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Impact of Bacterial Biomass on Contaminant Sorption and Transport in a Subsurface Soil†

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The objective of this study was to investigate the impact of bacterial biomass on the sorption and transport of three solutes (quinoline, naphthalene, and ^{45}Ca) in a subsurface soil. Miscible displacement techniques were employed to measure sorption and transport of the above compounds during steady, saturated water flow in sterile and/or bacterium-inoculated soil columns. The soil was inoculated with either a quinoline-degrading bacterium, *Pseudomonas* sp. 3N3A isolate, or its mutant isolate, B53, which does not degrade quinoline. In soil columns inoculated with the B53 and 3N3A isolates, quinoline sorption was reduced by about 60 and 20%, respectively. In contrast, ^{45}Ca sorption was minimally reduced, which indicated that biomass did not significantly alter the cation-exchange capacity of the soil. Biomass impacts on sorption were solute specific, even when the sorption mechanism for both quinoline and ^{45}Ca was similar. Thus, the differential response is attributed to biomass-induced changes in quinoline speciation; an increase in pH at the sorbent-water interface would result in a larger proportion of the neutral species and a decrease in sorption. Sorption of naphthalene was reduced by about 30%, which was attributed to accessibility of hydrophobic regions. Minimal biosorption of all solutes indicated negligible biofacilitated transport. Alteration of the soil surfaces upon addition of bacterial biomass reduced sorption of quinoline and naphthalene, thereby enhancing transport.

Bioremediation practices attempt to increase microbial activity or populations in order to degrade organic contaminants present in soils or aquifers. Indigenous microbial activity and/or populations may be increased by providing nutrients essential for bacterial growth, or axenic bacterial cultures known to degrade specific compounds may be injected directly into contaminated sites. Growth or addition of bacteria may drastically alter the chemical and physical characteristics of solid surfaces (17). Therefore, the impact of bacterial biomass on contaminant behavior in porous media near hazardous waste sites is of interest.

In addition to contaminant biodegradation, addition of bacteria to porous media may result in (i) bacterial growth or transport through the porous media, leading to pore clogging as a result of physical straining; (ii) biosorption and bacterial migration, facilitating contaminant transport; and (iii) bacterial sorption onto soil surfaces, altering the sorption capacity. Although bacterial migration through sandy soils and aquifers is well documented, bioremediation attempts have failed, among other reasons, because of the inability of injected bacteria to reach contaminated sites (18). Physical, chemical, and microbial factors controlling bacterial transport in porous media have recently been summarized (20, 30, 45). Bacterial transport may be limited by physical constraints imposed by the porous media, such as soil structure and pore size distribution (30). Straining or filtration occurs in soils and aquifers when bacteria are too large to pass through soil pores; this results in pore clogging, which restricts further penetration of bacteria (20, 22). Once bacteria become clogged in the soil pores, water flow is also restricted, and the path of water flow can be altered (48).

Chemical constraints, such as adsorption of bacteria, may also limit bacterial migration through soils and aquifers (1, 20, 21, 45). Bacteria that are hydrophobic and are minimally charged have the greatest potential to sorb onto surfaces; however, many other factors may influence bacterial attachment (51). Because of bacterial adsorption by soils (15) and clay minerals (43), the contaminant sorption capacity of the soil may be altered. Bacteria grow after they attach to surfaces if essential carbon and energy sources are available. Growth and development of bacterial colonies generally coincide with the production of extracellular polysaccharides and promote the formation of bacterial biofilms (17, 51). Bacterial biomass, therefore, contains live and dead cells and cell extracts (extracellular polymers). Under nutrient- and substrate-rich conditions, as may be the case near waste sites, biofilm formation may create diffusional barriers leading to nonequilibrium sorption of contaminants. This is generally the case for wastewater treatment by filtration through activated carbon beds (35, 41). Bacterial biomass may physically alter the accessibility of sorption sites, thereby reducing contaminant sorption. To further complicate the problem, bacterial biomass may act as an additional sorbent, thereby increasing contaminant sorption.

Sorption by various microorganisms in aquatic systems has been shown for hydrophobic organic chemicals (HOCs) (2, 47), metals (37), and organic amines (14). A consensus on biosorption mechanisms has not been reached, and usually no distinction is made between sorption onto extracellular regions and absorption into the cells. Properties such as aqueous solubility and log K_{ow} (K_{ow} = octanol water partition coefficient) for the contaminant (38) and bacterial lipid content (5) have been correlated to biosorption of HOCs. Biosorption of trace metals has been shown to occur via adsorption onto extracellular bacterial capsules with minimal intracellular uptake (37). Sorption of organic amines by algae has also been described by mechanisms including ion exchange and hydrophobic bonding (14). Occurrence of biosorption and bacterial migration, regardless of the underlying mechanisms, suggests the potential for biofacilitated transport of contaminants. Lindqvist and Enfield (29) dem-

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onstrated bacterium-facilitated transport of two HOCs (dichloro-diphenyl-trichloroethane and hexachlorobenzene) in sand columns. Biosorption technology has been commercialized to mobilize metals in the mining industry (16). However, biofacilitated transport of ionizable organic bases has yet to be demonstrated.

The objective of this study was to investigate the impact of bacterial biomass on the sorption and transport of three solutes (naphthalene, ^{45}Ca, and quinoline) in a subsurface soil. These compounds were selected because of their known specific interactions in soil: (i) naphthalene was selected to probe hydrophobic interactions with the nonpolar organic phase; (ii) ^{45}Ca was selected to probe electrostatic interactions with the cation-exchange sites; and (iii) quinoline, a N-heterocyclic organic base, was selected because it can exist as a neutral organic compound interacting with the organic phase or as a quinolinium ion interacting with cation-exchange sites. Miscible displacement techniques were used to measure sorption and transport of the above compounds during steady, saturated water flow conditions through homogeneously packed, sterile or bacterium-inoculated, soil columns. A fine-texture silt loam soil (Norborne; fine-loamy, mixed, mesic Typic Argiudoll) was chosen for these experiments because of the extensive characterization of quinoline sorption by this soil (53, 54). Sorption of naphthalene by the organic fraction of soil is well documented (13, 24). Preinoculation of the Norborne soil with bacteria (10^8 CFU g^{-1}) simulates contaminated subsurface soils and aquifers where bacterial populations may be high.

MATERIALS AND METHODS

Soil. The Norborne subsurface soil sample used in this study was collected from the C horizon (112- to 165-cm depth) at a field site in Missouri. This soil sample has an organic carbon content of 0.16%, a cation-exchange capacity (CEC) of 11.9 cmol(+) kg^{-1}, and a pH of 6.8 in 0.005 M $CaCl_2$. The cation-exchange capacity is predominately due to smectite-type clay minerals with the cation-exchange sites located in clay interlayer positions. The soil (<2 mm) was incubated for 24 h at 15% water content and sterilized by steam autoclaving for 30 min. The process was repeated two additional times while maintaining the initial soil water content. The autoclaved soil was confirmed to be sterile by plating autoclaved soil slurries on tryptic soy agar plates. The soil was then dried at 60°C for 24 h and used in all experiments.

Sorbates. Pentafluorobenzoic acid (PFBA; 150 μg ml^{-1}) was used as a conservative, nonsorbing tracer to assess the hydrodynamic dispersion and extent of physical nonequilibrium conditions prevailing during transport through the soil columns (12). Quinoline and naphthalene concentrations in the influent solutions for the column studies ranged from 4 to 10 μg ml^{-1}. Isotopic exchange of ^{40}Ca and ^{45}Ca (6,000 dpm ml^{-1}) was also investigated. Aqueous solutions of the chemicals were prepared in filter-sterilized (pore size, 0.2 μm) 0.005 or 0.05 M $CaCl_2$.

Bacterial strains and culture conditions. A strain of *Pseudomonas* sp. 3N3A capable of degrading quinoline and a mutant strain (B53) derived from the 3N3A strain were obtained from Brockman et al. (7). Incorporation of two proteins for bacterial enumeration rendered the organism incapable of degrading quinoline (32). The B53 isolate was used to determine the impact of biomass on sorption and transport of quinoline where degradation was not a factor.

The B53 and 3N3A strains were grown to mid-exponential phase (17.5 h) on tryptic soy broth (3 g $liter^{-1}$) at 28°C on a rotary shaker (100 rpm). Bacterial cells were harvested by centrifugation, washed two times and diluted to the desired bacterial density with the appropriate background matrix solution (0.005 or 0.05 M $CaCl_2$). Bacteria were allowed to equilibrate overnight in the desired matrix prior to each experiment. Standard plate count techniques were used to quantify bacterial populations. Plate counts were verified by visual inspection of bacterial suspensions with a hemacytometer. Bacteria tryptic soy agar plates were incubated at 28°C. A phase-contrast microscope (Wild Neenbrugg) was used for counting the bacteria in the hemacytometer.

Bacterial inoculation. A 0.5-ml aliquot of the appropriate bacterial suspension was placed in an aspirator. The sterile soil (50 g) was thinly spread on aluminum foil, and the bacterial suspension was sprayed on the soil in a fine mist. The soil sample was mixed thoroughly to ensure homogeneous distribution of the bacteria. The aspirator was rinsed with a 0.5-ml aliquot of filtered (pore size, 0.2 μm) $CaCl_2$, and the rinsate was sprayed on the soil. The initial inoculation rate was 10^6 CFU g of $soil^{-1}$ unless otherwise indicated. The soil was mixed again, and a subsample was taken for water content determination. The soil-water content following bacterial addition ranged from 5 to 10%.

Column studies. Miscible displacement techniques, similar to those described by Brusseau et al. (8), were used to characterize the transport of PFBA, ^{45}Ca, quinoline, and naphthalene. The sterile or bacterium-inoculated soil was packed into a Kontes glass column (5 cm long, 2.5 cm inside diameter). Bed supports on both ends of the column consisted of a Teflon diffusion mesh with a glass membrane porous filter (pore size, 1 μm). The high-performance liquid chromatography (HPLC) pumps (Gilson model 302) and tubing were disinfected by rinsing with methanol. Glass columns and solution vessels were sterilized by autoclaving. After packing, approximately 150 pore volumes of 0.005 or 0.05 M $CaCl_2$ solution was pumped through the column to achieve saturated, steady water flow conditions and uniform bacterial populations (10^8 CFU g^{-1}). Dissolved oxygen (DO) in the soil column effluent was measured at different pore velocities from 0.6 to 90 cm h^{-1}. A vessel was purged with N_2, effluent from the column was introduced, and DO was measured with a DO electrode (Yellow Springs Instruments 5750).

Soil columns varied in bacterial density and type (sterile or inoculated with either B53 or 3N3A isolate) and in ionic strength (0.005 or 0.05 M) of the displacing solution. Solute concentrations were monitored continuously or by collecting column effluent fractions. Quinoline and naphthalene were analyzed continuously with a flow-through UV detector (Gilson Holochrome or Milton Roy LDC) at 230 and 274 nm, respectively. Detector response was recorded with a strip chart recorder (Fisher series 5000). Effluent samples were collected intermittently and analyzed by HPLC-UV techniques (Gilson 115 UV detector, Gilson model 302 pump, Waters WISP 710B autosampler, and HP333492A integrator) to verify sample purity and to compare the initial solute concentration to the maximum effluent concentration. Quinoline was eluted from a reversed-phase column (Supelco LCPAH column) at a flow rate of 1 ml min^{-1} with a mobile phase of 10:10:80 (vol/vol/vol) methanol, acetonitrile, and water adjusted to pH 2 with HCl. On the same column, naphthalene was eluted at 2 ml min^{-1} with a mobile phase of 50:50 (vol/vol) acetonitrile and water at 2 ml min^{-1}. Soil column effluent pH was monitored on-line with an Ingold

microelectrode (27). Effluent fractions of the radiolabeled compounds were collected with an automatic sample collector (ISCO model 273). The activity of each radiolabeled compound was assayed with a liquid scintillation counter (Searle Delta 300). Low quenching required no correction for counting efficiency, and all counts were corrected for background counts.

Biofacilitated transport. (i) Bacterial migration. Bacterial movement in the Norborne soil was determined by packing the outlet half (2.5 cm) of a column with sterile soil, while the inlet half (2.5 cm) was packed with B53-inoculated soil (10^6 CFU g^{-1}). The column effluent was sampled, diluted, and plated to determine bacterial populations. After 7 days of flow, the column was sectioned into 1-cm segments, and bacteria were extracted with a pH 7.3 phosphate saline solution, diluted, and plated (which was recommended as a standard microbial technique) (52).

(ii) Biosorption studies. Sorption of quinoline by live cells of the B53 and 3N3A isolates was measured at a bacterial density of 10^8 CFU ml^{-1}. The initial quinoline concentrations were 1, 4, and 8 μg ml^{-1}. Bacterial suspensions were equilibrated with quinoline at 5°C for 1 h to minimize intracellular uptake and possible biodegradation by the 3N3A isolate. Biosorption of ^{45}Ca was measured at room temperature (22 to 25°C). Samples were centrifuged for 20 min at 1,250 × *g* at 5°C to separate the cells from the aqueous phase. Quinoline solution concentrations were measured by HPLC to monitor for possible biodegradation products. Biosorption was calculated as the difference in the initial and final solution concentrations. Miscible displacement techniques described earlier were employed to measure biosorption by bacteria attached to glass microbeads. Glass microbeads (average diameter, 150 μm; Alltech Associates) were inoculated with 10^7 CFU g^{-1}, packed into a column, and saturated with 0.05 M $CaCl_2$ for 48 h at a pore-water velocity of 13.5 cm h^{-1}. Breakthrough curves (BTCs) for quinoline, ^{45}Ca, and naphthalene were measured simultaneously by injecting a mixture of these three solutes on the column; this was done so that BTCs for all three solutes were obtained under identical hydrodynamic and microbial conditions. Effluent fractions were collected and monitored by HPLC-UV for quinoline and naphthalene and by radioassay techniques for ^{45}Ca.

Surface alteration. Electrophoretic mobility and hydrophobicity of the bacteria were measured in a mineral salt solution described by Brockman et al. (7) to assess the potential for sorption of the two isolates. The zeta potential of the 3N3A and B53 isolates was measured with a Laser Zee Meter (PENKEM model 501). The bacterial buffer solution (≈30 ml) was placed in an electrophoresis chamber consisting of two electrodes and a connecting chamber. The rate of bacterial movement in a known electric field was monitored through a microscope with a 20× objective lens and a 15× ocular lens. All measurements were made at the stationary layer to avoid flow in the boundary layers. Zeta potential was converted to electrophoretic mobility by the Helmholtz-Smoluchowski equation (39).

The hydrophobic character of the bacterial isolates were determined by partitioning the bacterial isolates between hexadecane and a phosphate buffer solution by procedure used by Rosenberg et al. (36). Bacterial cells which partition into the hexadecane phase from the aqueous phase indicated that bacterial surfaces are hydrophobic.

Surface accessibility. Sorption nonequilibrium can be described with the bicontinuum model (11). Constraints to sorption equilibrium may arise from transport-related processes (e.g., solute transfer from the mobile and immobile regions of a heterogeneous flow domain), or sorption-related processes (e.g., rate-limited sorption reactions between the chemical and the soil). Organic solute sorption has been represented as a two-step process in which the sorbent has two regions of sorption. Sorption in the first region is instantaneous, while the rate of sorption in the second region is kinetically constrained. The two-region or bicontinuum model is conceptualized as follows: $C \leftrightarrow S_1 \leftrightarrows S_2$, where C is the solution-phase concentration (M $liter^{-3}$), S_1 is the concentration in the instantaneous region (M M^{-1}), and S_2 is the concentration in the kinetically controlled region (M M^{-1}).

The bicontinuum model has been coupled to the advective-dispersive solute transport equation (9), with the following four dimensionless model parameters to define transport and sorption:

$$P = [vL/D]$$

$$R = [1 + (\rho/\theta)K_d]$$

$$\beta = \{[1 + (F\rho/\theta)K_d]/R\}$$

$$\omega = \{[k_2(1 - \beta)RL]/v\}$$

where v is the average pore-water velocity ($L\ T^{-1}$), L is the length of the column, D is the hydrodynamic dispersion coefficient ($L^2\ T^{-1}$), ρ is the soil bulk density ($M\ L^{-1}$), θ is the volumetric soil water content, K_d is the equilibrium sorption coefficient ($L^3\ M^{-1}$), F is the fraction of sorption in the instantaneous regions, and k_2 is the first-order rate coefficient (T^{-1}). Of the four dimensionless parameters, hydrodynamic dispersion in the column is represented by the Peclet number (P), equilibrium sorption is represented by the retardation factor (R), β is the fraction of instantaneous retardation, and ω is the Damkohler number, which is proportional to the ratio of hydrodynamic residence time ($L\ v^{-1}$) to the time constant for sorption ($1\ k_2^{-1}$).

Retardation factors were calculated from area above the BTC for quinoline and naphthalene (33); a linear extrapolation technique was used to extend the BTCs to $C/C_o = 1$ in order to estimate the area above the BTC. For ^{45}Ca pulses, the R was calculated by moment analysis techniques (8). The curve-fitting program CFITIM (49), which is based on nonlinear least-squares optimization techniques, was used to estimate the Peclet number (P) from the BTC for PFBA. For nonsorbed solutes ($R = 1$), two model parameters can be optimized: P, and the solute pulse size (J). Since the pulse size was determined experimentally, only the value for P was estimated by fitting to the measured BTC for PFBA. For sorbed solutes ($R > 1$), five model parameters can be optimized: P, R, β, ω, and J. For ^{45}Ca, naphthalene, and quinoline BTCs, R was fixed (estimated as described above), J was experimentally determined, P was fixed as the value estimated from PFBA BTCs, and the values of nonequilibrium sorption parameters (β and ω) were estimated from parameter optimization with the CFITIM program.

A comparison of the estimated values of the model parameters for the sterile and inoculated soil columns were used for a quantitative assessment of (i) the hydrodynamic impacts, based on P; (ii) the changes in equilibrium sorption capacity, based on K_d; and (iii) the accessibility of sorption regions, based on F and k_2.

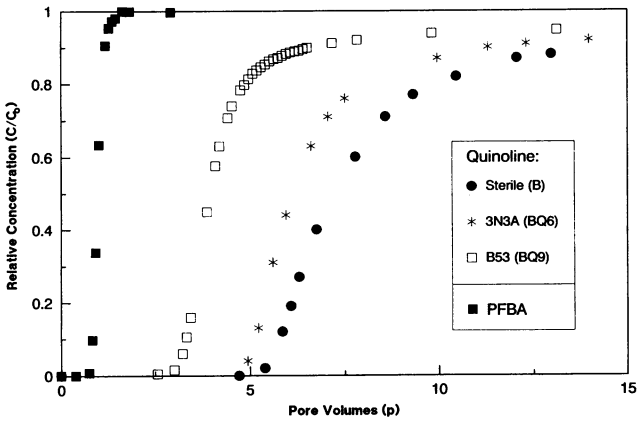

FIG. 1. Measured BTCs for PFBA in a sterile soil column and for quinoline in a sterile, 3N3A-inoculated, and B53-inoculated soil column. Column designations given in parenthesis correspond to those in Table 1.

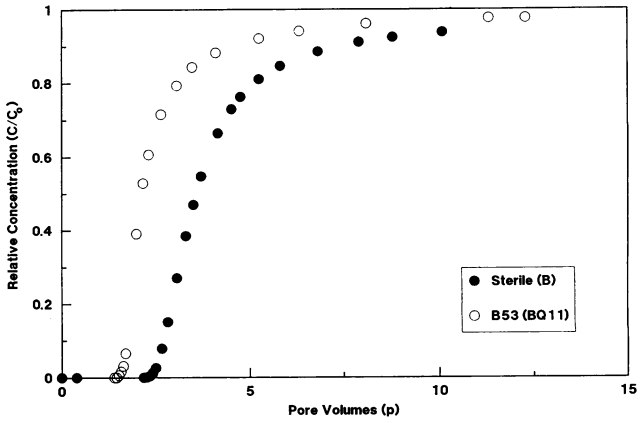

FIG. 3. Measured BTCs for naphthalene in a sterile and a B53-inoculated soil column. Column designations given in parenthesis correspond to those in Table 1.

RESULTS

The behavior of PFBA in sterile and bacterium-inoculated columns is represented by the PFBA BTC in Fig. 1. BTCs for quinoline (0.005 M $CaCl_2$) in sterile and inoculated (B53 and 3N3A isolates) columns are also shown in Fig. 1. BTCs for ^{45}Ca and naphthalene (0.05 M $CaCl_2$) in sterile and inoculated B53 columns are shown in Fig. 2 and 3, respectively. The PFBA BTCs for all soil columns were symmetrical and sigmoidal in shape, which suggests the absence of physical nonequilibrium (11), and $P > 98$ is indicative of minimal hydrodynamic dispersion.

Quinoline and naphthalene sorption was reduced in inoculated soil columns (Fig. 1 and 3). ^{45}Ca sorption (Fig. 2) was not reduced in the B53-inoculated soil columns. The shift in the ^{45}Ca BTC in the two bacterium-inoculated soil columns (BQ11 and BQ11-2) and the sterile column (B) resulted from differences in the bulk densities (ρ) and volumetric water contents (θ) of the various columns (Table 1). Therefore, direct comparison of R for different columns is misleading. The impact of bacteria on sorption and transport of quinoline, ^{45}Ca, and naphthalene was assessed by comparing the K_d values in sterile and inoculated columns. The K_d values verified that sorption of quinoline and naphthalene was reduced in inoculated columns, whereas ^{45}Ca sorption was not significantly different.

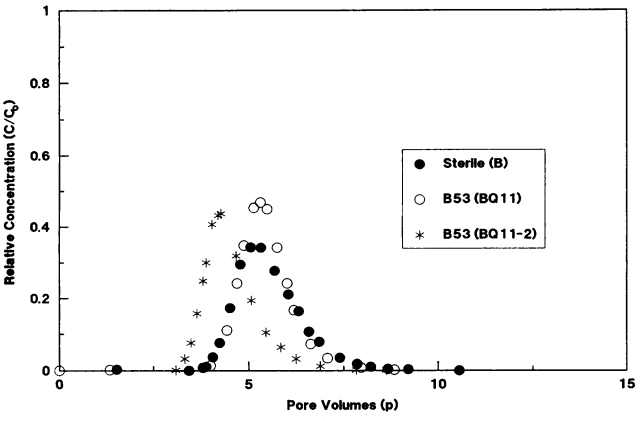

FIG. 2. Measured BTCs for ^{45}Ca in a sterile and B53-inoculated soil column. Column designations given in parenthesis correspond to those in Table 1.

The following results are from a series of experiments that were conducted to deduce the causes of reduced quinoline and naphthalene sorption. Experiments focused on distinguishing between the processes that may influence contaminant sorption and transport, including altered water flow resulting from pore blockage, biofacilitated contaminant transport, and/or altered sorption capacity of soil.

Pore blockage. Pore blockage or straining of bacteria was investigated by measuring BTCs for a nonadsorbed tracer (PFBA) once a day for 7 days following bacterial inoculation. Variations in pore volume determinations or asymmetrical BTCs would indicate changes in physical characteristics of the column. In all cases, the BTCs measured for PFBA were symmetrical (indicative of no changes in hydrodynamic characteristics) and the pore volume determined by PFBA remained constant (indicative of no blockage or exclusion of some pores). Therefore, early breakthrough of quinoline and naphthalene was not the result of pore blockage by bacterial biomass.

Biofacilitated transport. (i) Bacterial migration. Biofacilitated transport required verification of bacterial migration and biosorption. The appearance of 300 CFU ml^{-1} in effluent fractions after displacement of 75 pore volumes verified bacterial migration through the half-sterile and half-inoculated Norborne soil column. Bacterial counts were similar by using plate count techniques and by visual inspection with a hemacytometer. Therefore, bacterial populations were subsequently determined by plate counts. The bacterial density after 7 days of flow (13.5 cm h^{-1}) was 10^8 CFU g^{-1} at the inlet end of the column, 10^7 CFU g^{-1} in the three center sections of the column, and 10^6 CFU g^{-1} at the end of the column. Three observations were (i) increased bacterial densities verified bacterial growth, (ii) populations decreased from the inlet to the outlet end of the column in response to inoculation of the inlet 2.5 cm of the column, and (iii) bacteria migrated and populated the entire column, with the maximum population reaching 10^7 to 10^8 CFU g^{-1}. Maximum bacterial effluent concentrations ranged from 10^4 to 10^5 CFU ml^{-1}, confirming bacterial transport.

The bacterial population in the soil column was supported by nutrients and organic carbon released from the soil

TABLE 1. Column parameters and K_d values in Norborne C soil columns

Column designation	$CaCl_2$ (mol liter^{-1})	pH	ρ (g cm^{-3})	θ (cm^3 cm^{-3})	K_d (ml g^{-1})		
					Quinoline	Naphthalene	^{45}Ca
Sterile, BQ5	0.005	7.0	1.48	0.440	3.11		10.8
Sterile, B	0.05	6.2	1.54	0.415	3.11	0.946	1.17
B53, BQ9	0.005	6.8	1.46	0.449	1.39		
B53, BQ11	0.05	6.6	1.44	0.410	1.05	0.555	1.05
B53, BQ11-2	0.05	6.7	1.44	0.458			1.06
3N3A, BQ6	0.005	6.9	1.39	0.464	2.42		

matrix. Analysis of the column effluent confirmed the presence of trace quantities of essential elements for bacterial growth. Therefore, additional nutrients were not supplemented. Energy was likely derived from the dissolved organic carbon in the soil solution. Assuming a maximum bacterial population of 10^8 CFU^{-1}, bacterial dry weights of 16×10^{-13} g CFU^{-1} (19), and 50% of the bacterial cell is organic carbon (6), 8×10^{-5} g of organic carbon is required to maintain this population. The available dissolved organic carbon from soils has been estimated to be about 1% of the total organic carbon (34). Therefore, about 1.6×10^{-5} g of DOC per ml of soil solution may have been available, which can provide adequate energy for bacterial cell production.

Water flow may alter bacterial movement and the DO content, which in turn may influence the activity of microorganisms (28, 40, 46). In the Norborne soil columns, DO ranged from 0.5 to 2 mg liter^{-1} in the column effluent and increased with an increase in velocity (6 to 90 cm h^{-1}). As a result, subsequent experiments were conducted at about 15 cm h^{-1}. Transport of bacteria through the soil column is a necessary, but not a sufficient, condition for claiming biofacilitated transport of contaminants. It was also necessary to establish that the contaminant was sorbed to an appreciable extent by the bacterial biomass.

(ii) Biosorption. Quinoline and ^{45}Ca biosorption by the 3N3A isolate or its mutant B53 was not measurable by using batch techniques. However, variations in pH, nutrients, and availability of surfaces may alter the sorptive characteristics of microbial surfaces (4). Therefore, biosorption of quinoline and ^{45}Ca was determined directly in column experiments. Filtration (pore size, 0.2 μm) of the column effluent to separate biosorbed (trapped with the biomass on the filter) and free species (in the filtrate) showed no reduction in the solution concentration or accumulation on the filter. Therefore, biofacilitated transport of quinoline and ^{45}Ca by bacteria in the solution phase was not likely.

The extent of ^{45}Ca, quinoline, and naphthalene biosorption by adsorbed bacteria was determined by BTCs measured in a column packed with glass microbeads and inoculated with the B53 isolate (10^7 CFU g^{-1}). Miscible displacement techniques are preferred for estimating sorption parameters, especially in low-sorptive systems (11) (i.e., small K_d). The R for quinoline, ^{45}Ca, and naphthalene in a sterile, glass bead column was approximately 1, indicating no sorption of these solutes by glass bead surfaces. Thus, any retardation measured in the inoculated glass bead column is attributed to biosorption by the attached bacteria. Biosorption was small for ^{45}Ca (R = 1.15) and quinoline (R = 1.16), corresponding to a $K_d \approx 0.04$ ml g^{-1}, while naphthalene biosorption was slightly greater (R = 1.29; $K_d \approx 0.06$ ml g^{-1}) (Fig. 4). These results suggest that biofacilitated transport of ^{45}Ca, naphthalene, and quinoline is not likely to be important in our studies, unless high densities (>10^8 CFU ml^{-1}) of bacterial biomass are sloughed off into the column effluent.

Bacterial populations in the effluent of glass bead columns were 10^6 to 10^7 CFU ml^{-1}, which was higher than populations in the Norborne soil columns (10^5 CFU ml^{-1}). The increased bacterial populations may have been due to a larger pore size or a reduction in the sorption capacity of the glass beads versus the Norborne soil. Sorption of bacteria on glass surfaces and mechanisms of attachment have been documented (23, 31, 42, 51, 55). Therefore, larger pore size within the glass bead column likely reduced physical constraints and facilitated bacterial migration. Thus, biofacilitated transport may predominate in porous sandy aquifer material. Enhanced bacterial migration caused coatings to form on the UV cell which interfered with flowthrough detection of the column effluent. This suggests that fraction collection is essential to avoid analytical complications in highly porous media which are inoculated with bacteria.

Surface alteration. Alterations of soil surfaces by the addition of microorganisms may occur directly by bacterial sorption onto surfaces. Therefore, the potential for 3N3A and B53 to adhere onto surfaces was determined by measuring their electrophoretic mobility and hydrophobicity. The electrophoretic mobility of the 3N3A isolate ranged from -1.0 to -1.5 10^{-8} m V^{-1} s^{-1} from pH 4 to 8.5. Over the same pH range, the electrophoretic mobility of the B53 isolate ranged -0.5 to -1.0 10^{-8} m V^{-1} s^{-1}. These values are in agreement with electrophoretic mobilities measured over the same pH range (26) and electrophoretic mobilities (-0.42 to -3.42 10^{-8} m V^{-1} s^{-1}) measured for 23 bacterial isolates (50). The lower negative charge of the B53 isolate

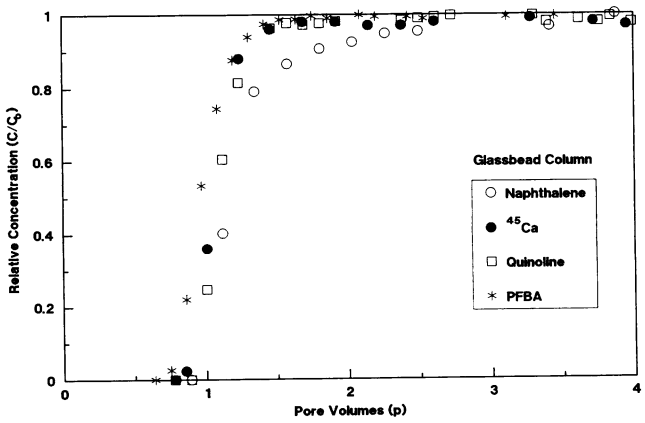

FIG. 4. Measured BTCs for PFBA, ^{45}Ca, quinoline, and naphthalene in a B53-inoculated glass bead column.

suggests that it has greater potential than 3N3A to approach the soil surface and attach.

The relative bacterial hydrophobicity (e.g., adsorption potential) of the two isolates was determined by measuring the bacterial distribution coefficient between the hexadecane phase and the aqueous phase (D_{HW}). At pH 7.5, the D_{HW} was 3 times larger for the B53 isolate (D_{HW} = 0.39 ml ml^{-1}) than the 3N3A isolate (D_{HW} = 0.12 ml ml^{-1}); however, at pH 6.5, the D_{HW} was 10 times larger for the B53 isolate (D_{HW} = 0.11 ml ml^{-1}) than the 3N3A isolate (D_{HW} = 0.01 ml ml^{-1}). The hydrophobicity of the B53 isolate is greater than the 3N3A isolate in the pH range of the soil columns. Both hydrophobic and electrostatic interactions favor sorption of the B53 isolate. Given that bacteria may attach, grow, and colonize the surface, the potential to alter the soil surface and more specifically the soil sorption capacity exists.

Evidence for alteration of the soil surface by bacterial biomass was suggested in an inert quartz sand (<2-mm) column. The effluent pH from a B53-inoculated quartz sand column was 4.65 upon introduction of PFBA (pK_a = 1.59), while the pH of PFBA passing through the sterile quartz sand column was pH 3.2. The quartz sand has no appreciable buffer capacity for maintaining the pH of the acidic PFBA solution. Therefore, the pH increase in the bacterium-inoculated column suggests that the bacterial biomass altered the soil surface environment (i.e., bacteria have an inherent buffer capacity). Changes in bulk solution pH were not observed for the experiments with the Norborne soil column because of the larger buffer capacity of this soil. This does not, however, preclude the possibility that alteration of pH had occurred within the interfacial regions for the Norborne soil. Since it is difficult to measure any changes in interfacial pH directly, we can only infer here the trends based on observed effects on quinoline sorption by the Norborne soil.

The addition of bacteria influenced quinoline retention more than the sorption of naphthalene or ^{45}Ca. Therefore, quinoline was used to further investigate the differences between the effects of 3N3A and B53 isolates on sorption and transport. The presence of the B53 isolate reduced the sorption of quinoline by about 60%, and 3N3A reduced quinoline sorption by about 20% (Table 1). Despite the differences in inoculation rate (10^6 CFU of 3N3A g^{-1}, and 10^5 CFU of B53 g^{-1}), the early quinoline breakthrough in inoculated (B53 versus 3N3A) columns was not likely due to variations in bacterial populations. To test the above hypothesis, soil columns were inoculated with the B53 isolate at 10^5, 10^6, and 10^7 CFU g^{-1}. Quinoline BTCs measured in each case were similar. Saturation of the soil columns prior to conducting the quinoline BTCs resulted in growth and colonization of soil surfaces. Bacterial densities of 10^8 CFU g^{-1} were supported in the Norborne soil supported independent of the initial inoculation rate and bacterial isolate. As a result, the differences in quinoline BTCs measured in the inoculated (3N3A and B53) soil columns were attributed to microbial surface characteristics and their impact on the soil surfaces.

Surface accessibility. An attempt was made to use the bicontinuum model to quantitatively assess the impacts of bacterial biomass on the physical accessibility of sorptive regions in the soil. However, bicontinuum sorption model analysis of the BTC data was attempted only for the naphthalene BTC data for the following reasons: (i) unpublished data suggest that quinoline sorption dynamics are more complicated than that conceptualized in the bicontinuum model, and (ii) cation-exchange kinetics are rapid enough that the bicontinuum model is not needed to describe ^{45}Ca BTCs; an equilibrium sorption model provides an adequate description (9).

The bicontinuum sorption model was used to fit the naphthalene BTC data and to evaluate the alterations in accessibility to sorptive regions of soil. About 60% (F = 0.63) of naphthalene sorption was surmised to have occurred instantaneously in the sterile soil, while F decreased to 0.33 in the inoculated column. About 50% reduction in the F value suggests that the accessibility of sorption regions to naphthalene had been reduced because of the presence of bacterial biomass. The k_2 (1.66 h^{-1}) and K_p (0.946 ml g^{-1}) from the sterile column are in agreement with the log-log-linear inverse relationship between log k_2 and log K_d values (log k_2 = 0.301 − 0.668 log K_p) reported for sorption of HOCs (10). However, the k_2 value estimated from the naphthalene BTC measured in the inoculated soil column was about a third of that for the sterile column (0.52 versus 1.66 h^{-1}), which is indicative of further constraints on naphthalene sorption. The analysis of model parameters suggests the following: (i) an overall reduction in naphthalene sorption (decrease in K_d), and (ii) a decrease in accessibility of sorption regions (decrease in both F and k_2).

DISCUSSION

The specific sorption mechanism for a solute may influence the impact of the microbial biomass on contaminant sorption and transport. For example, a compound undergoing electrostatic interactions, such as cation exchange, would exhibit reduced sorption if the specific exchange sites were inaccessible. Similarly, HOC sorption may be reduced if the biomass is less hydrophobic and reduces access to organic regions in which a nonpolar compound is sorbed. On the other hand, sorption of HOCs may increase if hydrophobic biomass remains on the soil surface and increases the overall hydrophobic nature of the soil. If, however, hydrophobic biomass is transported in the solution phase, biofacilitated transport may occur. For ionogenic compounds, bacterial biomass may cause interfacial variations in pH which would alter their sorptive behavior. The premise that bacterial biomass alters sorption of contaminants requires further definition of the locations of contaminant sorption as well as the bacterial colonies. This question is of great interest in remediation of contaminated soils and bioavailability of contaminants. However, the answer is not readily available. To facilitate the discussion, the following assumptions will be made: (i) HOC sorption occurs within the organic fraction of the soil; (ii) sorption of cations occurs predominantly on cation-exchange sites located within clay interlayers and aggregates; (iii) bacteria colonize soil surfaces as microcolonies (31, 48), and (iv) bacteria are assumed to adhere to soil surfaces in collocation with the energy and nutrient sources (e.g., organic matter and clay). The presence of bacterial biomass in soil may impact contaminant sorption directly by decreasing the accessibility of sorptive regions or indirectly by changing the interfacial properties of the soil.

Biofacilitated transport. Biosorption of quinoline and ^{45}Ca was not measurable; therefore, biofacilitated transport did not likely reduce retardation of these compounds. Naphthalene biosorption may have occurred in the column studies, resulting in biofacilitated transport in the inoculated columns. HOCs possessing a more hydrophobic nature are more apt to undergo biosorption and, therefore, biofacilitated transport. For example, DDT, a highly hydrophobic chemical, was strongly sorbed in sterile sand columns (R =

59.8), but displacement of bacterial solutions containing DDT through the sand columns demonstrated biofacilitated transport in which R was reduced eightfold (29). Biofacilitated transport may be most important in contaminated sites, where bacterial populations and chemical concentrations are high, and for chemicals exhibiting high biosorption potential. However, initial incubation of a contaminant with the soil prior to bacterial addition may reduce the potential for biofacilitated transport because of rate-limited contaminant desorption.

Surface alteration. Assuming that quinoline and ^{45}Ca access the same cation-exchange sites, sorption of ^{45}Ca and quinoline should be reduced to the same extent if bacterial microcolonies developed and access to exchange sites was inaccessible. Quinoline and ^{45}Ca sorption was not reduced in a similar manner, suggesting that biomass did not substantially alter the cation-exchange capacity of the soil. Biomass impacts on sorption were solute specific, even when the sorption sites for both quinoline and ^{45}Ca are similar. Thus, the differential response is attributed to biomass-induced changes in quinoline speciation; an increase in pH at the sorbent-water interface would result in a larger proportion of the neutral species and a decrease in sorption. Stucki et al. (44) suggested that microbial biomass modifies the redox status of clay minerals, resulting in the collapse of clay layers. Alteration of soil properties and the soil-solution interface by bacterial biomass may impact the behavior of ionogenic and inorganic compounds. The combination of biomass-induced changes in quinoline speciation and inaccessibility of sites likely reduced quinoline sorption.

Bacterial biomass may contribute to the measured increase in naphthalene sorption by adding hydrophobic microbial biomass to the soil. Bacterial populations in the soil columns were about 10^8 CFU g of soil^{-1}. Although plate counts possibly underestimated the total number of cells, it was reasonable to believe that 10^8 CFU g^{-1} were produced in the soil columns. The corresponding bacterial f_{oc} was 8×10^{-5}, and the soil had an f_{oc} of 0.0016. In this case, bacterial biomass added about 1% to the soil sorption capacity. Therefore, the bacterial biomass may not have contributed substantially to the organic carbon content. However, in low-organic-matter soils or sandy aquifer materials, contaminant sorption may be increased upon bacterial additions. Whereas quinoline and ^{45}Ca sorption was only reduced by the addition of bacterial biomass, naphthalene sorption may have been decreased by surface inaccessibility and biofacilitated transport or slightly increased by the addition of organic matter to the soil. The counteracting effects of these mechanisms likely decreased the magnitude of enhanced naphthalene transport.

Surface accessibility. Addition of bacterial biomass and production of extracellular bacterial polymers (3, 25, 48) may alter the ability of Norborne soil to sorb chemicals. This soil has a low organic carbon content (0.16%), and electrostatic interactions are primarily associated with the 2:1 clay interlayer positions. Bacterial biomass may have altered sorption of quinoline in intra-aggregate and interlamellar regions. Reduced accessibility of organic matter by bacterial biomass decreased naphthalene sorption.

The results presented here suggest that bacterial biomass can alter soil surfaces, thereby impacting contaminant sorption and transport. Earlier investigations generally focused on individual bacterial and contaminant properties; however, this study demonstrates the need for considering the interrelationship among microorganisms, surfaces, and contaminant behavior.

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