Pergamon

0043-1354(94)00338-6

Wat. Res. Vol. 29, No. 7, pp. 1673–1680, 1995
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0043-1354/95 $9.50 + 0.00

BACTERIAL TRANSPORT IN LABORATORY COLUMNS AND FILTERS: INFLUENCE OF IONIC STRENGTH AND pH ON COLLISION EFFICIENCY

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(First received June 1994; accepted in revised form November 1994)

Abstract—The influence of ionic strength and pH on the transport of *Pseudomonas fluorescens* P17 in porous media was investigated using continuous-flow laboratory columns and a rapid screening technique in which radiolabeled cells were applied to large-pore, glass-fiber filters. Colloid-filtration theory was used to interpret P17 transport results in the two systems. Bacterial retention was directly related to the ionic strength of the carrying solution. Decreasing the ionic strength from 10^{-1} to 10^{-5} M caused the bacterial collision efficiency, α, to decrease nearly 90% (from 0.18 to 0.026 in screening experiments and from 0.12 to 0.015 in column experiments). This change in α is qualitatively consistent with double-layer theory, but suggests that very large changes in ionic strength are needed to influence transport. Bacterial transport was unaffected by changes in pH in the range of $5.5 < pH < 7.0$ in both systems. This suggests that changes in surface properties of the bacteria and the silica collectors over the pH range that is commonly encountered in soil and ground water are not sufficient to influence cell attachment. The screening technique provided a rapid alternative to column experiments. Even though estimated α's were different in the two systems, the responses to changes in ionic strength and pH were similar.

Key words—ionic strength, pH, *Pseudomonas fluorescens*, continuous-flow laboratory study, collision efficiency, bacterial transport, colloid-filtration

INTRODUCTION

Past research on bacterial transport in porous media focused on understanding pathogen migration following land application of wastewaters (Canter and Knox, 1985; Gerba, 1985). A primary goal was to ensure that potentially harmful microorganisms in septic tank and municipal effluents did not contaminate ground-water resources. Studies have shown that the majority of bacteria in wastewaters are readily removed by filtration and adsorption to aquifer material (Gerba and Bitton, 1984; Harvey, 1991). More recently, research has focused on enhancing, not limiting, the extent of bacterial transport. For a microorganism with novel metabolic traits to be useful for *in situ* bioremediation it must be readily transported from the point of application (i.e. injection well) to the zone of contamination. Bacterial adsorption to the solid matrix reduces the area over which the microorganism can be dispersed, thereby restricting the extent of biorestoration. Biofilm growth in the vicinity of injection wells is also a common problem (Molz *et al.*, 1986; Nelson *et al.*, 1985), leading to well-screen and aquifer clogging.

Previous investigators have suggested that microbial transport can be enhanced by chemically modifying the carrying solution (Abbot *et al.*, 1983; Fontes *et al.*, 1991; Gannon *et al.*, 1991; Scholl *et al.*, 1990; Scholl and Harvey, 1992). For the most part, these studies did not provide parameters that could be used for quantitative modeling of transport.

Bacteria and virus transport parameters have been estimated using filtration theory in both field and column studies (Bales *et al.*, 1991, 1993; Harvey and Garabedian, 1991; Kinoshita *et al.*, 1993; Martin *et al.*, 1992; McCaulou *et al.*, 1994). However, larger-scale experiments are time and labor intensive and become impractical when screening the mobility of numerous bacterial species or establishing functional relationships between surface characteristics and cell transport. Several alternative reactor configurations, such as rotating disk experiments (Abbot *et al.*, 1983; Martin *et al.*, 1991) and commercially prepared filters (Hilbert, 1992; Logan *et al.*, 1993), have been used to estimate bacterial transport parameters. In the latter technique, radiolabeled cells were passed through large-pore laboratory filters. Filtration theory and assumptions regarding filter geometry were used to relate fractional cell retention

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to bacterial attachment efficiency. The procedure offered a rapid method for establishing bacterial transport properties as a function of independent variables of interest.

The primary objective of this study was to evaluate the effects of ionic strength and pH on the retention of *Pseudomonas fluorescens* P17 in porous media (silica). The second objective was to establish the correspondence between bacterial transport in commercially available glass-fiber filters and transport through laboratory columns. We examined the effects of ionic strength and pH in the suspending solution on bacterial transport using both filter and column reactors. Fractional bacterial penetration through the two experimental systems was measured, and bacterial collision efficiencies were calculated using filtration theory.

THEORY

Bacterial removal in a packed bed at steady state, assuming dispersive transport and cell desorption are negligible, can be described using the one-dimensional filtration equation (Yao *et al.*, 1971)

$$\frac{dC}{dL} = -\frac{3}{2}(1-\theta)\alpha\eta\frac{C}{d_c} \tag{1}$$

where C is the particle concentration, L the thickness of the filter bed, $(1-\theta)$ the solid fraction (θ is the filter-bed porosity), α the collision efficiency, η the collector efficiency, and d_c the collector diameter. Integrating over the thickness of the packed bed yields

$$F_p = \frac{C_e}{C_0} = \exp\left[-\frac{3}{2}\alpha\eta(1-\theta)\frac{L}{d_c}\right] \tag{2}$$

where F_p is the fractional penetration, or the ratio of effluent bacteria concentration (C_e) divided by the influent concentration (C_0). Since this is a steady-state solution, F_p represents a balance between cell adsorption and desorption.

Physical factors that account for particle collisions with the porous matrix (collectors) are incorporated into the collector efficiency, η. The collector efficiency is the ratio of the number of bacteria that approach a collector to the number that collide with the collector. The single-collector efficiency model (Rajagopalan and Tien, 1976; Rajagopalan *et al.*, 1982) will be used in this study. This is a semi-empirical approach that combines particle trajectory analysis (Payatakes *et al.*, 1974) and the sphere-in-cell porous media model (Happel, 1958) to determine η. The collector efficiency is calculated as

$$\eta = A_s N_{Lo}^{1/8} N_R^{15/8} + 0.00338 A_s N_G^{6/5} N_R^{-2/5} + 4A_s^{1/3} N_{Pe}^{-2/3} \tag{3}$$

N_{Lo}, N_R, N_G and N_{Pe}, are dimensionless quantities that describe bacteria-filter media collisions due to London-van der Waals interactions, interception, sedimentation and diffusion, respectively

$$N_{Lo} = \frac{4H}{9\pi\mu d_p^2 U} \tag{4}$$

$$N_R = \frac{d_p}{d_c} \tag{5}$$

$$N_G = \frac{g(\rho_p - \rho)d_p^2}{18\mu U} \tag{6}$$

$$N_{Pe} = \frac{3\pi\mu U d_c d_p}{kT} \tag{7}$$

where d_p and ρ_p are the equivalent diameter and density of the bacteria, U the approach velocity, μ and ρ the kinematic viscosity and fluid density, T the temperature, H the Hamaker constant, g the gravitational constant, and k the Boltzmann constant. A_s accounts for the influence of neighboring collectors on flow and is calculated as

$$A_s = \frac{2(1-\gamma^5)}{2-3\gamma+3\gamma^5-2\gamma^6} \tag{8}$$

where $\gamma = (1-\theta)^{1/3}$.

The collision efficiency, α, is the ratio of the number of bacteria removed by the collector to the number of bacteria–collector collisions, or the probability that a collision results in an attachment. All chemical factors influencing bacterial attachment are incorporated into α, which must be determined experimentally. All terms in equation (2), except for α, are known values or can be calculated from independently measured quantities using equations (3)–(8). Rearranging equation (2) and solving for α yields

$$\alpha = -\frac{2}{3}\frac{d}{\eta(1-\theta)L}\ln F_p. \tag{9}$$

Values for filtration parameters used in calculating bacterial α's for filter and column experiments are presented in Table 1.

MATERIALS AND METHODS

Bacteria

The microbial culture used in this study was *Pseudomonas fluorescens* P17, provided by C. P. Gerba (Department of Microbiology and Immunology, University of Arizona). P17 is a Gram-negative, motile, rod-shaped bacterium isolated from tap-water. P17 can use a variety of organic compounds at low concentrations as sources of carbon and energy, readily assimilates radiolabel as ^{3}H-leucine, and tolerates a wide range of environmental conditions (such as ionic strength and pH). Attachment properties of *P. fluorescens* P17 have been compared to other bacterial species, including microorganisms with biodegradative capabilities, in other investigations (Gross *et al.*, 1995; Logan *et al.*, 1993). Cells were maintained on Bacto R_2A agar (Difco Laboratories) at 4°C. *P. fluorescens* P17 suspensions were grown on glucose (0.25 g l^{-1}) in a minimal salts medium consisting of (per liter): 0.5 g K_2HPO_4, 1 g NH_4Cl, 0.15 g $CaCl_2$, 0.2 g $MgSO_4 \times 7H_2O$, and 0.4 mg $FeCl_3$. The liquid culture was incubated at 23–25°C on a shaker table. Cells were harvested during exponential growth.

Table 1. Parameter values used in calculating bacterial collision efficiencies, α, for filter and column experiments

Parameter	Symbol	GF/C filter	Column
Column/filter characteristics			
Length (m)	L	3×10^{-4}	0.2 & 0.7
Porosity	θ	0.89	0.39
Bacteria/collector characteristics			
Bacteria diameter (m)	d_p	1.27×10^{-6}	1.27×10^{-6}
Bacteria density (kg m^{-3})	ρ_p	1070	1070
Collector diameter (m)	d_c	2.2×10^{-6}	5×10^{-4}
Fluid characteristics			
Temperature (°C)	T	293	279
Density (kg m^{-3})	ρ	998	999
Velocity (m s^{-1})	U	8.8×10^{-4}	3.0×10^{-5}
Viscosity (N s m^{-2})	μ	1.0×10^{-3}	1.45×10^{-3}
Physical constants			
Boltzmann constant (J K^{-1})	k	1.38×10^{-23}	
Gravity constant (m s^{-2})	g	9.8	
Hamaker constant (J)	H	1.0×10^{-20}	

Cell preparation for filter experiments consisted of transferring 10 ml of the cell suspension to a 20-ml test tube containing 10–25 μl of radiolabeled [^{3}H]2,3,4,5-leucine (116 Ci mmol^{-1}, 1 Ci ml^{-1}, ICN Laboratories). Radiolabeled leucine was used since it is rapidly assimilated into new cell protein (Kirchman *et al.*, 1985). The culture was placed on a test-tube rotator and incubated at room temperature for 0.5–1.0 h. Radiolabeled cells were transferred to sterile buffer solutions (typically 0.05–0.5 ml of the labeled suspension per 150 ml MSM) to dilute the growth substrate and produce a final concentration of 10^6 cells ml^{-1}.

For column experiments, 40 ml of the *P. fluorescens* P17 culture was centrifuged at 6000 *g*, 6°C for 10 min. The resulting pellet was resuspended in 0.5–1.0 l sterile buffer solution and filtered through a sterile 5-μm cullulose filter (Millipore Corp.). Centrifuging and resuspending the cells removed any remaining growth substrate, while filtering removed flocs. Cells were enumerated by conventional acridine-orange direct count (AODC) procedure (Hobbie *et al.*, 1977). The final microbial stock solution had a cell concentration on the order of 10^6–10^7 cells ml^{-1}.

Bacterial size was estimated from projected cell areas measured via image analysis (Cue-2, Olympus). Bacteria were prepared for AODC and viewed at 1000×. The projected surface areas of approx. 100 cells were averaged and converted to an equivalent cell diameter assuming circular geometry. Microbe density was measured by density gradient centrifugation. Bacteria were stained with acridine orange, washed, and centrifuged in a Percoll gradient (Pharmacia Inc.). Cell density was determined by comparing the post-centrifugation level of the cell suspension with stratified density marker beads.

Screening experiments

Logan *et al.* (1993) developed a rapid method for establishing bacterial attachment properties in porous media based on the retention of radiolabeled cells through a large pore diameter ($\gg$0.2 μm) filters. Radiolabeled cells were suspended in a sterile buffer solution (either potassium phosphate or MorpholinoPropaneSulfonate, MOPS; experimental results were independent of buffer selection—not shown) of known ionic strength and pH. Filtration experiments were conducted at ionic strengths of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-5} M (pH = 7.0) to evaluate the influence of salt concentration on attachment. The effect of pH (5.0, 6.0 and 7.0) was then investigated at constant ionic strength (10^{-2} M). Bacterial retention was calculated as the fraction of retained (particulate) radiolabel in large-pore filters after accounting for adsorption of dissolved radiolabel to filter material.

Glass fiber filters (Whatman Ltd) with a pore diameter of 4.1 μm (Logan *et al.*, 1993) were used in this study because of assumed surface similarity to column media (silica spheres). Filters were placed in a 10-site vacuum manifold box containing stainless steel funnels and bases and Teflon valves and rinsed with 5-ml of sterile buffer. 5 mls of the diluted, radiolabeled cell suspension was then filtered, followed by two 5-ml rinses with the sterile buffer solution. Filters were removed from the manifold box and combined with 10 ml of scintillation cocktail for counting in a liquid scintillation counter (Beckman 3801) using H-number quench correction. Since some cells were enmeshed in the filters, all samples were counted 24 h after addition of scintillation cocktail and then recounted every 12 h until a stable count was obtained, indicating that radiolabel had been completely leached from the filter matrix. The procedure for correcting filter counts for adsorption of dissolved, noncellular, radiolabel and the calculation of bacterial penetration have been previously described (Logan *et al.*, 1993). Mean cell penetration ($\bar{F}_p$) was calculated based on the results of "n" experiments (Table 2).

Column experiments

Transport properties of *P. fluorescens* P17 also were evaluated in short columns containing silica spheres to verify the effects of ionic strength and pH on microbial attachment and confirm the results of filter experiments. The electrolyte concentration and pH influences were tested at ionic strengths of 2×10^{-2}, 5×10^{-4}, and 2×10^{-5} M pH 7.0; and at pHs of 5.5 and 7.0, $I = 2.0 \times 10^{-2}$ M.

Glass chromatography columns (Spectrum Medical Industries Inc.), 20 cm or 70 cm (1.5 cm diameter), were packed with silica beads using the tap and fill method (Snyder and Kirkland, 1979). The longer columns were used in ionic strength experiments to provide sufficient bacterial attenuation for meaningful calculation of collision efficiencies (Jewett *et al.*, 1993). The 20-cm length was sufficient for the experiments with pH as a variable because bacterial retention was greater in high ionic strength solution. Columns were sterilized in a 3:1 HNO_3 acid bath and rinsed with sterile distilled water. Porous media consisted of 0.5-mm diameter silica beads (Potters Industries Inc.). Successive acid/base washes (Bales *et al.*, 1991) were performed to remove soluble oxides and organic carbon from bead surfaces prior to column packing. Washing also served as a sterilization process. Autoclaved silicone tubing (Cole Parmer Instrument Co.) was used to connect the input reservoir to the column. Sterile buffers and stock solutions were pumped to the columns using a peristaltic pump. Approximately 3–4 pore volumes of sterile buffer solution with the same pH and ionic strength as the stock solution

Table 2. Experimental conditions and results—Filters

Experiment	Ionic strength (M)	pH	$\bar{F}_p$[a]	α[b]
1-F	10^{-1}	7	0.11 ($\pm$0.04, $n = 14$)	0.18 (0.16–0.22)
2-F	10^{-2}	7	0.15 ($\pm$0.04, $n = 4$)	0.17 (0.15–0.20)
3-F	10^{-3}	7	0.25 ($\pm$0.04, $n = 4$)	0.12 (0.11–0.14)
4-F	10^{-5}	7	0.52 ($\pm$0.04, $n = 4$)	0.062 (0.052–0.066)
5-F	10^{-1}	5	0.13 ($\pm$0.04, $n = 8$)	0.20 (0.17–0.22)
6-F	10^{-1}	6	0.08 ($\pm$0.03, $n = 8$)	0.23 (0.20–0.27)

[a] $\bar{F}_p$, mean fractional cell penetration (C/C_0), 95% confidence interval for $\bar{F}_p$ and number of experiments conducted (n) in parentheses.
[b] α, bacterial collision efficiency; range of α's for 95% confidence interval of $\bar{F}_p$ in parentheses.

were passed through the column at the start of each experiment. All experiments were performed at 6°C to minimize bacterial growth and decay.

A bacterial stock solution was created by suspending a washed *P. fluorescens* P-17 culture in sterile buffer. The stock solution was passed through the columns at an interstitial velocity of 7.5×10^{-5} m s^{-1} (approach velocity of 3×10^{-5} m s^{-1}), similar to groundwater velocities in clean sand (Freeze and Cherry, 1979). Samples were collected continuously with a fraction collector (Gilson Medical Electronics Inc.). Flow rates were established by periodically measuring cumulative effluent volumes. Bacterial assays were conducted on several samples prior to breakthrough (to ensure system sterility), all samples collected on the ascending leg of the breakthrough curve, and approximately two samples every pore volume during steady-state penetration for the remainder of the experiment. Bacteria were enumerated using AODC procedures. The stock solution in the input reservoir was assayed periodically to verify that the influent cell concentration was constant. $\bar{F}_p$ and the 95% confidence interval for mean penetration were calculated as described by Jewett *et al.* (1993) based on "m" samples (Table 3) collected during steady-state.

Column pore volume was calculated by averaging the results of conservative tracer tests that were conducted after each column experiment. The column was flooded with a 0.1-M NaCl solution until the effluent conductivity became stable. Then, 0.2-M NaCl was added to the column for about 2 pore volumes before the influent was switched back to 0.1-M NaCl. Effluent conductivity was measured continuously with an on-line detector (Wescan Instruments Inc.) connected to a strip-chart recorder. The resulting porosity (θ) was 0.39.

RESULTS

Bacterial penetration through glass fiber filters was directly related to the ionic strength of the carrying solution (Table 2, experiments 1F–4F). At an ionic strength of 10^{-1} M, the 95% confidence interval for mean cell penetration ($\bar{F}_p$) was 0.11 ± 0.04. Decreasing the ionic strength to 10^{-3} M resulted in roughly twice the penetration ($\bar{F}_p = 0.25 \pm 0.04$), and at $I = 10^{-5}$ M, $\bar{F}_p$ increased to 0.52 ± 0.04 (about five times that at $I = 10^{-1}$ M). Varying the pH of the buffer solution had little effect on microbial transport (Table 2, experiments 1F, 5F and 6F). The fraction of bacteria that passed through the filter was lowest at pH 6.0, but only slightly lower than at pHs 5.0 and 7.0.

Experiment 1F was repeated 13 times to test the reproducibility of the screening technique. The resultant mean penetration and 95% confidence interval of $\bar{F}_p$ was 0.11 ± 0.04. The relatively narrow confidence interval indicates that reproducibility was good for the filter method. Experiments 2F–6F were repeated from 4 to 8 times.

The same trend in cell penetration as a function of ionic strength was observed for the column experiments (Table 3). At an ionic strength of 2×10^{-2} M, the 95% confidence interval for $\bar{F}_p$ was 0.24 ± 0.05 [Fig. 1(A)]. At $I = 5 \times 10^{-4}$ M, $\bar{F}_p$ increased to 0.55 [Fig. 1(B)]. Maximum P17 penetration [$\bar{F}_p = 0.84 \pm 0.10$; Fig. 1(C)] occurred at $I = 2 \times 10^{-5}$ M. Decreasing the pH of the carrying solution from 7.0 to 5.5, experiments 5C and 4C, resulted in a negligible increase in cell mobility (Fig. 2).

Bacterial α's corresponding to $\bar{F}_p$ and 95% confidence interval for $\bar{F}_p$ were calculated for the filter (Table 2) and column (Table 3) data using equations and parameters presented above. The α in experiment 1F ($I = 10^{-1}$ M) was 0.18, with the 95% confidence interval being $0.16 < \alpha < 0.22$. At $I = 10^{-5}$ M (experiment 4F), α was 0.062, with a 95% confidence interval on $\bar{F}_p$ of $0.056 < \alpha < 0.066$.

Calculated α's for the column experiments were highest at $I = 2.0 \times 10^{-2}$ M ($\alpha = 0.12$, for $\bar{F}_p$; range of $0.11 < \alpha < 0.14$) and decreased by about 90% to $\alpha = 0.015$ ($0.0053 < \alpha < 0.026$) at $I = 2.0 \times 10^{-5}$ M. Throughout the range of ionic strengths investigated, α values for the screening

Table 3. Experimental conditions and results—Columns

Experiment	Ionic strength (M)	pH	Column length (m)	$\bar{F}_p$[a]	α[b]
1-C	2×10^{-2}	7.0	0.7	0.24 ($\pm$0.05, $m = 12$)	0.12 (0.11–0.14)
2-C	5×10^{-4}	7.0	0.7	0.55 ($\pm$0.12, $m = 10$)	0.051 (0.034–0.072)
3-C	2×10^{-5}	7.0	0.7	0.84 ($\pm$0.10, $m = 10$)	0.015 (0.0053–0.026)
4-C	2×10^{-2}	5.5	0.2	0.47 ($\pm$0.08, $m = 8$)	0.22 (0.18–0.28)
5-C	2×10^{-2}	7.0	0.2	0.57 ($\pm$0.07, $m = 8$)	0.17 (0.13–0.21)

[a] $\bar{F}_p$, mean fractional cell penetration (C/C_0) based on m measurements at steady-state; 95% confidence interval for $\bar{F}_p$ and m in parentheses.
[b] α, bacterial collision efficiency; range of α's for 95% confidence interval of $\bar{F}_p$ in parentheses.

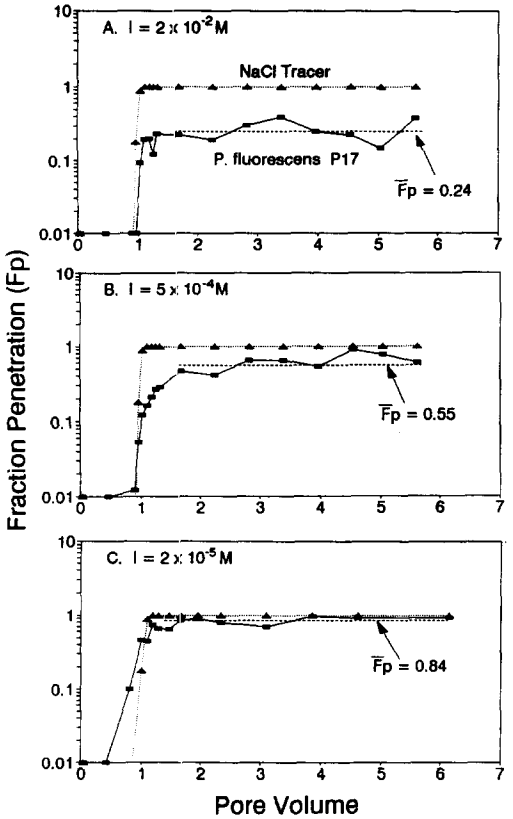

Fig. 1. Breakthrough curves for *P. fluorescens* P17 (■) and conservative tracer (▲) for column study evaluating ionic strength effects on bacterial transport: (A) experiment 1C, (B) experiment 2C, and (C) experiment 3C. Dashed horizontal lines indicate steady-state $\bar{F}_p$.

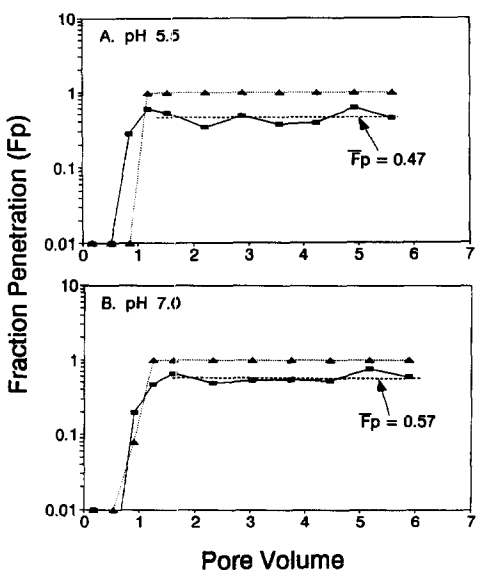

Fig. 2. Breakthrough curves for *P. fluorescens* P17 (■) and conservative tracer (▲) for column study evaluating pH effects on bacterial transport: (A) experiment 4C and (B) experiment 5C. Dashed horizontal lines indicate steady-state $\bar{F}_p$.

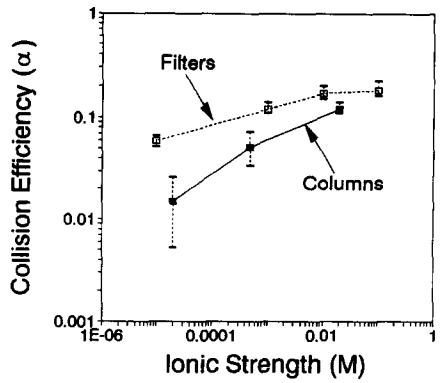

Fig. 3. Collision efficiencies (α) for *P. fluorescens* P17 as a function of ionic strength of buffer solution. Filter (□) and column (■) α values calculated using experimental parameters listed in Table 1, fractional penetrations given in Tables 2 and 3, and equation (8).

technique were about 1.5 times those found using the column method.

Altering the pH of the buffer had little effect on bacterial penetration in the filters, producing little variability in calculated α values (Table 2). Cell penetration was lowest at pH 6.0, where α corresponding to $\bar{F}_p$ was 0.23. Similar estimates of α for pHs 5.0 and 7.0 were 0.20 and 0.18, respectively. Likewise, no significant changes in α resulted from variation in pH for the column experiments (Table 3), $\alpha = 0.22$ at pH 5.5 and $\alpha = 0.17$ at pH 7.0.

Reproducibility of column data was established using experiments 1C and 5C. Both experiments were conducted at pH 7.0 and high ionic strength, but column lengths were 70 and 20-cm, respectively. Although cell penetration differed between the two experiments (Table 2), filtration theory accounts for differences due to column length, L, and comparable α values were expected. Bacterial α's were 0.12 and 0.17 for experiments 1C (70-cm) and 5C (20-cm), respectively.

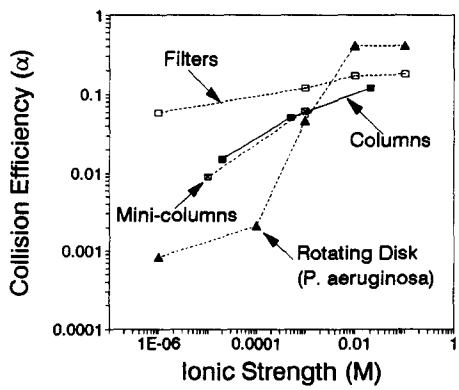

Fig. 4. Bacterial collision efficiencies (α) as a function of ionic strength of buffer solution determined in four different experimental systems: GF/C filters (□), this study; 70-cm columns (■), this study; mini-columns (☒), Gross (unpublished research, Univ. of Arizona); and rotating disks (▲), Martin *et al.* (1991).

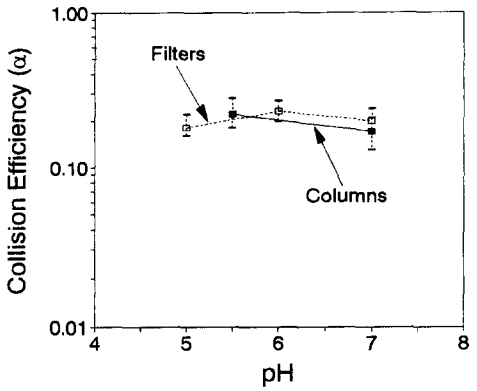

Fig. 5. Collision efficiencies (α) for *P. fluorescens* P17 as a function of pH of buffer solution. Filter (□) and column (■) α values calculated using experimental parameters listed in Table 1, fractional penetrations given in Tables 2 and 3, and equation (8).

The overlap in confidence intervals for calculated α values suggests that column experiments also were reproducible.

DISCUSSION

The results indicate that *P. fluorescens* P17 transport in porous media depended strongly on the ionic strength of the carrying solution with α decreasing from 0.12 to 0.015 in column experiments when ionic strength was decreased from 2×10^{-2} to 2×10^{-5} M. For filter experiments at high ionic strength, $\alpha = 0.18$, which is similar to α's calculated for other microorganisms (*Pseudomonas* sp. JS6, $\alpha = 0.11$; *Bacillus pumilus*, $\alpha = 0.20$) by Logan *et al.* (1993). These changes in α with ionic strength are consistent with electrical double-layer theory (Stumm, 1992). That is, a higher ionic strength compresses the electric double-layer and reduces electrostatic repulsion between like-charged particles at a given separation. Both the P17 cells and the filter/porous media collectors were negatively charged at the pH of the ionic strength experiments (Hilbert, 1992; Kinoshita *et al.*, 1993). Although the α's calculated for filter experiments were always about 1.5 times those determined under similar conditions in column experiments, varying the ionic strength produced the same trend in both systems (Fig. 3).

Other investigators have observed similar changes in cell penetration as a function of ionic strength, but not all studies support the calculation of α. Fontes *et al.* (1991) observed a 50–90% reduction in $\bar{F}_p$ for a subsurface isolate during passage through quartz sand columns in response to a 10-fold increase in ionic strength. Gannon *et al.* (1991) increased bacterial penetration from 0.022 to ~1.0 in a 0.3-m column containing representative aquifer sediment by suspending a *Pseudomonas* sp. in distilled water instead of a 10^{-2} M NaCl solution. Cell attachment to glass surfaces also increased with solution ionic strength in a rotating disk system (Abbot *et al.*, 1983).

Martin *et al.* (1991) used a rotating disk apparatus to calculate collision efficiencies for *Pseudomonas aeruginosa* with a glass surface, and Gross *et al.* (1995) experimented with radiolabeled *P. fluorescens* P17, estimating α on 40-μm glass beads. Bacterial α's reported by these investigators can be compared directly to our filter and column data. In all four experimental systems, α values decreased with decreasing ionic strength (Fig. 4). Values of α for the mini-column experiments are in excellent agreement with those of the 70-cm column, probably due to the similarity in geometric and hydraulic characteristics. While Martin's results show the same trend, α's for low-ionic-strength solutions are significantly lower than either filter or column data from this study. Differences may have arisen from the disparate geometries of rotating disks and flow-through systems or from differences in bacterial surface characteristics in the two studies.

P. fluorescens P17 transport through filters and columns was insensitive to variation in solution pH. The isoelectric point for most bacterial species lies between pH 1.5–4.0 (Harden and Harris, 1953), well below the lowest pH examined in this study. Therefore, pH likely had little effect on the surface charge density of *P. fluorescens* P17 or the collector. The pH experiments from this study were conducted at ionic strengths of 10^{-1} M (filters) and 2×10^{-2} M (columns). The results suggest that electrostatic effects due to the high ionic strength carrying solution dominated over changes in surface charges and hydrophobic properties associated with changes in pH.

Again, results for effects of pH on transport are consistent with the findings of other researchers. McEldowney and Fletcher (1986) found no clear relationship between pH and microbial retention at $5.0 < \text{pH} < 9.0$. Abbot *et al.* (1983) reported that bacterial attachment to a glass surface under defined flow conditions was unaffected by pH in the range $4.5 < \text{pH} < 7.5$. The retention of bacteria on cation- and anion-exchange beads and mineral surfaces was also unaffected by changes in pH in the range 4.0–9.0 (Stenstrom, 1989). However, Scholl *et al.* (1990) reported an increase in the retention of a ground-water bacterial isolate on quartz chips when the suspension pH was decreased from 7.5 to 5.0. Scholl and Harvey (1992) observed a similar trend among indigenous ground-water bacteria on glacial outwash sediment. These results are contrary to the findings of Kinoshita *et al.* (1993), who observed less efficient cell attachment to silica beads at pH 5.5 than pH 7.0.

CONCLUSIONS

Results suggest that bacterial dispersal in sandy aquifer material can be improved by introducing microorganisms into the subsurface in a low ionic strength carrying solution. Introducing a carrying

solution with a pH different from that of the soil or ground water should have no effect on dispersal.

Although the column experiments better simulate the hydraulic and geometric properties of subsurface porous media, they are time and labor intensive. Additionally, even highly reproducible column breakthrough data can produce excessive uncertainty in calculated α values if experiments are not carefully designed. As $\bar{F}_p$ approaches unity, small errors in the measurement of influent and effluent cell concentrations produce large errors in estimates of α (Fig. 3). Jewett *et al.* (1993) concluded that uncertainty inherent in measurements of culturable bacteria can preclude reliable estimations of low α's.

The filtration of radiolabeled microorganisms through laboratory filters is a rapid alternative to conventional column experiments. Nonetheless it has some general limitations. First, the removal of bacteria in filters by straining may be important when the collector diameter is less than five times the particle diameter (Tien and Payatakes, 1979). Because this criterion is not met in the filter experiments, straining may have contributed to differences in cell removals observed in filter and column experiments. However, Zierdt (1979) showed that rinsing filters with detergent (such as Tween 20) prior to their use in experiments reduced bacterial retention. Detergents would have little effect if straining were the primary mechanism of particle removal. Although straining does not seem to have a controlling effect on F_p, it may create a lower limit for the accuracy of the filter method and should be considered during experimental design. Second, collector and pore geometries are very different in porous media consisting of filter fibers and sediments or beads, contributing to difference in corresponding α values. Nevertheless, the experiments suggest that results from the filter method (F_p and α) correlate well with those of columns packed with silica beads, providing a convenient laboratory procedure for establishing bacterial transport characteristics and the effects of transport-enhancing treatments on cell attachment.

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