nitroaniline	2-NH ₂ -NB ⁱ	224	<-560 ^f	$(2.3 \pm 1.5) \times 10^{-3}$			0.080		
3-nitroaniline	3-NH ₂ -NB ⁱ	225	-500 ^f	$(4.3 \pm 0.14) \times 10^{-2}$			0.25		

^a Equation 1. ^b Column type II: $\tau = 30\text{--}40$ h, depending on the reactivity of the columns, porosity = 0.4. ^c Equation 2. ^d Column type III: $\tau = 48$ h, porosity = 0.4. ^e Supplier, Ems Chemie (Dottikon, CH). ^f The 95% confidence intervals for TNT and aminonitrotoluenes are about 6 mV. ^g The 95% confidence intervals ($n = 3\text{--}4$). ^h Promochem (Wesel, D). ⁱ Fluka AG, (Buchs CH). ^j References cited in ref 43. ^k Reference 55. ^l Data from ref 31. ^m References cited in Schwarzenbach et al. (33). ⁿ Reference 33.

prepared in oxygen-free methanol. Control experiments were prepared similarly except for the addition of juglone. All assays were stored in the dark in a water bath at 25 °C and were allowed to equilibrate at least 1 day before the start of the experiments.

Kinetic experiments were started by addition of the 20 mM methanolic (P)NAC solution (50 μ M initial (P)NAC concentration) to the assays. After the injection of a certain volume of N₂, an equal volume of aqueous sample was withdrawn with a syringe and was extracted with ethyl acetate (1:1; v/v) on a vortex mixer for 30 s. The extracts were analyzed by HPLC. The kinetics of (P)NAC reduction were pseudo first order with respect to both (P)NAC concentration and total juglone concentration (2–20 μ M; data not shown). The resulting second-order rate constants were corrected for the blank reaction of (P)NACs with hydrogen sulfide which was significant (2–35% of the overall reactivity) only for (P)NACs with $E_h^{1'} > -430$ mV (data not shown).

Batch Experiments in Fe(II)/ α -FeOOH Suspensions.

Experiments were set up in an anaerobic glovebox following a procedure described by Rügge et al. (28). The method was evaluated by Pecher et al. (35). The suspensions contained 11.2 m²/L (0.64 g/L) of goethite (α -FeOOH), 1.5 mM FeCl₂ and 25 mM 3-morpholinopropanesulfonic acid (MOPS) buffer (pH 7.22, ionic strength 20 mM). Goethite suspensions spiked with Fe(II) were equilibrated for 48 h before starting kinetic experiments by the addition of the methanolic (P)-NAC stock solutions with a gastight glass syringe (50 μ M initial (P)NAC concentration). The assays were kept in the dark in a water bath (25 °C) and were stirred at 600 rpm (4g) with a magnetic stirrer. Control assays were prepared similarly except for the addition of goethite or Fe(II), respectively. At given time intervals, aliquots of 500 μ L were withdrawn. Three hundred microliters of each sample was immediately filtrated (0.2 μ m, Spartan 13/A, regenerated cellulose, Schleicher & Schuell, Dassel, Germany) into an auto-sampler vial which contained 9 μ L of HClO₄ (60%) to avoid further reduction of the (P)NACs after sampling. Losses of (P)NACs due to filtration were small (<2%). Pseudo-first-order reaction rates of the (P)NACs were calculated from linear regression analysis.

General Description of the Column Experiments. The reduction of (P)NACs in column systems operated under iron-reducing conditions was investigated in three different column types following procedures described by Heijman et al. (31): type I, 24 cm length, 470 mL volume, seven sampling ports; type II, 22 cm length, 180 mL volume, two sampling ports; type III, 8.5 cm length, 12 mL volume, two sampling ports.

Zero-order rate constants, k_{obs} , calculated according to eq 1, were determined for all compounds in the column systems (for more details see ref 31):

$$k_{obs} = \frac{c_{in} - c_{eff}}{\tau} \quad (1)$$

c_{in} and c_{eff} are the stationary concentrations of (P)NACs in the influent and effluent, respectively, and τ is the residence time of the compound in the column. Binary competition coefficients of (P)NACs, Q_c , were calculated using the measured zero-order rate constants, k_{obs} , in a binary mixture of the (P)NAC and 4-chloronitrobenzene (4-Cl-NB; arbitrarily chosen reference compound) according to equation 2

$$Q_c = \frac{k_{obs}((P)NAC)}{k_{obs}(4-Cl-NB)} \quad (2)$$

Column experiments using FeOOH-coated sand and pure cultures of a dissimilatory iron(III)-reducing bacterium were conducted with *Geobacter metallireducens* strain GS-15 ((36), kindly provided by D. Lovley) and 10 substituted

NACs as model compounds. The experiments were performed in glass columns (type III) filled with acid-washed sand that was coated with a not further characterized iron oxyhydroxide (FeOOH) according to Scheidegger et al. (37). FeOOH was synthesized by neutralizing a solution of FeCl₃ following a method described by Lovley and Phillips (38). FeOOH-coated sand was washed with Nanopure H₂O and subsequently autoclaved before packing into the columns. The columns consisted of glass test tubes sealed with a Viton rubber stopper (Maagtechnik, Dübendorf, Switzerland) which had two drillings to guide the in-/outflow stainless steel tubes (1 mm i.d.). The inflow tube ended right after the stopper from where the solutes migrated through the matrix to the outflow tube at the bottom of the column. Flow was maintained at 2–3 mL/d with a peristaltic pump (ISMATEC, Model IPN, Zürich, Switzerland) which was connected to the outflow tube of the column.

The influent medium contained 5 g/L of NaHCO₃, 0.1 g/L CaCl₂, 0.1 g/L KCl, 1.5 g/L NH₄Cl, 10 mL/L of a vitamin solution (39), 10 mL/L of a trace mineral solution (39), and variable concentrations of sodium acetate (0–76 μ M). The final NAC concentration in this feeding solution was 150 μ M. The N₂/CO₂ gas mixture (90%/10%) used for buffering was led through an oxygen trap which consisted of an unbuffered 0.55 M FeCl₂ solution before entering the influent reservoir. Cultures of *G. metallireducens* were cultivated in an Fe(III)–citrate medium as described by Lovley and Phillips (39). The columns were inoculated by injecting through the Viton stopper 2 mL of a *G. metallireducens* culture that was in the late logarithmic growth phase.

Column Experiments Using Aquifer Sediments and an Indigenous Consortium of Iron-Reducing Bacteria. Experiments in aquifer columns (type I and II) were operated under iron-reducing conditions as described by Heijman et al. (31). The sandy matrix collected from the banks of a small river (Chriesbach, Dübendorf, Switzerland) contained 5.7 mg/g of Fe_{tot} and 0.88 mg C/g of total organic carbon (TOC) but negligible amounts of other electron donors (28). Prior to the addition of (P)NACs, all columns were run with oxygen-free NaHCO₃ solution (10 mM) for 15–20 days. Steady-state conditions with respect to (P)NAC transformation were reached 3 days after (P)NACs addition (31). To stimulate the activity of iron-reducing bacteria, acetate was fed to the columns (44 μ M) in experiments where the sequential reduction of TNT was studied (column type I). All other experiments were run acetate free. Initial (P)NAC concentrations in single solute assays are summarized in Table 2 and ranged from 79 to 146 μ M in binary mixtures.

Results and Discussion

One-Electron Reduction Potentials ($E_h^{1'}$) of TNT and ANTs. The reduction potential of the half-reaction shown in eq 3, i.e., the one-electron reduction potential, $E_h^{1'}$, of a given (P)-NAC, is an appropriate parameter for relating and/or evaluating reduction rates of a series of (P)NACs in a given system (28, 30, 31, 40).



The formation of the nitroaryl radical, i.e., the reversible transfer of the first electron to a (P)NAC (eq 3), is an endergonic process which determines the overall rate of the reduction of a (P)NAC to the corresponding aromatic amine (41). The differences of $E_h^{1'}$ s of a (P)NAC and a given reductant are proportional to the changes of the standard free energy of the transfer of the first electron (ΔG_1°) involved in the reduction of this (P)NAC in aqueous solution. By assuming a linear relationship between the free energy of activation and ΔG_1° , the $E_h^{1'}$ s can be a measure for the

TABLE 2. Reduction of TNT and Aminonitrotoluenes in Ferrogenic Aquifer Columns: Typical Substrate and Product Concentrations Measured in Single Solute Experiments^a

compound	no. of exp	c _{in} range ^b (μM)	reactant turnover ^c (%)	reaction products ^d (%)					mass balance ^e (%)
				2-A,4,6-DNT	4-A-2,6-DNT	2,4-DA-6-NT	2,6-DA-4-NT	2,4,6-TAT	
TNT	12	163–188	77–85	67	21	7	4	0	99
2-A-4,6-DNT	5	62–81	47–56			47		4	98
4-A-2,6-DNT	4	78–79	59–77			102		0	102
2,4-DA-6-NT	3	137–144	23–49					97	97
2,6-DA-4-NT	3	108–112	38–48					96	96

^a No acetate added to the influent, column type II, $\tau = 30$ –40 h, depending on the reactivity of the columns. ^b Variations of c_{in} due to the experimental procedure. ^c $(c_{in} - c_{eff})/c_{in}$. ^d In % of reactant reduced: $c_{eff}^{product}/(c_{in}^{reactant} - c_{eff}^{reactant})$. ^e $\sum c_{eff}^{products}/(c_{in}^{reactant} - c_{eff}^{reactant})$.

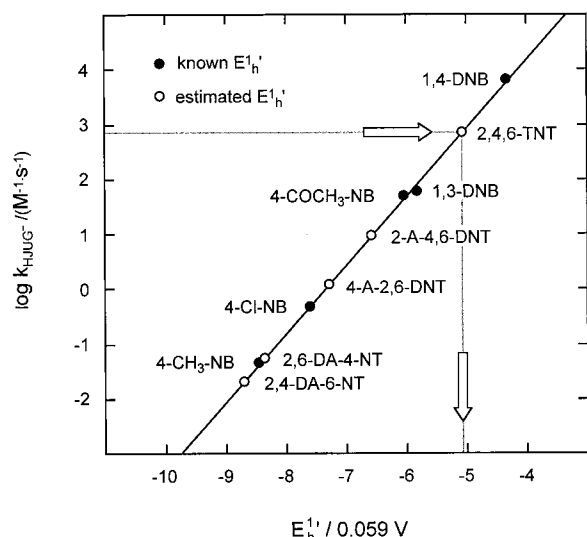


FIGURE 2. Plot of the second-order rate constants k_{HJUG-} of various (P)NACs (measured in 5 mM H_2S and variable concentrations of juglone (2–20 μM) at pH 6.60) versus the $E_h^{1'}$ of the compounds. Filled circles (●) represent reference compounds with known $E_h^{1'}$, whereas $E_h^{1'}$ s of the TNT and ANT (○) are calculated from the measured second-order rate constants using the LFER given by eqs 4a,b.

relative reactivity of a series of (P)NACs with a certain reductant (42). Since the $E_h^{1'}$ values of TNT and of its aminonitrotoluene reduction products were not available in the literature, we estimated them using a linear free energy relationship (LFER) established from five (P)NACs with known $E_h^{1'}$ values ((33, 43), eq 4a, see also Figure 2).

$$\log k_{HJUG-} = (1.25 \pm 0.03) \frac{E_h^{1'}(ArNO_2)}{0.059 \text{ V}} + (9.23 \pm 0.21) \quad r^2 = 0.998 \quad (4a)$$

In eq 4a, k_{HJUG-} is the second-order rate constants for the reduction of these compounds with a model hydroquinone (i.e., reduced juglone (8-hydroxy-1,4-naphthoquinone) in the presence of hydrogen sulfide). The excellent correlation of $\log k_{HJUG-}$ and $E_h^{1'}$ values over a range of almost 250 mV of $E_h^{1'}$ (i.e., more than 5 orders of magnitude in k_{HJUG-}) is due to the fact that under the experimental conditions chosen, the actual transfer of the first electron is the rate-determining step of the (P)NAC reduction (33). Table 1 summarizes the $E_h^{1'}$ values of TNT and aminonitrotoluenes calculated from eq 4 which is a rearranged form of eq 4a ($E_h^{1'}$ values in volts).

$$E_h^{1'}(ArNO_2) = 0.0476 \log k_{HJUG-} - 0.436 \quad (4b)$$

The $E_h^{1'}$ data calculated for TNT and the various aminonitrotoluenes cover a range of 215 mV (95% confidence interval of $E_h^{1'} = \pm 6$ mV), reflecting the changes of the electron acceptor properties of the compounds which occur upon sequential reduction of TNT. The $E_h^{1'}$ values decrease in the order TNT \gg 2-A-4,6-DNT $>$ 4-A-2,6-DNT \gg 2,6-DA-4-NT $>$ 2,4-DA-6-NT (Table 1, Figure 2). This sequence can be rationalized by the increased stabilization of a nitroaryl radical anion with an increasing number of electron-withdrawing nitro substituents. Note that consistent with the very small σ -effects of meta amino substituents (cf. Hammett $\sigma_m(NH_2)$ values of +0.02 to -0.20 (44)), the $E_h^{1'}$ value of 4-CH₃-NB and 2,6-DA-4-NT are very similar (Table 1). However, a more detailed analysis of the differences in the $E_h^{1'}$ values of aminodinitro and diaminonitro isomers would also have to include considerations of steric effects (i.e., steric interactions of the nitro group with substituents in ortho position) which is beyond the scope of this paper.

Finally, it should be noted that the juglone/ H_2S system used to determine the $E_h^{1'}$ values of (P)NACs is also a suitable model system to mimic the electron-transfer properties of natural organic matter (NOM) in the presence of H_2S/HS^- (40). As is evident from the data shown in Table 1 and Figure 2, rates for TAT formation from TNT by reaction with NOM would be about 5 orders of magnitude lower than the rate of TNT disappearance. These findings are consistent with those of Preuss et al. (45, 46), who observed a fast abiotic reduction of TNT to DANTs under sulfate-reducing conditions and showed that biotransformation of DANTs to TAT was faster than the reaction with the abiotic reductants present in the assays.

Reduction of (P)NACs by Surface-Bound Fe(II) in Aqueous Suspensions of Goethite. The reduction of TNT and of the aminonitrotoluenes (Figure 1) by surface-bound Fe(II) species was investigated in suspensions containing goethite (α -FeOOH), dissolved and adsorbed Fe(II) (Figure 3). No reactivity was observed within the time scale of the experiments in assays containing solely Fe(II) or goethite, respectively (Figure 3, insert). In the presence of both, Fe(II) and goethite, all (P)NACs were reduced by reactive Fe(II) species formed after adsorption of Fe(II) to the surface (for details about adsorption of Fe(II) to goethite, see ref 29). PNAC reduction followed pseudo-first-order kinetics as depicted in the insert of Figure 3. From the rate constants, $k_{obs,Fe(II)/goethite}$, listed in Table 1, it is evident that TNT, ADNTs, and DANTs can be reduced by surface-bound Fe(II) species within hours. Note that the differences in reactivities of the (P)NACs exhibiting very different $E_h^{1'}$ values (e.g., TNT and 2,4-DA-6-NT) are much less pronounced in Fe(II)/goethite system (slope of the LFER, $a = 0.6$) as compared to the juglone/ H_2S system ($a = 1.25$) discussed above. A similar picture of relative reactivities was found for mononitroaromatic compounds in Fe(II)/magnetite suspensions (30). These results suggest that similar reactive Fe(II) surface species were involved in

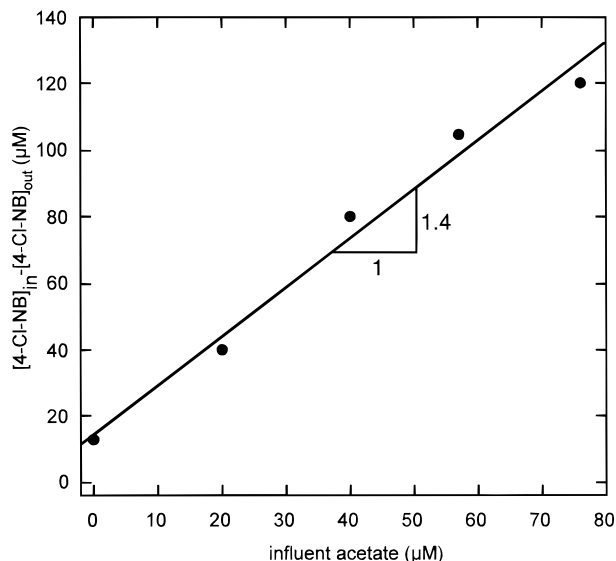
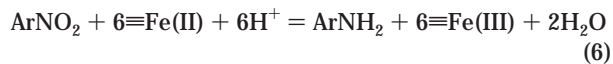
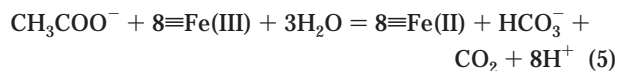


FIGURE 5. Effect of influent acetate concentration on the zero-order rate constant of 4-Cl-NB in columns (type III) containing *G. metallireducens* and FeOOH-coated sand. All acetate was consumed in the columns.

1.33 given in eqs 5 and 6 (39). The same influence of acetate on the reduction of NACs and similar electron balances was found in aquifer columns (31).



The reactivity pattern of the NACs in columns containing *G. metallireducens* and FeOOH-coated sand was essentially identical to that in the ferrogenic aquifer columns (Figure 4C,D). The rate constants, k_{obs} , pure culture, reported in Table 1 for the reduction of the 10 NACs were independent of the solute concentration (zero-order kinetics) and of the $E_h^{1'}$ values of the NACs (Figure 4C (□)). Similar to the results found for the (P)NACs (Figure 4B), the competition coefficients of the NACs correlated well with their $E_h^{1'}$ values except for the ortho-substituted compounds whose reactivity was underestimated by the established LFER (Figure 4D (□)).

The same pattern of (P)NAC reduction was found in aquifer sediment columns (Figure 4D (●), data from ref 31)

and in systems with Fe(II)/goethite (Figure 3) and Fe(II)/magnetite, respectively (30). This strongly suggests that Fe(II) surface species were the reductants of the (P)NACs in these experimental systems. The zero-order rate constants (Table 1) measured in the two sets of single solute experiments in iron-reducing column systems for the reduction of (P)NACs (Figure 4A, C) were very similar and independent of the $E_h^{1'}$ of the compounds. Therefore, the rate-determining step of the abiotic (P)NAC reduction with surface-bound Fe(II) was not the actual electron transfer but the microbial (re)generation of the limited number of reactive Fe(II) surface species (Scheme 1).

Complete Reduction of TNT to TAT in Ferrogenic Aquifer Columns. The reduction of 13 μM TNT was followed in aquifer columns with several lateral sampling ports. In the absence of acetate, TNT was reduced sequentially to ANTs (Figure 6A). With 44 μM of acetate in the feeding solution, TNT was rapidly and completely transformed to TAT, without detectable intermediates. TAT remained stable under the experimental conditions (Figure 6B), and mass balances were complete considering only TNT and TAT. The rapid reduction of TNT to TAT with acetate was probably due to the increased generation of reactive Fe(II) surface sites by iron-reducing bacteria. Hence, complete reduction of TNT in unamended column systems was limited by the availability of electron donors. As shown in Figure 6A, ANTs were not reduced before the complete disappearance of TNT. This observation is consistent with the competition of the (P)NACs for reactive Fe(II) surface sites measured in binary competition experiments (Q_c). Furthermore, these results agree well with earlier work using a complex mixtures of five (P)NACs, where strong competitors, i.e., (P)NACs with high Q_c values, strongly retarded the reaction of the other (P)NACs (28).

Environmental Significance

This work demonstrates that (P)NACs such as TNT and related ANTs can be completely reduced to the corresponding aromatic polyamines by Fe(II) present at Fe(III)(hydr)oxide surfaces or, less efficiently, by hydroquinone moieties such as juglone present in organic matter. The relative reactivity of (P)NACs, exemplified by TNT and ANTs in these systems, correlated well with their one-electron reduction potentials, $E_h^{1'}$, which we also determined in this work. Since the differences of the rate constants of the most and the least reactive (P)NACs (TNT and DANTs, respectively) was about 3 orders of magnitude smaller with surface-bound Fe(II) than with hydroquinone moieties of NOM, complete abiotic reduction of (P)NACs to the corresponding aromatic polyamines can be achieved easier by surface-bound Fe(II)

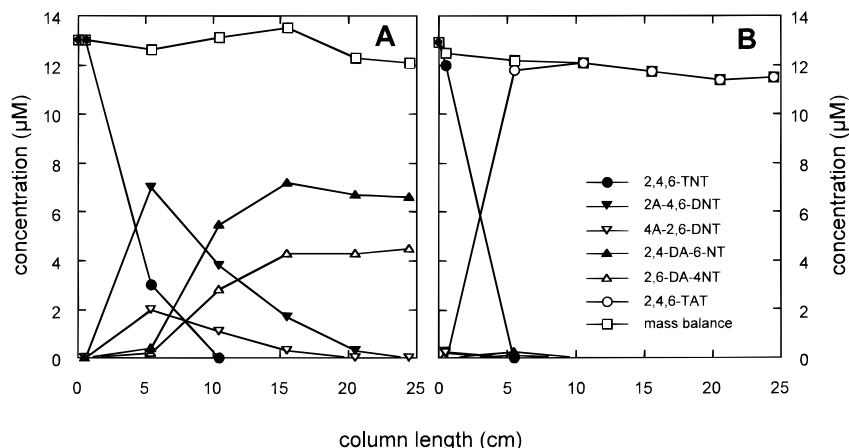


FIGURE 6. Reduction of TNT (13 μM initial concentration) to ADNTs, DANTs, and TAT in a column (type I, $\tau = 53$ h) filled with aquifer sediment and operated under iron-reducing conditions in the absence (A) and presence (B) of 44 μM of acetate in the influent.

rather than by reduced NOM under typical groundwater conditions (where the concentration of reduced NOM is not extraordinarily high (40)). In fact, surface-bound Fe(II) has been identified as the most active species with respect to nitroaryl reduction in a ferrogenic landfill leachate plume, exhibiting NOM concentrations as high as 100 mg/L (28).

Our work further demonstrates that at the given experimental conditions, the reactivity of surface-bound Fe(II) species with respect to kinetics and mechanisms of (P)NAC reduction is similar, irrespective of the processes that (re)-generated these surface sites. The processes can be adsorption of Fe(II) from aqueous solution or microbial reduction of Fe(III)(hydr)oxides (see Scheme 1). Both processes may contribute to the reactivity of Fe(II) surface species observed in natural systems (28). Since such reductants are almost ubiquitously present in subsoils, anoxic soils, and aquifers, contamination with (P)NACs will, even in the absence of microbial activity, inevitably result in pollution with problematic transformation products. The aromatic (poly)amines formed from the reduction of (P)NACs by reactive Fe(II) surface species were not only stable in our laboratory batch and column systems, but also in the iron-reducing zone of an anaerobic aquifer for at least 250 days (28). However, under aerobic conditions, dissolved aromatic (poly)amines can be eliminated very efficiently in soils and sediments by various processes, including oxidation and/or irreversible binding to mineral surfaces (24, 48–50), NOM (21–24, 51, 52), or by biotransformation (53, 54). Thus, creating or stimulating iron-reducing conditions may be an interesting option for supporting a two-stage anaerobic/aerobic bioremediation of (P)NACs. As observed in our column experiments, abiotic reduction of (P)NACs may also minimize the accumulation of (hydroxylamino)dinitrotoluene (HADNT) intermediates, which exhibit a high tendency to bind to the solid matrix of soils (24). Surface-bound HADNT, as shown by Daun et al. (24), may not be further reduced by microorganisms and may compromise the goal of complete nitro reduction and, thus, the applicability of anaerobic/aerobic remediation schemes. The reactivity of such surface-bound (P)NACs with respect to abiotic reduction is currently investigated in our laboratory.

Acknowledgments

We acknowledge Claudia Zraunig for her work with ferrogenic batch suspensions. Stephan J. Hug, Klaus Pecher, Ignaz J. Bürge, and Martin Elsner commented on the manuscript. This work was supported by the Swiss National Science Foundation (2000-045065.95).

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Received for review September 22, 1998. Revised manuscript received January 14, 1999. Accepted January 26, 1999.

ES9809760