Environ. Sci. Technol. **1998**, *32,* 1699−1708

Influence of Fluid Velocity and Cell Concentration on the Transport of Motile and Nonmotile Bacteria in Porous Media

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The effect of fluid velocity on the transport of motile and nonmotile bacteria was studied in saturated soil columns using radiolabeled cells. According to colloid filtration theory, decreasing the bulk fluid velocity in a porous medium increases the number of collisions of passive colloids with particles and, therefore, should result in increased colloid retention in porous media. However, for motile cells, there was a variation in cell retention significantly different from that predicted by filtration theory at low fluid velocities, leading to the conclusion that filtration theory is not applicable for this motile bacterial strain at low fluid velocities. As the pore velocity was decreased from 120 to 0.56 m/day, the fractional retention of motile cells (*Pseudomonas florescens* P17) decreased by 65%, and the collision efficiency (α) defined as the ratio of particles that attach to soil grains to particles that collide with the soil (calculated using a filtration equation) decreased from 0.37 (120 m/day) to 0.003 (0.56 m/day). For passive colloids, the fractional retention (if α is a constant equal to 0.01) would increase by more than 800% over this same velocity range. To support our conclusion that cell motility was the factor producing this change from filtration theory, we rendered P17 cells nonmotile and tested this strain and a second nonmotile strain [*Burkholderia* (*Pseudomonas*) *cepacia* G4] under the same conditions. Collision efficiencies for both nonmotile suspensions were constant. For nonmotile P17, α was equal to 0.018 ± 0.003 (0.56−590 m/day). Over a wide velocity range for nonmotile G4, α was equal to 0.22 ± 0.067 (11−560 m/day). Swimming cells were presumably able to avoid sticking to soil grains at low fluid velocities, but at high fluid velocities, cell motility did not reduce attachment. Two additional factors known to affect cell transport (solution ionic strength and cell concentration) were also examined with these two strains in porous media. Decreasing the ionic strength from 4.14 to 0.0011 mM (at a constant pH) decreased cell retention for motile P17 by 39 ± 12%, but this is less of a reduction than is typically observed for nonmotile strains. Increasing the cell concentrations of motile P17 increased the overall retention of cells, suggesting that previously deposited cells provided a more favorable surface for adhesion than the native soil (ripening). In contrast, increasing the cell concentrations of G4 resulted in lower retention, suggesting that deposited cells provided a less favorable collector surface (blocking). These results need to be further investigated with other motile and nonmotile species. However, our results do suggest that wider dispersal of cells during bioaugmentation than previously thought possible may be achieved by using a combination of motile cells, low pumping velocities, and low ionic strength solutions. Optimal cell concentrations to use for in situ bioaugmentation of contaminated soil will depend on the adhesion of the bacterial strains for soil grains and with each other, but in general blocking-type cells are capable of greater dispersal at higher concentration than ripening-type cells.

Introduction

A variety of microorganisms have been isolated that can degrade common chemical pollutants such as BTEX compounds and chlorinated aliphatics (*1, 2*). Some of these microorganisms show promise for subsurface bioremediation. However, to widely introduce these laboratory-grown microbes at high concentrations into contaminated aquifers, it may be necessary to facilitate their transport over larger distances (*3*). Large losses of cells due to attachment to soil grains have been observed in bioaugmentation tests (*4, 5*). Most laboratory strains investigated have high collision efficiencies (α values) at ionic strengths comparable to groundwater ($0.1 < \alpha < 1$; *6, 7*), where α is defined in filtration models as the fraction of colliding bacteria that attaches to the soil. Order of magnitude reductions in cell concentrations in a few centimeters can occur if α values are high (*8*).

Previous experiments on the transport of inorganic colloids, viruses, and bacteria in porous media suggest that bacterial transport in soil can be enhanced by altering physical factors such as water velocity and cell concentration (*9*−*12*). Column tests on nonmotile species support the trend of decreased colloid retention with increased velocities predicted by filtration models (*13*−*15*). However, previous column studies were designed to examine attachment at velocities produced by natural aquifer gradients, typically 0.1−10 m/day. During pump-and-treat soil remediation, high pumping rates may increase flow velocities by orders of magnitude (*5*), resulting in a flow range that has not been tested using filtration theory in subsurface applications, although high flow rates are common for water treatment filters (*16*). Clean-bed filtration theory predicts no effect of particle concentration on colloid retention (*17*), although high colloid concentrations are known to affect colloid retention in porous media (*11, 18*). Due to the large range of experimental conditions reported in the literature, it has not been satisfactorily explained whether the discrepancies between theory and experiments for the effects of colloid concentration and velocity on transport are due to violation of the clean-bed assumption or if in fact there are phenomena, such as blocking, ripening, or the orientation of cells and collectors during deposition with respect to velocity, that are not included in the theory. Rijnaarts et al. (*11*), using cell concentrations of 10^7 or 10^8 mL^{-1} and high surface coverages ($\geq$20%), proposed that some bacteria exhibit blocking where attached cells prevent further cells from attaching by blocking a portion of the collector surface (*17*). If colloidal interactions are favorable, increasing cell concentrations results in multilayer coverage (*18*), defined here as ripening.

The effect of fluid velocity on colloid transport is correctly predicted for passive particles, but it is not known whether

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S0013-936X(97)00996-6 CCC: $15.00
Published on Web 04/29/1998

the effect of velocity on the transport of nonpassive particles, such as motile bacteria, can be correctly predicted with colloid filtration theory. There are several reasons to believe that filtration theory may not apply to motile cells. First, dispersion is not accounted for in filtration theory, and cell motility could result in the cells transporting greater distances due to an increase in dispersion (*19*−*21*). Second, motile cells have been found to be more likely to attach to and colonize surfaces than nonmotile strains (*22*, *23*), resulting in higher retention of cells. Third, the behavior of motile cells at surfaces has been studied, and the results of these studies indicate that motile bacteria do not behave the same as passive particles when they are close to a surface. When motile cells come within 10 μm of a surface, they may swim along the surface at different orientations, either parallel to the surface, perpendicular to the surface (*24*, *25*), or tracing out circles along the surface (*26*, *27*). These three factors lead us to question whether filtration theory can accurately predict the transport of motile cells. However, there is a discrepancy in the literature of whether motility will enhance or deter cell transport since in some cases motile cells have been shown to transport further (*20*, *21*) and in some cases motile cells have been shown to attach to surfaces more than nonmotile cells (*22*, *23*).

The effects of water velocity and cell concentrations on bacterial transport in groundwater aquifers need to be better understood to develop the best strategies for enhancing soil remediation through bioaugmentation. Bacterial transport in laboratory columns has consistently been enhanced by applying low ionic strength (low IS) solutions (*8*, *28*−*30*). Unfortunately, most of these studies used highly cleaned surfaces as the porous media, not soils. In this study, we investigated the effects of flow velocity, cell motility, solution ionic strength, and cell concentration on bacterial transport in an unmodified soil. To compare our results with the broader literature on inorganic colloids and other bacterial transport studies, we analyzed our cell retention data using the clean-bed filtration model of Rajagopalan and Tien (R−T model; *31*−*33*). Transport in low IS water and artificial groundwater (AGW) was studied at pumping velocities between 0.56 and 590 m/day at constant cell concentrations (average = 2.0×10^7 mL^{-1}) using motile and killed (nonmotile) cells of the same bacterium and another nonmotile strain. Experiments were then conducted at a fixed fluid velocity, but at different cell concentrations, to determine whether cell retention was consistent with clean-bed filtration theory or if blocking or ripening produced deviations from filtration theory in regard to cell retention.

Materials and Methods

Filtration Theory and Calculation of Collision Efficiencies. Filtration theory (*31*−*33*) has been widely used to model the transport of bacteria in porous media (*7*, *8*, *30*, *34*). The measured fraction of bacteria that are retained in the column is used to calculate the collision efficiency, α. Rajagopalan and Tien's model (*31*; R−T model) can be used to relate the collision efficiency to the fraction of bacteria retained over a length, L, or the steady-state breakthrough concentration at the column effluent, C/C_o, by

$$F_R = \left(1 - \frac{C}{C_o}\right) = 1 - \left\{\exp\left[-\frac{3}{2}\frac{(1-\theta)}{d_c}\alpha\eta L\right]\right\} \quad (1)$$

where F_R is the fraction of bacteria retained in the column, C and C_o are the effluent and influent bacterial concentrations, θ is the porosity of the media, and d_c is the collector diameter. The collector efficiency, η, models diffusion and the physical factors affecting collisions: neighboring particles, London−van der Waals forces, interception, and gravitational settling. The collector efficiency includes a diffusivity term, which is the molecular diffusivity of the bacteria. The collision efficiency is often measured using the steady-state breakthrough concentration, C/C_o, over a whole column of length L (*21*, *35*). Instead, we calculated α by measuring the fraction of bacteria retained in 1-cm slices of the column, $F_{R,i}$, following the procedure used by Martin et al. (*36*). From the measurement of $F_{R,i}$, the collision efficiency (α_i) could be calculated at discrete locations in the column (*36*).

The dimensionless collision number, ξ, was used to scale column length based on collision frequencies (*36*) to compare particle removal at different velocities. The collision number is the ratio of the rate particles enter a column to the rate particles collide in the column and is calculated as

$$\xi = \frac{3}{2}\frac{(1-\theta)\eta}{d_c}L \quad (2)$$

Therefore, ξ equals the number of collisions a nonattaching bacterium undergos during flow through a column of length L.

Cultures. The bacteria used in these experiments were *Pseudomonas fluorescens* P17 and *Burkholderia* (*Pseudomonas*) *cepacia* G4. P17, provided by C. P. Gerba (Department of Microbiology and Immunology, The University of Arizona), was originally isolated from a water distribution system in The Netherlands. P17 is a motile, Gram-negative, rod-shaped bacterium with an average equivalent diameter of 0.8 μm (*37*). The transport properties of P17 have been previously described on quartz soil and using low IS water (*7*, *37*). The electrophoretic mobility of P17 was measured following the procedure of Baygents et al. (*38*), using a Beckman P/ACE System 2100 capillary zone electrophoresis (CZU) unit.

To compare the results of the motile P17 transport experiments with that of a nonmotile and potentially more relevant bacterium for soil remediation, the transport of G4 was also examined. G4 is able to cometabolically degrade TCE in an appropriate growth environment (*39*). G4, provided by D. F. Dwyer (Department of Civil Engineering, The University of Minnesota) is nonmotile, rod-shaped, and Gram-negative (*39*) and has an average equivalent diameter of 1.0 μm as measured using a microscope (Olympus BH-2) and image analysis system (Galai ScanArray).

Cultures were grown in MOPS/mineral salts media with 1.39 mM glucose (*37*; P17) or M9 buffer containing 20 mM lactate and a mineral salt solution (*40*, *41*; G4). After reaching late-log phase, bacteria were diluted to the desired experimental concentration and radiolabeled with 40 μL of [^{3}H]-2,3,4,5-leucine (116 Ci/mmol, 1 Ci/mL, ICN Laboratories) as previously described (*36*). Excess radiolabel was removed by filtering cells onto a 0.2-μm syringe filter and then backwashing the filter with either AGW or low IS water (the desired suspending phase) to recover cells at a final concentration of $2.0 \times 10^7 \pm 1.6 \times 10^7$ cells/mL, unless noted otherwise (*37*). Acridine orange direct counting (AODC) procedures (*42*) were used for cell counts. Cells were resuspended in either AGW or low IS water for column experiments. Temperature, growth media, viscosity of the media, and properties of the specific cell strain each affect cell motility (*43*). Removing cells from growth media and resuspending them into AGW and low IS water may have affected their motility in ways that we were not able to detect, even altering their swimming speed. However, cells were verified by microscope examination to be motile and verified to still be capable of growth by plating on 10% agar plates (data not shown). To conduct experiments with nonmotile cells, P17 was killed and therefore rendered nonmotile by adding an acridine orange solution containing 2% formaldehyde to a final acridine orange concentration of 0.001%. A similar DNA-specific stain, 4′,6-diamino-2-phenylindole (DAPI), has been used to label bacteria for

field tests (*3*, *44*), to prevent cells from growing, and to be able to identify added cells from indigenous microorganisms.

For the longest duration experiments (~5−25 h), we wanted to be sure that cells were not growing or dying and that no radiolabel was excreted by the cells. Therefore, the number of cells and the total amount of radioactivity present in the cells were each counted before and after the two longest experiments (0.56 and 2.7 m/day studies). Indigenous bacteria were not found to contribute to these overall counts, and growth was not a factor as no growth media was provided in the columns, and most experiments took only a few hours to complete.

Substantial deposition onto soil grains during a filtration experiment can influence the subsequent deposition rate of cells (*18*). For example, if cells do not stick to each other, they will block the subsequent deposition of other cells. Therefore, standard jar tests were performed on P17 and G4 to determine their cell-to-cell collision efficiencies (α_c values) relative to each other. Bacterial solutions were assumed to coagulate due to Brownian motion and shear in an additive manner, and α_c was calculated using

$$\frac{dN}{dt} = -\alpha_c (b_{br} + b_{sh})N \tag{3}$$

where N_o and N are the initial particle concentration and the concentration at any time, t; b_{br} is a constant accounting for the contribution of Brownian motion to coagulation ($b_{br} = 4kTN_o/(3\mu)$, where k is Boltzmann's constant, T is temperature, and μ is the viscosity of the fluid); and b_{sh} is a constant accounting for shear contributions ($b_{sh} = 2Gd_p^3N_o/3$, where G is the shear rate and d_p is the diameter of the bacterium) (*45*, *46*). Completely destabilized particles have maximum α_c values of only 0.5. The calculation of α_c was not meant to be used as a substitute for intra-column or overall (column) collision efficiencies but was instead measured to determine if blocking-type cells had lower α_c values than ripening-type cells.

Porous Media and Solutes. The soil used in these studies was a southern Arizona soil, collected 3−6 ft below the surface from the North Fallow Field at the University of Arizona farm. The soil has an average particle diameter of 127 μm (*47*); is composed of 90% sand, 7% silt, and 3% clay; and has an organic carbon content of 0.07%. The fraction of organic carbon was measured in the Soil, Water, and Plant Analysis Laboratory at the University of Arizona, Tucson, using a standard TOC analysis procedure (*48*). Soils were packed dry and without pretreatment into 7-cm × 1.5-cm glass columns (Omnifit) using a modified version of the tap and fill method (*37*, *49*) to a porosity of 0.41. We concluded that the media was packed uniformly and that there was no short-circuiting on the basis of a dye test and a [^{14}C]glucose tracer test, which showed little breakthrough before 1 pore volume and a low dispersion coefficient (0.005 cm^2/s; *50*). Low IS water (IS = 0.0011 mM) was sterile filtered, deionized water (Milli-Q water, Millipore Corp.). The artificial groundwater (AGW; IS = 4.14 mM) was prepared as described by Li (*47*).

Column Experiments. Packed columns were acclimated to AGW for 10 pore volumes (1 PV ≅ 4.5 mL) using a pediatric infusion pump (3M Health Care, velocities ≤ 20 m/day) or a peristaltic pump (Masterflex, velocities > 20 m/day). Low IS columns then received 10 PV each of low IS water; radiolabeled bacteria suspended in low IS water; and a cell-free, low IS water rinse. Separate columns were run for the same number of pore volumes using only AGW as controls for the effect of low IS water, so that results at groundwater ionic strengths could be separated from results at low ionic strength. The column effluent was sampled continuously using a fraction collector (Gilson) after radiolabeled bacteria were added.

After each transport experiment, the column media were extruded and sliced into 1-cm increments. To minimize interference of the soil with counting, these 1-cm slices were then divided into smaller increments, and only a portion of each 1-cm slice was used per sample in the scintillation counter. Subsections were weighed, mixed with 10 mL of scintillation cocktail (CytoScint, ICN Laboratories), and agitated for 18 h on a shaker table before analysis in a liquid scintillation counter (Beckman LS 3801). The radioactivity per soil sample was calculated using the mass of soil in each sample. Effluent (liquid) samples were mixed by hand and immediately analyzed.

Recovery of radiolabel from soil samples was lower when using soils, versus cleaned quartz, a trend that has been observed by others. Rogers (*51*) found that 44% of radiolabel could be recovered from Arizona soil, while 81% could be recovered from cleaned quartz. The interference caused by the soil may have been due in part to the color of the soil but also included other factors that were not identifiable. One method that has been used to account for this interference is the application of a spike correction factor (*50*, *51*). Samples were spiked with a known quantity of liquid radiolabel and analyzed for recovery. When a spike was added to a liquid solution, the full amount of the added spike could be measured. However, when a spike was added to a soil-containing sample, the full amount of the spike was not recoverable. Therefore, a correction factor was developed and calculated for each slice or portion of slice in the column:

$$CF_i = \frac{D_s}{D_{o+s} - D_o} \tag{4}$$

An average was taken over the samples in the column, which resulted in the overall column correction factor:

$$CF = \frac{\sum CF_i}{n} \tag{5}$$

where CF is the column correction factor, CF_i is the correction factor in the portion of slice that was analyzed, D_s is the number of dpm in the sample used for spiking, D_o is the dpm of the slice with the radiolabeled bacteria that deposited on it during the column experiment, D_{o+s} is the dpm of that same slice after the spike solution has been added, and n is the number of samples. For a typical experiment, the 7-cm column was sliced into 1-cm increments that were each quartered. Each quarter of a slice was spiked, and a CF_i was calculated, resulting in $n = 28$ samples. The overall column correction factor (CF) is a number greater than 1 that is multiplied by the uncorrected counts to obtain the corrected value of the counts.

Even after spiking and reanalzying the samples, the recovery of radiolabel was not 100%. Experimental results for overall α values and intra-column α_i values are reported using the values of α and α_i that were obtained after correcting the recovered radiolabel in the soil using a spike correction factor. A second method of calculating collision efficiencies, based on recovered label (equivalent to forcing the mass balance to 100%), was applied using

$$\alpha_{mb} = \frac{-2}{3} \frac{\ln (M_{eff}/M_{in})}{(1 - \theta)\eta L} \tag{6}$$

where α_{mb} is the overall collision efficiency based on a mass balance using the column effluent, M_{eff} is the total cell mass that came out of the column, and M_{in} is the total cell mass that was put into the column. The intra-column α_i values

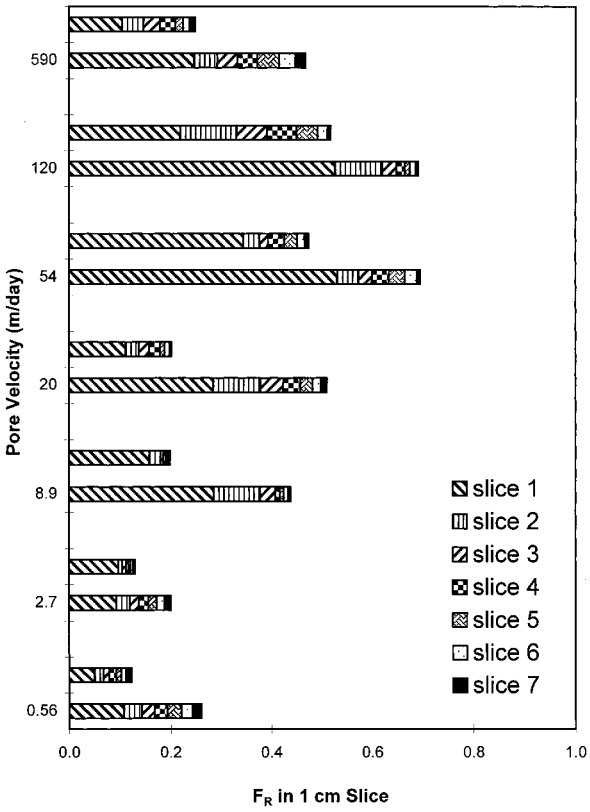

FIGURE 1. Fraction of bacteria retained in 1-cm slices over the length of a 7-cm column at different pore velocities. For each velocity pair, top bar is low ionic strength (IS) water, and bottom bar is artificial groundwater (AGW). The greatest retention occurs in the first slice, with the least retention in the last slice, as predicted by filtration theory.

could not be adjusted to 100% recovery since the quantity of label going into and coming out of each slice of the column could not be independently measured from liquid concentrations.

The procedure used for the experiments in which cell concentration was varied was the same procedure that was used for the velocity and ionic strength experiments, except that different influent cell concentrations and injection volumes were applied. P17 was injected at 10^7 cells/mL for 10 PV in the baseline experiment at 20 m/day. In other experiments, cells were injected at 10^7 mL^{-1} for 1 PV and at 10^6 mL^{-1} for both 1 and 10 PV (20 m/day). When 1 PV of bacteria was injected, the rinse was changed from 10 to 19 PV so that the total number of PV passed through the column was always the same. G4 was tested at 10^7 cells/mL for 10 PV and at 10^8 cells/mL for 1 and 10 PV (11 m/day). Experiments in which the concentration of cells was varied used AGW as the rinse and suspending solution.

Results

Transport of Motile and Nonmotile Cells. Seven different pore velocities (0.56−590 m/day) were tested with AGW and low IS water as the suspending phases using motile P17. The number of bacteria retained in each 1-cm slice decreased over the column length, a trend anticipated from filtration theory (Figure 1). Overall, low IS water treatment decreased the fraction of bacteria retained in a 7-cm column by 39 ± 12%.

According to filtration theory, the fraction of retained cells decreases with increasing velocity, and α is independent of velocity. Contrary to what is predicted using filtration theory if α is constant, the fraction of motile P17 retained increased as velocity increased over the range 0.56−120 m/day when either AGW or low IS water was used as the rinse solution (Figure 2A). To make the fraction retained consistent with filtration theory, α (the only adjustable parameter in eq 1) would have to have increased by more than 2 orders of magnitude from 0.003 (AGW, 0.56 m/day) to 0.43 (AGW, 590 m/day; Table 1). At the highest velocities (120 and 590 m/day), α was nearly constant (0.37 and 0.43 in AGW; 0.21 and 0.23 in low IS water; Figure 2B). The Reynold's (*Re*) numbers in these experiments approached one at the highest velocity tested. Although filtration equations were not developed for this flow range, filtration theory is applicable in water treatment filters, even with high values of *Re* (*16*).

Motility was hypothesized to be the cause of the discrepancy between filtration theory and the transport of P17 at different fluid velocities. Therefore, experiments were repeated using a nonmotile strain and killed (nonmotile) P17. For nonmotile G4 and the immobilized P17, F_R decreased as velocity increased and α was constant with velocity, in accordance with filtration theory (Figure 2C, D). For nonmotile P17, $\alpha = 0.018 \pm 0.003$ between 0.56 and 590 m/day; for G4, $\alpha = 0.22 \pm 0.067$ between 11 and 560 m/day. In comparison, α for motile P17 varied by more than 1 order of magnitude over a similar range (0.031 at 8.9 m/day, 0.43 at 590 m/day; Table 1). The collision efficiency of the nonmotile (dead) P17 was not the same as for motile P17 at high velocities, suggesting that the acridine orange solution that contained 2% formaldehyde modified the collision efficiencies of the cells, beyond simply making the cells nonmotile.

Comparison of Spike-Corrected Overall α and Mass Balance-Corrected Overall α_{mb}. The collision efficiencies reported in Table 1 and shown in Figure 2 for the motile and nonmotile species, calculated using fractional retention data with a spike correction factor (overall α), were also analyzed by assuming a complete mass balance based on total recovered label in the effluent (α_{mb}). For motile P17, the corrected α_{mb} was always higher than the spike-corrected α in both AGW and low IS water. For motile species, the trend of increasing collision efficiency with velocity still held. The mass balance-corrected recoveries for the nonmotile species, however, did not match the trends of the spike-corrected collision efficiencies: although spike-corrected α values were nearly constant with velocity, mass balance-corrected α_{mb} values were more variable and suggest a slight increase in the collision efficiency with velocity. Although we cannot explain the discrepancy between the two approaches to calculating collision efficiency, the spike-corrected α values were considered to more accurately reflect the effect of fluid flow because they are in accordance with filtration theory.

Intra-column Collision Efficiencies. Analysis of intra-column data, obtained by extruding and slicing the column, revealed significant deviations in cell transport from that predicted by filtration theory. They likely are part of the reason for differences between spiking and mass balance collision efficiencies. Within the column, the collision efficiency calculated on a slice-by-slice basis (α_i) was not constant for motile P17 (Figure 3), especially at low velocities. At higher velocities, α_i was high everywhere in the column. Motility was not the cause of the decrease in α_i over the length of the column for P17 since nonmotile strains P17 and G4 also exhibited decreasing α_i values along the length of the column (*50*).

Duplicate experiments at selected fluid velocities verified that the effect of fluid velocity on cell transport was reproducible. For example, for P17 in AGW and low IS water at 20 m/day, this same experiment was performed on three different days (Figure 4), and the results were shown to be reproducible. Although not all experiments were duplicated, certain phenomena were shown to be repeatable: retention

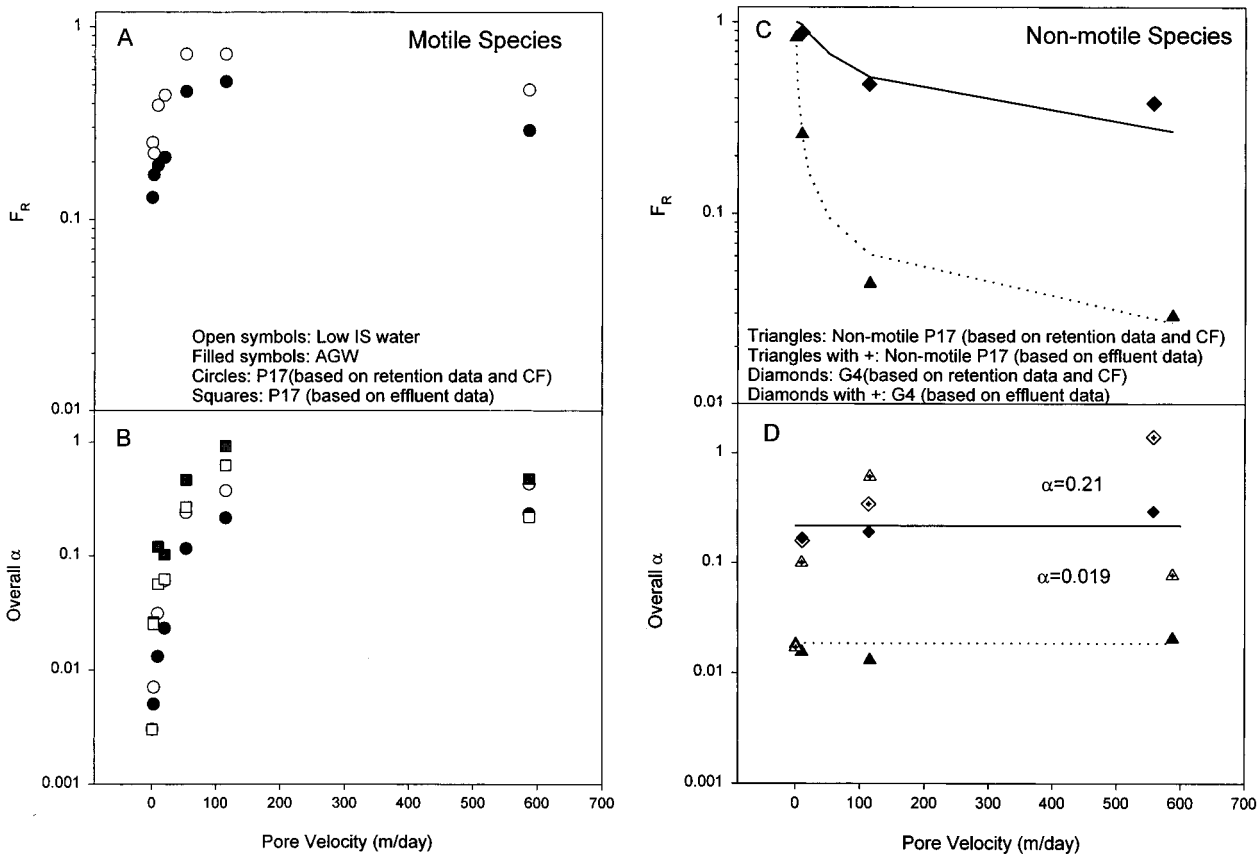

FIGURE 2. Fractional retention and collision efficiency data for motile and nonmotile species. (A) Cumulative fraction retained over column as a function of flow velocity (pore velocity) for motile P17. (B) Collision efficiency calculated using the cumulative fraction retained for motile P17 (circles) and collision efficiency based on effluent data (squares). (C) Same as panel A but for nonmotile species. (D) Same as panel B but for nonmotile species. In panels C and D, solid and dotted lines are best-fit filtration model predictions assuming $\alpha = 0.21$ for nonmotile G4 and $\alpha = 0.019$ for nonmotile P17. In panel D, predictions are shown only for α values calculated from retention data.

TABLE 1. Comparison of Overall α Values Calculated by Fractional Retention Corrected with Spike Factor versus Overall α Values Calculated by Forcing 100% Recovery Using Effluent Data[a]

	P17		P17 in low IS water		nonmotile P17		G4 (nonmotile)	
pore velocity (m/day)	α	α_{mb} (mass balance)	α	α_{mb} (mass balance)	α	α_{mb} (mass balance)	α	α_{mb} (mass balance)
0.56	0.003	0.003	0.001	0.003	0.018	0.017	–[b]	–
2.7	0.007	0.026	0.005	0.025	–	–	–	–
8.9 or 11[c]	0.031	0.12	0.013	0.056	0.020	0.10	0.17	0.16
20[d]	0.068	0.10	0.023	0.062	–	–	–	–
54	0.24	0.46	0.11	0.27	–	–	–	–
110 or 120	0.37	0.92	0.21	0.62	0.013	0.61	0.19	0.34
560 or 590	0.43	0.48	0.23	0.22	0.020	0.077	0.29	1.4

[a] All experiments on Arizona soil with artificial groundwater solutions or low ionic strength (IS) water. All influent concentrations = $2.0 \times 10^7 \pm 1.6 \times 10^7$ cells/mL. [b] Experiment not performed (–). [c] When two velocities are given, all three P17 experiments were at one velocity, and the G4 experiment was at the other velocity. [d] For P17 data, reported results are the average of three experiments.

was always less in low IS water and the collision efficiency always decreased with distance in the column. The experimental procedure we are using here is similar to the MARK test (*52*) except that the column is larger and flow is applied with a pump rather than inducing the flow with a vacuum. This method has been widely used and found to be reproducible (*8*, *47*, *52*). For example, Gross et al. (*52*) found MARK test results to be reproducible to within 4−7% when experiments were performed at least three times with the same bacterial culture. Also, Johnson and Logan (*53*) found that variation in overall retention for a culture on different days was larger than variation in replicate columns performed on the same day, but in general, these variations were not significant.

Blocking. Increased loading of P17 in column studies caused the overall α to increase from 0.013 to 0.068, indicating filter ripening (Table 2, Figure 5). In contrast, increased addition of G4 in column tests produced a blocking effect (Figure 6), resulting in a decrease in the overall α from 0.17 to 0.032.

Since deposited cells may affect the subsequent deposition of cells, we wanted to see if there was a qualitative trend in differences in cell retention in filtration experiments (i.e., blocking versus ripening) and cell-to-cell collision efficiencies (α_c values) measured in jar tests. Although we did not anticipate that the numbers would be identical, we hypothesized that cells with higher α_c values would be more likely to exhibit ripening and that cells with lower α_c values would

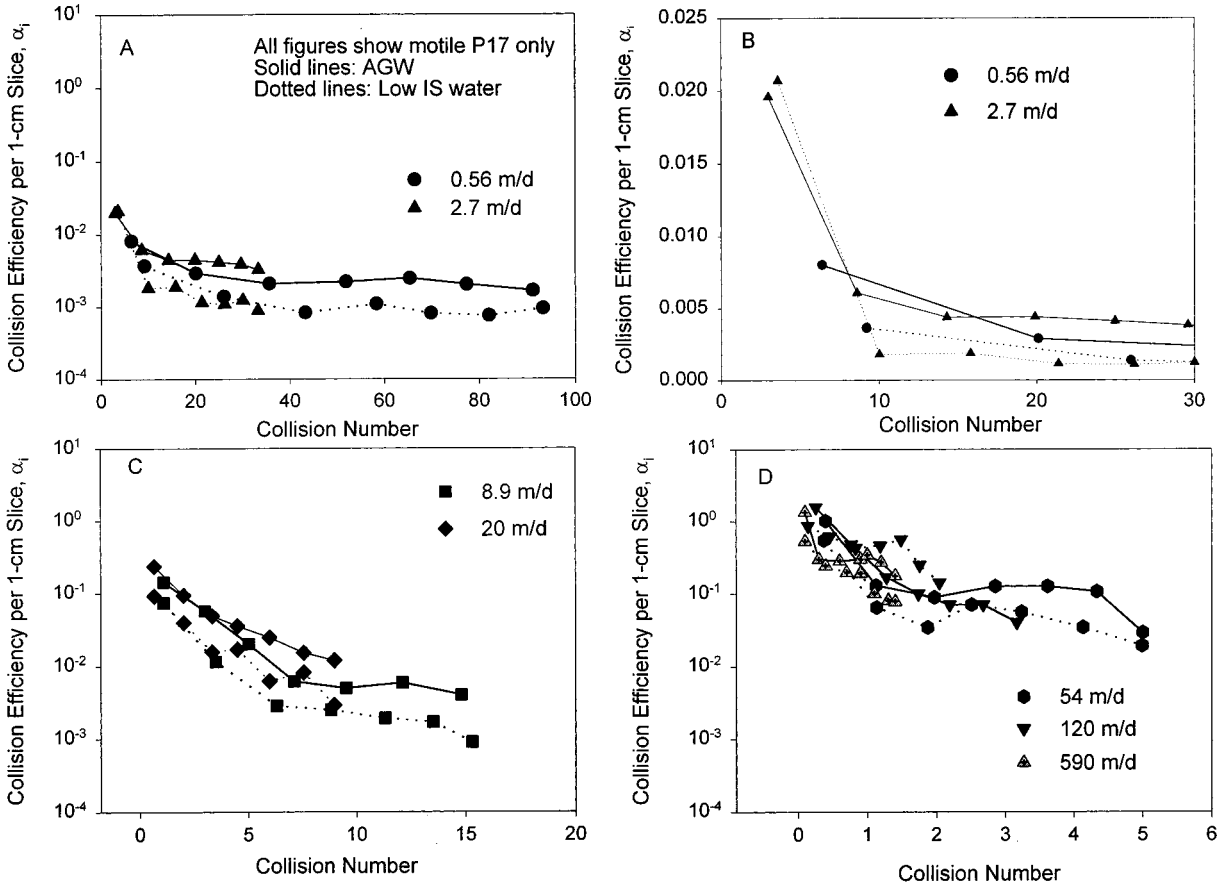

FIGURE 3. Impact of flow velocity (pore velocity) and chemical solution on the collision efficiency per 1-cm slice, for motile P17, scaled to the dimensionless collision number. If α is constant, all data should lie on a horizontal line. Refer to eq 3 for definition of collision number. (A) Lowest velocity range tested. (B) Same data as in panel A but early collision numbers are emphasized. (C) Middle velocity range tested. (D) High velocities.

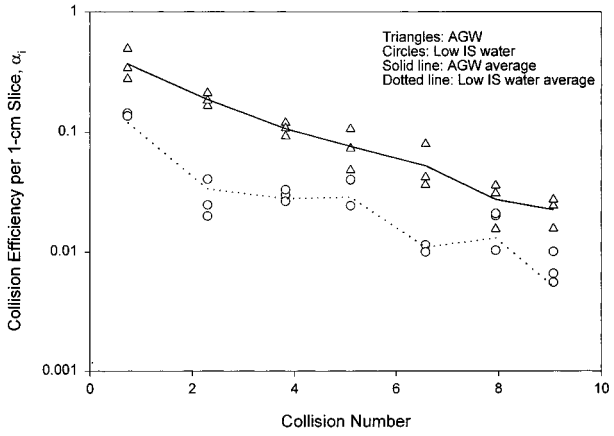

FIGURE 4. Triplicate data points for P17 at a pore velocity of 20 m/day in AGW and low IS water. Lines are averages for the three experiments. The amount of error in the individual experiments is small, and the trends in the data are reproducible. Collision number is defined in eq 3.

be more likely to exhibit blocking. Therefore, α_c values were measured for P17 and G4 in jar tests. The cell-to-cell α_c values did not vary appreciably with the ionic strengths tested (0.0011 mM low IS water and 4.14 mM AGW). For P17, α_c was 0.012 in either AGW or low IS water; for G4, α_c was 0.033 and 0.029 in AGW and low IS water, respectively. The results of the jar tests did not appear to support our hypothesis that the stickier cells should exhibit ripening behavior: G4 had a higher α_c than P17 but seemed to exhibit blocking in column studies; P17 has a lower α_c but appeared to exhibit ripening in column studies. In relative terms, however, neither of these α_c values are near the maximum value of 0.5 expected for completely destabilized particles. The different hydrodynamic conditions between the jar test and the columns may preclude any direct relationship between α_c in coagulation tests and α in filtration tests.

Two approaches to calculating collision efficiencies were again applied in the concentration experiments (Table 2). For P17, an increase in the collision efficiency with increasing concentration is less evident when examining α_{mb} values. For G4, α_{mb} decreased when the influent concentration was

TABLE 2. Overall α at Different Cell Loadings and Comparison of Spike Corrected α with Mass Balance Corrected α

bacterium	C_0	PV injected	total no. of cells injected	overall α (based on F_R)[a]	α_{mb} (mass balance)[b]
P17	1.08×10^6	1	4.86×10^6	0.013	0.083
	1.08×10^6	10	4.86×10^7	0.058	0.14
	1.00×10^7	1	4.50×10^7	0.042	0.081
	2.00×10^{7c}	10	8.55×10^8	0.068	0.10
G4	$1.13 \times 10^{7\ c}$	10	5.09×10^8	0.17	0.16
	1.90×10^8	1	8.55×10^8	0.077	0.18
	1.00×10^8	10	4.50×10^9	0.032	0.046

[a] α calculated from retention data, corrected using CF (eqs 1 and 6). [b] α_{mb} calculated from effluent data, 100% mass balance (eq 7). [c] Reported values are the average of three experiments. For P17, pore velocity = 20 m/day. For G4, pore velocity = 11 m/day.

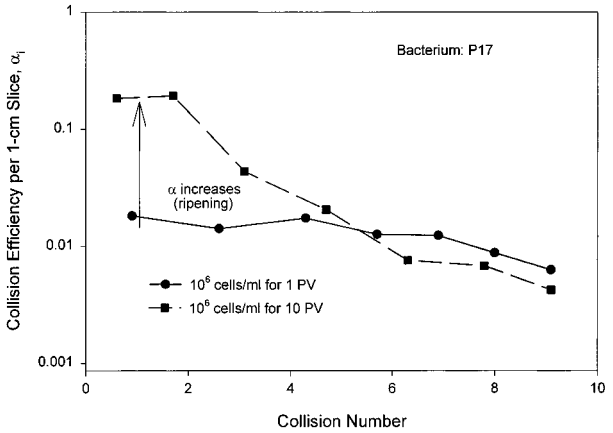

FIGURE 5. Collision efficiency per 1-cm slice for P17. All experiments conducted in AGW at a pore velocity of 20 m/day. Refer to eq 3 for definition of collision number.

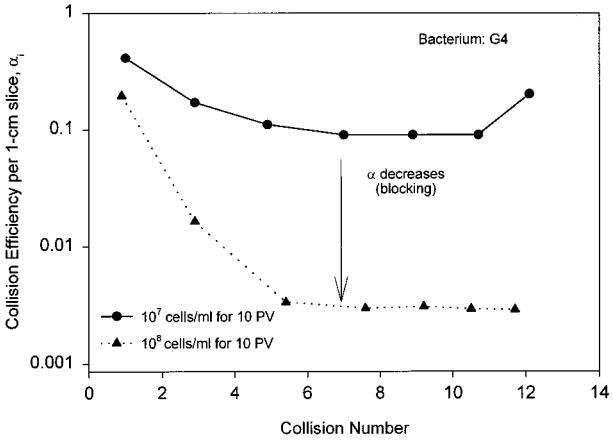

FIGURE 6. Collision efficiency per 1-cm slice for G4. All experiments conducted in AGW at a pore velocity of 11 m/day. Refer to eq 3 for definition of collision number.

decreased from 1.13×10^8 to 1.00×10^7 mL^{-1}, which is the same trend that was observed in the α values. However, since the collision efficiency is not constant throughout the column, the mass balance corrected α_{mb} values are probably not sensitive enough to account for the changes that occur in α_i over the length of the columns.

Discussion

The decrease in cell retention as fluid velocity increased, which was anticipated from clean-bed filtration theory and previous studies of nonmotile species (*21*), was not observed for a motile bacterium, P17. Instead, a large decrease in α was necessary to account for the lowered retention of motile P17 at low fluid velocities. At higher velocities, α was nearly constant and 2 orders of magnitude higher than α at low fluid velocities. We hypothesize that the discrepancy between theory and our observations is related to cell motility. Although we were not able to conduct these experiments with nonmotile mutants (as others have done, see refs *14*, *20*, *22*−*23*, and *54*), P17 rendered nonmotile and another nonmotile strain were removed at overall rates that were consistent with filtration theory in our experimental apparatus under otherwise identical conditions. The variation in α as a function of fluid velocity may be a result of the swimming velocity the cells relative to the fluid velocity. *P. fluorescens* has been observed to travel at velocities $\leq$7.3 m/day (85 μm/s; *22*). As a result, at typical groundwater velocities of $\sim$ 1 m/day, these bacteria can swim faster than the bulk fluid. At high velocities (> 100 m/day), motility apparently becomes unimportant since α was constant. Others have observed decreased retention in porous media by motile species (*14*, *21*), although the range of velocities previously examined has been limited to relatively low fluid velocities. Camper et al. (*14*) found that motile *P. fluorescens* CC-840406 (at 0.49 m/day) transported farther than a nonmotile mutant strain of the same bacterium. In their studies, transport of motile and nonmotile strains was similar at higher velocities (2.4 and 10.8 m/day). Apparently, there is a species-dependent velocity for which motility does not affect cell retention. The nonmotile P17 in this study were transported farther than motile P17 at high velocities, probably because the killed cells were smaller, sometimes lost their flagella, and may have had different surface properties than the motile cells because of the acridine orange addition. Although the nonmotile cells may not have been identical to the motile cells, the behavior of nonmotile P17 and nonmotile G4 was consistent with filtration theory, suggesting that any changes in fluid velocity were primarily a function of the loss of cell motility. Other methods of rendering cells nonmotile, such as heat killing, irradiation, or chemical poisoning, could also cause damage to the cell or induce other changes besides making the cell nonmotile. Developing nonmotile mutants may be an adequate way to study a nonmotile strain that is most similar to the motile strain (*14*, *20*, *22*−*23*, *54*), but there is no guarantee that the mutants will be identical to the parent strain. We did not further explore filtration of nonmotile cells since the control that we did use, the acridine orange treated cells, behaved in accordance with filtration theory predictions. However, experiments with other motile species will be necessary before establishing the generality of our findings.

The behavior of motile cells near a solid surface has been extensively studied in the absence of bulk flow (*24*−*26*, *55*), but relatively little is known about motile cell behavior in advective flow fields. Frymier and Ford (*25*) found that the speed of a bacterium slows as it approaches a solid surface and that the orientation of a swimming cell relative to the surface also affects the cell's speed. Cells that were swimming toward a surface slowed in speed; in some cases, cells swam parallel and along the surface at close approach and eventually regained the (higher) speed they had in the bulk fluid. Frymier et al. (*24*) hypothesized that an attractive−interaction potential was holding the cells in proximity to the surface. Using a 3-D tracking microscope, they also observed that motile cells swam in circles next to solid surfaces and left the surface only after tumbling. Berg and Turner (*26*) found that whether motile cells swam in circles or linearly depended on the size of the capillary the cells were swimming in. Cells in 10-μm capillaries could swim in only one dimension, but cells in 50-μm capillaries were able to swim in circles. Thus, cells in the smaller tubes can swim faster than cells in larger tubes. We estimate the average pore diameter in our experiments to be $\sim$20 μm (*56*), so it is not clear if the cells could swim in circles or not. Probably there are some larger pores where the cells can swim in circles and some smaller pores where the swimming is only one-dimensional. While it is still not clear how the results in the absence of bulk flow can be applied to bacteria swimming in fluid flowing through porous media, such research does suggest that cell motility will alter the behavior of motile cells in proximity to soil grains as compared to passive colloids. In our column experiments, the low α values measured for the motile species further suggest that cell motility may be a desirable attribute for bacterial species chosen for use in bioaugmentation.

Blocking. In the derivation of a clean-bed filtration theory, it is assumed that the column packing is clean, i.e., that deposited cells do not affect the rate of cell removal (*31*). If a collector becomes partly blocked by the presence of

attached cells, the overall α may either increase or decrease when additional cells are added, depending on whether cell−cell attachment is favorable or unfavorable.

Intra-column α_i values are affected by blocking due to variations in the number of deposited cells along the column. Blocking has been modeled (*11*) as

$$\alpha_i = \alpha_o(1 - B\Theta) \quad (7)$$

In this equation, α_i is the observed or apparent collision efficiency, α_o is the clean-bed collision efficiency, B is the blocking factor, and Θ is the surface coverage. The surface coverage decreases along the length of the column (see Figure 1) and B and α_o are assumed to be constant. Therefore, if blocking is important, α_i should increase with column length, not decrease as observed in our studies.

To account for our observations, an alternate model of filter ripening is suggested in which the collision efficiency is allowed to increase as the surface coverage increases. The trends in overall α values suggested that P17 exhibited filter ripening in porous media. Also, the intra-column α_i values support ripening for P17. When a low loading of P17 was applied (1.08×10^6 mL^{-1}, 4.86×10^6 total cells), a constant α_i was observed, suggesting that all sites in the column were equally available (Figure 5). Adding more bacteria (4.86×10^7 total cells) resulted in a nonconstant α_i, presumably because the sites at the beginning of the column were more filled than the sites at the end of the column. The further addition of bacteria (8.55×10^8 total cells) increased the overall α further, supporting the hypothesis that P17 is a ripening-type bacterium (Table 2). Intra-column and overall α values for P17 each indicate that P17 is a ripening-type bacterium.

For G4, there is an inconsistency between overall α values and intra-column α values, as far as blocking is concerned. Overall α values indicate that G4 blocked attachment (Table 2), but the intra-column α_i values for G4 (Figure 5) do not support blocking since α_i decreased along the length of the column (Figure 6), not increased as it should have according to eq 7. The decrease in retention observed at higher cell loadings cannot be explained by blocking, and we do not have an explanation for this decrease in retention that is consistent with a decreasing α_i with distance at this time. Rijnaarts et al. (*11*) reported blocking for a bacterial strain, and others have observed a blocking-type effect of cell concentration on bacterial transport (*15, 57*), but in these latter studies filtration theory was not applied, and retention was not measured inside the columns.

The decrease in α_i with distance has been explained by others (*32, 34, 58, 59*), but these explanations fail to completely explain the results observed in the current study. One of these explanations is that there may be two subpopulations of bacteria within a monoclonal population (*34*): a more sticky group and a less sticky group. Glynn (*58*) has hypothesized that such heterogeneities within a bacteria population are due to intrapopulation surface charge density variations. After performing capillary electrophoresis on several strains, he found that bacteria which exhibited variable α_i values with distance displayed two peaks in their electropherograms. Glynn has hypothesized that this meant the stickier bacteria attached at the beginning of the column, causing a high α_i at the entrance. After the stickier bacteria have attached, there are sites available farther down in the column, but only the less sticky bacteria remain, resulting in lower α_i values at the end of the column. If there is a surface charge density variation within a P17 population, it was not measurable by electrophoresis, even at high cell concentrations ($\sim 10^9$ mL^{-1}) since P17 exhibited only a single sharp electrophoretic mobility peak (*50*).

Second, there may be favorable and unfavorable sites or sites with high α values and sites with low α values within the column (*60*). If preferential filling of sites was occurring, α_i should have increased, not decreased, over the column length as preferred sites were filled in the early part of the column. Size heterogeneity of the soil grains could produce a nonuniform distribution of favorable sites within the column. However, α_i has been found to decrease with distance for P17, even using quartz with a narrow size distribution (*51*), suggesting that the decrease in α_i is not due to grain size heterogeneity. A surface charge distribution could also cause a nonuniform distribution of sites. Cleaning the collector surfaces to remove organic matter should result in a more homogeneous distribution of surface charges. However, Litton and Olsen (*35*) found that for soda-lime bead collectors cleaned with HCl the breakthrough concentration of particles (latex microspheres) increased over time, suggesting that, even for this clean media, not all of the collector sites had equal abilities to attract particles. Rogers (*51*) found that the transport of P17 on untreated Arizona soil and on rigorously cleaned quartz was similar in terms of α_i decreasing with distance. Therefore, either "cleaned" quartz is not homogeneous with respect to surface charge or surface charge heterogeneity cannot explain the decrease in α_i with distance. It is possible that even on cleaned quartz, however, surface charge heterogeneities exist. Cleaned glass beads have been found to contain trace concentrations of Fe_2O_3 (0.3%) and Al_2O_3 (0.2%) (*61*). Since these oxides have a surface charge opposite to bacteria, they are attractive sites for bacterial deposition.

Third, if dispersion was large enough it could produce a decrease in α_i. Calculated dispersion coefficients are smaller than necessary to explain the observed changes in α_i. Dispersion coefficients calculated for bacteria in Arizona soil using the Hiby correlation (*62*) are 10^{-3} to 10^{-6} cm^2/s. The dispersion coefficient for bacteria could be higher, as McCaulou et al. (*21*) found that an apparent dispersion coefficient of 2×10^{-2} cm^2/s was necessary to fit slowly rising bacteria breakthrough curves. Logan et al. (*59*) calculated that a dispersion coefficient of 0.1 cm^2/s in a similar column setup could cause a decrease in α_i of about 40% after 20 cm. Even if the effect of motility is taken into account, dispersion is not great enough to produce such a large change in α_i, according to the following calculation (R. M. Ford, personal communication). Using a velocity of 1 m/day and a typical dispersivity for a laboratory column of 0.035 cm (*63*), a dispersion coefficient of 4.05×10^{-5} cm^2/s is calculated. Accounting for cell motility requires adding a diffusion coefficient for motile bacteria to the dispersion coefficient. For *P. fluorescens* in bulk aqueous solution, the diffusion coefficient is 6×10^{-6} cm^2/s (*64*), resulting in a dispersion coefficient for motile bacteria of 4.65×10^{-5} cm^2/s, an increase of 15% as compared to the dispersion coefficient of the nonmotile strain. According to these calculations, dispersion was not large enough to account for either the decrease in the collision efficiency along the length of the column or the increase in transport of the motile species at low velocities.

None of these explanations is in itself enough to explain why α_i decreased with distance in these column experiments. Perhaps surface charge heterogeneity of the soil grains, dispersion, and blocking or ripening each contributed to the observed effects. Further work will be necessary to provide an answer.

Effect of Low Ionic Strength Solutions. The most consistently successful means of enhancing the transport of bacteria in porous media has been the use of low ionic strength solutions (*8, 28−30*). However, maintaining flow while applying low IS water may be difficult under certain geological and chemical conditions. Vaidya and Fogler (*65*) found that introducing low salinity water into Berea sand-

stone caused a drastic reduction in the permeability of the medium, attributed to the release of micron-sized kaolinite clay particles that were large enough to block fluid paths. They found that if the salinity was decreased gradually, rather than instantaneously, the release of fines did not occur. If low IS water is used in fieldwork, it may be necessary to gradually reduce the ionic strength of the groundwater prior to adding cell suspensions.

The ionic strength of the solution is not the only factor influencing the release of colloids. Weisner et al. (*66*) observed an initial drop in permeability in columns where either low IS water or groundwater was introduced. If low IS water is injected into an aquifer, whether colloids will be released from soils depends on how much colloidal material is present in the groundwater (*66*), the pH of the injected water (*65*), the composition of the porous media, and whether the ionic strength is changed gradually or instantaneously (*65*).

Implications for Bioaugmentation. Filtration theory cannot be solely used as a guide for predicting transport of bacteria in groundwater aquifers since there are intra-column changes in α_i not anticipated from filtration theory. The results of these studies suggest that the transport of bacteria through soil for bioaugmentation will be most successful for a motile bacterium at low flow velocity (0.56 m/day), in low IS water. At low velocities, after an initial period of attachment, the remaining bacteria were not likely to attach. After an attachment of 10% of injected cells in the first centimeter of the column, the collision efficiency per slice reached a constant value of $\sim 8 \times 10^{-4}$ (low IS water, 0.56 m/day). Applying filtration theory after this first centimeter, P17 could travel 11.5 m in an aquifer while undergoing a 2-log reduction in cell concentration ($\alpha = 8 \times 10^{-4}$). This scenario only applies to motile P17, as we have not yet examined other motile species or other soils. In practice, bacterial strains chosen for bioaugmentation are selected based on degradative capabilities, not necessarily for favorable transport properties. Optimization of the injection flow velocity, solution ionic strength, and cell loading can cause enhancements in bacterial transport, but column experiments must be performed in conjunction with filtration model calculations to ensure that increases in cell transport are sufficient to transmit cells into the aquifer and not just the location immediately surrounding the well.

Acknowledgments

This publication was made possible in whole by Grant ES-04940 from the National Institute of Environmental Health Sciences, NIEHS. Its contents are solely the responsibility of the authors and do not necessarily represent official views of the funding agency. We thank J. Glynn for help with the electrophoresis measurements, R. G. Arnold for discussions throughout this study, E. J. Bouwer and K. M. Unice for comments on an earlier manuscript, R. M. Ford for discussions on cell motility, and two anonymous reviewers for their suggestions.

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Received for review November 13, 1997. Revised manuscript received March 20, 1998. Accepted March 24, 1998.

ES970996M