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Spatial distribution of deposited bacteria following miscible displacement experiments in intact cores

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Abstract. Miscible displacement experiments were performed on intact sand columns ranging from 15 to 60 cm in length to determine whether bacterial deposition varies at the centimeter scale within aquifer sediments. A 1-pore-volume pulse of radiolabeled cell suspension was introduced into the columns followed by a 2-pore-volume flush of artificial groundwater. The columns were then drained and dissected along the axis of flow. At ~1-cm intervals, nine samples were removed for the enumeration of sediment-associated bacteria. Concentrations of sediment-associated (deposited) bacteria varied by up to 2 orders of magnitude in the direction perpendicular to flow demonstrating that bacterial deposition cannot be described mechanistically by a single rate coefficient. Incorporation of a distribution of sediment size and porosity values into Monte Carlo simulations indicates that physical heterogeneities are only partially responsible for the observed variability in deposited bacteria. A simple first-order model (classic filtration theory) adequately described the average spatial distribution of bacteria with depth within the 15-cm column. For the longer columns, however, the average concentration of deposited bacteria did not decrease exponentially with depth. A second-order model, modified to include an influent suspension of bacteria consisting of two subpopulations with separate sticking efficiencies (dual-alpha population), was required to describe the observed decreases of deposited bacteria with depth. A sensitivity analysis was performed with a first-order dual-alpha model to understand the effects of an influent suspension with two subpopulations of bacteria on the decrease of deposited bacteria with flow path length. Numerical simulations show that even for small fractions (0.01) of nonsticky bacteria, the decrease in deposited bacteria may deviate substantially from the exponential decrease expected from colloid-filtration theory. Results from experimental as well as numerical studies demonstrate the importance of column dissections for understanding bacterial deposition in saturated porous media.

1. Introduction

The successful use of microorganisms for the in situ restoration of polluted aquifers depends in large part on the ability to deliver pollutant-degrading bacteria to the area of contamination. One method of delivery currently being investigated is the use of bio-curtains: the establishment of a region of sediment-associated bacteria that is capable of contaminant degradation [*Starr and Cherry*, 1994; *Taylor et al.*, 1993]. A bio-curtain is established by injecting bacteria into the subsurface ahead of a migrating contaminant plume by a series of injection and withdrawal wells aligned perpendicular to the direction of groundwater flow. Ideally, as the contaminated water crosses the bio-curtain, the introduced bacteria degrade the contaminants with the result being that the exiting water meets preestablished standards. This method requires that a large number of the injected bacteria become associated with the aquifer sediment and that the bio-curtain be continuous. Therefore the efficient use of the bio-curtain method requires knowledge of the in situ spatial distribution of bacteria. Because in situ methods are by definition noninvasive, the in situ spatial distribution of bacteria must be inferred from either sediment-associated bacteria measured in column experiments or concentrations of effluent bacteria measured within the aquifer of interest. Such inference requires a fundamental understanding of the processes affecting both the transport and deposition (where deposition refers to all the processes resulting in bacteria becoming attached to the sediment surface) of bacteria within heterogeneous aquifer sediments.

The heterogeneous nature of aquifers is well established. Current theories of bacterial deposition, however, have been based on studies using well characterized homogeneous porous media [*Hornberger et al.*, 1992; *Kinoshita et al.*, 1993; *Martin et al.*, 1992] or repacked aquifer sediments [*Gannon et al.*, 1991; *Jackson et al.*, 1994; *Lindqvist and Bengtsson*, 1995; *McCaulou et al.*, 1995; *Tan et al.*, 1994]. The development of more realistic models that incorporate sediment heterogeneity has been slow owing to the limited amount of data from intact aquifer sediments [*Bales et al.*, 1997; *Harvey and Garabedian*, 1991; *Harvey et al.*, 1989; *Smith et al.*, 1985] and the lack of mechanistic models describing bacterial transport through heterogeneous porous media. In addition, our understanding of bacterial deposition has been limited by the fact that the majority of published studies have focused on inferring depositional processes from the analysis of bacterial breakthrough curves (BTCs). Only recently has the dissection of sandpacks and enumeration of deposited bacteria gained popularity, but, in the cases reported to date, only spatially homogeneous [*Albinger et al.*,

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1994; *Baygents et al.*, 1998; *Camesano and Logan*, 1998; *Gross et al.*, 1995; *Shonnard et al.*, 1994] or specifically constructed, spatially heterogeneous [*Murphy et al.*, 1997; *Silliman*, 1995] porous media have been considered.

As a result of our limited understanding of bacterial deposition in intact aquifer sediments, bacterial deposition is often assumed to be spatially constant. This assumption yields an expectation of a uniform distribution of deposited bacteria in the direction perpendicular to flow. At the microscopic level, the deposition of colloids may be expected to be nonuniform owing to differences in grain sphericity, flow velocity, and charge distribution due to lattice defects and chemical impurities [*Johnson et al.*, 1996; *Song et al.*, 1994]. Visualization of bacterial transport through a single pore throat using scanning confocal laser microscopy has shown that bacterial deposition at this scale is not uniform [*Hendry et al.*, 1997]. At scales of a meter to tens of meters, nonuniform deposition of bacteria may be expected because of stratigraphic variability in permeability [*Fontes et al.*, 1991; *Morley et al.*, 1998], grain size [*Fontes et al.*, 1991], and surface coatings [*Knapp et al.*, 1998; *Mills et al.*, 1994; *Scholl and Harvey*, 1992; *Scholl et al.*, 1990]. Indeed, the transport of bacteria has been observed to vary spatially over scales of <1 m in field experiments [*Harvey and Garabedian*, 1991; *Harvey et al.*, 1993]. To determine if heterogeneities at the centimeter scale are significant enough to create substantial spatial variations in concentrations of deposited bacteria, we focus our attention on the distribution of deposited bacteria within intact aquifer sediments following miscible displacement experiments.

Miscible displacement experiments were performed on columns containing intact aquifer sediments. Upon completion of the experiments, the columns were dissected and the sediment-associated (deposited) bacteria were enumerated. Concentrations of deposited bacteria (cells per grams of sediment) varied by as much as 2 orders of magnitude in the direction perpendicular to flow demonstrating that bacterial deposition is variable at this scale and therefore cannot be described mechanistically by a single deposition coefficient. Monte Carlo simulations incorporating physical heterogeneities were unable to account completely for this spatial variability. We observed that in all but one case the mean distribution of bacteria with depth could not be accounted for by simple colloid-filtration theory. More complex models incorporating second-order effects and a dual-alpha population of bacteria (where different values of sticking efficiencies (α) were assigned to each of two subpopulations of bacteria) were required to describe the nonexponential decrease in concentration of deposited bacteria with depth in these columns.

2. Experimental Methods

2.1. Column Experiments

Columns of intact sediments were obtained from an exposed vertical outcrop located near Oyster, Virginia, by driving 7.5-cm diameter aluminum pipes horizontally (parallel to groundwater flow direction) into the sediments. The columns were returned to the laboratory, and each column was cut to obtain a relatively undisturbed section. Column lengths of 15, 43, 45, and 60 cm were used in this study. End-caps made of PVC and fitted with 0.06-mm outlets were then placed on the columns and sealed air-tight with epoxy cement. The columns were mounted vertically on a rack and saturated with CO_2 gas from the bottom up. Degassed artificial groundwater (AGW) was slowly pumped into the columns in an upflow direction so that it took approximately 1–2 days to saturate the sediments. The AGW was prepared by dissolving the following salts in 1 L of deionized water: 60 mg $MgSO_4 \cdot 7H_2O$, 20 mg KNO_3, 36 mg $NaHCO_3$, 36 mg $CaCl_2$, 35 mg $Ca(NO_3)_2$, and 25 mg $CaSO_4 \cdot 2H_2O$. The columns were repeatedly tapped to assist any entrapped gas to escape. This process was continued until no more gas bubbles were observed and the *p*H of the effluent solution remained constant at ~6.2. A minimum of 20 pore volumes of AGW were run through each column (using this method with glass columns and packed sand has shown that within ~5 pore volumes all gas bubbles observable with a handheld magnifying glass disappear). After complete saturation, the flow direction was reversed for the introduction of tracer solutions into the column. The physical parameters (velocity and dispersivity) of the cores were obtained by fitting CXTFIT2 [*Toride et al.*, 1995] to the observed BTCs of a conservative tracer (Br^- and ^3H_2O).

A 1-pore-volume pulse of ^{14}C-labeled bacteria was passed through the columns at initial concentrations ranging from $\sim 1 \times 10^8$ cells mL^{-1} to 2×10^9 cells mL^{-1} as determined by acridine orange direct counts (AODC) of the influent suspension. The bacterial strain used, designated as W31, is a gram-negative coccus with an average diameter of 0.95 μm and was isolated from groundwater collected from the site and has been classified as hydrophilic according to the scheme of *Mozes et al.* [1986]. The organism was grown on R2A medium (Difco) amended with ^{14}C-glucose (1 μCi mL^{-1} of growth medium) and allowed to reach stationary phase. The cells were then washed 3 times in AGW and allowed to starve for 36–48 hours. Filtering samples from the influent cell suspension revealed that greater than 95% of the radioactivity was cell-associated. Dissolved radiolabel was observed to travel conservatively through these sediments. Effluent concentrations were collected every 0.1 pore volume with a fraction collector.

After flushing the columns with 2 pore volumes of AGW, the columns were drained and dissected. At each sampled depth (~1-cm intervals) 9 samples, each weighing between 2 and 3 g, were removed for the enumeration of sediment-associated bacteria. This method sampled ~40% of the sediment at each depth. Enumeration of sediment-associated bacteria was done by adding 10 mL of a cell-lysing solution containing 10 m*M* tris-Cl, 1.0 m*M* ethylenediamine tetraacetic acid (EDTA), and 1% sodium lauryl sulfate to the samples. The samples were shaken vigorously with a vortex mixer, and 1 mL of the solution was removed for liquid scintillation counting. The samples were drained and dried at 105°C, and the weight of the sand was determined. Concentrations of deposited bacteria are reported based on the weight of sand in each sample, the amount of ^{14}C in each sample, and the known amount of ^{14}C per cell (calculated as the total ^{14}C activity divided by the total number of bacteria as determined by AODC).

Prior to trimming the 15-cm column, sediment samples were removed (~2–3 g) and sieved by hand using mesh sizes of 4, 2, 0.71, 0.5, 0.35, 0.25, 0.177, and 0.125 mm to determine mean grain size and size distribution of the sand. Surface area analysis was also performed on these samples using the multipoint Brunauer, Emmett, and Teller (BET) method [*Hiemenz*, 1986].

2.2. Model Development

Assuming low surface coverage, bacterial transport through laboratory columns can be modeled by incorporating colloid-filtration theory into a one-dimensional advection-dispersion

equation [*Harvey and Garabedian*, 1991; *Hornberger et al.*, 1992; *McCaulou et al.*, 1995]

$$\frac{\partial c}{\partial t} + \frac{\rho_b}{\theta}\frac{\partial s}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - v\frac{\partial c}{\partial x} \tag{1}$$

$$\frac{\rho_b}{\theta}\frac{\partial s}{\partial t} = k_c c - \frac{\rho_b}{\theta}k_y s \tag{2}$$

where c is the aqueous concentration of bacteria (cells mL^{-1}), s is the concentration of bacteria on the solid phase (cells g^{-1}), x is the distance from the surface of the column (L), t is the time from initial input of bacteria (T), D is the dispersion coefficient ($L^2\ T^{-1}$), v is the interstitial pore water velocity ($L\ T^{-1}$), θ is porosity, ρ_b is the bulk density ($g\ L^{-3}$), k_c is the deposition coefficient (T^{-1}), and k_y is the entrainment coefficient (T^{-1}). The deposition coefficient is defined as [*Tien et al.*, 1979]

$$k_c = \frac{3(1-\theta)}{2d_c}\eta\alpha v \tag{3}$$

where d_c is the diameter of the sand grains, α is the sticking efficiency, and η is the single-collector efficiency. Using the model of *Rajagopalan and Tien* [1976], as modified by *Logan et al.* [1995], the single-collector efficiency can be calculated as

$$\eta = 4A_s^{1/3}N_{Pe}^{-2/3} + A_s N_{Lo}^{1/8}N_R^{15/8} + 0.00338A_s N_G^{1.2}N_R^{-0.4} \tag{4}$$

where $A_s = 2(1-\gamma^5)/(2-3\gamma+3\gamma^5-2\gamma^6)$, $\gamma = (1-\theta)^{1/3}$, $N_{Pe} = 3\pi\mu d_p d_c q/(kT)$, $N_{Lo} = 4H/(9\pi\mu d_p^2 q)$, $N_R = d_p/d_c$, $N_G = g(\rho_p - \rho_f)d_p^2/(18\mu q)$, d_p is the diameter of the bacteria (0.95 μm), μ is fluid viscosity (0.93×10^{-3} Pa s^{-1}), q is the specific discharge, k is the Boltzman constant (1.38048×10^{-23} J/K), T is temperature (298°K), H is the Hamaker constant (1×10^{-20} J), ρ_f is fluid density (998 kg m^{-3}), ρ_p is bacterial density (1080 kg m^{-3}), and g is gravitational acceleration. We define the dimensionless deposition coefficient as [*Bolster et al.*, 1998]

$$\kappa = \frac{3(1-\theta)}{2d_c}\eta\alpha L \tag{5}$$

where L is the length of the column.

Assumed in (2) is that deposition is a first-order kinetic process. If the rate of deposition is controlled both by the aqueous concentration as well as the concentration of deposited bacteria, a second-order model is appropriate. Second-order models have been developed from both mechanistic [*Johnson and Elimelech*, 1995; *Johnson et al.*, 1996; *Rijnaarts et al.*, 1996] and phenomenological [*Saiers et al.*, 1994; *Tan et al.*, 1994] viewpoints. Because of the inherent uncertainties in our system, we adopted the phenomenological approach. Equation (2) is modified to [*Saiers et al.*, 1994]

$$\frac{\rho_b}{\theta}\frac{\partial s}{\partial t} = k_c\psi c - \frac{\rho_b}{\theta}k_y s \tag{6}$$

$$\psi = \frac{X_{\max} - s}{X_{\max}} \tag{7}$$

where ψ is the fraction of the sediment available for deposition and $X_{\max}$ is the maximum retention capacity of the sediments (cells g^{-1}).

In addition to second-order effects, the presence of a distribution of sticking efficiencies within the influent bacterial population will lead to nonexponential decreases in deposited bacteria with depth [*Baygents et al.*, 1998]. The presence of a dual-alpha population of bacteria, where it is assumed that the influent suspension of bacteria is comprised of two distinct subpopulations, each with their own value of α, can be described simply by adding the fractional concentrations of deposited bacteria for the two subpopulations [*Simoni et al.*, 1998]. The concentration of deposited bacteria for a dual-alpha population of bacteria is

$$s_{\text{Total}}(x, t) = (F)s_{\alpha\text{high}}(x, t) + (1-F)s_{\alpha\text{low}}(x, t) \tag{8}$$

where F is the fraction of the influent bacteria with a high sticking efficiency (α_{high}), and $s_{\alpha\text{high}}$ and $s_{\alpha\text{low}}$ are the concentrations of deposited bacteria with high and low sticking efficiencies, respectively. Equation (8) is termed the dual-alpha model.

The first-order model was solved using the Crank-Nicolson finite-difference method and the second-order model was solved by the predictor-corrector finite-difference method [*Douglas and Jones*, 1963; *Remson et al.*, 1971]. Owing to mass-balance considerations, concentrations of deposited bacteria were calculated from resident concentrations using a third-type inlet boundary condition [*Kreft and Zuber*, 1978; *van Genuchten and Parker*, 1984]. All parameter values were obtained by fitting nondimensional versions of the appropriate models to the average concentration of deposited bacteria with depth using the Levenburg-Marquardt method. With data points spanning several orders of magnitude, data were log transformed prior to parameter estimation.

The model-selection criterion (MSC) was calculated to determine the information content of each model [*Koeppenkastrop and DeCarlo*, 1993]

$$\text{MSC} = \ln\frac{\sum_{i=1}^{n}(s_i - s_{\text{avg}})^2}{\sum_{i=1}^{n}(r_i)^2} - \frac{2p}{n} \tag{9}$$

where r_i is the ith residual between model prediction and observation, s_i is the ith observed concentration of deposited bacteria, s_{avg} is the average of the observed values, n is the number of observations, and p is the number of fitting parameters. The model with the largest MSC contains the highest information content and is generally considered the most appropriate model for describing the observed data. The four models tested include the first-order (Equations (1) and (2)), second-order (Equations (1), (6), and (7)), first-order dual-alpha (Equations (1), (2), and (8)), and second-order dual-alpha (Equations (1), (6), (7), and (8)) models. Model efficiency was used for determining the goodness of fit of the various models to the observed data. The model efficiency E is defined as [*Hornberger et al.*, 1992]

$$E = 1 - \frac{\sum_{i=1}^{n}(r_i)^2}{\sum_{i=1}^{n}(s_i - s_{\text{avg}})^2} \tag{10}$$

Values for both MSC and E were calculated from the log-transformed values of the observed and fitted data.

Table 1. Physical Parameters of the Columns

Column	v, m d^{-1}	θ	Pe	C_0, cells mL^{-1}	Total Number of Influent Bacteria
15 cm	2.5	0.35	9	1×10^8	2×10^{10}
43 cm	2.5	0.34	44	1×10^8	6×10^{10}
45 cm	0.78	0.35	113	2×10^9	1×10^{12}

2.3. Numerical Simulations

Monte Carlo simulations were performed (1000 realizations) with the first-order model (Equations (1) and (2)) to determine the effect of physical variability on bacterial deposition. Values for κ were calculated (using Equations (3)–(5)) from random values of d_c and θ generated by a pseudorandom number generator within the software package MATLAB®. Random values for d_c were based on the measured distribution from the 15-cm column. Random values for θ were based on the assumption that porosity was normally distributed [*Bennion and Griffiths*, 1966; *Law*, 1944] with 99% of the values falling between 0.24 and 0.48. The random distributions of d_c and θ were truncated at ± 1.9 standard deviations from the mean to prevent unrealistically high κ values. The sticking efficiency calculated from the 15-cm column was used in these simulations. Equations (1) and (2) were solved using the Galerkin formulation of the finite-element method [*Raffensperger*, 1996] with the appropriate boundary conditions. The resulting values for κ and s were log transformed to obtain a normal distribution so that the mean and a measure of dispersion (± 1.9 standard deviations from the mean) could be calculated.

A sensitivity analysis was performed with the first-order dual-alpha model (Equations (1), (2), and (8)). The dimensionless concentration of deposited bacteria (S) was calculated by

$$S = \frac{s\rho_b}{c_0\theta} \tag{11}$$

where s is the concentration of deposited bacteria determined by solving for s with equations (1), (2), and (8) for various values of the fraction of sticky bacteria (F), ratio of sticking efficiencies ($\lambda = \alpha_{high}/\alpha_{low}$), and the deposition coefficient for the sticky bacteria (κ_{high}). Values for the Peclet number (Pe) and pulse length were held constant at 45 and 1 pore volume, respectively.

3. Results

3.1. Column Experiments

BTCs of a conservative tracer eluted from 3 of the 4 columns (15, 43, and 45 cm) were adequately described by a one-region model (data not shown) suggesting that macroscopic physical heterogeneities were not present. Fitted values for average linear velocities ranged from 0.78 m d^{-1} for the 45-cm column to 2.5 m d^{-1} for the 15- and 43-cm columns (Table 1). Porosity estimates, as determined from discharge measurements and fitted velocity values, were consistent between columns with values ranging from 0.34 to 0.35. Fitted values of dispersivity were 1.65, 0.97, and 0.40 cm (Peclet numbers of 9, 44, and 113) for the 15-, 43-, and 45-cm columns, respectively. Inspection of the BTC of the conservative tracer through the 60-cm column, however, showed a step before reaching a normalized concentration of 1. Such behavior is indicative of multiple flow regimes, and quantitative analysis of the data from this column was not undertaken.

Recovery of bacteria in the effluent for all columns was <0.1% (obtained by integration of the BTC). The entrainment of bacteria into the fluid phase was deemed unimportant in these systems due to the minimal breakthrough of bacteria; therefore we set $k_y = 0$ for all model fits. Assuming a bulk density of 1.7 g cm^{-3}, integrating the measured concentrations of bacteria over the entire column yielded mass balances of ~80–95% of the influent bacteria for all columns. This represents an acceptable yield given the incomplete sampling and heterogeneous distribution of bacteria within the columns.

Dissection of the columns yielded 9 measurements of deposited-bacteria concentrations perpendicular to the direction of flow for each sampled depth (Figure 1). Spatial variations of 1–2 orders of magnitude in the measured concentration of sediment-associated bacteria (cells $gram^{-1}$ of sediment) were observed at several depths (Figure 2). For example, in the 45-cm column at a dimensionless depth (hereafter referred to only as depth) of $L = 0.27$, the number of cells $gram^{-1}$ of sediment varied from 2.8×10^7 to 1.8×10^9 (Figure 2c). In all columns, the variation in deposited bacteria within a section increased with depth from the inlet. In the 45-cm column the concentration of deposited bacteria ranged from 7.6×10^8 to 1.9×10^9 cells g^{-1} near the inlet ($L = 0.09$) but varied from 8.2×10^7 to 2.0×10^9 cells g^{-1} at $L = 0.24$. Deposited bacteria at a depth of 0.01 in the 60-cm column ranged from 1.1×10^8 to 3.7×10^8 cells g^{-1}, whereas, at $L = 0.19$, the concentration ranged from 1.4×10^7 to 1.1×10^8 cells g^{-1}

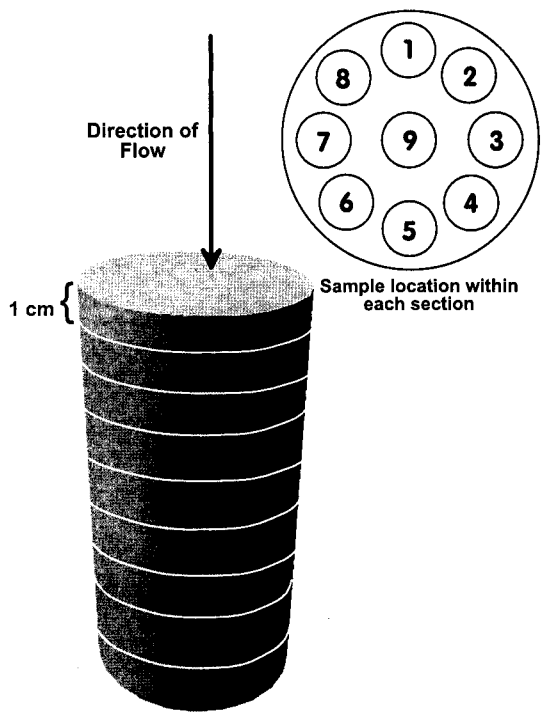

Figure 1. Sampling scheme for the intact cores. Approximately 40% of the sediment at each sectioned depth was sampled. Measured concentrations of sediment-associated bacteria were used to infer the total amount of bacteria retained within a given section. The diameter of the pipe was 7.5 cm.

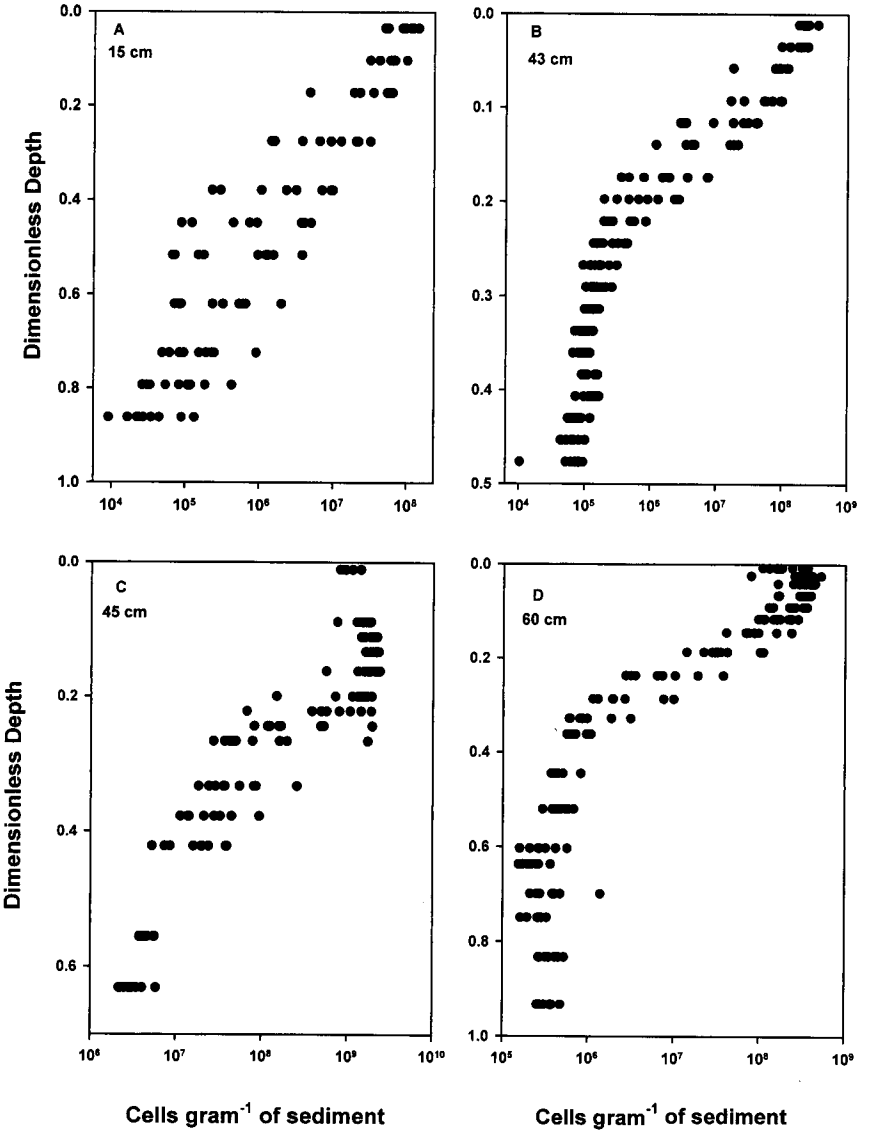

Figure 2. Distribution of deposited bacteria with dimensionless depth for intact columns of length (a) 15 cm, (b) 43 cm, (c) 45 cm, and (d) 60 cm. Dimensionless depth is defined as x/L, where x is the sampling location and L is the length of the column. Note differences in total sampled depth between columns.

(Figure 2d). In the 45-, 43-, and 15-cm columns, the variation in cells g^{-1} subsequently decreased closer to the outlet.

The trend in the overall distribution of deposited bacteria differed among the columns. For instance, in the 15-cm column the concentration of deposited bacteria decreased exponentially from the inlet, and the average behavior was well described by simple colloid-filtration theory (Figure 3a, Table 2). The effective sticking efficiency (so termed as it was determined by assuming homogeneous conditions in the calculation of the single-collector efficiency, η) was calculated to be 3.8 using the mean value of d_c and θ. Concentrations of deposited bacteria in the 43-cm column decreased exponentially (with the exception of a slight deviation near the inlet) away from the inlet to a depth of ~0.2 after which the rate of decrease with depth was minimal. The simple first-order decay model was insufficient for describing the overall mean behavior of the bacteria with depth for this column (Figure 3b, Table 2). Of the four models tested, the second-order dual-alpha model produced the best fit to the data ($E = 0.996$) and gave the greatest amount of information (MSC = 5.1). The first-order dual-alpha model fit produced a good fit to the data also ($E = 0.995$), but the information content was slightly less (MSC = 4.3). The fitted parameters indicate that for a monoclonal culture, 77% of the bacteria had an effective sticking efficiency of 4.3, whereas the remaining 23% had an effective sticking efficiency of 0.0012 for a ratio (λ) of greater than 3.4×10^3. In the 45-cm column (Figure 3c) the average concentration of deposited bacteria actually increased slightly from the inlet to a depth of 0.15 before decreasing. The second-order dual-alpha model produced the best fit ($E = 0.98$) to these data with the greatest amount of information (MSC = 3.3). The fitted parameters indicate that 99% of the bacteria had an effective sticking efficiency of 0.63, whereas the remaining 1% had an effective sticking efficiency of 0.031 ($\lambda = 20$). The average concentration in the 60-cm column remained constant to a depth of 0.1 after which the concentrations decreased exponentially to a depth of ~0.35. For the remainder of the column, the concentrations of deposited bacteria remained

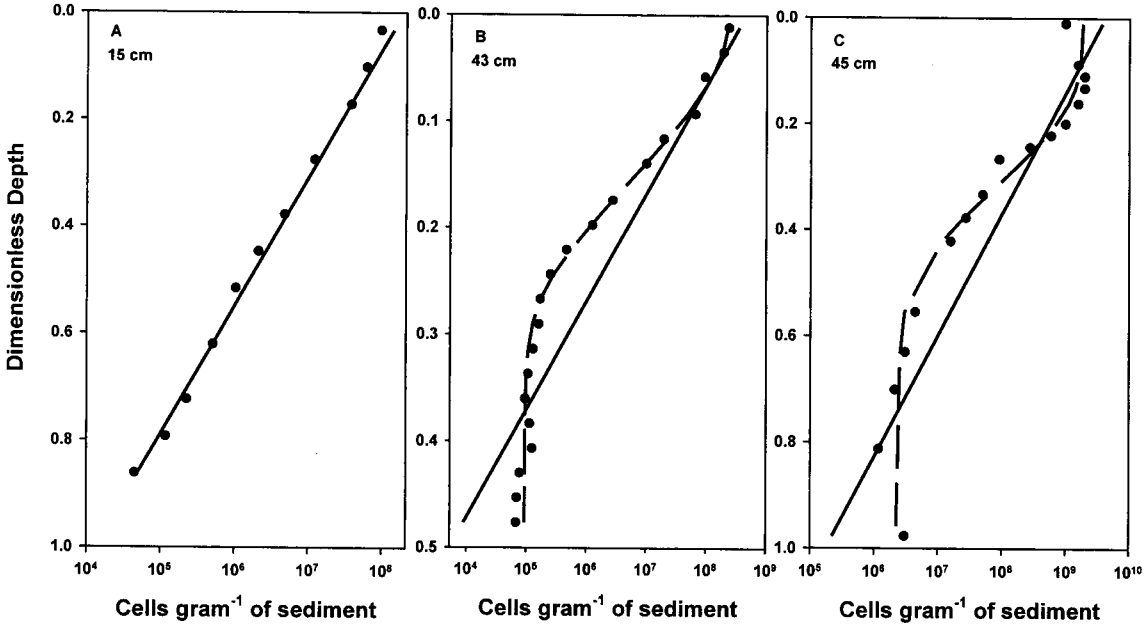

Figure 3. Observed (dots) and fitted values of the mean concentration of deposited bacteria for (a) 15-, (b) 43-, and (c) 45-cm columns. The solid line represents the best fit to the observed data using colloid-filtration theory, while the dashed line represents the best fit to the data using the second-order dual-alpha model.

relatively constant with depth. These data were not modeled due to the inference of the presence of multiple flow paths.

Measured mean grain diameter from samples taken from the 15- and 43-cm columns ranged from 0.4 to 1.2 mm (mean of 0.74 and standard deviation of 0.024) with uniformity coefficients (d_{60}/d_{10}) for these samples ranging from ~1 to 5. BET surface area analysis of the sediments from the 15-cm column gave a mean surface area of 0.64 $m^2\ g^{-1}$ with a standard deviation of 0.15 $m^2\ g^{-1}$ ($n = 10$).

3.2. Numerical Simulations

A normal distribution of d_c and θ was used for the Monte Carlo simulations; the resulting values of κ and the sediment-associated bacterial concentrations were log-normally distributed. Monte Carlo simulations using the measured distribution of sand grain diameters and an assumed distribution of porosity yielded concentrations of deposited bacteria varying by less than a factor of 6 for any given depth.

Simulations with a dual-alpha population of bacteria resulted in nonexponential decreases in concentrations of deposited bacteria with flow path length for high rates of deposition (Figure 4). The presence of a small fraction of nonsticky bacteria ($F = 0.99$) with a sticking efficiency ratio of 2 orders of magnitude ($\lambda = 100$) less than the remaining population yields substantially greater concentrations of deposited bacteria near $x = L$ in comparison to a bacterial suspension described by a single-sticking efficiency (Figure 4a). This increase in deposited bacteria can be attributed to the deposition of the nonsticky bacteria. The importance of the nonsticky bacteria in concentrations of deposited bacteria becomes important once the sticky bacteria have been removed from suspension. For the values used in these simulations, the effects of a small fraction of nonsticky bacteria, $F = 1$ versus $F = 0.99$, are substantially greater than the effects of a small fraction of sticky bacteria, $F = 0$ versus $F = 0.01$. As λ decreases from 1000 to 10, the concentration of deposited bacteria away from the inlet increases (Figure 4b) and further demonstrates the substantial deviation from a log linear decrease in deposited bacteria with flow path length for a dual-alpha population of bacteria. For low rates of deposition ($\kappa_{high} < 5$), exponential decreases with depth are observed for a dual-alpha population of bacteria when $\lambda = 100$ (Figure 4c) because, at these low rates of deposition, the nonsticky fraction of bacteria behaves conservatively ($\kappa_{low} < 0.05$) and thereby does not contribute to the concentration of deposited bacteria with depth.

4. Discussion

The results show that bacterial deposition is spatially variable at the centimeter scale for macroscopically homogeneous porous media. In accordance with colloid-filtration theory (Equations (3)–(5)), κ is dependent on several parameters, and variations in any of these parameters will result in variations in rates of deposition. At the centimeter scale, the most significant variations are likely to be those associated with grain diameter, porosity, sticking efficiency, and specific discharge (assumed constant in this study). Incorporating a distribution of both grain diameter and porosity yielded variability in concentrations of deposited bacteria by a factor of 6. This variability is much less than that observed within the columns and suggests that physical heterogeneity alone cannot explain the variation. We assumed that transverse dispersion was negligible in our Monte Carlo simulations. Although significant concentration gradients were likely present in the aqueous phase in our experimental columns (as a result of the distribution of deposited bacteria), at these flow rates and in a porous medium, we expect that the transfer of bacteria perpendicular to the direction of flow is inconsequential.

In addition to physical parameters, variability in the surface chemistry of the sediments may lead to variations in the sticking efficiency and, hence, the concentration of deposited bacteria. Analyses performed on sediments from this site have

Table 2. Results From Fitting the Various Models to the Observed Data

	First-Order Model (Equations (1) and (2))			Second-Order Model (Equations (1), (6), and (7))			First-Order Dual-Alpha Model (Equations (1), (2), and (8))				Second-Order Dual-Alpha Model (Equations (1), (6), (7), and (8))				
	κ	E	MSC	κ_{high}	X_{max}	E	MSC	κ_{high}	κ_{low}	F	E	MSC	κ_{high}	κ_{low}	X_{max}	F	E	MSC
15 cm	20.	0.994	4.9	20.	2.1×10^{13}	0.994	4.7	27.	10.	0.59	0.995	4.7	44.	17.	2.3×10^{8}	0.58	0.995	4.6
	(0.51)			(1.4)	(6.4×10^{10})			(45.)	(8.0)	(2.5)			(62.)	(5.5)	(2.7×10^{8})	(0.44)		
43 cm	35.	0.84	1.7	35.	1.2×10^{13}	0.840	1.6	56.	0.39	0.99	0.995	4.3	72.	0.021	3.3×10^{8}	0.77	0.996	5.1
	(2.39)			(6.0)	(5.8×10^{17})			(3.4)	(2.4)	(.068)			(5.2)	(0.0078)	(5.1×10^{7})	(0.087)		
45 cm	11.	0.85	1.8	11.	8.6×10^{13}	0.850	1.7	15.	0.16	0.97	0.94	2.5	27.	1.3	2.4×10^{9}	0.99	0.977	3.3
	(0.87)			(1.7)	(2.6×10^{18})			(2.4)	(2.0)	(0.32)			(4.9)	(1.9)	(4.1×10^{8})	(0.003)		

Standard errors are in parentheses. Second-order values are in cells g^{-1}.

shown appreciable amounts of Fe coatings [*Dobson*, 1997; *Knapp*, 1997]. The presence of these coatings can result in an electrostatic attraction, i.e., a negative Gibbs free energy for any separation distance, between the positively charged iron-coated sand grains (pH_{zpc} = 8.5 [*Stumm and Morgan*, 1981]) and the negatively charged bacteria at circumneutral pH values. The presence of Fe-oxyhydroxide coatings has been shown to increase bacterial attachment to sediment particles in both batch [*Mills et al.*, 1994] and transport studies [*Knapp et al.*, 1998; *Scholl and Harvey*, 1992; *Scholl et al.*, 1990]. Spatial variability in surface coatings would lead to variations in concentrations of deposited bacteria.

Although we took steps to ensure complete saturation of the cores, some entrapped air may have been present within the columns as small bubbles. Bacteria have been shown to sorb preferentially to the air-water interface [*Powelson and Mills*, 1996; *Wan and Wilson*, 1994], and a distribution of entrapped air bubbles could result in spatial variations of deposited bacteria. *Wan et al.* [1994], however, observed minimal retention of a hydrophilic bacterium in columns with entrapped air. Significant retention was not observed until a continuous gas phase was present (i.e., low water content). With the saturation method that we employed and the observed (fitted) porosity values of 0.35, the amount of entrapped air in these columns is likely to be negligible and therefore not responsible for the observed spatial variations in deposited bacteria.

The effect of straining on the removal of bacteria from the aqueous phase is most often assumed to be negligible in sandy aquifers because pore throats are much larger than the size of the bacteria. The presence of a distribution of sand sizes, however, will result in a heterogeneous distribution of pore throat diameters. If a significant number of pores are of the size range of the introduced bacteria, then straining becomes important [*McDowell-Boyer et al.*, 1986]. Uniformity coefficients (d_{60}/d_{10}) for samples from the 15-cm and 43-cm columns ranged from ~1 to 5, indicating a distribution in sand size as well as spatial variability in this distribution. *Matthess and Pekdeger* [1985] present a method for determining the importance of straining in porous media. The geometrical suffusion security, or straining, parameter is defined as

$$\eta_{sg} = \frac{d_p}{F_s d_k} \tag{12}$$

$$d_k = \sqrt[6]{U}\varepsilon d_{17} \tag{13}$$

where d_p is the diameter of the bacteria, F_s is the empirical transit factor for suffusion (a value of 0.6 was used [*Matthess and Pekdeger*, 1985]), d_k is the hydraulic equivalent diameter of the pore throats, U is the uniformity coefficient (d_{60}/d_{10}), and ε is the ratio of void volume to solid volume. Values of η_{sg} must be greater than 1.5 for mechanical filtration to be important. Calculation of η_{sg} from these samples yielded values of <0.2 indicating straining is not important in these sediments.

On the basis of colloid-filtration theory, the concentration of deposited bacteria is expected to decrease exponentially with flow path length. In three of the four columns, the concentration of deposited bacteria was nearly constant with depth in the region near the inlet. This behavior is expected for high-deposition rates if the sediments have a finite retention capacity that is exceeded by the aqueous concentration of bacteria [*Mills et al.*, 1994]. As the concentration of deposited bacteria approaches the retention capacity, fewer bacteria are depos-

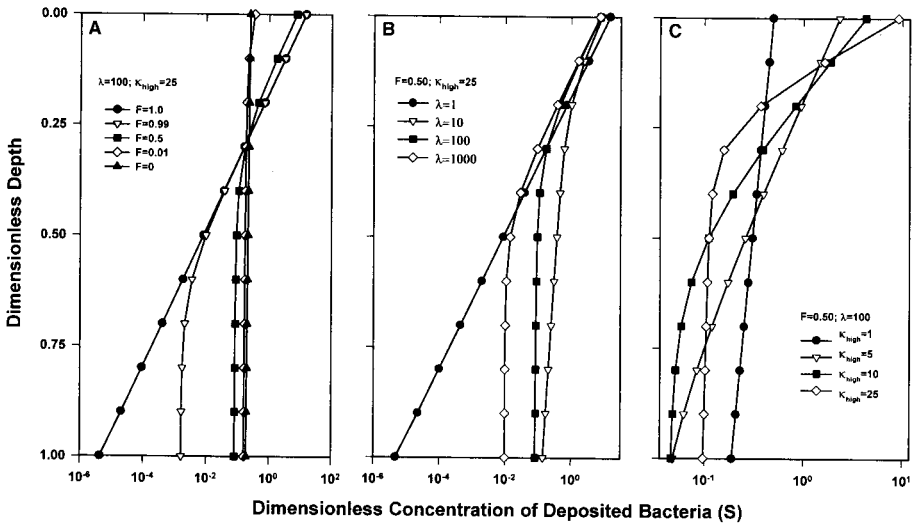

Figure 4. Effect of (a) fraction of bacteria with a high sticking efficiency (F), (b) sticking efficiency ratio (λ), and (c) deposition coefficient of sticky bacteria (κ_{high}) on the decrease of deposited bacteria with flow path length. In contrast to colloid-filtration theory, the presence of a dual-alpha population of bacteria may yield nonexponential decreases of deposited bacteria with depth.

ited. Instead, they travel farther through the column until they reach more reactive sediments. The result is a uniform concentration of deposited bacteria proximal to the inlet. This behavior is expected to become more pronounced as the total bacterial loading to the sediments increases and is in agreement with our findings.

Finite bacterial retention capacity of sediments has been inferred from the observed behavior of BTCs and has been modeled both phenomenologically [*Saiers et al.*, 1994; *Tan et al.*, 1994] and mechanistically [*Rijnaarts et al.*, 1996]. The latter study invoked a cell-blocking mechanism, where a deposited particle is believed to reduce deposition by blocking a part of the collector surface. If the BET-determined surface area of the sediments was similar among the columns, then the maximum (based on fitted values of X_{max}) surface coverage was 0.15 and 1.1% for the 43- and 45-cm columns, respectively, assuming full contact of the bacteria with the sand. Because bacteria are much larger than N_2 molecules, it is likely that the BET method may overestimate the surface area available for bacterial deposition if a significant fraction of the measured surface area is due to intraparticle porosity. Fractional surface coverage based on mean grain size is 27 and 200% for the 43- and 45-cm columns, respectively. The actual surface coverage is likely somewhere in between these two extremes. A finite retention capacity of the sediments for bacteria may also have the effect of decreasing the variability in deposited bacteria if there is less variability in carrying capacity (X_{max}) than in κ.

Minimal decreases in concentrations of deposited bacteria with depth also were observed farther away from the inlet for all but the 15-cm column. Nonexponential decreases in deposited bacteria with flow path length may be caused by physical or geochemical heterogeneities in the direction parallel to flow or by the presence of biological heterogeneities, as manifested by a distribution of sticking efficiencies within a single strain of bacteria. A distribution of sticking efficiencies has been observed for a number of organisms [*Albinger et al.*, 1994; *Baygents et al.*, 1998; *Martin et al.*, 1996; *Simoni et al.*, 1998; *Williams and Fletcher*, 1996]. *Albinger et al.* [1994] observed a 10-fold decrease in sticking efficiency with depth in a 1-cm sectioned core and concluded that a fraction of cells, even from a monoclonal culture, have surface characteristics more favorable for transport than the remaining cells. *Baygents et al.* [1998] found that a single value for α was insufficient to describe adequately the measured distribution of deposited bacteria in short columns (1 cm). A model invoking a dual-alpha distribution of sticking efficiencies (where it is assumed that the influent population of bacteria is composed of two subpopulations, each with distinct values of α) was found to be far superior in fitting the data. *Simoni et al.* [1998] also utilized a dual-alpha model when analyzing breakthrough data from columns varying in length from 0.3 to 6.4 cm. In both studies, it was observed that a small fraction (0.02–0.36) of the monoclonal culture had sticking efficiencies ranging from 83 to 348 times greater than the remaining population. We found that a small fraction of the monoclonal culture had sticking efficiencies from 20 to ~3400 times less than the remaining population. In conjunction with bacterial growth, the presence of a small fraction of a bacterial population with a very low sticking efficiency may explain why significant numbers of bacteria have been observed to be transported over great distances in the field [*Gerba and Bitton*, 1984]. The presence of biological variability suggests scaling of results from experiments conducted over short lengths may not be appropriate for predicting large-scale transport of bacteria, even for physically homogeneous porous media. Water protection agencies must therefore account for this enhanced transport due to distributed bacterial surface characteristics when determining setback distances for wellhead protection.

Although several researchers have observed sticking efficiencies to decrease with flow path length [*Baygents et al.*, 1998; *Martin et al.*, 1996; *Simoni et al.*, 1998], the mechanism for this behavior is still unknown. *Baygents et al.* [1998] observed dual peaks in surface charge density for the organisms used in their study. Since surface charge density has been shown to be a useful indicator of bacterial adhesion for hydrophilic organisms, the authors speculate that this may be the source of the

distribution. This explanation, however, is not conclusive since the fraction of bacteria with high- and low-sticking efficiencies and the corresponding peak areas in the electropherograms were not consistent for one of the two organisms studied. The authors, however, postulate that this inconsistency may be simply due to detection limitations of the method employed. Capillary electrophoresis measurements on the organism used in this study has shown that W31 does not exhibit surface charge variations [*Glynn et al.*, 1998] and therefore is unlikely to be the source of our observed dual-alpha population. *Simoni et al.* [1998] measured cell size, electrophoretic mobility, and hydrophobicity on bacterial suspensions before and after passage through columns and found no significant differences in these measurements indicating that these parameters were not responsible for the distribution found in their work. They also concluded that the fractionation of cells was not a result of differences in genotypes.

While biological explanations have been invoked to describe the apparent decrease in sticking efficiency with flow path length, physical mechanisms cannot yet be ruled out. For instance, the presence of cell clumping in the influent suspension would result in a distribution of the single-collector efficiency. This in turn could lead to results similar to a distribution of sticking efficiencies (Equation (3)). Also, *Camesano and Logan* [1998] observed an increase in variability in sticking efficiency with increasing bacterial loading to the sediments, suggesting filter ripening may be the source of the appearance of a distribution of sticking efficiencies in their study. The large range in sticking efficiency ratio (20 to ~3400) observed in this study suggests that this behavior may be due, at least in part, to physical causes.

It is unclear why the effects of an influent suspension containing two subpopulations of bacteria were not observed in the 15-cm column. One explanation may be that the presence of a dual-alpha population could not be detected in this column. Numerical simulations (data not shown) indicate that in some cases (for example, where a very small fraction of bacteria are described by low rates of deposition), the concentration of deposited bacteria for a dual-alpha population can be described satisfactorily with a single-alpha model. Increasing the column length would result in increased deposition coefficients and may reveal the presence of a dual-alpha population of bacteria.

The presence of a dual-alpha population of bacteria results in nonexponential decreases in deposited bacteria with flow path length for high rates of deposition. Because the spatial distribution of attached bacteria is often needed when designing bioremediation schemes, inferences of the spatial distribution of bacteria based on the assumption of a single value for the sticking efficiency are likely to be erroneous for influent suspensions with a distribution of sticking efficiencies. If bacterial deposition is greater at the inlet than expected, bioplugging of the injection well may occur sooner than expected, provided second-order effects are negligible. If a minimum concentration of deposited bacteria is needed to fulfill desired biodegradation requirements, then the efficient placement of injection wells will likely not be achieved if the effects of biological heterogeneities on bacterial deposition are ignored. Indeed, these results argue strongly for a priori knowledge of the presence of any biological heterogeneities of the influent suspension. Column dissections are a good method for obtaining this information.

Ultimately, limitations to colloid-filtration theory may exist which make it difficult to use these equations in describing bacterial deposition within heterogeneous porous media. Colloid-filtration theory grew out of the need to understand the operation of wastewater treatment processes. As a result, the constitutive equations were derived based on a uniform collector size. Aquifer sediments, however, consist of a distribution of grain sizes. *Martin et al.* [1996] found that d_{10} obtained from the volume size distribution was the most appropriate grain diameter to use in colloid-filtration theory for heterogeneous porous media due to the dominance of the smaller sand grains in controlling the number of collisions between the bacteria and sand grains. It is unclear, however, if this relationship is valid for more heterogeneous sediments as observed in this study. Further work is needed to develop a mechanistic model for describing bacterial deposition in heterogeneous porous media.

Through the use of column dissections, we were able to show that bacterial deposition at the centimeter scale is nonuniform within intact aquifer sediments. Our findings demonstrate that the assumption of spatially invariant deposition rates is invalid for field situations. Although our observations are at the centimeter scale and therefore not directly applicable to entire contaminant plumes, it is reasonable to expect that heterogeneities at the scale of a meter to tens of meters are, at a minimum, the same order of magnitude as observed within our columns. The use of a single deposition coefficient in modeling the transport behavior of bacteria through aquifer sediments is problematic in that the inferred and actual spatial distribution of deposited bacteria may differ significantly. Because the spatial distribution of bacteria will affect the biodegradation of contaminants, these findings indicate that variability in deposition rates, as well as the presence of biological heterogeneities, should be incorporated into numerical models for predicting contaminant biodegradation.

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