Transport of *Escherichia coli* in Sand Columns with Constant and Changing Water Contents

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ABSTRACT

Understanding how changes in volumetric water content (θ) affect bacterial adsorption could help reduce transport of pathogenic and indicator bacteria that may be present in infiltrating wastewater. Three flow regimes that simulated infiltration from a household septic system were evaluated: saturated, unsaturated with a constant volumetric water content θ (constant unsaturated flow), and unsaturated with cyclic changes in θ (variable unsaturated flow). *Escherichia coli* was suspended in artificial sewage (AS) and applied as step inputs to sand columns, with regular interruptions in input for variable unsaturated flow. A transport model was fit to the saturated and constant unsaturated flow breakthrough curves to determine retardation (R), the first-order filtration coefficient (μ), and the maximum outflow relative concentration (C_{max}). The total cells transported as a fraction of input (τ) in all three flow regimes was calculated. Constant unsaturated flow resulted in a significantly lower C_{max} (0.633) in comparison with saturated flow (0.803, $P \leq 0.05$), although unsaturated μ (0.0693 h^{-1}) was not significantly different from saturated μ (0.0259 h^{-1}). Constant unsaturated flow also resulted in a significantly smaller τ (0.617) than saturated (0.806) or variable unsaturated flow (0.734). In variable unsaturated flow, cell concentrations were out of phase with θ—as the column drained, cell concentrations in the outflow increased; and when a pulse of suspension was applied, cell concentrations decreased. Constant unsaturated flow is probably the best for removal of pathogenic bacteria because this regime resulted in lower maximum concentrations of *E. coli* and greater cell removal, in comparison with saturated and variable unsaturated flow.

AIR–WATER INTERFACES (AWIs) in unsaturated porous media may be important bacterial adsorption sites that are just beginning to be evaluated (Wan et al., 1994; Mills and Powelson, 1996). The areal extent and location of AWIs change in response to changes in θ, and this may have a major effect on bacterial adsorption. Variation in θ is the norm in soils and the shallow vadose zone as a result of infiltrating precipitation, evaporation, transpiration, flooding, irrigation, or discharge of wastewater. Understanding how these events affect bacterial adsorption could help reduce the transport of pathogenic bacteria that may be present in infiltrating wastewater, or help increase the transport of beneficial bacteria that are capable of degrading contaminants in the subsurface.

Bacterial transport in unsaturated flow is affected by adsorption to the AWI as well as to the solid phase. Wan and Wilson (1994) observed bacteria concentrating at the AWI, and predicted that for relatively hydrophobic bacteria, small amounts of residual air could dramatically reduce transport. Wan et al. (1994) found that bacterial transport via unsaturated flow at an average linear velocity ($v = Q/A/\theta$, where Q is the volumetric flow rate and A is the column cross-sectional area) of 10 cm h^{-1} decreased cell recoveries from 30-cm sand columns in comparison with saturated flow. The total recovery of transported cells, relative to recovery in saturated flow (τ/τ_{sat}), was 0.96 at 85% saturation and 0.77 at 44% saturation for a hydrophilic strain, and 0.63 at 85% saturation and 0.24 at 44% saturation for a relatively hydrophobic strain. Similar results were obtained by Schafer et al. (1998) in 20-cm sand columns with v between 84.6 and 119 cm h^{-1}; τ/τ_{sat} was 0.304 at 86% saturation and 0.100 at 69% saturation for the *Rhodococcus* strain; and 0.374 at 86% saturation and 0.413 at 63% saturation for the *Pseudomonas putida* strain. Jewett et al. (1999) found that recovery of *Pseudomonas fluorescens* in 10.5-cm sand columns with v between 11 and 48 cm h^{-1} also was directly proportional to θ; τ/τ_{sat} was 0.78 at 84% saturation and 0.10 at 46% saturation. Powelson and Mills (1998) applied suspensions of two ground water bacteria as step-inputs to 27-cm sand columns at an infiltration rate (Q/A) of 1.4 cm h^{-1}, resulting in an average v of 4.03 cm h^{-1} for saturated flow and 17.7 cm h^{-1} for unsaturated flow with an average saturation of 22.8%. A kinetic transport model was fit to the breakthrough curves, and this showed that the average bacterial adsorption rate coefficient was 18 times greater in unsaturated flow in comparison with saturated flow.

Three papers have experimentally evaluated bacterial transport in unsteady unsaturated flow. Rijnaarts et al. (1993) applied suspensions of three pseudomonads and seven coryneform bacterial strains in two ionic strengths (0.1 M and <0.0001 M) to 10-cm columns of sand-sized Teflon granules and then allowed the columns to drain. During drainage on the 0.1 M suspensions, passage of the AWI through the columns resulted in detachment of 7.5% or less of the adsorbed cells for 10 of the 11 strains. A higher level of desorption (22.5%) was observed for *Arthrobacter* sp. strain DSM 6687 (this strain was the most hydrophobic by contact angle measurement). Greater detachment was observed at the lower ionic strength, but all values remained below 15% except for *Arthrobacter* sp. strain DSM 6687, which again showed a 22.5% detachment. Powelson and Mills (1998) found that after partially desaturating columns that had reached complete bacterial breakthrough with saturated flow (described above), while continuing to apply the

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Published in J. Environ. Qual. 30:238–245 (2001).

Abbreviations: A, cross-sectional area; AS, artificial sewage; AWI, air–water interface; c, concentration; C_{max}, maximum relative concentration; D, dispersion coefficient; L, column length; pv, pore volume; N, number of cells; Q, volumetric flow rate; R, retardation coefficient; s, suspension volume; t, time; TPC, triple-phase contact; v, average linear velocity; x, distance; μ, first-order filtration coefficient; θ, volumetric water content; τ, fraction of cells transported.

bacterial suspension, the outflow cell concentrations declined by 78%. The outflow cell concentrations began to recover after about 10 pore volumes (pv) of unsaturated flow. The cell loss was attributed to adsorption to AWIs that formed during partial desaturation. Tan et al. (1992) used constant-head horizontal infiltration experiments to apply suspensions of *Pseudomonas fluorescens* strain 2-79 to slightly moist sand. After sectioning the columns, the bacterial profiles were found to scale in terms of distance divided by the square root of time. Retardation of transport was attributed to adsorption of bacteria onto the sand surfaces, which was measured independently in batch experiments. The distribution of bacteria was adequately described without consideration of adsorption to AWIs, although cell transport in saturated and unsaturated flow was not directly compared.

Microscopy of bacterial interactions near the triple-phase contact (TPC; where air, water, and solid meet) also may contribute to understanding how variable flow affects bacterial transport. Rijnaarts et al. (1993) microscopically observed cells of one bacterial strain (*Rhodococcus* sp. strain C125) accumulating at the AWI and preferentially attaching to the solid surface at the TPC. Pitt et al. (1993) employed video microscopy to observe the spatial distribution of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* adherent to glass and polymer substrates. When the surfaces were rinsed with saline solution, the TPC created by air bubbles disrupted the spatial distribution of bacteria, removing and redepositing the cells in clumps. From these observations it appears that movement of the TPC in response to a changing θ in porous media could either decrease suspended cell concentrations by spreading the enhanced adsorption at the TPC over a larger area, or increase concentrations by sweeping adsorbed cells from solid surfaces. The current study may help resolve the mechanisms involved.

Viruses also have been reported to interact with the AWI (Powelson et al., 1990; Poletika et al., 1995) and the TPC (Thompson et al., 1998), and these findings may be relevant to bacterial adsorption because both bacterial and viral surfaces have localized polar and nonpolar regions that may interact with the AWI or TPC in a similar manner. Thompson et al. (1998) found that mixing a suspension of bacteriophage MS2 in 15-mL polypropylene tubes for 3 h with 0.7 mL of air reduced virus survival to just 2% of that in full tubes, but mixing with air in glass tubes resulted in no pronounced viral inactivation. They proposed that virus proteins projecting into the gas phase also may interact with the hydrophobic polypropylene surface at the TPC, and that virus particles may be damaged as the AWI pulls away from the polypropylene.

Three models have been developed that may help quantify the effect of changing θ on colloidal transport. Corapcioglu and Choi (1996) developed a model to describe colloidal transport in transient unsaturated flow. One of their assumptions was that colloidal adsorption is directly related to the proportion of air in the porous media. This assumption was simulated but not evaluated in the laboratory. Schafer et al. (1998) used bacterial transport data under unsaturated conditions (described above) to test two models that included first-order filtration constants to account for bacterial adhesion to the AWI. They found that the model with a filtration constant that was a function of the air content and the cell concentration at the AWI was better suited for the description of the breakthrough curves than the model with a filtration constant that was a function of the air content alone. Wan and Tokunaga (1997) developed a film-straining model for unsaturated porous media that described colloidal entrapment by water rings around contact points of grains. The model was supported by experiments on transport of latex particles through sand columns with a range of θs. Variable flow, by their model, might be expected to alternately strain and release cells, although they did not specifically address this situation.

The studies listed above suggest different mechanisms and would make different predictions for the effect of variable flow on bacterial deposition and resuspension. The comparisons of saturated and unsaturated flow found less bacterial transport in unsaturated flow in comparison with saturated flow (Wan et al., 1994; Schafer et al., 1998; Powelson and Mills, 1998). In transient water-content conditions it would be reasonable to assume that the same relationship would hold; that is, that suspended cell concentrations and θ would increase or decrease in parallel. This conceptual model is supported by the saturated-to-unsaturated column experiment of Powelson and Mills (1998) and the mathematical models (Corapcioglu and Choi, 1996; Schafer et al., 1998; Wan and Tokunaga, 1997). The inverse model, that suspended cell concentrations would increase with decreasing θ and vice versa, is supported by the column-drainage experiment of Rijnaarts et al. (1993) and, possibly, by the microscopy of a moving TPC (Pitt et al., 1993).

The current study was initiated to contribute to a better understanding of bacterial deposition and resuspension due to changes in θ. Our working hypotheses were: (i) in variable unsaturated flow suspended cell concentrations and θ would increase and decrease in parallel and (ii) in variable unsaturated flow a smaller fraction of cells would be transported than in saturated or constant unsaturated flow. These hypotheses were tested by evaluating the transport of *Escherichia coli* in three different flow regimes: saturated, unsaturated with a constant θ, and unsaturated with a variable θ. The regimes were designed to simulate bacterial transport from a household septic system under three types of flow:

- Saturated flow may occur in a thin zone below a leach line and in the capillary fringe above the water table, as well as below the water table. It also was important to include saturated flow for theoretical reasons to evaluate the effect of the solid media in cell removal.
- Constant unsaturated flow occurs in the lower part of many vadose zones where matric forces and time serve to damp fluctuations in infiltration. It also was important to compare this relatively simple

interaction between cells and AWIs with the more complex interactions in variable flows.
- Variable unsaturated flow is typical in porous media receiving wastewater discharges due to repeated 12-h cycles of heavy household water use in the morning and evening.

In a preliminary study, six ground water solutions were evaluated to determine the one that was likely to result in the most efficient cell transport, and this solution was used in the flow regime experiments.

MATERIALS AND METHODS

Sand columns were contained in polybutyrate cylinders (25.4-cm-long by 7.6-cm-i.d. liners, AMS, American Falls, ID). An end plug that fit inside the cylinders was constructed to allow extraction of bacterial suspension through a porous stainless steel disk (10-μm pore size, Mott Corp., Farmington, CT). No cells were lost due to extraction though the disk in a preliminary saturated-flow test, where samples were taken from just above the disk as well as from the outflow tubing. The cylinders were filled with sand (Granusil, Unimin Corp., Portage, WI), that had been sieved to between 0.354 and 0.710 mm, washed in distilled water, and dried. The cylinders, with the end plug in place, were wet-packed by filling the cylinder with plain solution (see below), stirring in sand, and draining, if necessary, to the required θ. The final sand column lengths were 23.6 to 23.9 cm, and bulk densities ranged from 1.75 to 1.79 g cm^{-3}. The volumetric water content (θ) of each saturated column was determined by weighing. Two water-content probes (Thetaprobe system, Dynamax, Houston, TX) were inserted through the cylinder wall, 4 cm from the ends of each unsaturated column, and the voltage outputs recorded with a data logger at 10-min intervals. Thetaprobes generate a signal and measure changes in the voltage standing wave ratio to determine the apparent dielectric content of the soil (Gaskin and Miller, 1996). The probes were calibrated after installing them in a column that had been partially saturated with the experimental solution to a known θ. The column was turned over repeatedly until the probe voltages were equal, indicating a uniform θ. This voltage and the voltage for air-dry sand were used to create a column-specific equation relating θ to voltage as described in the Thetaprobe manual.

An initial saturated flow transport study used various solutions representative of wastewater or ground water. The name, pH, ionic strength (I, mM), and composition (mg L^{-1}) of the six solutions were:

- *KCl*, pH = 7.0, I = 10, KCl (704.8), $NaHCO_3$ (4.2), KBr (59.5);
- *KCl + phosphate*, pH = 7.0, I = 10, KCl (688), KH_2PO_4 (8.89), K_2HPO_4 (11.6), KBr (59.5);
- *KCl + surfactant*, pH = 7.0, I = 10, KCl (704.5), $NaHCO_3$ (2.1), KBr (59.5), sodium dodecylbenzene sulfonate (10);
- *$CaCl_2$*, pH = 7.0, I = 10, $CaCl_2$ (352), $NaHCO_3$ (4.2), KBr (59.5);
- *Phosphate*, pH = 7.0, I = 10, KH_2PO_4 (315), K_2HPO_4 (417.5), KBr (59.5);
- *Artificial Sewage (AS)*, pH = 7.3, I = 3, KH_2PO_4 (8.5), K_2HPO_4 (21.75), Na_2HPO_4 (17.7), $CaCl_2$ (27.5), $MgSO_4$ (11), NaCl (15), NaBr (51.5), Na–humic acid (112).

The KCl solution was considered a simple solution, to which phosphate was added as a pH buffer or surfactant was added to represent detergents and other dissolved organic matter in wastewater. The $CaCl_2$ solution was used to represent water dominated by Ca^{2+}. The phosphate solution was used to strongly buffer the pH. Artificial sewage, although chemically less well defined than the other solutions, is probably more representative of wastewater. Bromide was added to these suspending solutions as a conservative tracer, and its sample concentration was determined with a Br-and-reference electrode set (Orion, Boston, MA). Each of these bromide solutions had a corresponding *plain solution*, in which Cl^- substituted for Br^- on an equimolar basis. The plain solutions were used to precondition the columns before starting an experiment. Chloride did not interfere with the Br^- analysis, except possibly in the detection of initial Br^- breakthrough, because the maximum allowable molar ratio was $Cl^-/Br^- = 400$ (Orion instruction manual).

Artificial sewage was used instead of actual sewage because the composition of sewage is highly variable and bacterial transport may be influenced by many solution characteristics, including pH, ionic strength, and dissolved organic matter (Harvey, 1991). The low concentration range of average primary municipal sewage (Pettygrove and Asano, 1985) was approximated by AS, with dissolved organic matter provided by humic acid. The humic acid was purified from a commercial source (humic acid, sodium salt, Aldrich Chemical Co., Milwaukee, WI) to remove large colloids and components that are easily available for bacterial growth. This was accomplished by dissolving 1 g of the commercial product in 400 mL biological oxygen demand buffer (Part 5210B, American Public Health Association, 1995), inoculating with a mixed microbial culture from soil, incubating for 3 d at 24°C, centrifuging (15 000 × g), and filtering (5 μm) the supernatant. (The inoculation and incubation steps were used in preparing AS in all cases except for the batch used in the solution-comparison study.) A sample of this humic-acid concentrate was dried at 105°C to determine the solids concentration and the volume of concentrate needed to provide the 112 mg L^{-1} Na–humic acid in the AS, which corresponded to a dissolved organic matter concentration of 105 mg L^{-1} after accounting for the manufacturer's analysis of 6.2% Na^+.

The sand columns were preconditioned and pumping rates established with the plain version of the experimental solution. The inflow suspension was pumped through a 10-μm porous disk to insure no large cell clusters reached the soil, then through a manifold and seven microtubes (0.25 mm i.d.), thereby applying the suspension uniformly over the upper surface of the column (Fig. 1). For the saturated columns, a pond <1 cm deep was maintained on the column surface. After passing through the sand, suspension passed through the outflow porous disk and into a fraction collector. Peristaltic pumps and Tygon tubing were used to regulate inflow to and outflow from the column.

In saturated and constant unsaturated flow, matched inflow and outflow pumps were used to maintain steady-state flow and water content conditions. The water contents in the two constant unsaturated flow columns differed more than expected (average θs were 0.0745 for Column A and 0.0944 for Column B, Table 1). Nevertheless, these columns were sufficiently similar to be considered replicates for comparison with the saturated and variable unsaturated flow columns. The flow rate for the saturated columns was about 3.4 times that of the unsaturated columns to make average v approximately the same in all flow regimes (2.79 cm h^{-1} for saturated flow, 3.49 cm h^{-1} for constant unsaturated flow, and 2.47 cm h^{-1} for variable unsaturated flow, Table 1). At the beginning of an experiment (time zero), the plain solution was switched to the solution with Br^- and cells, thereby creating a step-input of the tracers. In the case of the saturated columns, the surface pond was allowed to drain without allowing air into the sand before starting the suspension of Br^- and cells. At the end of the saturated tests the inflow pump was turned off while the

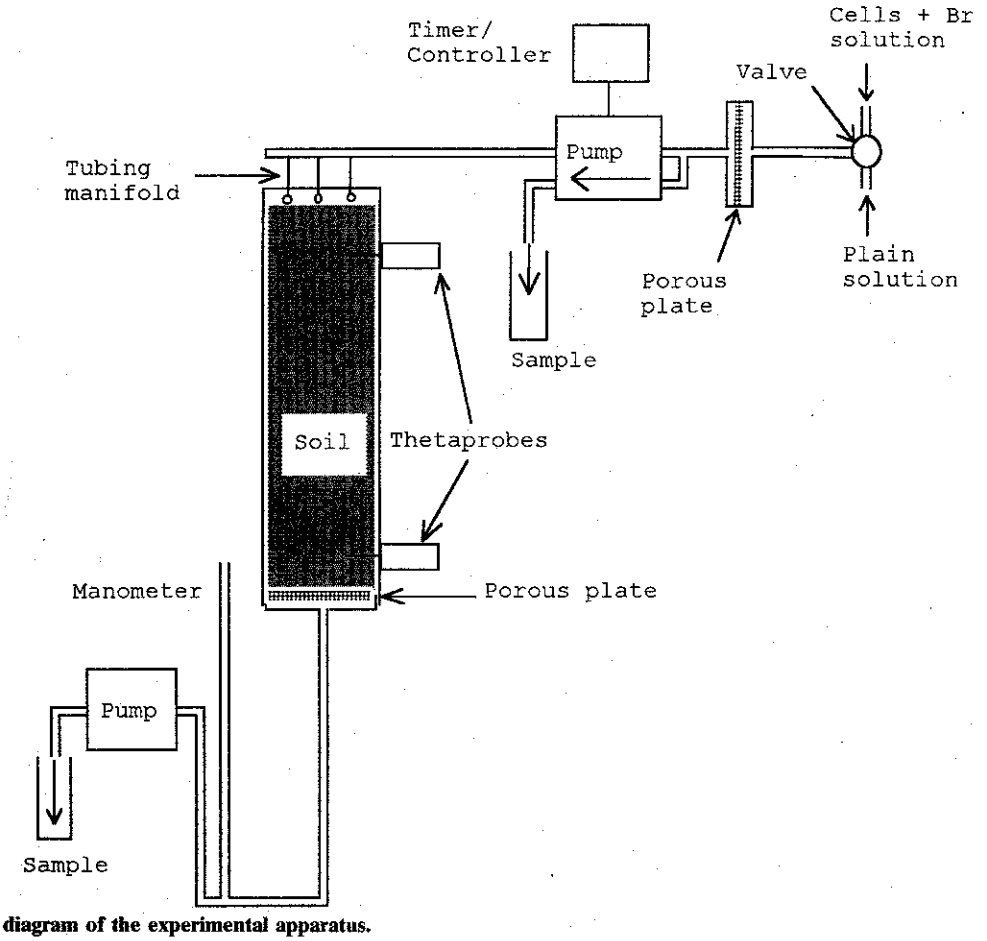

Fig. 1. Schematic diagram of the experimental apparatus.

outflow pump drained the column at a constant rate. Column methods for the solution-comparison preliminary study were the same as for the saturated columns described above and in Table 1, except that there was a wider range in flow rates between experiments (Q ranged from 35.7 to 42.7 mL h^{-1}).

In the variable unsaturated flow regime the inflow pump was turned on for 2 h and off for 10 h by a lab timer–controller, while the outflow was pumped at a constant rate (Table 1). The flow rates were set so that the total inflow and outflow volumes were equal in each 12-h cycle. By restricting outflow with a pump, the variation in θ was greater than would be expected under natural drainage. For example, outflow would normally increase after an inflow period, so that the increase in θ would be less than when the outflow is restricted with a pump. Nevertheless, the experimental variations in θ, although having a greater amplitude, were a reasonable approximation of natural variations, and especially for those soils that have a restrictive layer that limits outflow.

Table 1. Bacterial inflow concentrations and column water conditions.

Flow regime	Replicate	Inflow bacteria (c_o)†	Flow rate (Q)			Water content (θ)‡		Water velocity (v)§
		10^8 cells mL^{-1}	mL h^{-1}					cm h^{-1}
Saturated	A	1.31	37.7			0.326		2.76
	B	1.57	39.2			0.333		2.81
						Upper θ	Lower θ	
Constant unsaturated	A	2.18	12.3			0.0808	0.0682	3.94
	B	1.90	12.0			0.0768	0.112	3.04
Variable unsaturated			Inflow		Outflow (Q)			
			2 h¶	10 h¶	12 h¶			
			mL h^{-1}					
	A	2.60	67.9	0	11.3	0.0456	0.138	2.94
	B	1.97	54.1	0	9.02	0.0543	0.161	2.00

† Actual values equal reported values times the indicated factor.
‡ The average θ over the course of the experiment. In the unsaturated cases, upper θ was measured 4 cm from the top of the column, and lower θ was measured 4 cm from the bottom of the column.
§ Average linear velocity = $v = Q/A/\theta$, where A is the cross-sectional area (41.85 cm^2). In the unsaturated cases, average θ was used and the velocity was denoted by v_{avg}.
¶ Flow periods in each 12-h cycle.

Escherichia coli (ATCC 25922) was the experimental bacterial strain. An antibiotic-resistant mutant of this strain was used by Ijzerman et al. (1993) in a field study of bacterial transport from a septic leach line. *E. coli* is a well-characterized, Gram-negative, typical representative of the coliform bacteria, which are used as indicators of fecal contamination. The cells were motile and approximately 1 × 2 μm in size. To provide a uniform inoculum for each experiment, a stock culture was grown in nutrient broth (Difco, Detroit, MI) plus 10% glycerol to late log phase, dispensed in 1-mL aliquots in sterile capped tubes, and frozen at −10°C. The experimental culture for a column study was started from one of these frozen inocula in 100 mL nutrient broth with 1.9 × 10^6 Bq ^{14}C-acetate to radiolabel the cells, and grown to early stationary phase. Cells were separated from unincorporated ^{14}C and suspended in the experimental bromide solution by two cycles of centrifugation. To enumerate the cells, samples were filtered using 0.2-μm polycarbonate membranes and counted by liquid scintillation. Daily samples of the inflow suspension also were stained with acridine orange, filtered, and directly counted under the microscope so that the decays per minute mL^{-1} determined by scintillation counting could be converted to cells mL^{-1}, and to confirm that the cells looked normal.

Breakthrough curves from the saturated and constant unsaturated columns were compared by fitting the CXTFIT instantaneous-equilibrium model (Toride et al., 1995) to the data:

$$R\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - v\frac{\partial c}{\partial x} - \mu c \qquad [1]$$

where R is the retardation coefficient, c is concentration of Br^- (M) or cells (cells mL^{-1}), t is time (h), D is the dispersion coefficient (cm^2 h^{-1}), x is distance (cm), v is average linear velocity (cm h^{-1}), and μ is the filtration coefficient (h^{-1}). The equilibrium model was selected over kinetic models because outflow concentrations appeared to stabilize at steady-state values, which is consistent with first-order filtration. CXTFIT estimates transport parameters by using a nonlinear least-squares parameter optimization method. D for each column was determined from the Br^- breakthrough curve (setting $\mu = 0$), so that the fitted parameters for cell breakthrough were R and μ. The fitted cell-concentration curve was used to determine the maximum outflow relative concentration (C_{max}). The model of Corapcioglu and Choi (1996), which has the potential to describe bacterial transport in variable as well as steady-state flow regimes, was not used because of its assumption that suspended cell concentrations would decrease with decreasing θ, whereas the opposite was observed in the variable unsaturated flow experiments.

The total fraction of cells transported (τ) was calculated as the cumulative number of cells recovered in the outflow (N_{out}), divided by the cells added, less the cells remaining in the column:

$$\tau = \frac{N_{out}}{c_o(s_{tot} - s_{col})}$$

$$N_{out} = \sum_{n=1}^{m} (s_n - s_{n-1})(c_n + c_{n-1})/2 \qquad [2]$$

where c_o is the inflow cell concentration (cells mL^{-1}), s_{tot} is the total suspension volume added to the column (mL), s_{col} is the suspension volume remaining in the column at the end of the experiment (mL), s_n is the cumulative outflow suspension volume (mL) collected at sample number n, c_n is the cell concentration (cells mL^{-1}) at sample number n, and m is the total number of samples. For the case of the variable unsaturated flow columns, s_{col} is the volume remaining at the end of the last inflow period, and m is the sample total at the end of the last inflow period.

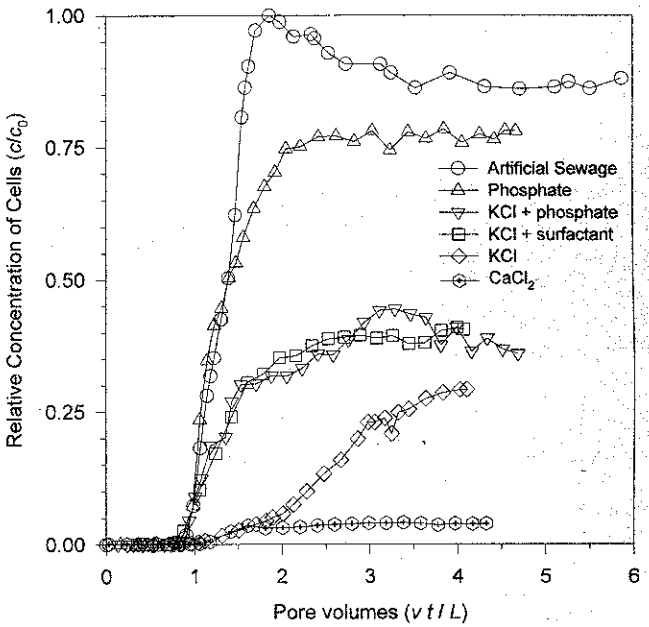

Fig. 2. Relative cell concentrations in six suspending solutions during saturated transport.

Significant effects of saturated and constant unsaturated flow on D, R, μ, and C_{max}, and of all three flow regimes on τ were determined by analysis of variance and Duncan's multiple range test (SAS Institute, 1990).

RESULTS AND DISCUSSION

Six solutions representative of ground water during wastewater infiltration were compared under saturated flow conditions to identify the one to use in the flow-regime study. Because of this limited objective, the treatments were not replicated. Use of AS resulted in the greatest relative concentration of cells transported (Fig. 2) and it was the most realistic solution; consequently, AS was used in the flow-regime study. Enhanced cell transport in AS may have been due to lower ionic strength and the presence of humic acid in comparison with the other solutions. The C_{max} reached with AS in this study (0.884) was greater than that achieved in the other saturated flow experiments (average C_{max} = 0.803, Table 2) that were part of the flow-regime study, possibly because the humic acid in this batch of AS was not pre-incubated to remove available nutrients, and growth of indigenous cells in the column may have reduced the number of available adsorption sites. Phosphate and surfactant enhanced the transport of *E. coli*, and Ca^{2+} reduced transport, relative to the simple KCl solution (Fig. 2).

In saturated flow using AS, cell breakthrough was slightly retarded relative to Br^- and had a small filtration coefficient (average R = 1.13 and average μ = 0.0259 h^{-1}, Table 2), resulting in less than complete breakthrough (Fig. 3). In Column A the maximum cell concentration was reached early in the experiment (c/c_o = 0.873 at 2.51 pv) and declined steadily to c/c_o = 0.676 at 7.79 pv. In Column B the cell concentration

Table 2. Results of model fits and cumulative cells transported.

Flow regime	Replicate and mean	Fitted values†				Cell-transport fraction‡
		D	R	μ	C_{max}	τ
		$cm^2\ h^{-1}$		h^{-1}		
Saturated	A	0.135	1.22	0.0292	0.780	0.765
	B	0.150	1.03	0.0226	0.826	0.847
	$\bar{x}$	0.142a	1.13a	0.0259a	0.803a	0.806a
Constant unsaturated	A	4.25	1.39	0.0826	0.616	0.605
	B	3.38	1.48	0.0560	0.651	0.629
	$\bar{x}$	3.82b	1.44a	0.0693a	0.633b	0.617b
Variable unsaturated	A					0.743
	B					0.724
	$\bar{x}$	nd	nd	nd	nd	0.734a

† Dispersion coefficient (D, from Br^- breakthrough curves), retardation (R), and filtration coefficient (μ) from fitting Eq. [1]; and the fitted maximum relative concentration (C_{max}). These parameters were not determined (nd) for the variable unsaturated flow study. Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

‡ Relative recovery of cells from the cumulative outflow (Eq. 2). Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

was nearly constant ($0.843 \leq c/c_o \leq 0.871$) from 2.33 to 7.46 pv. This behavior is consistent with a first-order filtration process, possibly enhanced in Column A by partial clogging of pores and increased filtration over the course of the experiment. When inflow was stopped and the columns became partially unsaturated, a sharp increase in cell outflow concentration was observed after one pv. In the case of Column B, the outflow increased to $c/c_o = 1.40$. It is likely that as AWIs invaded the saturated sand, some cells adsorbed to the sand were dislodged. Consequently, the advance of the *drying front* added cells to the suspension and resulted in an increase in outflow concentrations.

The constant flow unsaturated columns maintained a nearly constant outflow concentration from about 3 to more than 14 pv (Fig. 4), consistent with the first-order filtration model. The retardation and filtration coefficients were not significantly different from those determined in saturated flow (Table 2). The fitted maximum relative concentration was significantly less in unsaturated flow ($C_{max} = 0.663$) as compared with saturated flow ($C_{max} = 0.803$). The average fraction of cells transported (0.617) also was significantly less than in saturated flow (0.806). These results are consistent with the earlier studies that showed that the presence of AWIs reduced bacterial transport (Wan et al., 1994; Schafer et al., 1998; Powelson and Mills, 1998; Jewett et al., 1999).

It was surprising that the effect of unsaturated conditions on C_{max} was significant, while its effect on μ was not, because μ is approximately related to C_{max} in steady-state conditions ($\partial c/\partial t = 0$, and assuming $D = 0$, in Eq. [1]) by:

$$C_{max,ap} = \exp(-\mu L/v) \qquad [3]$$

where $C_{max,ap}$ is the approximate value of C_{max} and L is column length. There was some difference in v between the two unsaturated columns (Table 1), and, with μ constant, a greater v should have resulted in a greater C_{max}. But in the experiment, Column A had a greater v

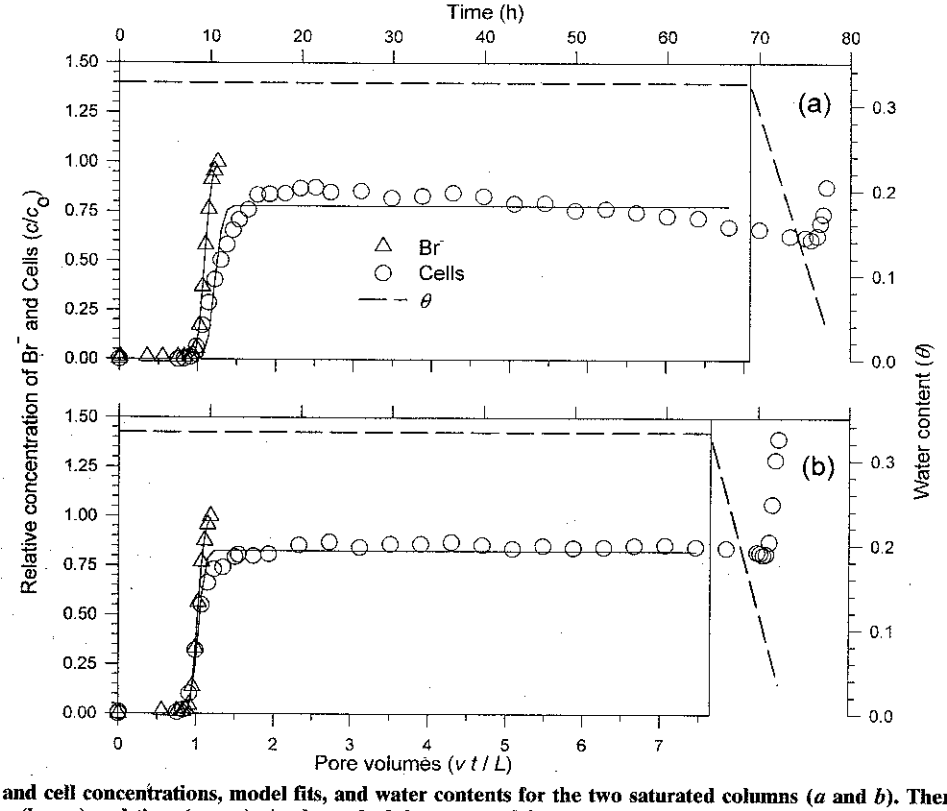

Fig. 3. Relative Br^- and cell concentrations, model fits, and water contents for the two saturated columns (*a* and *b*). There are two horizontal axes: pore volumes (lower) and time (upper). At the end of the saturated flow period inflow was stopped while outflow continued, resulting in a sharp drop in θ. After this point (indicated by the vertical line) the pore volume axis is not applicable.

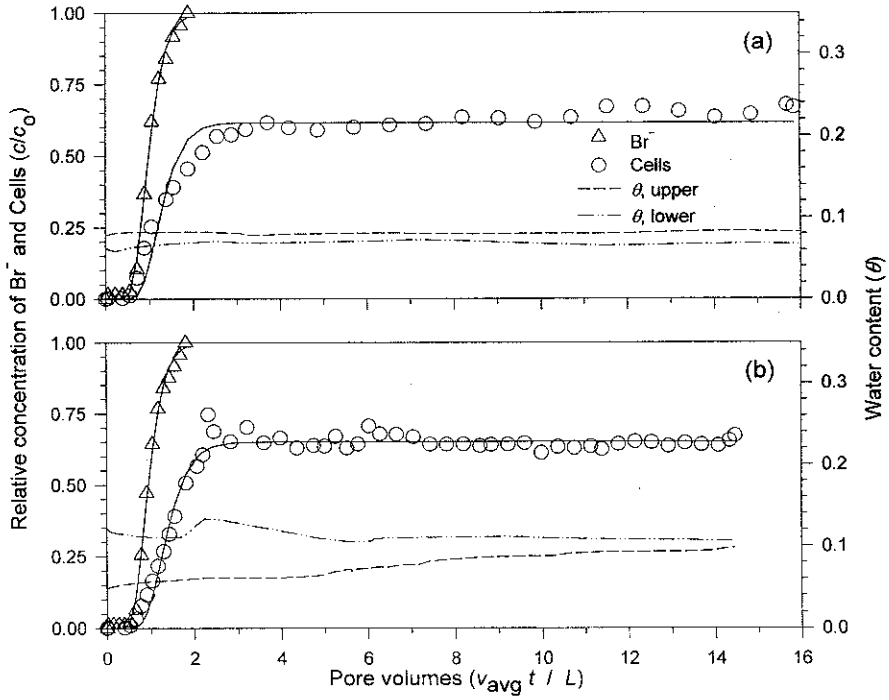

Fig. 4. Relative Br^- and cell concentrations, model fits, and water contents for the two constant unsaturated flow columns (*a* and *b*). The average residence time for one pore volume was 6.00 h for Column A and 7.83 h for Column B.

and a lower C_{max} (Table 2). This resulted in a large variance in unsaturated μ values and a nonsignificant effect. Additional studies are needed to determine if this discrepancy was due to experimental error, or if in fact that L is more important than residence time (L/v) in determining C_{max} and the model needs to be modified.

In variable unsaturated flow after the second inflow pulse, cell concentrations oscillated about average values (Fig. 5). For Column A the average during this period was c/c_o = 0.784, and for Column B it was

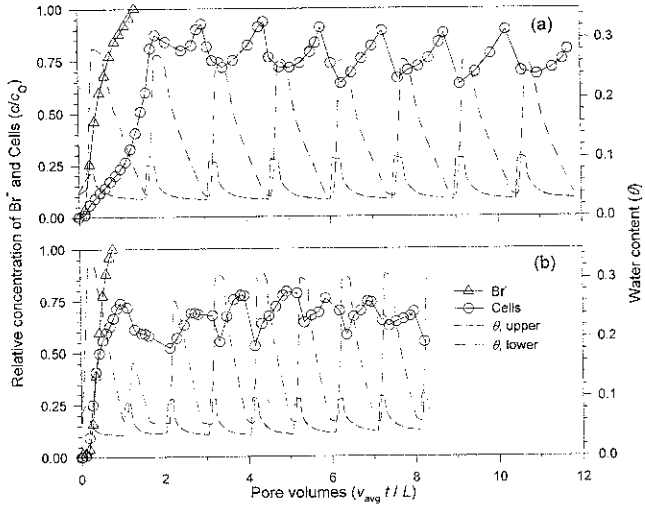

Fig. 5. Relative Br^- and cell concentrations and water contents for the two variable unsaturated flow columns (*a* and *b*). The average residence time for one pore volume was 8.13 h for Column A and 11.9 h for Column B. Both columns had 12-h cycles of inflow, but the pore volumes per cycle differed between columns due to differences in flow rate and water content (Table 1).

c/c_o = 0.671. Column B may have had a lower average concentration in part because the outflow had inadvertently increased to from 8.5 to 10.9 mL h^{-1} from 0.9 to 1.6 pv, resulting in a lower θ from 0.9 to 2.5 pv (Fig. 5b). Peak outflow concentrations in Column A (Fig. 5a) exceeded the peak concentration reached in saturated flow (c/c_o = 0.873, Fig. 3a), whereas peak concentrations in Column B were less, with c/c_o ranging from 0.690 to 0.792 (Fig. 5b). Outflow cell concentrations increased as the θ in the lower part of the column decreased. This is consistent with the increase in concentration observed at the end of the saturated studies as the AWI approached the outlet (Fig. 3). When the θ increased as a result of a new inflow pulse, cell concentrations decreased sharply. Fitted values of D, R, μ, and C_{max} could not be determined in variable conditions because CXTFIT requires steady-flow conditions. The fraction of cells transported in variable unsaturated flow (τ = 0.734) was significantly greater than that in constant unsaturated flow (τ = 0.617), and not significantly different from that in saturated flow (τ = 0.806) (Table 2).

The results did not support our first hypothesis, that in variable unsaturated flow suspended cell concentrations and θ would increase and decrease in parallel. In fact the opposite response was observed; that is, cells exhibited variable outflow concentrations that were out of phase with θ (Fig. 5). This observation may be important in understanding the mechanisms of cell adsorption and desorption in unsaturated flow. When a column was draining the TPCs advanced into the water phase. It is likely that this dislodged cells adsorbed to the solid surface, thereby increasing the suspended cell concentration. This mechanism is consistent with the observations of Pitt et al. (1993), and with the column results

of Rijnaarts et al. (1993). When a new pulse of water entered the column the TPCs reversed direction. It is likely that this allowed cells to contact adsorption sites on the solid surfaces that had been previously "swept clean" by the TPCs, thereby lowering the suspended cell concentration.

The inverse relationship between θ and suspended cell concentration during cycles of wetting and drying was unexpected because the results of most of the studies relating to the unsaturated transport of colloids were interpreted to support greater cell adsorption and filtration with lower θ (Wan et al., 1994; Schafer et al., 1998; Jewett et al., 1999). The results of the current study also appear to be in conflict with the observation in our previous study that after air entered a saturated column, cell concentrations declined sharply (Powelson and Mills, 1998). However, in our previous study unsaturated flow samples were taken after extracting 36 mL to partially desaturate the columns. The 36-mL volume was discarded, which was unfortunate because, based on the current results, this volume was likely to have had a high cell concentration. Some of the apparently contradictory results regarding AWIs and bacteria may be due to two opposing effects of air entry into a saturated region: adsorption of cells to the AWI, and the TPC sweeping cells from the solid surface. The net effect may depend on the frequency of changes in θ, with short cycles favoring the sweeping process and more cell transport.

The results did not support our second hypothesis, that variable unsaturated flow would result in a smaller proportion of cells transported than with saturated or constant unsaturated flow. Variable unsaturated flow was not significantly different from saturated flow in this regard, and it was not as effective as constant unsaturated flow in reducing cell transport. Because constant unsaturated flow resulted in significantly fewer *E. coli* cells transported, this flow regime is likely to be the best for soil-aquifer treatment of wastewater to achieve maximum removal of pathogenic bacteria. Variable flow, which may be more typical of household and municipal wastewater discharges, could be converted to constant flow by releasing stored wastewater at a steady rate.

There are many differences between real-world wastewater leach fields and the clean sand columns used in this study, including variations in soil texture and structure, the effects of extensive biofilms on bacterial adsorption, and protozoan predation (Harvey, 1991). Nevertheless, because these results indicate that flow regime has significant effects on bacterial transport, and because it may be feasible to change actual wastewater flow regimes, further laboratory and field testing of this effect is warranted.

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