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Effect of surface coatings, grain size, and ionic strength on the maximum attainable coverage of bacteria on sand surfaces

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Abstract

The injection of bacteria in the subsurface has been identified as a potential method for in situ cleanup of contaminated aquifers. For high bacterial loadings, the presence of previously deposited bacteria can result in decreased deposition rates—a phenomenon known as blocking. Miscible displacement experiments were performed on short sand columns (~ 5 cm) to determine how bacterial deposition on positively charged metal-oxyhydroxide-coated sands is affected by the presence of previously deposited bacteria. Approximately 8 pore volumes of a radiolabeled bacterial suspension at a concentration of $\sim 1 \times 10^9$ cells ml^{-1} were introduced into the columns followed by a 2-pore-volume flush of cell-free buffer. It was found that the presence of Al- and Fe-coated sand increased both deposition rates and maximum fractional surface coverage of bacteria on the sediment surfaces. The effect of grain size on maximum bacterial retention capacity, however, was not significant. Decreasing ionic strength from 10^{-1} to 10^{-2} M KCl resulted in noticeable decreases in sticking efficiency (α) and maximum surface coverage (θ_{max}) for clean silica sand—results consistent with DLVO theory. In columns containing positively charged Al- and Fe-coated sands, however, changes in α and θ_{max} due to decreasing ionic strength were minimal. These findings demonstrate the importance of geochemical controls on the maximum bacterial retention capacity of sands.

Keywords: Bacteria; Transport; Bioremediation; Metal hydroxides

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1. Introduction

The movement of bacteria in the subsurface is being investigated for several reasons, including the use of bacteria with novel degradation capabilities for the bioremediation of polluted aquifers. For some applications, high concentrations or large volumes of bacterial suspensions may be introduced into the subsurface. Large bacterial loadings can lead to high coverage of sediment surfaces resulting in a decrease in the deposition of bacteria—a phenomenon known as blocking (Privman et al., 1991). Time-dependent decreases in bacterial deposition rates can result in limiting the concentration of sediment-associated bacteria that can be attained for bioremediation purposes.

Because bacteria are colloidal in size, colloid-filtration theory (Yao et al., 1971)—the advection–dispersion equation with first-order deposition—is often used for describing their transport. In the original derivation of colloid-filtration theory (Rajagopalan and Tien, 1976; Yao et al., 1971), an assumption of a clean-bed was invoked; that is, the fraction of the collector surface covered by particles was assumed to be so low that it could not affect further deposition rates. It has been shown, however, that deposition rates often change with time indicating that the presence of previously deposited particles can indeed affect deposition rates. Although previously deposited particles have been observed in a few cases to increase deposition rates (a process referred to as filter ripening), more common is the observation that attached particles reduce deposition rates. To account for this observed behavior, the colloid-filtration model has been modified to account for decreasing deposition rates due to previously attached particles. In many instances, a Langmuir-type model is used in which deposition rates are modeled as decreasing linearly with the concentration of deposited particles, assuming monolayer coverage of particles on the collector surface. In this approach to modeling particle transport and attachment, a new parameter is introduced that quantifies the maximum available surface coverage of particles on the collectors. This modeling approach has been used successfully to describe the transport of both colloids (Johnson et al., 1996; Saiers et al., 1994; Song and Elimelech, 1993; Song et al., 1994) and bacteria (Bolster et al., 1999; Camesano et al., 1999; Deshpande and Shonnard, 1999; Lindqvist et al., 1994; Rijnaarts et al., 1996a,b; Saiers and Hornberger, 1996; Tan et al., 1994) when particle loadings are substantial enough to invalidate the clean-bed assumption.

While several studies have demonstrated the applicability of this approach in modeling bacterial transport and deposition, few systematic studies exist which address the factors controlling the effects of previously deposited bacteria on bacterial deposition rates. Tan et al. (1994) observed that increases in cell concentration and ionic strength increased bacterial retention capacity of aquifer sands. Rijnaarts et al. (1996a,b) determined that ionic strength, cell size, collector hydrophobicity, and bacterial surface coatings all affect the maximum bacterial surface coverage on the collectors. In each of the aforementioned studies, however, the bacteria and collectors were similarly charged.

The presence of metal-oxyhydroxide coatings on aquifer sediments at circumneutral pH can result in positively charged sediment surfaces (Hendershot and Lavkulich, 1983). Because bacteria are negatively charged at circumneutral pH, the presence of these coatings can result in increased deposition rates (Johnson and Logan, 1996; Knapp et al., 1998; Scholl and Harvey, 1992; Scholl et al., 1990). Whereas increases in sticking

efficiency due to the presence of positively charged Fe-oxyhydroxide coatings are well established in the literature, the effect of positively charged metal-oxyhydroxide coatings on bacterial retention capacity has not yet been explored.

In this paper, we evaluate the effects of surface coatings, grain size, and ionic strength on bacterial deposition and retention capacity. In particular, we address the following questions: (1) Does the presence of positively charged metal-oxyhydroxide coatings affect bacterial retention capacity? (2) Is the maximum available surface coverage dependent on grain size? (3) Does ionic strength affect deposition rates and retention capacity on metal-coated sands? We observed that the presence of positively charged metal-oxyhydroxide coatings increased the maximum amount of bacteria that can be deposited on a sand grain and that decreasing ionic strength had minimal effects on bacterial deposition rates and retention capacities on metal-coated sand grains. Our findings demonstrate the importance of geochemical controls on the maximum bacterial retention capacity of sands.

2. Methods

2.1. Sand preparation

Unimin™ quartz sand was sieved into two discrete size fractions, 0.42 to 0.50 mm (fine) and 0.707 to 0.850 mm (coarse). The sieved sand was then washed for 2 h in 10 M HCl and rinsed in distilled water (DW) until the rinse water became clear. The sand was then placed in a solution of 10% NaOH followed by rinsing in DW. The sand was then baked at 105°C for 24 h. Sand was coated with metal-oxides by a method similar to that employed by Mills et al. (1994). Briefly, sand was placed in a 1 M solution of $FeCl_3$ or $AlCl_3$. The pH was adjusted to > 9 by addition of 10 M NaOH. Sand was allowed to equilibrate for several days and pH was checked each day. In some instances, additional NaOH was added to the solution to maintain a pH of > 9. The sand was then rinsed in DW until the rinse water was clear and then baked at 105°C for 24 h. The sand was then rinsed again and baked for another 24 h at 105°C. Prior to packing columns, the dry sand was autoclaved at 121°C and 103.4 kPa for 20 min.

2.2. Column experiments

Columns were constructed by placing a 150-μm pore diameter fluorocarbon filter into a 60-cm^3 plastic syringe. CO_2 gas was introduced into the column prior to filling with a KCl solution. Columns were not packed until all observable gas bubbles in the filter had dissolved into the solution. The headspace of the columns was then flushed with CO_2 gas and sand was then slowly poured into 15 ml of the solution. The columns were periodically tapped and the sand stirred to release any entrapped gas in the columns. Columns were operated at a flow rate of ~ 3 m day^{-1}. The velocity was determined from measured values of flow rate and porosity. Measured porosity values ranged from 0.32 to 0.35. Column lengths ranged from 4.6 to 5.0 cm. Approximately 20 pore volumes of solution were eluted through the columns prior to any experiments to ensure equilibration of pH. Composition of the aqueous solution was 10^{-1} M KCl for

the high ionic strength experiments and 10^{-2} M KCl for the lower ionic strength experiments.

2.3. Preparation of bacteria

Two organisms were used in this study, W31 and E3W7. Both organisms were isolated from an unconfined aquifer located in Oyster, VA. W31 is a nonmotile, gram-negative coccus with a mean diameter of 0.95 μm and calculated projected surface area (i.e. equivalent two-dimensional surface area) of 7×10^{-9} cm^2. E3W7 is a nonmotile gram-negative rod with average dimensions of 1 μm × 2 μm and a calculated projected surface area of 1.8×10^{-8} cm^2. Electrophoretic mobility (-1.023 and -1.022 μm cm/V s for W31 and E3W7, respectively) and contact angle measurements (34° and 27° for W31 and E3W7, respectively) in 10^{-1} M KCl showed that both organisms have similar surface characteristics (Kerian Bicknell, personal communication).

Bacteria were grown in R2A broth by inoculating from a frozen culture and incubating for 36 h at 25°C, after which the culture was spiked with ^{14}C-amended glucose (~ 0.05 μCi/10^9 cells). After an additional 24 h of incubation, the bacterial culture was washed three times by centrifugation at $16{,}000 \times g$ and suspended in sterile KCl solution for a minimum of 24 h. Prior to the experiments, the bacterial suspensions were washed once more to remove any released label. Less than 1% of the label was found to pass a 0.2-μm filter; this value remained constant throughout the experiments. Bacterial suspensions were filtered through a 5.0-μm filter immediately prior to column experiments to remove any large aggregates. Initial concentrations of bacteria ($\sim 1 \times 10^9$ cells ml^{-1}) were determined by acridine orange direct counts. Effluent samples were collected with a fraction collector and counted by liquid scintillation counting.

2.4. Development of transport model

The one-dimensional transport and attachment of bacteria in laboratory columns can be described by (Rijnaarts et al., 1996a)

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

where θ is the fraction of the collector surface covered by deposited bacteria (total surface area of deposited cells/total surface area of sediment surface), a_b is the surface area of the bacteria (cm^2 cell^{-1}), c is the aqueous concentration of bacteria (cells ml^{-1}), x is the distance from the column inlet (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}), and f is the ratio of the total surface area of spherical collectors to the volume of pore fluid (L^{-1}) and is calculated by (Privman et al., 1991)

$$f = \frac{6(1-n)}{n d_c} \tag{2}$$

where n is porosity and d_c is the collector diameter.

The first term on the left-hand side of Eq. (1) represents the time rate of change of the aqueous concentration of bacteria and the second term on the left-hand side represents the time rate of change of the fractional surface coverage of the deposited bacteria. For high bacterial loadings, where the assumption of clean-bed conditions is not valid, the time rate of change of fractional surface coverage can be described by assuming that the rate of deposition is controlled both by the aqueous concentration as well as the concentration of deposited bacteria. In this study, we utilized a Langmuir-type blocking model where deposition is assumed to decrease linearly with the concentration of deposited bacteria (Privman et al., 1991)

$$\frac{\partial\theta}{\partial t} = \frac{q}{4} a_b \eta \alpha B(\theta) c \tag{3}$$

$$B(\theta) = \frac{\theta_{max} - \theta}{\theta_{max}} \tag{4}$$

$$\beta = \frac{1}{\theta_{max}} \tag{5}$$

where $B(\theta)$ is the blocking function, θ_{max} is the maximum attainable fractional surface coverage, β is the excluded area parameter and represents the normalized area blocked by a single cell (area blocked by cell/area of cell), q is specific discharge (L T^{-1}), 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

$$\eta = 4A_s^{1/3} N_{Pe}^{-2/3} + A_s N_{Lo}^{1/8} N_R^{15/8} + 0.00338 A_s N_G^{1.2} N_R^{-0.4} \tag{6}$$

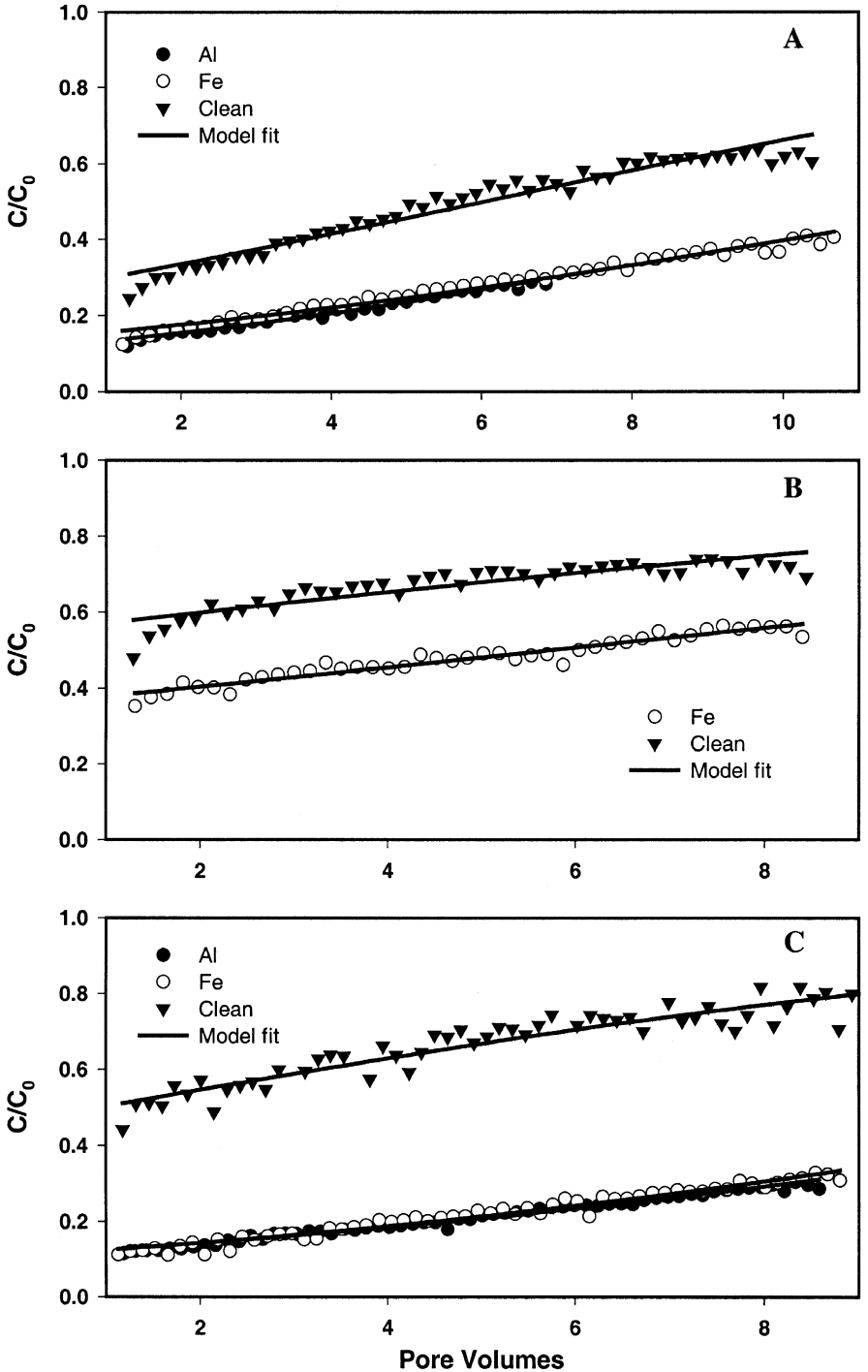
where $A_s = 2(1-\gamma^5)/(2-3\gamma+3\gamma^5-2\gamma^6)$, $\gamma = (1-n)^{1/3}$, $N_{Pe} = 3\pi\mu d_p d_c q/(kT)$, $N_{Lo} = 4H/(9\pi\mu d_p^2 q)$, $N_R = d_p/d_c$, $N_G = g(\rho_b - \rho_f)d_p^2/(18\mu q)$, d_p is the diameter of the bacteria, d_c is the diameter of the collector (sand grains), μ is the fluid viscosity (0.93×10^{-3} Pa s), q is the specific discharge, n is the porosity, k is the Boltzman constant (1.38048×10^{-23} J K^{-1}), T is the temperature (25 K), H is the Hamaker constant (1×10^{-20} J), ρ_f is the fluid density (998 kg m^{-3}), ρ_b is the bacterial density (1080 kg m^{-3}), and g is the gravitational acceleration.

By neglecting dispersion and transforming Eqs. (1), (3), and (4) to breakthrough coordinates, an analytical solution for these equations is given by (Kallay et al., 1987; Privman et al., 1991)

$$\frac{c}{c_0}(X,\tau) = \frac{\exp\left(\frac{q}{4}\eta\alpha a_b \beta c_0 \tau\right)}{\exp\left(\frac{q}{4}\eta\alpha a_b \beta c_0 \tau\right) + \exp\left(\frac{q}{4}\eta\alpha f X\right) - 1} \tag{7}$$

$$X = \frac{x}{v} \tag{8}$$

$$\tau = t - \frac{x}{v} \tag{9}$$


A
Al
Fe
Clean
Model fit
C/C0
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Clean
Model fit
C
Al
Fe
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Model fit
Pore Volumes

where c_0 is the aqueous concentration of bacteria at the column inlet (cells ml^{-1}). The initial and boundary conditions are

$$c(X,0) = 0 \tag{10a}$$

$$c(0,\tau > 0) = c_0 \tag{10b}$$

$$\theta(X,0) = 0 \tag{10c}$$

$$\theta(X,\tau \to \infty) = \theta_{max} \tag{10d}$$

Values of α and β were obtained by fitting Eq. (7) to the observed concentrations of aqueous bacteria with dimensionless time (τ) using the Levenburg–Marquardt method. Results are presented as aqueous concentrations of bacteria vs. pore volumes (vt/L). Goodness of fit was determined by calculating the model efficiency (E) (Hornberger et al., 1992)

$$E = 1 - \frac{\sum_{i=1}^{n} (r_i)^2}{\sum_{i=1}^{n} (c_i - c_{avg})^2} \tag{11}$$

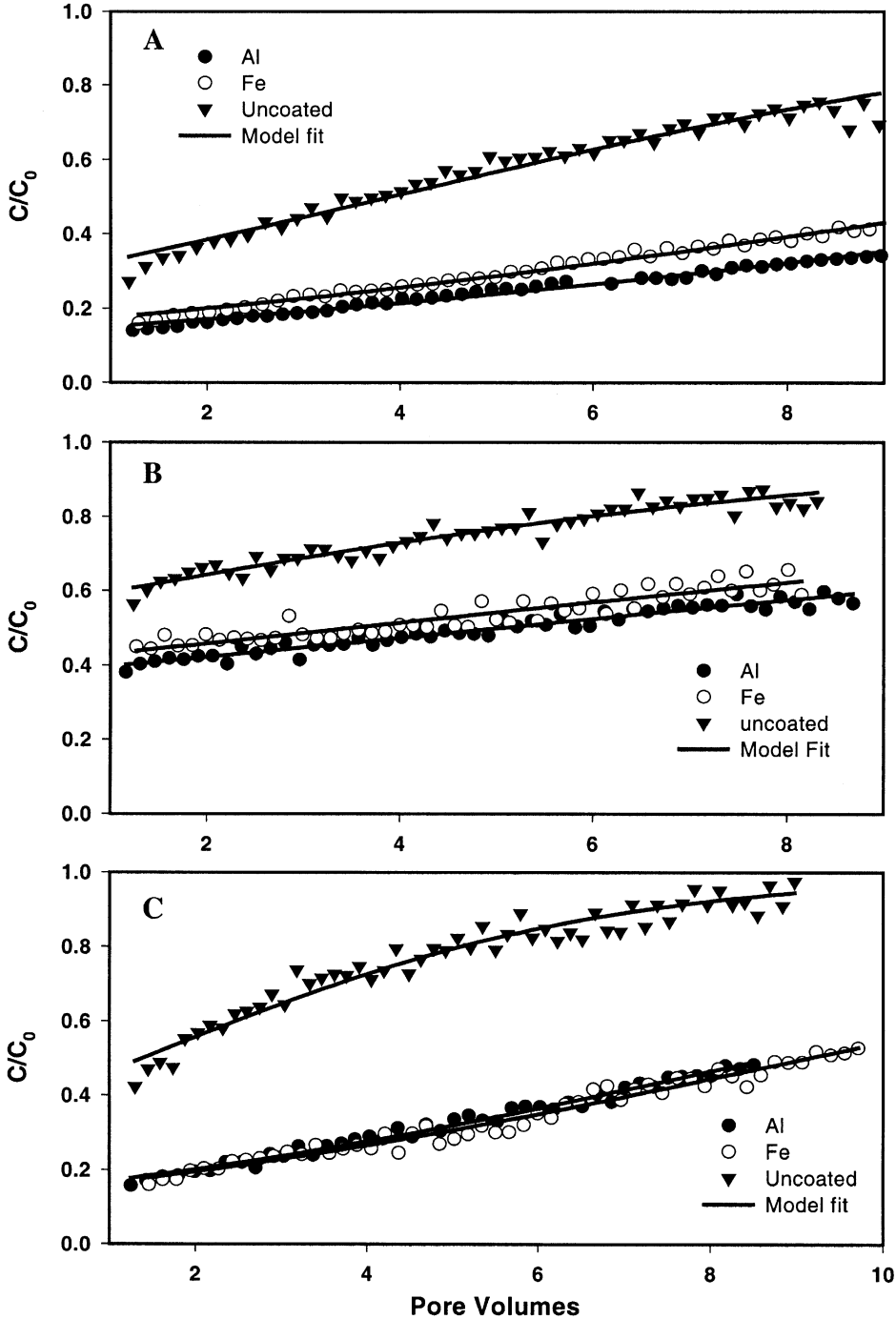
where r_i is the ith residual between model prediction and observation, c_i is the ith measured concentration of effluent bacteria, and c_{avg} is the average of the observed effluent concentrations.

Two potential limitations are inherent in the solution of Kallay et al (1987); the first being the omission of the dispersion term and the second being that Eq. (10b) does not describe accurately the actual boundary condition during the experiments in that it neglects the first pore volume of the tracer. These limitations, however, have been shown to be inconsequential for the breakthrough concentrations observed in this study (Bolster, 2000).

2.5. Uncertainty analysis

Uncertainties in fitted parameter values are often evaluated through the use of confidence intervals; however, this method is strictly valid for only one fitting parameter. When more than one parameter is fit to the data, joint confidence regions are appropriate for reflecting parameter uncertainties because this method reflects the joint variability between the parameters, therefore, we evaluated the uncertainties in our fitted values of α and β by calculating the 95% joint confidence regions. These joint

Fig. 1. Observed data (symbols) and model fits (lines) for breakthrough of W31 in columns packed with Al-coated, Fe-coated, and clean Unimin™ sand. Experimental conditions are (A) small grain size (0.42–0.50 mm) and high ionic strength (10^{-1} M KCl), (B) large grain size (0.707–0.850 mm) and high ionic strength (10^{-1} M KCl), and (C) small grain size (0.42–0.50 mm) and low ionic strength (10^{-2} M KCl). Differences in bacterial breakthrough between experiments with coated and uncoated sands become more pronounced when ionic strength is lowered from 10^{-1} M to 10^{-2} M.


A
Al
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Uncoated
Model fit
B
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Model Fit
C
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Uncoated
Model fit
C/C0
Pore Volumes

Table 1
Fitted values of α and β

Grain size	Ionic strength	Coating	W31				E3W7			
			α	β	θ_{max}	E	α	β	θ_{max}	E
Small	High	Al[a]	0.62	3.9	0.26	0.98	0.48	1.1	0.91	0.98
		Fe[a]	0.57	3.5	0.29	0.98	0.49	1.4	0.71	0.98
		Clean[a]	0.36	6.4	0.16	0.95	0.31	3.4	0.29	0.97
Large	High	Al[a]	ND	ND	ND	ND	0.60	0.88	1.1	0.94
		Fe[a]	0.68	2.8	0.36	0.93	0.54	1.1	0.91	0.86
		Clean[a]	0.45	4.9	0.20	0.78	0.34	3.2	0.31	0.93
Small	Low	Al[b]	0.50	3.1	0.32	0.98	0.52	1.1	0.91	0.98
		Fe[b]	0.53	3.2	0.31	0.96	0.48	1.2	0.81	0.97
		Clean[b]	0.18	11.0	0.091	0.87	0.22	5.0	0.20	0.95

[a,b]Treatments with the same letter denote experiments that were performed on the same day with the same influent suspension for each organism.

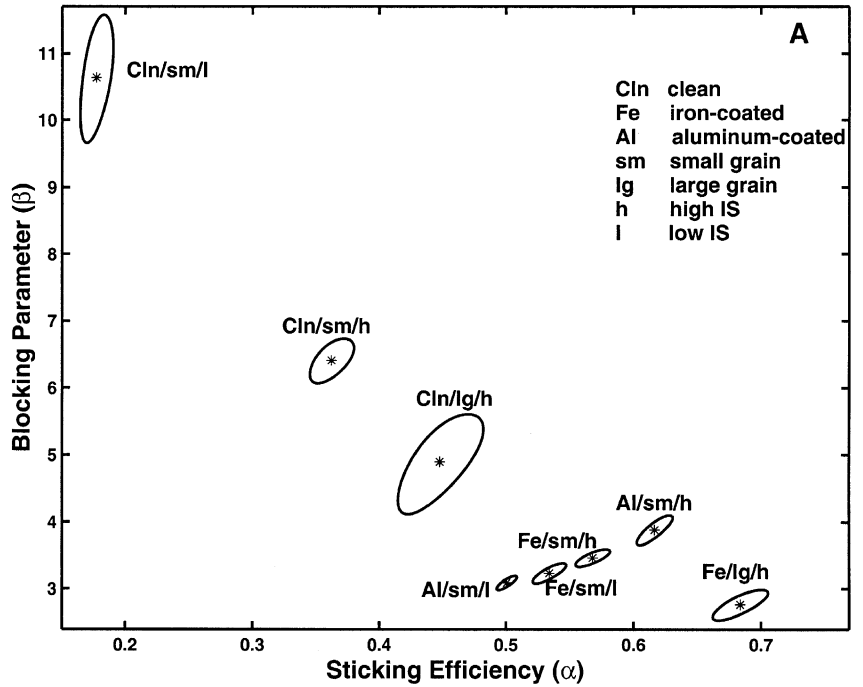
confidence regions are concentric ellipses centered on the best-fit parameter values and contain within them the true values of the fitted parameters within a 95% level of confidence. The shape of the ellipse (confidence region) is a measure of the uncertainty in the parameter values while the orientation is a representation of the correlation between the two parameters. When two parameters are correlated the axes of the ellipse are obliquely oriented with respect to the x- and y-axes. When two parameters are uncorrelated then the axes of the ellipse are parallel to the x- and y-axes.

Joint confidence regions can be evaluated by either exact or approximate methods (Ratkowsky, 1990). Because exact methods are more complicated and more difficult to solve, approximate methods are often used. Two approximate methods are available, the likelihood and linearized methods. Because of the nonlinearity in our governing equations, we chose the more robust likelihood-approximate method. The 95% likelihood confidence regions are calculated by finding values of α and β which satisfy (Draper and Smith, 1981)

$$\mathrm{SSQ}(\alpha,\beta) = \mathrm{SSQ}\left(\hat{\alpha},\hat{\beta}\right)\left[1 + \frac{p}{N-p}F(1,N-p,0.95)\right] \tag{12}$$

where N is the number of data points, p is the number of fitting parameters, $F(1, N-p, 0.95)$ is the tabulated value of an F-distribution with p degrees of freedom in the numerator, $N-p$ degrees in the denominator, and a specified significance level of 0.95,

Fig. 2. Observed data (symbols) and model fits (lines) for breakthrough of E3W7 in columns packed with Al-coated, Fe-coated, and clean Unimin™ sand. Experimental conditions are (A) small grain size (0.42–0.50 mm) and high ionic strength (10^{-1} M KCl), (B) large grain size (0.707–0.850 mm) and high ionic strength (10^{-1} M KCl), and (C) small grain size (0.42–0.50 mm) and low ionic strength (10^{-2} M KCl). Differences in bacterial breakthrough between experiments with coated and uncoated sands become more pronounced when ionic strength is lowered from 10^{-1} M to 10^{-2} M.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Fe iron-coated
Al aluminum-coated
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Al/sm/h
Fe/sm/h
Al/sm/l
Fe/sm/l
Fe/lg/h
Blocking Parameter (β)
Sticking Efficiency (α)
11
10
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8
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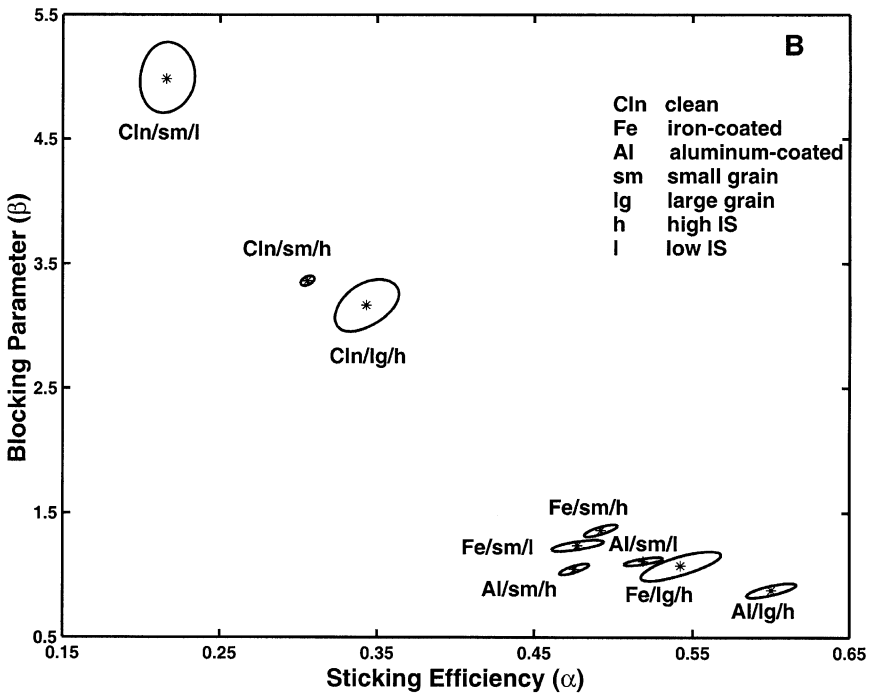
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Fe iron-coated
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Fe/sm/h
Fe/sm/l
Al/sm/l
Al/sm/h
Fe/lg/h
Al/lg/h
Blocking Parameter (β)
Sticking Efficiency (α)
5.5
4.5
3.5
2.5
1.5
0.5
0.15
0.25
0.35
0.45
0.55
0.65

Table 2
Percent change in sticking efficiency, α, and the blocking parameter, β, due to presence of metal-oxyhydroxide coatings

Grain size	Ionic strength	Coating	W31[a]		E3W7	
			α	β	α	β
Small	High	Al	72	−39	55	−68
		Fe	58	−46	58	−59
		Clean	–	–	–	–
Large	High	Al	ND	ND	76	−73
		Fe	51	−43	59	−66
		Clean	–	–	–	–
Small	Low	Al	178	−72	136	−78
		Fe	194	−71	118	−75
		Clean	–	–	–	–

[a]ND, not determined.

$\hat{\alpha}$ and $\hat{\beta}$ are the best-fit parameters, and SSQ($\hat{\alpha}$, $\hat{\beta}$) represents the minimum sum of squares corresponding to the least-squares parameter estimate calculated by

$$\mathrm{SSQ}\left(\hat{\alpha},\hat{\beta}\right)=\sum_{i=1}^{N}\left(c_{\mathrm{obs}_i}-c_{\mathrm{pred}_i}\right)^2 \tag{13}$$

where c_{obs} is the observed breakthrough concentration and c_{pred} is the predicted breakthrough concentration based on the best-fit estimates of α and β. For simplicity, we chose to assume that model errors were significantly greater than measurement errors.

2.6. Numerical simulations

The importance of decreasing deposition rates with time was investigated by comparing the results of two numerical simulations: in the first simulation no interaction of suspended with deposited bacteria was allowed (i.e. classic colloid-filtration theory), in the second simulation deposition rates were allowed to decrease linearly with the concentration of previously deposited bacteria (i.e. blocking model). For simulating fractional surface coverages within the columns, Eqs. (1), (3) and (4) were solved numerically by the predictor–corrector finite-difference method. Details of the model and boundary conditions are presented in Bolster et al. (1999). Parameters used in the simulations were similar to those found in this study ($\alpha=0.4$, $\eta=0.025$, $\theta_{\max}=0.28$, $a_{\mathrm{b}}=2\times10^{-8}$ cm^2 cell^{-1}). For these simulations, a 10-pore-volume (vt/L) pulse was

Fig. 3. Best-fit values of α and β (symbol) and approximate joint 95% confidence regions for the various treatments as denoted by coating/grain size/ionic strength for organisms (A) W31 and (B) E3W7. Colinearity between α and β, as represented by confidence regions with axes obliquely oriented to the x- and y-axes, can be seen for some experiments. For the clean sand, a clear inverse relationship exists between α and β. In general, the uncertainty associated with the fitted parameters was greater for the clean-sand experiments than with the experiments using the metal-coated sand.

applied at an inlet concentration (c_0) of 2×10^8 cells ml^{-1}. In addition to fractional surface coverage, we also calculate the concentration of bacteria on the sediment phase (Bolster, 2000)

$$s = \frac{\theta}{a_b} \frac{nf}{\rho_b} \tag{14}$$

where s is the concentration of bacteria on the sand grains (cells gram^{-1} of sediment) and ρ_b is the bulk density (1.6 g cm^{-3}).

3. Results

Preliminary tests showed that differences between replicate columns were minimal (Bolster, 2000); therefore, single columns were used for each treatment. This reproducibility is consistent with what has been seen in our lab as well as what has been reported in the literature (Deshpande and Shonnard, 1999; Rijnaarts et al., 1996b). Also, tailing was observed to be minimal in our experiments as determined by negligible effluent concentrations of bacteria following the pulse of cell-free buffer. Therefore, remobilization of attached bacteria (entrainment) was deemed not responsible for the observed increases in aqueous concentrations of bacteria with time thus validating the omission of entrainment in our model.

Visual inspection of the model fits and values for the model efficiency (E) indicate that the Langmuir-type blocking model provided relatively good fits to the data for bacterial transport through both metal-coated and clean sands (Figs. 1 and 2; Table 1). For some treatments, however, there was a significant amount of unexplained variability. For example, in several experiments the model fits were higher than observed values at the beginning and ending of the breakthrough curve; this phenomenon was more pronounced in the clean-sand experiments and likely explains the greater uncertainty in the fitted parameter values in most of these experiments. Inspection of the residual plots for these experiments shows both curvature and heteroscedasticity (non-constant variance) (Bolster, 2000). In addition, in some instances part of the unexplained variability may be a result of sampling, rather than model, error (c.f. Fig. 2C, clean-sand treatment).

The presence of metal-oxyhydroxide coatings resulted in greater surface coverage (θ_{max}), and thus decreased blocking (β), and increased deposition rates (higher α) in all experiments (Table 1). Specifically, the presence of metal-oxyhydroxide coatings increased α by a minimum of 50% and decreased β by a minimum of 39% for all treatments (Table 2). For both organisms, the 95% confidence regions for α and β clearly do not overlap between the metal-coated and clean sands (Fig. 3A and B) for each treatment (grain size and ionic strength). Increasing grain size from 0.42–0.50 mm to 0.707–0.850 mm yielded smaller values of β (larger values of θ_{max}) for all five treatments (Table 3). Comparing the 95% confidence interval range, however, shows that the values for β for the grain-size experiments with E3W7 and clean sand do overlap (Fig. 3B). Increasing grain size also resulted in increases in α for both organisms. Increasing values for α with grain size has been observed for both bacteria

Table 3
Percent change in sticking efficiency, α, and the blocking parameter, β, due to changes in ionic strength and grain size

Coating	Percent change due to increasing grain size				Percent change due to decreasing ionic strength			
	W31		E3W7		W31		E3W7	
	α	β	α	β	α	β	α	β
Al	ND	ND	25	−20	−19	−21	8	0
Fe	19	−20	10	−21	−7	−9	−2	−14
Clean	25	−23	10	−6	−50	72	−29	47

(Hornberger et al., 1992) and colloids (Saiers et al., 1994) and is likely a result of limitations in colloid-filtration theory.

Decreasing ionic strength one order of magnitude resulted in noticeable decreases in α and increases in β for experiments with clean sand (Table 3). In contrast, decreasing

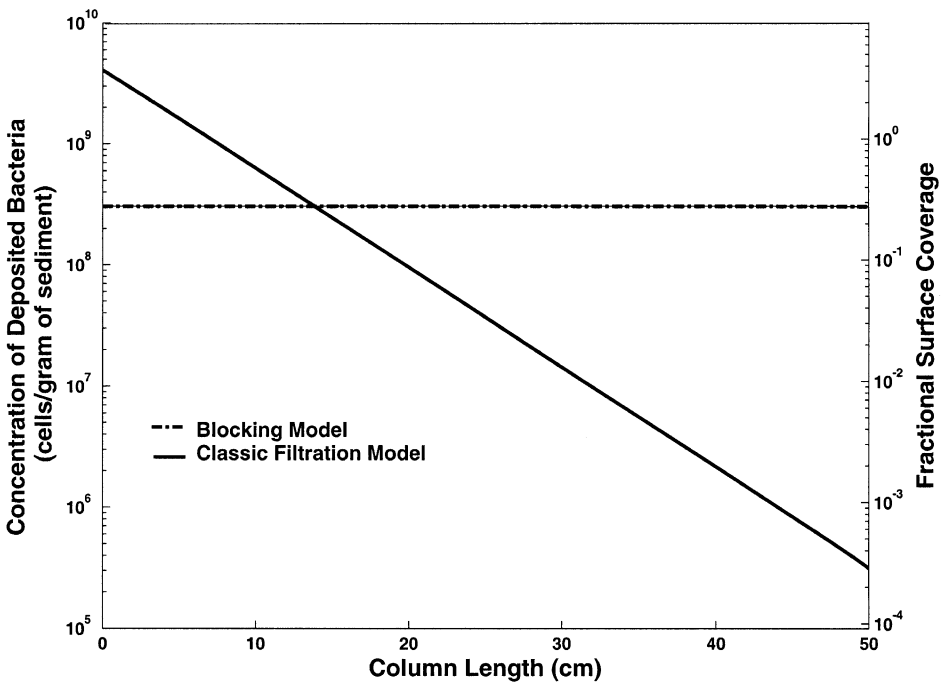

Fig. 4. Simulated distribution of fractional surface coverage and concentration of sediment-associated bacteria with flow-path length for a 50-cm column based on the blocking model and traditional colloid-filtration theory. Comparison between the two simulations reveals that when previously deposited bacteria decrease deposition rates, concentrations of deposited bacteria can remain relatively uniform with flow-path length. A more uniform distribution of bacteria in the subsurface will likely lead to more uniform biodegradation rates. In addition, the effect of blocking on the transport of bacteria can be demonstrated by the fact that the simulated fraction of influent bacteria recovered in the aqueous phase at 50 cm was 2.8×10^{-1} for the blocking model and 6.2×10^{-5} for the traditional filtration model.

ionic strength had minimal effects on α and β in experiments with metal-coated sands with the exception of W31 and Al-coated sand where α decreased by 19% and β decreased by 21%, however, the high ionic strength experiment was cut short due to equipment failure. In the other three experiments, the 95% confidence ranges in α and β either overlap or come quite close to each other (Fig. 3A and B). For all treatments, blocking of the larger E3W7 was considerably less than that of W31 (Table 1). In fact, values of β for E3W7 were between 50% and 75% less than those for W31 for all but one experiment.

Numerical simulations reveal the effect that decreasing deposition rates with time can have on the spatial distribution of sediment-associated bacteria (Fig. 4). When the fractional surface coverage (θ) approaches the maximum surface coverage (θ_{max}), the presence of previously deposited bacteria decreases deposition rates resulting in concentrations of deposited bacteria remaining relatively uniform with flow-path length. This is in direct contrast to classic colloid-filtration theory where concentrations of deposited bacteria decrease exponentially with depth.

4. Discussion

The results show that both surface coatings and ionic strength can affect the maximum bacterial retention capacity of sands. We observed that the presence of metal-oxyhydroxide coatings increased the maximum surface coverage of bacteria on sediments, thereby increasing the amount of bacteria capable of being removed from the aqueous phase. Rijnaarts et al. (1996b) observed that blocking on Teflon was more pronounced than blocking on glass, indicating that in addition to cell–cell interactions, cell–collector (hydrophobic) interactions were important in controlling the maximum surface area available for bacterial deposition. Our studies confirm this finding and indicate that surface coatings on the collectors can also affect the maximum surface coverage allowed.

A majority of studies looking at bacterial deposition and surface coatings have focused on Fe-coated sands. The presence of iron coatings has been shown to increase the attachment of bacteria to sediment surfaces in both column (Johnson and Logan, 1996; Knapp et al., 1998; Scholl and Harvey, 1992; Scholl et al., 1990) and batch studies (Mills et al., 1994) due to the charge reversal on the sediment surface by the Fe-oxyhydroxide coatings. Our finding that both sticking efficiency and maximum surface coverage are very similar between the Fe- and Al-coated sands suggests that the presence of any metal-oxyhydroxides may increase bacterial removal in aquifer sediments. Further studies should also look at other metal-oxyhydroxide coatings such as manganese.

Decreasing ionic strength was shown to decrease α and increase β in experiments using clean, negatively charged quartz sand (i.e. unfavorable deposition conditions). As ionic strength decreases, the thickness of the electrical double layer around the bacteria and collectors increases. According to DLVO theory, this increase in thickness of the double layer increases the amount of energy needed for the bacteria to overcome the potential energy barrier and enter into the secondary minimum where attachment can occur for like-charged surfaces. Similarly, increases in the electrical double layer will

also result in greater cell–cell repulsion. The observation that sticking efficiency decreased and blocking increased with decreasing ionic strength under unfavorable deposition conditions is consistent with DLVO theory and with what has been reported in the literature (Fontes et al., 1991; Deshpande and Shonnard, 1999; Rijnaarts et al., 1996b; Tan et al., 1994).

Decreasing ionic strength by an order of magnitude yielded minimal changes in α in three out of the four experiments with metal-coated sands indicating that no repulsive barrier exists between the positively charged sand grains and the negatively charged bacteria. In these same three experiments, we also observed minimal changes in blocking (β). Johnson et al. (1996) report that α values for silica colloids and iron-coated sands remained at $1 \pm 10\%$ for ionic strength changes from 10^{-5} to 10^{-2} M, results consistent with our findings. In contrast to our study, however, these same authors report decreases in β by a factor of ~ 2 for each order-of-magnitude increase in ionic strength. It is unclear what the source of the discrepancy between our findings and those of Johnson et al. (1996). One explanation may be the higher ionic strength carrier fluid used in our study.

Although the results show that both surface coatings and ionic strength affect the maximum bacterial retention capacity of sands, the effect of grain size on maximum bacterial retention capacity was not clear. Whereas the 95% confidence regions for β for four out of the five grain-size treatments do not overlap, because measurement error is not accounted for, it is unclear whether these observed decreases in β with increasing grain size are statistically significant. This is further highlighted by the fact that the ranges in β, while not overlapping, do in many cases come rather close to each other.

Based on our observations, it appears that the presence of positively charged metal-oxyhydroxide coatings would have a greater impact on deposition rates and maximum surface coverages in low ionic strength waters. In an earlier study (Bolster et al., 1999) using low ionic strength artificial ground water, we observed variations in concentrations of deposited bacteria in intact aquifer sediments of up to two orders of magnitude in the direction perpendicular to flow. We speculated that part of this variability was due to spatial heterogeneity in surface coatings. Our findings here that the effects of metal-coated sand on α and β are more pronounced at lower ionic strength waters support this conclusion. However, within contaminant plumes where high ionic strength water is likely, differences in sticking efficiency and maximum surface coverage between coated and uncoated sands will likely diminish. The presence of aqueous phase natural organic matter, also likely to be found within contaminant plumes, may also diminish the effect of metal-coated sands on bacterial deposition and retention capacity (Johnson and Logan, 1996).

In addition to solution chemistry, hydrodynamic factors have been shown to affect blocking. By solving an approximate solution to Stokes equation for two particles, Dabros (1989) observed that directly behind a deposited particle, a hydrodynamic shadow developed where deposition was prohibited. The size of the shadow was shown to increase with Peclet number

$$Pe = \frac{\xi r^3}{2D} \tag{15}$$

where ξ is the flow intensity parameter, r is the particle radius, and D is the particle diffusion coefficient. Both organisms used in this study had similar surface characteristics (e.g. surface charge and hydrophobicity) but blocking for the larger E3W7 was consistently 50–75% lower than for W31. Based on Eq. (15), however, we would expect that blocking would be greater for the larger E3W7 due to a larger hydrodynamic shadow (because E3W7 is not a sphere it is difficult to know what value to use for r and, hence, the magnitude of the increase in Pe). The fact that we observed greater blocking for W31 suggests that blocking may be a function of parameters not measured in our experiments. Simulations performed by Dabros (1989), however, were for clean-bed conditions—conditions clearly not representative of our work.

Based on geometric considerations, the maximum fractional surface coverage (θ_{max}) for non-overlapping spheres on a two-dimensional surface is 0.547 ($\beta = 1.83$) and is termed the jamming limit (Hinrichsen et al., 1986). Fitted values of θ_{max} for W31 were all below this jamming limit while fitted values of θ_{max} for E3W7 exceeded this theoretical limit in six of the nine experiments. Several reasonable explanations exist for this apparent discrepancy. Given the fact that the sand grains used in our studies were not perfect spheres, fitted values of θ_{max}—calculated by assuming spherical collectors—are higher than the actual surface coverage on the sand grains. Also, the numerical experiments performed by Hinrichsen et al. (1986) were for spherical particles, therefore, application of this value to rod-shaped bacteria is unwarranted. Finally, the presence of clumping in the influent suspension would allow for greater values of θ_{max} than the theoretical jamming limit. Although our bacterial suspensions were pre-filtered through a 5.0-μm filter, some clumping of cells may be expected at the ionic strengths used in our experiments.

Implicit in the calculated values of β in this study is the assumption of a homogeneous surface charge on the collectors. Surface charge heterogeneities on collector surfaces may be present owing to surface nonidealities (Song et al., 1994), lattice defects, surface-bound impurities introduced during sand preparation (Litton and Olson, 1993), and nonuniform flow around the surface of a spherical collector (Song and Elimelech, 1993). Surface charge heterogeneities may also be expected on the surfaces of metal-oxyhydroxide-coated sands. Hiemstra et al. (1989) report that several crystal faces of gibbsite ($AlOH_3$), hematite (Fe_2O_3), and goethite (FeOOH) do not develop surface charges over a relatively wide pH range. Based on these studies, as well as others, it has been widely accepted that collector surfaces are likely to be heterogeneously charged (Elimelech and O'Melia, 1990; Johnson et al., 1996; Song and Elimelech, 1994; Song et al., 1994). Surface charge heterogeneity results in nonuniform deposition rates and surface coverages on the collectors. As a result, blocking on more favorable sites may be less than the fitted value of β whereas blocking on less favorable deposition sites may be greater than the fitted value of β. In addition, it has been shown that within a bacterial population heterogeneity exists leading to a distribution of sticking efficiencies (Baygents et al., 1998; Bolster et al., 1999; Simoni et al., 1998). Because blocking is partly a function of bacterial surface characteristics, it may be feasible to expect changes in the maximum attainable fractional surface coverage (θ_{max}) with flow-path length due to the removal of the stickier bacteria near the injection point (Bolster et al., 2000).

Most colloidal studies looking at decreasing deposition rates with time have utilized successfully a Langmuir-type blocking model where deposition is assumed to be linearly dependent on surface coverage. Johnson and Elimelech (1995), however, argue that under favorable deposition conditions (i.e. oppositely charged particles and collectors) the Langmuir approach is inadequate to describe the processes involved and suggest the use of the more theoretically rigorous random sequential adsorption (RSA) model, a model specifically developed for colloids. These authors found that this non-linear model was superior to the Langmuir model for describing the attachment of positively charged colloids to negatively charged collectors. In another study with positively charged colloids and negatively charged collectors, however, the superiority of the RSA model was not clearly evident (Johnson et al., 1996). Based on visual inspection of the breakthrough curves, we found that the Langmuir-type blocking model could describe adequately the attachment of negatively charged bacteria to positively charged collectors under the conditions used in our study. Of course, a good fit to the data does not validate the assumptions behind the model.

5. Environmental significance

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 currently being investigated is the use of bio-curtains—the establishment of a region of sediment-associated bacteria capable of contaminant degradation (Duba et al., 1996; Dybas et al., 1998). Ideally, as contaminated water moves through the bio-curtain, metabolic processes result in the complete mineralization of the toxic compounds. To be effective, this method requires some minimum concentration of bacteria on the sediments and a continuous bio-curtain. Our results indicate that when large numbers of bacteria are introduced into the subsurface, sediment surfaces near the injection point may become "saturated" allowing for a more uniform distribution of sediment-associated bacteria with flow-path length thereby resulting in greater expansion of the bioactive zone (Fig. 4). This blocking phenomenon, however, may also limit the concentration of deposited bacteria available for metabolic transformation of contaminants and in turn limit biodegradation rates.

Conversely, high concentrations of introduced bacteria have also been shown to increase attachment rates (filter ripening). Although observed less frequently than blocking, filter ripening can ultimately result in permeability reductions due to microbial clogging of aquifer materials (Vandevivere et al., 1995). Deshpande and Shonnard (1999) observed filter ripening for influent concentrations of $\sim 3 \times 10^8$ cells ml^{-1} under conditions similar to those used in this study (ionic strength and grain size). Bolster et al. (1999), however, observed blocking rather than ripening for influent concentrations ranging from 10^8 to 10^9 cells ml^{-1} using intact sandy aquifer sediments indicating that blocking can be important in aquifer materials. Further research is needed to look at these two competing processes in aquifer sediments.

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