APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1988, p. 1182–1187
0099-2240/88/051182-06$02.00/0

Vol. 54, No. 5

Degradation of Polycyclic Aromatic Hydrocarbon Compounds under Various Redox Conditions in Soil-Water Systems

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Received 2 November 1987/Accepted 4 February 1988

This study evaluated the microbial degradation of naphthol, naphthalene, and acenaphthene under aerobic, anaerobic, and denitrification conditions in soil-water systems. Chemical degradation of naphthol and naphthalene in the presence of a manganese oxide was also studied. Naphthol, naphthalene, and acenaphthene were degraded microbially under aerobic conditions from initial aqueous-phase concentrations of 9, 7, and 1 mg/liter to nondetectable levels in 3, 10, and 10 days, respectively. Under anaerobic conditions naphthol degraded to nondetectable levels in 15 days, whereas naphthalene and acenaphthene showed no significant degradation over periods of 50 and 70 days, respectively. Under denitrification conditions naphthol, naphthalene, and acenaphthene were degraded from initial aqueous-phase concentrations of 8, 7, and 0.4 mg/liter to nondetectable levels in 16, 45, and 40 days, respectively. Acclimation periods of approximately 2 days under aerobic conditions and 2 weeks under denitrification conditions were observed for both naphthalene and acenaphthene. Abiotic degradation of naphthalene and naphthol were evaluated by reaction with manganese oxide, a minor soil constituent. In the presence of a manganese oxide, naphthalene showed no abiotic degradation over a period of 9 weeks, whereas the aqueous naphthol concentration decreased from 9 mg/liter to nondetectable levels in 9 days. The results of this study show that low-molecular-weight, unsubstituted, polycyclic aromatic hydrocarbons are amenable to microbial degradation in soil-water systems under denitrification conditions.

Polycyclic aromatic hydrocarbon (PAH) compounds have been found to exhibit toxic and hazardous properties. As a consequence, the U.S. Environmental Protection Agency has listed 16 PAH compounds, including naphthalene and acenaphthene, as priority pollutants to be monitored in industrial effluents. For these reasons, there is interest in understanding the movement and fate of these compounds in subsurface aquatic environments, such as groundwaters and soil-water systems (11). Before the fate of PAH compounds in these environments can be evaluated, there must be an understanding of the various mechanisms which may degrade PAH compounds through biotic or abiotic processes. Currently there is little detailed information available on natural degradation reactions of PAH compounds, particularly in the context of soil-water systems and groundwater contamination. The purpose of this study was to provide initial observations on the microbial degradation of PAH compounds in soil-water systems under different redox conditions and to compare these pathways with abiotic degradation through reaction with manganese oxide.

Various studies have identified specific microorganisms which may degrade PAH compounds. These organisms include algae (6, 7), fungi (5), and bacteria (12, 17, 18, 24). Although much has been published on microbial metabolism of PAH compounds and on the reaction products of microbial degradation, this information is generally limited to aerobic pathways for two- and three-ring PAH compounds including naphthalene (8, 9, 13, 23), acenaphthene (24), and phenanthrene (8, 23). The literature on microbial degradation and stability of PAH compounds in soil-water systems has been summarized by Atlas (2) and Sims and Overcash (25). It is evident (25) that many of the previous data were obtained without considering the specific mechanisms responsible for the decrease of aqueous-phase PAH concentrations in soil-water systems, e.g., apparent reduction in the aqueous-phase PAH concentration owing to the physical processes of organic solute sorption and volatilization versus depletion of PAH compounds via microbial processes.

Microbial degradation of PAH compounds in soil-water systems is influenced strongly by the redox environment and the nature of any substituent groups on the PAH compound. Microbial degradation of unsubstituted PAH compounds in aerobic soil-water systems has been reported (3, 10, 15, 16, 24). In contrast, unsubstituted PAH compounds are thought to be refractory in anaerobic soil-water systems. This has been documented in a study showing the stability of naphthalene and anthracene in anaerobic sediment-water systems for periods up to 16 weeks (3) and in a study in which naphthalene showed minimal degradation over 96 days in an anaerobic soil-water system (10), as well as in other work (14, 28). Naphthalene stability under denitrification conditions has been reported previously (4), and no significant degradation of naphthalene was evident over a time span of 11 weeks. It is recognized that the experimental conditions in that study were different from those reported here; particularly, the initial naphthalene concentrations were approximately 41 to 114 μg/liter, rather than several milligrams per liter, and the biological seed was primary sewage effluent, rather than a mixed soil population.

The chemical oxidation reaction of hydroxy- and carboxyl-substituted benzene compounds with manganese dioxide has been reported (26, 27). It was found that compounds with strong electron-withdrawing substituents lowered the reaction rate with manganese dioxide, whereas electron-donating groups increased the reaction rate.

The objectives of this study were to compare microbial degradation of naphthol, naphthalene, and acenaphthene by soil microorganisms under aerobic, anaerobic (i.e., no oxygen or nitrate), and denitrification conditions. Chemical

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degradation of naphthol and naphthalene in the presence of a manganese oxide was also studied.

MATERIALS AND METHODS

Soil. The soil used in this study was an undisturbed, subhumid grassland soil of the Barnes-Hamerly Association, obtained by William C. Dahnke, Agricultural Experiment Station, North Dakota State University, Fargo. The soil was collected from the A soil horizon at the SW1/4 of Section 16, T 139 N, R 55 W in Cass County, N.D. The soil was air dried, screened to pass a U.S. standard sieve no. 10 (2.0 mm), and then placed in refrigerated storage.

Mineral medium. The mineral medium used in the biodegradation tests was prepared in deionized water with salts to provide buffering capacity, microbial nutrients, and sustaining electrolyte. The mineral medium was prepared such that after dilution with stock PAH solution, the background electrolyte was 0.01 N $CaCl_2$ with the following salts (in milligrams per liter): KH_2PO_4, 8.5; K_2HPO_4, 21.75; $Na_2HPO_4 \cdot 7H_2O$, 33.4; $FeCl_3 \cdot 6H_2O$, 0.25; NH_4Cl, 1.7; and $MgSO_4 \cdot 7H_2O$, 22.5. The mineral medium pH was 6.80. At the initiation of an experiment, mineral medium was combined with soil and stock PAH solution. The purpose of the 0.01 N $CaCl_2$ electrolyte was to assist centrifugation and separation of the solid phase at the completion of a test.

Chemical reagents. Chemical reagents were obtained from Fisher Scientific Co., Pittsburgh, Pa., and Aldrich Chemical Co., Inc., Milwaukee, Wis. Scintillation grade or certified grade acenaphthene, naphthalene, and naphthol were obtained as crystalline powders. The name naphthol refers to the compound α-naphthol.

Instrumentation and analyses. Solute analyses were performed with a high-pressure liquid chromatograph system manufactured by The Perkin-Elmer Corp., Norwalk, Conn. A Series 3 liquid chromatograph unit and model 204-S fluorescence detector were used, and the results were recorded and integrated on an LC-100 Laboratory Computing Integrator. Shaking was performed with a wrist action shaker (model 74; Burrell Corp., Pittsburgh, Pa.), and centrifuging was performed at 875 × *g* with a clinical centrifuge (International Equipment Co., Needham Heights, Mass.).

The PAH compounds were analyzed on an LC-PAH column (Supelco, Inc., Bellefonte, Pa.) by direct aqueous injection of 1- to 10-μl samples which were eluted under isocratic conditions with 60% high-pressure liquid chromatography grade acetonitrile and 40% deionized water. Each sample point was injected at least three times. Prior to use in high-pressure liquid chromatography analysis, acetonitrile and water were filtered through Teflon and cellulose filters, respectively (pore size, 0.45 μm), degassed by vacuum treatment, and purged with helium. Acenaphthene and naphthalene were detected by setting the fluorescence excitation and emission wavelengths at 280 and 340 nm, respectively, whereas for naphthol the settings were adjusted to 310 and 340 nm, respectively. The PAH detection limit was 0.01 mg/liter.

Dissolution of PAH compounds. The PAH compounds used in these tests exist as crystalline solids at room temperature. Stock solutions of these compounds were prepared by placing a known amount of solid in deionized water and then dissolving it overnight by magnetic stirring in a closed glass container covered with aluminum foil. The solution was then filtered through an extra-thick glass fiber filter (no. 66077; Gelman Sciences Inc., Ann Arbor, Mich.) to remove any undissolved, suspended crystals. The filtered stock solutions were analyzed by high-pressure liquid chromatography techniques to assess the preparation procedures. Typically, these techniques resulted in stock solutions containing the PAH compounds in the desired range of about 1 to 10 mg/liter.

Sample preparation. Pyrex centrifuge tubes (50 ml; Corning Glass Works, Corning, N.Y.) were used for the test samples, controls, and blanks. Each tube was sealed with a Teflon-lined septum (model 2-3281; Supelco) and secured with an open-port screw cap. Test samples were composed of a mixture of mineral medium and PAH stock solution with 1.0000 g (dry weight) of soil and filled to zero headspace. Immediately after being filled, the tubes were secured with the Teflon-lined septum and open-port screw cap and covered with aluminum foil. The tubes were shaken for 4 h per day on a wrist action shaker.

Sterile blank and control samples were run concurrently with the test samples. Blanks contained mineral medium and PAH stock solution in sterilized glassware without soil. Sterile control samples contained mineral medium, PAH stock solution, and 1 g of soil. Sterilization was carried out by autoclaving the glassware and soil at 121°C under steam pressure of 20 lb/in^2 for 1 h and then adding $HgCl_2$ such that the concentration of $HgCl_2$ in the control samples was 400 mg/liter. Sample blanks were used to confirm no loss of PAH compounds through volatilization, photodegradation, or interaction with the Teflon-lined septum, whereas sample controls were used to confirm the results obtained from the sample blanks as well as to verify no loss of PAH compounds through abiotic degradation. The experiments were conducted in a batch mode with test samples, blanks, and controls being prepared simultaneously. Approximately 36 separate tubes were set up at the initiation of a single experiment. The tubes were withdrawn individually for analysis during the course of an experiment and then removed from service after being analyzed.

Soil-water samples were centrifuged for 1 h before the aqueous phase was analyzed for PAH compounds. The aqueous-phase samples were obtained by puncturing the septum with an injection syringe equipped with a removable gas chromatography needle (series 800; Hamilton Co., Reno, Nev.) and immediately analyzing the sample on the HPLC. Therefore, test samples, blanks, and controls remained closed and secure from the external environment from the initiation of the experiment through sample analysis. The samples were maintained at room temperature during the experiments.

Degradation test conditions. (i) Aerobic degradation. PAH stock solution and oxygen-saturated mineral medium were combined in a 50-ml centrifuge tube containing 1 g (dry weight) of soil. The relative volumes of mineral medium to PAH solution were 3:2, 4:1, and 4:1 for acenaphthene, naphthalene, and naphthol, respectively. The larger proportion of acenaphthene stock solution to mineral medium was necessary to provide sufficient aqueous-phase PAH concentration following sorption equilibrium of acenaphthene with soil.

Separate measurements were performed to determine the dissolved oxygen concentration in the test samples at the beginning of a run. Dissolved oxygen was measured by iodometric titration procedures (1). This confirmed the presence of sufficient oxygen for possible mineralization of the PAH compound. Selected test samples were analyzed for residual dissolved oxygen at the conclusion of an experiment after sampling for the PAH compound. The final dissolved oxygen measurement showed that dissolved oxygen was

present throughout the test. For example, in the aerobic test with the solute naphthol, the initial dissolved oxygen concentration was approximately 30 mg/liter, and this was decreased to approximately 5.3 mg/liter by the conclusion of the experiment.

(ii) Anaerobic degradation. Similar experimental protocols as in the aerobic tests were used in the experiments to assess PAH degradation under anaerobic conditions, except that oxygen was removed from the samples by first degassing the mineral medium by vacuum treatment and then purging the mineral medium with helium for 30 min. Mineral medium was then combined with PAH stock solution and purged further with helium for 1 h before it was transferred to 50-ml centrifuge tubes. Iodometric titration measurements for dissolved oxygen were conducted on selected samples at the beginning and at the conclusion of a test run. These measurements confirmed the absence of oxygen in the anaerobic samples.

(iii) Denitrification. The experiments to evaluate PAH compound degradation under denitrification conditions involved procedures similar to those used in the anaerobic experiments, except that $NaNO_3$ was added to the mineral medium. The initial nitrate concentration in the test samples was approximately 75 mg of NO_3^- as nitrate per liter. As in the anaerobic tests, the absence of dissolved oxygen was monitored by iodometric titrametric procedures.

(iv) Chemical degradation. Chemical degradation tests were done by experimental procedures described elsewhere (26). The manganese oxide suspension was prepared from oxygenation of Mn^{2+}, after which the oxide was deoxygenated by purging with nitrogen for 90 min. Then 10 ml of the oxide suspension was immediately combined with 40 ml of PAH solution. In these tests, the PAH solution consisted of a buffer and electrolyte solution containing (milligrams per liter) KH_2PO_4, 17.0; K_2HPO_4, 43.5; and $Na_2HPO_4 \cdot 7H_2O$, 66.8; sufficient NaCl to maintain an ionic strength of 0.01 N NaCl in the final sample was also added. The manganese oxide concentration in an individual sample, after being combined with PAH stock solution, was 2×10^{-3} M. The pH of the samples was adjusted to 5.9 for the test with the solute naphthol and to 5.0 and 8.0 for the tests with naphthalene. Blanks were prepared with PAH stock solution without manganese oxide. The centrifuge tubes were sealed with Teflon-lined septum tops, covered with aluminum foil, and rotated six times daily.

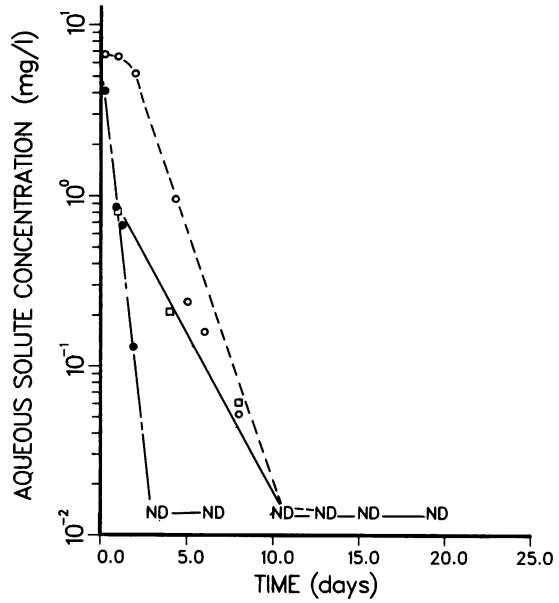

FIG. 1. Microbial degradation of naphthol (●), naphthalene (○), and acenaphthene (□) under aerobic conditions. Initial aqueous naphthol, naphthalene, and acenaphthene concentrations were 9, 7, and 1 mg/liter, respectively. ND, Nondetectable levels.

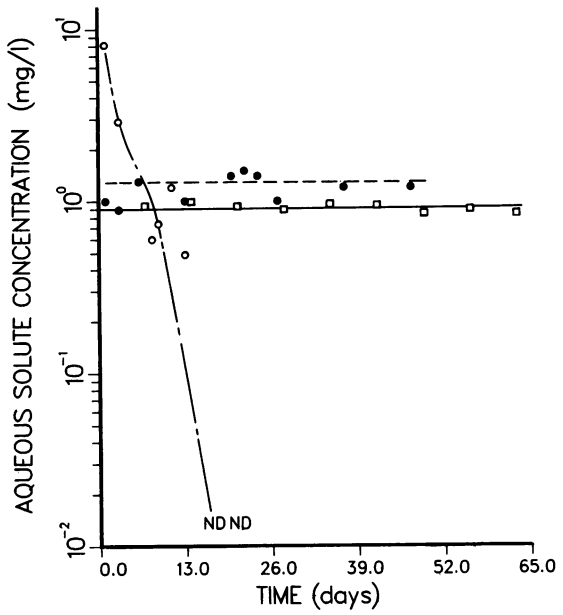

FIG. 2. Microbial degradation of naphthol (○), naphthalene (●), and acenaphthene (□) under anaerobic conditions. Initial aqueous naphthol, naphthalene, and acenaphthene concentrations were 8, 1.3, and 1 mg/liter, respectively. ND, Nondetectable levels.

RESULTS

In the following presentation of data, each sample point represents the average of two to five individual samples. After analysis of PAH compounds, selected samples were monitored for dissolved oxygen and pH. The individual samples were discarded after sampling for PAH compounds because the septum was punctured in the sampling process. Nondetectable levels of a PAH compound refer to an aqueous concentration of less than 0.01 mg of the PAH compound per liter.

Figure 1 shows microbial degradation of naphthol, naphthalene, and acenaphthene under aerobic conditions. The aqueous-phase naphthol concentration decreased from approximately 9 mg/liter to nondetectable levels in 3 days with no observed acclimation period. Naphthalene was degraded from approximately 7 mg/liter to nondetectable levels in 10 days, and acenaphthene degraded from approximately 1 mg/liter to nondetectable levels in 10 days. Naphthalene and acenaphthene showed acclimation periods of approximately 2 days before degradation occurred. Blanks containing no soil and controls which contained sterilized soil showed no significant loss in aqueous-phase PAH concentration through the duration of the tests (data not shown).

Figure 2 shows results of microbial degradation tests with naphthol, naphthalene, and acenaphthene under anaerobic conditions. Naphthol was degraded from an initial aqueous-phase concentration of approximately 9 mg/liter to nonde-

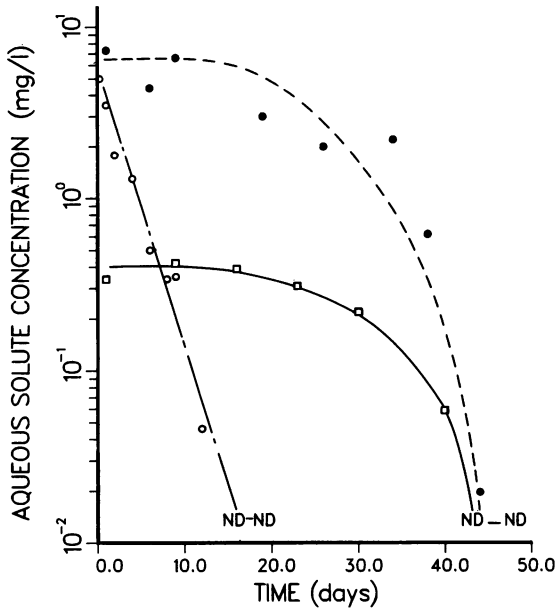

FIG. 3. Microbial degradation of naphthol (○), naphthalene (●), and acenaphthene (□) under denitrification conditions. Initial aqueous naphthol, naphthalene, and acenaphthene concentrations were 8, 7, and 0.4 mg/liter, respectively, and the initial nitrate concentration was 75 mg/liter in all tests. ND, Nondetectable levels.

tectable levels in 15 days. No acclimation period was evident for microbial degradation of naphthol. The aqueous-phase naphthalene concentration did not change significantly over a time span of approximately 50 days; it remained at approximately 1.3 mg/liter for the duration of the experiment. The aqueous-phase acenaphthene concentration did not change from approximately 1 mg/liter over the 70-day duration of the anaerobic experiment. Blanks and controls remained at constant concentration for each of the three sets of anaerobic tests.

Figure 3 shows microbial degradation under denitrification conditions for the solutes naphthol, naphthalene, and acenaphthene. These experiments were performed to assess the microbial degradation of PAH in the presence of nitrate, which may serve as an alternate electron acceptor to oxygen. At neutral pH values, the microbial process of oxidation of organic solute via denitrification is only slightly less favorable energetically than that via aerobic respiration, but considerably more favorable than the anaerobic processes of oxidation of organic solutes via sulfate reduction or methane fermentation (22). Naphthol was degraded from an initial aqueous-phase concentration of 8 mg/liter to nondetectable levels in less than 16 days (Fig. 3). In this test no acclimation period was evident. The aqueous-phase naphthalene concentration decreased from an initial concentration of 7 mg/liter to nondetectable levels in 45 days. In this test an acclimation period of approximately 10 days was observed prior to the occurrence of significant degradation. The initial aqueous-phase concentration of acenaphthene, approximately 0.4 mg/liter, decreased to nondetectable levels in 40 days with an acclimation period of approximately 15 to 20 days. As in the previous tests, the sample blanks and controls remained unchanged through the denitrification experiments. For example, for the test conducted with acenaphthene, blank concentrations were 0.45 and 0.48 mg/liter, whereas control concentrations were 0.39 and 0.32 mg/liter at time zero and 45 days, respectively.

Figure 4 shows the naphthol reaction in the presence of manganese oxide at a pH of 5.9. The abiotic degradation reaction began immediately, without a lag time, and the aqueous-phase naphthol concentration decreased from approximately 9 mg/liter to nondetectable levels in approximately 9 days. Analogous experiments were conducted to assess naphthalene reactivity in the presence of manganese oxide. Naphthalene exhibited no significant degradation reaction with manganese oxide over a period of 9 weeks at a pH value of either 5.0 or 8.0. Sample blanks remained constant for the experiments with either solute.

DISCUSSION

The experimental data gave results on the microbial degradation of naphthol, naphthalene, and acenaphthene under aerobic, anaerobic, and denitrification conditions in natural soil-water systems. Also presented were results on the chemical degradation of naphthol and naphthalene in the presence of a manganese oxide. These results demonstrate that the redox environment is an important factor in assessing the persistence of PAH compounds in soil-water systems.

Reaction with manganese oxide. The chemical processes by which manganese oxides are reduced and solubilized by reaction with aromatic hydrocarbons have been discussed by Stone and Morgan (26, 27). Reactive organic solutes include species which may form surface complexes with the oxide surface. Electron-donating substituents on aromatic solutes, such as -OH on naphthol, increase the reaction rate. The rate of the reaction depends on pH, solute concentration, and the concentration of the oxide suspension, as well as other parameters such as suspension age and the presence of competing sorbates. Data in Fig. 4 can be used to obtain an estimate of a second-order rate constant for degradation of naphthol in the presence of manganese oxide, assuming

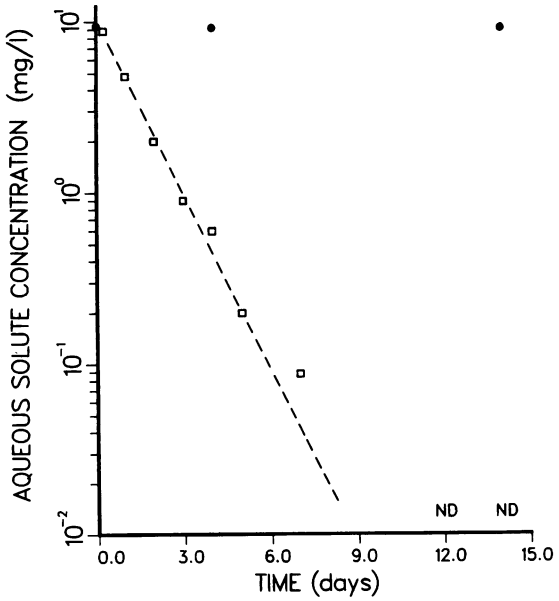

FIG. 4. Chemical degradation of naphthol (□) in the presence of manganese oxide. The initial concentrations of manganese oxide and naphthol were 2×10^{-3} M and 6.25×10^{-5} M, respectively. A sample blank (●) was used for comparison. ND, Nondetectable levels.

first-order dependence with respect to both the solute and the oxide (27). The experiment was performed with a 32-fold molar excess of manganese oxide, assuming a uniequivalent reaction between manganese and naphthol, and thus the manganese oxide concentration can be considered constant in the experiment. These assumptions provide a second-order naphthol reaction rate constant of approximately 3×10^{-3} liters/mol per s for pH 5.9 and other conditions specified earlier. This rate is in the range of that reported by Stone and Morgan (27) for reaction of resorcinol and salicylate with manganese oxide, although it is recognized that the results are not strictly comparable owing to the somewhat different experimental conditions.

The significance of the test results with manganese oxide is that naphthol may reduce and solubilize manganese from manganese oxide-bearing soils and sediments. However, the results of the microbial degradation tests indicate that the fate of naphthol in soil systems is more likely to be dependent on microbial degradation processes, regardless of aerobic, anaerobic, or denitrification conditions, rather than on abiotic degradation reactions with manganese oxide. This is because naturally occurring manganese oxide may exhibit reactivity different from that of the freshly prepared material used in these studies and because manganese is a minor constituent of soils, being present at about 0.1% by weight (20). However, the reactivity with manganese may be important even at low concentrations if manganese can be rapidly recycled between oxidized and reduced phases. Another complicating factor in understanding the role of manganese is the possibility of microbial reduction of manganese (W. C. Ghiorse, *in* J. B. Zehuder, ed., *Environmental Microbiology of Anaerobes*, in press).

Microbial degradation. Acenaphthene, naphthalene, and naphthol were degraded microbially under aerobic conditions to nondetectable levels in 10 days or less. These observations are consistent with the results of several other studies which have reported on the microbial degradation of naphthalene (3, 10, 15, 16) and anthracene (3, 16) in natural soils and sediments.

Naphthol was degraded under all microbial test conditions through aerobic, anaerobic, or denitrification processes. The aqueous naphthol concentration attained nondetectable levels most rapidly under aerobic microbial degradation conditions, with the compound depleted to less than 0.01 mg/liter in 3 days. Although naphthol was the only substituted PAH compound examined in this study, it is evident that the substituent hydroxyl group causes naphthol to be more biologically reactive than the unsubstituted parent compound, naphthalene. The higher reactivity of naphthol than naphthalene under aerobic conditions is expected, because the initial steps in microbial metabolism of naphthalene when catalyzed by microbial oxygenases is believed to entail dihydroxylation followed by cleavage of the aromatic ring (9, 13).

In contrast to naphthol, naphthalene and acenaphthene showed no microbial degradation under anaerobic conditions for test durations of up to 10 weeks. These observations are similar to the results of other studies which have reported the stability of PAH compounds such as naphthalene and anthracene in the absence of oxygen (3, 10). Hence, the data support the current view that anaerobic degradation of unsubstituted PAH compounds by microorganisms at best proceeds at low rates in sediments (2). However, the soil used in this study was obtained from an upper soil horizon and therefore may not be truly representative of the activity of microbial populations from anaerobic soils.

Previous research has shown that the presence of oxygen on the PAH aromatic ring or ring substituent is apparently a basic requirement for cleavage of the aromatic ring under anoxic conditions (2, 4). In this regard, the most significant result of this study is the demonstration that microbial degradation of naphthalene and acenaphthene may occur under denitrification conditions, although this may require acclimation periods of several weeks prior to the onset of microbial degradation. In this study it is important to note the stability of blanks containing no soil and of sterilized controls containing soil, which demonstrated no loss of compound across, or reaction with, the Teflon-lined septum. Furthermore, stable test samples under anaerobic conditions indicate that oxygen intrusion across the Teflon-lined septum was not occurring.

Although anaerobic degradation of oxygen-substituted benzenes, e.g., derivatives of phenol and benzoic acid, and oxygen-substituted PAH compounds has been demonstrated previously, this investigation presents the first evidence of microbial degradation of unsubstituted PAH compounds under denitrification conditions. Likewise, the degradation of *p*-, *m*-, and *o*-xylene by a mixed microbial population under nitrate-reducing conditions has recently been reported (19, 29). These studies demonstrated in an analogous manner that alkyl-substituted benzenes, which also were believed previously to be microbially inert in the absence of molecular oxygen, were degraded under denitrification conditions.

The results of this study and those of Kuhn et al. (19) and Zeyer et al. (29) show the significance of denitrification as a mechanism for microbial degradation of aromatic hydrocarbons containing no oxygen substituents. This work suggests pathways for microbial restoration of anoxic soils, sediments, and groundwater systems contaminated with PAH compounds. The companion paper (21) further examines the microbial degradation of acenaphthene and naphthalene under denitrification conditions, discusses the role of solute sorption-desorption on microbial degradation, and evaluates the roles of nitrate and naturally occurring soil organic carbon on the microbial degradation of PAH compounds.

ACKNOWLEDGMENTS

This investigation was supported by the U.S. Department of Energy, Coal Conversion Project Branch, contract no. DE-AC18-84FC10619. Leland Paulson was the project manager.

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