Natural organic matter (NOM) from a variety of sources has been shown to mediate the reduction of substituted nitrobenzenes in aqueous solution containing hydrogen sulfide. Pseudo-first-order rate constants were proportional to NOM concentrations and increased with increasing pH and decreasing reduction potential (Eh) of the solution. At fixed pH and Eh, the carbon-normalized rate constant (kNOM) of a given compound varied less than 1 order of magnitude among NOMs derived from various natural waters. The effect of substituents on the reaction rate could be described by a linear free energy relationship (LFER) of the general form log kNOM = aEh1'(ArNO2) + b, where Eh1'(ArNO2) is the one-electron reduction potential of the nitroaromatic compound. Such LFERs were applied successfully to predict the kNOM values of previously untested compounds. The results of this study suggest that hydroquinone moieties within the NOM may play a pivotal role in the mediation of electron-transfer reactions involving organic pollutants.

Introduction

Abiotic reduction of organic pollutants in reducing environments (e.g., sediments, aquifers, and hazardous waste sites) has recently drawn considerable interest, since it has been recognized that such reactions can lead to transformation products of similar or even greater concern than the parent compounds (1). Reactions of most interest include reductive dehalogenation of polyhalogenated hydrocarbons (2, 3), reduction of nitroaromatic compounds (2, 4-8), and reduction of azo compounds (9). Some advances in understanding the mechanisms and pathways of such reactions in homogeneous aqueous systems have been achieved using model reductants (e.g., hydroquinones, iron porphyrins, and other transition metal complexes). However, previous investigations utilizing environmental matrices (e.g., sediment and dissolved and sorbed organic carbon) have not adequately provided a process-level understanding of key factors controlling the reduction reactions.

One important and largely unanswered question is, which natural reductants are involved in the abiotic reduction of organic pollutants. Aside from biological electron donors, the most abundant natural reductants present in anaerobic environments include reduced inorganic forms of iron and sulfur [such as iron(II/III) oxides, iron(II) carbonates, iron(II) sulfides, and hydrogen sulfide]. Although it has been shown in various studies that these reductants react with organic pollutants (10-14), the reported reaction rates are often too slow to account for the much faster transformation rates observed in some natural systems. For example, for the reduction of the nitro group of parathion or methyl parathion in anaerobic soils and sediments, half-lives of as short as a few minutes have been observed (5, 6, 15). Hence, there must be other, much more reactive reductants available. These highly reactive species may not be present in large abundance but may play the role of electron-transfer mediators as illustrated by Figure 1. Thus, after electron transfer to the pollutant, the mediators may be rapidly reduced again by the bulk reductants present. Possible mediators include quinone-type compounds and a variety of transition metal complexes. Such species are not only well-known components of biological electron-transfer systems (16) but they are also very likely to exist as constituents of natural organic matter (17-20). The reduction potential of natural aqueous systems is generally controlled by microorganisms, thus “bulk” electron donors and reduced forms of electron-transfer mediators may also be replenished through microbial processes.

In this paper, we report the effect of natural organic matter (NOM) on the reduction kinetics of a series of monosubstituted nitrobenzenes in aqueous solutions containing hydrogen sulfide as the bulk electron donor. The major goals of this investigation were (1) to evaluate and quantify the effect of organic matter from different natural sources on the reduction kinetics of nitroaromatic compounds in aqueous solution, (2) to derive relationships for quantification of the effect of substituents on the rate of reduction of the nitro group, and (3) to gain insights into the type of structural moieties within the NOM that may be involved in mediating the reduction reactions. The results of this investigation are interpreted in light of the results of our previous investigation (8), which used two hydroquinones and an iron porphyrin as model electron-transfer mediators.

Experimental Section

Chemicals. Nitrobenzene (NB), 2-chloro-, 3-chloro-, and 4-chloronitrobenzene (CINB); 2-methyl-, 3-methyl-, and 4-methyl nitrobenzene (MeNB); 2-chloro-, 3-chloro-, and 4-chloroaniline; 2-methyl-, 3-methyl-, and 4-methylaniline; 8-hydroxy-1,4-naphthoquinone (juglone); and 2-hydroxy-1,4-naphthoquinone (lawson) were purchased from Fluka AG (Buchs, Switzerland). 2,4-Dinitrotoluene, 2-nitrophenol (2-NP); 6-methyl-2,4-dinitrophenol (DNOC); 2-nitro-, 3-nitro-, and 4-nitrosoacetophenone (ACNB); Methyl Red (2-carboxy-4'-(dimethylamino)azobenzene); 2-amino-, 3-amino-, and 4-aminoacetophenone; nitrosobenzene; and
were virtually identical, thus validating the latter method measured for the synthesized 3-chlorophenylhydroxylamine and calculated from the FeS mass balance method. Experiments found that the phosphate buffer systems yield as Fe, Cu, Mn, etc. (22). Prior to the use of sulfonic acid-based buffers, phosphate buffer solutions were used (prepared from commercial KH₂PO₄). Preliminary experiments found that the phosphate buffer systems yield poor reproducibility for ortho-substituted nitrobenzenes.

Characterization of Selected Waters Containing Natural Organic Matter. Various types of NOMs were evaluated for their ability to mediate the reduction of the substituted nitroaromatic compounds. The sources included two anaerobic groundwaters (MW-6 and MW-10 from Colorado, described in ref 23), four waters from streams draining bog areas (Hyde County, NC, Georgetown, SC, Suwannee River, GA, and Great Dismal Swamp, VA) two landfill leachates (hazardous waste site in Kölliken, Switzerland, and municipal landfill (methanogenic phase) in Bayreuth, Germany), and two “laboratory-prepared” extracts from walnut and elm tree materials. The two latter NOMs were obtained from a suspension containing distilled water, leaves, wood chips, and tree bark from each variety of tree (walnut or elm). Each suspension was incubated at room temperature (20-25 °C) for 2-3 months, resulting in a dark brown supernatant which was decanted and stored at 10 °C until use. A summary of the total organic carbon (TOC) concentrations and selected transition metal content of these waters is given in Table I. Both organic carbon and metal concentrations were determined on filtered samples (0.20-μm sterile filter; Schleicher & Schuell; Fellbach, Germany) and tree bark from each variety of tree (walnut or elm). Each suspension was incubated at room temperature (20-25 °C) for 2-3 months, resulting in a dark brown supernatant which was decanted and stored at 10 °C until use. A summary of the total organic carbon (TOC) concentrations and selected transition metal content of these waters is given in Table I. Both organic carbon and metal concentrations were determined on filtered samples (0.20-μm sterile filter; Schleicher & Schuell; Fellbach, Switzerland) and used in the experiments are 0.2-μm filtrates. All NOMs were used unaltered with the exception of pH adjustment with dilute NaOH immediately prior to use.

Experimental Procedures. Experiments were conducted in 60-mL serum flasks sealed with Teflon-coated, gray butyl rubber stoppers (Wheaton; Millville, NJ). The pH was controlled by either sodium phosphate- or sulfonic acid-based buffers (refer to the Chemicals section for exact buffer compounds and pH ranges). Buffer concentrations ranged from 5.5 to 8.5 solutions), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES; for pH 7.2, 7.5, and 7.9 solutions), and N-[tris(hydroxymethyl)methyl]-3-amino-propanesulfonic acid (TAPS; for pH 8.6 solutions), were purchased from Fluka BioChemika (Buchs, Switzerland). These buffers were selected because of their low complexation of metals, such as Fe, Cu, Mn, etc. (22). Prior to the use of sulfonic acid-based buffers, phosphate buffer solutions were used (prepared from commercial KH₂PO₄). Preliminary experiments found that the phosphate buffer systems yield poor reproducibility for ortho-substituted nitrobenzenes.
(typically 0.3 mL) of 0.5 M Na₂S solution were added via a syringe. Total hydrogen sulfide concentration was usually 5 mM; however, higher concentrations were used in some experiments. Based on a total concentration of 5 mM hydrogen sulfide, the E₀ of the systems ranged from -0.19 (at pH 5.5) to -0.22 V (at pH 8.6). After the addition of Na₂S, solutions were equilibrated in a 25 °C water bath for at least 15 h. The final aqueous volume in each vial was usually 30 mL and resulted in a 1:1 dilution of the original TIC and total metal concentrations shown in Table I. Similarly, two types of blank solutions were prepared to measure the disappearance of substituted nitrobenzenes in the absence of either hydrogen sulfide or NOM. One system contained distilled water, pH buffer, HCl (to compensate for sodium sulfide addition), and sodium sulfide, but no NOM was added. Another blank system contained distilled water, NOM, and pH buffer, but no sodium sulfide.

A kinetic experiment was initiated by adding 25 μL of a 0.1 M methanolic solution of a nitroaromatic compound to the reaction flask (final concentration = 80 μM). Flasks were slightly pressurized with nitrogen in order to minimize leakage of oxygen during sampling, returned to the water bath, and sampled at regular time intervals, depending on the expected reaction rates. Experiments were typically monitored through three reaction half-lives. Samples (0.5 mL) were taken with sterile 1-mL syringes and added to 3-mL vials containing 0.5 mL buffer solution (0.1 M aqueous hydroxylamine hydrochloride, pH 6.0), an internal solution (100 μM of a structurally related nitrobenzene compound), and 0.5 mL of ethyl acetate. The mixture was shaken for 30 s and the ethyl acetate extract analyzed by HPLC for the nitroaromatic compound, internal standard, and transformation product(s) (i.e., the corresponding aniline and phenylhydroxylamine).

HPLC analysis was performed on a RP-18 reversed-phase column (stainless steel cartridge LiChrocart 125 × 4 mm, 5-μm spheres; Merck, Darmstadt, Germany) connected to a pumping system (Spectra Physics; San Jose, CA) supplemented with an injector and a variable-wavelength UV/visible detector. The detector wavelength was adjusted to either 254 or 280 nm, depending on the specific compounds of interest. Methyl Red, 3,4-DNT, 2-NP, and DNOC were measured at 420 nm. The mobile phase was 0.01 M hydroxylamine hydrochloride buffer (pH 6.0), an internal standard (100 μM of a structurally related nitrobenzene compound), and 0.5 mM of ethyl acetate. The mixture was shaken for 30 s and the ethyl acetate extract analyzed by HPLC for the nitroaromatic compound, internal standard, and transformation product(s) (i.e., the corresponding aniline and phenylhydroxylamine).

Validation of the Experimental System. An important prerequisite for studying the kinetics of reduction reactions is the maintenance of a stable experimental system with respect to pH and E₀. As a test of our systems, the pH and total hydrogen sulfide concentration were monitored over 41 days in systems containing 5 mM H₂S and pH ranging from 5.5 to 8.6. Systems of interest included (1) distilled water (pH adjusted with dilute HCl), (2) distilled water and phosphate buffer, (3) distilled water and sulfonic acid-based buffer, and (4) NOM, distilled water, and sulfonic acid-based buffer. Results showed that no change in pH (±0.02 pH unit) or total H₂S concentration occurred over this time period for any of the systems. Furthermore, no unexpected changes in pH or H₂S concentration were observed in solutions used in nitroaromatic reduction experiments. Thus, the experimental techniques described above allowed the study of nitro reductions under stable pH and E₀ conditions, with the reproducibility between rate constants varying on average ±5%.

Results and Discussion

Reduction of Substituted Nitrobenzenes in NOM
analysis are $5.2 \times 10^{-2}$ h$^{-1}$ for the reaction in the presence of NOM and $0.08 \times 10^{-2}$ h$^{-1}$ for the reaction in the absence of NOM. Similar rate constants, as found for the NOM-free 5 mM hydrogen sulfide solutions, were also observed in buffered distilled water and in solutions containing buffer and NOM but no hydrogen sulfide. The absence of significant amounts of reduction products (see below) in these blank systems indicates that most of the disappearance of the nitroaromatic compounds was probably due to losses to the experimental apparatus (e.g., diffusion into the septum). These results also confirm our earlier findings that the reaction of hydrogen sulfide with nitroaromatic compounds is rather slow (8). The fact that pseudo-first-order kinetics were observed over more than 3 half-lives, even in solutions with very low NOM concentrations (7 mg of C/L; data not shown), indicates that the reactive NOM constituents were reduced by the hydrogen sulfide at a faster rate than they reacted with the nitroaromatic compound. Note that if one assumes that a reactive NOM constituent contains at least 10 carbon atoms, 7 mg of C/L would correspond to a maximum reductant concentration of only 50 μM. Thus, these NOM constituents indeed acted as effective electron-transfer mediators (refer to Figure 1).

**Reaction Products.** The reduction of an aromatic nitro group to the corresponding aniline is commonly assumed to occur by a series of electron addition and protonation steps (12)

$$\text{NO}_2^- + 2e^- + 2H^+ \rightarrow \text{NH}_2$$

where aniline is the final product and nitrosobenzene and phenylhydroxylamine are the intermediates, respectively.

Figure 2b shows the disappearance of the parent compound (3-chloronitrobenzene) and the appearance of products for the same experimental system used in Figure 2a. Major products, 3-chlorophenylhydroxylamine and 3-chloroaniline, were identified on the basis of comparison of the retention times with authentic standards. The data points (× symbols) across the top of the figure represent the sum of the parent and product concentrations present in the reaction vials as a function of time and illustrate the mass balance in the system. Independent experiments with unsubstituted nitrosobenzene showed that this compound reacted completely and rapidly (half-life < 1 min) in both NOM and blank solutions containing hydrogen sulfide to form primarily phenylhydroxylamine and aniline. The mass balance illustrated in Figure 2b, and observed for other compounds, suggests that all substituted nitrosobenzenes were rapidly converted to products.

The kinetics of product formation are presented in Figure 2b as an illustration only. The shape of the product formation curves were found to be system-specific, varying with NOM source, the pH of the system, and the nitroaromatic compound under investigation. While the disappearance of ArNO$_2$ compounds followed first-order kinetics, the formation of products did not appear to follow a series of first-order reactions. For the faster reacting compounds (e.g., COCH$_3$ substitutions), and at high pH's for many other compounds, the phenylhydroxylamine product was more rapidly formed and less of the corresponding aniline was produced during the experiment. In fact, for 3- and 4-acetyl nitrobenzenes, no significant concentration of the aniline product was detected after 3 half-lives of reaction. For the slower reacting compounds, lower accumulation of the phenylhydroxylamine products was observed. For the very slow reaction of the methyl-nitrobenzenes, no phenylhydroxylamine intermediates were observed. Due to the slow reactions of these compounds (2- and 4-Me), disappearance in blank solutions became an appreciable factor, and reactions were monitored only through 1 half-life.

**Reaction Rate as a Function of Organic Carbon Concentration and NOM Source.** As illustrated in Figure 3, at fixed pH and hydrogen sulfide concentration, pseudo-first-order rate constants were found to be a linear function of the total organic carbon (TOC) concentrations in the experimental solutions. Thus, for a given compound and for given system conditions, a carbon-normalized second-order rate constant, $k_{\text{NOM}}$

$$k_{\text{NOM}} = \frac{k_{\text{obs}}}{[\text{TOC}]}$$

can be calculated. The $k_{\text{NOM}}$ values for 3-chloronitrobenzene with various NOM sources investigated at pH 7.0 and 5 mM H$_2$S are summarized in Figure 4.

A striking feature of these results is the similarity of the carbon-normalized rate constants for the NOMs originating from natural waters and from the dump sites, which are all in the same order of magnitude. The NOM source with a reactivity somewhat outside of this range can be classified as a clear exception. Walnut trees are known to produce high concentrations of quinone derivatives [such as juglone (25)], and we have demonstrated in earlier work (8) that this compound mediates the reduction of nitrobenzenes in aqueous hydrogen sulfide solutions. Thus, the high reactivity of this extract, relative to most sources is not surprising.

**Effect of Substituents on Reaction Rates: Linear Free Energy Relationships (LFERs).** Table II summarizes the $k_{\text{NOM}}$ values determined for a series of substituted nitrobenzenes in Hyde County NOM water (66 mg of C/L, 5 mM hydrogen sulfide, 25 °C) at various pH values. The data in Table II show that, for all compounds, the $k_{\text{NOM}}$ values depend strongly on the pH (and $E_h$) of the system and that substituents have a significant effect on the reduction rate. Similar results were also found for other NOM waters but are not included in Table II. We will address the pH and $E_h$ dependence of the $k_{\text{NOM}}$ values later and first discuss the effect of substituents on reaction rates.

The data in Table II illustrate that the most reactive compound (4-acetylnitrobenzene) was reduced up to more
Table II. Compound Names, One-Electron-Reduction Potentials (E_{1/2}), and Carbon-Normalized Second-Order Rate Constants (k_{NOM}) for Reactions of Substituted Nitrobenzenes with Hyde County NOM Water in 5 mM H_{2}S at Various pH Values (25°C)

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH 5.50</th>
<th>pH 6.50</th>
<th>pH 7.20</th>
<th>pH 7.50</th>
<th>pH 7.90</th>
<th>pH 8.50</th>
</tr>
</thead>
<tbody>
<tr>
<td>nitrobenzene</td>
<td>-0.485</td>
<td>E_{1/2}</td>
<td>E_{o} = -0.117 V</td>
<td>E_{b} = -0.173 V</td>
<td>E_{b} = -0.207 V</td>
<td>E_{b} = -0.218 V</td>
</tr>
<tr>
<td>2-methylnitrobenzene</td>
<td>-0.590</td>
<td>E_{1/2}</td>
<td>E_{o} = -0.117 V</td>
<td>E_{b} = -0.173 V</td>
<td>E_{b} = -0.207 V</td>
<td>E_{b} = -0.218 V</td>
</tr>
<tr>
<td>3-methylnitrobenzene</td>
<td>-0.475</td>
<td>E_{1/2}</td>
<td>E_{o} = -0.117 V</td>
<td>E_{b} = -0.173 V</td>
<td>E_{b} = -0.207 V</td>
<td>E_{b} = -0.218 V</td>
</tr>
<tr>
<td>4-methylnitrobenzene</td>
<td>-0.500</td>
<td>E_{1/2}</td>
<td>E_{o} = -0.117 V</td>
<td>E_{b} = -0.173 V</td>
<td>E_{b} = -0.207 V</td>
<td>E_{b} = -0.218 V</td>
</tr>
<tr>
<td>2-chloronitrobenzene</td>
<td>-0.485</td>
<td>E_{1/2}</td>
<td>E_{o} = -0.117 V</td>
<td>E_{b} = -0.173 V</td>
<td>E_{b} = -0.207 V</td>
<td>E_{b} = -0.218 V</td>
</tr>
<tr>
<td>3-chloronitrobenzene</td>
<td>-0.405</td>
<td>E_{1/2}</td>
<td>E_{o} = -0.117 V</td>
<td>E_{b} = -0.173 V</td>
<td>E_{b} = -0.207 V</td>
<td>E_{b} = -0.218 V</td>
</tr>
<tr>
<td>4-chloronitrobenzene</td>
<td>-0.450</td>
<td>E_{1/2}</td>
<td>E_{o} = -0.117 V</td>
<td>E_{b} = -0.173 V</td>
<td>E_{b} = -0.207 V</td>
<td>E_{b} = -0.218 V</td>
</tr>
<tr>
<td>2-acetylnitrobenzene</td>
<td>-0.475</td>
<td>E_{1/2}</td>
<td>E_{o} = -0.117 V</td>
<td>E_{b} = -0.173 V</td>
<td>E_{b} = -0.207 V</td>
<td>E_{b} = -0.218 V</td>
</tr>
<tr>
<td>3-acetylnitrobenzene</td>
<td>-0.405</td>
<td>E_{1/2}</td>
<td>E_{o} = -0.117 V</td>
<td>E_{b} = -0.173 V</td>
<td>E_{b} = -0.207 V</td>
<td>E_{b} = -0.218 V</td>
</tr>
<tr>
<td>4-acetylnitrobenzene</td>
<td>-0.380</td>
<td>E_{1/2}</td>
<td>E_{o} = -0.117 V</td>
<td>E_{b} = -0.173 V</td>
<td>E_{b} = -0.207 V</td>
<td>E_{b} = -0.218 V</td>
</tr>
</tbody>
</table>

a E_{1/2} is the one-electron-reduction potential of the half-reaction ArNO_{2} + e^{-} → ArNO_{2}^{+}; data from ref 8 and references cited therein. Values are the average of duplicate pseudo-first-order rate constants (from eq 2) divided by the total organic carbon concentration (66 mg of C/L in all experiments). Pseudo-first-order rate constants were corrected for disappearance in blank solutions when necessary. The reduction potentials for each pH condition were calculated using eq 7.

For our purposes, k in eq 5 is the carbon-normalized second-order rate constant (k_{NOM}) determined at given conditions (e.g., fixed pH and E_{b}; note that at 25 °C 2.3RT/F = 0.059 V), while a and b are constants. A relationship as described by eq 5 can be expected for k_{NOM} values if the transfer of the first electron from the reductants present in NOM to the nitroaromatic compound is rate-determining. For the reaction of the same 10 compounds with two model hydroquinones [reduced 8-hydroxy-1,4-naphthoquinone (juglone) and 2-hydroxy-1,4-naphthoquinone (lawnone)], it was found that the dissociated hydroquinone species (e.g., monophenolate and biphenolate) were effective mediators, while the nondissociated species were quite unreactive (8). For the reaction of the nitroaromatic compounds with the two hydroquinones, good linear correlations as described by eq 5 were found, where k was the second-order rate constant for the reaction with the monophenolate species. In both cases, a slope of close to 1.0 was obtained, indicating that the reaction occurred by an “outer-sphere” mechanism (26); that is, precursor complex formation was not a major factor in determining differences in the overall reaction rates. In contrast to the results with the hydroquinones, a slope of 0.6 was obtained for the reaction of the meta- and para-substituted nitrobenzenes with the reduced iron porphyrin, while the ortho-substituted compounds reacted much faster than expected from their E_{1/2}(ArNO_{2}) values. Finally, competition between different substituted nitrobenzenes was observed in the case of the iron porphyrin, while no effects were found with the hydroquinones. From these findings it was concluded that, for the reaction with the iron porphyrin, precursor formation was a more important factor in determining the relative reaction rates as compared to the reaction with the hydroquinones.

Figure 5 shows an illustrative plot of log k_{NOM} vs E_{1/2}(ArNO_{2})/0.059 V for the reduction of the 10 compounds in the Hyde County NOM water system at pH 7.50 (data from Table II). The open square symbols represent the data from Table II, while the solid squares represent rate constants for other compounds which will be discussed later (Table IV). As can be seen, although there is some scatter in the data, a reasonably good linear correlation was found between reactivity and one-electron-reduction potential of the compounds. Table III summarizes the slopes a and intercepts b of the LFERs established for this NOM at various pH values. Very similar LFERS were obtained for pH values above pH 7, suggesting that in this pH region similar types of reactive NOM compounds were responsible for the reduction of the nitrobenzenes. Further...
thermore, the slopes of the regression lines are close to 1.0, suggesting an outer-sphere electron-transfer mechanism as was found for the reactions with model hydroquinones (see above). In addition, as is illustrated in Figure 6 for the three chloronitrobenzene isomers, no competition was found between different compounds in any of the investigated NOM water systems (Hyde County, Elm, MW-6). Thus, the NOM moieties responsible for the reduction of the nitroaromatic compounds showed a reaction behavior very similar to the ones observed for the two hydroquinones. This is actually not too surprising since quinone-type structures have been postulated to be present in diverse sources of NOM (17-20).

A final aspect that needs to be addressed is the effect of the compound hydrophobicity on $k_{NOM}$. In a study in which the rates of reduction of a series of 4-$n$-alkynitrobenzenes (alkyl chain lengths of 1, 2, 4, and 8) in anaerobic sediment/water slurries were determined, Wolfe et al. (27)

found that the observed pseudo-first-order reaction rate decreased with increasing alkyl chain length, that is, with increasing hydrophobicity of the compound. For example, a difference in reaction rate of more than 1 order of magnitude was found between 4-methylnitrobenzene and 4-n-octynitrobenzene. Note that very similar $E^\text{a}_{1/2}(\text{ArNO}_2)$ values can be assumed for all 4-$n$-alkynitrobenzenes, which is corroborated by our findings that 4-ethylnitrobenzene (4-Et), 4-$n$-butynitrobenzene (4-Bu), and 4-$n$-octynitrobenzene (4-0c) reacted with juglone at a rate very similar to that found for 4-methylnitrobenzene (see below, discussion on prediction of $k_{NOM}$ values). Wolfe et al. (27) attributed the observed decrease in reactivity with increasing compound hydrophobicity to the increased sorption of the compound to nonreactive sediment NOM. In our study with aqueous-phase NOM, no apparent effect of hydrophobicity on $k_{NOM}$ was observed. For two different NOMs, no significant difference in $k_{NOM}$ were found for 4-Me, 4-Et, 4-Bu, and 4-0c, and there was no evidence of an inverse relationship between $k_{NOM}$ and hydrophobicity of the compounds.
$E_b$ and pH Dependence of $k_{NOM}$. In Figure 7, the $k_{NOM}$ value of 3-chloronitrobenzene is shown as a function of system $E_b$ at fixed pH (pH 7.00) for three different NOM water systems. The $E_b$ of each system was adjusted by changing the total hydrogen sulfide concentration and was calculated by (28)

$$E_b = +0.144 \text{ V} + (2.3RT/2F) \log ([H^+]/([H^+] + K_a)/[H_2S]_{total}) \quad (7)$$

where $[H^+] = 10^{-7.0}$ and $K_a$ (acidity constant of H$_2$S from ref 29) = $10^{-7.0}$. As seen in Figure 7, a significant increase in the $k_{NOM}$ of 3-chloronitrobenzene was observed with decreasing $E_b$ for all three NOM water systems. Solutions of Hyde Country NOM not containing hydrogen sulfide were also reduced by bubbling hydrogen gas through a suspension containing NOM and platinum-coated beads. Measured $k_{NOM}$ values were slightly less than the results for the 50 mM sulfide system shown in Figure 7, but indicate that reduced constituents of the NOM, independent of the reduction method employed, are capable of mediating the reduction of nitroaromatic compounds. The increase in $k_{NOM}$ with decreasing $E_b$ is most likely due to the additional reduction of reactive NOM components exhibiting a more negative reduction potential. The extent of the increase in $k_{NOM}$ with decreasing $E_b$ then reflects the distribution of the reduction potentials of the reactive NOM species. When postulating that quinone-type structures are the predominant electron-transfer mediators present in the NOM, one would expect that at pH 7.0 all of these moieties should be reduced when the $E_b$ of the system reaches approximately $-0.3$ V (30). Thus, one would anticipate a leveling off of the $k_{NOM}$ values near this $E_b$. Unfortunately, with the hydrogen sulfide redox buffer, it is not possible to reach such low $E_b$ values. Additional experiments using other bulk reductants which do not directly reduce nitroaromatic compounds are necessary to check this hypothesis.

In order to evaluate the pH dependence of $k_{NOM}$, several factors have to be considered. First, at a given hydrogen sulfide concentration, the $E_b$ of the system changes with pH (see eq 6 and Table II). Second, the reduction potentials of many of the reactive NOM components, particularly of the quinones, may exhibit a very strong pH dependence (8, 30). For example, in 5 mM hydrogen sulfide at pH 7.0 (calculated $E_b$ = $-0.20$ V), over 95% of 2-hydroxy-1,4-naphthoquinone is reduced, while at pH 8.0 (calculated $E_b$ = $-0.24$ V) only 35% is present in reduced form. The complex relationship between pH, $E_b$, and reduced quinone species is due to the much stronger pH dependence of the reduction potential of the lawsone quinone/hydroquinone couple as compared to the pH dependence of the hydrogen sulfide redox buffer. Finally, as discussed above, if hydroquinone species were the predominant electron-transfer mediators present in NOM, an increasing number of reactive species (i.e., the phenolate species) would be created with increasing pH due to the depoxygenation of phenolic groups, which would lead to an increase in $k_{NOM}$. Thus, when considering the pH dependence of $k_{NOM}$ in 5 mM hydrogen sulfide as illustrated in Figure 8, one should realize that the observed increase in $k_{NOM}$ with increasing pH is the net result of an increase in the concentration of reactive species (e.g., phenolic groups) due to proton-transfer reactions as well as a possible decrease in reactive species due to the strong pH dependence of their reduction potentials.

One interesting feature of the NOM-mediated reductions as mentioned earlier is that nonpseudo-first-order kinetics were observed in the initial phase of experiments at high pH's in some NOM water systems. An illustrative example is shown in Figure 9 for the reduction of 3-chloronitrobenzene at pH 8.6 in the Hyde County NOM system. As can be seen, the rate continued to increase with time until a final reaction rate, which is consistent with pseudo-first-order reaction kinetics, was observed. The time required to reach pseudo-first-order reaction kinetics was dependent on the type of compound and type of NOM used, and the duration of the transition period increased with increasing pH. When such a solution was respiked after completion of the initial reaction, pseudo-first-order behavior was again observed and the reaction rate corresponded to the final reaction rate obtained in the initial experiment. Furthermore, when the solution was spiked prior to an experiment with nitrosobenzene at a concentration similar to that used for the nitro compound, pseudo-first-order kinetics were observed with a $k_{obs}$ value (0.15 h$^{-1}$; data not shown in Figure 9) similar to that found for the respiked solutions (0.14 h$^{-1}$). Note that all $k_{NOM}$ values at higher pH conditions (Table II) have been derived from experiments in which nitrosobenzene was added prior to the kinetic experiment or from the analysis of results obtained with respiked solutions. It is also interesting to note that very similar observations were made in experiments with hydroquinones (e.g., juglone and lawsone) but not with iron porphyrins as electron-transfer mediators (data not shown; also see data in ref 31). Again, these findings are consistent with the hypothesis that
Table IV. Predicted One-Electron-Reduction Potentials ($E_{\text{b}}^{\text{b}}$), Predicted $k_{\text{NOM}}$ Values, and Experimental $k_{\text{NOM}}$ Values for Selected Pollutants in Hyde County NOM Solutions at pH 7.50, 5 mM H$_2$S, and 25 °C

<table>
<thead>
<tr>
<th>cmpd</th>
<th>$k_{\text{NOM}}$ h$^{-1}$ (mg of C/L)$^{-1}$</th>
<th>$E_{\text{b}}^{\text{b}}$ /V</th>
<th>$E_{\text{b}}^{\text{b}}$ (calc) /V</th>
<th>$E_{\text{b}}^{\text{b}}$ (meas) /V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl Red</td>
<td>1.5 $\times$ 10$^{-1}$, 1.3 $\times$ 10$^{-1}$</td>
<td>-0.309</td>
<td>186</td>
<td></td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>2.0 $\times$ 10$^{-2}$, 6.5 $\times$ 10$^{-3}$</td>
<td>-0.380</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>(2,4-DNT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-methyl-2,4-dinitrophenol (DNOC)</td>
<td>1.4 $\times$ 10$^{-5}$, 5.0 $\times$ 10$^{-4}$</td>
<td>-0.430</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>2-nitrophenol (2-NP)</td>
<td>1.2 $\times$ 10$^{-4}$, 2.3 $\times$ 10$^{-4}$</td>
<td>-0.492</td>
<td>0.16</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated using $\log k_{\text{NOM}} = 7.53 + 1.00E_{\text{b}}^{\text{b}}(\text{ArNO}_2)/0.059$ V; 100 μM juglone. 

A plausible explanation for the kinetic behavior observed at higher pH and Eh conditions of the system is the following. The transfer of the second electron to the nitroaromatic compound yielding the nitroso species is most probably preceded by a proton transfer

$$\text{ArNO}_2^- + \text{H}^+ = \text{ArNO}_2\text{H}^-$$

$$\text{ArNO}_2\text{H}^- + e^- + \text{H}^+ = \text{ArNO}_2 + \text{H}_2\text{O}$$  \hspace{1cm} (8)$$

this step could become rate-determining at higher pH. This could lead to an accumulation of the ArNO$_2^-$ species, and the back-reaction (eq 6) could become important, thus leading to a decrease in the observed rate constant. If, in the course of the reaction, one or several intermediates are formed (e.g., products from reactions of the nitroso compound) that consume the ArNO$_2^-$ species, one would expect the rate constant to increase with increasing time (an autocatalytic effect since more and more reactive species are formed) until the transfer of the first electron becomes rate-determining again. A further investigation of this phenomenon is presently in progress and will be the subject of a future paper.

Prediction of $k_{\text{NOM}}$ Values. Considering the complex mixture of possible electron-transfer mediators in NOM, and the rather complex dependence of the reactivities of these mediators on the pH and $E_{\text{h}}$ of the system, it can be easily concluded that a priori predictions of $k_{\text{NOM}}$ will be difficult, if not impossible. However, as is illustrated by the data given in Tables II and III, relative reactivities among a series of compounds do not change significantly with changing pH and $E_{\text{h}}$ conditions. Thus, if $k_{\text{NOM}}$ values have been determined for a series of model compounds in a given NOM water system, LFERs such as those given in Table III may be used to predict $k_{\text{NOM}}$ values of untested compounds. A prerequisite is, of course, that the one-electron-reduction potential of the compound of interest is known or can be estimated. Unfortunately, such reduction potentials are usually not available. In a previous study, we have shown that one-electron-reduction potentials of substituted nitrobenzenes can be estimated with reasonable success from rate constants determined for the reaction of the compounds with a model hydroquinone (8) using a LFER of the type given by rearrangement of eq 5

$$E_{\text{h}}^{1/2}(\text{ArNO}_2) = [2.3RT/F(\log k - b)]/a$$  \hspace{1cm} (9)$$

In eq 9, $a$ and $b$ have been determined previously with a set of model compounds and $k$ is, for example, the second-order rate constant for the reaction with the hydroquinone monophenolate or the $k_{\text{NOM}}$ value at given pH and $E_{\text{h}}$ conditions.

Table IV gives the $E_{\text{b}}^{1/2}(\text{ArNO}_2)$ values estimated for four compounds not included in our previous set of model compounds [2,4-dinitrotoluene, 2-nitrophenol, 6-methyl-2,4-dinitrophenol (DNOC), and Methyl Red [2-carboxy-4'- (dimethylamino)azobenzene]]. These $E_{\text{b}}^{1/2}(\text{ArNO}_2)$ values were determined from reaction rate data using juglone as the model mediator and the substituted nitrobenzenes included in Table II as model compounds for determining $a$ and $b$ in eq 8 (from eq 5). With these $E_{\text{b}}^{1/2}(\text{ArNO}_2)$ data, $k_{\text{NOM}}$ values of the test compounds were predicted for the Hyde County NOM system at pH 7.50 using the corresponding LFERs given in Table III and are compared to the experimentally determined values in Table IV. As can be seen from data in Table IV and Figure 5, there is a very satisfactory agreement between predicted and experimental values even for $E_{\text{b}}^{1/2}(\text{ArNO}_2)$ values outside of the established $E_{\text{b}}^{1/2}(\text{ArNO}_2)$ range (−0.360 to −0.590 V). Thus, approaches such as this can be used to predict reaction rate constants for other environmental pollutants.

Environmental Relevance and Conclusions

The results of this study clearly demonstrate that components of natural organic matter can effectively mediate the reduction of nitroaromatic compounds in reducing environments. As is illustrated in Figure 10, in natural waters exhibiting typical NOM concentrations of 1–10 mg of C/L and at low reduction potentials (i.e., $E_{\text{h}} < -0.2$ V), very short half-lives (minutes to hours) may be expected for nitroaromatic compounds carrying electron-withdrawing substituents. Such compounds include a variety of pesticides and other environmentally relevant chemicals, above all substituted dinitrobenzenes and dinitrophenols. Thus, under redox conditions typical for sulfate-reducing and, particularly, for methanogenic environments where reduction potentials well below −0.2 V have been measured (32, 33), the abiotic reduction of nitroaromatic compounds by reduced NOM constituents may be a significant transformation process. Various results of this study suggest that the reactive NOM constituents may be qui-

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*Figure 10. Plot illustrating the log half-lives of the nitroaromatic compounds vs their $E_{\text{b}}^{1/2}(\text{ArNO}_2)/0.059$ V for NOM-mediated reductions. Lines were generated for each condition based on a NOM of average reactivity (Hyde County) at pH 7.0. Note the large increase in half-life with increasing $E_{\text{b}}$ above an $E_{\text{b}}$ value of about −0.1 V; the ability of the NOM to mediate reductions may not be important even for very reactive compounds.*
none-type moieties which have recently been shown to be present in aqueous fulvic and humic acid samples (34).

Considering the large diversity of NOMs and the rather complex $E_0$ and pH dependence of the reactivities of the NOM constituents involved in the electron-transfer reactions, it can be concluded that a priori predictions of rates of NOM-mediated reduction of nitroaromatic compounds is very difficult. However, as has been shown by this study, LFERs relating rate constants with one-electron-reduction potentials of a set of model compounds may be used to predict reduction rates of previously untested chemicals. In addition, one-electron-reduction potentials may be estimated from reaction rate data using a model hydroquinone as reductant. Future work will reveal to what extent this approach can be extended to other compound classes. The fact that the reduction rate of even an azo compound (Methyl Red; see Table IV and Figure 5) was accurately predicted by LFERs derived for substituted nitrobenzenes is promising.

The results of this study also have implications for the interpretation of results obtained from studies of reductive transformations of organic pollutants in natural soils and sediments. First, organic matter associated with particles should not be viewed necessarily as a nonreactive compartment in which reducible organic compounds are partitioned/sorbed and thus protected from reaction, as has been suggested in other studies (9, 27). Second, if NOM constituents are involved in the reaction, particularly under high-pH conditions, measurements of initial reaction rates in batch experiments may not yield representative kinetic values due to the use of data which do not truly follow first-order rate expressions. Finally, initial nonpseudo-first-order kinetics that have the appearance of lag phases, as often observed in microbial systems, may not always imply that a reaction is catalyzed by an adapting population of microorganisms but may result from the product catalysis of an abiotic process.

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