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Chemical- and Sediment-Mediated Reduction of the Azo Dye Disperse Blue 79

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Disperse Blue 79, a large volume disperse azo dye, and 2-bromo-4,6-dinitroaniline (BDNA), an important intermediate in the preparation of Disperse Blue 79, were readily reduced chemically and in three anoxic sediment–water systems studied; half-lives were on the order of minutes to hours. No reduction of Disperse Blue 79 or BDNA was observed however in a sediment–water system containing sediment with low organic carbon. The reaction kinetics of Disperse Blue 79 in the reducing sediments are biphasic, that is, the initial rapid loss of dye is followed by a much slower rate of transformation. The reaction pathways for the chemical and sediment-mediated reduction of Disperse Blue 79 were quite similar, suggesting that the chemical reduction of such complex chemicals can provide valuable insight into their reaction pathways in environmental systems. For Disperse Blue 79, a number of reaction products resulting from the reduction of both the azo linkage and aromatic nitro groups were formed. The sediment-mediated reduction of BDNA was regioselective resulting in the formation of a 3-bromo-5-nitro-1,2-diaminobenzene, which was further reduced at a much slower rate to 6-bromo-1,2,4-triaminobenzene. These results suggest that Disperse Blue 79 and BDNA may undergo reduction in some natural anoxic sediments, resulting in the subsequent release of potentially hazardous aromatic amines to the water column.

Introduction

In 1980, approximately 111 million kg of synthetic organic dyestuffs were produced in the United States. In addition, another 13 million kg were imported. The textile industry is the largest consumer of these products, accounting for two-thirds of the dyestuff market (*1*). Recent estimates indicate that approximately 12% of the synthetic textile dyes used each year are lost to waste streams during manufacturing and processing operations and that 20% of these losses will enter the environment through effluents from wastewater treatment plants (*2*). Thus, approximately 3 million kg of organic dyestuffs are discharged into surface waters each year in the United States. Although textile dyes represent a potentially important class of organic pollutants, little is known about their environmental fate. Furthermore, available information on the physical and chemical properties needed to predict dye transport and transformation in the environment is not specific for the dyestuffs currently in use.

Of the dyes available on the market today, approximately 50% are azo compounds (*1*). Azo dyes, which can be divided into monoazo, diazo, and triazo classes, are found in six application categories: acid, basic, direct, disperse, azoic, and pigments. Of these various application categories, the disperse dyes have shown the most significant growth in use since the early 1970s. Their rapid growth is attributed to the fact that they are the only dyes that can be used on polyester fibers. Within the category of disperse dyes, the monoazo class has seen the greatest growth. Monoazo dyes currently account for 70% of the disperse category (*3*).

In recent years, concern over the environmental fate of the disperse azo dyes in natural water systems has grown. This concern arises from the fact that these dyes are hydrophobic compounds, suggesting they will partition strongly to bottom sediments where reductive cleavage of the azo linkage may occur. This transformation process could result in the release of potentially hazardous aromatic amines to the water column. Weber and Wolfe (*4*) demonstrated that the reductive cleavage of simple, substituted azobenzenes in anoxic sediment–water systems is a facile process. Furthermore, Yen et al. (*5*) and Baughman and Weber (*6*) reported the rapid reduction of two nitroazobenzene disperse dyes in sediments. The important role of the sediment–water interface in determining the fate of organic compounds in aquatic systems has been discussed by Zepp and Wolfe (*7*) and Macalady et al. (*8*).

Among the family of disperse dyes, there is particular concern over the fate of Disperse Blue 79, which is one of the largest volume dyes currently on the market (*9*). As illustrated in Scheme 1, reductive cleavage of the azo linkage of Disperse Blue 79 results in the formation of 2-bromo-4,6-dinitroaniline (BDNA), which has been shown to be both toxic and mutagenic, and the *N*-substituted 1,4-diaminobenzene, **1** (*10*).

Both Disperse Blue 79 and BDNA have been detected in water and sediment downstream from textile mills on the Yamaska River in Quebec, Canada (*11*). Gardner et al. (*12*) demonstrated in pilot studies that the treatment of Disperse Blue 79 in conventionally operated aerobic biological sludge processes will be problematic, and ad-

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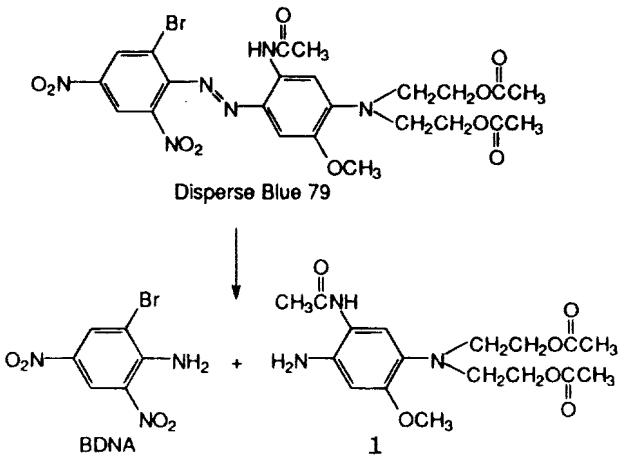
SCHEME 1



ditional treatment with an anaerobic sludge digestion system is required for effective removal of the dye from textile wastewaters.

In addition to reduction of the azo linkage, the nitro groups on Disperse Blue 79 (those on BDNA as well) will be susceptible to reduction in anoxic systems. Numerous studies have demonstrated the facile reduction of aromatic nitro groups in anoxic soils and sediments (*5, 13–18*). Of course, reduction of either of the nitro groups of Disperse Blue 79 prior to the cleavage of the azo group would preclude formation of BDNA.

In this study, we report on the chemical- and sediment-associated reduction of Disperse Blue 79 and BDNA. In particular, we were interested in determining whether reduction of Disperse Blue 79 results in the formation of BDNA and in establishing the degree to which BDNA persists in anoxic sediment–water systems. Toward this goal, we found that the chemical reduction of Disperse Blue 79 provided a great deal of insight into the reaction pathways of the dye in the reducing sediments. In addition to providing analytical standards for identification and quantification purposes, the chemical reduction studies were invaluable for identifying the initial site for reduction of Disperse Blue 79, establishing the regiochemistry of the diaminobenzenes being formed, and establishing the pathway for the formation of the benzimidazole.

Experimental Section

Materials. Disperse Blue 79 (presscake sample) and BDNA (95% pure; Aldrich) were purified by flash chromatography on silica gel (32–63 mm, Universal Adsorbents) with mixtures of ethyl acetate and hexane as eluents (*19*). Iron (40 mesh, Fisher Scientific), ferric chloride (reagent, J. T. Baker), and sodium dithionite (purified, J. T. Baker) were used without further purification. All solvents used were of high purity (Burdick and Jackson).

Sediments were collected from two ponds, Cherokee Park and Bar-H, and two streams, Beaver Dam and Rocky Creek, in the Athens, GA, area. The Cherokee Park, Bar-H, and Beaver Dam sites are not known to be impacted by xenobiotic chemicals. Sediment samples from Rocky Creek were collected approximately 20 m upstream from the entry point of the effluent of a wastewater treatment plant receiving dye waste from two textile mills. The sediment and associated water were collected by scooping up the first 5–10 cm of the sediment surface in 1-L glass jars at a depth of 30–60 cm below the water surface. The jars were capped after being brought to the surface. The samples then were transported to the laboratory, passed through a 1-mm sieve on the bench top in the open laboratory, and stored in an anaerobic chamber (95% N_2/5% H_2) until used. Control studies demonstrated that processing the sediments in this manner as compared to strict anaerobic techniques (e.g., sieving in the anaerobic chamber) had negligible effects on reaction kinetics. Sediments that were not used within 1 week were discarded. The water pH varied from 6.5 to 7.0. The percent organic carbon (air-dried sediment) and sediment-to-water ratio (w/w) for each sediment was as follows: Cherokee Park (3.3 ± 0.5%, 0.3); Bar-H (5.6 ± 0.3%, 0.3); Beaver Dam (2.2 ± 1.0%, 0.4); Rocky Creek (0.37 ± 0.05%, 0.3).

Analytical Methods. The HPLC system used consisted of a Gilson 305 gradient chromatographic pump equipped with an Applied Biosystems 783 programmable wavelength detector, a Rheodyne 7161 injector (200-μL sample loop), an Alcott 728 autosampler, and a Hewlett Packard 3396A integrator. The detection wavelength was 285 nm. The analytical column was an Alltech RSIL C18 (25 cm long × 4.6 mm i.d., 5-mm particle size). The preparative column was a Whatman ODS M9 (25 cm long x 9.4 mm i.d., 10 mm particle size). The columns were protected with an Alltech-Applied Science Adsorbosphere C18 cartridge guard column. Mixtures of acetonitrile and water, ranging from 40 to 100% acetonitrile, were used to ensure elution times of 8.0–20.0 min for the parent compounds and their reaction products.

Solid probe mass spectra were recorded on a Finnigan 4500 GC/MS using a DB-5 (30 m × 0.25 mm) capillary column. High-resolution mass spectrometry (HR/MS) was conducted on a VG 70 SEQ hybrid mass spectrometer with a VAX-based OPUS data system operated at a resolution of 7000. Low-resolution mass spectra were recorded on a Hewlett Packard 5970 with a Hewlett Packard Chemstation using an HP-1 (12m x 0.20 mm) capillary column. The GC conditions were as follows: 40 °C for 2 min, 12 °C/min, final temperature of 280 °C. Infrared spectra were recorded on a Bio-Rad FTS-60 with KBr pellets. Absorption spectra were measured on a Perkin Elmer Lambda 4 UV/visible spectrophotometer and recorded on a Perkin Elmer Chemstation.

Kinetic Studies. Homogeneous aliquots (5 mL) of sediment–water, drawn while stirring the sediment in a glovebox (nitrogen atmosphere), were transferred to a series of 15-mL, screw-capped test tubes. The tubes were capped with Hungate (open top) screw caps fitted with Teflon-lined septa and brought outside of the glovebox. The tubes were then spiked through the septa with an acetonitrile solution of the desired compound while vortex-mixing. The spiked tubes were placed in a constant temperature room at 25 °C. At selected time intervals, 3 mL of acetonitrile was added to a tube from the series followed by vortex-mixing for 60 s. At the same time, the remaining tubes were inverted to mix the contents. The tube containing acetonitrile was then centrifuged (tabletop centrifuge, 2500 rpm for 20 min). The supernatant was transferred to a 15-mL, screw-capped test tube. After centrifuging again (2500 rpm for 20 min), the sample was ready for analysis. All kinetic runs were performed in duplicate. The pseudo-first-order disappearance rate constants, k_{obs}, for Disperse Blue 79 and BDNA were determined from the slope of the line fitted by the least-squares regression after inspection of ln C_t/C_o versus time plots. The reduction kinetics of

Disperse Blue 79 were quantified by determining a first-order rate constant, k_{obs}, from only the first 0.5–1.0 half-life of the ln C_t/C_o versus time plots.

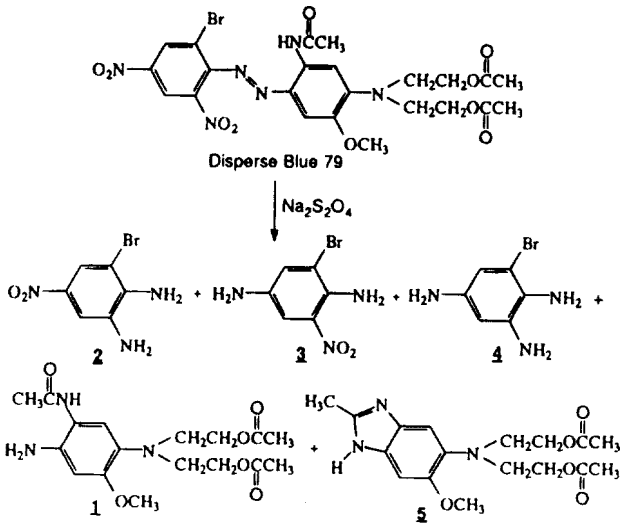
Identification and Quantification of Reaction Products in Sediments. Identification of reaction products was performed by GC/MS analysis and by comparison with standards on the LC and GC/MS that were independently prepared by chemical reduction. For GC/MS analyses, studies were performed at elevated concentrations of Disperse Blue 79 and BDNA. A series of 50-mL, screw-capped test tubes, capped with Hungate (open top) screw caps fitted with Teflon-lined septa, containing 25-mL homogeneous aliquots of sediment–water were spiked with 125 μL of acetonitrile solutions of Disperse Blue 79 or BDNA (1.0×10^{-3} M) while vortex-mixing. At selected times, the tubes were extracted with acetonitrile and centrifuged as described above. The supernatant was transferred to 15-mL screw-capped tubes and centrifuged a second time. The aqueous phase was extracted with two 10-mL portions of methylene chloride. The extracts were combined and rotary-evaporated. The residue was analyzed by GC/MS.

Quantification of reaction products in the sediment–water systems was performed by comparisons with standards by LC analysis that were independently prepared by chemical reduction. The experimental design was identical to that described for the kinetic studies of Disperse Blue 79 and BDNA. Concentrations were calculated from standard curves that were prepared from at least three analytical standards.

Reduction of Disperse Blue 79 with Iron. To a suspension of 25 mg (0.04 mmol) of purified Disperse Blue 79 and 222 mg (4.0 mmol) of iron powder (40 mesh) in 10 mL of a methanol/water mixture (9:1) was added 5 mg of ferric chloride. The suspension was heated to a gentle reflux for 1.5 h. The dark yellow reaction mixture was poured into 20 mL of water and extracted with three 25-mL aliquots of ethyl acetate. The extracts were individually washed in series with one 10-mL portion of saturated NaCl solution (26%). The extracts were combined, dried ($MgSO_4$), and evaporated. The residue was flash chromatographed on silica gel with ethyl acetate/hexane mixtures of increasing polarity to give 5.0 mg (21% yield) of **6** as red crystals and 2.1 mg (9% yield) of **7** as orange crystals. Compound **6**: Anal. Calcd for $C_{23}H_{27}BrN_6O_8$: *m/e* 594.10857, measured *m/e* 594.10737. LC R_t = 16.3 min.

Reduction of BDNA with Iron. To a suspension of 200 mg (0.76 mmol) of BDNA and 852 mg (15.3 mmol) of iron powder (40 mesh) in 20 mL of methanol/water mixture (4:1) was added 50 mg of ferric chloride. The suspension was heated to a gentle reflux for 3 h. The dark yellow reaction mixture was poured into 20 mL of water and extracted with three 25-mL aliquots of methylene chloride. The extracts were individually washed in series with one 10-mL portion of saturated NaCl solution. The extracts were combined, dried ($MgSO_4$), and evaporated. The residue was flash-chromatographed on silica gel with ethyl acetate/hexane mixtures of increasing polarity to give 53 mg (30% yield) of **2** as red crystals, 2 mg (1.1% yield) of **3** as a dark pasty solid, and a trace amount of **4**. Compound **2**: Anal. Calcd for $C_6H_6BrN_3O_2$: *m/e* 232.96229, measured *m/e* 232.96218; LC R_t = 11.4 min, GC R_t = 10.8 min. Compound **3**: LC R_t = 16.3, GC R_t = 10.8 min. Compound **4**: Anal. Calcd for $C_6H_8BrN_3$: *m/e* 202.98811, measured *m/e* 202.98839; LC R_t = 10.5 min, GC R_t = 9.7 min.

SCHEME 2



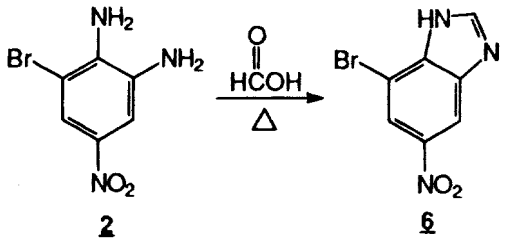
Reduction of Disperse Blue 79 with Sodium Dithionite. To a suspension of 25 mg (0.08 mmol) of purified Disperse Blue 79 in 25 mL of methanol was added 3 mL of 0.016 mmol sodium dithionite in water. The suspension was heated to a gentle reflux for 15 min. The orange-yellow mixture was poured into 20 mL of water and extracted with three 25-mL aliquots of methylene chloride. The extracts were individually washed in series with one 10-mL portion of saturated NaCl solution. The extracts were combined, dried ($MgSO_4$), and rotary-evaporated. The residue was analyzed by HPLC and GC/MS. The residue was flash-chromatographed on silica gel with ethyl acetate/hexane mixtures of increasing polarity to give 4 mg of **2**, trace amounts of **3** and **4**, 5 mg of **1** and 5 mg of **5**. Compound **1**: Anal. Calcd for $C_{17}H_{25}N_3O_6$: *m/e* 367.17434, measured *m/e* 367.17333; LC R_t = 9.7, GC R_t = 13.9 min. Compound **5**: Anal. Calcd for $C_{17}H_{23}N_3O_5$: *m/e* 349.16377, measured *m/e* 349.16360; LC R_t = 11.4, GC R_t = 10.8 min.

Treatment of 3-Bromo-5-nitro-1,2-diaminobenzene, 2, with Formic Acid. A suspension of 3-bromo-5-nitro-1,2-diaminobenzene, **2**, (2.0 mg) in 1.0 mL of formic acid was heated to a gentle reflux for 2 h. After cooling to room temperature, the pH of the reaction mixture was adjusted to 6.0 by the addition of 10% NaOH and extracted with two 5-mL aliquots of methylene chloride. The extracts were combined and rotary-evaporated. The residue was analyzed by GC/MS. MS (70 eV): 243 (M^+), 241, 212, 210, 197, 195, 170, 168, 116; GC R_t = 11.36 min.

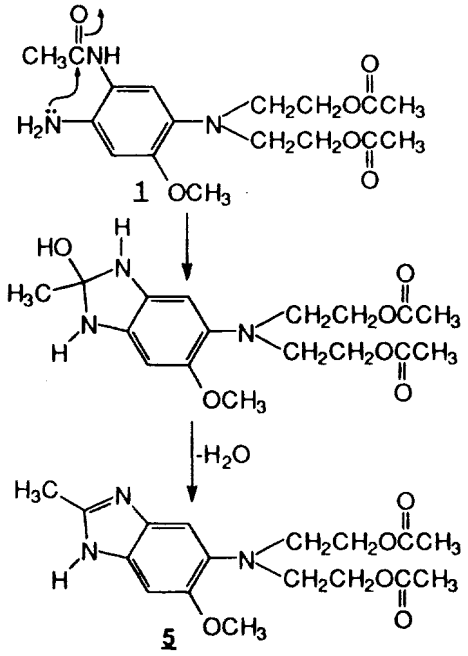
Results and Discussion

Chemical Reduction of Disperse Blue 79 and BDNA. To provide insight into potential reaction pathways in anoxic sediments and to aide in the identification of reaction products, initial product studies focused on the chemical reduction of Disperse Blue 79 and BDNA. The chemical reduction of azo dyes and subsequent analysis of the reaction products have been proposed as a method for the screening of azo dyes in complex industrial wastes and to assess environmental risk of the waste (*20*). Reduction of Disperse Blue 79 with sodium dithionite affected reduction of the azo linkage as well as the nitro groups, resulting in the formation of BDNA; the *N,N*-disubstituted diaminobenzene, **1**; 3-bromo-5-nitro-1,2-diaminobenzene, **2**; 2-bromo-6-nitro-1,4-diaminobenzene, **3**; 3-bromo-1,2,5-

SCHEME 3



SCHEME 4



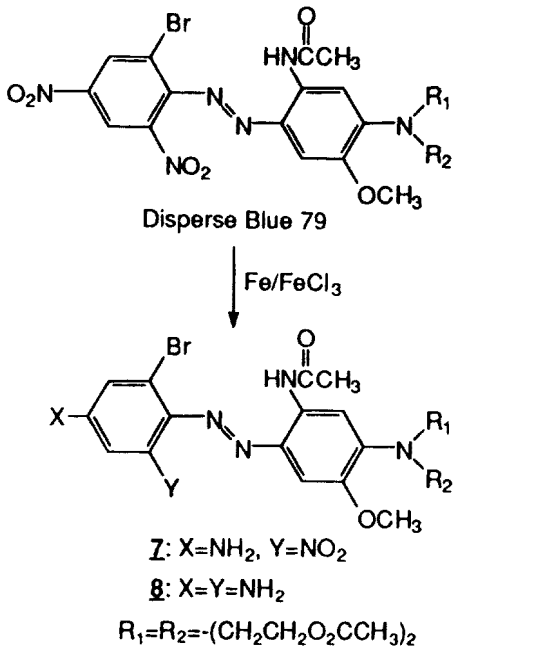
triaminobenzene, **4**; and the benzimidazole, **5**, as illustrated in Scheme 2.

The structures of **2** and **3** were confirmed by comparison of their mass and infrared spectra to those of the known compounds, 4-nitro-1,2-diaminobenzene and 2-nitro-1,4-diaminobenzene. Further confirmation of the identify of the regioisomers **2** and **3** was provided by treatment of **2** with formic acid under reflux conditions, which resulted in the formation of the benzimidazole, **6** (21) (Scheme 3). Of course, a 1,4-orientation of the amino groups would preclude formation of the benzimidazole. Formation of the benzimidazole, **5**, in Scheme 2 results from intramolecular nucleophilic addition of the unsubstituted amino group to the carbonyl moiety and subsequent loss of water (Scheme 4).

The observation that ethyl acetate extracts containing mixtures of **1** and **5** become enriched in **5** upon standing at room temperature suggested that cyclization was occurring spontaneously and not on the heated injection port of the GC/MS. Furthermore, introduction of the product mixture to the GC/MS by cool on-column injection had no effect on the ratio of **1** to **5** as compared to introduction of the sample through the heated injection port.

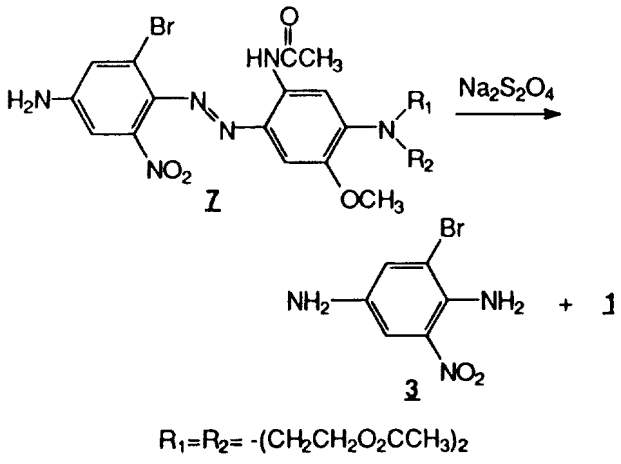
To selectively reduce the aromatic nitro groups of the dye without affecting reduction of the azo linkage, Disperse Blue 79 was reduced with metallic iron (*22*). Halogenated nitro compounds are known to undergo dehalogenation under some reducing conditions (*23, 24*). Treatment of Disperse Blue 79 with metallic iron and a catalytic amount

SCHEME 5



of ferric chloride afforded the azo compounds **7** and **8**, as shown in Scheme 5.

SCHEME 6

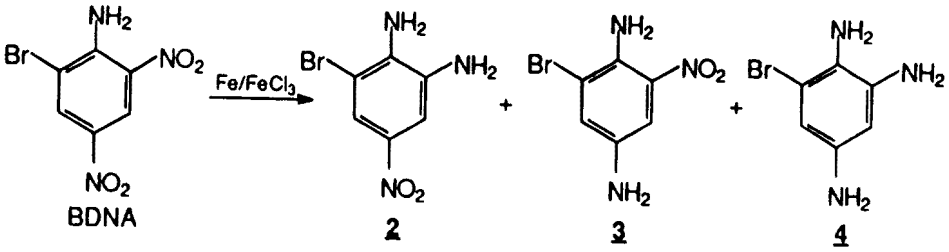


The chemical formulas of **7** and **8** were confirmed by HR/MS. The regiostereochemistry of **7** was established by reduction of the azo linkage with sodium dithionite, which resulted in the formation of 2-bromo-6-nitro-1,4-diaminobenzene, **3**, and **1**, as illustrated in Scheme 6. None of the 3-bromo-5-nitro-1,2-diaminobenzene, **2**, could be detected in the reaction mixture.

Treatment of BDNA with metallic iron gave the aromatic amines **2** and **3** in a 20:1 ratio (Scheme 7). Only a trace amount of the triaminobenzene, **4**, resulting from reduction of both nitro groups was detected.

Kinetic and Product Studies of Disperse Blue 79 in Sediment–Water Systems. Reduction kinetics for Disperse Blue 79 were measured in two pond and two stream sediment–water systems. The initial concentrations of Disperse Blue 79 in these experiments ranged from 1.46×10^{-6} to 3.13×10^{-6} M. At higher concentrations of Disperse Blue 79, we observed significant scatter in the kinetic data, which we attributed to incomplete mixing of Disperse Blue 79 in the sediment–water system. In the Cherokee Park,

SCHEME 7



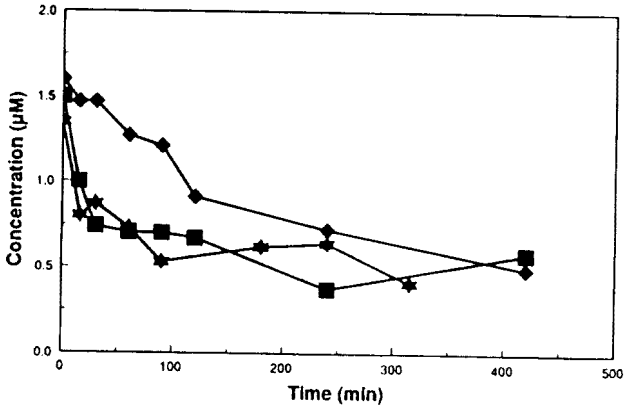


FIGURE 1. Plot of concentration versus time for the reduction of Disperse Blue 79 in a Bar H (■), Cherokee Park (◆), and Beaver Dam (★) sediment–water system.

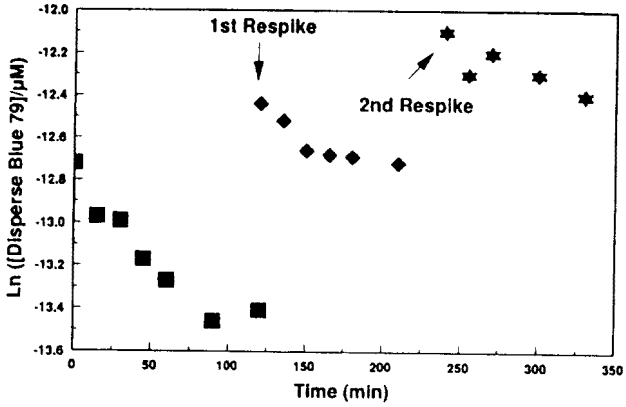


FIGURE 2. Plot of ln concentration versus time for the reduction of Disperse Blue 79 in a Beaver Dam sediment–water system. Initial reduction of Disperse Blue 79 (■); reduction of Disperse Blue 79 after respiking with second equivalent of dye (◆); reduction of Disperse Blue 79 after respiking with third equivalent of dye (★).

Bar-H, and Beaver Dam sediment–water systems, the reaction kinetics of Disperse Blue 79 were extremely fast, degradation half-lives were measured on the order of minutes to hours. At the other extreme, however, no disappearance of Disperse Blue 79 could be detected in the Rocky Creek sediment–water system over a period of several days. An obvious difference between the nonreactive sediment and the reactive sediments is that Rocky Creek sediment contains significantly less organic carbon than the others (i.e., 0.37% vs 2–5%). Reduction rate constants for a series of halogenated aromatics (*25, 26*) have been found to correlate with sediment organic carbon content. Laboratory studies suggest that natural organic matter may play the role of an electron carrier or mediator between the bulk reductant (e.g., reduced iron and sulfur species) and the organic pollutant (*27, 28*). Furthermore, and perhaps of greater significance, is the fact that the Rocky Creek sediment was sampled from a fast flowing stream with a well mixed water column that probably results in an aerobic bottom sediment.

The reduction rate constants for the initial reduction of Disperse Blue 79 in the reactive sediment–water systems vary, at most, by a factor of 10. The mean values and standard deviations for k_{red} in Beaver Dam, Cherokee Park, and Bar-H sediment–water systems were $(4.44 \pm 8.17) \times 10^{-3}$ min^{-1}, $(5.83 \pm 7.97) \times 10^{-3}$ min^{-1}, and $(2.63 \pm 3.95) \times 10^{-3}$ min^{-1}, respectively. We found no clear dependence of k_{red} on the time of year at which the sediment samples were collected. We also found that sieving the sediments in the open air versus in the anaerobic chamber had no significant effect on k_{red}. Removal of the sediment from these systems by filtration provided a filtrate that had little or no reactivity for Disperse Blue 79, suggesting that the reducing capacity of the sediment–water systems is associated with the sediment phase. This observation appears to be a general phenomenon for the reduction of organic chemicals in anoxic sediment–water systems (*4, 7, 18, 28*).

The concentration versus time plots in Figure 1 for the reduction of Disperse Blue 79 in the reactive sediment–water systems illustrate a significant decrease in the rate of disappearance of the dye after 1–1.5 half-lives. In fact, after the initial rapid loss of Disperse Blue 79 had occurred, it was often difficult to discern whether further reduction of the dye was occurring. The initial rapid loss of dye could be positively attributed to a transformation process because of the concurrent formation of reaction products. The rapid reduction of Disperse Blue 79 with no lag phase as well as the observation that considerable loss of the dye was observed in heat-sterilized sediments strongly suggest that chemical or cometabolic processes were responsible for transformation of the dye.

The observed decrease in the rate of reduction of Disperse Blue 79 after its initial rapid loss indicates a change in the rate-limiting step controlling the disappearance of the dye. We propose two possible scenarios that would account for this type of kinetic behavior: (1) saturation of reductive sites, or species, associated with the sediment phase or (2) sorption of the dye to the sediment such that only the dissolved fraction of the dye can be reduced. In an attempt to distinguish between these two scenarios, we respiked a Bar-H sediment–water system with a second equivalent of Disperse Blue 79 after the initial rapid loss of dye had occurred. As with the first spike, the second equivalent of dye had an initial rapid degradation (Figure 2). In fact, respiking the sediment–water system with a third equivalent of Disperse Blue 79 resulted in the initial rapid loss of dye again. Although a significant increase in the concentration of reaction products was observed with dye loss after each respike, the saturation hypothesis cannot be clearly refuted because the data do not indicate simple first-order kinetics. Rather, following each respike it appears as if the dye rapidly disappears followed by a leveling of the dye concentration. With each successive

spike, the duration of the initial phase of rapid decrease in dye concentration appears to lessen.

The alternative hypothesis that partitioning of the dye to the sediment inhibits reduction was not tested experimentally. Although we did not attempt to measure sorption kinetics or the sediment–water partition coefficient for Disperse Blue 79 because of its extremely low water solubility, it was possible to estimate the fraction of Disperse Blue 79 in the water column of a Bar-H sediment–water system using eq 1 and a value of 1.26×10^5 for the organic carbon-normalized partition coefficient, K_{oc}, for Disperse Blue 79, which was calculated by Hou et al. (*29*):

$$F_{sol} = 1/(1 + [S]K_{oc}f_{oc}) \quad (1)$$

where [S] is the sediment concentration and f_{oc} is the fraction of organic carbon in the sediment phase. Equation 1 gives a value of 1.43×10^{-4} for the predicted fraction of Disperse Blue 79 in the water column of a Bar-H sediment–water system, indicating that at equilibrium Disperse Blue 79 is virtually totally sorbed to the sediment.

In light of this finding, it is quite surprising that we observed such fast reaction kinetics for the initial reduction of Disperse Blue 79 in the anoxic sediments based on the kinetic model of Weber and Wolfe, which describes the reduction of substituted azobenzenes in anoxic sediment–water systems (*4*). This model predicts that the rate of reduction is controlled by the amount of partitioning onto the sediment, with increased partitioning inhibiting the reduction process. For a hydrophobic azo compound such as Disperse Blue 79, the model would predict a half-life on the order of weeks, not minutes as was observed in this study. The observed increased reactivity of Disperse Blue 79 in high organic sediment–water systems over predicted results may be attributable to the activation of the azo group by the strong electron-withdrawing bromo and nitro groups in the *ortho* and *para* positions. The facile reductive transformation of disperse azo dyes in anoxic sediment–water systems appears to be a general phenomenon (*5, 6*). Clearly, our current understanding of the reaction kinetics of highly functionalized disperse azo dyes such as Disperse Blue in sediment–water systems is not complete. Greater insight into the nature and availability of the reducing sites that are associated with the sediment and a more thorough understanding of how sorption processes effect reduction kinetics will be required before useful predictive models can be developed.

Product studies of Disperse Blue 79 were conducted primarily in Cherokee Park sediment–water systems. Analysis of acetonitrile extracts of Cherokee Park, Bar-H, and Beaver Dam sediment–water systems by HPLC and GC/MS indicated that Cherokee Park sediment contained the fewest background components that could potentially interfere with reaction product detection and identification. Although detailed comparative studies were not performed, it was evident from the kinetic studies of Disperse Blue 79 that the sediment source, as long as the organic carbon content was high, had little effect on the formation or distribution of the major reaction products. Reaction products formed in the sediment–water systems were identified by GC/MS and/or HPLC by comparisons with standards that were prepared from the chemical reduction of the dye. The major reaction products identified in Cherokee Park sediment, which included the *N,N*-disubstituted 1,4-diaminobenzene, **1**, the 1,2-diaminobenzene,

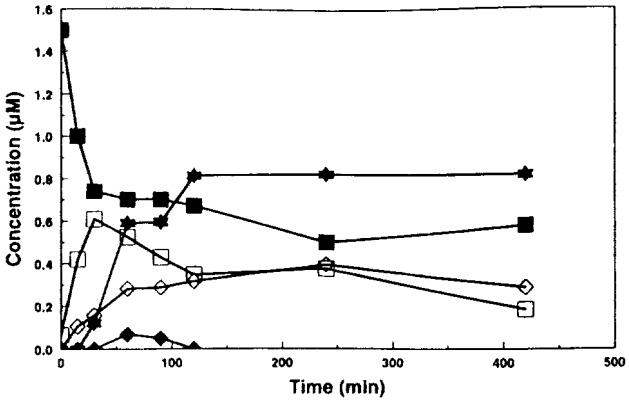
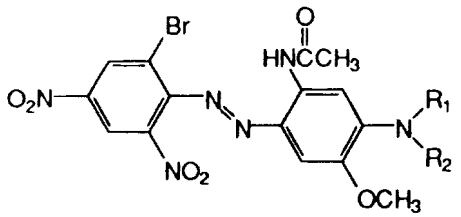


FIGURE 3. Plot of concentration versus time for the reduction of Disperse Blue 79 (■) and the formation of BDNA (◆), 1 (□), 2 (★), and 5 (◇) in a Cherokee Park sediment–water system.

SCHEME 8



9: R_1=CH_2CH_2OH, R_2=$CH_2CH_2CO_2CH_3$
10: R_1=R_2=CH_2CH_2OH

2, and the benzimidazole, **5**, were the same as those observed in the chemical reduction of Disperse Blue 79 by sodium dithionite (see Scheme 7). We found that sediment extracts containing mixtures of **1** and **5** became enriched in **5** upon standing at room temperature or at 4 °C.

A concentration versus time plot illustrating the rapid disappearance of Disperse Blue 79 and the formation of reaction products in a Cherokee Park sediment–water system is illustrated in Figure 3. The formation of BDNA, the reaction product of most concern, and the azo compounds **7** and **8** were somewhat sporadic. We found that in those sediment–water systems in which the reduction of the parent compound, Disperse Blue 79, was extremely fast, we were less likely to observe the formation of these reaction products. In the experiments in which BDNA was detected, its concentration never accounted for more than a few percent of the amount of parent dye that had degraded (see Figure 3).

The mono- and dialcohols, **9** and **10**, resulting from the hydrolysis of the acetate groups were observed at trace levels in one experiment with a sample of Cherokee Park sediment (Scheme 8). In the Rocky Creek sediment–water system, where reduction of the dye did not occur, hydrolysis of the acetate groups was not observed either.

Kinetic and Product Studies of BDNA. Reduction kinetics for BDNA also were measured in the Cherokee Park, Bar-H, Beaver Dam, and Rocky Creek sediment–water systems and additionally from filtered waters of the Cherokee Park and Beaver Dam sediment–water systems. The reactivity of BDNA in the sediment–water systems was very similar to that of Disperse Blue 79. Rapid disappearance of BDNA was observed in the Cherokee Park, Bar-H, and Beaver Dam sediment–water systems. In contrast, no reduction of BDNA was observed in the Rocky Creek

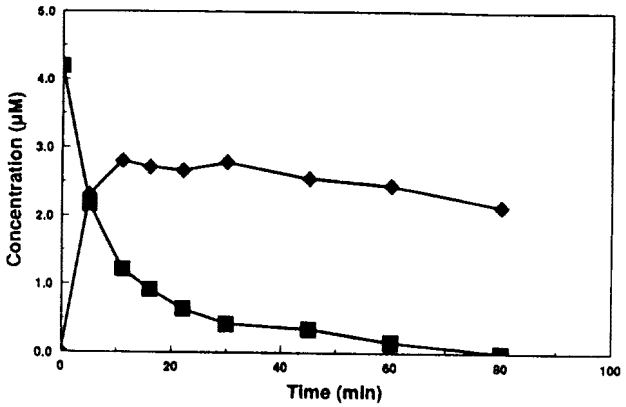


FIGURE 4. Plot of concentration versus time for the reduction of BDNA (■) and the formation of 3-bromo-5-nitro-1,2-diaminobenzene (◆) in a Beaver Dam sediment–water system.

sediment–water system or the filtered Cherokee Park or Beaver Dam water. The mean values and standard deviations for k_{deg} in Cherokee Park, Bar-H, and Beaver Dam sediment–water systems were $(4.95 \pm 0.44) \times 10^{-2}$ min^{-1}, $(1.24 \pm 0.41) \times 10^{-2}$ min^{-1}, and $(4.65 \pm 1.45) \times 10^{-2}$ min^{-1}, respectively.

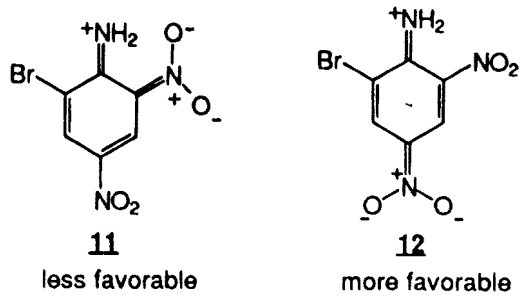
The concentration versus time plot for the disappearance of BDNA in a Beaver Dam sediment–water system is shown in Figure 4. The decay curve of BDNA is best described by a first-order kinetic expression. The only major reduction product that could be detected in the sediment–water system treated with BDNA was the 1,2-diaminobenzene, **2**. The 1,4-diaminobenzene, **3**, and the triaminobenzene, **4**, were detected also but at trace levels (see Scheme 7). The rate of formation of **2** in relation to the disappearance of BDNA is illustrated in Figure 4. The concentration of **2** reached a maximum at $t = 11$ min and remained nearly constant over the lifetime of the experiment, accounting for 55–65% of the parent compound loss.

Reduction of the more sterically hindered nitro group in the *ortho* position of BDNA observed in the high organic sediment–water systems (and by treatment with metallic iron) appears to be a general phenomenon for the chemical (*31–33*) and enzymatic reduction (*34*) of 2,4-dinitrobenzenes substituted with resonance electron-donating groups (e.g., -NH_2 or -OH) in the 1-position. By contrast, for 2,4-dinitrobenzenes with substituents in the 1-position that are not resonance electron-donating (e.g, -CH_3, as in 2,4-dinitrotoluene), the less sterically hindered nitro group in the *para* position is preferentially reduced (*32, 34*). A plausible explanation for the observed regioselectivity of these aromatic nitro reductions is the existence of an unfavorable dipole–dipole interaction in resonance structure, **11**, due the close proximity of like charges. A more favorable dipole–dipole interaction exists in resonance structure, **12** (Scheme 9).

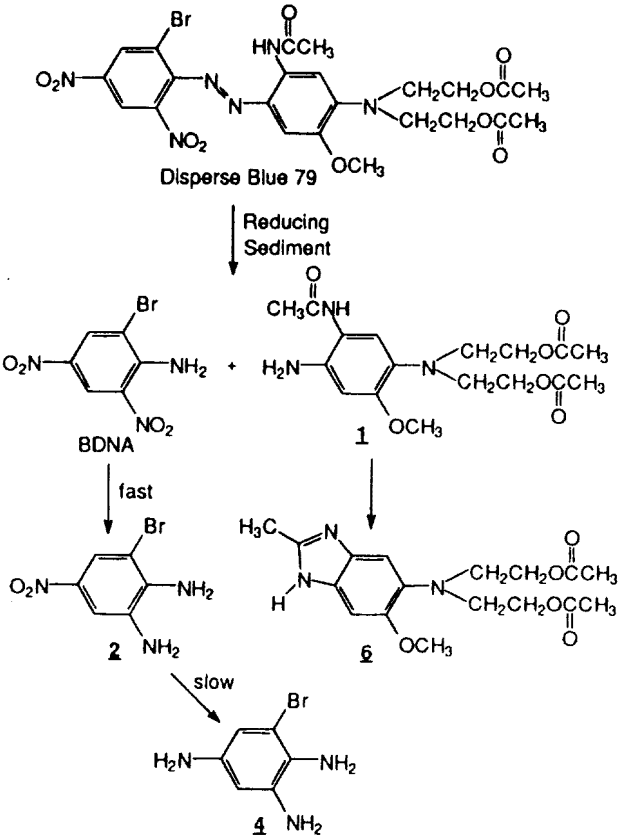
Furthermore, deactivation of the *o*-nitro group by through-resonance is minimized because the 1-substituent forces the *o*-nitro group out of the plane of the ring, a phenomenon referred to as steric inhibition of resonance (*35*). The result is that resonance electron-donating groups deactivate *p*-nitro groups toward reduction to a greater extent than *o*-nitro groups. In the case of 2,4-dinitrotoluene, steric interactions appear to be the dominant factor controlling the regioselectivity of nitro reduction (*32, 34*).

Reaction Pathways of Disperse Blue 79 and BDNA in Anoxic Sediment–Water Systems. The results of the

SCHEME 9



SCHEME 10



kinetic and product studies of Disperse Blue 79 and BDNA are consistent with a reaction pathway in which reductive cleavage of the azo linkage of Disperse Blue 79 occurs initially, resulting in the formation of BDNA and the *N,N*-disubstituted 1,4-diaminobenzene, **1** (Scheme 10). Subsequently, facile reduction of the 2-nitro group of BDNA occurs to give the 1,2-diaminobenzene, **2**, which in turn is slowly reduced to the triaminobenzene, **4**. The *N,N*-disubstituted 1,4-diaminobenzene, **1**, undergoes a cyclization reaction through nucleophilic attack of the unsubstituted amino group on the adjacent amide group to give the benzimidazole, **5**.

An alternative reaction pathway for the formation of **2** that involves the initial reduction of the *o*-nitro group of the parent dye with subsequent reduction of the azo linkage appears not to be an important process. Although formation of trace levels of the reaction products **7** and **8** indicates that reduction of the nitro groups on Disperse Blue 79 can occur prior to cleavage of the azo linkage, the fact that the azo compound resulting from reduction of the nitro group in the *ortho* position was not observed in either the chemical reduction or product studies of Disperse Blue 79 in

sediments suggests that reduction of the nitro group in the *para* position is preferred. Furthermore, 3-bromo-6-nitro-1,4-diaminobenzene, **3**, which is the expected reaction product if reductive cleavage of the azo linkage of **7** occurs, was either not observed or observed at only trace levels.

Conclusions

Disperse Blue 79 and BDNA were readily reduced chemically and in three (high organic carbon content) of four anoxic sediment–water systems studied; half-lives were on the order of minutes to hours. The reaction kinetics of Disperse Blue 79 in the reducing sediments are biphasic, that is, the initial rapid loss of dye is followed by a much slower rate of transformation. The reaction pathways for the chemical- and sediment-mediated reduction of Disperse Blue 79 were quite similar, suggesting that the chemical reduction of such complex chemicals can provide valuable insight into their reaction pathways in environmental systems. The product studies demonstrated that the nitro groups and azo linkage of Disperse Blue 79 are susceptible to reduction, resulting principally in the formation of the *N,N*-disubstituted 1,4-diaminobenzene, **1**, and 3-bromo-6-nitro-1,2-diaminobenzene, **2**, and the benzimidazole **5** resulting from cyclization of **1**. The azo compounds, **7** and **8**, the 1,4-diaminobenzene, **3**, and the triaminobenzene, **4**, were formed in trace amounts. On average, these reduction products accounted for approximately 40% of the parent compound in Cherokee Park sediment–water systems. The *N,N*-disubstituted 1,2-diaminobenzene, **1**, the 1,2-diaminobenzene, **2**, and the benzimidazole, **5**, could be detected in both the aqueous and sediment phases of a Cherokee Park sediment–water system, whereas the azo compounds **7** and **8** were detected in only the sediment phase. Reduction of BDNA in a Beaver Dam sediment–water system gave only the 1,2-diaminobenzene, **2**. This product was further reduced to the triaminobenzene, **4**, but at a rate much slower than the parent compound. The results of this study suggest that disperse dyes such as Disperse Blue 79 can undergo rapid reductive transformation in anoxic bottom sediments, resulting in the release of aromatic amines to the water column

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