Effect of Intra-Population Variability on the Long-Distance Transport of Bacteria

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Abstract

Recent experimental work has suggested that within a single bacterial strain there may exist two distinct subpopulations, each with its own sticking efficiency. We performed a sensitivity analysis using an advection-dispersion model to elucidate the effects of an influent suspension of bacteria composed of two subpopulations, each with distinct sticking efficiencies (dual-alpha population), on the removal and transport of bacteria over distances of tens of meters. In the simulations, we assumed idealized conditions (i.e., one-dimensional transport through physically and geochemically homogeneous porous media). Results demonstrate that in cases where a small fraction of the influent bacteria have surface characteristics favorable for transport, the prediction of field-scale transport based on laboratory-derived parameters from short column experiments will tend to overestimate substantially the amount of bacterial removal. Results demonstrate the importance of a priori knowledge of the presence of intra-population variability when predicting the field-scale transport of bacteria.

Introduction

The transport of pathogenic viruses and bacteria in the subsurface poses a potential threat to public health as evidenced by reports of fecal contamination in a substantial number of tested ground water wells (Macler 1995). As required by the Safe Drinking Water Act, the U.S. Envioronmental Protection Agency (EPA) is developing the Groundwater Disinfection Rule (GWDR) to protect consumers from the contamination of ground water supplies by microorganisms (Macler 1995, 1996). One concern is the determination of adequate setback distances between sources of contaminants (i.e., septic tanks) and wellfields so that desired reductions in aqueous concentrations of pathogens are achieved. Currently, setback distances are determined by the states. Setback distances should be based on an understanding of the transport behavior of the pathogens, or indicator organisms, through the aquifer of interest. Due to difficulties in conducting field-scale experiments, prediction of microbial transport at the field scale often involves the extrapolation of laboratory-derived transport parameters to the field.

Difficulties in predicting bacterial transport through intact aquifer sediments from laboratory columns have been recognized and attributed mainly to the heterogeneous nature of aquifer properties, including variations in ground water velocity (Hendry et al. 1999), grain size (Fontes et al. 1991), and surface coatings (Mills et al. 1994). Fontes et al. (1991) showed that regions of high hydraulic conductivity could serve as conduits for enhanced bacterial transport due to increases in grain size and velocity. Camesano and Logan (1998) observed that the transport of nonmotile bacteria varied with velocity in accordance with colloid-filtration theory. Smith et al. (1985) observed greater transport of bacteria within intact cores than in repacked cores of the same material suggesting preferential flowpaths can enhance transport. In addition, because bacterial deposition depends on the surface coatings of the sediments (Knapp et al. 1998; Mills et al. 1994; Scholl and Harvey 1992; Scholl et al. 1990), spatial distributions of metal-oxide coatings may also contribute to the difficulty experienced in scaling laboratory results to field-scale settings.

In addition to the presence of physical and geochemical heterogeneities within aquifer sediments, the presence of biological heterogeneities, as manifested by a distribution of sticking efficiencies within a single strain of bacteria (where the sticking efficiency is a measure of the affinity of the bacteria to "stick" to the aquifer sediments), may also limit the ability to make predictions in the field. A distribution of sticking efficiencies has been observed for a number of organisms (Albinger et al. 1994; Baygents et al. 1998; Bolster et al. 1999b; Simoni et al. 1998). Several researchers have used a dual-alpha model (where it is assumed that the influent population of bacteria is composed of two subpopulations, each with distinct values of α) for describing both bacterial transport (Simoni et al. 1998) and deposition (Baygents et al. 1998; Bolster et al. 1999b). To recognize the presence of biological heterogeneity requires either the enumeration of sediment-associated bacteria with depth from column dissections (Baygents et al. 1998; Bolster et al. 1999b) or measuring the fractional recovery from several columns of different lengths (Simoni et al. 1998). Typically, however, observed bacterial breakthrough curves from columns of a set length are fitted with a transport model using a single deposition coefficient (e.g., Hornberger et al. 1992; Bolster et al. 1998;

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McCaulou et al. 1994; Martin et al. 1992). This approach essentially neglects the role biological heterogeneities play in the transport of bacteria. As a result, sticking efficiencies obtained from this method may not describe adequately the transport of bacteria over long distances.

We examined the role that biological heterogeneity plays in predicting and understanding bacterial transport over distances important in the field (tens of meters). Numerical simulations, assuming one-dimensional transport through physically and geochemically uniform porous media, were performed with a model that accounts for a dual-alpha population of bacteria. Results indicate that predictions based on assumptions of a uniform, or single-alpha, population of bacteria will tend to overpredict greatly the removal of bacterial cells from the aqueous phase. The results demonstrate the potential for transport of bacteria over tens to hundreds of meters in the subsurface and suggest that a priori knowledge of biological heterogeneity is required for predicting adequate setback distances.

Materials and Methods

Model Development

Neglecting the effects of entrainment and assuming low surface coverage, the transport of bacteria in one-dimensional flow can be described by incorporating colloid-filtration theory into the advection-dispersion equation (Bolster et al. 1998; Hornberger et al. 1992; Logan et al. 1995; Martin et al. 1992; McCaulou et al. 1995)

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - v\frac{\partial c}{\partial x} - k_c c \tag{1}$$

where c is the concentration of aqueous bacteria (cells mL^{-1}), D is the coefficient of hydrodynamic dispersion ($L^2\,T^{-1}$) where $D = \alpha_L v$, α_L is dispersivity (L), x is distance from point of injection (L), v is the pore water velocity ($L\,T^{-1}$), and k_c is the deposition coefficient (T^{-1}), which is defined as (Tien et al. 1979)

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

where θ is the porosity, d_c is the diameter of the sand grains, v is the average linear pore water velocity, α is the sticking efficiency, and η is the single-collector efficiency, which can be calculated using the model of Rajagopalan and Tien (1976), as modified by Logan et al. (1995). Some researchers (Harvey and Garabedian 1991; Hendry et al. 1997) have modeled bacterial transport using a two-site model where both reversible and irreversible deposition is allowed. In this study, we consider only irreversible deposition.

Integrating the analytical solution to Equation 1 over time yields an expression for the fractional recovery (Bolster et al. 1998, 1999a; Harvey and Garabedian 1991)

$$fr = \exp\left[\frac{xv}{2D}\left(1 - \sqrt{1 + 4k_c Dv^{-2}}\right)\right] \tag{3}$$

where fr is the fractional recovery defined as the number of bacteria recovered at distance x normalized to the total number of bacteria introduced into the system. Rearranging Equation 3 to solve for the sticking efficiency from measured values of the fractional recovery yields

$$\alpha = \frac{2d_c}{3(1-\theta)x}\frac{1}{\eta}\left[-\ln(fr) + \frac{\alpha_L \ln(fr)^2}{x}\right] \tag{4}$$

The presence of a dual-alpha population of bacteria, where it is assumed that the influent suspension of bacteria is composed of two distinct subpopulations, each with its own value of α, can be described simply by weight-averaging the fractional recoveries of the two subpopulations. Inserting the solution for fractional recovery into a model incorporating a dual-alpha population of bacteria yields (Simoni et al. 1998)

$$fr = F\exp\left[\frac{xv}{2D}\left(1 - \sqrt{1 + 4k_{c(high)}Dv^{-2}}\right)\right] + (1-F)\exp\left[\frac{xv}{2D}\left(1 - \sqrt{1 + 4k_{c(low)}Dv^{-2}}\right)\right] \tag{5}$$

where F is the fraction of bacteria in the influent suspension with a high sticking efficiency (α_{high}), $k_{c(high)}$ is the deposition coefficient for the sticky bacteria, and $k_{c(low)}$ is the deposition coefficient for the less sticky bacteria.

Sensitivity Studies

The effect of a dual-alpha distribution of sticking efficiencies on the ability to make field-scale predictions was studied numerically. A length scale of 30.5 m was chosen because state-established setback distances are commonly this distance (Yates 1997). We assumed one-dimensional transport through physically and geochemically uniform porous media. Simulations were performed where (1) dispersivity was scale invariant, and (2) dispersivity was allowed to increase uniformly with distance. This approach allowed us to focus our attention on the role biological heterogeneities play in predicting and understanding bacterial transport over long distances. The sensitivity studies were conducted using the following three steps:

1. Equation 5 was used to calculate the fractional recovery (fr) of a dual-alpha population of bacteria for a given set of parameters for sampling points located every 0.1 m for 30.5 m. Values of F, α_{high}, and α_{low} were chosen to be in the range reported in the literature (Baygents et al. 1998; Bolster et al. 1999b; Simoni et al. 1998). Physical parameters (α_L = 2.2 cm; v = 0.335 m day^{-1}; θ = 0.35; d_c = 0.59 mm; $\eta = 5.0 \times 10^{-2}$) were chosen to be representative of a sandy aquifer (Bouwer and Rittman 1992; Harvey and Garabedian 1991).
2. Apparent sticking efficiencies were calculated from the fractional recovery at each sampling point by

$$\alpha_{apparent} = \frac{2d_c}{3(1-\theta)x}\frac{1}{\eta}\left[-\ln(fr) + \frac{\alpha_L \ln(fr)^2}{x}\right] \tag{6}$$

where $\alpha_{apparent}$ represents the apparent sticking efficiency that describes the overall transport behavior of the bacterial population assuming a uniform population with a single α for the experimental length (Simoni et al. 1998).
3. From these calculated sticking efficiencies, predictions of the fractional recovery were made for a travel distance of 30.5 m. The predicted fractional recovery assuming a uniform population of bacteria is

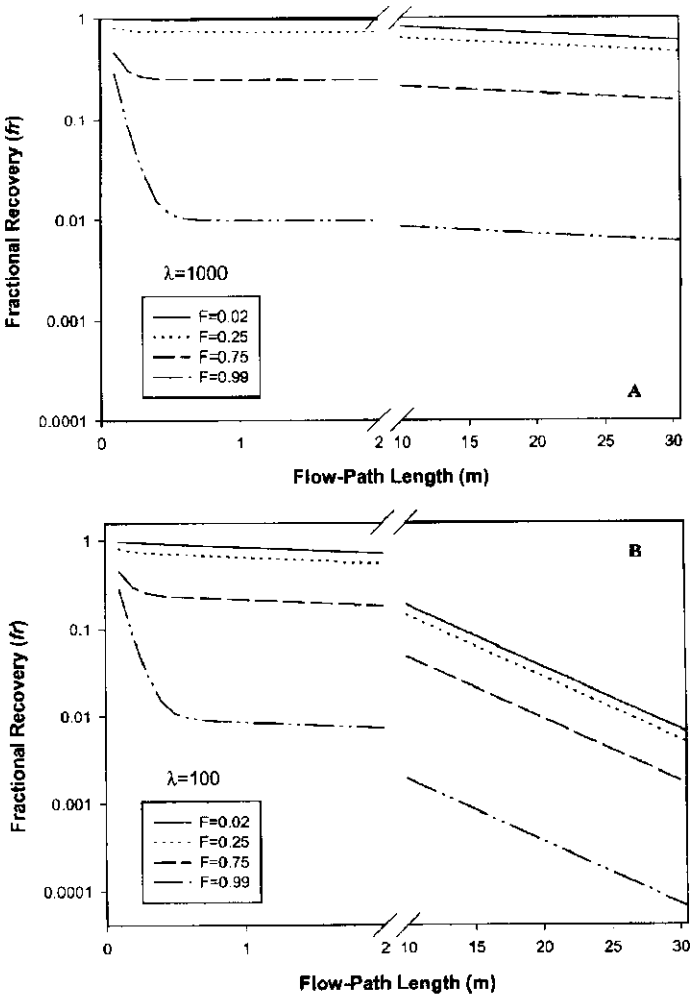

Figure 1. Effect of fraction of sticky bacteria (F) and sticking efficiency ratio ($\lambda = \alpha_{high}/\alpha_{low}$) on the removal of bacteria with flowpath length for (a) $\lambda = 1000$ and (b) $\lambda = 100$. Nonexponential decreases in fractional recovery with depth are seen for high values of F. In cases where a small fraction of bacteria (high F) is relatively unreactive with the sediment surface, the majority of bacterial removal occurs near the injection point. Subsequent decreases with flowpath length are minimal. Decreasing λ results in more noticeable removal of bacteria with flowpath length.

$$fr_{predict} = \exp\left[\frac{xv}{2D}\left(1 - \sqrt{1 + 4k_{predict}Dv^{-2}}\right)\right] \quad (7)$$

$$k_{predict} = \frac{3(1-\theta)v}{2d_c}\eta\alpha_{apparent} \quad (8)$$

where x = 30.5 m and $\alpha_{apparent}$ is the sticking efficiency calculated by Equation 6. The predicted fractional recovery (calculated from the fractional recovery at each sampling point using $\alpha_{apparent}$) was then compared to the actual fractional recovery (that calculated with the dual-alpha model) at x = 30.5 m. This method simulates the use of short columns, or transport over short distances in the field, for calculating α and making predictions of the large-scale transport of bacteria.

Results

Differences between simulations where dispersivity was held constant and where dispersivity was allowed to increase with scale were minimal (data not shown), therefore only the results obtained with scale-invariant dispersivity are presented below. The presence of a dual-alpha population yielded nonexponential decreases in aqueous concentrations of bacteria with flowpath length. These results are substantially different from the exponential decrease expected for a single-alpha population. For values of F greater than 0.75, a substantial amount of removal occurs over the first 50 cm of transport based on the parameter values used in this study (Figure 1). Further decreases in aqueous concentrations of bacteria with distance are minimal. As the ratio of sticking efficiencies ($\lambda = \alpha_{high}/\alpha_{low}$) decreases from 1000 to 100 (resulting in increases in α_{low}), reductions in aqueous concentrations of bacteria become more noticeable (Figure 1b). Minimal decreases in aqueous concentrations of bacteria can be explained by the decrease in the apparent sticking efficiency with length (Figure 2).

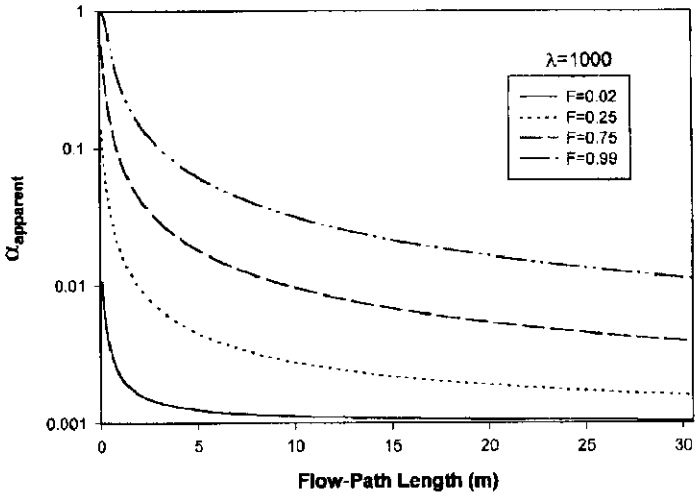

Figure 2. Changes in the apparent sticking efficiency with flowpath length. For low values of F, $\alpha_{apparent}$ remains constant after a short distance suggesting that reasonably sized REVs may be defined, thereby increasing the ability to make adequate predictions at scale based on assumptions of a uniform α.

The effect of a dual-alpha population on the ability to scale deposition parameters from short transport experiments to longer flow paths is dependent on the fraction of the influent suspension that is sticky (i.e., has a high α). As the fraction of bacteria with a high sticking efficiency increases, the ability to predict bacterial transport at greater distances decreases (Figure 3). For an F value of 0.02, the predicted fractional recovery ($fr_{predict}$) is about 90% of the actual $fr_{actional}$ recovery (fr_{actual} at x = 30.5 m when based on sample lengths of greater than 1 m or greater. Depending on the acceptable tolerance, this indicates that the presence of a tiny fraction of sticky bacteria may not preclude the ability to predict accurately bacterial transport based on assumptions of a uniform sticking efficiency. Predicting bacterial transport based on assumptions of a uniform sticking efficiency, however, becomes essentially impossible when influent suspensions are composed of a substantial fraction of sticky bacteria (Figure 3). For adequate predictions at scale, the sample size must be a representative elementary volume (REV) for the parameter of interest. The REV is defined as a sufficiently large sample where the parameter of interest is scale invariant (Bear 1972). For cases of high F, the apparent sticking efficiency continues to decrease with increasing flowpath length (Figure 2). For low values of F, however, the decrease of α with sample size is short-lived, indicating that a reasonably sized REV may be defined under these idealized conditions.

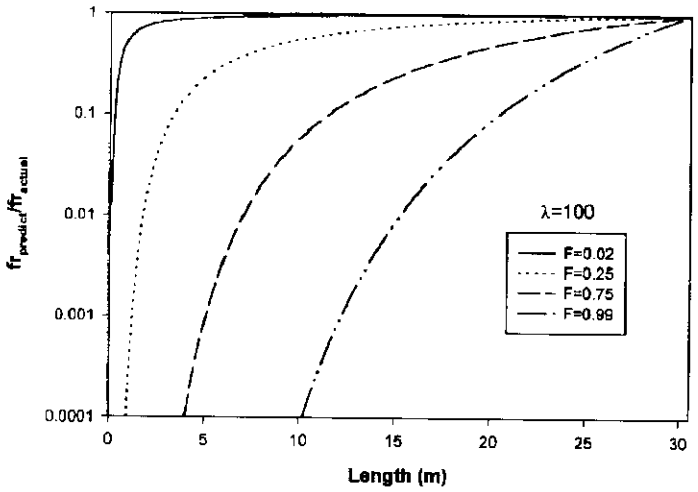

Figure 3. Effect of sample size on predicting the transport of a dual-alpha population of bacteria over a 30.5 m distance where $fr_{predict}$ represents the predicted fractional recovery based on the assumption of a uniform population of bacteria and fr_{actual} is the actual fractional recovery at x = 30.5 m for the dual-alpha population. The x-axis represents the sample length. For low values of F, assumptions of a uniform population of bacteria do not preclude accurate estimates of bacterial deposition at scale. As F increases, however, the ability to accurately predict bacterial deposition declines for reasonably sized sample lengths.

Discussion

The results demonstrate that the presence of a dual-alpha population of bacteria makes scaling of laboratory-derived parameters to the field problematic, even for the idealized situations assumed in this study. This finding is a result of the overall transport behavior near the point of injection being dominated by the sticky bacteria. Farther downgradient, the sticky fraction is removed from suspension resulting in the transport behavior being dominated by the less-sticky bacteria. The presence of a small fraction of an influent population with surface characteristics favorable for transport may yield substantially greater concentrations of bacteria than would be expected based on the assumption of a uniform sticking efficiency. Substantial decreases over such short travel distances may leave the false impression that significant numbers of bacteria will not travel very far in the field.

In addition to bacteria, the presence of intra-population variability may also exist for viruses. Modeling the unadsorbed fraction of virus with time, Burge and Enkiri (1978) found that an assumption of a heterogeneous population of viruses was best able to describe the data. Powelson et al. (1990) observed that a single decay coefficient could not describe adequately the transport of viruses through unsaturated soil columns. Pieper et al. (1997) report field-derived sticking efficiencies for bacteriophage PRD1 (at travel distances of 1.8 m and greater) to be much lower than laboratory derived values (as calculated from 10 to 15 cm columns). The authors attribute this difference to the presence of dissolved organic matter in the aquifer. Another potential explanation for this behavior is the presence of a distribution of sticking efficiencies. The presence of a distribution of sticking efficiencies is further supported by the finding of these same authors that the relative breakthrough of PRD1 over the first 1.8 m of transport decreased substantially but minimal decreases were observed for the remaining 1.8 m of transport. Bales et al. (1995) observed a 10^4 to 10^7 attenuation of PRD1 over the first meter of transport yet observed only a tenfold attenuation over the following meter of transport in a field study. Schijven et al. (1999) observed sticking efficiencies to decrease from .0024 to .00043 for travel lengths of 2.4 and 30 m in a field study. Such decreases of virus attenuation with flowpath length are consistent with the hypothesis that a distribution of sticking efficiencies may be present within the influent suspension. Both Schijven et al. (1999) and Pieper et al. (1997) recognized this possibility. Research is needed to determine whether intrapopulation variability may be present within single viral isolates.

Our findings demonstrate that a priori knowledge of the presence of an influent population with nonuniform sticking efficiencies is required to obtain reasonable estimates of pathogen removal in ground water systems. It has been argued that column dissections, where sediment-associated bacteria are enumerated at various depths, are a good method for obtaining this type of information (Baygents et al. 1998; Bolster et al. 1999b; Logan et al. 1999). Column dissections allow data from a single column to be used in determining and quantifying the presence of nonuniform sticking efficiencies within an influent suspension of bacteria. One limitation to this approach is that current methods of removing and enumerating sediment-associated bacteria are incomplete (Bolster et al. 1999b; Camesano and Logan 1998). Another approach is to use effluent data from columns of varying length (Simoni et al. 1998), a method that requires numerous column experiments. In either case, to determine and quantify accurately the presence of a microbial population with a distribution of sticking efficiencies requires the use of columns that are of sufficient length so that the transport behavior of the less-sticky population is observed (Figure 4).

Although several researchers have observed sticking efficiencies to decrease with flowpath length (Albinger et al. 1994; Baygents et al. 1998; Bolster et al. 1999b; Martin et al. 1996; Simoni et al. 1998), the mechanism for this behavior is still unknown. Baygents et al. (1998) concluded that the modeled dual-alpha population in their study was a result of intra-population differences in bacterial surface properties. This conclusion was based on the existence of two substantial peaks in electrophoretic mobility for the bacterial strains used in their study. Variations in cell size, electrophoretic mobility, and hydrophobicity were ruled out by Simoni et al. (1998) as being the source of the observed variability for the organism used in their study. Similarly, surface charge variations were not observed (Glynn et al. 1998) for the organism used in the study of Bolster et al. (1999b). Different culturing methods, however, were employed in the studies of Glynn et al. (1998) and Bolster et al. (1999b).

In addition to biological explanations, physical mechanisms cannot be ruled out to explain the apparent decrease in sticking efficiency with flowpath length. 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. Bolster et al. (1999b) observed the sticking efficiency ratio (λ) to vary between 20 and ~ 3400 for the same organism in different porous media, suggesting that biological explanations may not be solely responsible for the observed dual-alpha population in their study. Thus, based on conflicting data from a variety of studies, it appears that there is currently no definitive explanation for the source of the nonsingular sticking efficiencies observed by a number of investigators.

Clearly the approach we have taken is an oversimplification of the factors limiting the prediction and understanding of field-scale transport of bacteria in the subsurface. In addition to assumptions

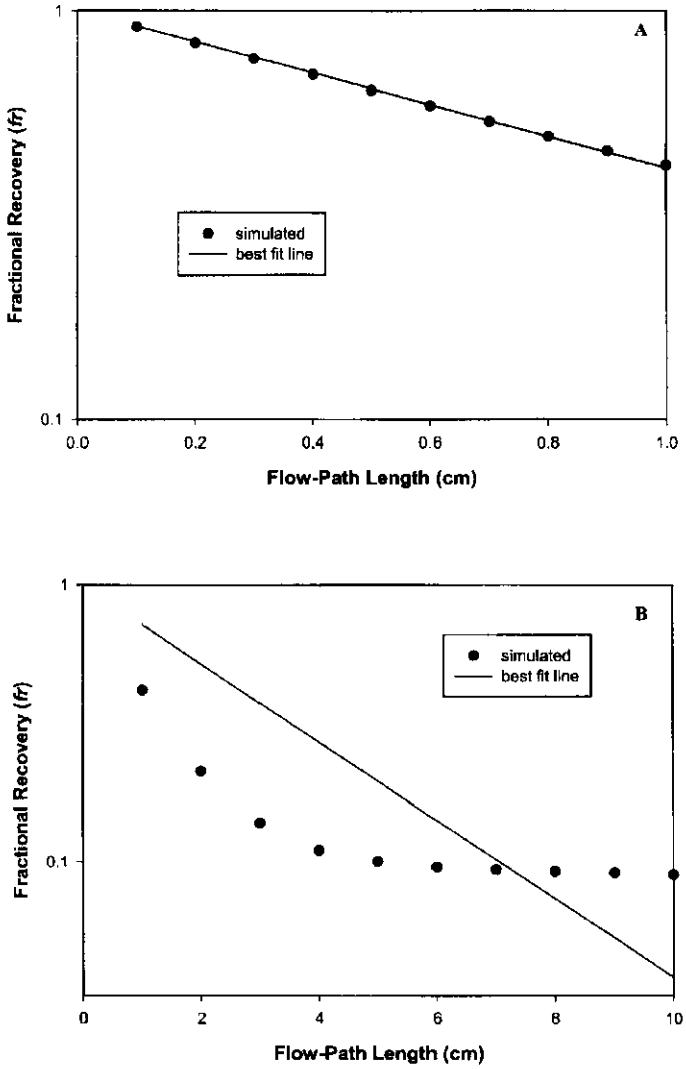

Figure 4. Effect of column length on elucidating the presence of a dual-alpha population of bacteria for column lengths of (a) 1 cm and (b) 10 cm. Symbols are fractional recoveries generated by the dual-alpha model (Equation 5) and solid lines are the best fit line obtained by fitting a single-alpha model (Equation 3) to the data generated by Equation 5. Results show that, in some cases, the existence of a distribution of sticking efficiencies may go unrecognized when short columns are used.

of physical and geochemical homogeneity, we neglected the effects of growth, death, and remobilization (entrainment) of bacteria in our analysis. We recognize the potential importance of these processes in predicting and understanding the long-distance transport of bacteria in the subsurface. For instance, the transport of just a few microorganisms to regions favorable for growth may significantly increase the numbers of pathogenic microorganisms in the aqueous phase. Additionally, the slow release of microorganisms from the sediment phase into the aqueous phase represents a potential source for continuing contamination of subsurface water supplies. Nevertheless, our results indicate that the presence of intrapopulation variability may be a significant reason why bacteria have been observed to travel such great distances in ground water systems. The results demonstrate the importance of incorporating the presence of biological heterogeneities, in conjunction with physical and geochemical heterogeneities, in numerical models for understanding and predicting microbial transport in the field.

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