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Use of Colloid Filtration Theory in Modeling Movement of Bacteria through a Contaminated Sandy Aquifer

Ronald W. Harvey*

U.S. Geological Survey, Water Resources Division, Menlo Park, California 94025

Stephen P. Garabedian

U.S. Geological Survey, Water Resources Division, Marlborough , Massachusetts 01752

A filtration model commonly used to describe removal of colloids during packed-bed filtration in water treatment applications was modified for describing downgradient transport of bacteria in sandy, aquifer sediments. The modified model was applied to the results of a small-scale (7 m), natural-gradient tracer test and to observations of an indigenous bacterial population moving downgradient within a plume of organically contaminated groundwater in Cape Cod, MA. The model reasonably accounted for concentration histories of labeled bacteria appearing at samplers downgradient from the injection well in the tracer experiment and for the observed 0.25-μm increase in average cell length for an unlabeled, indigenous bacterial population, 0.6 km downgradient from the source of the plume. Several uncertainties were apparent in applying filtration theory to problems involving transport of bacteria in groundwater. However, adsorption (attachment) appeared to be a major control of the extent of bacterial movement downgradient, which could be described, in part, by filtration theory. Estimates of the collision efficiency factor, which represents the physicochemical factors that determine adsorption of the bacteria onto the grain surfaces, ranged from 5.4×10^{-3} to 9.7×10^{-3}.

Introduction

The recognition that water-supply well contamination by microbial pathogens is a major cause of waterborne disease outbreaks in the United States (1) and the increasing interest in the use of genetically altered (engineered) and "waste-adapted" bacteria for aquifer restoration are leading to efforts to model more accurately the transport of bacteria through porous media. The ability of bacteria that are capable of degrading highly refractory compounds to reach contaminant-affected areas is critical to the success of several proposed schemes involving in situ treatment of organically contaminated groundwater. Theoretical models that describe bacterial movement through porous media have been developed (2-4). However, few attempts have been made to model data resulting from controlled transport experiments involving bacteria in groundwater, and several aspects of bacterial migration through the subsurface are not well understood.

An important determinant of the extent of bacterial movement in sandy aquifers is the interaction between the bacteria and solid surfaces, which can involve a number of complex and interactive processes that are difficult to describe mathematically (5). In recent small-scale (2 m), forced-gradient tracer experiments, many of the labeled, indigenous bacteria that were reintroduced into sandy aquifer sediments became immobilized at particle surfaces (6). It was also observed that immobilization of bacteria-sized microspheres was inversely related to size, suggesting that sorption and not straining was the important control over the extent of transport. Matthess and Pekdeger (7) have suggested that filtration theory (8), which has been used to describe the removal of colloidal particles during packed-bed filtration in water-treatment applications, may be employed in models of bacterial transport in groundwater. An important parameter in the filtration model is the so-called collision efficiency factor, α, which must be experimentally determined. Little information is available on the values of α for sorptive removal of microbes moving through aquifer sediments.

The objective of our study was to evaluate the advantages and limitations of using filtration theory in mathematical descriptions of bacteria transport through sandy aquifer sediments and to compare resulting estimates of α with other published values. The experimental work involved a small-scale (7 m), natural-gradient tracer test in which fluorochrome-labeled groundwater bacteria were reintroduced into a sandy, sewage-contaminated aquifer and their concentration histories at samplers downgradient compared with those of a conservative tracer (Br-). Observed bacterial transport behavior was simulated with a filtration model that was modified to include advection, storage, physical dispersion, and reversible adsorption. The filtration model was also used to predict the effect of the aquifer sediments upon the size distribution of bacterial populations moving through it. This involved comparing predicted and observed changes in the size frequency distribution for a morphologically diverse, free living (unattached) bacterial population moving downgradient along a 0.6-km-long transect through a plume of organically contaminated groundwater.

Methods

Injection Experiment. A small-scale, natural-gradient groundwater tracer experiment was run in October 1987 at U.S. Geological Survey well site F347 in a sandy aquifer on Cape Cod, MA, using Br- and labeled bacteria. An earlier tracer test at this site involving bacteria-sized microspheres is described by Harvey et al. (6). Contaminated groundwater was collected with a stainless-steel submersible pump from a screened (250-μm slot width) PVC observation well that was located 100 m downgradient from an on-land, treated-sewage infiltration bed. A morphologically diverse population of bacteria was concentrated on-site from 600 L of contaminated groundwater to 8 L (final volume) by using a hollow-fiber, tangential flow filtration device at a processing rate of 2 L/min (9). Recovery of bacteria was ~33%. Recovered bacteria (0.2-1.4 μm; 0.6-μm average cell length) were stained with a DNA-specific fluorochrome, 4',6-diamidino-2-phenylindole (DAPI), using a previously described procedure (6). The stained bacteria were diluted with groundwater collected at the injection test site to a final volume of 90 L. The consequential dilution of the DAPI stain precluded staining other bacteria in the aquifer. Bromide was added to the injectate to a final concentration of 150 mg/L.

Bacteria and Br- were added slowly (0.85 L/m) to the aquifer at 8.5 and 9.1 m below land surface (BLS) and monitored as they moved with the natural flow of

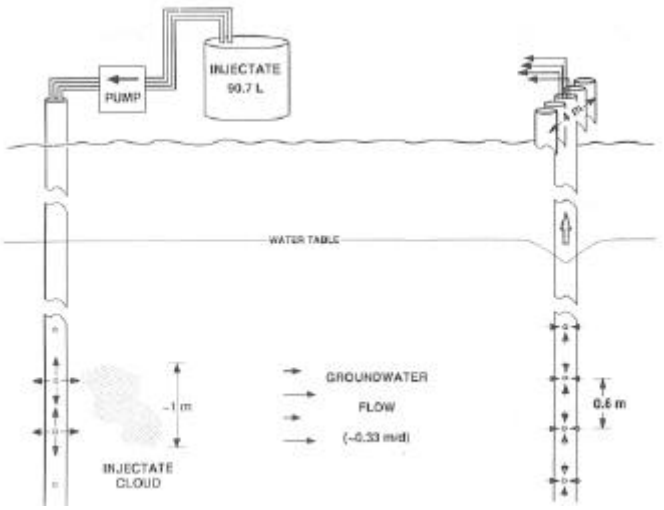

Figure 1. Schematic representation of the small-scale, natural-gradient tracer experiment with Br- and labeled bacteria.

Table 1. Transport Parameters for the Small-Scale Tracer Experiment

param'	9.1 m BLS		8.5 m BLS
A		0.51 m^2	
α_L	2.2 cm		2.2, 5.3 cm[b]
C_{mez}/C_0	0.093		0.035
d		0.59 mm	
d_p		0.2–1.4 μm	
g		9.81 m/s^2	
k		1.38 × 10^{-16} g cm^2/s^2 K	
μ		1.14 × 10^{-2} g/cm s	
η		(1.0–3.5) × 10^{-2}	
RB	0.21		0.15
ρ		0.999 g/cm^3	
ρ_p		1.001 g/cm^3	
ρ_b		1.72 g/cm^3	
T		2.88 × 10^2 K	
θ		0.35	
V		9.1 × 10^{-2} m^3	
v	0.335 m/day		0.33, 0.25 m/day[b]
x		6.8 m	
K_d		0.061	
k_f		0.002	
k_r		0.4	

'Estimates for d and 0 were determined in a previous study [LeBlanc (11)]. 'Estimates are for the faster and slower zones, respectively.

groundwater past a row of multilevel sampling devices (MLSD) set perpendicular to the direction of groundwater flow 6.8 in downgradient (Figure 1). Experimental parameters and conditions are listed in Table I. Groundwater samples (500 mL were collected daily from sampling ports located in the path of injectate travel. Bromide was measured with a specific-ion electrode and confirmed by ion chromatography (Waters ICP-A column with borate-gluconate buffer at 1.2 mL/min at 25 'C). Preparations for enumeration of DAPI-stained bacteria were made with 100-200 mL of sample to obtain accurate counting statistics. The DAPI-stained bacteria in these samples were clearly visible by epifluorescence microscopy (340-380-nm excitation) after the month-long tracer test and after a 2-month, 12 *C test incubation in filter-sterilized Cape Cod groundwater. The bacteria were enumerated on black polycarbonate membrane filters (0.2-gm pore size, 25-mm diameter) by using a microscope that was fitted for epifluorescence (10). The possibility of growth of the DAPI-stained population during the tracer experiment was investigated by monitoring abundance of DAPI-stained bacteria in a control suspension and in tracer test samples for a 30-day period after collection.

Contaminant Plume Study. The plume study involved modeling changes in the population of free-living bacteria during transport downgradient in a 640-m-long section of the contaminant plume. The plume of organically contaminated groundwater (~4 km in length) was formed by the on-land disposal of secondary sewage effluent from a sewage treatment plant at Otis Air Force Base, MA. The contaminant plume is characterized by elevated levels of specific conductivity (up to 400 μS/cm), dissolved organic carbon (DOC; up to 4 mg/L), and temperature (up to 18 ºC) relative to adjacent uncontaminated groundwater (<80 μS/cm, <1 mg/L, and 10 ºC, respectively) (11). Groundwater samples were taken from observation wells at 0, 0.38, and 0.64 km downgradient from the sewage-infiltration beds along a longitudinal transect through the core of the plume. Samples were immediately fixed with formaldehyde (2% w/v final concentration) and stored at 4 ºC. Bacterial abundances were determined with acridine orange stain and epifluorescence microscopy (12). Bacterial size distributions were determined from scaled photomicrographs by a previous described procedure (13).

Formulation. A colloid filtration model was incorporated into a simple transport model to explain the movement, losses due to adsorption, and spreading of DAPI-stained bacteria in the small-scale injection test. A one-dimensional equation for the transport of bacteria, which contains terms for storage, reversible and irreversible adsorption, dispersion, and advection can be written as

$$\theta \frac{\delta c}{\delta t} + \rho_b \frac{\delta s}{\delta t} = D\theta \frac{\delta^2 c}{\delta x^2} - v\theta\left(\frac{\delta c}{\delta x} + k_p c\right) \qquad (1)$$

where 0 is the porosity; c the concentration of bacteria in solution; t the time after injection; ρ_b the sediment bulk density; s the concentration of reversibly adsorbed bacteria on the solid surface; D the dispersion coefficient, which is equivalent to the product of the longitudinal dispersivity (α_L) and the fluid velocity (v); x the spatial coordinate; and k_p the irreversible adsorption constant. The latter may be described by the colloid filtration model (8) used to explain the removal of colloidal-sized material during filtration in packed-bed systems:

$$k_p = \frac{3}{2}\frac{(1-\theta)}{d}\alpha\eta \qquad (2)$$

where d is the diameter of the porous media grains, a is the collision efficiency factor, and η is the single- collector efficiency.

The methods used to handle adsorption of bacteria during transport are analogous to the first-order reversible and two-site adsorption models commonly used in virus transport literature (14). Two different forms of the reversible adsorption term in eq 1 were used; the first assumes a linear isotherm and relatively fast adsorption with respect to advection, i.e.:

$$s = K_d c \qquad (3)$$

If the adsorption constant (K_d) does not vary with time, the time variation of bacteria on the solid and in solution can be related directly:

$$\frac{\delta s}{\delta t} = K_d \frac{\delta c}{\delta t} \qquad (4)$$

The other type of reversible adsorption term assumes a first-order kinetic reaction for the rates of adsorption and desorption:

$$\frac{\delta s}{\delta t} = k_f c - k_r s \quad (5)$$

Where k_f and k_r are the forward and reverse adsorption rate constants, respectively.

Our approach in modeling transport of bacteria involved use of reported values for θ and ρ_b estimation of α_L and v by calibrating model solutions to the observed breakthrough curves of the nonreactive solute (Br-), and determination of the remaining parameters (K_d, k_f, k_r, αη) by calibrating different solutions of eq 1 to observed bacteria breakthrough curves. The parameter pair (αη) was treated differently because the effect of changing either parameter on the model output is identical. This is because the two parameters only appear in eq 2 together and, therefore, cannot be separately calibrated. The approach used here was to estimate η by use of a published equation (8), since the factors determining α are less well understood, and then use a as the calibration parameter.

The single-collector efficiency, η, is the rate at which particles strike a single porous media grain divided by the rate at which particles move toward the grain and represents the physical factors determining particle collision. If close-approach effects are neglected, its value can be estimated by the following (8):

$$\eta = \eta_D + \eta_I + \eta_G = 0.9\left[\frac{kT}{\mu d_p v}\right]^{2/3} + 1.5(d_p/d)^2 + \frac{(\rho_p - \rho)g d_p^2}{18\,\mu v} \quad (6)$$

Where η_D is the colloid collector collision caused by Brownian motion, η_I the colloid collector collision caused by interception, η_G the colloid collector collision caused by settling, *k* the Boltzman constant, T the solute temperature, μ the fluid viscosity, d_p the bacterial diameter, ρ the fluid density, ρ_p the bacterial density (specific gravity of bacterial biomass), and g the gravitational constant. Because each of the parameters in eq 6 can be independently measured, η was estimated for each size class of bacteria and the subsequent size-specific breakthrough curves were superposed to obtain a composite breakthrough curve for the entire bacterial population.

The collision efficiency factor, α, in eq 2 is the efficiency with which collisions between colloidal-sized particles and the stationary solid surfaces result in the immobilization of the bacteria and represents the physicochemical factors determining bacterial attachment to the solid surface. α was calibrated by assuming that the irreversible adsorption term in eq 1 was the only process that significantly and permanently reduced the total mass of bacteria in solution. In comparison, there is no loss of bromide from solution and a can be estimated by using the ratio of the relative masses of bacteria to bromide (RB) at the sampling well:

$$\mathrm{RB} = \int_{t_0}^{t_f} \frac{C(t)}{C_0} dt \Big/ \int_{t_0}^{t_f} \frac{\mathrm{Br}^-(t)}{\mathrm{Br}^-_0} dt \quad (7)$$

where C_0 and Br_0 are the bacterial and bromide concentrations, respectively, at point of injection and t_0 and t_f are the respective times at the beginning and end of breakthrough.

If it is assumed that the mass entered the system at one time (i.e., a pulse input), than a can be found by using a time moments analysis (15):

$$\alpha = \frac{d[[1 - 2(\alpha_L/x_1)\ln(\mathrm{RB})]^2 - 1]}{6(1-\theta)\eta\alpha_L} \quad (8)$$

where x_1 is the distance from the injection point to the MLSD. Equation 8 can be simplified when the dispersivity is small with respect to the travel distance ($\alpha_L/x_1 < 0.01$):

$$\alpha = \frac{-d \ln(\mathrm{RB})}{1.5(1-\theta)\eta x_1} \quad (9)$$

Equations 8 and 9 can be used to estimate a from known parameters *(d, q, h,* x_1), and the measured loss of relative mass, RB.

Solutions to eq 1, with appropriate boundary and initial conditions, were compared with breakthrough curves and used to explain the transport of bromide and bacteria. The two different reversible adsorption models produce different solutions to eq 1. The solution for the linear adsorption model (eq 3), with a pulse input above (or below) background concentration, is a well-known solution for relative concentration with a first-order decay term

$$\frac{C(t)}{C_0} = \frac{V_0}{A\theta[4\pi\alpha_L x']^{1/2}} \exp\left[\frac{-(x_1 - x')^2}{4\alpha_L x'} - k_p x'\right] \quad (10)$$

where V_o is the initial injection volume, A the cross-sectional area of flow through the porous media, x' the spatial coordinate for the center of mass *(vt/R)* and *R* the retardation coefficient *(1 +* $r_b K_d/q$*)*

For bromide, there is no adsorption *(*$K_d = k_p = 0$*)* and *x'= vt.* Therefore, a simplified version of eq 10 (assuming α_L is small relative to x_1) was used to model its breakthrough curves. It is apparent from eq 10 that the peak bromide concentration occurs when the center of mass passes by the sampler (i.e., x'= x, v ~ x_1/t_{peak}), and the fluid velocity can be directly obtained from the known distance between injection and sampling points and the time to the peak bromide concentration. The dispersivity parameter α_L is obtained from the bromide curve by use of the following relation based on eq 10:

$$\alpha_L = \frac{x_1(\Delta t/t_{peak})^2}{16 \ln 2} \quad (11)$$

where t is the duration of breakthrough when [Br -](t) > 1/2[Br-]$_{max}$; $[Br]_{max}$ the peak bromide concentration, and t_{peak} the time to peak concentration. The area of the porous media through which the solutes or particles flow, A, can be found by calibration using the peak bromide concentration ($[Br]_{max}$) and eq 10, when x' = x_1:

The solution for eq 1 with the kinetic adsorption term (eq 5) and a pulse input is found by using a modified form of a solution by Valocchi (15)

$$\frac{c(t)}{c_0} = L^{-1}\left[\frac{V_0}{\theta A x_1}\exp\left[\frac{x_1}{2\alpha_L}\left[1 - \left[1 + \frac{4\alpha_L}{x_1}\left[p + k_p x_1 + \frac{p\rho x_1 k_f}{\theta(vp + k_r x_1)}\right]\right]^{1/2}\right]\right]\right] \quad (13)$$

where L^{-1} indicates the inverse Laplace transform for the quantity inside the outermost bracket and p represents the Laplace domain parameter. The Laplace transform (eq 13) was numerically inverted by a method described by Crump (16) and available in the IMSL mathematics library. Equations 10 and 13 form the basis for the quantitative modeling of the bacterial breakthrough and explanation of the bacterial transport.

Results

Theoretical estimations of collector efficiency (η) and experimentally determined values of collision efficiency (α), which are the two key parameters in the filtration

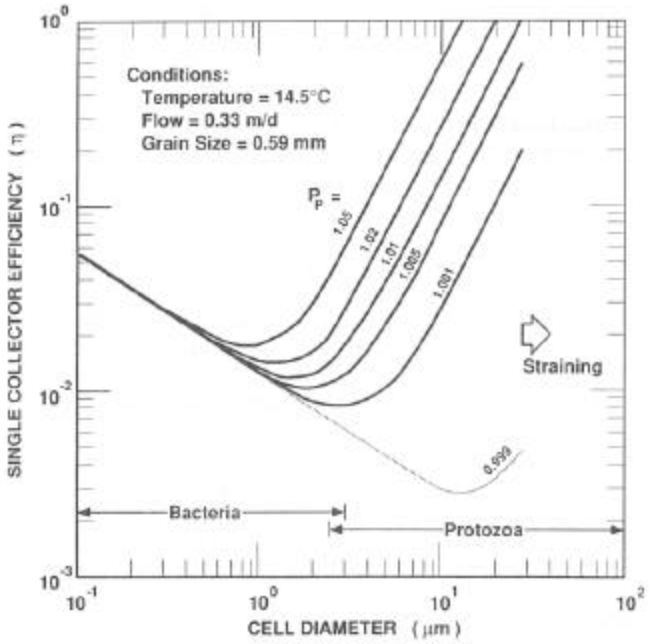

Figure 2. Theoretical estimates for the single-collector efficiency factor versus cell diameter for microorganisms traveling with the groundwater in the Cape Cod aquifer. Different curves represent different specific gravities of the biomass.

portion of the transport model, are given in Figure 2 and Table II, respectively. Collector efficiency, calculated by using eq 6 and measured physical parameters (Table I), was determined as a function of buoyant density (not measured in this study) and microbial size. At near-neutral buoyancy (1.001 g/cm^3), diffusion was the primary determinant of collector efficiency for the bacterial size class (0.2-2 μm), but accounted for less than half of η for ~4-5 μm (diameter) protozoa that inhabit the aerobic portions of the contaminant plume; contributions of physical interception (η_I) were generally insignificant. The contribution of settling (η_G) to collector efficiency was generally significant for the larger microbial size classes (1 μm and larger), but was highly sensitive to small changes (e.g., 0.01 g/cm^3) in buoyant density. A decreases in buoyant density from 1.05 to 1.001 g/cm^3 corresponded to a shift in the optimal microbial size for transport (theoretically at the η function minimum) from ~0.8 to ~3 μm.

Table II. Estimates of the Collision Efficiency Factor (α) for Bacteria and Carboxylated, 0.23-μm (Diameter) Microspheres Being Transported through Sandy Sediments of the Cape Cod Aquifer

	depth, m BLS	distance, m	collision effic factor (×10^{-3}) bacteria	collision effic factor (×10^{-3}) microspheres
injection test	9.1	6.8	8.1[a] 5.4[b]	26.4[a]
	8.5	6.8	9.7[a] 5.4, 8.5[b]	ND[d]
contaminant plume		800	0.3[c]	ND[d]

[a] Estimated by using eq 9. Microsphere data from ref 9. [b] Determined by calibration (eq 10). Values at the 8.5-m depth are for the faster and slower zones, respectively. [c] Estimated by Reynolds (17) from the data of Harvey et al. (12). Estimate not corrected for bacterial growth. [d] ND, not determined.

Estimates of the collision efficiency factor, a, for bacteria in aquifer sediments are given for the two depths employed in the injection experiment and for the upgradient part of the contaminant plume (Table II). An estimate of a for carboxylated, bacteria-sized microspheres employed in an earlier experiment at the same site (6) is also listed for comparative purposes. It was found that estimated values of a were up to 30% higher for the shallower layer (8.5 in BLS) as compared with the deeper layer (9.1 in BLS) of aquifer sediment. Estimates of a for indigenous bacteria in the injection experiment are more than 20-fold higher than that estimated by Reynolds (17), who used previously reported bacterial distribution data for the contaminant plume (12). However, the estimates are ~3-fold lower than that calculated for the carboxylated microspheres.

The dimensionless concentration histories of Br- and DAPI-stained bacteria at 6.8 m downgradient from point of injection are depicted in Figure 3 and 4 for the 9.1- and 8.5-m depths, respectively. Breakthrough of tracers at other sampled depths and at adjacent MLSD were not significant. For both depths, the patterns of breakthrough for stained bacteria and Br- were similar. Breakthrough of Br (Figures 3A and 4A) and of stained bacteria (Figures 3B,C and 4B,C) exhibited single peaks and followed similar temporal patterns. However, substantial reversible behavior ("tailing") was observed for the stained bacteria at the 9.1-m depth, but not for Br-. Peak abundances in

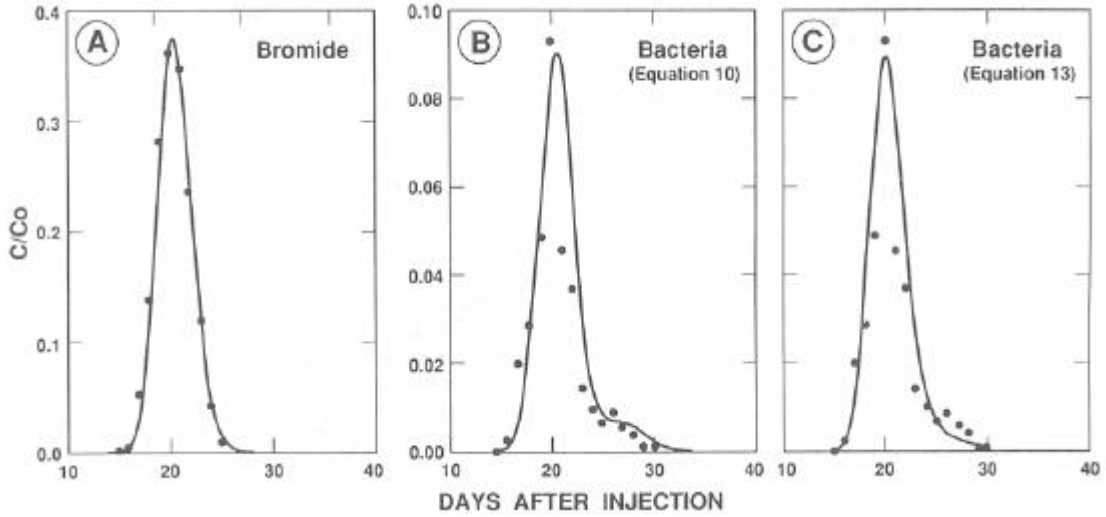

Figure 3. Observed dimensionless concentration histories (data points) and theoretical computer simulations (curves) for breakthrough at the 9.1-m depth (small-scale tracer experiment) of bromide (A) and stained bacteria (B and C). Retarded and unretarded (nonreactive) segments of the bacterial population are assumed in B and a uniformly reactive population with different constants describing sorption and desorption is assumed in C.

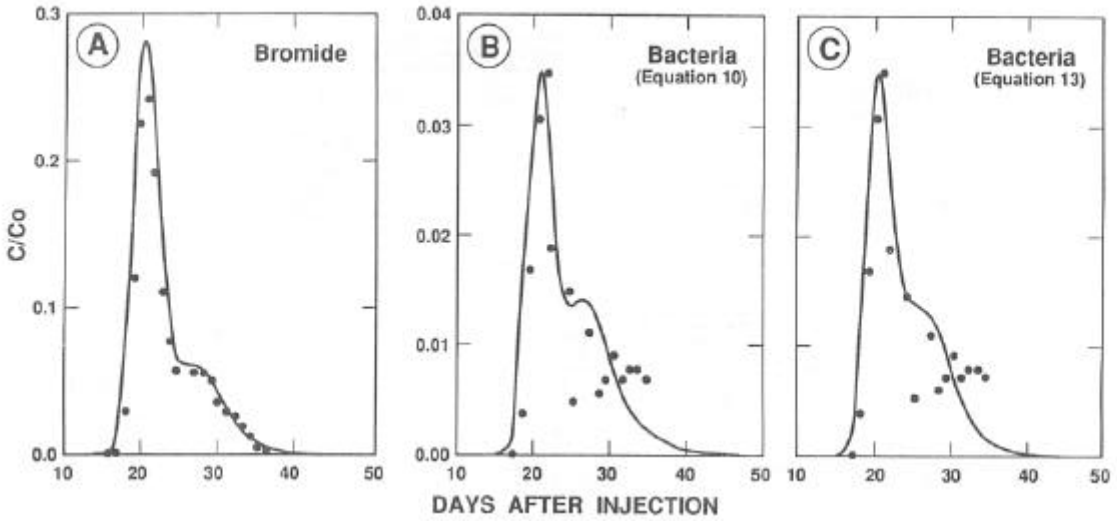

Figure 4. Observed dimensionless concentration histories (data points) and theoretical, two-zone computer simulations (curves) for breakthrough at the 8.5-m depth (small-scale tracer experiment) of bromide (A) and stained bacteria (B and C). Retarded and unretarded (nonreactive) segments of the bacterial population are assumed in B and a uniformly reactive population with different constants describing sorption and desorption is assumed in C.

stained bacteria and Br- occurred within ~0.5 days of each other, but maximum dimensionless concentrations for stained bacteria were only 26 and 14% of that observed for Br- at the 9.1- and 8.5-m depths, respectively. The ratios of relative masses of bacteria with respect to bromide (eq 7) were 21 and 15%, respectively.

A comparison of observed and predicted dimensionless concentration histories for bromide and stained bacteria is also shown in Figures 3 and 4. Dimensionless concentration histories for Br- were simulated by use of the transport models described by eq 10, assuming no adsorption. Values and estimates for other parameters used are listed in Table I. Dimensionless concentration history for Br- at the 9.1-m depth was simulated by assuming a single conductive zone. However, the physical heterogeneity that was apparent at the 8.5-m depth necessitated a two-zone (layer) application of the model for the shallower depth, as depicted in Figure 5. It was assumed that 25% of the sampled solute traveled through a less conductive (slower) zone in the aquifer, whereas the other 75% traveled through a more conductive zone. The final solution represents a superposition of individual solutions of eq 10 for the faster and slower zones.

At both depths, predicted dimensionless concentration histories for the stained bacteria from eqs 10 and 13 were reasonably close to what was observed in the small-scale experiments. However, the "tailing" in the observed concentration history of bacteria relative to Br- was simulated in the model described by eq 10 by making the assumption that a small portion (7%) of the nonimmobilized, stained population interacted with surfaces of stationary particles in a reversible manner. The two-zone approach that was employed for Br- to account for the physical heterogeneity at the 8.5-m depth was also used for the bacteria, although computer simulations for the latter portion of bacterial breakthrough were less accurate (Figure 4B). Simulations of bacterial breakthrough curves using eq 13, which assumes that all moving bacteria interact with surfaces, are shown in Figures 3C and 4C for the 9.1- and 8.5-m depths, respectively. Since little retardation was observed, little difference in accuracy was observed between the fast-adsorption (eq 10) and kinetic, nonequilibrium (eq 13) models in spite of the difference in approach.

Predicted and observed changes in the cell size frequency distribution for the indigenous bacterial population

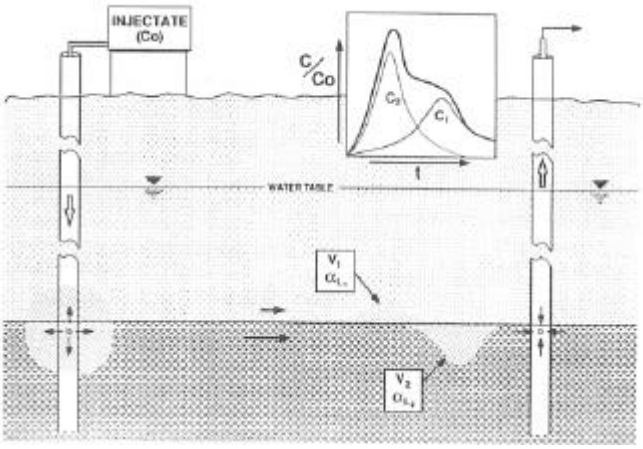

Figure 5. Schematic representation of the effect of adjacent zones of differing conductivities upon observed breakthrough of injectate transported through a layered aquifer.

being transported downgradient along a 680-m transect through the contaminant plume are depicted in Figure 6. Predicted changes that would be expected due to sorption were calculated by using eq 2 and were based upon the observed cell size distribution at the head of the plume (USGS well S314). The filtration model tended to underpredict the relative abundance of bacteria in the smallest size class (0.2 μm). There was also an overestimation of relative abundance in several of the larger size classes, particularly for the 1.2- and 1.4-μm classes. However, the predicted increase in the average cell size of the bacterial population with travel downgradient were within 0.06 μm of the observed value at 380 m downgradient (USGS well F347) and the same as the observed value at 640 m (USGS well F349).

Discussion

Model Parameters. Our approach and the one generally employed in laboratory studies involving colloid retention in porous media columns (18) was to first calculate collector efficiency (η) based upon physical measurements and then empirically determine the collision efficiency (α) based upon observed removal efficiencies and the theoretical value of η. The rationale is that η involves physical aspects of the filtration model that are better

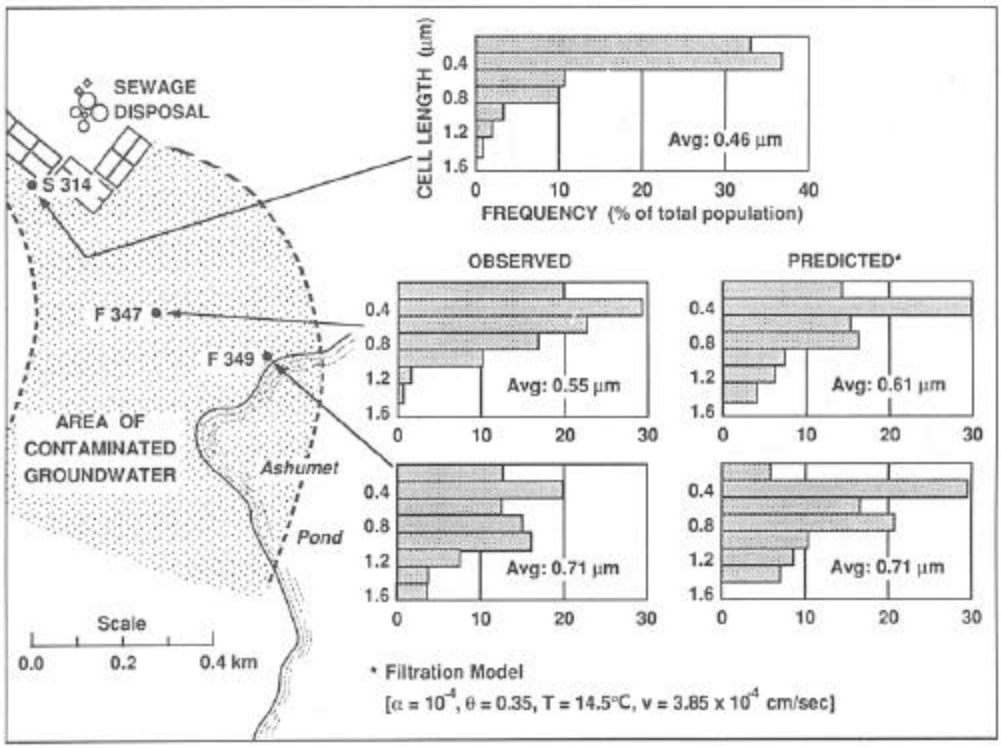

Figure 6. Observed and predicted changes in the size frequency distribution of the indigenous bacterial population that is being transported downgradient in the upper 640 m of the contaminant plume.

understood and more easily measured than are the chemical factors that affect α (18).

There are, however, several uncertainties in estimating collector efficiency for bacteria moving through aquifer sediments. One involves the effect of bacterial motility (locomotion by means of flagella), which can result in more rapid movement than that caused by random thermal (Brownian) motion (19). Chemotaxis (movement in response to chemical gradients) of motile bacteria toward or away from stationary surfaces further complicates estimates of η. Bacterial motility may not be a problem in our small-scale injection experiment, since the DAPI stain adversely affects bacterial activity (20). However, a substantial fraction (over half in some samples) of the free-living bacterial population in the contaminant plume appears to be motile. Therefore, more accurate estimates of the collector efficiency factors for indigenous bacteria in sandy aquifers may require inclusion of a term that accounts for the small-scale movement due to taxis. A simple expression describing the contribution of taxis is included in at least one theoretical model of microbial transport through porous media (2), but its validity in these applications has yet to be determined.

Another uncertainty involves the range of buoyant densities (ρ_p) which affects the magnitude of settling and the optimal size for transport (Figure 2). The more accurate measurements have involved centrifugation techniques based upon equilibrium sedimentation using density gradients created with colloidal silica (21). Unfortunately, such determinations involve pure cultures, typically fast-growing bacteria of clinical interest. Little information is available on the range of buoyant densities for indigenous bacterial populations in low-nutrient (oligotrophic) aquatic systems. However, it may be assumed that many bacteria in the Cape Cod aquifer are near-neutral bouyancy, judging from their slow sinking velocities. Although it is commonly assumed that the optimal diameter for transport through coarse, sandy sediment is ~1 um, the optimal size for microbial transport through the Cape Cod aquifer at near-neutral buoyancy (e.g., $\rho_p = 1.001$) may be 3-4 μm (Figure 2). This would not be the case for microbes with much higher specific gravities ($\rho_p > 1.1$), since the 3-4-μm class would predictably "settle out" onto grain surfaces.

Other uncertainties and complications in using eq 6 for microorganisms moving through sandy aquifers involve the effect of differences in both microbial and grain size morphology. The colloid- filtration model assumes spherical colloids and collectors. Deviation of individual sand grains from spherical morphology may result in some degree of error. Although there are a number of spherical (coccoidal) bacteria present in the Cape Cod aquifer, most appear to be rod shaped. There are also a number of spiral and filamentous forms (12). Morphological differences among microbes can result in different rates of attachment (22). However, the colloid filtration model has been successfully applied to sand-bed filtration where there is morphologic variation in both collector and colloid.

Collision efficiency factors for other in situ studies involving bacterial transport through sandy aquifer sediments are not available. The estimates in Table 11 ($5 \times 10^{-3} - 1 \times 10^{-2}$) compare favorably with an a value of 4.6×10^{-2} reported for laboratory experiments with *Pseudomonas aeruginosa* and glass surfaces at 10^{-1} M NaCl (23). However, propensity for attachment to surfaces can vary among bacterial species, with solution conditions, and with the nature of the solid surface (24). In a previous transport experiment with bacteria-sized microspheres, differences in the rate of immobilization were observed for microspheres having approximately the same diameter, but different surface characteristics (6). This suggests that the collision efficiency for bacteria may vary even among species of similar size and morphology. Since some aquatic bacteria may alter their propensity for attachment to solid surfaces in response to changes in nutrient conditions (25, 26), temporal changes in the apparent value of a would not be unreasonable for bacteria moving with contaminated groundwater. Consequently, comparison of labo

ratory- and field-derived estimates of a values may be difficult to interpret.

The discrepancy between collision efficiency factors estimated from our injection test data and the value estimated earlier for the indigenous population in the contaminant plume (17) may be due to bacterial growth. Recent estimates of in situ growth rates for the unattached bacteria that are being transported through the most contaminated parts of the plume range up to 0.042 h^{-1} (16-h average doubling time) (27). However, growth was not observed for the DAPI-stained population in the injection test, due to the inhibitory effect of the DAPI stain or to differences in nutrient and environmental conditions between the groundwater at the injection site and the highly contaminated groundwater where the injectate population was collected. On the other hand, losses of bacteria during transport from nonsorptive processes such as straining, grazing by Protozoa, or lysis in response to unfavorable conditions, parasitism from bacteriophages, or *Bdellovibrio* may lead to an overestimation of collision efficiency. Losses of stained bacteria due to straining may not be significant, judging from the large median grain size of the aquifer sediments (0.59 mm) relative to the bacteria (0.6 μm). In general, straining becomes significant when the diameter of the suspended particles are >5% of that of the media (28), although physical heterogeneity makes the application of this criterion less accurate. Also, the low level of dissolved oxygen (<0.1 mg/L) may preclude the establishment of a substantial population of bactiverous protozoa. Lysis did not appear to be much of a factor in the apparent attenuation of DAPI-stained bacteria in the injection experiments, since it was not significant during the subsequent month-long, 12 °C sample incubations.

The differences in estimated values of collision efficiency for the two depths for which the injection test was run (8.5 and 9.1 m BLS) suggest that substantial changes in the apparent value of a may occur over relatively small distances in the aquifer. Spatial variability in the collision efficiency factor due to heterogeneity in the aquifer may necessitate a stochastic treatment of a in models of bacterial transport models. It should be recognized, however, that the colloid filtration model is very sensitive to the value of α and it is difficult to modify this parameter value without significantly changing the model output.

Filtration Theory in Modeling. Use of filtration theory appeared to give reasonable approximations of attenuation for the stained bacteria in the small-scale injection experiment (Figures 3B,C and 4B,C). However, the higher dimensionless concentrations of bacteria relative to Br- during days 25-30 following injection at the 9.1-m depth suggest reversible adsorption behavior and the need for a model that allows for retardation of at least a portion of the bacterial population. Retardation was more extensive than that observed earlier in the forced-gradient experiment performed at another location in the aquifer (6). The role of physical heterogeneity in the observed differences in transport behavior of bacteria at different locations within the same aquifer complicates interpretation of data resulting from these field experiments.

Given the above cautionary statements, there is nonetheless a good fit to the breakthrough curves for both bromide and bacteria by the solute transport model that was modified to include irreversible and reversible adsorption. The successful employment of a solute transport model to simulate observed transport behavior of nonsorbing MS2 bacteriophage in columns of saturated sediment has been reported (29). However, adsorption appears to be a major mechanism controlling bacterial transport in our in situ experiments and accounted for removal of most (up to 85%) nongrowing bacteria within a 7-m travel distance. It is not clear from the data how the reversible component of bacterial adsorption should be represented in the overall model. Although the linear, instantaneous (fast relative to advection) adsorption model is appealing because of its simplicity, it is based upon the unverifiable assumption that only a small percentage (7%) of the bacterial population undergoes reversible adsorption. In contrast, the model involving kinetic limitation allows for the reversible adsorption of all unattached bacteria and has the same first-order form as the filtration portion of the model. A limitation in this approach is that the average characteristics that the rate constants represent are likely a continuum of different site types that may be poorly represented by a single average value. However, more accurate mathematical descriptions of sorption for a diverse bacterial population onto aquifer sediment surfaces is beyond the scope of this study, and more controlled experimentation needs to be done to delineate bacterial sorption and desorption behavior in the presence of aquifer sediments.

The relationship of bacterial size upon the likelihood of sorption during transport downgradient is suggested in Figure 6. The observed and predicted increase in average cell size with increasing distance downgradient suggests that transport favors larger bacteria (>0.6 μm). Several biological factors may also lead to changes in the size distribution, such as changes in nutrient levels or the abundance of bactiverous protozoa. The effect of groundwater protozoa on average bacterial size in the contaminated groundwater is not well understood, but preliminary surveys suggest that protozoa may be less abundant in the anoxic core of the plume (N. Kinner, personal communication) where the samples used in this study were taken. Decreases in average cell size of bacterial populations in response to a severe carbon limitation (oligotrophic conditions) are well documented. Levels of dissolved organic carbon range from ~4 mg/L immediately downgradient to ~1 mg/L at 700 m downgradient. Therefore, the observation that bacteria were larger in nutrient-depleted groundwater at 380 and 640 in downgradient from the outfall as compared with those found in highly contaminated groundwater further upgradient is difficult to explain on the basis of nutrient conditions.

Increases in average diameter of a polydispersed population of carboxylated, bacteria-sized microspheres as a consequence of travel downgradient through the aquifer also have been observed (6). The relative breakthrough C/Co of the largest [1.35-μm (diameter)] carboxylated microspheres was observed to be over 10-fold higher than for the smallest [0.23 μm (diameter)]. This may be predicted by using eqs 6 and 9, a specific gravity for the microspheres of 1.05, and a calculated α value of 2.6×10^{-2} (Table II). Therefore, an abiotic explanation for observed increases in average cell size of bacteria traveling downgradient through the contaminant plume seems reasonable. The predicted preference for transport of larger versus smaller bacteria may explain the relatively large size of free-living bacteria in uncontaminated groundwater at our site relative to "dwarf" bacterial populations often found in other nutrient-depleted aquatic environments such as the open ocean (30). The apparent effect of cell size upon immobilization occurring during transport downgradient has important implications in the proposed use of introduced, nonindigenous bacteria in aquifer cleanup. However, the size dependency of bacterial immobilization in

groundwater would also depend on the phenomenon of "filter ripening" (31) and average pore size. In aquifers where straining is an important determinant of bacterial transport, larger bacteria would be at a disadvantage.

In summary, there appear to be a number of advantages in using colloid filtration theory in modeling transport of bacteria through contaminated, sandy aquifers. The colloid filtration model is relatively simple, accounts for the abiotic mechanisms by which bacteria contact stationary solid surfaces, and can reasonably predict the effect of cell size upon the rate of immobilization. Some of the uncertainty in this application involves the buoyant densities of indigenous bacteria, the effect of bacterial motility on collector efficiency, and how to mathematically treat some of the physical differences between situations of groundwater flow through sandy aquifers and packed-bed filtration for which the model was developed. In addition, filtration theory does not account for differences in surface characteristics among bacteria or the effect of nonequilibrium adsorption or nonsorptive interactions of bacteria with particles. The effect of aquifer heterogeneity, growth, grazing by protozoa, lysis, and detachment from solid surfaces would necessitate the use of a more complex model for large-scale transport experiments with bacteria. However, our results suggest that filtration theory may be useful in a multi-component description of immobilization in transport models involving bacteria in groundwater.

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