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Mobile Bacteria and Transport of Polynuclear Aromatic Hydrocarbons in Porous Media

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Sorption of hydrophobic pollutants such as polynuclear aromatic hydrocarbons (PAHs) to soil and aquifer materials can severely retard their mobility and the time course of their removal. Because mobile colloids may enhance the mobility of hydrophobic pollutants in porous media and indigenous bacteria are generally colloidal in size, bacterial isolates from soil and subsurface environments were tested for their ability to enhance the transport of phenanthrene, a model PAH, in aquifer sand. Batch isotherm experiments were performed to measure the ability of selected bacteria, including 14 isolates from a manufactured gas plant waste site, to sorb ^{14}C-phenanthrene and to determine whether the presence of the suspended cells would reduce the distribution coefficient (K_d) for phenanthrene with the sand. Column experiments were then used to test the mobility of isolates that reduced the K_d for phenanthrene and to test the most mobile isolate for its ability to enhance the transport of phenanthrene. All of the isolates tested passively sorbed phenanthrene, and most but not all of the isolates reduced the K_d for phenanthrene. Some, but not all, of those isolates were mobile in column experiments. The most mobile isolate significantly enhanced the transport of phenanthrene in aquifer sand, reducing its retardation coefficient by 25% at a cell concentration of $\sim 5 \times 10^7$ ml^{-1}. The experimental results demonstrated that mobile bacteria may enhance the transport of PAHs in the subsurface.

Soil and groundwater contamination has become a common problem in many locations. Many organic pollutants are hydrophobic and sparingly soluble. Polynuclear aromatic hydrocarbons (PAHs) are exemplary compounds of this type and are known components of coal tars, gasoline, and jet fuel (38). Since the PAH benzo[*a*]pyrene is a known carcinogen and PAHs such as naphthalene and phenanthrene are priority pollutants listed by the Environmental Protection Agency, the presence of these compounds in groundwater and soil constitutes a health hazard, and their removal from contaminated sites is desirable.

PAHs strongly sorb to soils; this action decreases their transport rate and increases the time required for the remediation of contaminated soils. In addition, sorption of degradable hydrophobic pollutants has been implicated as a major factor governing their biodegradation by subsurface microorganisms (30, 32). Overcoming the limiting effects of sorption of hydrophobic pollutants on their removal from porous media and their bioavailability in soil has recently been identified by the Environmental Protection Agency Bioremediation Action Committee as a priority research area (1).

Some studies have indicated that hydrophobic chemicals have been transported greater distances and at higher concentrations than predicted on the basis of their sorptive distribution coefficients (K_ds) (4, 10, 11, 20, 39). One possible explanation for this "facilitated" transport is the presence of colloidal solids or dissolved macromolecules that may act as carriers for hydrophobic pollutants (27, 37). Bacteria are colloidal in size; they are indigenous to soil, subsurface, and aquifer environments and therefore represent a natural means by which pollutant mobility may be enhanced. Sorption of hydrophobic compounds to microorganisms has been examined by several investigators, is generally assumed to be a passive process, and is treated in a manner analogous to sorptive partitioning (5, 22, 36). Sorption of organic pollutants to cells is reversible, although the kinetics of desorption may differ from those of sorption (22). It cannot be assumed, however, that all microorganisms passively sorb hydrophobic compounds with the same affinity. Some components of a microbial community may more readily sorb hydrophobic contaminants than others. In addition, organisms that sorb phenanthrene must also be mobile in soil to have a significant effect on the facilitated transport of phenanthrene.

Magee et al. (25) developed a three-component model for the interaction among a nonionic hydrophobic pollutant, a porous medium, and a carrier that interacts with the medium by sorption. As a first approximation, this model may be extended to experimental systems in which the carrier is a colloidal solid. In this case, the sorption of bacteria to the porous medium (or their removal by filtration) must be considered a reversible process. At the steady state, the ratio of the rates of colloid removal and release would result in a linear colloid "K_d" that would replace the sorption partition coefficient for the dissolved carrier in the model (Fig. 1). The model suggests that at least two conditions must be met for colloidal solids, such as bacteria, to increase the mobility of hydrophobic pollutants: (i) the pollutant must bind to the carrier, and (ii) the carrier must be more mobile than the contaminant. The mobility of bacterial cells in porous media is therefore one of the criteria for bacterially facilitated transport of hydrophobic pollutants in the environment.

Models for filtration (40) suggest that colloidal particles with diameters of ≈ 1 μm (the size of many soil bacteria) are of an optimal size for transport. Field studies have indeed demonstrated the mobility of bacteria in soil (15) and groundwater systems (17). There is, however, little information on the efficacy of soil bacteria as carriers for hydrophobic pollutants.

The objectives of this study were to characterize selected bacteria, many isolated from subsurface environments, for

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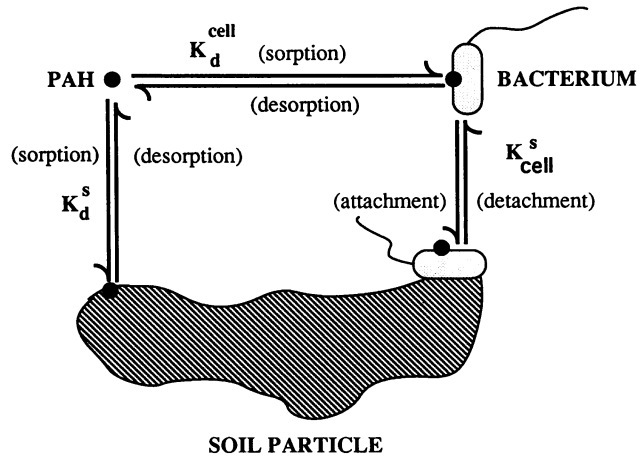

FIG. 1. Partitioning of a PAH, such as phenanthrene, and a bacterial cell in soil.

their ability to sorb phenanthrene (a model PAH), to screen the bacterial isolates for their ability to decrease the K_d for phenanthrene with a natural sorbent (aquifer sand), to test selected isolates for mobility in porous media, and to determine whether a mobile isolate capable of sorbing phenanthrene would enhance the mobility of phenanthrene.

MATERIALS AND METHODS

Bacterial cultures and growth conditions. The bacterial isolates examined in this investigation are listed in Table 1. The *Bacillus subtilis* strain, isolates N1 and Nd9, and all of the isolates prefixed by "97" were obtained from W. Ghiorse (Section of Microbiology, Cornell University). Isolates B121, B550, B649, and B693 were obtained from P. Baveye (Department of Soil, Crop, and Atmospheric Science, Cornell University). Isolate A100 was obtained from M. Alexander (Department of Soil, Crop, and Atmospheric Science, Cornell University). Peptone-tryptone-yeast extract-glucose (PTYG) medium consisting of (per liter) 5 g of peptone, 5 g of tryptone, 10 g of yeast extract, 10 g of glucose, 0.6 g of $MgSO_4 \cdot 7H_2O$, and 0.07 g of $CaCl_2 \cdot 2H_2O$ and with the pH adjusted to 7 was used for initial cell cultures. A single colony of an isolate from a fresh full-strength or 5% PTYG agar slant or plate was used to inoculate a small volume of either full-strength or 5% PTYG broth, which was then incubated at 25°C on a rotary shaker at 180 rpm until the stationary growth phase. A 10-ml volume of this culture was used to inoculate 250 ml of PTYG broth, which was then incubated as described above. This volume of cells was harvested and used in experiments. The cells were centri-

TABLE 1. Bacterial isolates used in experiments

Bacterial isolate	Depth (m)	Gram reaction	Catalase	Oxidase	Source or reference
Arthrobacter globiformis ATCC 8010	NA[a]	+	+	ND[b]	ATCC[c]
Acinetobacter calcoaceticus ATCC 31012	NA	−	+	−	ATCC
Bacillus subtilis	NA	+	+	+	W. Ghiorse, Cornell University
Pseudomonas cepacia 249-100	NA	−	+	+	29
P. fluorescens ATCC 13524	NA	−	+	+	ATCC
Zoogloea sp. strain WNJ8	NA	−	+	+	29
A100[d]	Surface	−	+	+	18
B121[e]	ND	−	+	+	35
B550	ND	+	+	+	35
B649	259	+	+	+	35
B693	259	+	+	+	35
N1[f]	Surface	−	+	−	24
Nd9[g]	Surface	−	+	+	W. Ghiorse, Cornell University
9701A-2[h]	1.8	−	+	+	24
9702A-2	4.0	−	−	+	24
9702M-4	4.0	−	−	+	24
9703A-1	11	+	+	−	24
9703A-5	11	+	+	−	24
9706M-2	4.8	+	+	−	24
9707M-3	7.0	+	+	−	24
9709A-3	9.4	+	+	−	24
9710M-3	3.5	+	+	−	24
9711A-2	5.8	+	+	−	24
9711A-4	5.8	+	+	−	24
9712M-1	8.1	+	+	−	24
9712M-3	8.1	+	+	−	24
9714A-4	9.1	+	+	−	24

[a] NA, not applicable.
[b] ND, no data.
[c] ATCC, American Type Culture Collection, Rockville, Md.
[d] Rhizosphere isolate obtained from experimental soybean plants cultured in Honeoye silt loam obtained from the Aurora Research Farm of Cornell University, Aurora, N.Y.
[e] Isolates prefixed by "B" were obtained from the Department of Energy (DOE) field site (site P24) at the Savannah River Plant, Aiken, S.C. (DOE Subsurface Microbial Culture Collection at Florida State University).
[f] A naphthalene-metabolizing strain isolated from a surface soil environment at a forested site contaminated with coal tar residues.
[g] A naphthalene-metabolizing strain isolated from a DOE field site in Hanford, Wash.
[h] Strains prefixed by "97" were isolated from an Electric Power Research Institute manufactured gas plant waste site in the midwestern United States.

TABLE 2. Characteristics of aquifer sand

Characteristic	Value
Organic carbon, %[a]	0.04
Clay and silt, %[b]	1.2
Sand, %	98.7
Very fine (0.1–0.05 mm)	3.7
Fine (0.25–0.1 mm)	47.2
Medium (0.5–0.25 mm)	47.6
Coarse (>0.5 mm)	0.2
pH in water	7.9
Phenanthrene K_{oc}, ml g^{-1}	5.3×10^4

[a] Analysis was done by the Walkley-Black method (2).
[b] See reference 25.

fuged at 8,000 to 10,000 × *g* for 20 to 30 min and washed twice in 100 ml of a sterile 5 mM solution of $CaSO_4$ with or without 0.02% NaN_3 (sodium azide). After the second wash, the cells were resuspended in 100 ml of sterile 5 mM $CaSO_4$ and starved for 24 to 48 h on a rotary shaker at 180 rpm and 25°C. Starved cells were harvested by centrifugation and resuspended in 5 mM $CaSO_4$ with or without 0.02% NaN_3. Cell densities were determined with a Coulter Counter, by measuring cell dry weights, or by total organic carbon (TOC) analysis with an O.I. Corp. model 700 TOC analyzer.

Batch isotherm experiments. In all batch isotherm experiments, funnel-top borosilicate ampoules (Wheaton) were used to contain a maximum volume of 12 ml, 11 ml of a 5 mM $CaSO_4$–0.02% NaN_3 solution, with or without a suspension of bacterial cells, and 1 ml of 1.5 ± 0.1 μM ^{14}C-phenanthrene (13.1 mCi of 9-^{14}C-phenanthrene $mmol^{-1}$; Sigma Chemical Co.). The purity of the ^{14}C-phenanthrene was tested by high-performance liquid chromatography, and the radiolabeled phenanthrene was found to be free of labeled or unlabeled contaminants. The ampoules were flame sealed immediately after the addition of the ^{14}C-phenanthrene. Sealed ampoules were equilibrated at a constant temperature of 25°C on a slowly rotating tumbler. After equilibration, cells were removed from the suspension by centrifugation (~330 × *g* for 30 min), the ampoules were opened, 1 ml of the electrolyte was transferred to 10 ml of scintillation fluid (ScintiVerse; Fisher Scientific), and the ^{14}C activity for each sample was determined on a Beckman LS 9800 scintillation counter. ^{14}C activity was corrected for background and quench. The cell densities of the dilutions pipetted into the ampoules were measured before and after the period of equilibration. In all instances, there was no indication of changes in cell mass. NaN_3, a respiratory inhibitor, was used to prevent the active uptake and metabolism of phenanthrene. The constancy of cell mass in the experiments was taken as evidence that azide also inhibited replication.

In experiments with sand present, the sand was separated from suspensions containing phenanthrene (with or without added cells) by sedimentation for 24 h. The aquifer sand used in the experiments was from Newfield, N.Y., and is characterized in Table 2. Sorption of phenanthrene to the aquifer sand in the presence and absence of suspended cells was evaluated by use of the following linearized form of the mass balance equation (25):

$$(C_oV_o)/(C_sV_s) = 1 + [K_d(M/V_s)] \quad (1)$$

where C_o is the initial concentration, in disintegrations per minute, of ^{14}C-phenanthrene; V_o is the volume of the solution; C_s is the concentration of the sorbate in equilibrium with the sorbent; V_s is the volume of the solution containing the sorbent; and M is the mass of the sorbent. Plotting M/V_s on the x axis and $(C_oV_o)/(C_sV_s)$ on the y axis yields a linear slope, the value of which equals the K_d for the solute with the sorbent.

Soil isolate 9710M-3 was used to examine the kinetics of the sorption of phenanthrene (sorbate) to bacterial cells (sorbent). Duplicate samples were removed at 0, 4, 8, 12, 24, and 48 h, and the ^{14}C activity was determined. Twenty-four hours proved sufficient for reaching equilibrium. Isotherm experiments with three concentrations of cells (9710M-3) with and without NaN_3 indicated that sodium azide did not affect the K_d of phenanthrene with the cells. Thus, NaN_3 was used in all subsequent batch isotherm experiments. For determining the effect of the cells on the K_d of phenanthrene with the aquifer sand, three quantities of sand (generally, 1, 2, and 3 g) and three concentrations of cells were used, with two or three replicates of each treatment.

Test for cell surface hydrophobicity. The hydrophobicity of selected bacterial isolates was quantified by the microbial-adhesion-to-hydrocarbons (MATH) test as described by Rosenberg and coworkers (33, 34). The following organisms were tested: *Acinetobacter calcoaceticus* (ATCC 31012) and soil isolates 9701A-2, 9702A-2, 9703A-1, 9709A-3, 9711A-2, 9712M-3, A100, Nd9, and N1. All glassware used in this test was acid washed and autoclaved. Bacterial cells were grown and harvested as described above with the following changes. The cells were washed twice in 0.15 M phosphate buffer at pH 7, resuspended in phosphate buffer, and adjusted to an optical density at 600 nm of 1.5. A 2.4-ml volume of cell suspension was aseptically pipetted into round-bottom screw-cap culture tubes. In duplicate, 100, 200, and 400 μl of hexadecane (Sigma) were pipetted into the cell suspensions. The tubes were incubated in a water bath at 30°C for 15 min. After the tubes were vortexed for 120 s, they were placed in the 30°C water bath for 15 min to allow the two phases to separate. The final optical density at 600 nm of the cell suspensions was determined with a Bausch & Lomb Spectronic 20 instrument.

Bacterial cell mobility in porous media. The isolates were grown and harvested as described above. Plate counts and the TOC contents of the cell suspensions were determined. The dimensions of the starved cells were determined with a Zeiss universal transmitted-light microscope. An autoclavable syringe transfer pipette was dry packed with aquifer sand after Whatman no. 40 filter paper had been placed over the outflow orifice. Another piece of filter paper was placed over the exposed sand, and a no. 2 rubber stopper with a 6-cm length of 0.064-cm (outer-diameter) stainless steel syringe tubing through it was used to cap the sand column. The column was mounted in a vertical position. The bulk density of the aquifer sand was determined gravimetrically. After the column was saturated with a solution of 5 mM $CaSO_4$, it and the tubing connecting it with the reservoir and fraction collector were autoclaved for 2 h. The sterility of the system was checked before the initiation of an experiment by pumping sterile 5 mM $CaSO_4$ through the tubing and column and plating the effluent on 5% PTYG agar medium. These plates were incubated at 25°C; the absence of growth after 2 days was considered grounds to initiate a mobility experiment. By use of a peristaltic pump at a flow rate of 6 to 12 ml h^{-1}, a 5 mM $CaSO_4$ solution was pumped upward through the column. Autoclavable Nalgene tubing was used to deliver the effluent to a fraction collector (Gilson model 222). The length of the packed column was 6.5 cm, and the bulk density of the packed sand was ~1.60 g cm^{-3}.

The column apparatus was set up under a laminar-flow

transfer hood to decrease the probability of contamination. A chloride breakthrough curve (BTC) was determined separately before determination of the BTC for the cells. A sterile 0.085 M solution of NaCl in 5 mM $CaSO_4$ was applied to the column for 2 h, and then several pore volumes of a sterile 5 mM $CaSO_4$ solution were applied (1 pore volume is defined as the volume of the empty column multiplied by the porosity). As with the chloride BTC, the cell suspension was injected into the column as a 2- or 6-h pulse; the pulse was followed by a continuous injection of sterile 5 mM $CaSO_4$.

The chloride concentration was determined by standard argentometric methods (3). The cell density was determined by use of plate counts and TOC analysis. By use of the chloride BTC and CXTFIT, a model developed by Parker and van Genuchten (31), the dispersion coefficient and pore water velocity were estimated. The retardation coefficient (R) for the isolates was estimated by use of the first temporal moment of the BTC:

$$R = \left\{ \{\Sigma(C/C_o)\theta d\theta / [\Sigma(C/C_o)d\theta]\} - (\theta_p/2) \right\} \quad (2)$$

where C is the effluent cell concentration, C_o is the influent cell concentration, θ is the pore volumes, and θ_p is the duration in pore volumes of the pulse of cells.

The K_d for bacterial cells with sand (K^s_{cell}) was calculated from R with the equation

$$R = 1 + [K^s_{cell}(\rho/\eta)] \quad (3)$$

where ρ is the bulk density of the sand and η is porosity.

Phenanthrene BTCs determined with a glass column packed with aquifer sand. An all-glass column assembly comparable to that described by Lion et al. (23) was acid washed, and the dead volume (water-filled space in the apparatus that was not part of the column, e.g., tubing) was determined gravimetrically. Whatman GF/D glass microfiber filter paper (2.7-μm-pore size) was combusted at 550°C to remove carbonaceous material and placed at the inlet end of the column before it was dry packed, under conditions of constant tamping, with aquifer sand. The length of the packed column was ~5 cm, and the bulk density was determined by weight to be between 1.5 and 1.6 g cm^{-3}. The column was connected to a continuous-flow syringe pump (Pharmacia LKB pump P-500) and a fraction collector (Gilson model 222). The column was housed in a constant-temperature chamber that was kept at 25°C. Before initiation of a BTC determination, the column was saturated with a solution of 5 mM $CaSO_4$–0.02% NaN_3. A chloride BTC determination preceded the phenanthrene BTC determination.

For the phenanthrene BTC determination, 0.42 μM ^{14}C-phenanthrene (~12,000 dpm ml^{-1}) was applied to the column for 6 h, and then 5 mM $CaSO_4$–0.02% NaN_3 was applied. When a bacterial culture was incorporated into the column experiments, washed and starved cells of a known cell concentration were mixed with the ^{14}C-phenanthrene used in the pulse, which was then chased with a suspension of cells of the same cell concentration. The flow rate of the syringe pump was 5 ml h^{-1}. The concentration of the cell suspension was ~65 μg of TOC ml^{-1} (~5 × 10^7 cells ml^{-1}) in all experiments. One-milliliter fractions of the eluate were collected directly into scintillation cocktail. The concentration of the radiolabel was determined with a Beckman LS 9800 liquid scintillation counter. CXTFIT (31) was used to determine the pore water velocity and the dispersion coefficient on the basis of data from the chloride BTC. The retardation of phenanthrene was estimated by use of equation 2 for the first temporal moment.

TABLE 3. Distribution coefficients for phenanthrene with bacterial cells (K^{cell}_d)[a]

Bacterial isolate	K^{cell}_d (ml/g of cell carbon) (±SD) (10^3)
Acinetobacter calcoaceticus ATCC 31012	5.6 ± 0.1
Pseudomonas cepacia 249-100	8.1 ± 0.3
P. fluorescens ATCC 13524	8.4 ± 0.2
A100	13.2 ± 0.3
B121	10.5 ± 0.3
B550	7.3 ± 0.2
B649	7.9 ± 0.2
B693	6.2 ± 0.1
9702A-2	11.1 ± 0.1
9703A-1	3.0 ± 0.03
9703A-5	3.3 ± 0.1
9710M-3	10.0 ± 0.2

[a] No r^2 value for the linear isotherms was <0.940.

Model predictions. The mathematical model developed by Magee et al. (25) for predicting the effect of organic matter on the transport of PAHs in a porous medium was adapted to predict the effect of bacterial cells on PAH transport. The model has the following form:

$$R = \frac{(1 + K^{cell}_d[\text{cell}]) + (K^s_d\rho/\eta)}{1 + \{K^{cell}_d[\text{cell}]/(1 + K^s_{cell})\}} \quad (4)$$

where K^{cell}_d is the K_d for the sorption of phenanthrene to cells (milliliters per gram of cell carbon), [cell] is the concentration of cells (milligrams of cell carbon per milliliter), and K^s_d is the K_d for phenanthrene sorption to aquifer sand (in the absence of cells). R values calculated from the model were converted to "apparent" K_d values by use of equation 3 for comparison with observations of batch sorption experiments conducted with phenanthrene in the presence of cells and aquifer sand.

RESULTS

Batch isotherm experiments. The batch isotherms for the bacterial isolates tested were linear, and significant differences between organisms were evident (Table 3). The K^{cell}_d values were lower than the K_d value normalized for the organic carbon content (K_{oc} [$K_{oc} = K_d^s/F_{oc}$, where F_{oc} is the weight fraction of organic carbon]) for phenanthrene with the aquifer sand (average sand K_{oc}, 5.3 × 10^4 ml/g of organic C).

The presence of suspended bacteria at cell densities of >100 μg of TOC ml^{-1}, in all instances but one (n = 20), decreased the K_d value for phenanthrene with the aquifer sand. Most isolates (70%) at concentrations of 20 to 55 μg of TOC ml^{-1} significantly decreased (>10%) the K_d value for phenanthrene with the aquifer sand, as measured by the level of radioactivity in suspension after settling of the sand. At a cell concentration of 65 μg of TOC ml^{-1}, 40% of the isolates reduced the estimated K_d value by more than 30% (Table 4).

Bacterial cell hydrophobicity. MATH test results indicated that *A. calcoaceticus* ATCC 31012 behaved as it has for others (34); i.e., it displayed hydrophobic properties. The gram-positive soil isolates 9703A-1 and 9711A-2 showed moderate hydrophobicity. None of the other isolates se-

TABLE 4. Bacterial isolates that most decreased the K_d value for phenanthrene with the aquifer sand at a cell concentration of 65 μg of TOC ml^{-1}[a]

Strain	% Decrease in K_d
9701A-2	48
9702A-2	46
9702M-4	40
9703A-1	57
9709A-3	38
9711A-2	65
9712M-3	40
A100	34

[a] Observed values for K_d over a range of cell concentrations were fit by nonlinear regression to a hyperbolic equation of the form $K_d = AB/(B + [cell])$. The K_d value was calculated for a cell concentration of 65 μg of TOC ml^{-1} for purposes of comparison.

lected for testing appeared to possess hydrophobic tendencies. Neither of the naphthalene-degrading isolates (Nd9 and N1) appeared to be hydrophobic, suggesting that there may be no physiological connection between cell surface hydrophobicity and the ability to degrade sparingly soluble PAHs or other hydrocarbons, as previously suggested (33). There also appeared to be no relationship between the ability of a bacterial cell to sorb phenanthrene and cell surface hydrophobicity as measured in the MATH test. The rhizosphere isolate A100 had a K_d^{cell} value of >13,000, while *A. calcoaceticus* had a K_d^{cell} value of <6,000.

Bacterial cell mobility. Five subsurface isolates that most reduced the K_d value for phenanthrene (Table 4), 9711A-2, 9703A-1, 9701A-2, 9702A-2, and the rhizosphere isolate A100, were examined further for their mobility through a packed-sand column. Fitting of the chloride BTC yielded pore water velocities in the column experiments ranging from 0.1 to 0.2 cm min^{-1}. Results of the BTC determination for 9711A-2 indicated that this spore-forming, gram-positive bacillus had negligible mobility. In contrast, the other isolates tested appeared to be somewhat mobile (Fig. 2). Gram-negative A100 had a length of 1 μm which, on the basis of a consideration of filtration theory (40) and the effect of particle size (28), would lend itself to transport. However, only 13% of the biomass applied to the column was recovered over the duration of the experiment. Although the length of gram-positive bacillus 9703A-1 was twice that of A100, the mass recovery of the former was significantly higher (68%). The mass recovery of isolate 9701A-2, a gram-negative rod that was ~1 μm long, was 25%. Compared with the other isolates, isolate 9702A-2, a gram-negative rod that was ~1 μm long, was by far the most mobile, with an R value of 1.0 and a mass recovery of 86%. Despite the variable mass recovery, the results of these BTC determinations showed some bacterial mobility. The K_{cell}^{Σ} values (based on the R value calculated for the portion of the cell mass recovered in the BTC determination) of 0.53, 0.65,

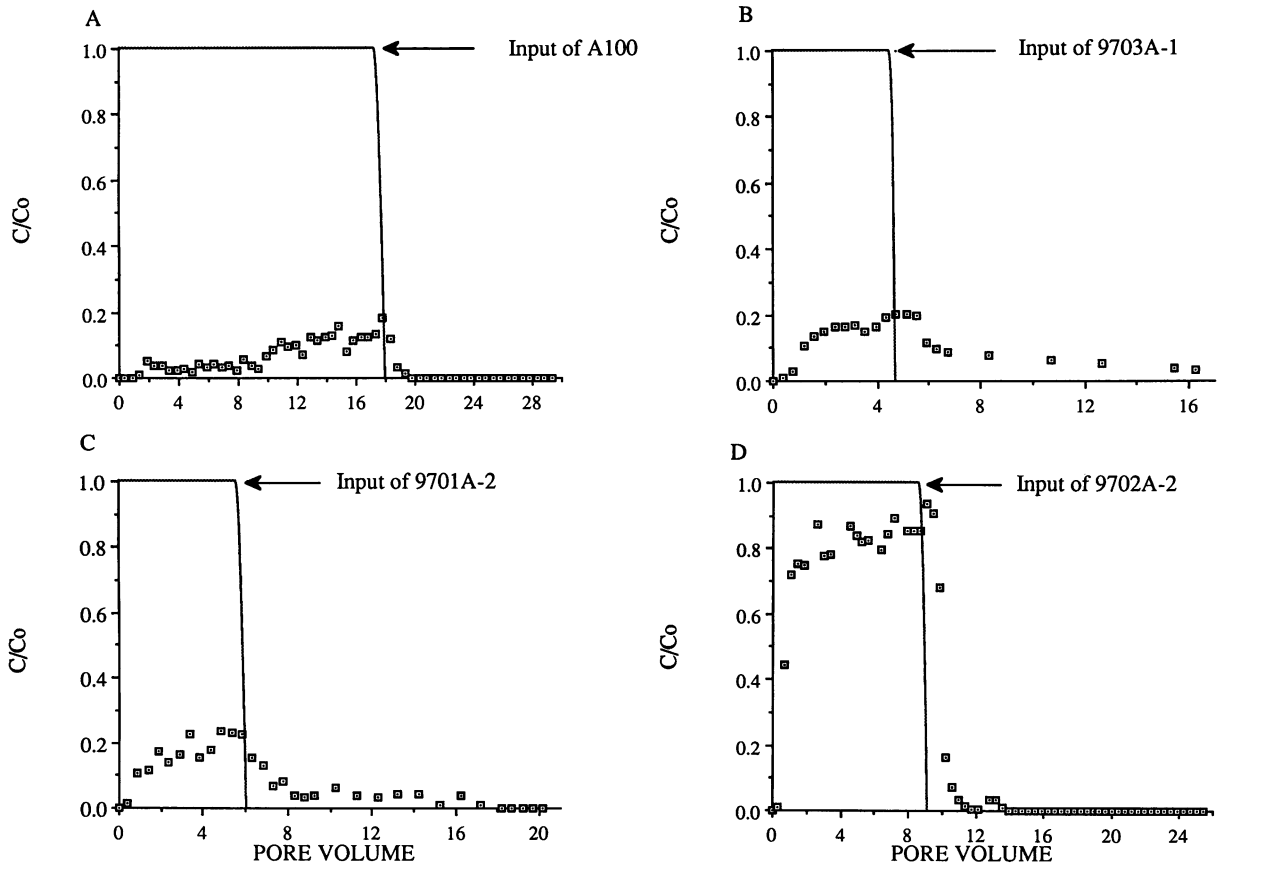

FIG. 2. BTCs for bacterial isolates A100 (A), 9703A-1 (B), 9701A-2 (C), and 9702A-2 (D). Lines indicate the duration (in pore volumes) of the input of cells to the sand column.

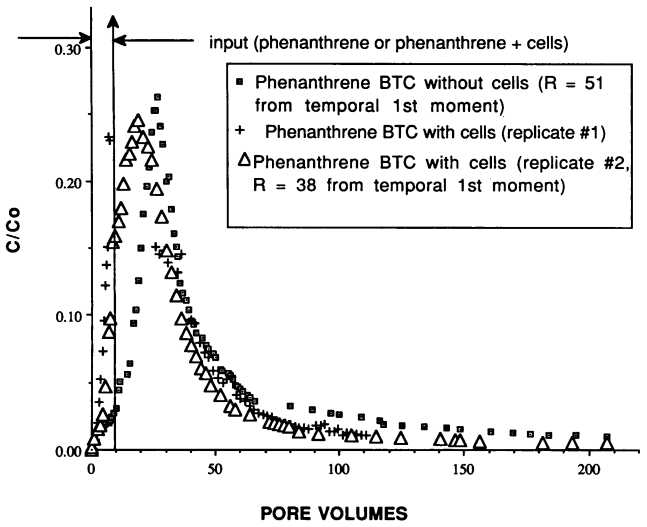

FIG. 3. BTCs determined for phenanthrene with and without the presence of isolate 9702A-2. Datum points represent the relative phenanthrene concentrations in the column effluent. Phenanthrene retardation in the first experiment with cells (replicate 1) was not calculated because of a gap in the BTC resulting from a malfunction in the fraction collector.

0.41, and 0 for A100, 9703A-1, 9701A-2, and 9702A-2, respectively, were significantly lower than the K_d value for phenanthrene with the aquifer sand. K^s_{cell} values of >0 were indicative of reversible sorption, and slow desorption kinetics were indicated by the tailing of the BTCs.

Phenanthrene BTCs determined with and without a bacterial carrier. Replicate BTCs indicated that the presence of 65 μg of TOC ml^{-1} (equivalent to ~5×10^7 starved cells ml^{-1}) of isolate 9702A-2 reduced the R value for phenanthrene in the aquifer sand by approximately 25% (Fig. 3). These data show that the interaction between the ability of a cell to sorb hydrophobic compounds, such as phenanthrene, and the mobility of the cell in porous material can result in the enhanced transport of hydrophobic pollutants. Although the partition coefficient for phenanthrene with isolate 9702A-2 was about four times less than the K_{oc} for phenanthrene with the aquifer sand and 14% of the cells were retained in the column, the combined effects of the sorption of phenanthrene to 9702A-2 and the mobility of 9702A-2 facilitated the transport of phenanthrene.

Model predictions. The predicted reduction in the K_d value for phenanthrene with subsurface isolate 9702A-2 was compared with observed values (Fig. 4A). The predictions for enhanced transport of phenanthrene in the presence of isolate 9702A-2, which had a K^s_{cell} value of 0 (R = 1), conformed closely to the values observed in both the batch and the column experiments. Model calculations were also performed to predict the batch experiment results for bacterial isolates with lower mobilities (isolates A100 and 9703A-1). Irreversible removal of cells was not considered by the model and, as anticipated, the model predictions did not conform as closely to the values observed for these isolates. The observed reduction in the K_d value for phenanthrene with isolate A100 was between the model calculations in which cells were assumed to be entirely mobile (K^s_{cell} = 0) and reversibly sorbed (K^s_{cell} = 0.53), as was observed in the cell mobility experiments (Fig. 4B).

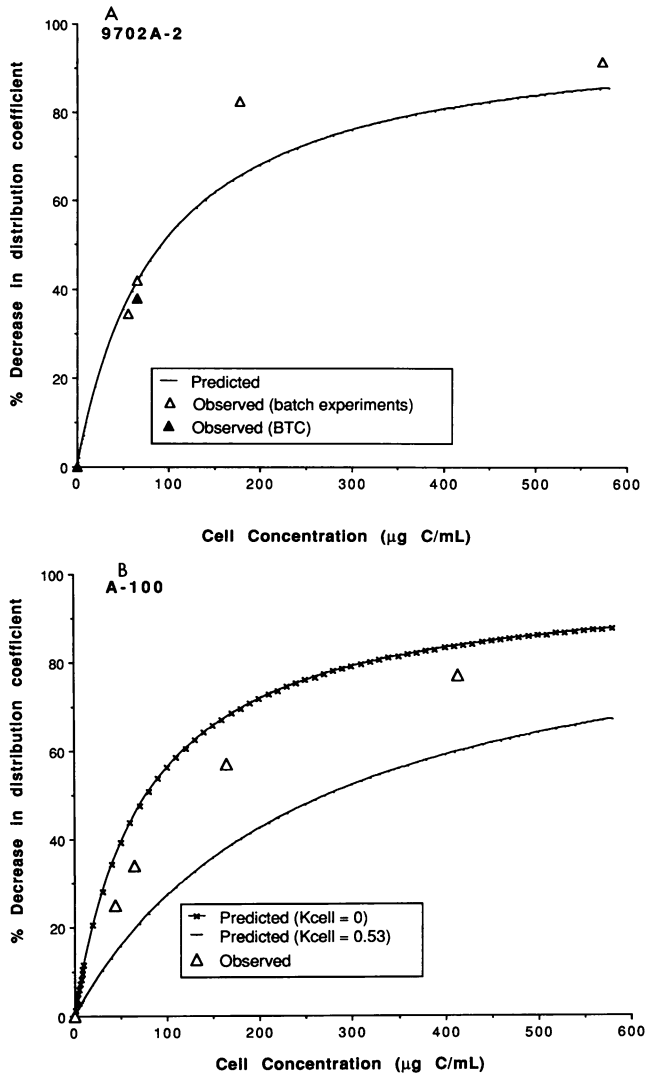

FIG. 4. Predicted and observed decreases in K_d values for phenanthrene with aquifer sand, determined by use of the model developed by Magee et al. (25). Percent reduction was calculated as $[(K^s_d - K_d$ observed or predicted$)/K^s_d] \times 100$. Kcell = $(R - 1)(\eta/\rho)$.

DISCUSSION

Sorption of phenanthrene by bacterial cells was observed in this research and has been reported by other investigators (36). Hydrophobic contaminants, such as hexachlorobenzene (HCB) and dichlorodiphenyltrichloroethane (DDT), are also known to sorb to cells (5). In contrast, Bellin and Rao (6) recently reported negligible sorption of naphthalene by a bacterial biomass of ~10^7 cells g of $soil^{-1}$. It is important for the purposes of prediction of pollutant transport to be acquainted with the distribution coefficients for specific pollutants, such as phenanthrene, with a spectrum of bacterial types, including those isolated from soil and subsurface environments (5). The data in Table 3 contribute to this scant data base.

Steen and Karickhoff (36) showed differences in K^{cell}_d values between several mixed cultures but not individual isolates. Other researchers showed such differences in sorption to selected bacterial strains for environmental pollut-

ants, such as DDT (5, 7, 19, 22). Previously reported K_{oc} values for phenanthrene with organic matter derived from soils (25) (K_{oc} = 43,000) and sediments (21) (K_{oc} = 22,900) were higher than the K_{oc} value for phenanthrene with cells (K_{oc} = 3,000 to 11,100) observed in this research. The disparity in the K_{oc} values for phenanthrene with bacterial cells and soil organic matter may indicate a difference between these two potential carriers of nonpolar pollutants. Similarly, Chin and Gschwend (8) noted significant differences in the K_{oc} values for pyrene with organic colloids from different sediments. This observation indicates that generalizations regarding various colloidal carriers, including cells, may lead to erroneous conclusions.

On the basis of the MATH test (33, 34), cell surface hydrophobicity appeared not to be a factor in the ability of isolates to sorb phenanthrene. Lindqvist and Enfield (22) made similar observations regarding the sorption of HCB and DDT. In fact, two of the isolates that most reduced the K_d value for phenanthrene with the aquifer sand were less hydrophobic than the *A. calcoaceticus* strain. Many isolates that reduced the K_d value for phenanthrene with the aquifer sand to a greater extent than did *A. calcoaceticus* were hydrophilic, as defined by the MATH test. Although the reported differences between organic colloids in their ability to sorb the PAH pyrene have been attributed to differences in lipid content or nonpolar character (8), there appeared in this research to be no connection between the affinity of phenanthrene for a bacterial isolate and its cell surface hydrophobicity.

The mobility of bacterial cells through a porous medium is likely to have a major impact on their ability to enhance the transport of nonpolar compounds. Two factors may affect the transport or mobility of bacteria through a porous medium: (i) filtration, or physical blockage by pores smaller than the cells (9), and (ii) sorption, or the reversible attachment to and detachment from the surface of the porous medium (26). The ionic strength of the soil solution may influence both of these factors. Some bacterial cells may behave like an inert tracer and thus have an R value of 1. Such was the case with isolate 9702A-2, which appeared to show no indications of sorption to the aquifer sand. The lack of complete mass recovery of this isolate may have been indicative of filtration. In contrast, isolate 9711A-2 was "irreversibly" retained (over the duration of the experiment) by the packed aquifer sand. The incomplete mass recoveries of isolates 9703A-1, 9701A-2, and A100 were presumed to be the result of either filtration or sorption, as evident in the tailing of the BTCs for 9703A-1 and 9701A-2 (22). Lindqvist and Enfield (22) suggested that irreversible sorption, not physical straining or filtration, was significant in cell retention. Harvey and Garabedian (16) also maintained that straining of their tracer bacteria in field tests was likely to be insignificant because of the relationship between the median grain size of the porous material and the dimensions of the bacteria. If the diameter of a colloid is <5% of the diameter of the particles in a porous medium, straining is generally considered to be insignificant (28). The cell dimensions of the isolates tested in this research were significantly less than 5% of the diameter of the particles constituting 95% of the aquifer sand.

The isolates tested for mobility were chosen on the basis of their capacity to reduce the K_d value for phenanthrene with the aquifer sand used in this study. The differences in mobility among the isolates are probably attributable to multiple factors. Gannon et al. (12, 13), for example, observed no correlation between cell surface hydrophobicity, net surface charge, presence of capsular material, or flagella and the variability in transport noted for several genera of bacteria. They found that the apparent filtration of cells was correlated with cell size and was dependent on ionic strength. Too few isolates were tested in this study to make meaningful correlations; however, the variable mobility of isolates 9703A-1 and 9711A-2, both of which displayed some hydrophobicity and were two times larger than isolates 9701A-2, 9702A-2, and A100, suggested that factors other than cell surface hydrophobicity and size affected bacterial mobility. A significantly larger fraction of 9701A-2 than of 9702A-2 was retained by the aquifer sand. These differences also suggest that factors other than cell dimensions governed retardation or filtration (at a constant ionic strength).

Isolate 9702A-2, like the other bacterial isolates tested, showed a capacity for binding phenanthrene. Because of its mobility, 9702A-2 moved through the packed aquifer sand more quickly than phenanthrene and consequently enhanced the transport of phenanthrene that was sorbed to these mobile cells. The decrease in the R value for phenanthrene in the presence of 9702A-2 shows that components of indigenous bacterial communities may significantly enhance the transport of phenanthrene and, by extension, perhaps other PAHs in aquifer environments. The cell concentration of 65 μg of TOC ml^{-1} used in the column experiments corresponded to $\sim 5 \times 10^7$ cells ml^{-1}, a value on the high end of the natural range of total bacterial densities associated with contaminated subsurface soil environments (14). Recent research by Bellin and Rao (6) indicates that the presence of bacteria in soils may also alter PAH transport by modification of the sorptive properties of the soils.

The comparison between predicted and observed values for the decrease in the K_d value for phenanthrene with the aquifer sand indicated that the three-component model developed by Magee et al. (25) may be successfully applied to mobile bacterial cells. The calculated reduction in pollutant retardation is related to cell concentration, with increased mobility being anticipated at higher cell concentrations. Engineered forms of aquifer remediation that introduce high concentrations of mobile cells or stimulate their growth in situ may merit further research.

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