BACTERIA TRANSPORT IN A POROUS MEDIUM: RETENTION OF BACILLUS AND PSEUDOMONAS ON SILICA SURFACES

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Abstract—Pseudomonas fluorescens and Bacillus subtilis transport was studied in water flowing through laboratory columns packed with 0.5-mm silica beads. The rate of bacterial attachment to the silica was shown to reach steady state after about one pore volume of bacteria-containing solution had passed through the column. Column-outlet concentrations were 6–80% of inlet concentrations. There was less P. fluorescens retardation at pH 5.5 versus 7.0; since the pH was (isoelectric point) for both bacteria are at lower pHs, this difference indicates that the change in electrostatic repulsion over this pH range was relatively unimportant in determining bacteria retention in the porous medium. Transport of both species was highly retarded, suggesting that neither could be quickly transported over long distances in sandy soils. Results were adequately described using a steady-state transport model with bacterial attachment to soil as a pseudo-first-order process. Sticking efficiency (ξ), calculated using the steady-state colloid-filtration model, was near one. The continued appearance of bacteria in the column effluent for several pore volumes after switching to a bacteria-free inlet solution shows attachment to be irreversible, though slowly reversible.

Key words—bacteria, bacteria transport, biocolloid, filtration, colloid transport, groundwater, soil, column experiments

INTRODUCTION

The movement of bacteria is of interest in groundwater because: (i) subsurface migration of bacteria is an important determinant of subsurface ecology, (ii) introduction of mobile bacteria or enzymes into contaminated aquifers is a potential in situ cleanup strategy, (iii) bacteria can contribute to aquifer clogging and (iv) bacterial contamination of drinking-water wells is a potential public health problem. The ability of bacteria that are native to subsurface environments to migrate in laboratory columns (Fontes et al., 1991) and over distances of several meters (Harvey and Garabedian, 1991) in the field have been demonstrated. As of yet, little work has been done with laboratory species that may have engineering application for bioremediation of waste sites. Most previous research on biocolloid transport in the subsurface has centered on the travel of disease-causing bacteria and viruses (Gerba and Bitton, 1984). This work has shown that microorganisms are capable of long-distance transport in the subsurface under certain environmental conditions. How the surface-chemical and microphysical properties of the porous media control the transport is not well understood, however. It has been shown that bacteriophage transport in porous media composed of silica is greater at pH 7.0 than at 5.5; but even small amounts of hydrophobic material immobilized on the silica in greater retention and less transport on the phage (Bales et al., 1993). Phage attachment to the silica is reversible (Bales et al., 1991).

A transport and colloid-filtration model using a single-collector removal efficiency (η) and sticking efficiency (ξ) to interpret the rate of bacteria removal in soil was applied to modeling bacterial movement at a field site on Cape Code by Harvey and Garabedian (1991). The same approach has been used to describe bacteriophage transport through laboratory columns (Bales et al., 1991). During the initial stages of filtration, before deposited particles build up to an extent large enough to affect the particle removal and resuspension rates, particle removal from suspension is approximately constant (dC/dt ≈ 0); and a mass balance on the porous medium can be simplified as:

\[ \frac{dC}{dx} = -k_1C \]  

where \( u \) is velocity and \( k_1 \) is a filtration coefficient, i.e. a pseudo-first-order rate coefficient. Using a single-collector model (O'Melia, 1980), \( k_1 \) is:

\[ k_1 = \frac{31 - \theta}{2d} \eta \xi \]  

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where $\theta$ is porosity and $d$ is the collector diameter. Colloidal particles are transported from the moving fluid to collector surfaces by Brownian diffusion, interception due to fluid stream lines and gravitation setting. The respective $\eta$s are (O'Melia, 1985):

$$\eta_0 = 0.9 A^{1/3} \left( \frac{kT}{\mu_d d_d} \right)^{1/3}$$

(3)

and

$$\eta_1 = \frac{3}{2} A_1 \left( \frac{d_p}{d} \right)^2$$

(4)

and

$$\eta_0 = \frac{(\rho_p - \rho) g}{18 \mu u} d_p^2$$

(5)

where $\mu$ is water viscosity, $k$ is the Boltzmann constant, $T$ is temperature, $A_1$ is a parameter that accounts for the effects of adjacent media grains on the flow about a collector, $d_p$ is the bacteria diameter, $\rho_p$ is water density and $g$ is the gravitational constant. Equivalent expressions derived for non-spherical particles differ only slightly (Bales, 1984). For a spherical collector, $A_1$ has been given as:

$$A_1 = \frac{1 - \varepsilon^3}{1 - 1.5\varepsilon + 1.5\varepsilon^2 - \varepsilon^3}$$

(6)

where $\varepsilon = (1 - \theta)^{1/3}$ (O'Melia, 1985).

Substitution of $\eta$ into equation (1) for $k_1$ and integration gives:

$$\log \frac{C}{C_0} = -\frac{1}{2.303} \frac{31 - \theta}{d} \eta \alpha L$$

(7)

where $L$ is column length.

The purpose of the work reported here was to determine the relative extent and rate at which two bacteria attach to and detach from silica surfaces during transport through porous media. The pHs and physical conditions studied were designed to mimic those in a sandy soil or groundwater environment. Silica was chosen as a model for sandy soil as its surface is well defined and contains essentially no organic carbon.

**Table 1. Experimental conditions and results**

<table>
<thead>
<tr>
<th>Exp.</th>
<th>pH</th>
<th>$d$ (mm)</th>
<th>Average $u$ (cm s$^{-1}$)</th>
<th>log $C_0$</th>
<th>log $C_1/C_0$</th>
<th>$\eta$</th>
<th>$\alpha$</th>
<th>Fraction detached$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.0</td>
<td>0.5</td>
<td>0.0078</td>
<td>5.4</td>
<td>-1.2</td>
<td>0.0070</td>
<td>1.2</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>7.0</td>
<td>0.5</td>
<td>0.0111</td>
<td>4.4</td>
<td>-1.1</td>
<td>0.0055</td>
<td>1.2</td>
<td>0.94</td>
</tr>
<tr>
<td>3</td>
<td>5.5</td>
<td>0.5</td>
<td>0.0090</td>
<td>5.4</td>
<td>-0.4</td>
<td>0.0063</td>
<td>0.4</td>
<td>0.19</td>
</tr>
<tr>
<td>4</td>
<td>7.0</td>
<td>0.5</td>
<td>0.0088</td>
<td>3.4</td>
<td>-0.7</td>
<td>0.0050</td>
<td>0.9</td>
<td>0.10</td>
</tr>
<tr>
<td>5</td>
<td>7.0</td>
<td>0.5</td>
<td>0.0119</td>
<td>4.6</td>
<td>-0.2</td>
<td>0.0041</td>
<td>0.3</td>
<td>0.37</td>
</tr>
</tbody>
</table>

$^*$Number of bacteria retained in column during period of bacteria feed divided by number released during period of no bacteria feed. Numbers dervied from respective areas above and below breakthrough curves.

$^\dagger$High C values suggest that growth occurred upon adding beef extract.

**MATERIALS AND METHODS**

**Column-experiment procedures**

Five continuous-flow column experiments were conducted to develop breakthrough curves for bacterial attachment to and detachment from 0.5-mm silica beads (Table 1). Experiments were done at 7°C in order to minimize bacterial growth and death. Applied bacteria were *Pseudomonas fluorescens* P17 and *Bacillus subtilis* TF-32. Calcium-phosphate buffer adjusted to pH 5.5 and 7.0 was used for attachment (bacterial loading) and to pHs 7.0 and 8.2 for detachment (bacterial release) portions of the experiments. Stock solutions of bacteria were pumped to the column using peristaltic pumps. The experimental set-up was similar to that reported previously for bacteriophage column studies (Bales et al., 1991).

Glass-chromatography columns (Spectrum Medical Industries Inc., Los Angeles, Calif.) 20 x 1.4-cm diameter, were packed by the tap and fill method (Snyder and Kirkland, 1979) and the weight of media recorded to estimate dry bulk density. Columns were packed with fresh beads for each experiment. Teflon tubing was used from the input reservoir up to the peristaltic pump and 0.76-mm inner diameter Tygon tubing from the peristaltic pump to the column. Prior to each experiment, a column was wrapped with aluminum foil and sterilized by autoclaving for about 30 min, then flooded from the bottom at a low flow rate to minimize air entrapment. Buffer at the same pH as the stock solution was passed through the column for about 5–8 pore volumes overnight prior to the beginning of the experiment. Effluent pH was the same as that of the input buffer after this process. All tubing, reservoir, buffers and collection vessels were also sterilized by autoclaving.

To determine if any bacterial inactivation occurred, samples from the reservoir were collected at regular intervals and assayed for viable bacteria. The results indicated that no significant die-off or growth of the test bacteria occurred during the course of an experiment.

During an experiment, 1.4–2.1 pore volumes per hour (velocity of 0.008–0.012 cm s$^{-1}$; Table 1) of solution was passed through the columns. These velocities are higher than generally observed under natural gradients in groundwater. Samples were collected for 5 min at hourly intervals over a period of 2–3 days. Sample-collection time was determined to provide sufficient volume for two assays. Flow rate was monitored at the column outlet throughout the experiment. Fluctuations in flow rates for experiments 1 and 2 were less than 24% and for experiments 3–5 were less than 6%. In addition, it was observed that switching from one pump channel to the other caused a 32% velocity increase in experiment 5, a 5–8% increase in experiments 2 and 4 and an 8% decrease in experiment 3; no change was observed in experiment 1. Monitored flow rates were used to calculate the pore volumes of solution that had passed through the column. To verify the buffering, effluent pH from the column was measured periodically.

Column pore volume was calculated from the average of
two conservative-tracer breakthrough curves. A column was flooded with 0.1-M NaCl solution until the outlet conductivity became stable. Then, 0.2-M NaCl was passed through the column for about 2 pore volumes; then the inlet was switched back to 0.1-M NaCl. Conductivity was monitored with a Wedeco model 213a conductivity detector (Wedeco Instruments Inc., Santa Clara, Calif.) connected to a strip-chart recorder. A tracer experiment was done prior to each experiment. The resulting \( \theta \) was 0.43.

Calcium-phosphate buffer (0.02 M) was used for experimental stock solutions. To induce more complete bacterial detachment a solution of 2.5% by weight of beef extract was passed through the column in experiment 1. No growth of the test organism occurred in a beef extract control at 7°C during the time it took to perform the experiment.

_Bacillus subtilis_ TF-32 was isolated from Tumacoc Hills, Tucson, Ariz. in 1987 by K. Duncan (University of Arizona, Department of Ecology and Evolutionary Biology). _Bacillus subtilis_ are Gram-positive, rod-shaped bacteria. They are aerobic, motile and produce heat-resistant endospores (0.8 × 1.5-1.8 μm). Gram-positive bacteria have a thick cell wall of polymeric composition, which has various functional groups such as –COOH, –CH₃ (aliphatic), –NH₂ that may be significant in the adhesion of bacteria to surfaces. _Pseudomonas fluorescens_ P17 was obtained from The Metropolitan Water District of Southern California (Los Angeles, Calif.). _Pseudomonas fluorescens_ are Gram-negative bacteria. They are also rods, 0.7-0.8 × 2.3-2.8 μm during exponential growth and may be shorter and thinner in old cultures; they are motile with polar multistrand flagellation, occasionally non-motile, and are obligately aerobic. Optimal temperature for growth ranges from 25 to 30°C with no growth at 4°C or below.

Silica beads, nominally 0.5 mm in diameter (Potters Industries Inc., Hasbrouck Heights, N.J., 0.600-0.425 mm), were used for most experiments. In order to reduce effects of soluble oxides of calcium and sodium, the beads were washed to leach these compounds from their surfaces by the following steps: (i) approx. 200 ml of beads were wetted with deionized water, rinsed in 1 liter of 1-M NH₄OH, and rinsed to below pH 11 with deionized water, (ii) beads were refluxed in 1 liter of 2-M HCl for 4 h, HCl was drained, and the beads were rinsed in deionized water, (iii) they were twice refluxed in fresh 2-M HCl for 2 h and drained; (iv) rinsed in deionized water until the rinse-water pH rose above pH 4; (v) refluxed in deionized water for 1 h to remove excess H⁺ from the surfaces; and (vi) finally rinsed in deionized water and oven-dried overnight at 105°C.

**Assay procedures**

Bacteria were detected by formation of colonies on R2A agar Caifico, Detroit, Mich. using the spread plate method (APHA, 1985).

Tris-buffered saline solution (TRIS) was used for dilution of samples before assay. First, 63.2 g of trisma hydrochloride (Sigma Chemical Co., St Louis, Mo.), 163.6 g NaCl, 7.46 g KCl and 1.13 g Na⁺ were dissolved in 1.61 of distilled water and the pH adjusted to 7.2-7.4 with 12-N HCl. Next, 160 ml of this solution was diluted to 2.1 with distilled water. Then, 2.7-ml volumes of this diluted solution were dispensed into glass test tubes and capped. Finally, these tubes were autoclaved for 15 min at 121°C and 15 psi, then stored at 7°C until needed.

_Pseudomonas fluorescens_ was prepared for an experiment by suspension of one loopful of stock culture solution into 9 ml of phosphate buffer. This solution was then vortexed and serially diluted 1000-fold with phosphate buffer. 4 ml of this dilution were added to 36 ml of phosphate buffer. 50 ml of the 1 mg l⁻¹ sodium acetate solution and 40 ml of the P17 solution were then mixed in a sterile 125-ml screw-top Erlenmeyer flask and incubated at room temperature for 4 days.

_Bacillus subtilis_ was prepared by streaking a stock culture suspension on R2A plates and incubating at 37°C overnight. An isolated colony was transferred to a bottle containing 50-100 ml of tryptase soy broth (TSB) and incubated for 3 h at 37°C with continuous shaking. The TSB culture was then centrifuged at 8000 g at 10°C for 15 min. The resulting pellet was resuspended in filtered well water and centrifuged again at the same condition. Again, the pellet was resuspended in filtered well water and stored at 7°C.

_Pseudomonas fluorescens_ was incubated at room temperature for 3 days and _B. subtilis_ incubated at 37°C overnight before counting. Size, shape and grain staining of the cultures was confirmed after addition to the buffer to ensure that contamination had not occurred and that size and shape were in the range reported for the test organisms at regular intervals during the course of this project.

**Microelectrophoresis**

The electrophoretic mobility of bacteria at various pHs was measured by using a Rank Brothers Mark II particle-electrophoresis apparatus (Rank Brothers Ltd, Cambridge, England) equipped with a flat cell. For each measurement, 20 particles were timed under constant voltage, 5 times for each polarity at each stationary level. Mean mobility (\( \mu_w \)) was calculated by using the average velocity: \( \mu_w = D/V/L \), where \( D \) is the distance between electrodes (cm), \( V \) is applied voltage (V), and \( L \) is equivalent cell length (cm) and \( V \) is applied voltage (V). Electrophoretic mobility of the silica beads was reported previously (Bales et al., 1991).

**RESULTS**

The _B. subtilis_ (Fig. 1) were negatively charged at the pHs of our experiments, with an isoelectric point (pHₐ) lower than pH 3, the lowest pH of our measurements. _Bacillus subtilis_ had an electrophoretic mobility of about \(-2 \mu m \cdot s^{-1} \cdot V^{-1} \cdot cm^{-1}\) at both pH 5.5 and 7.0. Silica used in the experiments had a mobility of about \(-4 \mu m \cdot s^{-1} \cdot V^{-1} \cdot cm^{-1}\) at both pHs (Bales et al., 1991).

Concentration of the bacteria at column outlet achieved a steady-state value about one pore volume after the passage of bacteria into the column (Figs 2 and 3). Column outlet divided by inlet concentration (\( C/C_0 \)) values then dropped slightly in the experiments with _P. fluorescens_ about one pore volume after switching the column feed to a bacteria-free solution. In the _B. subtilis_ experiments, \( C/C_0 \) dropped to approx. 1% of the previous steady-state value changing to a bacteria-free feed solution. Overall,
10–94% of the retained bacteria were released during the times that bacteria-free solutions were passed through the columns (Table 1).

In experiments 1 and 2, at pH 7.0 with P. fluorescens, the steady-state C/C₀ s were approx. 0.06 and 0.01, respectively [Fig. 2 (a) and (b)]. The first experiment has a small amount of air entrapped in the column, but that did not appear to have had a significant effect when compared to experiment 2 without entrapped air. One pore volume after switching to a bacteria-free buffer, C/C₀ dropped to about 10% of the prior value in experiment 1 and to about 30% in experiment 2; it then gradually increased to about the same level as before the drop

![Fig. 3. Breakthrough curves of column experiments with B. subtilis through 0.5 mm silica beads; experimental conditions noted in Table 1. Regions separated by vertical dotted lines as noted on Fig. 2. C₀ indicates bacteria concentration in column inlet.](image)

(C/C₀ = 0.09). After 32 pore volumes, introduction of a pH-7.0 beef-extract solution in experiment 1 caused an increase in C/C₀ to 15; followed by a steady decline over the next 5 pore volumes. The main reason for adding the beef extract was to determine if there were viable bacteria remaining in the column. Beef extract is a high-ionic-strength protein solution, and has been shown to induce bacteriophage detachment under similar conditions (Bales et al., 1991). Addition of beef extract apparently stimulated growth, so it was not used in subsequent experiments. In experiment 2, increasing the column-inlet pH to 8.0 after 45 pore volumes resulted in a slight decline in column-outlet concentration followed by a gradual increase. Thus detachment rate was relatively insensitive to pH in this range.

In experiment 3 at pH 5.5, C/C₀ was about 0.4, or 4–6 times that at pH 7.0 [Fig. 2(c)]. That is, greater bacteria removal occurred at the higher pH. The bacteria were first detected after 0.6 pore volume of water had passed through the column. Upon switching to a bacteria-free-buffer, C/C₀ dropped from 0.4 to 0.05, then dropped slowly over 11 pore volumes to a C/C₀ of 0.03. pH was then increased to 8.2, causing another drop in C/C₀ to about 0.005, followed by a further steady decline.

In experiments 4 and 5, at pH 7.0 with B. subtilis, the steady-state C/C₀ values were about 0.2 and 0.8, respectively. Bacteria were first detected after 0.6–0.8 pore volumes of water had passed through the
column. In both experiments, the column-outlet concentrations dropped to less than 1% of the previous steady-state value upon switching to a bacteria-free solution. This gave \( C/C_0 \) values below the detection limit in experiment 4, and \(< 0.001\) in experiment 5. Increasing the pH to 8.0 caused a noticeable increase in \( C/C_0 \) and thus in the rate of detachment in both experiments.

**DISCUSSION**

Experiment 1 had a steady state \( C/C_0 \) value that was 65% of that for experiment 2; but using equation (7) to estimate equivalent \( C/C_0 \) values for the two experiments at the same velocity (0.0090 cm s\(^{-1}\)) gives \( C/C_0 \) values that are within 10%. The respective means and 95% confidence intervals are 0.067 ± 0.009 and 0.074 ± 0.014. Thus the experiments showed good reproducibility for *P. fluorescens*. The differences in log \( C_0 \) (5.3 vs 4.3) for the two experiments should not affect the results. In experiment 3, the steady state \( C/C_0 \) was 25 times that for the average of experiments 1 and 2 (0.381 ± 0.061 vs 0.070 ± 0.016), suggesting that the pH effect was significant.

Experiments 4 and 5 with *B. subtilis* were also replicates, and experiment 4 had a \( C/C_0 \) value that was 30% of that for experiment 5. Using equation (7) to estimate equivalent \( C/C_0 \) values at \( u = 0.0090 \) cm s\(^{-1}\) gives \( C/C_0 \) values and 95% confidence intervals of 0.20 ± 0.01 and 0.51 ± 0.12, respectively. Again, differences in log \( C_0 \) (3.4 vs 4.6) were not thought to be responsible for the differences. The two-experiment mean \( C/C_0 \) was 0.34 ± 0.10.

The between-experiment differences between observed and relative \( C/C_0s \) reflect the normal variability expected in biocolloid experiments. At a minimum, differences in \( C/C_0 \) of one-half log unit (factor of 3) should be considered significant.

*Pseudomonas fluorescens* attachment to silica beads was greater at higher pH, with \( C/C_0 \) averaging 0.08 at pH 7.0 vs 0.4 at pH 5.5. Detachment rates in a bacteria-free buffer were greater in the pH-7 experiments, as evidenced by the lack of a drop in \( C/C_0 \) upon switching to a bacteria-free buffer in experiments 1 and 2. This greater detachment rate at higher pH suggests that either hydrophobic effects or specific chemical interactions were of primary importance. We discount the importance of electrostatic repulsion between the negatively charged silica and bacteria because there was a relatively small change in the electrophoretic mobility of both silica and bacteria in going from pH 5.5 to 7.0. The relative values for bacteria were −2.0 and −2.1 \( \mu \text{m cm V}^{-1} \text{s}^{-1} \) (Table 1) and for silica were −2.2 and −2.8 \( \mu \text{m cm V}^{-1} \text{s}^{-1} \) (Bales et al., 1991). Electrostatic repulsion between two like-charged surfaces should increase at higher pHs; differences in this case were apparently not significant. The enhanced bacterial elution upon raising pH from 7 to 8 is consistent with electrostatic repulsion being important.

Bacteria surfaces contain hydrophobic substances such as lipids, giving them the ability to exhibit hydrophobic interactions. In general, both hydrophobic and hydrophilic factors influence bacterial attachment to surfaces (Van Loosdrecht et al., 1990). The pH dependence of hydrophobic effects should be small relative to that for electrostatic or specific-chemical effects. Attachment of both *B. subtilis* and *P. fluorescens* to silica beads should also involve hydrophobic effects. Doyle et al. (1984) showed the greater hydrophobic nature of spores as compared to vegetative cells of *B. subtilis* by demonstrating the stronger adherence of the spores to (hydrophobic) hexadecane. But of nine *Bacillus* species tested, *B. subtilis* was the least hydrophobic.

*Pseudomonas fluorescens* attachment was more easily reversible than was *B. subtilis*, as evidenced by the significant drops in \( C/C_0 \) upon switching to a bacteria-free buffer in experiments 4 and 5. The change in log \( C/C_0 \) was −0.7 in experiments 1 and 2, versus at least −2 in experiments 4 and 5.

Calculation of \( \alpha \) from the experimental results was done using equations (3)–(7). Values used in the calculation were: \( T = 280 \text{ K}, \mu = 0.014 \text{ g cm}^{-1} \text{ s}^{-1}, k = 1.4 \times 10^{-16} \text{ cm}^3 \text{ s}^{-1} \text{ K}^{-1}, d = 2.5 \mu \text{m} \text{ for } *P. fluorescens* \text{ and } 1.7 \mu \text{m} \text{ for } *B. subtilis*, \( d = 0.05 \text{ cm}, \theta = 0.43, \rho_s = 1.1 \text{ g cm}^{-3} \text{ and } g = 980 \text{ cm} \text{ s}^{-2}. \) The largest dimension of the bacteria was used, which assumes that rotational diffusion should be fast relative to advection in our experiments. For a spherical particle, the distance that a point on the surface moves by translational and rotational diffusion are approximately equal (Lykelma, 1976). \( A \), was calculated to be 31.7.

For the observed steady-state \( C/C_0 \) values, \( \alpha \) was calculated to be near 1.0 (Table 1). That is, bacterial attachment was essentially that predicted for no repulsion between colloid and collector, despite the presence of negative charges on both. For *P. fluorescens* at pH 7, \( \alpha \) was slightly greater than 1.0 (1.2 ± 0.2); the difference is not statistically significant, however. Possible causes for \( \alpha > 1 \) could be a greater effective diameter of bacteria owing to appendages or polymeric coatings, or hydrophobic exclusion from the water. Using the smallest size of the bacteria (0.8 \( \mu \text{m} \)) gave \( \alpha \) within 13% of those in Table 1. Including a hydrodynamic correction (Rajagopalan and Tien, 1976) gave \( \alpha \) about 50% higher than those in Table 1. Given the relative bacteria and silica sizes, physical straining should be unimportant relative to filtration. Martin et al. (1991) also reported complete or nearly complete breakthrough of *P. arugans* in 30-cm columns packed with glass beads, suggesting an \( \alpha \) near one.

Harvey and Garabedian (1991), reported \( \alpha \) of \( 5 \times 10^{-3} - 1 \times 10^{-2} \) for transport of bacteria (0.2–1.4 \( \mu \text{m} \)) in their Cape Cod field experiments; Bouwer and Rittmann (1992) noted that the \( \alpha \)
should be 80% lower using the same model and assumptions that we apply here. Both estimates are less than 1% of those for the bacteria column experiments in this study. The difference in buffer could be in part responsible for greater removal in our columns. One main difference is thought to be Harvey and Garabedian (1991) use of native bacteria vs our use of laboratory cultures. C/Cb was 0.1–0.8 over 20 cm in our columns, and 0.1–0.04 over 6.8 m in their field studies. This could reflect large differences in size and hydrophobicity of the different bacteria; note that Harvey and Garabedian (1991) did not distinguish different species. They also point out the uncertainties due to bacterial motility, chemotaxis (movement in response to chemical gradients), buoyant densities and microbial and grain-size morphology (Harvey and Garabedian, 1991). Indigenous bacteria may be better adapted to transport than our laboratory strains. A second difference is our use of packed laboratory columns of uniform media, versus their in situ experiments, natural heterogeneities in flow paths should be important. Smith et al. (1985) noted up to two order-of-magnitude differences in Escherichia coli movement in disturbed vs intact soil cores. In general, lab and field tests are not directly comparable for this reason. The importance of macropore flow for biocolloid transport has been noted in several natural soils (Rahe et al., 1978). Two physical factors were significantly different between the studies: (i) flow velocity and (ii) distance from inlet to outlet. First, the velocity in this study (≈1 × 10^{-2} cm s^{-1}) was about 25 times that of Harvey and Garabedian (1991) (0.33 m day^{-1} = 3.8 × 10^{-4} cm s^{-1}, resulting in one order of magnitude smaller ηS for our study. Second, our column length was only 20 cm, while their distance from the injection well to the monitoring well was 6.8 m (about 35 times). In principle, these differences should not affect the results.

Smaller ηS were also reported for bacteriophage (0.023 and 0.069 μm diameter) transported through the same glass-bead columns as used in our current work, with α values on the order of 0.001–0.01 (Bales et al., 1993).

In a series of experiments using sand columns, it was recently reported that transport of Rhodotorula, a species of yeast found in groundwater, was significantly retarded (Marlow et al., 1991). Using our previously described approach for estimating ηS from non-steady-state experiments (Bales et al., 1991), we estimated ηS from their breakthrough curves to be 0.1–0.2. Their column length was not reported, so we assumed 20 cm.

CONCLUSIONS

Differences in pH of 5.5 vs 7.0 had a small but significant effect on P. fluorescens transport, suggesting that hydrophobic factors or specific chemical interactions dominated over electrostatic repulsion in bacterial adhesion to silica beads. Laboratory strains of P. fluorescens and S. subtilis had high α values, i.e. they were not efficiently transported through silica beads. Both should be retained over even short distances in sandy soil. Bacteria attachment was slowly reversible, with changes in pH from 7.0 to 8.0 having a small effect on bacterial detachment.

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