Simultaneous Transport of Two Bacterial Strains in Intact Cores from Oyster, Virginia: Biological Effects and Numerical Modeling

Hailiang Dong,^{1*} Randi Rothmel,² Tullis C. Onstott,¹ Mark E. Fuller,² Mary F. DeFlaun,²
Sheryl H. Streger,² Robb Dunlap,³ and Madilyn Fletcher³

Department of Geosciences, Princeton University, Princeton, New Jersey 08544¹; Envirogen, Inc., Lawrenceville, New Jersey 08648²;
and Earth Water and Science, University of South Carolina, Columbia, South Carolina 29208³

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The transport characteristics of two adhesion-deficient, indigenous groundwater strains, *Comamonas* sp. strain DA001 and *Erwinia herbicola* OYS2-A, were studied by using intact sediment cores (7 by 50 cm) from Oyster, Va. Both strains are gram-negative rods (1.10 by 0.56 and 1.56 by 0.46 μm , respectively) with strongly hydrophilic membranes and a slightly negative surface charge. The two strains exhibited markedly different behaviors when they were transported through granular porous sediment. To eliminate any effects of physical and chemical heterogeneity on bacterial transport and thus isolate the biological effect, the two strains were simultaneously injected into the same core. DA001 cells were metabolically labeled with ^{35}S and tagged with a vital fluorescent stain, while OYS2-A cells were metabolically labeled with ^{14}C . The fast decay of ^{35}S allowed deconvolution of the two isotopes (and therefore the two strains). Dramatic differences in the transport behaviors were observed. The breakthrough of DA001 and the breakthrough of OYS2-A both occurred before the breakthrough of a conservative tracer (termed differential advection), with effluent recoveries of 55 and 30%, respectively. The retained bacterial concentration of OYS2-A in the sediment was twofold higher than that of DA001. Among the cell properties analyzed, the statistically significant differences between the two strains were cell length and diameter. The shorter, larger-diameter DA001 cells displayed a higher effluent recovery than the longer, smaller-diameter OYS2-A cells. CXTFIT modeling results indicated that compared to the DA001 cells, the OYS2-A cells experienced lower pore velocity, higher porosity, a higher attachment rate, and a lower detachment rate. All these factors may contribute to the observed differences in transport.

Understanding bacterial transport in porous media is of great importance for successful implementation of bioremediation strategies in subsurface environments. One vital requirement for successful implementation of bioaugmentation, the injection of bacteria with degradative capabilities into the subsurface to remediate a contaminated aquifer (30, 36, 43), is the delivery of selected bacteria to and through the contaminated zone of an aquifer. In most bacterial transport studies, bacterial attachment to mineral grains significantly impairs effective dispersion of the introduced bacteria throughout the aquifer (8, 23, 25). Bacterial attachment to mineral grain surfaces is influenced by biological factors associated with the bacterial cells and by physical and chemical factors associated with a granular aquifer (12, 17, 22, 34).

The physical, chemical, and biological effects are typically grouped into two probabilities, as described in filtration theory: the probability of bacterial collision with a sediment grain collector upon approach (collector efficiency) and the probability of bacterial attachment to the collector upon collision (collision efficiency). The collector efficiency accounts for the physical factors (interception, diffusion, and gravitational settling processes) that control the frequency of bacterial collisions with grain surfaces relative to the flux of bacteria toward the collectors. The collision efficiency accounts for the chemical and biological factors described by the Derjaguin-Landau-

Verwey-Overbeek theory (10, 48). This theory describes the total interaction force between bacterial and mineral grain surfaces as the sum of the double-layer, London-van der Waals, and acid-base type (hydrophobic interaction) potential energies over the distance separating the surfaces (41). The injected bacteria are more likely to attach to mineral surfaces if the total interaction force is attractive than if the force is repulsive.

Previous studies have indicated that there are four major biological properties that affect bacterial attachment to mineral grain surfaces, bacterial surface charge (21, 47), hydrophobicity (32, 47), cell size (15, 19), and cell motility (16, 29, 33, 49). Bacterial surface charge is typically measured by electrophoresis (20, 46). The electrostatic interaction predicts that a negative cell surface charge promotes bacterial attachment to positively charged Fe and Al oxide surfaces but inhibits attachment to negatively charged quartz surfaces (14). In reality, this simple relationship is complicated by the presence of organic matter, which can alter the surface charges of both bacteria and sediment (27, 42).

Cell hydrophobicity has been investigated in relation to bacterial transport, and the results have been somewhat inconsistent. Whereas Gannon et al. (19) found no correlation between bacterial transport and cell hydrophobicity in soil columns, Mueller et al. (35) found a positive relationship between collision efficiency and hydrophobicity. Likewise, Mills (34) found an approximate positive correlation between the percentage of cells retained and hydrophobicity. Experiments on the effects of cell motility have also resulted in different outcomes. In some cases, motile cells have been shown to be transported

* Corresponding author. Present address: Department of Geology, Miami University, Oxford, OH 45056. Phone: (513) 529-2517. Fax: (513) 529-1542. E-mail: dongh@muohio.edu.

further (33), and in other cases, motile cells have been shown to attach to mineral grain surfaces more than nonmotile cells (29). This discrepancy may be related to substrate surface characteristics. Comesano and Logan (5) observed that motile cells were retained less in sediments due to the ability of the bacteria to swim and avoid collisions with grain surfaces.

Evidence that cell size has an effect on bacterial transport has been scarce, although it is generally recognized that smaller cells are transported more readily than larger cells. On the basis of a regression analysis of the relationship between the percentage of cells transported and cell surface properties, Gannon et al. (19) concluded that cell size was the only statistically significant parameter responsible for the observed differences in transport of 19 strains through soil columns. Fontes et al. (15) performed bacterial transport experiments by injecting two strains that had the same hydrophobicity but were different sizes (a 0.75- μm -diameter coccus and a rod that was 0.75 by 1.8 μm) into a number of columns of clean quartz sand in which the grain sizes were different (<0.44 and 1 to 1.14 mm) and the ionic strengths of the pore water were different. They concluded that cell size and ionic strength were of equal importance but were less important than grain size. However, they did not measure surface charge or the presence or absence of specific reactive groups on the cell surface, and it was therefore difficult to conclude if cell size was the most important factor controlling transport. These authors did imply that if the physical and chemical factors were kept equal, the most important biological factor was cell size.

Well-controlled bacterial transport studies have been conducted with columns filled with either glass beads or homogenized sediments. Because the homogenization and repacking process destroys the natural structure (i.e., physical and chemical heterogeneity), results obtained with repacked cores may not accurately represent the transport behavior of bacteria in the subsurface (22) and are difficult to extrapolate to field studies. This study is part of a U.S. Department of Energy-supported bacterial transport project being conducted on both laboratory and field scales. The main purpose of the project is to evaluate the relative importance of physical, chemical, and biological effects in bacterial transport. In preparation for field-scale experiments, extensive laboratory experiments with intact cores and a variety of indigenous groundwater bacterial strains have been performed (12, 13, 17). In previous work, we addressed the relative importance of physical and chemical factors in bacterial attachment to mineral grains. This study expanded on that work and addressed the importance of bacterial properties in the transport of two bacterial strains through a number of intact cores. Intact cores are often the most predictive means of assessing rates and distances of bacterial transport prior to injection in the field, so understanding core-scale processes and their relationship to field-scale transport is an essential step in predicting field-scale transport. This research can be distinguished from previous work because we used (i) intact cores, (ii) simultaneous transport of two bacterial strains so that any effect due to physical or chemical heterogeneity of cores could be eliminated, and (iii) extensive characterization of bacterial surface properties so that responsible biological effects could be identified.

MATERIALS AND METHODS

Field site and intact core collection. The Oyster site is located on the southern Delmarva Peninsula on the eastern coast of the United States between the Chesapeake Bay and the Atlantic Ocean (8). The sediments at this site consist of unconsolidated to weakly cemented, well-sorted, medium- to fine-grained Late Pleistocene sands and pebbly sands. The upper 5 to 9 m is in the Wachapreague Formation, and the sediments below 9 m are in the Butlers Bluff Member of the Nassawadox Formation. The procedure used for intact core retrieval is described elsewhere (12, 17). The three cores used in this study, HS54, SG11, and SG48, represent two of the three sedimentary facies identified at the site. The horizontally stratified facies (core HS54) has a mean grain size of less than 200 μm and a terrigenous gravel content of less than 10%. The shelly-gravelly facies (cores SG11 and SG48) has a mean grain size of 280 μm and abundant lithic fragments (>30%), as well as calcium carbonate shell material. The HS54 and SG11 experiments were performed with one bacterial strain (DA001), and the SG48 experiment was performed with two strains used simultaneously (OYS2-A and DA001).

Groundwater geochemistry. The groundwater at the Oyster site is oxygenated (6 to 8 mg of dissolved oxygen per liter); the pH values range from 5.4 to 6.0, and the temperature is $\sim 15^\circ\text{C}$. The dissolved organic carbon content ranges from 1 to 4 mg/liter (8). Oyster artificial groundwater was made based on the Oyster site groundwater chemistry but did not contain colloids and organic carbon (8). The artificial groundwater was used to culture bacteria for injection. Because of an insufficient supply of Oyster natural groundwater, natural groundwater from approximately 0.25 mile away at the Narrow Channel flow cell was used for the transport experiments in this study. Because of their close proximity, the groundwaters from the two flow cells were virtually identical (8, 17).

Bacterial strain isolation, culturing, and radiolabeling. The indigenous groundwater strains *Erwinia herbicola* OYS2-A and *Comamonas* sp. strain DA001 were isolated from the Oyster and Narrow Channel bacterial transport field sites, respectively. Isolation and identification of adhesion-deficient variants of these strains have been described elsewhere (12, 17). The inocula were grown and radiolabeled as described previously (17, 18). The DA001 cells injected into cores HS54 and SG11 were labeled with ^{35}S . The DA001 and OYS2-A cells injected into SG48 were labeled with ^{35}S and ^{14}C , respectively. It has been demonstrated previously (7, 18) that these radiolabels stay cell associated until the end of bacterial breakthrough (up to 250 h).

Cell surface characterization. (i) Electrostatic interaction chromatography. Electrostatic interaction chromatography was performed by using the protocol described by Dahlback et al. (6) with cells suspended in artificial groundwater.

(ii) Electrophoretic mobility. A laser Doppler velocimetric instrument (DELSA 440SX 2.03; Beckman-Coulter, Fullerton, Calif.) was used to measure the electrophoretic mobilities of DA001 and OYS2-A cells (14). The measurements were made under the following conditions: temperature, 25°C ; conductivity of the artificial Oyster groundwater, 0.29 mS/cm; frequency range, 500 Hz; voltage, 6 V; and on time and off time, 2.5 and 0.5 s, respectively. Although it would have been more relevant to measure electrophoretic mobility at 15°C , the in situ groundwater temperature at which bacterial transport experiments were run, it was not possible to place the DELSA 440SX 2.03 instrument inside an environmental chamber. The mobility values measured at 25°C were assumed to be the same as those at 15°C .

(iii) Bacterial cell size and morphology. Cell size and morphology were characterized by transmission electron microscopy using a procedure described previously (11). Cell size was measured on photographic negatives, and the values and errors reported below are averages based on 20 measurements.

(iv) Bacterial cell density. Cell density was determined by using a Percoll gradient method similar to that described by Harvey et al. (23). Brightly colored density marker beads (Sigma-Aldrich Company, St. Louis, Mo.) were used to indicate specific buoyant density values. The density marker beads and a sample (1 ml) of either a DA001 or OYS2-A cell suspension were carefully layered on top of a Percoll solution in 15-ml round-bottom test tubes. The tubes were centrifuged at $15,000 \times g$ for 1 h at room temperature. The density of cells was determined from the position of the bacterial band relative to the positions of the bands formed by the beads.

(v) Cell hydrophobicity. Hydrophobic interaction chromatography (6), bacterial adherence to hydrocarbons (BATH) (40), and contact angle measurements (4) were used to determine the hydrophobicities of OYS2-A and DA001 cells. The BATH procedure was performed with hexadecane, octane, and *p*-xylene as the hydrocarbon phases. Radiolabeled and starved OYS2-A and DA001 cells were washed and suspended in PUM buffer (40) at an optical density at 550 nm of 0.2. Samples (1.2 ml) of a cell suspension were added to round-bottom test tubes. For hexadecane, four different volumes (0.025, 0.05, 0.1, and 0.2 ml) were

TABLE 1. Transport experimental parameters

Core	Pore vol (ml)	Tracer pulse length (h)	Tracer flow rate (ml/h)	Bacterial pulse length (h)	Bacterial flow rate (ml/h)	Tracer run duration (h)	Bacterial run duration (h)	Organism	Isotope	Influent cell concn (cells/ml)	Total cells injected	Cells/dpm
HS54	754	4.18	90.0	4.63	82.4	24.75	343.75	DA001	³⁵ S	3.65×10^7	1.38×10^{10}	52
SG11	562	4.50	62.7	4.50	62.6	24.75	343.50	DA001	³⁵ S	3.65×10^7	1.03×10^{10}	52
SG48	530	5.67	50.1	5.67	50.1	238	238	DA001	³⁵ S	2.47×10^7	6.88×10^9	167
								OYS2-A	¹⁴ C	8.03×10^7	2.24×10^{10}	1,120
								Mixed		1.05×10^8	2.93×10^{10}	533

then added to triplicate tubes. The tubes containing the cell-hexadecane mixtures were incubated for 10 min at 30°C, after which they were vortexed for 2 min and then incubated at room temperature for 15 min, which allowed the hydrocarbon phase to rise to the top of the suspension. Known volumes of the hexadecane and of the aqueous phase (top and bottom portions of the suspension, respectively) were removed and counted with a model 1209 Rackbeta scintillation counter (Pharmacia LKB Nuclear, Gaithersburg, Md.). The experiment was then repeated using octane and *p*-xylene in duplicate with two different volumes of each hydrocarbon (0.2 and 0.05 ml). Contact angles were measured by the method of Brown (4) by placing droplets of 1-bromonaphthalene on a bacterial lawn and subsequently determining the angle of contact between each droplet and the lawn.

(vi) Characterization of LPS. Bacterial lipopolysaccharide (LPS) was prepared by a modification of the method of Hitchcock and Brown (24) for whole-cell lysates and proteinase K digestion. Bacteria were grown to the early stationary phase in 0.2% (wt/vol) sodium acetate in a basal salt medium at room temperature and washed, and the optical density at 550 nm in Narrow Channel site groundwater was adjusted to 1.0. Samples (6 µl) of LPS preparations were electrophoresed on a sodium dodecyl sulfate–15% polyacrylamide minigel at a constant voltage (200 V). The LPS gels were then silver stained and observed.

Intact core setup, conservative tracer and bacterial injections, and effluent sampling. The procedure used for intact core setup and injection is described elsewhere (9, 12, 17). Prior to bacterial injection, 0.5 core pore volume (Table 1) of bromide (Br) (~50 mg/liter) was injected into cores HS54 and SG11. After being starved for 48 h by shaking in Narrow Channel artificial groundwater at 15°C, ³⁵S-labeled DA001 cells were washed once, suspended in Narrow Channel site groundwater at the desired concentration, and injected into HS54 and SG11 in a 15°C environmental chamber. For conservative tracer and cell injection into SG48, starved OYS2-A and DA001 cells were premixed with ³H₂O (conservative tracer) to obtain a final total cell density of 1.05×10^8 cells/ml; 0.5 core pore volume of this cell suspension was injected into SG48. Once injection was complete, Narrow Channel site groundwater was continuously pumped into the cores until the effluent conservative tracer and bacterial concentrations were below the background levels (<1 mg/liter, ~30 dpm/ml, and ~30 dpm/ml for Br, ³H₂O, and bacteria, respectively), at which time the experiment was terminated. This time was approximately 30 h for the conservative tracers and 250 to 350 h for the bacteria (Table 1).

The procedures used for effluent processing and termination of the transport experiments are described elsewhere (12, 17). The radioactivities of effluent samples were counted with a Rackbeta scintillation counter. For SG48, the ³H radioactivity was counted in a window different from that used for counting ³⁵S and ¹⁴C, and there was no interference between ³H and either ³⁵S or ¹⁴C. Separate tests showed that the count for a sample containing both ³H and ¹⁴C (or ³⁵S) was the same as the sum of the counts for individual ³H and ¹⁴C samples. However, ³⁵S and ¹⁴C were counted in the same window, and the radioactivity represented a mixture of ³⁵S and ¹⁴C signals. A deconvolution procedure was therefore necessary to obtain individual concentrations of DA001 and OYS2-A (see below). To test the validity of the deconvolution method, 10 controls were used; these controls included pure DA001 (³⁵S only), pure OYS2-A (¹⁴C only), and mixtures of the two strains in various proportions (90% DA001 plus 10% OYS2-A, 80% DA001 plus 20% OYS2-A, 70% DA001 plus 30% OYS2-A, 60% DA001 plus 40% OYS2-A, 50% DA001 plus 50% OYS2-A, 40% DA001 plus 60% OYS2-A, 30% DA001 plus 70% OYS2-A, 20% DA001 plus 80% OYS2-A, and 10% DA001 plus 90% OYS2-A). To verify the reliability of the deconvolution method, DA001 cells injected into core SG48 were also stained with 5- and 6-carboxyfluorescein diacetate, succinimidyl ester (CFDA/SE) so that independent effluent breakthrough data could be obtained (18). It has been shown that the CSDA/SE stain does not affect transport characteristics of the bacteria (7, 28). The effluent fractions were also routinely plated on R2A agar and counted to verify the radioactivity and staining results.

Enumeration of bacteria attached to sediment. At the end of the transport experiment, large fractions of the injected bacteria were retained within the cores. To assess cell retention within a core, the aluminum casing was cut, and the core was split longitudinally. The concentration of bacteria retained within the core was determined in both core halves by using the sediment subsampling (17) and phosphorimaging (13) techniques. The subsampling method provides a direct measurement of radioactivity (and hence bacterial concentration in a sediment). The phosphorimaging method provides direct high-resolution visualization of bacterial retention in sediment (spatial resolution, 88 µm). Attached bacteria were also extracted from the sediment (via gentle vortexing in a pyrophosphate buffer saline solution) and plated. Plate counts indicated that bacteria were viable, and the amount of radioactivity per cell did not change, as would have been the case if the bacteria divided during the experiments.

For subsampling, a core half was divided into a grid of 28 rows longitudinally and five columns laterally (17). The sediment in each grid volume was homogenized, and the radioactivity was measured with a scintillation counter. Multiple measurements of the same sample gave rise to an analytical error of approximately 5%.

The storage phosphorimaging technique was employed to directly image the distribution of radioactivity (and therefore bacteria) in the sediment. Six or seven epoxy-fixed thin sections (lateral distance, 5 cm; longitudinal distance, 7.5 cm) were obtained from one core half and exposed to a tritium imaging screen, and the recorded radioactivity was read with a Molecular Dynamics PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.).

Characterization of core sediments. Characterization of physical and chemical heterogeneity provides a baseline with which a biological effect can be assessed. Porosity, grain size, and positively charged Fe, Al, and Mn hydroxides are the major physical and chemical factors that influence bacterial transport. Thin sections were analyzed for these parameters by using a Philips XL-30 field emission gun scanning electron microscope (SEM) fitted with back-scattered and secondary electron detectors and an IMIX X-ray energy dispersive spectrum analytical system. The electron microscope was equipped with a computer image analysis program (particle segmentation and feature analysis) (Princeton Gamma Tech, Princeton, N.J.) to allow image processing. The physical and chemical parameters were determined for selected sections of the cores at an areal resolution of 5 mm (longitudinal) by 4 mm (lateral) (12, 13).

Data analysis. (i) Deconvolution of DA001 from OYS2-A. Although scintillation counting could not distinguish the ¹⁴C (OYS2-A) and ³⁵S (DA001) isotopes, the short half-life of the ³⁵S isotope allowed determination of the concentrations of the individual isotopes. Specifically, the following equations were used:

$$C_{t_1}^{tot} = C_{t_1}^s + C_{t_1}^c = C_0^s \exp(-\lambda_s t_1) + C_0^c \exp(-\lambda_c t_1) \quad (1)$$

$$C_{t_2}^{tot} = C_{t_2}^s + C_{t_2}^c = C_0^s \exp(-\lambda_s t_2) + C_0^c \exp(-\lambda_c t_2) \quad (2)$$

where $C_{t_1}^{tot}$ and $C_{t_2}^{tot}$ are the measured radioactivities of a mixture of the ³⁵S and ¹⁴C isotopes at times t_1 and t_2 , respectively; $C_{t_1}^s$ and $C_{t_1}^c$ are the radioactivities of ³⁵S and ¹⁴C, respectively, in a mixture at time t_1 ; $C_{t_2}^s$ and $C_{t_2}^c$ are the radioactivities of ³⁵S and ¹⁴C, respectively, in a mixture at time t_2 ; C_0^s and C_0^c are the radioactivities of ³⁵S and ¹⁴C, respectively, in a mixture at time t_0 ; and λ_s and λ_c are decay constants of ³⁵S (7.95×10^{-3} day⁻¹) and ¹⁴C (3.32×10^{-7} day⁻¹), respectively. The two unknowns, C_0^s and C_0^c , can be solved based on two measurements at two times. The radioactivities of mixtures of ³⁵S and ¹⁴C were obtained at four times from the start of the experiment (3, 38, 79, and 163 days) for breakthrough data and at two times (44 and 163 days) for the retained bacterial concentrations in the sediments. The effluent samples used for counting the radioactivity at four times were fixed in 1% formaldehyde and stored in the dark so that cell respiration did not occur. The radioactivities of 10 controls were measured at each time when the effluent samples were analyzed.

TABLE 2. Strain characteristics

Strain	Electrophoretic mobility ($\text{m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$, 10^{-8}) ^a	Surface potential (mV)	Contact angle (degrees)	Density (g/ml)	Cell diam (μm)	Cell length (μm)
OYS2-A	-0.37 ± 0.01^b	-5	28.6 ± 0.5	$1.075\text{--}1.087 (\pm 0.001)^c$	0.46 ± 0.02	1.56 ± 0.11
DA001	-0.45 ± 0.01	-6	29.8 ± 0.3	$1.075\text{--}1.087 (\pm 0.001)$	0.56 ± 0.02	1.10 ± 0.06

^a Electrophoretic mobility was measured in Narrow Channel artificial groundwater with an ionic strength of 0.003 M.

^b Mean $\pm$ standard error.

^c The values in parentheses are standard errors.

(ii) **Modeling.** The factors controlling transport of bacteria can be investigated by using numerical simulations to derive transport parameters for both conservative tracers and the bacteria so that these factors can be quantitatively compared. To constrain the range of parameters to be derived from the CXTFIT model (45) and therefore to obtain a unique set of parameters, the effective porosity and dispersivity of the conservative tracer were first estimated by using advection-dispersion equations assuming no sorption (25, 32). Pore velocity was then estimated from the experimental approach velocity and effective porosity. The values for estimated pore velocity and dispersivity were then used as initial inputs for the CXTFIT model with the one-site nonequilibrium model option. Given the total flow rate and the volume of the core, the effective porosity was then computed. The CXTFIT model with the same model option was used to independently estimate parameters for the observed bacterial breakthrough curves (velocity, dispersivity, and attachment and detachment rates). Pore velocity and dispersivity values obtained with the conservative tracer were used as the initial input for the CXTFIT model. CXTFIT model runs were also made to predict the profiles of the attached bacterial concentrations using the same set of parameters derived from the breakthrough data, and the results were compared to the observed attached bacterial concentrations. It was assumed that all of the missing mass for the observed data (mass balance error) was in the sediment (17) and that the missing mass was proportional to the observed concentration. The model was simulated for a partial duration of the transport experiments (30 h or ~ 2 core pore volumes), at which time significant mass transport was complete. Because of a possible tailing effect at times beyond 30 h, the model simulation was also run for the entire duration of the experiments (~ 240 to 350 h) to evaluate if the derived transport parameters were different.

Despite our efforts to constrain the range of the parameters used as inputs for the CXTFIT model, a possibility of overparameterization existed. Because four parameters were fitted to the data sets, it was possible that the solution might not be unique. For this reason, different initial inputs were used to test the uniqueness of the model solution. It was found that as long as the initial inputs were within reasonable ranges, the solution was unique. This uniqueness was reflected in the covariance matrix (i.e., low values for individual elements in the matrix).

(iii) **Filtration theory and calculation of collision efficiency.** Transport of two strains in the same core can ultimately be compared in terms of collision efficiency because collision efficiency is a direct measure of cell-mineral interactions. For SG48, because the two strains used interacted with the same mineral surfaces, any difference in collision efficiency must reflect a difference in cell properties. Calculation of collision efficiency is a two-step process. The first step calculates the collector efficiency from known grain and bacterial sizes, porosity, approach velocity, bacterial density, water density, and viscosity at 15°C (13). The second step calculates collision efficiency given values for collector efficiency and other measured parameters. The collector efficiency value is the sum of diffusion, London-van der Waals force, interception, and sedimentation terms, and the expressions can be found elsewhere (13, 31, 38). The procedures for calculating

the collision efficiency value using the sediment data are described elsewhere (12). Briefly, the cores were divided into five grid columns, and each grid column had 28 grid rows. The collision efficiency values were calculated for each grid by using the following equations:

$$R_i = \frac{S_i}{c_0 - \sum_{j=1}^{i-1} S_j} \quad (3)$$

$$\alpha_i = -\frac{2}{3} \frac{d_i}{(1 - \theta_i)\eta_i L_i} \ln(1 - R_i) \quad (4)$$

where S_i is the bacterial concentration in grid i ; c_0 is the initial cell concentration injected into the cores; R_i is the fraction of cell retention in grid i (the ratio of the number of cells retained in the grid to the number of cells that entered the grid); α_i is the collision efficiency; d_i is the collector diameter (i.e., grain size); θ_i is the porosity; η_i is the collector efficiency; and L_i is the thickness of the grid in the flow direction (1.6 cm). A set of parameters used for these calculations is shown below.

RESULTS

Bacterial properties. OYS2-A and DA001 cells are rod shaped, are the same density, and are slightly negatively charged on their surfaces (Table 2). The widths and lengths of OYS2-A and DA001 cells are different, and t tests indicate that both dimensions are statistically significantly different ($P = 0.008$ and $P = 0.01$ for length and width, respectively). Approximately 10% of the OYS2-A cells possess flagella, whereas DA001 cells do not have any flagella. Hydrophobic interaction chromatography data indicated that both DA001 and OYS2-A are strongly hydrophilic. The BATH results showed different patterns for the two strains. Neither strain partitioned into hexadecane. Approximately 3 to 9% of the DA001 cells partitioned into p -xylene but not octane. OYS2-A cells did not partition into either p -xylene or octane. However, approximately 10 to 28% of OYS2-A cells were detected at the interface between groundwater and either p -xylene or octane. Contact angles of 29.8 ± 0.9 and 28.6 ± 1.4 degrees were obtained for DA001 and OYS2-A, respectively. These angles were consistent with hydrophilic cell membranes and indicated that

TABLE 3. Experimental core results

Core	Avg grain size (μm)	Avg porosity (%)	Avg metal hydroxide content (%)	Organism	Effluent recovery (%)	Retention (%) ^a
HS54	154 ± 4^b	38 ± 1	11 ± 1	DA001	67.5 ± 0.1	9.5 ± 0.3
SG11	286 ± 33	30 ± 3	7 ± 1	DA001	67.8 ± 0.1	8.4 ± 0.4
SG48	281 ± 25	30 ± 3	4 ± 1	DA001	55.4 ± 0.2	21.8 ± 2.3
				OYS2-A	29.9 ± 0.1	40.8 ± 3.1
				Mixed	35.9 ± 0.2	36.3 ± 2.6

^a A previous study (17) indicated that poor mass balance was due to incomplete counting of radioactivity in sediment subsamples by scintillation counting, and grinding of sediments resulted in recovery of all missing biomass. Therefore, all missing biomass in the cores was assumed to be in sediment.

^b Mean $\pm$ standard error.

FIG. 1. Breakthrough curves for the conservative tracer and bacteria in HS54 (A), SG11 (B), and GS48 (C). The pore volume was determined by multiplying the total volume of an intact core by the effective porosity for the conservative tracer.

there was no significant difference between the two strains. Both DA001 and OYS2-A possessed LPS, although their electrophoretic profiles were different. Specifically, the band intervals in the ladder-like pattern for OYS2-A were larger than those observed in the pattern for DA001, suggesting that the repeating units of O-antigen were larger for OYS2-A than for DA001 (data not shown).

Sediment properties. Core HS54 was dominated by quartz and feldspars and contained minor amounts of Al and Fe hydroxides in decreasing order of abundance. A similar mineralogy dominated the shelly-gravelly facies (cores SG11 and SG48), and there were abundant bands; back-scattered elec-

tron imaging and energy dispersive spectrum analyses identified these bands as Al hydroxides, and there was Mn in some of the Al hydroxides. Systematic measurements of grain size, porosity, and areal abundance of the metal hydroxides (i.e., Fe, Al, and Mn hydroxides) in a 5- by 4-mm rectangle revealed that SG11 and SG48 had similar porosities and grain sizes but slightly different metal hydroxide contents (Table 3). The HS54 core had greater porosity, smaller grain size, and more abundant metal hydroxides than the other two cores (Table 3).

Bacterial breakthrough and effluent recovery. The shapes of the bacterial breakthrough curves for HS54 and SG11 were similar, and these curves were characterized by a single peak

FIG. 2. Radioactivities of ^{14}C , ^{35}S , and a representative mixture of ^{14}C and ^{35}S (50:50) decay with time. The solid symbols represent the radioactivity measured with a scintillation counter, and the open symbols represent the radioactivity predicted by the radioactive decay method according to equations 1 and 2. The close match demonstrates that the deconvolution method is valid and that neither isotope was respired by cells during sample storage and analysis. The error bars are smaller than the symbols.

with no significant tailing (Fig. 1A and B). The bacterial breakthrough occurred before the Br breakthrough for both cores. The effluent recoveries for these two cores were similar (Table 3). For SG48, the measured radioactivities of ^{14}C , ^{35}S , and mixtures of these two isotopes in various proportions matched those predicted by radioactive decay of the two isotopes (Fig. 2), demonstrating that the deconvolution method was valid and that neither ^{14}C nor ^{35}S was respired during sample storage. The breakthrough peak of the DA001–OYS2-A cell mixture in SG48 occurred before that of ^3H . The shapes of the deconvoluted individual bacterial breakthrough curves for OYS2-A and DA001 were similar (Fig. 1C), but DA001 had higher effluent recovery. The breakthrough curves obtained by plate counting (for both DA001 and OYS2-A) and CFDA/SE staining (for DA001) were similar. This similarity suggested that there was no cell division during the transport experiments, because if there had been cell division, the stain intensity would have been diluted and the breakthrough curve obtained from the stained cells would have been different from that obtained from plate counts.

The CXTFIT model fit the breakthrough peak for both the conservative tracer and the bacteria (Fig. 1). The fitting parameters for 30 h and for the entire duration of the experiment were very similar, and the parameters for 30 h are shown in Table 4. The pore velocity and dispersivity of the conservative

tracers were different from those of the bacteria, and thus the effective porosities were different (Table 4). The effective porosity derived from the bacterial breakthrough curves was similar to that obtained from SEM measurements (Tables 3 and 4), suggesting that the SEM measured only the macroporosity that the bacteria experienced. Early breakthrough of the bacteria could be quantified in terms of the reduction in the effective porosity for the bacteria compared to that for the conservative tracers. We defined a differential advection factor to be the ratio of the advective pore velocity of the bacteria to the advective pore velocity of the conservative tracer adjusted for different flow rates for the bacteria and the conservative tracer (equivalent to the ratio of the effective porosity for the conservative tracer to the effective porosity for the bacteria) (Table 4). When DA001 cells traveled alone (HS54 and SG11), they broke through faster in HS54. When DA001 and OYS2-A cells traveled together in SG48, the shorter, larger-diameter DA001 cells broke through faster than the longer, smaller-diameter OYS2-A cells.

Bacterial retention in core sediment. The concentration profiles of attached DA001 cells in the sediment revealed a decrease in the longitudinal direction and some lateral variability in HS54 and SG11 (Fig. 3). The concentration of attached DA001 and OYS2-A cells in SG48 increased from the influent end to a distance of 7 to 12 cm, and this was followed by a

TABLE 4. Model output parameters

Core	Output	Organism	Pore velocity (m/h)	Dispersivity (m)	Effective porosity	Permeability (darcy)	Differential advection factor	Attachment rate (1/h)	Detachment rate (1/h)	Collision efficiency ^a
HS54	Br tracer		0.043 (1×10^{-4}) ^b	0.003 (8×10^{-4})	0.50					
	Bacteria	DA001	0.049 (1×10^{-4})	0.003 (8×10^{-6})	0.40		1.25	0.13	0.03	0.006
SG11	Br tracer	DA001	0.038 (6×10^{-4})	0.003 (7×10^{-4})	0.40	39				
	Bacteria	DA001	0.043 (3×10^{-4})	0.016 (5×10^{-5})	0.34		1.18	0.10	0.02	0.009
SG48	^3H tracer		0.012 (3×10^{-5})	0.007 (3×10^{-3})	0.45					
	Bacteria	DA001	0.041 (5×10^{-4})	0.010 (2×10^{-5})	0.29		1.55	0.07	0.02	0.003
		OYS2-A	0.037 (6×10^{-5})	0.013 (3×10^{-5})	0.32		1.41	0.08	0.01	0.004

^a Collision efficiency was calculated by using fitted values for the attachment rate, and the data compare well with the values obtained with the sediment data (Fig. 7).

^b The values in parentheses are standard errors

FIG. 3. Contour plots of the distribution of the retained DA001 cells in HS54 (A), SG11 (B), and SG48 (C) and of OYS2-A cells in SG48 (D) as determined by scintillation counting of sediment subsamples.

gradual decrease toward the effluent end. Although the patterns of the two concentration profiles were similar, the retained cell concentration of OYS2-A was much higher than that of DA001. Because 3.3 times more OYS2-A cells were injected, the retained OYS2-A concentrations were divided by this factor so that the two strains were normalized to the same influent concentration. The normalized concentrations of OYS2-A in the sediment were still higher than those of DA001 by a factor of two.

The distribution of bacteria in the thin sections revealed by the phosphorimaging technique was more variable than the distribution determined by scintillation counting. The concentration profile for retained DA001 cells revealed an overall decrease in the longitudinal direction for both HS54 and SG11 (Fig. 4B and D). The metal hydroxide bands (Fig. 4A) correlated with high retention of bacteria (Fig. 4B). The phosphorimages of SG48 also revealed a high level of retention 7 to 12 cm from the influent end (Fig. 4D).

Although the CXTFIT model fit the breakthrough curves reasonably well, the model was not capable of accurately predicting concentration profiles in the sediments using the same

set of parameters (Table 4) when the model was run for 30 h (Fig. 5). The model tended to underpredict the attached bacterial concentration near the influent end (Fig. 5A and B). When the model simulation was run for the entire duration of the experiment (250 to 300 h), the model tended to overpredict the tailing portion of the breakthrough curve, and the modeled concentration in the sediment increased from the influent end to the effluent end (Fig. 6). Because the model considered only reversible attachment, longer times tended to flush out the injected pulse of bacteria. The natural system is more complex, and the reality may be between the two extremes (30 h and the entire duration of the experiment), as discussed below.

Collision efficiency. In previous studies workers have used parameters (pore velocity, dispersivity, and effective porosity) derived from conservative tracer breakthrough to calculate bacterial attachment and detachment rates and collision efficiency (2, 17). However, when the bacterial breakthrough curves were fitted independently, a different set of parameters was derived. It appeared that it was more realistic to use bacterium-derived parameters to estimate attachment and detachment rates and collision efficiency. To test the effects of the

FIG. 4. Photographs of thin sections (A and C) and corresponding phosphorimages (B and D) of cores SG11 and SG48. Preferential attachment of bacteria to the metal hydroxide bands is apparent in SG11 but not in SG48. For example, in panel A curved Fe and Al oxide bands with fine grains were revealed by SEM, and the corresponding images show enhanced radioactivity signals (arrows).

two sets of parameters on collision efficiency, the parameters derived from both the conservative tracers and the bacteria were used to calculate collision efficiency using the average grain size from the SEM measurements. The following general parameters were used in the calculations: bacterial size, 1.10 and 1.56 μm for DA001 and OYS2-A, respectively; viscosity of water at 288 K, $1.14 \times 10^{-3} \text{ N s/m}^2$; fluid temperature, 288 K; bacterial density, 1,080 kg/m^3 for both strains; water density at 288 K, 997 kg/m^3 ; and Hamaker constant, $4 \times 10^{-21} \text{ J}$. The collision efficiency values calculated by using the velocity and porosity values derived from the bacterial breakthrough curves were slightly different from the collision efficiency values obtained when velocity and porosity values from the conservative tracer breakthrough curves were used (Fig. 7A and B). These two sets of collision efficiency values overlapped within the errors, however. For SG48, porosity and velocity values derived from the bacteria were used to calculate collision efficiency.

The collision efficiency values of DA001 decreased along the length of cores HS54 and SG11 (Fig. 7A and B). Intercore variability was observed in the distribution of collision efficiency values. The DA001 cells injected into core SG11 had higher collision efficiency values at the influent end than the DA001 cells injected into core HS54 at the equivalent distances. The collision efficiency values of OYS2-A cells were systematically greater than the collision efficiency values of DA001 in core SG48 (Fig. 7C).

DISCUSSION

Development of an experimental method. This research successfully demonstrated that it is possible to study the simultaneous transport of two bacterial strains using ^{14}C and ^{35}S . Because the two strains experienced identical physical and chemical conditions, this approach enabled comparisons of the biological factors between the two strains. Taking advantage of the short half-life of ^{35}S , we found that the deconvolution method is reliable and robust. The reliability of the method was independently verified by the staining method and by routine culturing. The slightly lower effluent recovery of DA001 in SG48 than in SG11 (two cores from the same sedimentary facies) was probably due to cell-cell interactions between DA001 and OYS2-A in SG48. Because the isotope method was not specific to the two bacterial strains studied, it should be applicable to any bacteria.

Modeling. Although the CXTFIT model is a linear, first-order, kinetically limited model with no irreversible attachment term, it was adequate to describe bacterial breakthrough and to approximate sediment retention. Within a short time (30 to 50 h), the attachment process dominated transport, and the irreversible nature of attachment and detachment was negligible. The model was not capable of describing long-term tailing of the breakthrough curve or the dynamic evolution of the concentration profile of attached bacteria in the sediment (Fig. 6). Specifically, the model overestimated the bacterial concentration observed during long-term tailing, and the

FIG. 5. Concentration profiles of attached bacteria averaged over the lateral direction for HS54 (A), SG11 (B), and SG48 (C). Both experimental and model-predicted profiles are shown for comparison.

model values deviated from the experimental profile of the retained bacterial concentration in the sediment. These inadequacies were due to the fact that in the long term, when the attachment processes diminished and detachment became dominant, irreversible attachment played an important role. Compared with fully reversible attachment, irreversible attachment reduces the rate of release of bacteria to the aqueous phase (resulting in faster tailing-off than the model prediction) and results in retention of more bacteria in the sediment (resulting in a decrease in the retained bacterial concentration from the influent end to the effluent end instead of an increase, as the model predicted). Nevertheless, the attachment and

detachment rates derived from either short-term (Table 4) or long-term (data not shown) observations were consistent and indicated that these rates were meaningful. These rates may be useful in making predictions in large-scale, field-oriented bacterial transport studies. For long-term transport (such as extended tailing), numerical models, which account for time-dependent desorption or bacterial subpopulations (3, 26), may be better able to represent the experimental data. Close examination of the experimental data obtained in this study revealed that a second-order, kinetically limited model with two subpopulations might better fit the concentration profiles of the retained bacteria in the sediment (3). Recent modeling

FIG. 6. Breakthrough curve (A) and concentration profile for attached bacteria (B) for SG11, showing the entire set of data (343.5 h or 30 core pore volumes). Superimposed are CXTFIT model fits for the breakthrough data and model prediction for the concentration profile of attached bacteria. The model overestimates the tailing portion of the breakthrough curve and poorly predicts the experimental profile for attached bacteria in the sediment.

advances even suggest that there may be a distribution of collision efficiencies in a single culture, and it may be necessary to incorporate such a distribution into modeling considerations (B. J. Mailloux, T. C. Onstott, J. Hall, M. E. Fuller, D. F. DeFlaun, and H. Dong, abstract from the American Geophysical Union Fall Meeting 2000, vol. 81, p. F181, 2000). A particle-based model (T. D. Scheibe, submitted for publication) is capable of incorporating these complexities into the model formulation and yielding a robust fit for both effluent breakthrough and the profile of attached bacteria in sediment (51).

Differential advection. Early breakthrough of microorganisms compared to the breakthrough of a conservative tracer has been observed in several studies (1, 37, 44), and the primary mechanisms include (i) volume size exclusion (exclusion of colloids from smaller pores due to the inability of the colloids to fit into the pores), (ii) preferential flow path through high-conductivity regions, and (iii) hydrodynamic retardation (or chromatography) (exclusion of colloids from the lower-velocity regions of a pore throat due to the size of the colloids) (T. R. Ginn, Letter, *Water Resour. Res.* **36**:1981–1982, 2000; L. L. C. Rehmann, C. Welty, and R. W. Harvey, Author's Reply, *Water Resour. Res.* **36**:1983–1984, 2000). It is likely that one or more of these mechanisms are operative, and our data provide a basis for a mechanistic interpretation.

The modeling efforts indicated that a reduction in the effective porosity for the bacteria relative to the conservative tracer

of up to 55% (Table 4) was necessary to account for the observed differential advection. The dispersivity and pore velocity for the conservative tracers were also significantly different from the dispersivity and pore velocity for the bacteria. These observations strongly suggest that the conservative tracer and the bacteria were transported via different flow paths and experienced different pore spaces, and therefore the parameters derived from the conservative tracer (such as velocity, dispersivity, and effective porosity) could not be used to model bacterial transport. However, the collision efficiency was relatively insensitive to the observed reduction in effective porosity and enhanced pore velocity, suggesting that previously described practices (2, 17) in which conservative tracer-derived parameters are used to calculate collision efficiency and the attachment rate for bacteria are valid.

Experimental reproducibility and cell-cell interactions. SG11 and SG48 were from the same sedimentary facies and had similar porosities and grain sizes but slightly different metal hydroxide contents (Table 3). When DA001 cells were injected into these two cores, the effluent recovery and sediment retention data were expected to be similar. Likewise, SG13, which was used by Fuller et al. (17), and SG48 had similar porosities, grain sizes, and metal hydroxide contents, and the effluent recovery and sediment retention data for OYS2-A were expected to be similar with these two cores. The difference in effluent recovery between SG11 and SG48 for DA001 or between SG13 and SG48 for OYS2-A was not conclusive. A more systematic comparison using collision efficiency values could be made. The collision efficiency values of DA001 near the influent end of SG11 were much higher than those at the equivalent distance in SG48 (Fig. 7). Likewise, the collision efficiency values of OYS2-A near the end of SG13 (17) were higher than those at the equivalent distance in SG48. One likely mechanism for such a difference may be cell-cell interactions between DA001 and OYS2-A in SG48, where the presence of one cell type may have inhibited attachment of the other cell type to the same mineral surface. The interactions among different types of cells may be different from the interactions among cells of the same type, a phenomenon referred to as cell blocking (39). Further work is necessary to study this effect.

Biological effect on bacterial transport. When DA001 and OYS2-A cells were injected individually into intact cores of the same facies type, major differences in both effluent recovery and sediment retention were observed (OYS2-A transport in horizontally stratified and shelly-gravelly cores is described by Fuller et al. [17]). The effluent recovery rate for DA001 was much higher than that for OYS2-A (~68 versus 10 to 15%). Whereas most of the retained OYS2-A cells were near the influent end, the DA001 cells were evenly distributed throughout the entire lengths of the cores. When DA001 and OYS2-A were simultaneously injected into the same core (SG48), the shapes of their breakthrough curves were similar, but the concentrations and timing of breakthrough compared to that of ^3H were different. The effluent recovery rates of the two strains were very different (55 versus 30%).

Among the cell surface properties measured, cell size was the parameter identified that was statistically different (1.56 ± 0.33 and $1.10 \pm 0.19 \mu\text{m}$ for OYS2-A and DA001, respectively). Cell size has previously been observed to be a dominant

FIG. 7. Distribution of collision efficiencies along the lengths of HS54 (A), SG11(B), and SG48 (C). The error associated with each data point was 20 to 30% and is not shown for clarity.

controlling factor in bacterial transport (15, 19) and was probably responsible for the large difference observed in this study. Compared to the DA001 cells, the longer, smaller-diameter OYS2-A cells exhibited low pore velocity, high porosity, a high attachment rate, and a low detachment rate. All of these factors may contribute to the lower effluent recovery rate for OYS2-A cells. If cell transport were transverse, the longer OYS2-A cells would be excluded more from small pores in a core. The modeling results indicate otherwise, however. Two explanations are possible. First, the cell diameter of DA001 is larger than that of OYS2-A. If cells are transported longitudinally, the DA001 cells should be excluded more from the available pore spaces, resulting in lower effective porosity and higher velocity. Second, 10% of the injected OYS2-A cells possessed flagella, and flagella may be able to penetrate into small pore spaces, accounting for the higher effective porosity for OYS2-A. In addition, OYS2-A cells have significantly higher charge density (surface charge/surface area) than DA001 cells and therefore are expected to have a higher attachment

rate than OYS2-A cells, which is consistent with the experimental observations.

The flagellated OYS2-A cells (10% of the total injected population) probably facilitated attachment of this strain to the sediment, impeding transport. The different BATH results for the two strains may also be related to differences in attachment of the two strains. It was difficult, however, to directly compare the results and relate the results to transport. It is not clear if the percentages of OYS2-A (10 to 28%) at the hydrocarbon-aqueous phase interface correspond to the levels of retention in the sediment, although the data appear to suggest that OYS2-A cells may be more hydrophobic. The LPS gel patterns indicated that there are differences in the surface properties of the two organisms, but it is not possible to assess their relevance to adhesion without more analyses. Previous studies have shown that attenuation of the O-antigen can be related to changes in attachment properties and transport of different strains of the same species through porous media (50). There are a number of other parameters that could contribute to the

observed differences in the transport of the two strains, including exopolymeric substances. Certain bacteria (e.g., *Shewanella algae* BrY) are capable of producing these surface polymers in response to Fe oxides (M. M. Urrutia and J. K. Fredrickson, unpublished data). Extracellular polymers produced by bacteria can extend up to 10 μm from the cell surface and can bind to sediment surfaces, significantly enhancing the attachment rate.

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