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WATER SATURATION AND SURFACTANT EFFECTS ON BACTERIAL TRANSPORT IN SAND COLUMNS

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Bacterial breakthrough curves from clean sand columns were used in two related studies of the effects of unsaturated flow and added surfactant on cell transport. In the first study, step-inputs of bacteria were used to evaluate the effects of water saturation (saturated and unsaturated condition), surfactant concentration (0 and 20 μmol/L sodium dodecylbenzene sulfonate (DDBS)), and bacterial strain (aquifer isolates E3W7 and W31) on the kinetics of cell deposition and entrainment. These surfactant concentrations and bacterial strains did not have significant effects on the rate coefficients. Unsaturated columns, however, always had delayed cell breakthrough compared with saturated columns, and the deposition rate coefficient was significantly greater in unsaturated conditions compared with saturated conditions. After the outflow concentration of E3W7 from the saturated columns reached the inflow concentration, the columns were partially desaturated. This resulted in a rapid drop in outflow concentration—a decline of 29% with surfactant and 78% without surfactant. A second study was conducted to investigate the mechanism of surfactant-enhancement of bacterial transport by applying 100 μmol/L DDBS before a pulse of W31, concurrent with the pulse, following the pulse, at all times, or at no time. Only the presence of surfactant at all times and concurrently with the cell pulse resulted in significantly greater cell recovery compared with the no-surfactant control. This suggests that 100 μmol/L DDBS interacted with the cells to reduce their adsorption to gas-water interfaces in unsaturated sand. (Soil Science 1998;163:694–704)

Key words: Bacterial transport, bioaugmentation, unsaturated transport, surfactant, gas-water interface, adsorption kinetics.

UNDERGROUND gas-water interfaces (GWIs) may be important microbial habitats that have not been well characterized (Wan et al. 1994; Mills and Powelson 1996, Powelson and Mills 1996). In addition to unsaturated soil and the vadose zone, GWIs are associated with gas bubbles trapped in the capillary fringe, and they may develop in regions of biogenic gas production deeper in an aquifer. The understanding of basic microbial ecology, soil treatment of waste water, bioremediation of ground-water contaminants, and modeling of microbial transport may be improved as more information is gained on the interaction of bacteria and GWIs.

Many factors could influence cell-GWI interactions and, consequently, bacterial transport in porous media. These include the degree of water saturation, bacterial strain, and presence of surfactants. In porous media, unsaturated water conditions have usually been found to reduce the survival and transport of microbes (Powelson and Gerba 1995). Recovery of pulses of bacteria from unsaturated sand columns was 21 to 52% less than recovery from saturated columns (Wan et al. 1994). Different bacterial types adhere to surfaces to different degrees and by different mechanisms (Fletcher 1996). Percolation of 19 strains of bacteria through saturated 5-cm columns of loam resulted in recoveries of 0.01 to 15% (Gannon et al. 1991). The surfactant sodium dodecylbenzene

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sulfonate (DDBS) was found to increase bacterial transport in saturated soil (Jackson et al. 1994) and to reduce adsorption of bacterial cells to the GWI in a glass dish (Powelson and Mills 1996).

It is unclear how surfactant affects bacterial adsorption. Possible mechanisms for surfactant-enhanced bacterial transport in unsaturated soil include: (i) blocking adsorption sites at the GWI, in which case surfactant treatment before arrival of bacteria should be most effective in improving transport; (ii) blocking hydrophobic sites on bacterial cell surfaces and making the cell less likely to adsorb to the GWI, in which case surfactant application concurrent with cells should be most effective; or (iii) loosening cells bound to the GWI, in which case surfactant treatment following a pulse of bacteria should be most effective.

The research presented here evaluated the effects of water saturation, bacterial strain, and surfactant on bacterial transport through sand columns. In the first study, the effects of two levels of these three variables were evaluated using a step-input of cells. A one-site kinetic transport model was fitted to bacterial breakthrough data (Hornberger et al. 1992), and the fitted first-order rate coefficients for deposition (k_c) and entrainment (k_y) were used as dependent variables in a three-way multivariate ANOVA. In the second study, the mechanism of surfactant-enhancement of cell transport was further evaluated by changing the timing of surfactant application relative to pulses of bacteria and comparing the cumulative cell recovery in the column outflow.

MATERIALS AND METHODS

Bacteria

Two bacterial isolates were obtained from a sandy, coastal plain aquifer (1 km north of Oyster, VA). Cells in stationary phase were used for all characterizations and experiments.

Strain E3W7 was selected from among the first series of aquifer isolates because it was nonmotile. E3W7 is a Gram-positive rod, 2 μm × 1 μm in size. Its electrophoretic mobility was −1.0 μm cm/(V s) in NaH_2PO_4 buffer adjusted to pH 5.7 and 8.3 mmol/L ionic strength. A drop of distilled water formed a contact angle of 32° with a layer of E3W7 cells. Liquid cultures were started by inoculation from single colonies into half-strength peptone-yeast extract (1/2 PYE: 0.125 g peptone, 0.125 g yeast extract, 15 mg $MgSO_4 \cdot 7H_2O$, 1.3 mg $CaCl_2$ in 1 L of distilled water) and grown for 3 days at 20 to 25 °C on a rotary table at 125 rpm. This dilute growth medium was used to approximate the nutrient conditions found in the aquifer. Cells were suspended in an experimental buffer (described below in "Experimental Solutions and Sand") by two cycles of centrifugation (16,000 × *g*) and resuspension. Bacterial suspensions were adapted overnight in the experimental buffer. Before beginning an experiment, suspensions were filtered through a 5-μm porous stainless steel plate to limit the size of any cell aggregates. Bacteria were enumerated for the first study by the acridine orange direct-count procedure (Murray et al. 1994).

Isolate W31 was selected from a later series of aquifer isolates because the nonmotile cells were smaller than those of E3W7 and because W31 would be used in a future field injection experiment. W31 is a Gram-positive coccus with a diameter of 0.9 μm. Its electrophoretic mobility was −0.44 μm cm/(V s) in NaH_2PO_4 buffer adjusted to pH 5.7 and 9.0 mmol/L ionic strength. A drop of water formed a contact angle of 25° with W31 cells. W31 was grown in the same liquid medium and in the same conditions as E3W7 except that the resistance of W31 to nalidixic acid was utilized to reduce the possibility of contamination (50 mg nalidixic acid was dissolved in 3.3 mL of 0.1 mol/L NaOH and added to 1 liter of 1/2 PYE, and the pH was adjusted to 7.2 with about 0.4 mL 0.05 mol/L H_2SO_4). Centrifugation and resuspension of W31 was optimal at 5800 × *g*; otherwise, preparation and enumeration of W31 was the same as for E3W7.

Some indigenous cells (< 0.001 of column inflow concentration, c_o) were observed in the column outflow before introduction of E3W7 or W31 because the sand and some of the equipment, although cleaned as described below in "Apparatus and Procedure," were not sterile. A control experiment was conducted using mixed-batch reactors to determine if the indigenous cells or the experimental strains would grow in the experimental sand and buffer (DDBS-plus-Br^- buffer, described below). Inoculation of the indigenous cells was accomplished by passing buffer through the experimental cylinder, porous plate, and tubing and adding it to nonsterile sand. W31 and E3W7 suspensions were prepared as described above, and inoculated into sterile buffer and sand. Cell concentrations decreased slightly over 6 days (the maximum time for an experiment), but the regression slopes were not significantly different from zero ($P = 0.05$), indicating that the indigenous, W31, and E3W7 cells did not grow in the experimental columns. The lack of indigenous cell growth and the level of

precision imposed by volumetric measurements (two significant figures) meant that any differential treatment effects on the indigenous cell concentration could be ignored. Consequently, the initial indigenous cell concentration was subtracted from the measured acridine-orange cell concentrations to determine the outflow cell concentrations (c) of W31 or E3W7.

Bacteria were enumerated for the second study using cell-associated ^{14}C determined by liquid scintillation counting. Radiolabeling was used to improve resolution in these shorter-term tests. Only strain W31 was used in the second experiment. Cell suspensions were prepared in the same way as for the first study except that a culture was started by adding 1 mL of a frozen culture to 100 mL PYE plus 0.1 mCi of uniformly labeled ^{14}C-D-glucose (NEC-042B, DuPont, Boston, MA). Cell-associated radioactivity was determined by filtering a 1-mL sample (0.2 μm polycarbonate membrane, Osmonics, Livermore, CA), washing the filter with distilled water, and measuring ^{14}C activity in dpm (LS6500, Beckman Instruments, Inc., Fullerton, CA). The input concentration was also determined by the acridine orange direct-count procedure so that cells/dpm could be calculated and cell concentrations could be reported as cells/mL.

Experimental Solutions and Sand

Three buffers were used in the first study. Plain buffer, 8 mmol/L NaH_2PO_4 and 0.5 mmol/L $NaNO_3$ (ionic strength adjustor), was used in the columns before introducing cells. The Br^- buffer consisted of 8 mmol/L NaH_2PO_4 and 0.5 mmol/L NaBr, used as a conservative tracer. The Br^--plus-DDBS buffer consisted of Br^- buffer plus 0.02 mmol/L DDBS surfactant. DDBS (sodium dodecylbenzene sulfonate, Sigma Chemical Co., St. Louis, MO) is an anionic surfactant with a critical micelle concentration of 1.5 mmol/L. The molarity of DDBS was calculated by assuming that the material obtained from the supplier (20% other alkylbenzenesulfonates) had an average molecular weight equivalent to that of DDBS, i.e., 348.5 g/mol. All buffers were adjusted to pH 5.7 with NaOH, resulting in a final ionic strength of 9 mmol/L, and then autoclaved. This pH was selected to approximate that of the groundwater where the bacterial strains were obtained.

Two buffers were used in the second study. The plain buffer consisted of 6.60 mmol/L KH_2PO_4 and 1.20 mmol/L K_2HPO_4; the DDBS buffer consisted of 6.04 mmol/L KH_2PO_4, 1.52 mmol/L K_2HPO_4, and 0.10 mmol/L DDBS. All buffers were adjusted to pH 5.7 with H_3PO_4, resulting in a final ionic strength of 9 mmol/L, and then autoclaved.

A single batch of sand (Granusil, Unimin Corporation, Portage, WI) to fill all of the columns was sieved to 354 to 710 μm in diameter. Ten percent of the sieved sand by mass was less than 460 μm in diameter. The sand was cleaned thoroughly to remove reactive materials that could enhance cell adsorption, thereby improving resolution of other removal mechanisms in unsaturated flow. The sand was first soaked in 10% HNO_3 for 24 h and then rinsed in distilled water; next it was soaked in 0.5 mol/L NaOH for 2 h and rinsed in distilled water; finally, any remaining iron oxyhydroxides were removed by citrate-dithionate extraction (Kunze and Dixon 1986), followed by thorough rinsing with distilled water and drying.

Apparatus and Procedure

Each sand column was contained in a cylinder constructed of two 60-cm^3 polypropylene syringe barrels (Fig. 1). The inside of the cylinder was roughened with sandpaper to reduce water flow along the cylinder wall in the saturated tests. A porous stainless steel disk (22-mm diameter, 10-μm pore size, Mott Metallurgical Corporation, Farmington, CT) was glued inside the funnel end of each syringe barrel with epoxy. All tubing was polyvinyl chloride (Tygon® R-3603), and plastic 3-way valves directed flow in the tubing. The inflow tube was connected to a stainless steel infusion needle that discharged the suspension against the sand side of the inflow porous disk. A manometer tube was attached to the syringe fitting on the other side of the inflow disk. The outlet tube and sampling valve were attached to the outlet syringe fitting; the outlet tube also served as a manometer. A gasket was sealed between the open ends of the syringe barrels with silicone grease. Preliminary transport experiments with E3W7 and W31 using the apparatus without sand were conducted to ensure that there was no loss of cells as a result of the porous disk or other components. Between experiments, the apparatus was cleaned and disinfected with 70% ethanol, except for the valves and assemblies containing epoxy glue, which were soaked for 2 h in 15% HNO_3 and rinsed with sterile distilled water.

The cylinder was filled with air-dry sand by pouring it through a port in the side of the cylinder, resulting in a bulk density (ρ_b) of 1.75 ± 0.03 g/cm^3 (mean ± SD). The cylinder was weighed before and after filling with sand to determine ρ_b, and daily during the experiment, to determine the volumetric water content, θ (water vol-

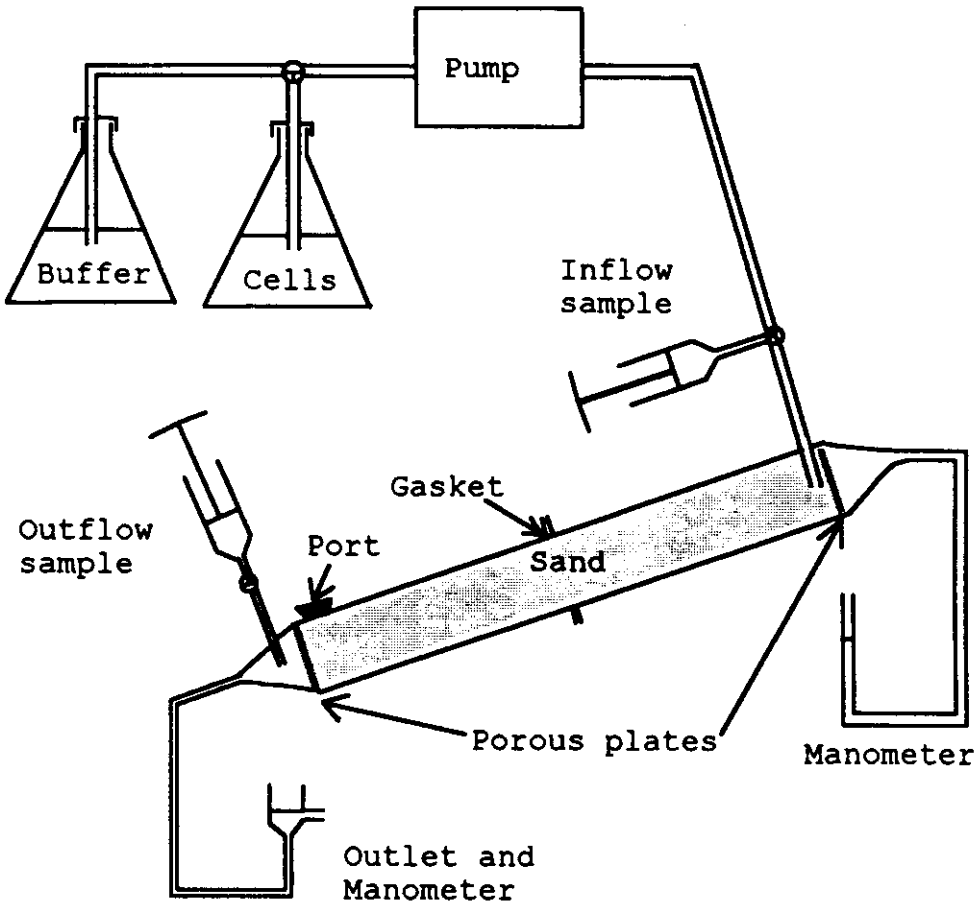

Fig. 1. Schematic of the experimental apparatus. The column is shown in the tilted position used for unsaturated flow; for saturated flow the column was vertical with the inlet at the bottom.

ume/bulk sand volume). The sand columns had a length (L) of 26.8 cm and a diameter of 2.6 cm.

All columns were run with a flow (q) = 7.4 mL/h, which provided a flux (J_w) = 1.4 cm/h. To obtain saturated conditions, the dry sand in the column was flushed with CO_2 and saturated from the bottom with plain buffer, degassed by autoclaving immediately before use. Saturated columns were run vertically, with the inlet at the bottom and the tubing outlet 2 cm above the top of the column to assure that the pressure potential (h_p) was >0 throughout the column. Saturated θ ranged from 0.37 to 0.33 (Table 1). The unsaturated water content was made reasonably uniform along the column by adjusting the angle of the column (to about 27° from horizontal, Fig. 1) so that the inflow h_p equalled the outflow h_p. For the unsaturated experiments, the average column h_p was -13 ± 2.7 cm, and θ ranged from 0.059 to 0.099 (Table 1). Columns were equilibrated overnight at the experimental θ with plain buffer.

To start an experiment in the first study, steady-state flow conditions were established with plain buffer, and at time-zero, the inflowing buffer was switched to the experimental cell-buffer suspension (step-input method). Samples were taken in plastic syringes from three-way valves located in the inflow tubing and just past the column outflow disk (Fig. 1). To determine average c_o, two inflow samples were taken daily; outflow samples were taken more frequently, especially during initial breakthrough. Air was removed from the sample syringes, and the cells were counted within 24 h. Br^- concentrations were determined with a Br–and-reference electrode set (Orion, Boston, MA). After the saturated columns with E3W7 reached complete cell breakthrough, the columns were partially desaturated by putting the columns in the unsaturated configuration while maintaining the cell-suspension inflow. This resulted in a rapid outflow of about 36 mL, which was disregarded when plotting the breakthrough curves.

TABLE 1
Variables and model-fitted parameters for the first study

Strain	DDBS (μM)	Water†	Rep.	c_o (10^6 cells/mL) (mean ± SD)	θ‡	α§ (cm)	k_c (/h)	k_y (/h)	R^2¶
E3W7	0	sat	a	15 ± 8.6	0.35	0.16	0.19	0.16	0.96
			b	13 ± 4.1	0.33	0.20	0.19	0.15	0.87
E3W7	0	unsat	a	18 ± 4.1	0.083	3.1	4.2	0.30	0.95
			b	17 ± 5.6	0.083	3.0	1.6	0.032	0.71
E3W7	20	sat	a	22 ± 3.2	0.34	0.39	0.049	0.19	0.94
			b	28 ± 2.8	0.36	0.71	0.13	0.53	0.96
E3W7	20	unsat	a	19 ± 2.1	0.059	3.3	0.98	0.14	0.88
			b	20 − na‖	0.099	2.0	2.1	1.7	0.95
W31	0	sat	a	39 ± 6.2	0.34	0.16	0.19	0.046	0.92
			b	36 ± 2.6	0.34	0.10	0.20	0.035	0.90
W31	0	unsat	a	29 ± 5.8	0.086	2.0	2.0	0.026	0.89
			b	30 ± 11	0.083	4.6	2.1	0.028	0.71
W31	20	sat	a	25 ± 2.5	0.37	0.12	0.054	0.0082	0.90
			b	29 ± 5.7	0.34	0.10	0.13	0.084	0.86
W31	20	unsat	a	14 ± 1.8	0.066	5.1	4.0	0.033	0.84
			b	14 ± 1.2	0.076	1.4	3.3	0.050	0.93

†Column water condition: sat = saturated, unsat = unsaturated.
‡Volumetric water content.
§Dispersivity (D/v) determined from the column Br^- breakthrough curve.
¶Coefficient of determination for regression of observed versus predicted values.
‖Not applicable. This experiment lasted only 6 hours, and only one c_o sample was taken.

The apparatus and procedures were the same for the second study with the following exceptions. A column was given one of five types of DDBS-buffer treatment: treated before the cell pulse (Pre), treated concurrently with the cell pulse (Con), treated following the cell pulse (Fol), treated at all stages (All), and never treated (Non). Plain buffer was used at the other stages (Table 2). The columns were equilibrated with the prepulse buffer to an unsaturated θ of 0.16, and maintained at $0.15 \leq \theta \leq 0.19$ during the course of the experiment. Column outflow was pumped into a fraction collector (30 min/tube). An experiment was started by switching the inflow from the prepulse buffer to the cell-buffer suspension, and after about 1.6 pore volumes, the inflow was switched to the after-pulse buffer (pulse-input method). Inflow samples were taken at the beginning and end of the pulse and averaged to determine c_0.

Transport Modeling and Statistics

The breakthrough curves from the first study were compared by fitting the deposition-entrainment model of Hornberger et al. (1992) to the data. The model is a one-site kinetic formulation shown to be applicable to describing bacterial transport (Hornberger et al. 1992; Lindqvist and Enfield 1992). The model was selected because it fit the data better than an instantaneous equilibrium model, and it fit as well as a two-site kinetic model but with fewer fitting parameters. The model, using deposition (k_c) and entrainment (k_y) coefficients as fitting parameters, is

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

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

where c is cell concentration, t is time, D is the dispersion coefficient, x is distance, v is the average linear velocity (J_w/θ), θ is volumetric water content, ρ_b is bulk density, and s is the concentration of deposited cells (cells/g). The program CXTFIT (Toride et al. 1995), which optimizes parameters by nonlinear least-squares, was used to fit models to breakthrough curve data. The CXTFIT fitting parameters ω and R were used to calculate k_c and k_y ($k_c = \omega$ v/L; $k_y = k_c/(R-1)$). D for each column was determined from the Br^- breakthrough curve using the CXTFIT instantaneous-equilibrium model. To facilitate comparison of the results, c and t were made nondimensional; c was converted to relative concentration (c/c_o), and t to water-filled pore volumes (vt/L).

TABLE 2

Variables and cell transport for the second study

Exp. ID	Buffer			Rep.	Water (θ)	c_o (10^6 cells/mL)	Pulse length (pv)†	Cells transported‡		
	Before pulse	During pulse	After pulse					Rep. (%)	Avg. (%)	
Non	Plain	Plain	Plain	a	0.19	1200	1.3	46	53	a§
				b	0.16	1500	1.6	61		
Fol	Plain	Plain	DDBS	a	0.16	1600	1.7	45	52	a
				b	0.16	1300	1.7	58		
Pre	DDBS	Plain	Plain	a	0.16	1200	1.6	55	63	ab
				b	0.15	1100	1.8	71		
Con	Plain	DDBS	Plain	a	0.17	1400	1.5	87	82	b
				b	0.16	1300	1.8	77		
All	DDBS	DDBS	DDBS	a	0.16	1100	1.6	90	86	b
				b	0.16	370	1.6	82		

†Pore Volumes (vt/L).

‡Total cells transported through the column as a percentage of total cells added to the column.

§Values with the same letter are not significantly different by Duncan's multiple range test ($P \leq 0.05$).

Sixteen column breakthrough experiments were conducted in the first study. The fitted k_c and k_y values were used as dependent variables in a three-way multivariate ANOVA and Duncan's multiple range test (SAS/STAT, SAS Institute, Cary, North Carolina). A complete factorial design was used to evaluate the effects of three independent variables with two levels each: (1) bacterial strain (E3W7 and W31), (2) presence of surfactant DDBS (0 and 20 μM), and (3) water saturation condition (saturated and unsaturated). There were two independent replicate columns for each level.

In the second study, the dependent variable was the cell-recovery fraction, calculated by dividing the total cells recovered in the column outflow by the total cells added in the inflow. The outflow cell-total was defined as the cumulative number of cells collected from the beginning of the pulse to 3 pore volumes after the end of the pulse and was calculated by integrating the area under the curve of cell concentration versus outflow volume. The inflow cell-total was the average of the two inflow sample concentrations multiplied by the pulse volume. Each treatment had two replicates for a total of 10 column experiments. Significant differences in recovery caused by the timing of DDBS application were determined with an ANOVA and Duncan's multiple range test (SAS/STAT).

RESULTS

Bacteria passed through the columns with relatively little delay under saturated conditions (Fig. 2 and 3). This is reflected in the values of k_c and k_y (Table 1), which showed that for E3W7, deposition was approximately the same as or less than entrainment (average saturated k_c/k_y = 0.73). W31 in saturated columns showed some delay (average k_c/k_y = 4.5). Unsaturated conditions resulted in later breakthrough and increased kc in every case (average unsaturated k_c/k_y = 51). The greatest delay occurred with W31 in unsaturated flow; in one case c/c_o = 0.36 after 54 pore volumes (Fig. 3d), and k_c/k_y = 122. The multivariate ANOVA showed that the overall average unsaturated k_c (2.5/h) was significantly greater than the average saturated k_c (0.14/h)(P = 0.0002, Table 3). Water saturation did not have a significant effect on k_y (P = 0.51). The ANOVA indicated there were no significant interactions among the variables.

The effect of surfactant was not as clear as the effect of water content. Cell transport was increased in the presence of surfactant, i.e., breakthrough occurred sooner, except for W31 under unsaturated conditions (Fig. 3d). Although DDBS tended to increase entrainment of cells (greater k_y, Table 3), the surfactant effect was not significant (P = 0.26). The average deposition coefficients were not affected by DDBS (P = 0.96, Table 3).

W31 tended to be less readily transported than E3W7; however, bacterial strain did not have a significant effect on k_c (P = 0.40) or k_y (P = 0.11). There was some indication that the strains differed primarily in entrainment: E3W7 was 11 times more easily removed than W31

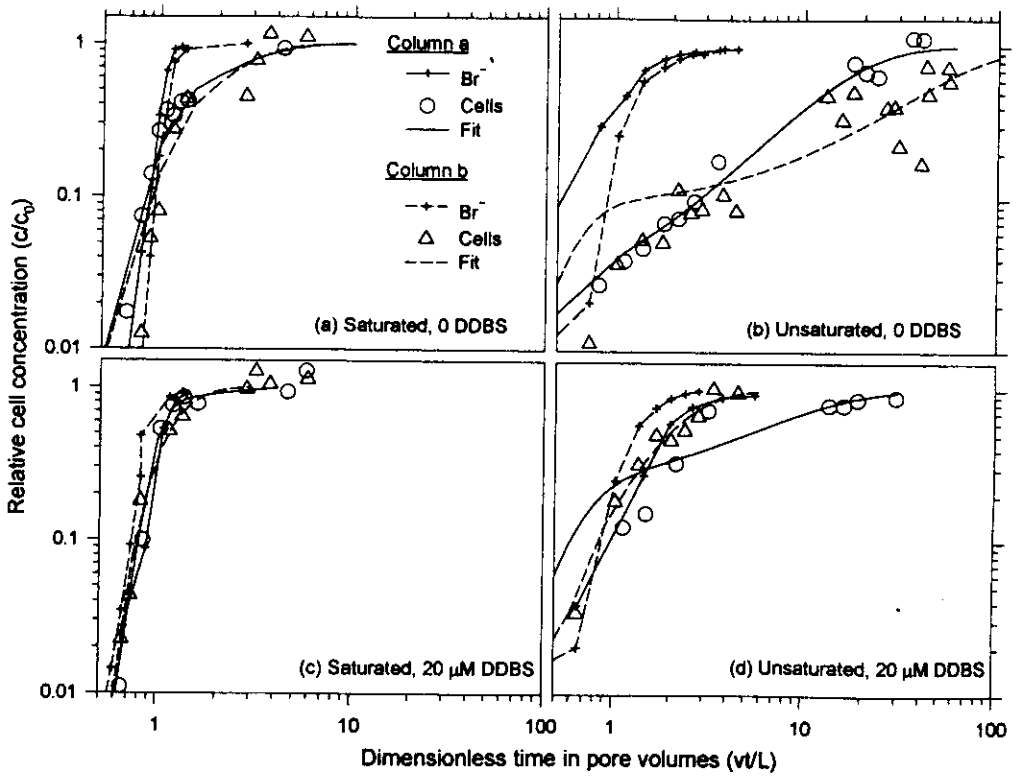

Fig. 2. Strain-E3W7 relative concentrations from the first study, fitted 1-site kinetic model curves, and relative Br^- concentrations. The effects of two variables are shown: water saturation (saturated and unsaturated) and surfactant (with and without DDBS).

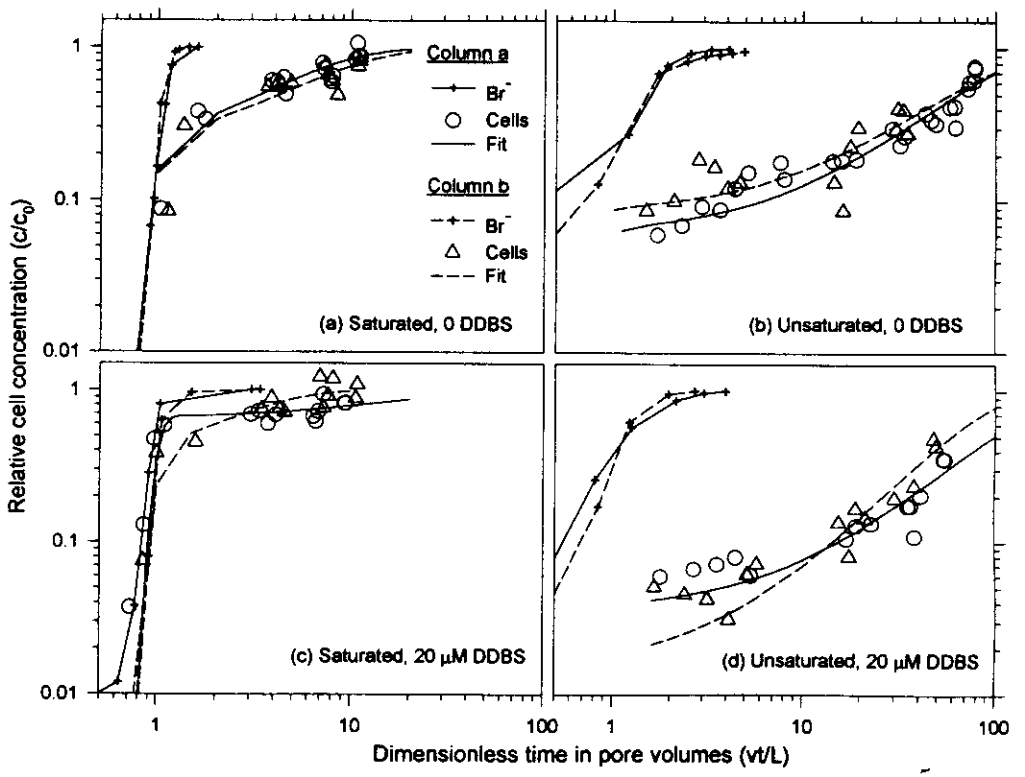

Fig. 3. Strain-W31 relative concentrations from the first study, fitted 1-site kinetic model curves, and relative Br^- concentrations. The effects of two variables are shown: water saturation (saturated and unsaturated) and surfactant (with and without DDBS).

TABLE 3

Treatment effects on the rate of coefficients (first study).

Treatment levels	k_c (/h)		k_v (/h)	
Unsaturated	2.5	a†	0.29	a
Saturated	0.14	b	0.15	a
DDBS	1.3	a	0.35	a
Plain	1.3	a	0.097	a
W31	1.5	a	0.039	a
E3W7	1.2	a	0.41	a

†Within each treatment, k_c or k_v values with the same letter are not significantly different by the Duncan's multiple range test ($P \leq 0.05$).

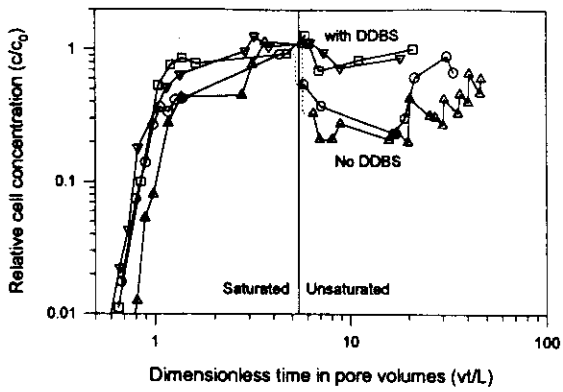

Fig. 4. The response of strain-E3W7 concentrations in saturated columns to the introduction of air, with and without DDBS. The data in the saturated region are also shown in Fig. 2.

(k_v/k_v), whereas the k_c values were not much different (Table 3).

The effect of adding air to saturated columns that had achieved complete bacterial breakthrough was tested with E3W7 (Fig. 4). Following air entry, outflow bacterial concentrations immediately declined. DDBS reduced the decline: with DDBS, the minimum c/c_o in the unsaturated region was 0.71; without DDBS, the minimum was 0.22. Recovery toward $c/c_o = 1$ was also faster with DDBS than without.

The breakthrough curves of the second study (Fig. 5) show a clear trend of low recovery with Non-treatment to high recovery with All-treatment. In every case, cell breakthrough began at about 0.5 pore volume. The Non and Fol curves had a less steep rising limb than the Pre, Con, and All treatments, indicating more adsorption when DDBS was lacking. The Pre curves rise more rapidly but reach about the same peak value as Non and Fol curves. Con and All curves rise rapidly and reach higher peak values than the other treatments. All curves show tailing, or a long period of low-concentration cell elution, after the end of the pulse.

The second-study ANOVA of cell recovery showed a significant treatment effect ($P = 0.036$). The Non and Fol treatments had significantly lower recovery (53% and 52%) than the Con and All treatments (82% and 86%, Table 2). Pre-treatment recovery (63%) was not significantly different from the other treatments.

DISCUSSION

Two possible explanations for enhanced removal of bacteria in unsaturated flow are: increased interaction between suspended cells and sand grains, and bacterial adsorption to GWIs. Cell-solid interactions might be expected to increase in unsaturated conditions due to the draining of the largest pores, thereby diverting cells through small pores. Flow tortuosity also increased in unsaturated conditions, as indicated by the greater dispersivity ($\alpha = D/v$) of unsaturated columns in comparison with saturated columns. The average dispersivity in the unsaturated columns was 13 times greater ($\alpha = 3.1$ cm) in comparison with the saturated columns ($\alpha = 0.24$ cm)(Table 1). However, the fact that the saturated columns quickly reached complete breakthrough (Fig. 2 and 3) indicated that the relatively few deposition sites on the sand particles, in large pores and small, were quickly filled. It is unlikely, therefore, that the small-pore flow and increased tortuosity in unsaturated conditions increased adsorption to sand. It is more likely that the introduction of air into the columns created new adsorptive sites on GWIs. We expected that bacteria would be deposited to a greater extent in unsaturated as compared with saturated conditions based on the results of using filters to collect bacterially-enriched GWIs (Powelson and Mills, 1996), and the direct observation of bacteria accumulating at GWIs in pores (Wan et al., 1994). The result of the unsaturated versus saturated comparison in the first study was as expected, and the greater loss of cells in the outflow of the unsaturated columns was attributed to a greater deposition rate coefficient (k_c).

The effect of GWIs on cell adsorption was also demonstrated by allowing air to partially replace water in columns that had achieved complete cell breakthrough, resulting in a sharp decline in outflow concentrations (Fig. 4). The added GWIs apparently provided additional adsorption sites, and DDBS interfered with this adsorption. The recovery of outflow concentrations following this decline was presumably

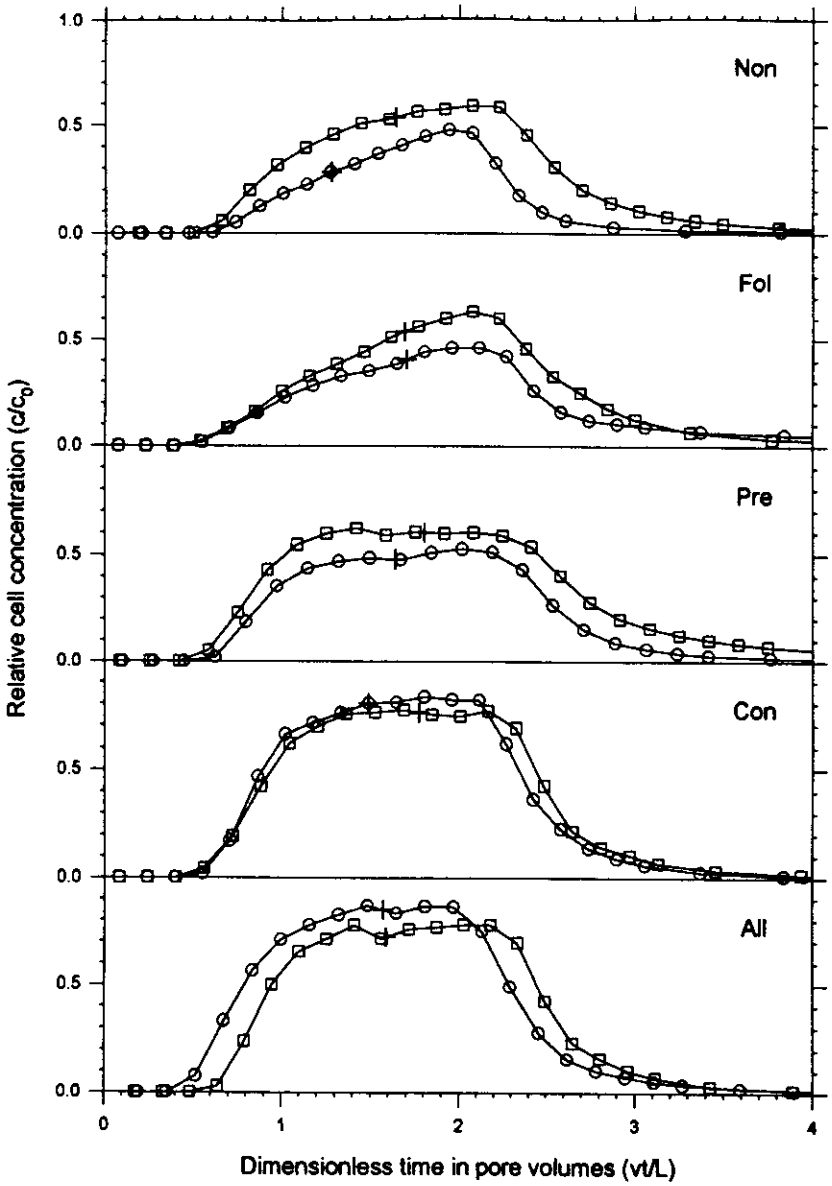

Fig. 5. Relative concentrations of strain-W31 from pulse-input experiments (the second study). Surfactant was applied: at all times (All), concurrently with the cell pulse (Con), before the pulse (Pre), following the pulse (Fol), and at no time (Non). The two replicate column breakthroughs [a (○) and b (□)] and the pulse endpoint (+) are shown.

attributable to filling of GWI adsorption sites, which allowed the continued inflow of cells to pass through the column without adsorbing.

The lack of a significant surfactant effect on k_c and k_v was partly the result of a minimal response of W31 to 20 μmol/L DDBS. The 20 μmol/L DDBS concentration used in this study was close to the lowest concentration that reduced adsorption of E3W7 to the GWI (Powelson and Mills 1996). This low concentration was selected to maintain the surface tension and avoid possible air-breakthrough in the porous disks, and because low concentrations of surfactant were expected to be more realistic in the field. The 5-times greater DDBS concentration used in the second experiment greatly improved unsaturated transport of W31. The average maximum W31 concentration reached in unsaturated flow with 20 μmol/L DDBS was $c/c_0 = 0.42$ after more than 48 pore volumes (Fig. 3d), whereas in 100 μmol/L DDBS, $c/c_0 = 0.42$ was reached in less than 1 pore volume (Fig. 5). Much greater surfactant concentra-

tions have been used to influence bacterial adsorption: concentrations as great as 1.3 mmol/L DDBS were used to evaluate adsorption of E3W7 to GWIs (Powelson and Mills 1996), and a 1.5 mmol/L DDBS concentration was found to improve transport of *Pseudomonas pseudoalcaligenes* through soil (Jackson et al. 1994).

The ability of the multivariate ANOVA to find significant differences attributable to the DDBS and bacterial strain treatments in the first study was limited by the variability between replicates and by having only two replicates for each treatment level. In addition, the DDBS and strain treatment levels may not have different enough to show significant effects.

In all step-input experiments, the relative concentration of cells reached complete breakthrough ($c/c_0 = 1$) or was increasing toward complete breakthrough when the experiment ended (Figs. 2 and 3). Although many of the pulse-input breakthroughs appeared to stabilize at $c/c_0 < 1$ (Fig. 5), it is likely that a longer pulse would have shown complete breakthrough. Because complete breakthrough was indicated, cell removal was not attributed to irreversible, continuous adsorption or filtration. Instead, it was likely that the number of deposition sites in a column was limited and that deposition was caused by adsorption to these sites and entrainment to desorption.

In the second study, the improved bacterial transport when DDBS was applied concurrently with the cells, compared with before or after the cells, suggests that the most important effect of surfactant was to mask hydrophobic binding sites on the cell surface thereby reducing cell adsorption to the GWI. Presumably, the hydrophobic "tails" of surfactant molecules associated with hydrophobic regions of the cell surface, thereby rendering the cell surface more hydrophilic. The cell-surfactant complexes would be more likely to stay in suspension than to adsorb to the hydrophobic GWI. The lack of significant differences between Pre-treatment and the other treatments may indicate that DDBS diffused from GWIs and sand surfaces into the cell suspension to create an intermediate effect. The low recovery when DDBS was applied after the cell pulse showed that surfactant is not very effective in loosening and resuspending adsorbed cells.

Peak values for the Non and Fol curves ($c/c_0 \approx 0.5$, Fig. 5) were greater than the concentrations reached in the first study in similar conditions ($c/c_0 \approx 0.1$, Fig. 3b at 2 pore volumes). This may have been caused by wetter conditions in the second study (average $\theta = 0.17$ for Non and Fol, Table 2) compared with the first study (average $\theta = 0.085$, for W31, no DDBS, unsaturated conditions; Table 1).

The effects of surfactant and unsaturated conditions on transport of two bacterial strains through clean sand columns were evaluated. In every case, unsaturated conditions resulted in later breakthrough of bacteria. By fitting a transport model to the data, the delayed breakthrough was attributed to a significantly greater cell deposition rate constant in unsaturated conditions. The other variables—presence of surfactant and bacterial strain—did not have significant effects on cell deposition or entrainment. In a second study using a higher surfactant concentration, cell transport through unsaturated sand was significantly greater when surfactant was included in a pulse of cells compared with the no-surfactant control. It is likely that cells adsorb to gas-water interfaces in unsaturated soil and that surfactant interferes with this adsorption.

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