(4) The acidity of the groundwater is the controlling factor in reducing the solubility of pentachlorophenol and its relative significance as a contaminant in this aquifer.

(5) The occurrence of fatty acids and methane, not components of either creosote or PCP formulations, is further evidence of biological activity in the aquifer.

Registry No. PCP, 87-86-5; indene, 95-13-6; naphthalene, 91-20-3; benzothiophene, 11095-43-5; 2-methylnaphthalene, 91-57-6; 1-methylnaphthalene, 90-12-0; biphenyl, 92-52-4; acenaphthalene, 208-96-8; 1,2-dihydroacenaphthalene, 83-32-9; dibenzofuran, 132-64-9; fluorene, 244-36-0; phenanthrene, 85-01-8; carbazole, 86-74-8; phenol, 108-95-2; 2-methylphenol, 95-48-7; 3-methylphenol, 108-39-4; 4-methylphenol, 106-44-5; 2,6-dimethylphenol, 576-26-1; 2-ethylphenol, 90-00-6; 2,4-dimethylphenol, 105-67-9; 2,5-dimethylphenol, 95-87-4; 3,5-dimethylphenol, 108-68-9; 2,3-dimethylphenol, 526-75-0; 3,4-dimethylphenol, 95-65-8; 2,4,6-trimethylphenol, 527-60-6; 2,3,6-trimethylphenol, 2416-94-6; ethylmethylphenol, 30230-52-5; 2,3,5-trimethylphenol, 697-82-5; 2,3,5,6-tetramethylphenol, 527-35-5; 1-naphthol, 90-15-3; 2-naphthol, 135-19-3; acetic acid, 64-19-7; formic acid, 64-18-6; propionic acid, 79-09-4; 2-methylpropionic acid, 79-31-2; butanoic acid, 107-92-6; 3-methylbutanoic acid, 503-74-2; pentanoic acid, 109-52-4; benzoic acid, 65-85-0; 2(1*H*)-quinolinone, 59-31-4; methane, 74-82-8.

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Microbial Transformations of Substituted Benzenes during Infiltration of River Water to Groundwater: Laboratory Column Studies

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■ The microbial transformations of dimethyl- and dichlorobenzenes have been studied in laboratory aquifer columns simulating saturated-flow conditions typical for a river water/groundwater infiltration system. The behavior of all compounds in the laboratory was qualitatively the same as that observed at a field site. Both dimethyl- and dichlorobenzenes were biotransformed under aerobic conditions, the dimethylbenzenes faster than the chlorinated analogues. Evidence is presented that dimethylbenzenes are degraded by denitrifying bacteria under anaerobic conditions. Dichlorobenzenes were not transformed under these conditions. Significant differences in the rates of transformation between isomers were found. The dimethylbenzenes and *p*-dichlorobenzene appeared to be utilized by bacteria as sole carbon and energy sources. Transfer of rate constants determined in the laboratory at elevated substrate concentrations to the field was difficult.

Of the various processes that govern the subsurface movement of xenobiotic organic chemicals, microbially mediated reactions are possibly the most complex and certainly the least investigated. To date, little progress has been made in the study of the microbial metabolism of trace organic pollutants under natural conditions in groundwater systems. "Natural conditions" infers relatively constant but low concentrations of organic compounds (nM to μM), low temperatures, spatial and/or temporal variations in both redox conditions, and flow-through porous media.

One experimental approach which has been recently applied to groundwater systems is the biofilm or fixed-film kinetic approach (*1-3*). Using fixed-film reactors, Bouwer and McCarty (*1-3*) have demonstrated that a number of chlorinated benzenes and halogenated aliphatic compounds are degradable as secondary substrates under appropriate redox conditions. In an attempt to bring laboratory investigations closer to conditions in natural groundwater systems, Wilson et al. (*4, 5*) have obtained aquifer material from uncontaminated cores and looked for transformation of a variety of xenobiotic contaminants commonly found in groundwater. Due to novel techniques developed by Ghiorse and Balkwill (*6*) and White et al. (*7-10*), some quantitative information about microbial life in the subsurface is now available. By use of both direct microscopic methods (*6*) and biochemical indicators of biomass (*11*), a previously unknown population of subsurface-dwelling bacteria has been found, even at sites believed free of organic substrates (*4*). The results of these studies indicate that bacteria indigenous to the subsurface are extremely small (*6*), generally oligotrophic (*5*), capable of degrading some pollutants commonly detected in groundwaters (*4*), and that they tend to attach to surfaces (*12*).

Our research on the transport and fate of xenobiotic organic compounds in the subsurface has been focusing mainly on natural river water/groundwater infiltration

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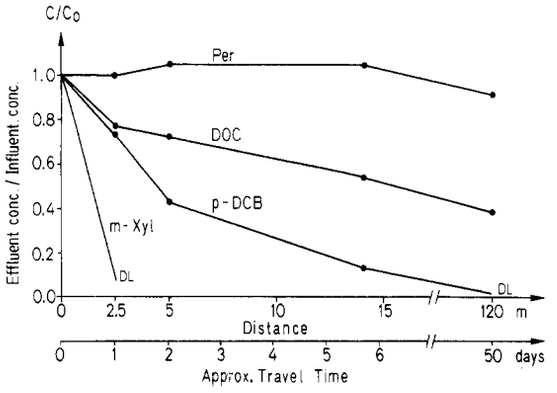

Figure 1. Average concentration profiles of selected organic compounds in the River Glatt infiltration zone under mean flow conditions. Average concentrations in River water of [Per] = 0.005 μM, [*m*-Xyl] = 0.002 μM, [*p*-DCB] = 0.002 μM, and dissolved organic carbon [DOC] = 4 mg C/L. DL = detection limit.

systems. In previous papers, we have presented the results of laboratory studies conducted to investigate the sorption behavior of neutral (*13*) and ionizable (*14*) hydrophobic organic compounds in aquifers, and we have reported on a field study (*15*, *16*) in which we demonstrated the behavior of such compounds during infiltration of river water to groundwater. These field measurements revealed that, during infiltration of river water to groundwater, certain trace organic contaminants (e.g., alkylated benzenes) were always rapidly removed, although they are only weakly sorbed to the aquifer material. Other compounds (e.g., 1,4-dichlorobenzene) were eliminated at much slower rates and only in the presence of oxygen. Some compounds (e.g., 1,1,1-trichloroethane and tri- and tetrachloroethylene) were never removed under any conditions (see examples given in Figure 1). The observed eliminations were postulated to be mediated by biological processes, and laboratory column studies were initiated to confirm this hypothesis and to further investigate the microbial transformation of selected model compounds under conditions typical for the infiltration site. To this end, two columns were constructed, filled with aquifer material taken from the interface of a well-characterized river/groundwater infiltration site (*16*), and continuously operated under saturated-flow conditions. The columns were fitted with a series of sampling ports, thus allowing analyses to be made over the length of the column. In this paper, we report the results of studies in which tetrachloroethylene and several alkylated and chlorinated benzenes were continuously provided as potential substrates for microorganisms from the infiltration site and compare these results with those obtained from the field investigation.

Experimental Section

Column Design. Experiments were conducted with two different columns. Column 1 (20 cm × 4 cm i.d.) was a preliminary design and was made of borosilicate glass. Three sampling ports, positioned at distances of 1.5, 4.7, and 9.1 cm from the inlet, were made from stainless steel tubes projecting about 2 cm into the center of the column. Fine mesh nylon screens were placed in the ends of the tubes to prevent clogging with sediment. The inlet of the column consisted of a glass-fritted filter and a 0.45-μm membrane filter (Nuclepore Corp., Pleasanton, CA, USA), the latter added to prevent microbial contamination in the inlet tubing. Column 2 (100 cm × 5.0 cm i.d.) was constructed of transparent Plexiglas, a material that was found not to adsorb hydrophobic organic compounds; i.e., no detectable sorption could be measured in a series of batch experiments (unpublished data). The sampling ports were Plexiglas stoppers fitted with stainless steel capillary tubes with headpieces of sintered stainless steel positioned in the center of the column. The first port was located at a distance of 2.7 cm from the column inlet with 19 additional ports placed at intervals of 5 cm. Small channels in the Plexiglas stoppers leading to the sampling tubes served as flow-through conductivity cells, which made it possible to measure conductivity during conservative tracer experiments.

Preparation and Characterization of Column Sediments. Column 1 was filled with aquifer material from the interface of a river/groundwater infiltration site (*15*, *16*). Column 1 served primarily to test methodological aspects and to evaluate the feasibility of the column approach to investigating microbial transformations of trace organic pollutants. Therefore, in order to ensure microbial activity, this column was inoculated with xylene-degrading bacteria (see below). Column 2 was packed with material from the same site but was not inoculated. For column 1, the sediment was wet sieved to pass a mesh of 63–125 μm and wet packed into the column. Column 2 sediment was air-dried and then dry sieved to pass a mesh of 63–125 μm and wet packed into the column.

BET surface areas of the sieved sediments were 1.8–1.9 m^2/g and were determined on a Carlo Erba Model 1800 Sorptomatic (Carlo Erba, Milan, Italy) using nitrogen as the adsorbing gas (0.16 nm^2/N_2). The samples were outpassed for 48 h at 50° and at 0.005 ± 0.002 torr. Adsorption and desorption isotherms were determined at 77 K. The measured pore volumes were always smaller than 0.02 cm^3/g, indicating that the sediment particles were nonporous. The porosity of the packed columns was determined with NaCl and tritiated water (^3H_2O) as conservative tracers in step-function input experiments in which conductivity and radioactivity were measured. The porosities were ~0.50 for column 1 and ~0.40 for column 2. The differences in porosities were probably due to the different packing techniques used.

Determination of the particle size distribution of the sediment material in the columns was made by visual accumulation analysis and was performed by the Institut für Grundbau und Bodenmechanik, Swiss Federal Institute of Technology, Zürich. The dry-sieved 63–125-μm fraction also contained 2 wt % clay (<2-μm diameter), probably caused by adhesion of smaller clay particles on sand grains during sieving.

The organic carbon (OC) content of the sediment materials was determined by the method of Baccini et al. (*17*). The detection limit of this technique was 0.1 mg of OC/g for sediment samples. Column 1 sediment contained approximately 2 mg of OC/g and column 2 contained 5 mg of OC/g.

Xylene-Enrichment Cultures. The composition of enrichment culture medium for xylene-degrading bacteria was as follows: 0.1 g of $MgSO_4 \cdot 6H_2O$, 0.02 g of $CaCl_2 \cdot H_2O$, 0.3 g of NH_4Cl, 1.0 g of $NaNO_3$, 40 mL of 0.2 M Na_2HPO_4, 27 mL of 0.2 M KH_2PO_4, and 0.5 mL of trace element solution (*18*), added to double-distilled water for a total volume of 1 L. One hundred milliliters of medium was placed into each of three 500-mL serum bottles and autoclaved at 121 °C for 15 min. Each of the three xylenes was individually added to a bottle of sterile medium. The substrate was added aseptically (Acrodisc filter, Gelman Corp., Ann Arbor, MI) with a 1-mL syringe through the septum of the bottle to give a final "concentration" of 5 g of xylene/L. (Note, the solubility of xylenes in water at

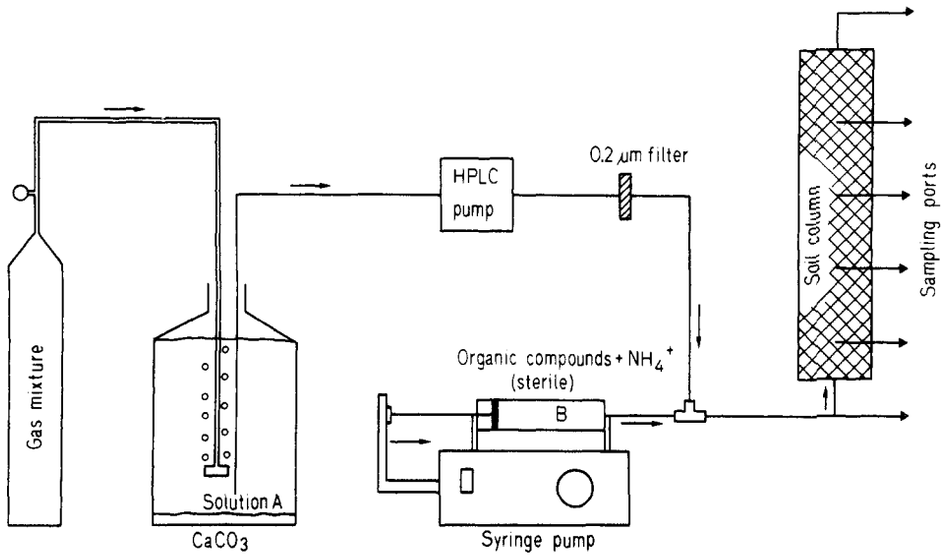

Figure 2. Experimental setup of the saturated-upflow sediment columns. (A) Reservoir containing mineral salts solution. (B) Syringe containing concentrated organic compounds and ammonia.

20–25 °C is less than 200 mg/L.) Each serum bottle was then inoculated with 5 mL of activated sludge and incubated in the dark at 25 °C on a shaker. After four successive transfers, 10 mL from each of the three enrichment cultures was mixed with the sediment before it was wet packed into the column.

Chemicals and Solutions. *p*-Dichlorobenzene (*p*-DCB), *m*-dichlorobenzene (*m*-DCB), *o*-dichlorobenzene (*o*-DCB), tetrachloroethylene (Per), *p*-xylene, (*p*-dimethylbenzene, *p*-Xyl), *m*-xylene (*m*-Xyl), and *o*-xylene (*o*-Xyl) were obtained from Fluka (Buchs, Switzerland) and had chemical purities of at least 99%. All other chemicals used were analytical grade and purchased from Merck (Darmstadt, West Germany).

The trace organic compound solutions were prepared in volumetric flasks with ground glass stoppers. Maximum concentrations of 15 mg/L for the DCB isomers, 12 mg/L for the xylenes, and 30 mg/L for Per were dissolved in organic-free, double-distilled water by stirring overnight. These solutions could be stored at 4 °C for up to 2 months in a flask without significant losses. Storage of solutions in 50-mL glass syringes was possible for up to 10 days.

It was desirable to use water with a pH and major ion composition similar to that of the infiltrating river water at the field site (*15*, *16*). Therefore, a synthetic river water for the aerobic experiments and a synthetic groundwater for the anaerobic experiments were prepared according to the following procedure. Double-distilled water (DOC < 0.2 mg of C/L) with an excess of granulated marble (Merck, Darmstadt, W. Germany) was stirred and bubbled with either air (0.03% CO_2) for aerobic experiments (resulting in a pH typical for the river water) or with N_2 gas containing 0.5% CO_2 for anoxic experiments (yielding a pH typical for the infiltrate after a few meters of infiltration) until the solution composition was stable. Equilibrium was obtained after about 1 day as indicated by a constant pH of 8.3 ± 0.1 or 7.35 ± 0.1, respectively. These solutions were used in all column experiments and are referred to as $CaCO_3/CO_2$ water. The synthetic solutions also contained nutrient concentrations similar to those in the Glatt River: 0.68 mM Na^+, 0.13 mM K^+, 0.62 mM Mg^{2+}, 0.26 mM NO_3^-, 0.03 mM HPO_4^{2-}, 0.21 mM SO_4^{2-}, and 1.34 mM Cl^-.

Experimental Apparatus. Figure 2 shows the experimental setup for these studies. The $CaCO_3/CO_2$ waters were prepared in the reservoir labeled A. Before each experiment, the columns were rinsed with clean $CaCO_3/CO_2$ water until only background concentrations of the compounds of interest were detected in the effluent. An high-performance liquid chromatography (HPLC) pump (Kontron, Rotkreuz, Switzerland) was used to attain low flow rates in the columns. The velocity of liquid flowing through the packed columns was, if not otherwise stated, 4.0 cm/h in column 1 and, due to the different geometry, 7.6 cm/h in column 2, rates that are typical for the flow velocities found in the Glatt River infiltration zone. A syringe pump (Braun Perfusor, Melsungen, West Germany) was used to deliver an accurate dosage of organic chemicals and ammonium into a stainless steel T-joint connnection to the $CaCO_3/CO_2$ water at the base of the columns. The syringe (B) was aseptically filled as follows: the autoclaved NH_4Cl solution (0.7 mg of NH_4^+–N/L) was mixed with a 0.2-µm filter-sterilized solution containing the volatile organic compounds. This mixture was then taken up in an autoclaved, 50-mL glass syringe. For anaerobic experiments, the NH_4Cl solution was autoclaved, oxygen was removed by stripping with 0.5% CO_2–99.5% N_2, and the organic-containing solutions were added and stirred for 24 h. The columns were operated in the saturated, upflow mode. In order to prevent losses of the volatile organic compounds due to either volatilization or adsorption, the systems were constructed entirely of glass, Plexiglas, and stainless steel.

All experiments were conducted as step-input experiments in which the concentrations of organic compounds were kept constant for a specified period of time. The concentration ranges of the various compounds used (Per, xylene isomers, and DCB isomers) were 0.2–0.8 µM for aerobic experiments and 0.4–7.0 µM for anaerobic experiments. The columns were operated in a thermostatically controlled room at 20 ± 0.5 °C in the dark. A temperature of 20 °C was chosen since it represents the water temperature at the field site during the summer months during which anoxic conditions were observed in the groundwater in the near field of the river (*15*).

Analytical Methods. Influent, port, and effluent samples were taken with a peristaltic pump at controlled flow rates of 0.1 mL/min. The sample was collected in a stainless steel tube which was inserted between the port and the pump. Thus, depending on the length of the tubing, typically 3–6 mL of liquid was obtained and flushed into a preweighed, 120-mL gas washing flask containing

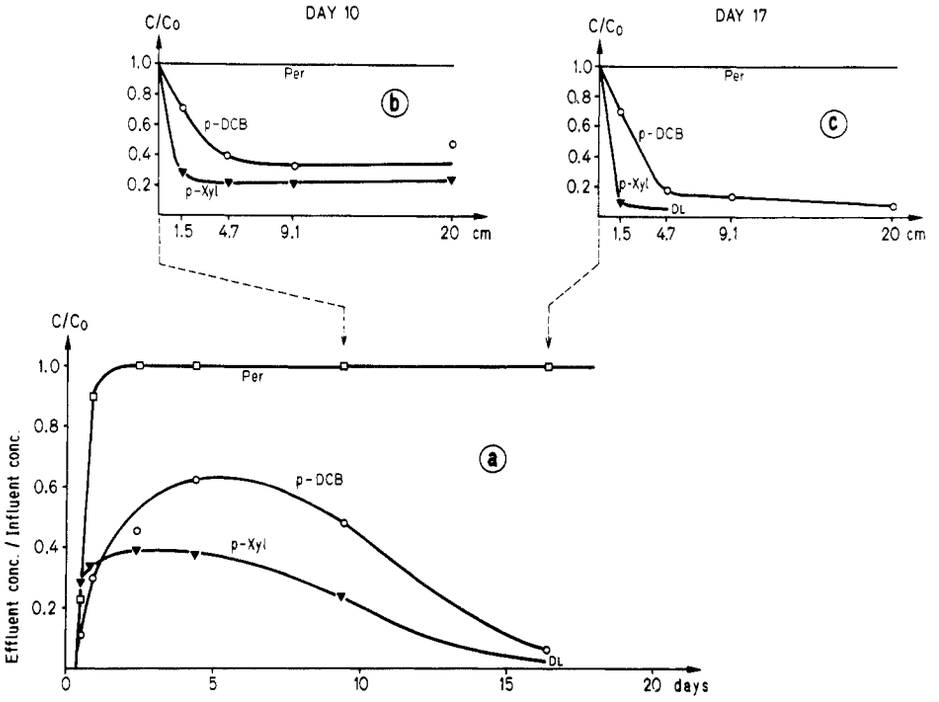

Figure 3. (a) Typical transformation behavior of *p*-Xyl, *p*-DCB, and Per in the sediment columns following initial breakthrough. Data are plotted as the ratios of the effluent (C_{20cm}) to input (C_0) concentrations vs. time in days. DL = detection limit. (b) Concentration profiles of *p*-Xyl, *p*-DCB, and Per over length of the column (column 1) determined after 10 days of continuous operation. (c) Concentration profiles determined after 17 days of operation. Influence concentrations of [*p*-Xyl] = 0.5 μM, [*p*-DCB] = 0.2 μM, and [Per] = 0.5 μM.

50-mL of organic compound free, double-distilled water. The exact volume of sample obtained was determined by weighing. The sample was then spiked with an internal standard (1-chlorohexane) and concentrated immediately by the closed-loop stripping technique of Grob and Zürcher (*19*). Because Per was not transformed under any conditions, it served as an internal standard for the whole procedure (i.e. sampling, concentration, and analysis). Analyses were made by high-performance capillary gas chromatography. Including errors due to sample collection, a relative standard deviation of ±10% is obtained with this method.

Ammonia was determined by a modification of the method as described in ref 20. Nitrite and nitrate were determined by the method described in ref 21. Dissolved oxygen was routinely monitored in a flow-through microcuvette with an oxygen electrode (Type IL 213, Dr. W. Ingold AG, Zürich, Switzerland). For aerobic experiments, the influent solution was saturated with oxygen (8.35 mg of O_2/L at 20 °C); the effluent concentration was always greater than 3.3 mg of O_2/L.

Results and Discussion

Column Studies under Aerobic Conditions. Figure 3 illustrates the typical breakthrough behavior of three of the compounds used in this study following their initial input to column 1. The ordinate on each graph Figure 3a–c is the ratio of the effluent concentration (C) to the input concentration (C_0) for each compound, plotted vs. time (days) in Figure 3a and vs. column length (cm) in Figure 3b,c. It should be noted that although the initial rates of transformation differed somewhat, very similar results were obtained from both the inoculated (column 1) and noninoculated column (column 2). After breakthrough, Per was always recovered at input levels ($C = C_0$), suggesting that under aerobic conditions it was not affected by any removal mechanism. In 9 months of continuous operation of this column (column 1) and of column 2, which was run under aerobic conditions followed by 9 months under denitrifying conditions, the output concentrations of Per never decreased. Results of other studies (*22*, *23*) also suggest that Per is resistant to microbial degradation under these conditions.

Parts b and c of Figure 3 are concentration profiles of the model compounds over the length of the column on two sampling dates. The disappearance of all xylene isomers (e.g., *p*-Xyl) was typically rapid, with 70% removal within the first 1.5 cm of the column after 10 days of operation (see Figure 3b). After 17 days (Figure 3c), the rate of removal increased slightly so that all xylenes were no longer detectable (detection limit = 0.01–0.02 μM) at the second sampling port at 4.7 cm. Interestingly, with increasing removal of *p*-DCB (see below), the rate of *o*-Xyl elimination was observed to decrease with time relative to removal of the para and meta isomers of xylene (see profiles in Figure 4b). The degradation of *p*-, *m*-, and *o*-xylenes under aerobic conditions has been previously described (*24–27*).

As is evident from Figure 3a, *p*-DCB elimination in the column was initially less rapid than that of the xylenes. Likewise, as seen in Figure 3b, the total percentage removal over the length of the column was also lower, with almost 50% of the original *p*-DCB recovered in the effluent after 10 days of operation. However, as shown in Figure 3c, the percentage of *p*-DCB removal and the rate of its transformation gradually increased with time, so that after almost 3 weeks, the overall *p*-DCB level in the effluent was reduced to less than 10% of the input value. Most of the *p*-DCB removal (~80%) occurred within the first 4.7 cm of the column.

Bouwer and McCarty (*1–3*) also observed *p*-DCB elimination in their biofilm reactors. They suggested that the removal mechanism was a secondary metabolism, rather than use of *p*-DCB as a sole carbon and energy source by bacteria. In a later experiment conducted in this study, in which *p*-DCB and Per were the only added sources of organic carbon in column 1, the rate of *p*-DCB elimination

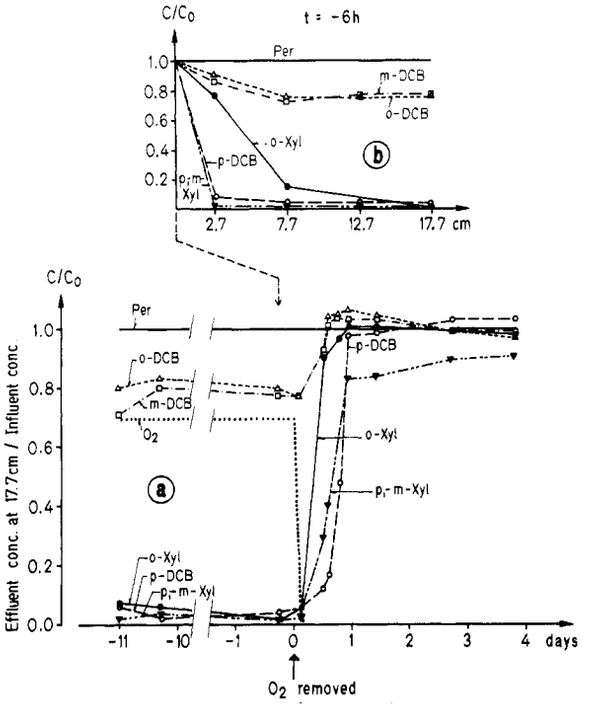

Figure 4. (a) Transitional transformation behavior observed in column 2 (after 17.7 cm) upon removal of oxygen (denitrifying conditions). (b) Concentration profiles of *p*, *m*- and *o*-Xyl *p*-, *m*-, and *o*-DCBs, and Per 6 h before oxygen removal. Influent concentrations of [*p*-Xyl] = [*m*-Xyl] = [*o*-Xyl] = 0.5 μM, [*p*-DCB] = 0.4 μM, [*m*-DCB] = [*o*-DCB] = 0.5 μM, and [Per] = 0.8 μM.

continued to increase with time. Additional studies in our laboratory confirm that *p*-DCB may be used as a sole substrate by bacteria during batch culture growth (unpublished data). Thus, the continuous supply of *p*-DCB at a concentration of 0.2 μM seemed to support growth and/or enhance the activity of *p*-DCB-degrading organisms. Transformation of *o*- and *m*-DCBs under aerobic conditions in both columns, however, never exceeded 25% of the input concentration (see, e.g., Figure 4) and occurred only in that region of the column where *p*-DCB and the xylenes were transformed.

In summary, the increases in removal rates both of *p*- and *m*-Xyl and of *p*-DCB with time indicate that the bacterial population in the columns and/or their activity increased over time; that is, the organisms were growing and/or had acclimated to the xenobiotic compounds provided as substrates.

Enumeration of Bacteria in Column 1. The number of organisms present in column 1 after 9 months of operation was estimated by the method of Ghiorse and Balkwill (*6*). The numbers obtained (2.0×10^7 bacteria/g dry weight) corresponded well with values reported for groundwater aquifer sediments (*5*, *6*, *11*) and were evenly distributed with depth in the column. Most of the cells appeared to be associated, perhaps attached, to particles of the silt–clay size fraction. However, since column 1 was originally inoculated with xylene-degrading bacteria, it was not possible to determine what percentage of the observed organisms were indigenous to the infiltration site. Likewise, it was not possible to assess what percentage of the microbial population transformed the xenobiotic compounds. However, as stated above, the transformation behavior of all compounds in the uninoculated column was very similar to that in the inoculated column.

Column Studies under Anoxic, Denitrifying Conditions. Figure 4a shows the response in column 2 upon removal of oxygen from salt solution A. A 99.5% N_2–0.5% CO_2 gas mixture was substituted for equilibration with solution A, but nitrate (NO_3^-) was still provided and now available as an alternative electron acceptor in the inflowing medium. For a few hours after oxygen removal, *p*-DCB and *p*- and *m*-xylene continued to be eliminated, due perhaps to traces of oxygen trapped as microscopic gas bubbles within the pores of the sediment. A few days after removal of oxygen then, all previously observed biotransformations ceased. No further removal of Per, the xylenes, and DCB isomers was observed over the next 90 days.

After 3 months of feeding *p*-DCB (0.4 μM), Per (0.8 μM), and the three xylenes (0.5 μM each) in the absence of oxygen, some removal of *p*- and *m*-Xyl but not *o*-Xyl was observed (see Figure 5). To exclude the possibility that traces of oxygen could have been reponsible for the metabolism of these compounds and to allow a balance of electron acceptor and donors, the concentrations of all organic compounds in the feed solution were increased by a factor of 8.8. Two weeks later, after a short transition period, about 70% of the *p*- and *m*-xylene was eliminated.

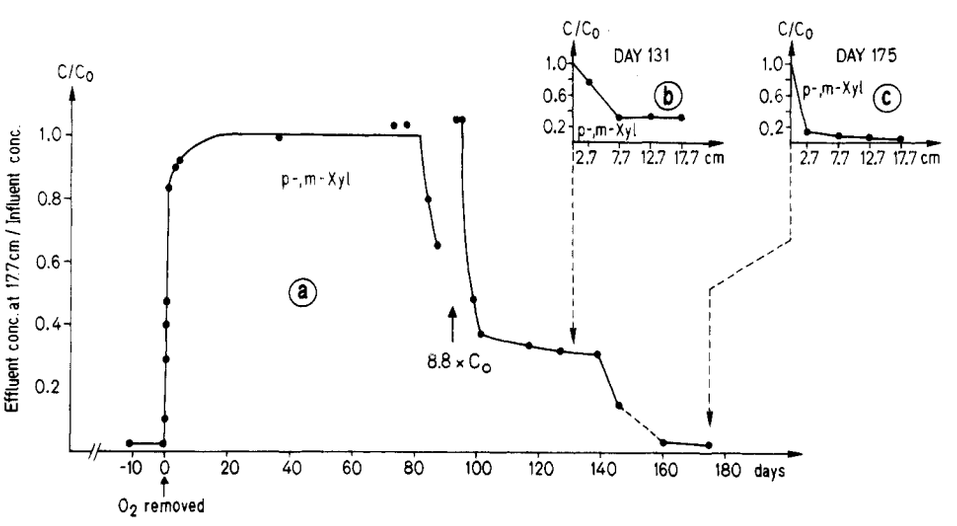

Figure 5. Disappearance of *p*- and *m*-Xyl in column 2 under denitrifying conditions. Input concentrations of *p*- and *m*-Xyl were increased by a factor of 8.8 on day 92 (8.8 C_0). (b) Profile of *p*- and *m*-Xyl concentrations 131 days after oxygen removal and 7 weeks after *p*- and *m*-Xyl transformation under denitrifying conditions first observed in the column. (c) Profile of *p*- and *m*-Xyl 175 days after oxygen removal and about 13 weeks after *p*- and *m*-Xyl elimination under denitrifying conditions was observed. Influent concentrations (C_0) are identical with those presented in the legend of Figure 4.

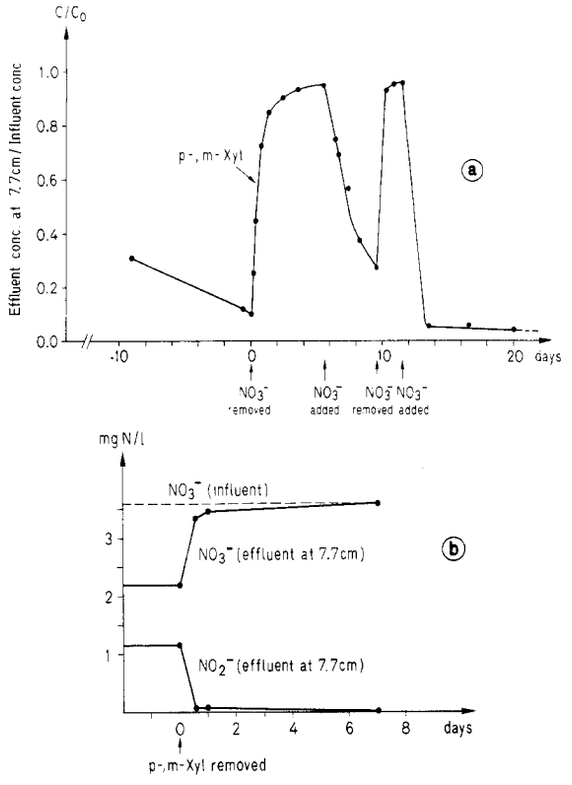

Figure 6. (a) Sequential response of *p*- and *m*-Xyl to removal (and addition) of nitrate. (b) Nitrate (NO_3^-) and nitrite (NO_2^-) concentrations 7.7 cm from the influent following removal of *p*- and *m*-Xyl from the inflowing medium. Influent concentration of [*p*-Xyl] = [*m*-Xyl] = 4.5 μM.

During 40 days, the profile was stable (Figure 5), and degradation occurred in the first 7.7 cm. After a second transition, nearly all of the *p*- and *m*-Xyl was removed, and activity was also observed after the sampling port at 7.7 cm (Figure 5).

As may be seen in Figure 6, the degradation of *p*- and *m*-Xyl in column 2 was clearly mediated by denitrifying bacteria. Removal of nitrate from the feed solution resulted in a cessation of *p*- and *m*-Xyl degradation. Subsequent additions of nitrate resulted in resumed transformation of the compounds (Figure 6a). When *p*- and *m*-Xyl, in turn, were removed from the feed solution, nitrate was not eliminated and nitrite was no longer formed (see Figure 6b). Addition of ^{14}C-uniformly labeled *m*-Xyl to column 2 resulted in recovery of 48% of the label as $^{14}CO_2$ in the effluent. Another 27% of the label was present in the particulate organic carbon, probably incorporated in the attached biomass, with the residual label possibly bound in the sediment as solid $CaCO_3$ (data not shown).

It is interesting to point out that between days 100 and 140, the nitrate was more or less stoichiometrically reduced to nitrite in the region of xylene degradation, i.e., the nitrite concentration in the effluent at 7.7 cm corresponded to the decline in nitrate between the influent and this port. On the basis of this nitrogen balance, only after day 140 were significant amounts of (nonidentified) gaseous denitrification products produced, accompanied by a concomitant increase in the rates of *p*- and *m*-xylene transformation (Figure 5). The observed delay, i.e., a lag in synthesis of the necessary enzymes responsible for the production of gaseous products from nitrate, is not uncommon among denitrifiers (*28*, *29*). Furthermore, many microorganisms possess nitrate reductase as a constitutive enzyme (*30*) but lack any other reductases involved in the various steps of

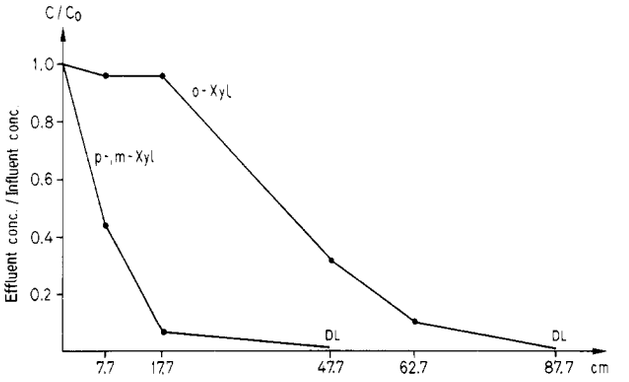

Figure 7. Concentration profiles of *p*-, *m*-, and *o*-Xyl under denitrifying conditions over the length of column 2. DL = detection limit. Influent concentration of [*p*-Xyl] = [*m*-Xyl] = [*o*-Xyl] = 4.5 μM.

denitrification (i.e., nitrite, nitric oxide, and nitrous oxide reductase).

Six months after the removal of oxygen from column 2, *o*-Xyl was also transformed under denitrifying conditions, but only after the *p*- and *m*-Xyl had been temporarily removed from the feed solution. The reason for this behavior is unclear since when the *p*- and *m*-Xyl were later added to the column, *o*-Xyl disappearance continued. However, *o*-Xyl transformation occurred after the zone where most of the *p* and *m*-Xyl had already been removed (Figure 7). In addition, as already observed under oxic conditions (see above), the rate of *o*-Xyl transformation was significantly slower than that of the two other isomers.

The finding that aromatic hydrocarbons such as the xylenes are degraded under denitrifying conditions is the most significant result of this study. Such compounds were previously believed to be inert in the absence of molecular oxygen (*31*), although anaerobic catabolism of aromatic compounds with substituents containing oxygen (e.g., benzoate, phenol, phthalic acid, aminobenzoate) is well established (*32–35*). Thus, it is the initial step(s) in the anaerobic transformation of aromatic hydrocarbons with no such functional groups that is (are) of particular interest. Further work to investigate this question is in progress.

Comparison of Laboratory and Field Results. The column experiments conducted in this study were aimed primarily at confirming observations made in the field, to evaluate the likelihood of biotransformations of the various xenobiotic model compounds (i.e., their potential to serve as carbon and energy source) under oxic and anoxic, denitrifying conditions, and to investigate the influence of minor structural dissimilarities (i.e., isomerism) on the rate of transformation. To answer these questions, the input concentrations used, although still representative for contaminated groundwaters, were much higher (i.e., a factor of ~100) than the concentrations typically detected in the river water at the natural infiltration site (Figure 1). A quantitative comparison between transformation rates observed in the field with those obtained in the laboratory is, therefore, very difficult, particularly since microbial activity in the columns was observed to increase substantially during the experiments. Due to the experimental boundary conditions, a microbial community different from that at the field site was probably enriched in the columns. Despite these difficulties, some valid conclusions can be drawn from a comparison of the laboratory and field results. All compounds investigated showed qualitatively the same behavior in the column as at the field site. Compounds that were rapidly eliminated during natural infiltration were readily removed in the

columns, whereas compounds that were found more persisent in the field showed slower transformation rates in the laboratory. Furthermore, when a simple standard advective dispersion transport model was applied, which included both sorption and a first-order kinetic term for degradation to the initial profiles for DCB (after a few days) in both columns, rate constants of the order of 0.5–1 day^{-1} were obtained. These rate constants are in the same order of magnitude as those derived from DCB profiles measured at the field site ($\sim$0.4 day^{-1}) during the warmer period of the year at times when no anoxic conditions prevailed. Thus, even when elevated pollutant concentrations are applied, initial transformation rates obtained in laboratory columns containing aquifer materials from a given field site might give reasonable "order of magnitude" estimates of actual field rates at that site, provided that the "right" physical and chemical environment (i.e., redox conditions, temperature, flow rate, pH, major ion composition, etc.) is maintained in the column. However, the results of this study clearly indicate that the concentration of the xenobiotic compound of interest as well as the concentrations of other xenobiotic compounds present are important variables that have to be carefully investigated when rate constants determined in laboratory systems are to be applied to the field.

Summary and Conclusions

A laboratory column system was developed to simulate the transport and transformation of trace organic compounds under saturated groundwater flow conditions. The system was used to study the microbial transformations of dimethyl- and dichlorobenzenes under conditions typical for a river water/groundwater infiltration zone. The most important conclusions of this study are as follows:

(1) Under both aerobic and denitrifying conditions, the behavior of all compounds investigated was qualitatively the same as that observed in the field. Xylenes (dimethylbenzenes) were microbially transformed under both aerobic and anaerobic, denitrifying conditions, dichlorobenzenes were transformed only under aerobic conditions, and tetrachloroethylene was not transformed in either case.

(2) Our results suggest that all three xylene isomers may be used by bacteria as sole carbon and energy sources under aerobic and anaerobic, denitrifying conditions. This is the first clear evidence that alkyl-substituted benzenes are microbially transformed under anoxic conditions. *p*-Dichlorobenzene (*p*-DCB) possibly also served as a sole carbon and energy source for bacteria under aerobic conditions.

(3) Significant differences were found in the rates of transformation between isomers. Under both aerobic and anaerobic conditions, *p*- and *m*-xylenes were degraded at equal rates but significantly faster than the ortho isomer of xylene. The transformation rate of *p*-DCB was much faster than the rates of disappearance of the meta and ortho isomers under aerobic conditions.

(4) The laboratory experiments allowed investigation of the temporal and spatial distribution of trace organic compounds during microbial growth and transformation. As expected, however, the difficulty in transferring rate constants from laboratory column studies in the field was evident. While the pseudo-first-order rate constant for *p*-DCB transformation was initially small (0.5–1 day^{-1}), it increased during the experiment from 20 day^{-1} at 10 days to 110 day^{-1} at 39 days. On the basis of the substantial increase in substrate utilization observed during the experiment, microbial biomass and/or activity in the column must have increased. Therefore, since microbial growth on some xenobiotic compounds as sole carbon and energy sources is possible, even at moderate concentrations, it is suggested that input concentrations to laboratory columns be very similar to those found in the field, particularly if one expects to apply the laboratory-derived rate constants to field conditions.

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Registry No. *p*-DCB, 106-46-7; *m*-DCB, 541-73-1; *o*-DCB, 95-50-1; Per, 127-18-4; *p*-xyl, 106-42-3; *m*-xyl, 108-38-3; *o*-xyl, 95-47-6.

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Reactions of *o*-Cresol and Nitrocresol with NO_x in Sunlight and with Ozone–Nitrogen Dioxide Mixtures in the Dark

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■ Studies relevant to the atmospheric chemistry of *o*-cresol were carried out using FEP Teflon outdoor chambers. These studies included sunlight irradiations of *o*-cresol–NO and nitrocresol–NO mixtures in purified air, reactions of *o*-cresol and of nitrocresol with O_3–NO_2 mixtures in the dark, and photolysis as well as NO_x photooxidation of the dicarbonyls pyruvic acid (CH_3COCOR, R = OH) and biacetyl (R = CH_3). Products identified included gas-phase nitrocresols in both cresol–NO–sunlight and cresol–O_3–NO_2–dark experiments and dinitrocresol as an aerosol product of nitrocresol–NO_x reactions. Yields of gas-phase and aerosol products, including gas–aerosol partition of nitrocresol isomers, are presented and are discussed in terms of reactions with NO_3 (dark) and with OH (sunlight). Photolysis rates for pyruvic acid and biacetyl were determined, and the corresponding product yields were measured.

Introduction

Owing to their abundance in urban air (*1*, *2*), aromatic hydrocarbons continue to receive substantial attention as major precursors of ozone and organic aerosol formation. Several critical aspects of the atmospheric transformations of aromatic hydrocarbons are still poorly understood. Reactions of their polar *aromatic* products (cresols, nitrocresols) have received limited attention (*3*). The nature, yields, and subsequent reactions of the *aliphatic* dicarbonyls produced following opening of the aromatic ring are, for both aromatic hydrocarbons and their polar aromatic products, the object of considerable uncertainty (*4*).

In a recent study (*3*), we have carried out sunlight irradiations of *o*-cresol–NO_x mixtures in air and obtained some information on the nature of gas-phase and aerosol products. We present here additional results relevant to specific aspects of the atmospheric chemistry of *o*-cresol. Because *o*-cresol may react with both OH and NO_3 radicals, we attempted to separate the two chemistry regimes and carried out experiments with cresol–NO_2–O_3 mixtures in the dark (NO_3–N_2O_5 chemistry) and cresol–NO in sunlight (mostly OH chemistry). Nitrocresols were formed in both cases, and their gas/aerosol-phase partition was investigated. Subsequent reactions of nitrocresols were also studied in the dark (O_3–NO_2 mixtures) and in sunlight (NO_x photooxidation). Experiments were also carried out with the dicarbonyl pyruvic acid (CH_3COCOR, R = OH), a product of *o*-cresol photooxidation (*3*, *5*), and with its structural homologue biacetyl (R = CH_3).

Experimental Methods

Experiments were carried out in outdoor chambers constructed from FEP 200A Teflon film. Experimental protocols and measurement methods have been described before (*3*, *5–10*) and are only listed here in summary form (Table I). New methods and protocols are described below.

Gas-Phase Measurements. The ultraviolet photometer (Dasibi 1003 AH) we employed in previous studies of organic–NO_x reactions responds to polar aromatics such as cresols and nitrocresols in purified, ozone-free air (*11*). In this work, we employed an ethylene chemiluminescence instrument (McMillan 1100-2) that is not subject to these interferences. The instrument was calibrated on test ozone atmospheres against a "transfer standard" UV photometer (Dasibi 1008 PC), which in turn, was periodically checked against the UV photometer maintained by the California Air Resources Board at their El Monte, CA, laboratory.

Biacetyl was measured by electron capture gas chromatography using conditions identical with those previously described for peroxyacetyl nitrate (PAN) (*8*). The two compounds are well resolved when present together at ppb levels in air.

Experiments with Ozone and NO_2 in the Dark. The chamber containing purified air was covered with black plastic film which removed ≥99% of the incident sunlight. Ozone was introduced first from the diluted output of an O_3 generator. The hydrocarbon was injected next by using a glass bulb flushed with dry N_2. Bulb and Teflon carrier gas lines were heated to facilitate injection. NO_2 (from 100 ppm in a N_2 cylinder and using two nylon filters in series to remove nitric acid impurity, if any) was introduced last by syringe injection. Control experiments were conducted with cresol alone in purified air and with mixtures of NO_2 and O_3 (no *o*-cresol added) in purified air.

Nitrocresols. The isomer selected for study was 4-hydroxy-3-nitrotoluene. The two nitrocresol isomers formed as major products of *o*-cresol photooxidation (*3*) are not commercially available. Of the available isomers, the one selected has the lowest melting point, 32 °C, an important consideration for ease of injection into the chamber and for minimizing loss to the chamber walls. This isomer is also relevant to atmospheric chemistry as

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