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Importance of Unattached Bacteria and Bacteria Attached to Sediment in Determining Potentials for Degradation of Xenobiotic Organic Contaminants in an Aerobic Aquifer

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The bacterial abundance, distribution, and degradation potential (in terms of degradation versus lack of degradation) for four xenobiotic compounds in an aerobic aquifer sediment have been examined in laboratory and field experiments. The xenobiotic compounds studied were benzene, toluene, *o*-xylene, and naphthalene (all at concentrations of approximately 120 μg/liter). The aerobic degradation experiments ran for approximately 90 days at 10°C, which corresponded to the groundwater temperature. At the end of the experiment, the major part of the microbial biomass, quantified as acridine orange direct counts, was attached to the groundwater sediment (18×10^6 to 25×10^6 cells per g [dry weight]), and only a minor part was unattached in the groundwater (0.6×10^6 to 5.5×10^6 cells per ml). Experiments involving aquifer sediment suspensions showed identical degradation potentials in the laboratory and in the field. However, laboratory experiments involving only groundwater (excluding aquifer sediment) showed less degradation potential than in situ experiments involving only groundwater, indicating that the manipulation or approach of the laboratory experiments could affect the determination of the degradation potentials. No differences were observed between the groundwater-only and the sediment compartments in the in situ experiments in the ability to degrade the compounds, but the maximum degradation rates were substantially lower in the groundwater-only compartment. Preparations used in laboratory experiments for studying the degradation potential for xenobiotic organic contaminants should contain sediment to obtain the highest numbers of bacteria as well as the broadest and most stable degradation. When only the fine (silt- and clay-size) particles of the sediment were used, nearly the same advantages were gained without seriously complicating the sampling procedures by the occurrence of sand and gravel fractions.

According to the literature, the main portion of the bacteria in aquifers is attached to the aquifer material, and only a small fraction is suspended unattached in the groundwater (e.g., see references 4, 12, 13, and 15). However, the unattached bacteria may potentially reflect the total microbial biomass in the aquifer. Hirsch and Rades-Rohkohl (14) showed that when a vessel with sterilized aquifer material was inserted in the aquifer, it obtained, via groundwater flowing through, a microbial population representing a part of the morphotypes found in the surrounding pristine aquifer material. With respect to the potential (in terms of degradation versus lack of degradation) for degradation of xenobiotic organic contaminants in the aquifer, however, hardly any convincing reports on the significance of unattached and sediment-attached bacteria have been published. Thomas et al. (20), however, did compare degradation potentials in groundwater and aquifer sediment but concluded that the groundwater used might have contained a microflora not representative of the subsurface. The lack of investigations on this topic is striking, since many different approaches have been adopted for studying degradation potentials, ranging from laboratory experiments with groundwater (e.g., see reference 1) to laboratory experiments with aquifer sediment suspended in groundwater (e.g., see reference 21) and flowthrough laboratory columns (e.g., see reference 7) to, in a few cases, actual field experiments (e.g., see reference 5). The choice of experimental approach has probably been governed by the project scope and available resources, but it may also have biased the results if the groundwater alone did not reflect the total degradation potential of the aquifer. Groundwater samples are often easier to obtain than sediment cores or sediment material, but the achieved saving does not match the loss in scientific value if the larger sediment-associated biomass has a greater potential or higher maximum rates of degradation of xenobiotic organic contaminants and thus gives more accurate biodegradation information than the groundwater alone.

Recent investigations have shown that the sediment-attached bacterial biomass is found primarily in the fine-particle fraction, typically the silt- and clay-sized range. Harvey et al. (11) showed by acridine orange direct counting (AODC) of bacteria that 67% of all bacteria in the sediment were attached to the silt-and-clay fraction, though this sediment fraction accounted for only 1% of the total sediment by weight. Bacteria attached to the silt fraction have also been found to be viable and metabolically active, as they were able to take up [^{14}C]acetate (2). This indicates that in laboratory studies, it may not be necessary to include the complete sediment in order to include the complete (or representative) biomass. This is an important point, since experiments excluding the coarse fraction are easier to handle. Furthermore, replicate systems may be easier to establish in the laboratory with fine-particle suspensions than with suspensions of complete sediment. Coarse fractions (sand and gravel) make representative subsampling

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difficult because of rapid settling, and they complicate extraction procedures.

Because of their simplicity and controllability, laboratory experiments are often preferred over other experimental approaches (e.g., in situ microcosms or field injection experiments) for determining degradation potentials for xenobiotic organic contaminants in aquifers, but introduction of artifacts (so-called "bottle effects") due to the high degree of manipulation in the laboratory systems may compromise the ability of laboratory microcosms to simulate aquifer conditions (21). The value of a well-documented comparison of groundwater and aquifer sediment suspensions with respect to degradation of xenobiotic organic contaminants may be dubious if both systems are significantly affected by artifacts. This has necessitated use of a new in situ experimental approach which isolates the degradation potential of the groundwater from that of the sediment with interstitial water (16).

The purpose of this study was to compare the potentials of attached and unattached bacteria for degradation of four xenobiotic organic trace contaminants in an aerobic aquifer. The degradation is evaluated in terms of degradation potentials (compounds being degraded) and maximum degradation rates. Three problems are examined: (i) comparison of groundwater-only and sediment suspensions in laboratory experiments with respect to biomass and degradation potential, (ii) comparison of suspensions involving the complete sediment and those involving only the fine particles, and (iii) comparison of laboratory and in situ results with respect to groundwater only and sediment with interstitial water.

MATERIALS AND METHODS

Field site description. The field site is located 400 m downstream of the municipal landfill of the city of Vejen in Jutland, Denmark. The samples were collected from the shallow upper aquifer, a glaciofluvial sand-and-gravel aquifer consisting of 1.6% clay (<2 μm), 2.3% silt (2 to 20 μm), 10.1% fine sand (20 to 200 μm), and 86.0% coarse sand (200 to 2,000 μm).

The groundwater samples had an oxygen content of 6 mg/liter, a specific conductivity of 290 μS/cm, a pH of 4.6, and a total organic carbon content of 3.9 mg of C per liter. The concentration of nitrate was 12 mg of NO_3^--N per liter, and that of chloride was 31 mg of Cl^- per liter. The results of all of the geochemical measurements were consistent with the geochemistry of the aquifer outside the plume (6), and the field site was considered uncontaminated.

Field experiment. (i) In situ microcosm. Three in situ microcosms (A, B, and C [control]) were installed in the Vejen aquifer. The in situ microcosm concept was originally described by Gillham et al. (9) but was modified for this study (16) (see Fig. 1). The in situ microcosm isolates 2 volumes of the aquifer: one lower compartment with aquifer sediment and groundwater and one upper compartment with groundwater only. The upper compartment consists of a coil (internal diameter, 8 mm) for groundwater only, while the lower compartment is a bottomless column for sediment with interstitial water. Both compartments are connected separately to the ground surface by Teflon tubes (see Fig. 1). The microcosm is made of high-quality stainless steel. Detailed information on the in situ microcosm is available elsewhere (16).

(ii) Installation. The in situ microcosms (Fig. 1) were installed in cased boreholes drilled with a hand auger and a hand-operated bailer boring auger (Eijkelkamp, Giesbeek, The Netherlands). The microcosm was driven down with a Cobra jackhammer, filling the lower compartment with aquifer material and groundwater. During installation, the casing was withdrawn. A minimum of 5 liters of groundwater was pumped (approximately 1 liter/h) from the microcosm for development before sampling for groundwater geochemical characterization (500 ml). The three in situ microcosms were installed with the bottoms 2 to 4 m below the groundwater table.

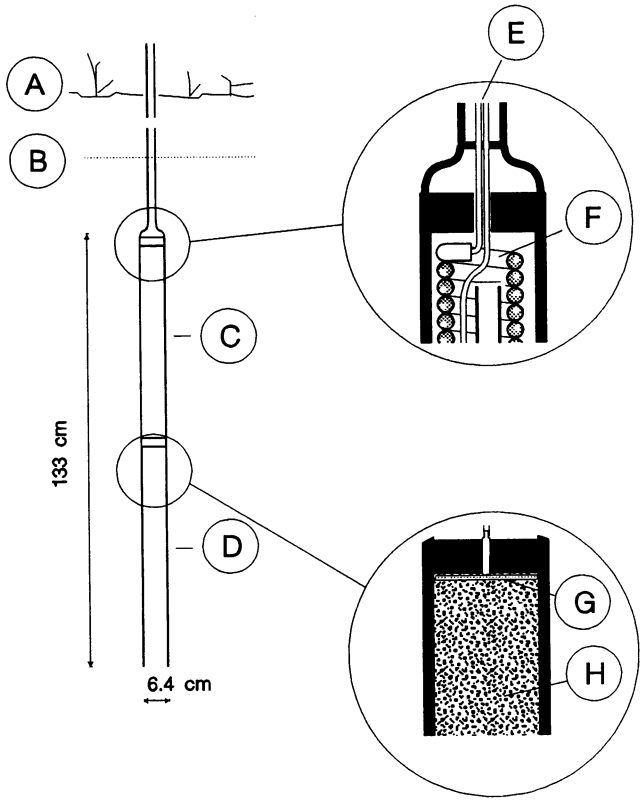

FIG. 1. In situ microcosms. A, ground surface; B, groundwater table; C, upper compartment containing coil for groundwater only; D, lower compartment for sediment with interstitial water; E, tubes for loading and sampling; F, coil with total volume of 350 ml; G, screen in the top of the lower compartment; H, aquifer material (sediment with interstitial water) in the lower compartment.

(iii) Loading of in situ microcosm. The in situ microcosms were loaded the day after the installation. A Tedlar bag (5 liters) was filled with groundwater from the lower compartment by a peristaltic pump and spiked with 200 ml of distilled water containing ^3H_2O as a hydraulic tracer and a mixture of 23 organic contaminants, all in analytical quality. The 23 organic contaminants were benzene, toluene, *o*-toluene, naphthalene, 1,1,1-trichloroethane, tetrachloromethane, trichloroethene, tetrachloroethene, 1,1,2,2-tetrachloroethane, 1,2-dichlorobenzene, 1,4-dichlorobenzene, biphenyl, fluorenone, dibenzothiophene, phenanthrene, nitrobenzene, phenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 4,6-*o*-dichlorocresol, pentachlorophenol, *o*-nitrophenol, and *p*-nitrophenol. The concentration of each of the 23 compounds in the bag was approximately 120 μg/liter. The compounds focused on in this article are benzene, toluene, *o*-xylene, and naphthalene, each of which is commonly detected at waste disposal site pollution plumes (17–19). Four liters of spiked

water was pumped (2 liters/h) into the lower compartment of the microcosm, and 1 liter was pumped into the coil of the upper compartment. Both compartments in the control in situ microcosm (microcosm C) were poisoned by adding formaldehyde (250 mg/liter) to the Tedlar bag.

(iv) Sampling. Samples (10 ml) from the microcosm were transferred directly into a 10-ml measuring flask by a syringe connected to the sampling tube (for more details, see reference 16). During the experimental periods, which varied from 89 to 94 days, samples were collected 10 times.

Laboratory experiment. (i) Field sampling. Aquifer material was obtained directly from the borehole where in situ microcosm A was installed afterwards. Aquifer sediment was withdrawn with a bailer boring auger and transferred to dark plastic bags. After the in situ microcosm was installed but before it was loaded, groundwater (15 liters) was collected from the lower compartment of the microcosm for the laboratory experiments. Sediment and groundwater were stored for fewer than 10 days at 4°C until they were transported to the laboratory. Samples were processed within 2 days.

(ii) Incubation. The laboratory experiments were conducted in eight 2-liter glass bottles, each equipped with a sampling gate. Four different sample types, all in duplicate, were incubated: (i) groundwater only (2.0 liters of groundwater), (ii) a sediment suspension (1.1 kg [dry weight] [0.73 liters] of unfractioned sediment and 1.4 liters of groundwater), (iii) a fine-particle suspension (45 g of the fine-particle fraction [silt-sized and smaller] of 1.1 kg of sediment, suspended in 2.0 liters of groundwater), and (iv) an abiotic control (same as the fine-particle suspension, but formaldehyde was added to a final concentration of 250 mg/liter). For the fine-particle suspension, the 45 g of particles was obtained by decanting approximately 0.7 liters of a suspension of groundwater and particles from a slurry of 1.1 kg (dry weight) of sediment and 1.0 liters of water after 0.5 min of sedimentation. This procedure was repeated once and was followed by addition of groundwater to a total volume of 2.0 liters.

All vessels were enriched with the mixture of organic contaminants used in the in situ microcosms, resulting in concentrations of approximately 120 μg of each compound per liter. All vessels were incubated for 90 days in the dark at 10°C (the actual groundwater temperature) under aerobic conditions. The oxygen content was 8.4 to 8.7 mg of O_2 per liter at the end of the experiments, confirming aerobic incubation. During incubation, the bottles were placed in a box for top-over-bottom rotation (10 times per h).

(iii) Sampling. At various times, a slight pressure was introduced into the vessels by addition of air from a syringe through the sampling gate. After sedimentation of suspended particles for 30 min, water samples (10 ml) were obtained by releasing the pressure through the sampling gate.

Analytical procedures. (i) Contaminant analysis. The samples (10 ml) were preserved by adding 10 μl of 10 M NaOH and extracted by 100 μl of pentane with an internal standard (isopropylbenzene). Concentrations of organic compounds were measured on a Carlo Erba Mega 500 gas chromatograph equipped with a 0.32-mm (inner diameter) DB-5 capillar column and a flame ionization detector. For more details, see reference 16.

(ii) Groundwater geochemical analysis. Samples were preserved by addition of concentrated sulfuric acid (final concentration, 10 ml/liter) for analysis of total organic carbon and by addition of 1 droplet of 25-g/liter mercury chloride per 10-ml sample for analysis of nitrate. Samples for Cl^- analysis were kept at 4°C until analysis. Specific conductivity and pH were measured in the field by electrodes (pH, WTW SenTix 96; specific conductivity, Hanna HI 8733). Dissolved oxygen was measured in the field by Winkler titration (modified for 12-ml volumes). Total organic carbon analysis was performed with a Dohrmann total organic carbon analyzer, while analysis of NO_3^- and Cl^- was done by a standard autoanalyzer routine (Technicon Autoanalyzer II). ^3H_2O was quantified by liquid scintillation counting on a Packard Tri-Carb 2000 CA Liquid Scintillation Analyzer.

(iii) Texture. The texture of sediment used for the laboratory experiments was determined. Texture analysis for gravel and sand was based on wet sieving. Silt and clay fractions were measured in a dispersion solution (4 mM $Na_2P_2O_7$ with 3 droplets of pentanol) by the hydrometer technique.

(iv) Bacterial counting. At the end of the incubation period, subsamples of the water phase were collected from the laboratory vessels as described above. From the suspension vessels, samples of the sediment phase were collected with a spoon after 24 h of sedimentation and decanting of the water. The samples were preserved by addition of phosphate-buffered formaldehyde (final concentration, 2%) for bacteria counting or were frozen at −80°C for ATP analysis.

The bacteria were counted by AODC as described by Albrechtsen and Winding (3). Dilutions of the samples were mixed thoroughly, filtered onto a black 0.2-μm-pore-size Nuclepore filter, and stained with acridine orange (final concentration, 10 μg/ml). Fading of the fluorescence was reduced by washing the filter with 0.3 M DABCO (1,4-diazabicyclo[2,2,2]octane [Merck]) for 0.3 min. All liquids were filtered through a 0.2-μm-pore-size filter before use. The bacteria were counted with an epifluorescence microscope.

ATP contents were determined by the luciferin-luciferase method with purified enzyme reagent from Lumac BV or Boehringer GmbH. Water samples (100 μl) were supplied with 100 μl of nucleotide-releasing reagent (NRB; Lumac) to extract the ATP. A parallel water sample was supplied with 20 μl of ATP solution (5 ng/ml) as an internal standard. ATP was extracted from sediment samples (2.5 g) with 5 ml of H_2SO_4-Na_2HPO_4 extractant (8). After extraction and centrifugation, 1 ml was transferred to 4 ml of Tris-EDTA buffer (37.7 g of Trizma per liter, 1.5 g of EDTA [Titriplex III] per liter) and neutralized to a pH of 7.7 to 7.8. The ATP content in the neutralized extract was determined in the same way as for water samples, except that the internal standard was added to each sample after the enzyme was added and the ATP content was measured and the ATP content was measured again without further enzyme addition. The light output was converted to ATP on the basis of a standard curve prepared for each batch of enzyme reagent with ATP purchased from Lumac.

RESULTS

Comparison between groundwater-only and sediment suspensions in laboratory experiments. In the laboratory experiments, a higher ATP content was found in the water phases (0.037 to 0.041 ng of ATP per ml) from the sediment suspensions than in the groundwater replicates (0.017 ng of ATP per ml) (Table 1). Unfortunately, the ATP concentrations in the sediment phase in the sediment suspensions were below the ATP detection limit in sediments (0.09 ng of ATP per gram [dry weight] of sediment).

The total number of bacteria, counted as the AODC (Table

TABLE 1. Cell numbers (AODC) and ATP concentrations[a]

Sample	AODC in:			Concn or amt of ATP in:		
	Water (10^6/ml)	Sediment (10^6/g [dry wt])	Vessel[b] (10^8)	Water (ng of ATP/ml)	Sediment (ng of ATP/g [dry wt])	Vessel (ng of ATP)
Groundwater A	0.6 ± 0.3	—[c]	11 ± 6	0.017 ± 0.004[d]	—	34 ± 1
Groundwater B	1.4 ± 0.7	—	28 ± 14	0.017 ± 0.004	—	34 ± 1
Sediment suspension A	5.5 ± 0.5	18 ± 7	273 ± 87	0.041 ± 0.001	BD[e]	NA[f]
Sediment suspension B	5.3 ± 1.1	25 ± 10	353 ± 129	0.037 ± 0.001	BD	NA
Fine-particle suspension A	1.2 ± 0.5	537 ± 80	259 ± 38	0.029 ± 0.001	2.28 ± 0.03	158 ± 2
Fine-particle suspension B	1.2 ± 0.5	356 ± 115	203 ± 66	0.062 ± 0.002	3.51 ± 0.16	300 ± 9

[a] Data are means ± standard deviations.
[b] Whole vessel at end of experiment.
[c] —, no sediment phase in bottle.
[d] Standard deviation for water-phase ATP calculated from a general coefficient of variance for ATP determinations in water.
[e] BD, below detection limit (0.09 ng of ATP per g [dry weight]).
[f] NA, not available because the ATP content in the sediment phase cannot be estimated.

1), was highest in the sediment phase (18×10^6 to 25×10^6 cells per g [dry weight] or 27×10^6 to 38×10^6 cells per ml of wet sediment). On a volume basis, the AODC of the sediment was 5 to 7 times higher than in the water phase in sediment suspensions and 20 to 67 times higher than in the vessels containing groundwater only. Considering the volumes of the water and sediment phases, the total number of bacteria in each vessel was calculated, and the vessels with sediment contained 10- to 32-fold-higher AODCs (273×10^8 to 353×10^8 cells) than the vessels with water (11×10^8 to 28×10^8 cells).

Benzene, toluene, *o*-xylene, and naphthalene concentrations in the sediment suspensions and in groundwater only are presented as a function of time in Fig. 2. In the abiotic controls (data not shown), the concentrations remained constant during the incubation. All four aromatic compounds were degraded in all of the laboratory experiments, except for groundwater replicate A (Fig. 2), which showed little or no potential for degrading benzene, toluene, or *o*-xylene. Naphthalene was completely degraded in replicate A within the first 60 days of the experimental period, but a lag phase of at least 15 days was observed before degradation began. The reasons for this difference in degradation kinetics and for the lag phase are unknown.

The degradation of the easily degradable compounds benzene, toluene, and naphthalene was slower in the beginning and at the end of the degradation period than in the middle. *o*-Xylene was the most slowly degraded of the four compounds, and the rates of its degradation were nearly constant over time.

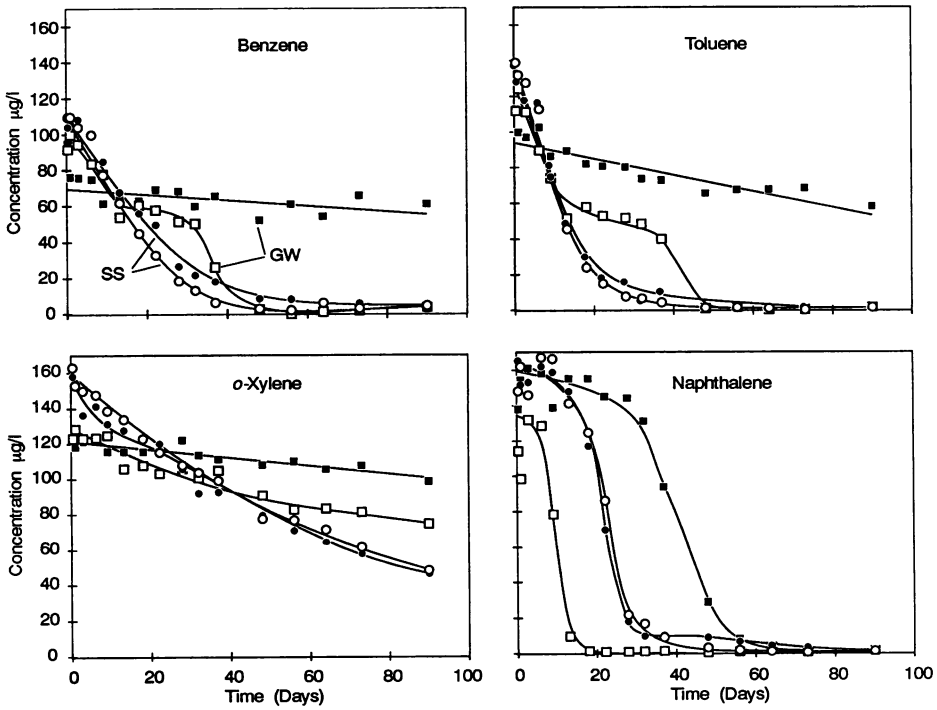

FIG. 2. Degradation of benzene, toluene, *o*-xylene, and naphthalene in laboratory experiments compared for sediment suspensions (SS) (●, replicate A; ○, replicate B) and groundwater only (GW) (■, replicate A; □, replicate B). Concentrations are shown as a function of time during aerobic incubation at 10°C.

TABLE 2. Maximum degradation rates measured in laboratory suspensions containing groundwater, fine-particle suspensions, or whole sediment suspensions

Compound	Maximum degradation rate (μg liter^{-1} day^{-1}) in indicated suspension and replicate					
	Groundwater only		Fine-particle suspension		Sediment suspension	
	A	B	A	B	A	B
Benzene	0.2	4	7	5	4	4
Toluene	0.3	6	10	8	6	6
o-Xylene	0.2	1	3	2	2	2
Naphthalene	7	13	12	13	10	9

TABLE 3. Maximum degradation rates observed in the groundwater and sediment compartments of in situ microcosms A and B

Compound	Maximum degradation rate (μg liter^{-1} day^{-1}) in:			
	Water		Sediment	
	A	B	A	B
Benzene	0.8	0.8	7.0	4.0
Toluene	0.7	0.8	5.0	4.0
o-Xylene	0.2	0.4	1.0	1.3
Naphthalene	0.5	0.8	2.0	1.5

The patterns of degradation in the two vessels with sediment suspensions resulted in very similar curves, whereas the patterns of degradation in the vessels with groundwater only were more variable. The degradation curves of groundwater replicate B (Fig. 2) roughly follow the curves of the sediment replicates (Fig. 2) but with lower rates of degradation, except for that of naphthalene.

Evaluation of laboratory systems with fine-particle suspensions. The total amounts of sediment particles in a volume of 2 liters were 1,100 g (dry weight) in the sediment suspensions and 45 g (dry weight) in the fine-particle suspensions. The particles of the fine-particle suspensions made up 4.0% of total sediment and included the silt- and clay-sized fractions. According to texture, silt- and clay-sized fractions accounted for 3.9% of the sediment. The total number of bacteria and the ATP content in the fine-particle suspensions are included in Table 1. These vessels contained 203×10^8 to 259×10^8 cells, corresponding to 58 to 95% of the total number of bacteria found in the sediment suspensions.

The overall degradation showed the same pattern (in terms of the shape of the degradation curves for each compound) in sediment suspensions and in fine-particle suspensions. The maximum degradation rates of the four aromatic compounds were estimated as the decrease in concentration of compounds (in micrograms per liter per day) at the steepest part of the curve and compared for the sediment suspensions and the fine-particle suspensions (Table 2). Generally, for all compounds the maximum degradation rates were comparable in the fine-particle suspensions (e.g., 8 to 10 μg liter^{-1} day^{-1} for toluene) and in the sediment suspensions (6 μg liter^{-1} day^{-1} for toluene). However, the maximum degradation rates of the two fine-particle suspensions were not as similar as those of the two sediment suspensions.

Comparison of sediment and groundwater in the field experiments. In the field experiments, the concentration of the hydraulic tracer (^3H_2O) was constant over time for all the in situ microcosms. For example, in the groundwater compartment, the initial concentration was 161 Bq/ml and the final concentration was 157 Bq/ml; in the aquifer compartment, the initial concentration was 162 Bq/ml and the final concentration was 155 Bq/ml. As there was no evident dilution effect, the tracer results were not used in any degradation calculations.

During the first 7 to 10 days, the concentrations of xenobiotics decreased in the poisoned control in situ microcosms (data not shown). In the groundwater-only compartment, the concentrations decreased by approximately 10%; in the sediment compartment, the concentrations decreased by approximately 15%, except for that of naphthalene, which decreased by approximately 50%. This was ascribed to sorption to the sediment. After the first 7 to 10 days, the concentration of xenobiotics remained constant in the control microcosm in the groundwater-only compartment as well as in the sediment compartment. The system, therefore, did not seem to be subject to any unaccounted loss of xenobiotics by sorption, volatilization, or chemical degradation after the initial period.

The field results from the in situ microcosms are summarized in terms of maximum degradation rates in Table 3. Benzene, toluene, *o*-xylene, and naphthalene were all degraded in both microcosms in the groundwater compartment as well as in the sediment compartment. For each compound, the maximum degradation rates were lower (by a factor of 1.9 to 8.8) in the groundwater-only compartments than in the sediment compartments (Table 3).

The maximum degradation rates were on the same order of magnitude in the sediment suspensions in the laboratory (2 to 10 μg liter^{-1} day^{-1}) (Table 3) and in the sediment compartment of the in situ microcosms (1.0 to 7.0 μg liter^{-1} day^{-1}) (Table 2). In the laboratory experiment, the maximum degradation rates in the groundwater were generally higher (0.2 to 13 μg liter^{-1} day^{-1}) than in the groundwater compartment of the in situ microcosms (0.2 to 0.8 μg liter^{-1} day^{-1}), but the compounds were removed to nearly the same extent.

DISCUSSION

The 23 different contaminants added in all of the experiments were selected because they are commonly detected in waste disposal site pollution plumes (17–19) and have been used in many degradation studies in our laboratory. The results of the degradation experiments with the four aromatics are the focus of this article mainly in order to compare the investigated systems rather than to investigate the degradation of these specific compounds. The other contaminants may have affected the rate and extent of biodegradation of the four aromatics, but this possible effect was not investigated. If there were such an effect, it might have been of minor importance, as the same mixture was used in all experiments.

A higher biomass (i.e., a higher AODC) was found in the sediment suspensions than in groundwater alone (Table 1). The ATP content verifies that at least a part of the enumerated population was alive. The bacterial numbers found in the sediment phases are in agreement with values found by Albrechtsen and Winding (3) in sediment from the same aquifer, indicating that the laboratory systems were not enriched during incubation.

Microbial biomass per unit volume measured by AODC in the sediment suspensions was five to seven times higher in the sediment than in the groundwater. Relating the biomass

concentration in the sediment phase to the biomass in the groundwater-only vessels revealed ratios of 20:1 to 67:1. These ratios are consistent with ratios reported in the literature; for example, Harvey et al. (11) found 26- to 2,000-fold-higher bacterial numbers in sediment than in pore water from a groundwater sediment. In our study, the fact that numbers of unattached bacteria in the water phase of the sediment suspensions were higher than those in groundwater only at the end of the experiment could be explained by insufficient separation of the water and sediment phases during sampling. However, this is unlikely because the water phase had no visible suspended particles after the 24-h sedimentation period before sampling. A more likely explanation would be that some bacteria were released from the sediment to the groundwater during the experiment either by mechanical disturbance during the shaking of the suspensions or by physiological adaptation to a higher substrate concentration (10).

Degradation of all four tested aromatic compounds (benzene, toluene, *o*-xylene, and naphthalene) was observed in the laboratory experiments in the sediment suspensions, while in the groundwater only, the potential to degrade all four compounds was found in only one of the two replicates (replicate B [Fig. 2]). In the other groundwater-only replicate (replicate A [Fig. 2]), only naphthalene was degraded. These observations indicate that the higher biomass in the sediment suspensions than in the groundwater experiments implies a higher degradation potential, higher maximum rates, and more-similar degradation patterns in the sediment suspensions than in the groundwater-only experiments (Table 2). Contrasting conclusions were reported by Thomas et al. (20), who found that three out of four soils had low viable-cell counts (below the detection limit) and showed no degradation potential for naphthalene, whereas all groundwater samples harbored culturable bacteria and had naphthalene degradation potential. In their experiment, however, the water was collected from an existing well, and although the well was developed before sampling, the water might not represent the subsurface, whereas we collected the water immediately after drilling. Factors other than the higher biomass could influence the rates and the degradation potentials in the sediment; the attached population may be distinct from the unattached population (14), or the attached bacteria may have higher levels of metabolic activity.

The fine-particle suspensions showed a potential for degrading all four aromatic compounds that was similar to that observed when whole sediment was used. This reflects the similar bacterial abundances in the two different systems at the end of the experiment. When the variation of maximum degradation rates between the duplicates of fine particle suspensions is larger than that between the sediment suspensions (Table 2), it can be ascribed to small variations in the amount and composition of the fine particles (approximately 45 g) separated out from the whole sediment (1,100 g).

The field experiment, which attempted to eliminate the effects of transport, storage, and manipulations in the laboratory, showed that the groundwater-only compartments actually had a potential for degrading all four aromatic compounds similar to that of the sediment compartments, though the maximum degradation rates were substantially lower in the groundwater compartments (Table 3).

The surface area of the inner side of the coil isolating the water compartment in the field experiment was relatively large compared with the surface areas of the laboratory vessels, and growth of biomass on the surface of the walls of the coil was observed after the end of the experiment. However, if surface-associated bacteria in the water compartment participated in the degradation of the organic xenobiotic compounds, they were originally transferred as unattached bacteria during the initiation of the experiment. This is in accordance with previous observations of in situ colonization of sterile sediment by groundwater bacteria (14).

The laboratory experiments including sediment differed from the experiments using the sediment compartment in the in situ microcosm in the solid-to-water ratio. In the laboratory sediment suspensions, the sediment-to-water (volume) ratio was 1:2; in the sediment in the in situ microcosm, it was 2:1. This difference might be the reason for the significant sorption observed in the in situ microcosm during the initial period (discussed elsewhere [16]) but not in the laboratory systems.

The degradation potential found in the sediment compartment of the in situ microcosm was very similar to the degradation potential in the laboratory sediment as well as in the fine-particle suspension, while the degradation potential differed in the groundwater only. This could be due to greater protection of bacteria attached to sediment during handling, transport, storage, and setting up of laboratory experiments or to a scarcity of surface area in the laboratory groundwater-only experiments. The actual cause cannot be determined from the reported experiment.

Conclusions. On the basis of laboratory and field experiments on degradation of four xenobiotic organic contaminants in an aerobic sandy aquifer, the following conclusions can be made.

Most of the bacteria were associated with grain surfaces, especially the fine-particle fractions (silt and clay sized); few bacteria were free-living. In the laboratory, degradation experiments showed more similar degradation curves and higher degradation potentials in sediment suspensions than in groundwater only. The field experiments, however, showed that the abilities to degrade the compounds of interest were identical in the groundwater and in the sediment compartments, although the latter exhibited much higher degradation rates, as expected from the higher biomass present.

Transferring the fine-particle fraction (4% by weight) of the groundwater aquifer material into a separate vessel with groundwater resulted in a system equivalent to a sediment suspension with respect to biomass and degradation potential for the xenobiotic organic compounds. To obtain reliable results in laboratory degradation tests, it seems important to include sediment, but it seems that the coarse aquifer solids can be omitted from laboratory incubations with little effect on the rate and extent of biodegradation of contaminants.

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