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Effects of transverse mixing on transport of bacteria through heterogeneous porous media

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Abstract. In this paper, we examine two questions: (1) can the effects of transverse mixing of bacteria in a system constructed to have a permeability discontinuity in the direction parallel to the flow be measured; and (2) if the effects are measurable, can they be calculated using a transverse dispersion coefficient estimated from experiments using a conservative tracer? Pulses of chloride and bacteria were transported downward through heterogeneous columns constructed with a tubule of coarse, quartz sand surrounding an annulus of fine, quartz sand. Pulses of each were also transported through homogeneous columns of the two sands. Doubly peaked breakthrough curves resulted from the columns containing two distinct sand sizes. Modeling of the breakthrough curves was performed taking into account advection, dispersion, deposition, entrainment, and pore-size exclusion. The results revealed that transverse mixing does occur during transport of bacteria through heterogeneous material and that this mixing can be estimated using a conservative tracer.

1. Introduction

Processes affecting the transport of bacteria through aquifers have been the focus of increased scientific investigation recently. As with the transport of dissolved constituents through soils and rocks, the transport of bacteria by ground water is conditioned strongly by heterogeneities in the medium. In many aquifers, flow is mainly parallel to the dip of hydrostratigraphic units that define major heterogeneities. The flow-parallel interfaces between units are known to be important sites of bacterial activity [*Smith et al.*, 1991; *McMahon and Chapelle*, 1991]. Transport processes near interfaces that delimit discontinuities in physical and chemical aquifer properties are likely to be of considerable importance both for a basic understanding of bacterial movement and for application of remediation technologies at sites of ground-water contamination. The necessary quantification will come through determination of parameters that describe advective and dispersive transport of bacteria in ground waters.

Most of the work to date on models for transport of bacteria by ground water has been related to homogeneous porous media. Even these models for transport through porous media are relatively complex, containing a plethora of parameters that can be difficult to estimate [*Corapcioglu and Haridas*, 1984]. To help resolve the parameter estimation problem, investigators have used transport parameters (dispersivity, in particular) determined from data on a conservative tracer and have assumed that these can be applied to colloid transport as well [e.g., *Harvey and Garabedian*, 1991; *Saiers et al.*, 1994; *Saiers and Hornberger*, 1996a]. Although a theoretical basis for such an assumption is lacking, in practice, the application of longitudinal dispersion coefficients estimated with solutes to colloid transport has yielded reasonable results, at least when compared to data from laboratory experiments on columns packed with relatively uniform sand [*Saiers et al.*, 1994; *Saiers and Hornberger*, 1996a].

Even with relatively small laboratory columns, however, the effects of heterogeneities often are evident. Breakthrough curves of solutes and colloids (including bacteria) in natural materials often fail to be characterized using the simplest form of a model for describing advective and dispersive transport processes [*Brusseau and Zachara*, 1993; *Lindqvist and Enfield*, 1992; *Saiers and Hornberger*, 1996b]. One explanation for the observed asymmetric breakthrough of materials in a medium is the concept of mobile and immobile water, with exchange between the two regions. *Bales et al.* [1989], for example, modeled the transport of virus through a fractured tuff using a mobile-immobile model. The conceptual model underlying this approach is that flow occurs only in a subset of pores; in another subset of pores, water and solutes are stagnant with exchange with the mobile region taking place by a diffusion-like process. Although the mobile-immobile water model has proved to be valuable in the interpretation of transport through soil columns, it cannot be expected to do justice to the case of flow through a heterogeneous material where advection takes place in all different regions, albeit at different rates. That is, in some instances, it is necessary to use a model that allows water movement in multiple transport zones [*Gwo et al.*, 1995].

Transport in shallow sandy aquifers often is seen to depend on strong contrasts in hydraulic conductivity in layers of sediment [e.g., *Harvey et al.*, 1993]. In such cases, primarily horizontal movement of ground water can occur in the different layers at different velocities. Transverse mixing across layer interfaces is, in some ways, conceptually similar to exchanges among multiple mobile zones. *Harvey et al.* [1993] speculated that the effects of such transverse mixing were negligible for their experimental conditions, finding that the composite breakthrough in a fully penetrating well could be reproduced by simply adding the contribution of three separate layers ignoring communication among them. The data from the field experiments described in *Harvey et al.* [1993] are not precise

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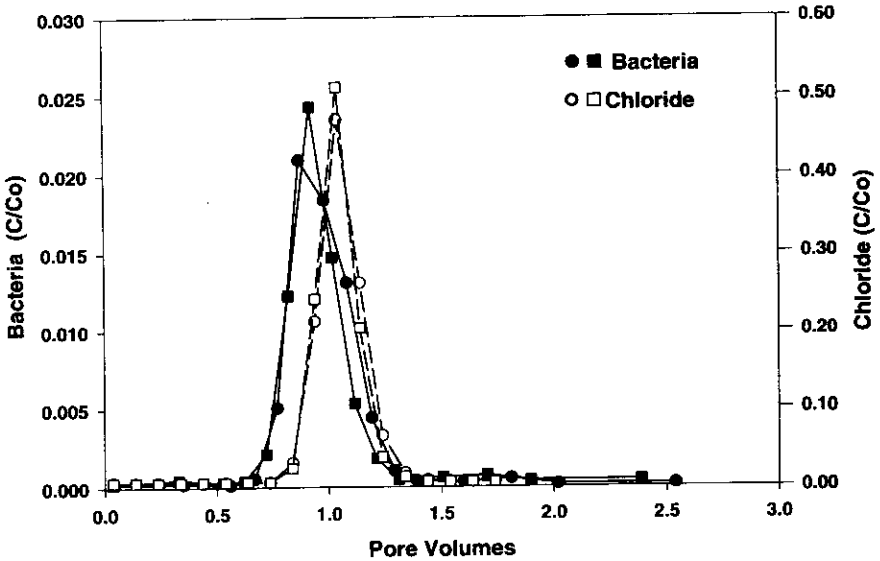

Figure 1. Breakthrough curves for bacteria and for chloride in replicate columns of homogeneous fine sand.

enough to determine to what extent mixing across the interfaces did occur, however. The questions of the extent of transverse mixing that may occur and whether a transverse dispersivity can characterize the process remain open.

In this paper, we examine two questions: (1) can the effects of transverse mixing of bacteria in a system constructed to have a permeability discontinuity in the direction parallel to the flow (what we will call a structured heterogeneity) be measured; and (2) if the effects are measurable, can they be calculated using a transverse dispersion coefficient estimated from experiments using a conservative tracer? Our approach is to use packed-sand columns with a vein of relatively coarse material surrounded by a matrix of relatively finer material. Results using a pulse input of bacteria show that the two peaks in the observed breakthrough curve are due to transport through the vein and the annulus, respectively, but that a saddle between the peaks represents the effects of transverse mixing. The observations are consistent with a model that assumes transverse mixing of bacteria can be described using a transverse dispersion coefficient determined from the measured breakthrough of chloride through the column.

2. Methods

2.1. Column Experiments

Methods used are similar to those described by *Saiers et al.* [1994]. Glass chromatography columns (Kontes) with an inner diameter of 4.8 cm were wet packed with acid and base washed sand to a column length of ~13.5 cm (13.4–13.8 cm) and a total pore volume of ~93 ml (90–96 ml). Two sand sizes were used in the column experiments, a "coarse" sand (in a relative sense) with diameters ranging from 1.0 to 1.19 mm and a "fine"

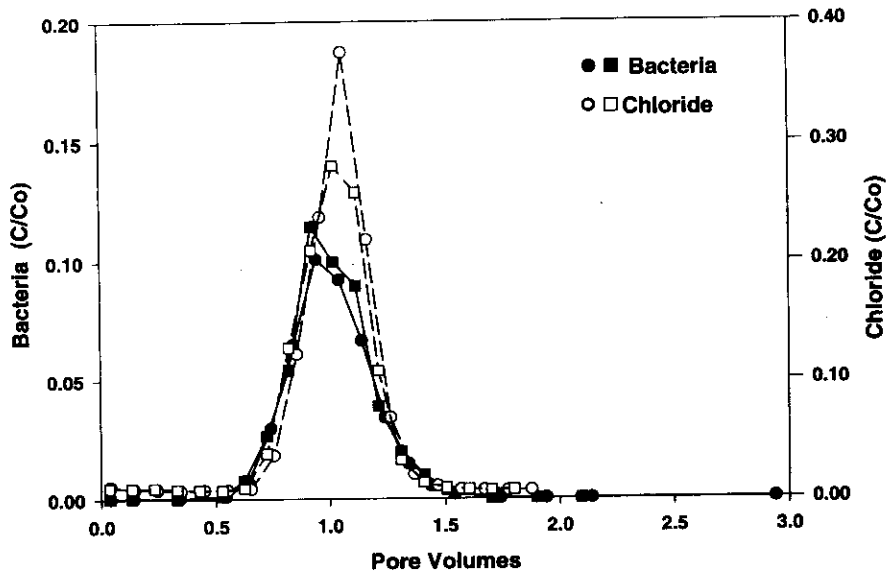

Figure 2. Breakthrough curves for bacteria and for chloride in replicate columns of homogeneous coarse sand.

Table 1. Percent Recovery of Bacteria

Experiment	First Peak	Second Peak	Total
Homogeneous, fine sand, W8	13%, 12%	NA	13%, 12%
Homogeneous, coarse sand, W8	80%, 85%	NA	80%, 85%
Heterogeneous column, W8	49%, 44%	8%, 13%	57%, 57%
Injection into heterogeneous column fine sand, W8	NA	12%, 13%	12%, 13%
Injection into heterogeneous column coarse sand, W8	81%, 94%	NA	81%, 94%

(The two values are the results from the A and B columns, which are replicate columns.) NA, not available.

sand with diameters ranging from 0.5 to 0.6 mm. Columns with a coarse vein of material running the length of the column were packed by first pouring fine sand around a glass tube with an inner diameter of 1.6 cm. After the fine sand had been added, coarse sand was poured into the glass tube, slowly removing the tube while adding sand. Columns without a central vein were packed using the same method without the glass tube.

Transport experiments were performed at flow rates of ~100 ml h^{-1} (89–107 ml h^{-1}). The flow was adjusted using a variable-flow peristaltic pump. Inflow of artificial ground water (AGW) with an effective ionic strength of 0.00089 m was regulated with a flow adapter such that a constant head of AGW was maintained on the top of the sand throughout the experiments.

Chloride was used as a conservative tracer in all experiments. A 10-ml pulse of 10^{-2} M chloride solution was used as input. In all experiments, chloride (and bacteria) were added as discrete, constant-concentration pulses. After adjusting flow rates in the columns to ~100 ml h^{-1}, the AGW was drained to the top surface of the sand and the flow was stopped. Water containing the chloride tracer was then added to the top of the column. Flow was initiated until once again the water level reached the surface of the sand. Flow again was stopped, 10 ml of AGW was added to the top of the column to reestablish the original head, the flow adapters were refitted onto the column, and flow was restarted. Effluent samples were collected on 0.1 pore volume fractions and analyzed using a chloride-specific electrode.

A bacterial strain (W8) isolated from ground water from a shallow coastal-plain aquifer on the eastern shore of Virginia was used to study transport of bacteria. This bacterium is a nonmotile, nonspore-forming, hydrophilic (classification of *Mozes et al.* [1987]), gram-positive rod, with dimensions of 0.3 × 1.1 μm. The bacteria were inoculated in sterilized peptone-yeast extract (DIFFCO) solution on a rotary shaker for 48 hours. The cells were then centrifuged for 20 min at ~27,000 g, resuspended in AGW, and left on a rotary shaker for 24 hours [*Fontes et al.*, 1991]. The bacteria were in a resting state for the transport experiments.

A 5-ml pulse of bacterial suspension was introduced into the columns after the completion of the chloride breakthrough again using the procedure described above. Bacterial concentrations in the influent and effluent were determined using acridine orange direct counts (AODC) [*Hobbie et al.*, 1977]. Samples were diluted such that from 20 to 300 bacteria appeared on each slide.

Separate experiments, in which bacteria were injected into either the fine-sand annulus or the coarse-sand vein, were performed to estimate directly the amount of mixing between the two regions. Pulses were injected using a 3-ml disposable syringe with a 4-cm-long needle. After adjusting flow rates in

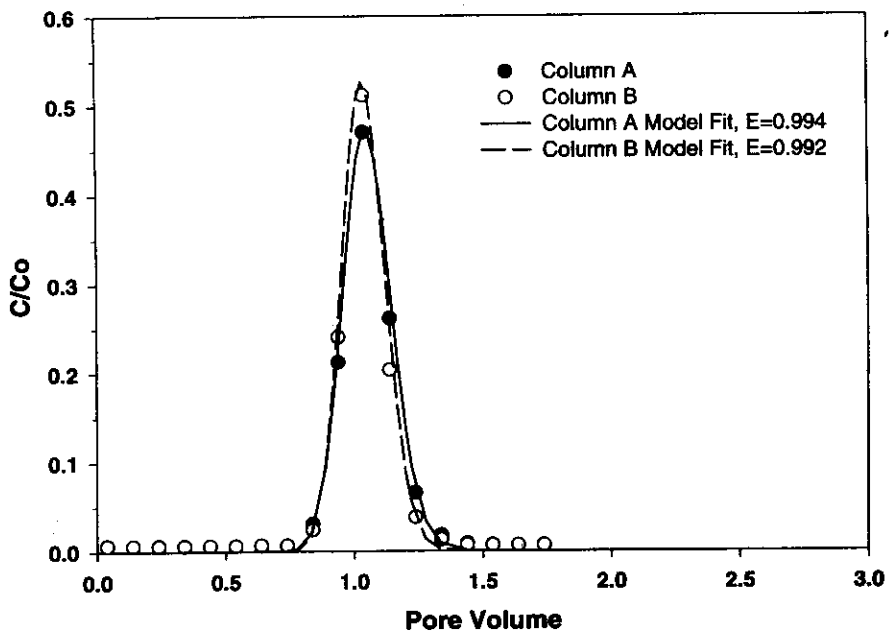

Figure 3. Observed and modeled chloride breakthrough in replicate columns of homogeneous fine sand.

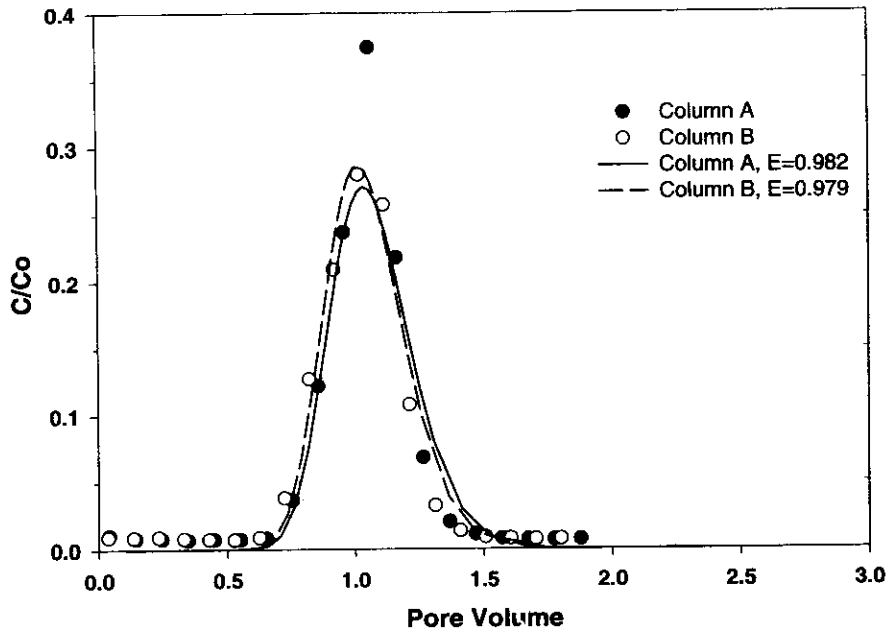

Figure 4. Observed and modeled chloride breakthrough in replicate columns of homogeneous coarse sand.

the columns to ~100 ml h^{-1}, the AGW was drained to the top surface of the sand and the flow was stopped. The entire column of sand was always kept below the free water surface. An empty syringe was inserted to a depth of 2 cm into either the fine sand at ~0.5 cm from the outside wall or into the center of the coarse sand. A pulse of influent solute or suspension was pipetted into the syringe and injected into the sand. After removing the syringe, 10 ml of AGW was added to the top of the column to reestablish the original head, the flow adapters were refitted onto the column, and flow was restarted.

Subsequent to experiments on transport of chloride and bacteria using a heterogeneous column, injections of methylene blue dye into either the fine annulus or the coarse vein were conducted to visualize lateral dispersion. The procedure for syringe injection described above was followed. When dye first could be seen in the effluent, the flow was stopped, the column carefully inverted, and the Teflon™ end fitting removed. Because the dye stained the sand as it moved through the column, the lateral spreading of the dye could be discerned easily. The extent of lateral spreading of the dye at the exit to the column was observed and then material was removed in stages so the spreading could be observed at locations along the axis of the column.

2.2. Transport Modeling

Transport in homogeneous columns was modeled using a one-dimensional advection-dispersion equation. Model parameters for homogeneous columns were estimated with CFIT [*van Genuchten*, 1981]. Transport of chloride was modeled using

$$R\frac{\partial c}{\partial t} = \frac{q}{n}\alpha_L\frac{\partial^2 c}{\partial z^2} - \frac{q}{n}\frac{\partial c}{\partial z} \tag{1}$$

where R is the retardation factor, c is aqueous concentration, t is time, q is Darcian velocity, n is porosity, α_L is longitudinal dispersivity, and z is the spatial coordinate in the flow direction. Retardation of chloride is not expected in these columns, but the form allowing for $R \neq 1$ was used to account for timing errors in the experiments.

Transport of bacteria was modeled using

Table 2. Homogeneous Column Model Parameters

	Chloride		Bacteria-W8	
	Fine	Coarse	Fine	Coarse
Pulse Length, h	0.099, 0.099*	0.097, 0.104*	0.048, 0.050*	0.049, 0.051*
Peclet number, unitless	260 (12.4), 325 (19.1)	101 (8.7)[a], 84.4 (7.2)	260, 325*	84.4, 84.4*
Retardation factor (unitless)	0.997 (.002), 0.983 (.003)	1.010 (0.005), 0.982 (0.008)	15.6 (6.8), 14.6 (5.7)	1.62 (.26), 1.36 (.21)
Omega (unitless)	NA	NA	2.36 (0.10), 2.41 (0.09)	0.31 (.03), 0.23 (.04)
Porosity	0.363, 0.367*	0.376, 0.370*	0.330, 0.325	0.365, 0.360
Efficiency[b]	0.994, 0.992	0.982, 0.979	0.854, 0.869	0.989, 0.980

The two values are the results from the A and B columns, which are replicate columns. Standard errors, supplied in the model outputs, are shown in parentheses. Asterisk indicates that items were input to the model. Items without the asterisk were fit to the data.

[a]This Peclet number was determined after eliminating one point from the data.

[b]The efficiency, a measure of goodness of model fit, is one minus the variance of the residuals divided by the variance of the data.

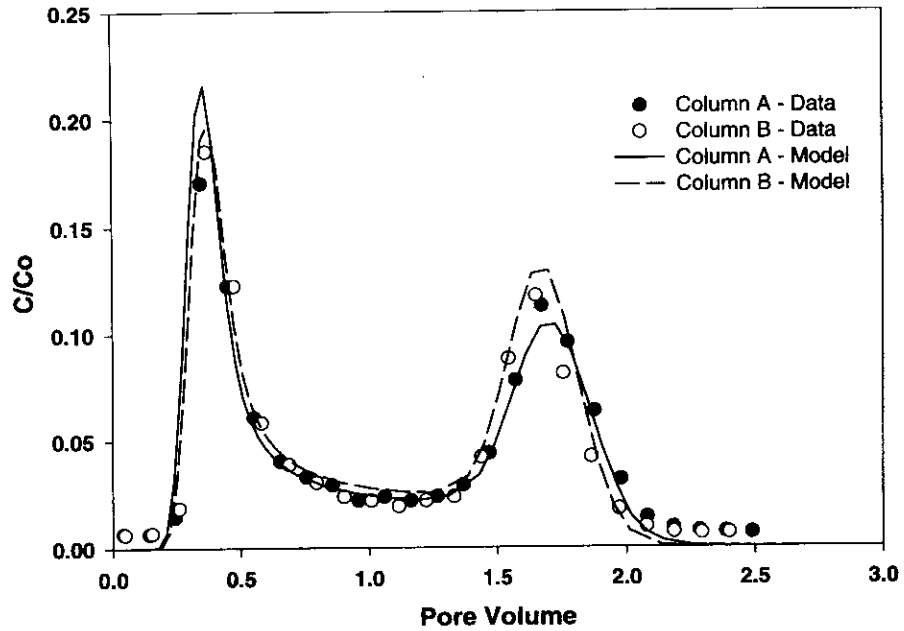

Figure 5. Observed and modeled chloride breakthrough in replicate heterogeneous columns.

$$R\frac{\partial c}{\partial t} = \frac{q}{n}\alpha_L\frac{\partial^2 c}{\partial z^2} - \frac{q}{n}\frac{\partial c}{\partial z} - k_c c + k_y s\frac{\partial s}{\partial t} = k_c c - k_y s \tag{2}$$

where k_c is the deposition coefficient, k_y is the entrainment coefficient, c is the concentration of suspended bacteria, and s is the concentration of bacteria on the mineral surfaces expressed per volume of pore water. CFIT uses dimensionless forms of the equations above. The parameters estimated are the Peclet number (qL/nD_L), where D_L is the longitudinal dispersion coefficient, a retardation factor, R, and a dimensionless kinetic coefficient, ω. (Note that R in this case is part of the representation of the deposition-entrainment process [*van Genuchten*, 1981; *Hornberger et al.*, 1992] and not a separate "sorption" mechanism.) The deposition and entrainment coefficients for bacteria are related to the dimensionless coefficients by $k_c = \omega(qL/n)$ and $k_y = k_c/(R - 1)$.

Transport of chloride and bacteria through heterogeneous columns was simulated using a two-dimensional advection-dispersion equation assuming radial symmetry. For bacterial transport, the equation takes the form

$$R\frac{\partial c}{\partial t} = \frac{q}{n}\alpha_L\frac{\partial^2 c}{\partial z^2} + \frac{q}{n}\alpha_T\frac{\partial^2 c}{\partial r^2} + \frac{q}{n}\frac{\alpha_T}{r}\frac{\partial c}{\partial r} - \frac{q}{n}\frac{\partial c}{\partial z} - k_c c + k_y s \tag{3}$$

where r is the radial spatial coordinate and α_T is the transverse dispersivity. SUTRA [*Voss*, 1984] was used to compute breakthrough curves for the two-dimensional model. Chloride data were used to estimate (via visual fits to the breakthrough curves) relative hydraulic conductivities of the fine-grained annulus and the coarse-grained vein [*Saiers et al.*, 1994]. All other parameters except the transverse dispersivities were either measured or were taken from the values determined using homogeneous columns. The transverse dispersivities were selected by visual fits to the measured breakthrough in the heterogeneous columns.

3. Results

The peaks in breakthrough curves of bacteria from replicate homogeneous columns ("A" and "B") consistently preceded the peaks in breakthrough curves of chloride by a small amount (Figures 1 and 2). About 12% of the injected bacteria was recovered in the effluent from the column packed with fine sand while >80% was recovered from the columns packed with coarse sand (Table 1).

Modeled concentrations of chloride fit the measured breakthrough curves reasonably well, and the replicate columns were consistent when one measurement for column A for the coarse sand was ignored (Figures 3 and 4). All of the measured data for columns A and B for the coarse sand were very close except for the one high value measured in column A. We decided to accept the parameters determined after dropping the one data point in that this yielded consistency between replicates. With the Peclet number fixed at the value determined from the chloride breakthrough curves, the parameters for the bacterial breakthrough experiments were determined. Again, replicate columns gave very similar results (Table 2).

Breakthrough curves from the heterogeneous columns had two peaks. The chloride was conservative; 49.2% of all chloride was recovered in the first peak, and 50.8% was recovered in the second peak. (The areas beneath the first and second peaks were defined using time at the minimum in the saddle in the breakthrough curve between the peaks as the dividing time.) Although approximately half of the flow passed through each of the domains, significantly more bacteria were recovered in the first peak than in the second (Table 1). On the basis of the results from the homogeneous columns, the (approximate) expected recoveries of bacteria associated with each flow path can be calculated by multiplying the fractions recovered in the homogeneous columns (~83% in the coarse sand and ~12% in the fine sand) by the respective fractional flow rates (49.2% and 50.8% respectively). These expected recoveries (41% and 6%) are close to those measured in the heterogeneous columns

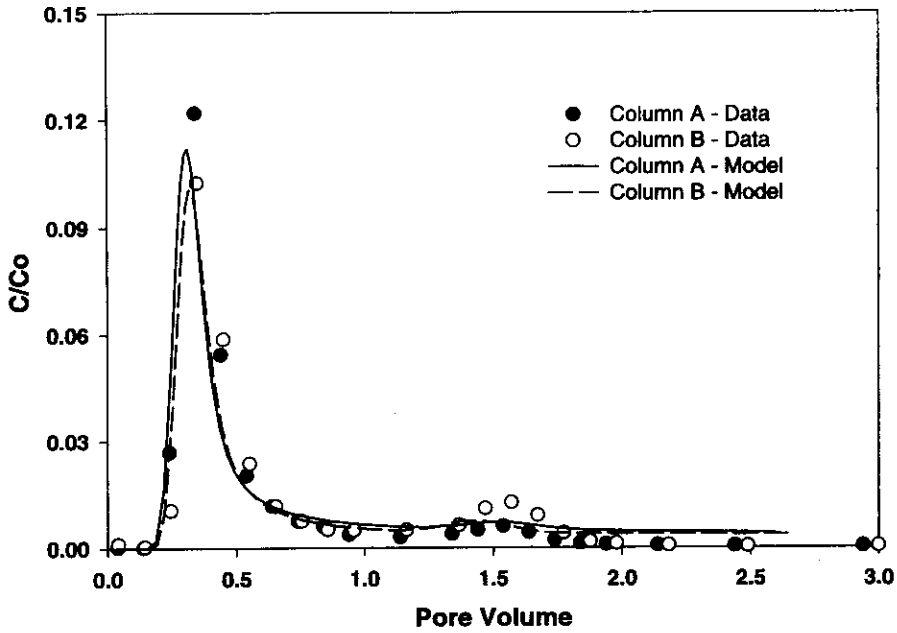

Figure 6. Observed and modeled breakthrough of bacteria in replicate heterogeneous columns.

(Table 1). Evidence supporting this interpretation of the data comes from the experiments in which bacteria were injected directly into either the fine annulus or into the coarse vein of the heterogeneous (Table 1).

Modeled breakthrough curves for the heterogeneous columns made using SUTRA reproduced the essential attributes of the measured breakthrough curves (Figures 5 and 6). Values for the hydraulic conductivities and the transverse dispersivities were adjusted to obtain a fit to the chloride breakthrough (Table 3). Other parameters in the model either were measured directly (e.g., pulse length) or were taken from model fits to data from homogeneous columns (e.g., longitudinal dispersivities). The physical parameters determined from the chloride experiments were taken to be representative of the bacterial transport as well. Deposition and entrainment rates were taken from the experiments on the homogeneous columns.

The methylene blue injected into the fine-sand annulus formed a spot ~1.3 cm in diameter at the injection point. At the exit to the column the dyed spot, entirely in the fine domain, was ~1.7 cm in diameter. These observations can be used to obtain a crude estimate of lateral dispersivity. The theory of diffusion indicates that the second spatial moment of the dye in the plane perpendicular to the flow should equal twice the product of the transverse dispersion coefficient and time. If we take the square of the radius of the dye spot as a surrogate for the second spatial moment, we have

$$(r_2^2 - r_1^2) = 2D_T(t_2 - t_1)$$

where r_2 and r_1 are, respectively, the radii of the spot at times t_2 and t_1. Taking time t_1 to be zero and time t_2 to be the time at which the dye reached the end of the column (the column length divided by the average linear pore velocity) gives

$$(0.85\text{ cm})^2 - (0.65\text{ cm})^2 = 2\alpha_T\left(\frac{q}{n}\right)(11.6\text{ cm})$$

or

Table 3. Heterogeneous Column Parameters

	Chloride	Bacteria-W8
Pulse length, h	0.100, 0.095*	0.048, 0.048*
Vein porosity, dimensionless	0.380, 0.370*	0.365, 0.360*
Annulus porosity, dimensionless	0.36, 0.37*	0.33, 0.325*
Vein deposition coefficient, 1/h	0.0, 0.0*	0.33, 0.24*
Annulus deposition coefficient, 1/h	0.0, 0.0*	2.70, 2.63*
Vein entrainment coefficient, 1/h	0.0, 0.0*	0.532, 0.310*
Annulus entrainment coefficient, 1/h	0.0, 0.0*	0.185, 0.193*
Vein longitudinal dispersivity, cm	0.165, 0.163*	0.165, 0.163*
Annulus longitudinal dispersivity, cm	0.0527, 0.0422*	0.0527, 0.0422*
Vein transverse dispersivity, cm	0.0194, 0.0194	0.0194, 0.0194*
Annulus transverse dispersivity, cm	0.03, 0.03	0.03, 0.03*
Vein hydraulic conductivity, cm/h	7200, 7200	7200, 7200*
Annulus hydraulic conductivity, cm/h	1150, 1300	1150, 1300*
Hydraulic head, cm	0.0392, 0.0388*	0.0405, 0.0388*

The two values are the results from the A and B columns, which are replicate columns. Asterisk indicates that items were input to the model. Items without asterisk were fit to the data.

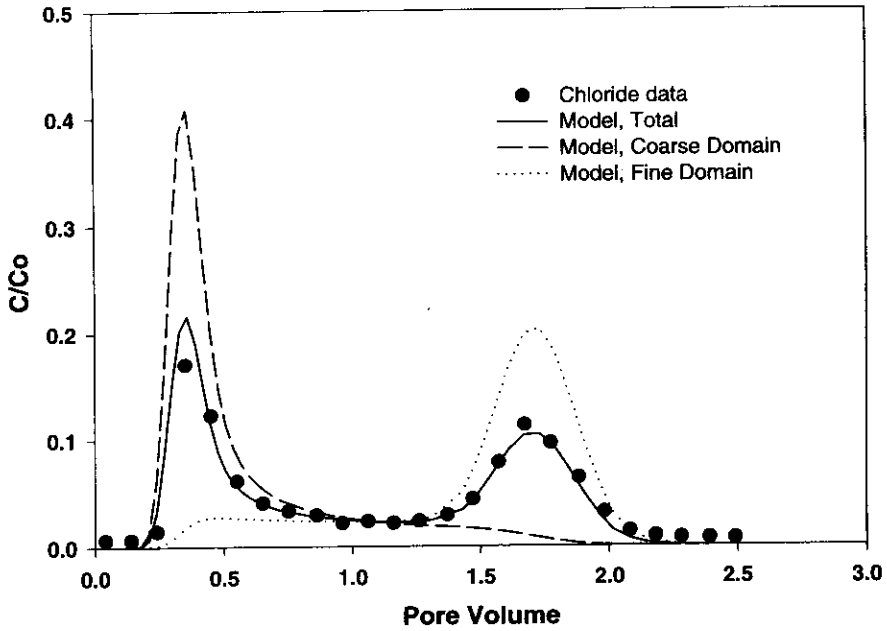

Figure 7. Calculated breakthrough of chloride from the fine-sand and coarse-sand domains of a heterogeneous column.

$$\alpha_T = 0.026 \text{ cm}$$

This value, rounded appropriately, is the same as the value for transverse dispersivity selected for the two-dimensional model fit to the chloride data, 0.03 cm.

4. Discussion

Breakthrough experiments using homogeneous columns showed slightly earlier arrival of bacteria relative to chloride. This early arrival may be due to a size exclusion effect, an effect called upon by several others to explain early breakthrough of bacteria [*White*, 1985; *Harvey et al.*, 1993]. Estimated effective porosities for bacterial transport suggest that bacteria may have been excluded from ~9% of the pores in the fine-sand columns and from 3% of the pores in the coarse-sand columns (Table 2). For a dense packing of spherical grains, pore throats in the fine sand should be ~0.084 mm and in the coarse sand ~0.17 mm. Given that the length of the bacteria used was ~0.001 mm, it is difficult to reconcile the observations with a physical size exclusion. Nevertheless, our results,

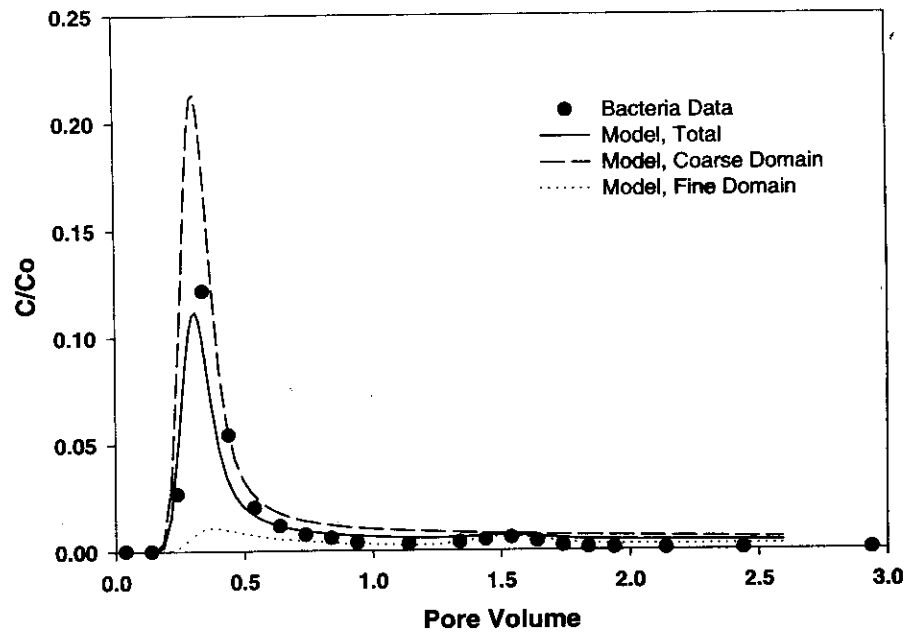

Figure 8. Calculated breakthrough of bacteria from the fine-sand and coarse-sand domains of a heterogeneous column.

and those of *Harvey et al.* [1993], suggest that there is an apparent size exclusion for bacteria in sands. One possible explanation is that there is an as yet unmeasured correlation between cell size and the surface properties that affect attachment to mineral surfaces.

Our results indicate that transverse mixing between layers of sands of different texture does occur for bacteria. Furthermore, the transport appears to be described reasonably well using the standard approach of including a transverse dispersivity in the transport equation. The results from the two-dimensional model, which can be arranged to yield outflows from each separate domain, show that chloride effluent from the coarse central vein is responsible for the first peak in the breakthrough curve and that effluent from the fine annulus is responsible for the second peak (Figure 7). Note, however, that the calculated breakthrough curves from the separate domains are asymmetrical due to interaction. The breakthrough from the central vein has an elongated tail while the breakthrough from the annulus begins soon after initial breakthrough in the central vein and maintains a long leading shoulder relative to the peak in the breakthrough curve from the annulus. Calculations for bacterial transport indicate a similar pattern except the size of the second peak is much reduced because of the strong retention of bacteria in the fine sand (Figure 8).

Our results are consistent with inferences drawn from injections into sandy aquifers that varying flow rates through "layers" affect transport much more strongly than does dispersion, at least transverse dispersion [*Molz et al.*, 1986; *Harvey and Garabedian*, 1991]. That is, if what is desired is a prediction of a composite breakthrough curve, a simple, flow-weighted superposition of the breakthrough for each layer calculated independently captures a large fraction of the total variance. Our results also indicate, however, that cross-strata exchange will occur and that it may be described adequately using the standard transverse dispersion formulation for transport. Although this cross-strata exchange may indeed be of limited importance in predictions of breakthrough over short distances, it may be of critical importance for understanding other processes, the higher microbiological activities reported at permeability interfaces for example. Further work in the field is required to determine whether our inferences gained from a controlled column study are applicable to glaciofluvial aquifers.

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