Physical and Chemical Factors Influencing Transport of Microorganisms through Porous Media

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Resting-cell suspensions of bacteria isolated from groundwater were added as a pulse to the tops of columns of clean quartz sand. An artificial groundwater solution (AGW) was pumped through the columns, and bacterial breakthrough curves were established and compared to test the effects of ionic strength of the AGW, cell size (by using strains of similar cell surface hydrophobicity but different size), mineral grain size, and presence of heterogeneities within the porous media on transport of the bacteria. The proportion of cells recovered in the effluent ranged from nearly 90% for AGW of a higher ionic strength ($I = 0.0089$ versus 0.00089 m), small cells (0.75-μm-diameter spheres versus 0.75 by 1.8-μm rods), and coarse-grained sand (1.0 versus 0.33 mm) to $<1\%$ for AGW of lower ionic strength, large cells, and fine-grained sand. Differences in the widths of peaks (an indicator of dispersion) were significant only for the cell size treatment. For treatments containing heterogeneities (a vein of coarse sand in the center of a bed of fine sand), doubly peaked breakthrough curves were obtained. The first peak represents movement of bacteria through the transmissive coarse-grained vein. The second peak is thought to be dominated by cells which have moved (due to dispersion) from the fine-grained matrix to the coarse-grained vein near the top of the column and thus had been retarded, but not retained, by the column. Strength of effects tests indicated that grain size was the most important factor controlling transport of bacteria over the range of values tested for all of the factors examined. Cell size and ionic strength were about equal in importance and were lower in importance than the grain size. The results indicate that significant numbers of bacteria can move through porous media, even when the percentage retained is very high, and the data suggest that manipulation of groundwater environments to control the transport of bacterial cells may be feasible.

Interest in the transport of microorganisms in porous media is motivated by concerns over microbes as pollutants, as in the contamination of drinking water by sewage or septic waste (15); microbes as disseminators of pollutants, as in the case of enhancement of the mobility of radionuclides by association of the nuclides with microbial cells (5, 36); the fate of genetically manipulated microbes released to groundwater aquifers intentionally or inadvertently (30); and the role of microorganisms in the biorestoration of contaminated aquifers (9, 22, 34, 38). The interest has been further piqued by the recognition of abundant, active, and diverse microbial communities deep in saturated sediments that make up some aquifers, a region many have considered to be generally devoid of life (1, 2, 11, 12, 16, 37). Although all of these concerns demand quantitative information on the potential, and real, distances over which microorganisms can travel and the time required for them to span those distances, the ability to predict accurately the rate and extent of microbial transport in the subsurface is particularly critical in the area of bioremediation. In situ bioremediation can involve either the stimulation of the existing microbiotic community that possesses the ability to degrade the contaminant or the introduction of microbes with the specialized metabolic capabilities necessary to degrade the pollutant contaminating the site. The latter approach requires that the introduced microbes be transported throughout the site of contamination, which in some cases may be deep underground. Thus, quantitative understanding of microbial transport will provide the physical link between the promise of biorestorative miracles afforded by those who examine the physiological abilities of microbes collected from or groomed for groundwater environments and the reality of effective cleanup of contaminated aquifers.

While it is important to gain an understanding of movement of bacteria in soils, sediment, and bedrock, theories currently available are arguably incomplete (9, 24), and tests of existing models against laboratory or field data are few. The approaches currently used for modeling bacterial transport are phenomenological; that is, transport is described by using an advection-dispersion equation modified to include growth, death, and a number of other processes through the incorporation of phenomenological coefficients (6, 7, 27), with limited ability to specify the coefficients on theoretical grounds. The full models are very difficult to test empirically, due to their reliance on a large number of parameters describing geological and bacterial variability, the determinations of which are impractical at best. It is therefore necessary to test first bacterial transport processes under carefully controlled laboratory conditions.

In an effort to provide an adequate theoretical basis for quantitative models of bacterial transport, we have focused our initial attention on advective transport in the absence of biological processes such as growth, death, and predation. Bacteria are capable of movement by nonadvective processes in porous media at penetration rates approximating the flow velocities observed in many aquifers. Penetration as a result of growth and motility can occur at rates of up to 0.5 cm h^{-1} in packed-sand cores under no-flow conditions (29). To avoid the confounding of quantitative results by changes in total cell number or changes in position of cells due to growth, death, motility, etc., the study described here relied on cells in the resting state (no change in numbers over the time period of the experiments). Such an approach permits

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the conceptual use of models formulated for transport of colloidal particles (e.g., see references 8, 21, and 28).

This paper examines a number of different factors, each being varied in turn while keeping the general experimental setup constant. The experimental variables chosen were ionic strength of the eluent, grain size of the porous medium, bacterial cell size, and heterogeneities within the medium. Ionic strength is known to have a significant effect on the deposition of cells from the fluid to solid phase (17, 31) and was therefore expected to affect the efficiency of cell transport through the biphasic porous medium. The process of pore clogging by bacteria should also affect microbial transport, and the extent to which such clogging occurs should depend directly on the size of the grains in the porous medium and the size of the cells themselves (14). Previous studies have utilized columns packed with soils of nonuniform size (39), confounding analysis of the effects of mineral grain size on bacterial transport. The dramatic effects of structural heterogeneities in porous media on hydrological transport have been repeatedly demonstrated (e.g., see reference 3), but bacterial transport studies involving heterogeneities are especially rare, and the heterogeneities involved were not characterized because they were either natural heterogeneities, in intact soil cores (33), or heterogeneities generated randomly, as by burrowing earthworms (23).

The results of the present study indicate that, even in cases of efficient filtration, significant numbers of bacteria can be transported through porous media. All of the variables examined significantly affected bacterial transport, but mineral grain size had the strongest effects over the ranges examined. The existence of preferred flow paths within a medium dramatically changed the transport profiles, confirming the speculation that heterogeneities (macropores, fractures, etc.) in the subsurface environment may be responsible for much of the long-range transport of microbes. Differences in transport behavior with changes in ionic strength and strain (presumably due to cell size differences) suggest that manipulation of some contaminated sites may permit or enhance bacterial transport to improve the prospects for effective in situ renovation of groundwater aquifers.

MATERIALS AND METHODS

Bacterial strains. Bacteria were collected from the bulk sediments of a freshly hand-augered well on the eastern shore of Virginia, and pure strains were isolated by plating on half-strength peptone-yeast extract agar (per liter: peptone, 125 mg; yeast extract, 125 mg; $MgSO_4 \cdot 7H_2O$, 150 mg; $CaCl_2 \cdot H_2O$, 1.75 mg; agar, 15 g). A number of pure cultures were isolated from the sample; some of the isolates were associated with the aqueous phase, while others were associated with the solid-phase sediment particles and were released into suspension only after vigorous shaking. Strains W6 and W8 were selected from the cultures isolated from the aqueous phase for use in these experiments on the basis of their similarities in cell surface hydrophobicity and Gram reaction and their differences in cell size and shape. Strain W6 is a nonsporeforming, gram-negative coccus (approximately 0.75 μm in diameter). Strain W8 is a nonsporeforming, gram-negative rod (approximately 0.75 by 1.8 μm). The hydrophobicity of each strain was determined by measuring the contact angle of a droplet of deionized water on a lawn of the bacteria. Both organisms had a contact angle of 20°,

indicating that the cell surfaces are highly hydrophilic, according to the classification scheme of Mozes et al. (25).

Media and growth conditions. Strains W6 (coccus) and W8 (rod) were grown for experimental use in half-strength peptone-yeast extract broth at 21°C aerated by gentle swirling on a rotary shaker for 2 to 3 days. The cells were removed from suspension by centrifugation at 18,000 × g for 10 min. The pellet was resuspended in artificial groundwater (AGW) (31) (1.5×10^{-5} M KNO_3, 1.4×10^{-4} M $MgSO_4 \cdot 7H_2O$, 7.0×10^{-5} M $CaSO_4 \cdot 2H_2O$, 8.0×10^{-5} M NaCl, 1.4×10^{-4} M $NaHCO_3$, pH 6.8) for experiments involving a low-ionic-strength eluent (I = 0.00089 m) or in a 10× solution of AGW for experiments involving a higher-ionic-strength eluent (I = 0.0089 m). The cells were left in AGW for a period of 24 to 36 h to ensure that they had entered a resting stage and had ceased growth. Preliminary experiments indicated that no change in cell numbers (as determined by acridine orange direct counts) occurred during the period of the experiment.

Columns and sand. Glass chromatography columns (4.8-cm inside diameter Kontes) were autoclaved prior to each experiment and then wet packed with rounded quartz sand (Unimin Corp.) which had been washed in 10% nitric acid, thoroughly rinsed with deionized water, autoclaved, and dried. The sand had been separated into two size classes: fine sand was that which passed through a 0.40-mm sieve but not a 0.33-mm sieve; coarse sand was that which passed through a 1.14-mm sieve but not a 1.00-mm sieve. Four hundred grams of a single type of sand was packed into each column for experiments involving homogeneous porous media. For those experiments involving structured heterogeneities, a preferred flow path was created by inserting a glass tube (1.6-cm inside diameter) in the center of the column and packing 370 g of fine sand around it; the inside of the glass tube was then packed with 30 g of coarse sand, and the tube was carefully removed. In all cases, dry sand was poured into the columns (partially filled with AGW) in increments of about 2 to 2.5 cm which were allowed to settle before adding more sand. This practice, combined with the careful size fractionation, minimized size-based stratification of the sand in the columns.

A standing pool of AGW was maintained above the sand surface to ensure the equal distribution of solution throughout the column at all times. These procedures yielded sand columns which were 14 cm long with a pore volume of 88 cm^3. The porosity of both fine- and coarse-grained homogeneous columns was determined to be 0.35. The hydraulic conductivities, determined by a falling head test, were 0.37 and 2.0 cm s^{-1} for the fine- and coarse-grained homogeneous columns, respectively.

Experimental procedures. Combinations of column and solution conditions were established to test the effect on bacterial breakthrough of grain size, ionic strength, and bacterial strain in a full factorial design. The grain size treatment included both fine and coarse sand and the presence of the structured heterogeneity (tested statistically as a third grain size category). Each type of column was set up and run in duplicate with each organism (to test the effect of bacterial strain) and each ionic strength of eluent. Columns were run on the bench top at ambient laboratory temperatures (20 to 22°C).

The flow of eluent from a sterile reservoir through the column was regulated by a variable-flow peristaltic pump. Once a constant flow rate of 88 ml h^{-1} (1 pore volume h^{-1}, or 14 cm h^{-1}) was established, 2 ml of a bacterial suspension containing approximately 10^9 cells ml^{-1} was introduced into

the pool of AGW on the top of the column. This was accomplished by the use of a valve which changed the source of input to the column from the AGW reservoir to a graduated cylinder containing the bacterial suspension. When exactly 2 ml of suspension had been removed from the cylinder and had passed the valve, the valve was returned to its original position, and AGW flow resumed. Eluent samples were collected from the base of the column in 0.25-pore-volume intervals and analyzed for bacterial concentration by using the acridine orange direct count method of Hobbie et al. (20). Because the cells used in these experiments were uniform in size (as determined by measurement of 200 cells of each type with an eyepiece micrometer), the abundance of bacterial cells is directly related to the biomass in any given suspension. Thus, the term mass is used interchangeably with abundance throughout this discussion. In columns containing a preferred flow path (structured heterogeneity), breakthrough of bacteria occurred very rapidly. To characterize this sharp initial peak in the heterogeneous columns more accurately, the first four samples in these experiments were collected at 0.125-pore-volume intervals. Experimental runs were continued until at least 3 pore volumes of AGW had passed through the columns.

In some early experiments, a pulse of 0.1 M NaCl was introduced into the column (0.25 pore volume) prior to the introduction of the bacteria. Determination of the specific conductivity of each eluent sample helped to elucidate the transport characteristics of the columns during the initial experiments.

Statistical analysis. Acridine orange direct count data were used to create breakthrough curves (cells per milliliter in the eluent versus pore volumes eluted) describing the transport of bacteria through the columns. To compare the breakthrough curves and to permit appropriate statistical analysis, a number of response variables were generated from the curves.

The effect of the various treatments on the response variables was examined by using the MANOVA procedure in the SPSS-PC statistical software package (SPSS Inc., Chicago, Ill.). The response variables examined for the homogeneous cases included the percentage of total cells introduced into the column that were recovered in the eluent (percent recovery), the width of the peak at one-half its height, and the bacterial retention time (the time in pore volumes to the maximum measured concentration). Also, the centroidal volume (CV) was computed. The centroid represents the center of mass, that is, the point at which the mass of cells in the early part of the breakthrough is exactly balanced by the mass of cells in the rest of the breakthrough; it is expressed as a volume because of the units plotted on the x axis. The long tail of the breakthrough tends to move the centroid of the breakthrough curve to the right on the plots. The height of the peak was also determined. The peak height was expressed as the ratio of the highest measured concentration of cells eluted to the number of cells put into the column. This somewhat unorthodox ratio of a concentration to a mass was necessary because the actual concentration of cells entering the sand was not known due to the inability to measure accurately the volume of the standing pool of AGW above the sand. The variations in concentration were assumed to be unimportant based on results of early experiments in which equal numbers of cells were put into different volumes of AGW pooled at the top of the sand columns (i.e., experiments were run at different input concentrations of bacterial cells). No measurable difference in peak height was observed over an order of magnitude
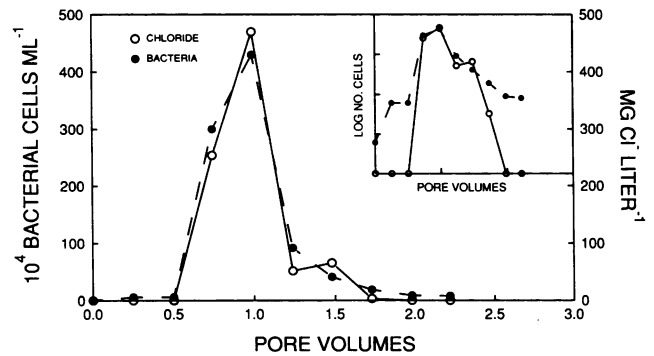

FIG. 1. Breakthrough curve for bacteria and chloride in a column packed homogeneously with clean quartz sand. (Inset) Same data plotted as the $\log_{10}$ of the measurement variable versus the pore volume.

difference in input concentration. Since only the concentration and not the total mass of cells entering the column was changed, the results suggest that the penetration of cells into the column behaved as though the injection was instantaneous. Concentrations in the actual experiments are believed to differ by no more than a factor of 2.

Because the heterogeneous cases yielded breakthrough curves with two peaks instead of one, a different set of response variables was necessary to describe the breakthrough curves. Only two of the variables, percent recovery and centroidal volume, coincided with the variables computed for the homogeneous cases. Other variables examined for use as quantitative descriptors of the doubly peaked curves included the area ratio (area of peak 1/area of peak 2), height ratio (height of peak 1/height of peak 2; height calculated as described for the homogeneous cases), width at half height for peak 1, and the width at half height for peak 2.

To examine the relative importance of the various treatments on the recovery of bacteria from the columns, the treatments were ranked in order of percent recovery and the rankings were assessed. Then a "strength of effects" analysis was employed, using the methods detailed by Box and Draper (4) for a two-level, full factorial design. In this analysis, the heterogeneous cases were not included, because they do not truly represent a grain size category (although this treatment could have been included if hydraulic conductivity were the measured treatment variable). This analysis can be thought of as a simple form of linear response-surface analysis in which the average difference in the level of response is sought as one moves from the low to the high level of a particular variable. A pictorial representation of the analytical design is given in Fig. 6. Average responses for each level of each variable are found by computing the mean of the values on each face of the cube (the cube is valid only for the three-factor case; more factors would prohibit expression in a graphical format) and determining the difference between the mean values obtained at each level of the treatment examined.

RESULTS

Breakthrough curves generated from homogeneous columns were all similar in shape to that shown in Fig. 1 (cf. Fig. 3 and 4). In all cases, a "tailing" effect was seen. After reaching a peak concentration at or near 1 pore volume,
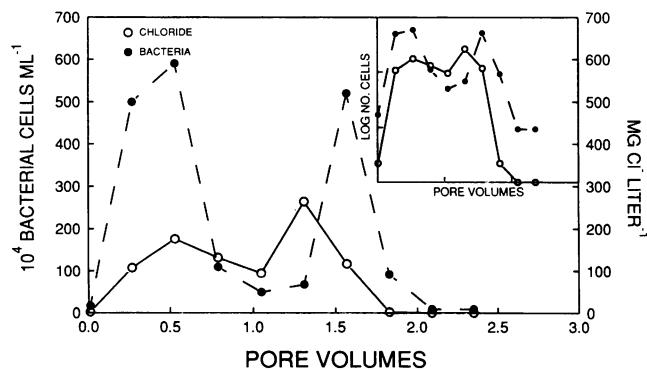
FIG. 2. Breakthrough curve for bacteria and chloride in a column packed with clean quartz sand. This column was packed to include a structured heterogeneity. (Inset) Same data plotted as the $\log_{10}$ of the measurement variable versus the pore volume.

TABLE 1. Values of the response variables calculated for the breakthrough curves: homogeneous case[a]

Organism[a]	Ionic strength	Mineral grain size[b]	%R	Peak width at half ht	Retention time[c]	Peak ht (10^4)	CV
W6	Low	F	14.5	0.397	1.125	37.85	1.14
W6	Low	C	88.4	0.568	1.000	166.	1.05
W6	High	F	2.75	0.630	0.875	3.92	1.25
W6	High	C	49.3	0.270	1.125	186.5	1.15
W8	Low	F	3.90	0.447	1.125	8.68	1.17
W8	Low	C	43.6	0.758	0.875	98.55	1.08
W8	High	F	0.335	0.791	0.875	0.33	1.29
W8	High	C	4.45	0.677	1.125	6.545	1.24

[a] W6, coccus; W8, the larger rod.
[b] F, fine-grained sand; C, coarse-grained sand.
[c] Time of maximum measured concentration.

bacterial cell number declined and leveled off at a non-zero baseline concentration. Earlier work in this laboratory has shown the tail to persist for at least 7 pore volumes (31). The tail was not seen in the breakthrough curves of chloride ion, which declined to zero concentration. Except for the presence of the tail in the bacterial curves, breakthrough curves for bacteria and chloride were very similar in shape in all cases, although the recovery of bacteria was always less than the recovery of chloride, which consistently exhibited 100% recovery. That the peak breakthrough for Cl^- occurred at about 1 pore volume and that the peak was generally symmetrical suggest that there was no preferred flow along the edges of the columns.

Breakthrough curves generated from columns containing a preferred flow path exhibited a doubly peaked pattern (Fig. 2). The first peak in concentration occurred well in advance of 1 pore volume (between 0.188 and 0.312 pore volume). The second peak occurred at around 1.5 pore volumes. This was true of breakthrough curves for both bacteria and chloride, although in many cases it appeared that the initial breakthrough of bacteria slightly preceded that of chloride. It should be noted that the pore volume of the central vein was 12% of the total pore volume of the column (based on the porosity and the diameter of the tube used to construct the heterogeneity). The similarity of this value and the volume of the observed breakthrough suggests that the first peak arises from cells travelling primarily through the structured heterogeneity.

Behavior of the response variables. Some of the response variables examined exhibited a very wide range of values, while others seemed to be relatively unchanged by any of the treatments (Tables 1 and 2). The percentage of cells recovered from the column (%R) ranged from <1% in the case of the larger strain W8 in fine-grained homogeneous columns at high ionic strength (Fig. 4B) to nearly 90% for the smaller strain W6 in coarse-grained homogeneous columns at low ionic strength (Fig. 3A). Peak height also showed a wide range of values, from approximately 1.7×10^{-2} for the coccus (W6) in coarse sand at either ionic strength (Fig. 3A and 4A) to around 3.0×10^{-5} for W8 (the rod) in fine sand at high ionic strength (Fig. 4B). The width of the curves at one-half height (an indicator of dispersion of the bacterial cells as they passed through the column) ranged from around 0.27 pore volume for W6 in coarse sand at high ionic strength (Fig. 4A) to about 0.79 pore volume for W8 in fine sand at high ionic strength (Fig. 4B).

On the basis of examination of the response variables along with the results of the MANOVA analysis (Table 3), the response variable that could be most meaningfully interpreted was the percent recovery (%R). Although interesting information could be gained from several of the variables, %R gave an integrated view of the behavior of each of the columns in terms of the total number of cells that passed through the column (i.e., mass recovery) and could be compared for both the heterogeneous and the homogeneous cases. Thus, most of the conclusions about the comparative behavior of the columns were based on this variable. Peak height behaved similarly to %R (all main effects were significant), and there was a strong relationship between the two variables ($r^2 = 0.84$).

The CV also showed significant differences among the treatments (Table 3), with the CV of the fine-grained treatments always exceeding that of the equivalent coarse-grained treatments, CV of the high-ionic-strength treatments exceeding that of the low-ionic-strength analogs, and the CV for W8 (larger cells) exceeding the CV for equivalent treatments with W6 (smaller cells) (Tables 1 and 2). Larger CVs represent more of the mass recovered in the later portions of the curve (i.e., in the tail).

Values of retention time were always about 1 pore volume, and none of the main effects were significant. Because of the resolution of the sampling, small differences in retention times, although not expected, could not have been detected.

Effect of changes in ionic strength. The ionic strength of the AGW was inversely related to the percent recovery, and the effect of ionic strength on the recovery of cells was signifi-

TABLE 2. Values of the response variables calculated for the breakthrough curves: heterogeneous case

Organism[a]	Ionic strength	Mineral grain size[b]	%R	A1/2[c]	H1/2[d]	PW1[e]	PW2[e]	CV
W6	Low	H	79.3	1.16	2.78	0.272	0.639	0.88
W6	High	H	19.7	1.14	2.23	0.263	0.530	0.76
W8	Low	H	39.1	0.449	0.806	0.474	0.568	1.2
W8	High	H	11.7	0.940	1.26	0.523	0.520	0.82

[a] W6, coccus; W8, the larger rod.
[b] H, heterogeneous.
[c] A1/2, area of peak 1/area of peak 2.
[d] H1/2, height of peak 1/height of peak 2.
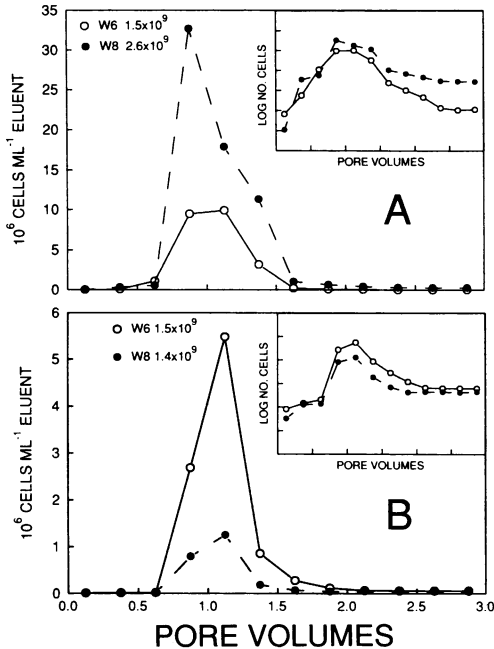[e] PW1, PW2, width (at half-height) of peak 1 or 2, respectively.

FIG. 3. Breakthrough of bacteria (two sizes) in low-ionic-strength eluent through homogeneously packed columns containing (A) coarse-grained and (B) fine-grained clean quartz sand. Log_{10} plots (insets) are provided to illustrate the tailing associated with the bacterial transport. Numbers associated with the symbols indicate the total number of each strain added to each column. Although panel A appears to show that more of the larger cells (W8) than the smaller cocci (W6) eluted from the column, the %R was greater for the smaller than for the larger cells.
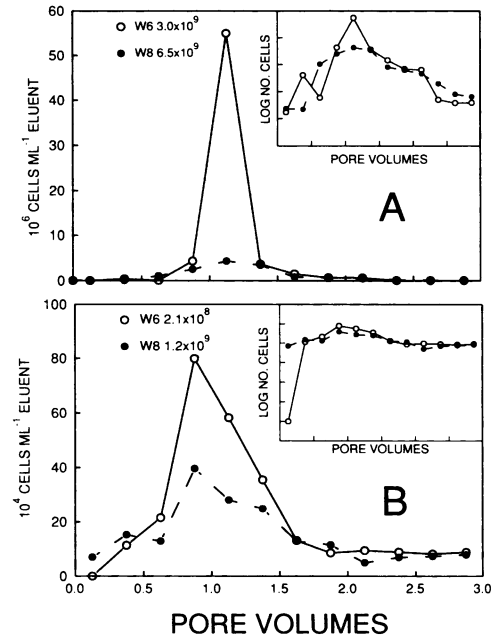

FIG. 4. Breakthrough of bacteria (two sizes) in high-ionic-strength eluent through homogeneously packed columns containing (A) coarse-grained and (B) fine-grained clean quartz sand. Log_{10} plots (insets) are provided to illustrate the tailing associated with the bacterial transport. Numbers associated with the symbols indicate the total number of each strain added to each column.

cant (Table 3). A 10-fold increase in ionic strength cut the recovery of strain W6 (coccus) in coarse homogeneous columns nearly in half. Other experiments showed even more marked effects: the recovery of strain W8 in fine homogeneous columns was reduced by an order of magnitude by the same 10-fold increase in ionic strength.

Effect of differences in strains. In all treatments, the larger cells (strain W8) exhibited a smaller percent recovery and a lower peak height than the smaller cells (strain W6), and the effect on %R was significant for the homogeneous, heterogeneous, and combined cases. In the homogeneous case, the width of the peak (PW) in the breakthrough curves of strain W8 was significantly different from that of the smaller strain W6 (Table 3), with the measured width of the peak for W8 always being greater than that for W6. In the heterogeneous case, differences in the width of the first peak, but not the second, were significant. Peak width is an indication of the amount of dispersion that the cells have undergone as they traveled through the column. It is interesting to note that the larger cells were more strongly dispersed.

Effect of grain size. In all treatments, the height of the peak and the percent recovery of cells from coarse-homogeneous columns was greater than that from fine-homogeneous columns (Tables 1 and 2). Peak width did not differ significantly between fine- and coarse-grained columns (Table 3).

Effect of structured heterogeneities. The existence of a preferred flow path in the porous media drastically altered the transport behavior. Breakthrough curves in these columns were doubly peaked (Fig. 5). Significant effects (α = 0.05) of the organism used were observed for the width of the

first peak (the coccus displayed a sharper initial peak than did the rod), the ratio of peak areas (that of the coccus was consistently greater than that of the rod), CV (the rod was always greater than the coccus), and percent recovery (the coccus always exceeded the larger rod). In comparison with the homogeneous case, the response variable CV tended toward lower values, reflecting the early breakthrough (compared with total pore volume) in columns containing a preferred flow path. Ionic strength exhibited a significant effect only on percent recovery, an effect which mirrored that observed in the homogeneous columns.

DISCUSSION

Homogeneous columns. The percentage of cells recovered from the columns (%R) was the best of the response variables in describing the integrated effects of the various treatments. The treatments produced breakthrough curves that behaved largely as would be predicted; that is, more organisms came through the columns under conditions of low ionic strength, coarse grains, and small cells. Significant differences in %R were observed for all treatments. Differences in grain size of the porous media were associated with large effects. The coarse sand used was approximately three times the diameter of the fine-grained sand, and recoveries from coarse columns were often in excess of an order of magnitude higher than recoveries from fine columns. This effect is likely the result of (i) a 3-fold-lower total sand-surface area in columns containing coarse sand as compared with those containing fine sand, resulting in a proportionately lower number of sites available for bacterial cell adhesion, and (ii) a 5.5-fold increase in the hydraulic conductivity in the coarse-grained columns as compared with the fine-grained columns, indicating the larger average pore
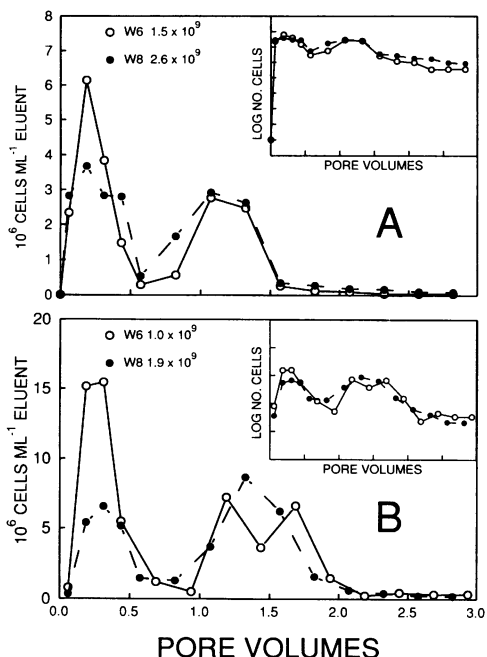
FIG. 5. Breakthrough of bacteria (two sizes) in (A) high- or (B) low-ionic-strength eluent through columns containing a structured heterogeneity. Log plots (insets) are provided to illustrate the tailing associated with the bacterial transport. Numbers associated with the symbols indicate the total number of each strain added to each column.

diameter in columns containing coarse sand that lowers the likelihood of pore clogging by the bacterial cells.

It is important to note that differences in the behavior of the two strains examined were assumed to be due largely, if not wholly, to difference in cell size. That assumption was based on the observation that the cell surface hydrophobicity was identical for each strain. There are a number of other parameters that could contribute to the observed differences in transport of the two strains, including electrostatic charge

differences, presence or absence of specific reactive groups on the cell surfaces, presence or absence of sticky coatings, etc. While other factors could indeed have contributed to the strain effect that was interpreted to be a result of cell size, size is certainly among the most important of the factors under the experimental conditions imposed. Indeed, a survey of a large number of isolates by Gannon et al. (14) led to the conclusion that surface charge and hydrophobicity were not significant factors in the transport of bacterial cells in soil columns; cell size was the only factor demonstrated to have a significant effect. Further work with more strains under the conditions imposed combined with more detailed cell surface characterization will help elucidate the relative importance of size to other potentially important factors.

Ionic strength of the eluent also had a large effect on the percentage of cells recovered from the column. Increasing ionic strength enhances the ability of bacteria to adhere to the quartz sand substrate by increasing the availability of ions in solution which can form bridges between charged sites on the sand surface and on the cell surface and by decreasing the thickness of the double layer, allowing a closer approach to the mineral surface by the bacterial cells. Increases in ionic strength were associated with increased attachment of suspended bacteria to smooth quartz surfaces in batch studies carried out by Scholl et al. (31). Ionic strength may also enhance the aggregation of bacterial cells, leading to increased pore clogging and, therefore, decreased recovery. Electrostatic interactions between bacterial cells and solid surfaces (including other cells) play an important role in bacterial attachment and transport (25, 32, 35), so the large effect of ionic strength on overall cell recovery is not surprising.

The tailing of the bacterial breakthrough curves is believed to arise from the constant flushing of cells which had previously been retained by the column, by either pore clogging or adhesion. The presence of the tail indicates that at least some percentage of the retained cells was not irreversibly attached to the sand grains. The response variable CV gives a direct indication of the skewing of the mass transport due to the tailing observed. Ionic strength had a significant effect on CV. The relationship between these

TABLE 3. Summary of MANOVA results from comparisons of breakthrough curve response variables[a]

Case	Response variable[b]	Organism	Ionic strength	Mineral grain size	Organism × ionic strength	Organism × grain size	Ionic strength × grain size	Organism × ionic strength × grain size
Homogeneous	%R	0.0001	0.0001	0.0001	0.242	0.0001	0.0001	0.237
	PW	0.0001	0.054	0.925	0.006	0.002	0.0001	0.26
	RT	0.347	0.347	0.347	0.347	0.347	0.0001	0.347
	PH	0.0001	0.006	0.0001	0.023	0.0001	0.374	0.002
	CV	0.017	0.0001	0.001	0.274	0.426	0.628	0.343
Homogeneous and heterogeneous	%R	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Heterogeneous	%R	0.0001	0.0001		0.0001			
	A1/2	0.013	0.093		0.071			
	H1/2	0.031	0.916		0.324			
	PW1	0.0001	0.205		0.092			
	PW2	0.618	0.36		0.709			
	CV	0.003	0.001		0.011			

[a] Separate analyses were conducted for the homogeneous and heterogeneous cases. Values presented are the P values obtained for the contrast examined (significance of F for the effect listed). For the variable percent recovery (%R), an analysis was run on the pooled homogeneous and heterogeneous cases with the presence of the heterogeneity as a "treatment" condition included in the "grain size." Significance was defined at $P \le 0.05$.

[b] PH, peak height; A1/2, area of peak 1/area of peak 2; H1/2, height of peak 1/height of peak 2; PW1, and PW2, width (at half-height) or peak 1 or 2, respectively.

variables may relate to desorption of cells from mineral surfaces or deflocculation of aggregated cells in pore throats too wide to prevent the flow of single cells.

The response variable retention time, a measure of the time of transport, varied little (always about 1 pore volume, i.e., 1 h under the experimental flow rates), although small differences would not have been detected. This indicates that, while some cells were retained by the column, a large majority of those cells that were not retained moved directly through the column with little or no retardation. The concept of retention with minimal retardation is not consistent with equilibrium sorption coupled with advection-dispersion theory, although the results obtained in the present study are corroborated by field studies performed by Harvey and George (19), who observed bacterial breakthrough coincident with or slightly in advance of bromide breakthrough in a sandy Cape Cod aquifer. Also, Pekdeger and Matthess (26) reported retardation factors for bacteria as low as 1 (i.e., no retardation) in some of their field experiments. In neither case were all of the bacteria recovered.

The peak width variable displayed a strong effect of the organism, with the large rods of W8 having broader peaks than the smaller cells of W6. The argument of size exclusion is often used to provide explanation for differences in transport of different sized particles. The size exclusion principle dictates that larger cells will be the first to break through a column with a distribution of pore diameters, because only the wide pores are available to the bigger particles, and the velocity in these pores is higher than that in smaller pores.

Mechanical dispersion is a result of mixing of waters that follow "paths" of various velocities (13). In a porous medium velocity varies as the square of the pore size. Because the smaller organism can be transported through a wider variety of pore sizes than the larger organism, the pore size argument would appear to predict that smaller-sized particles (i.e., W6) should be the most influenced by dispersion. Such was not the case, however; the larger cells were most strongly influenced by dispersion in our experiments. An explanation may be found in the hypothesis of Gvirtzman and Gorelick (18) which asserts that dispersion actually depends on mixing of paths with significantly different velocities. In a porous medium the average distance between mixing points (pore intersections) is related to the sizes of the paths. Furthermore, because velocity is related to the square of the pore diameter, velocity differences between two intersecting paths will be related to size. Thus, Gvirtzman and Gorelick argue that the longer the distance between mixing points, the greater will be the dispersion. If this hypothesis is true, greater dispersion effects in the treatments with the larger-diameter bacterial cells would be expected, because the larger cells will pass only through the larger pores, which have longer distances between mixing points.

Heterogeneous columns. The doubly peaked breakthrough curves of the heterogeneous columns were apparently the result of differential flow rates within the preferred flow path and the surrounding fine-grained matrix. Initially, it was presumed that the first peak is dominated by cells which were transported through the central vein of coarse sand, while the second peak largely represented cells which passed through the fine-grained portion of the column. The height of the second peak is somewhat puzzling, in that it was expected (based on the results of the homogeneous columns) that more cells would be retained by the finer-grained matrix, and the second peak would be much smaller than the

first one; mass recoveries in the second peak always exceeded those for the fine-grained homogeneous columns with the same organism and ionic strength. In contrast to our expectations, the two peak heights were more evenly matched (Tables 1 and 2; Fig. 5).

It is important to note that included in the height of the second peak is the height of the tail of the first peak. Although the tail heights observed in the homogeneous columns are fairly negligible compared with the peak heights, the tail of the first peak in the heterogeneous columns may be higher (or at least slower to reach a low level) due to interactions between the two domains of flow. For example, cells which had been initially retained by the fine-grained matrix could be diffusing into the preferred flow path, becoming, in effect, a part of the first peak's tail. Yet reduction of the area of the second peak by the area that should be included in the tail of the first peak cannot account for the difference in the number of cells observed in the second peak and the number of cells predicted on the basis of the results with homogeneous columns.

In general, the response variables determined for the breakthrough curves of the heterogeneous cases behaved in a manner consistent with the homogeneous case (where comparisons can be made), with the notable exception being the variable relating the area of the two peaks (A1/2). The peaks are roughly equivalent in area, even though the ratio of the cross-sectional areas of the two flow domains is about 1:6 (coarse/fine). Computation of flow volumes (based on comparisons of porosity, cross-sectional area, and hydraulic conductivity for the two domains) indicates that the ratio of flow in the coarse vein to flow in the fine bed is 40:60%. Thus, the expected value of A1/2 should be approximately 0.67 if the two peaks represent transport that is proportional to the flow in the two domains. The actual ratios do not approximate the ideal value of 0.67 (Tables 1 and 2). The ratio for W6 is approximately 1.15 for both ionic strengths, whereas the values for W8 are much more variable but always <1. Also, the response variable CV demonstrated a significant organism effect, and the values for the larger cells (W8) were always greater than those for the smaller cells (W6), in analogous treatments, indicating the observed relative shift in mass to the second peak. Obviously, the response variable A1/2 indicates that the relative mass of cells in each peak is controlled by some factor other than the flow in the two domains.

As pointed out above, if the second peak represents only flow through the fine-grained portion of the column, the recovery could not exceed that in the fine-grained homogeneous treatments; thus, some major portion (if not all) of the cells in the second peak must also pass through the preferred flow path. We hypothesize (somewhat speculatively) that the mechanism for the generation of the relatively large second peak involves a radially distributed capture of randomly patterned flow paths of various lengths. In essence, the transport of cells through the matrix is "short circuited" whenever the flow path happens to intersect the tubule. Some of the paths are short circuited very early, while others do not intersect the tubule at all. Thus, there would be a distribution of times at which the cells would reach the tubule with a peak in the distribution later than the transport time for the tubule itself. The greatest contribution of such a mechanism, therefore, would occur later in the flow sequence. Note that this is a dispersive mechanism calling for mixing along flow paths having different velocities.

The organism effect on this variable (Table 3) suggests that the hypothesized mechanism is specific to the organism. The
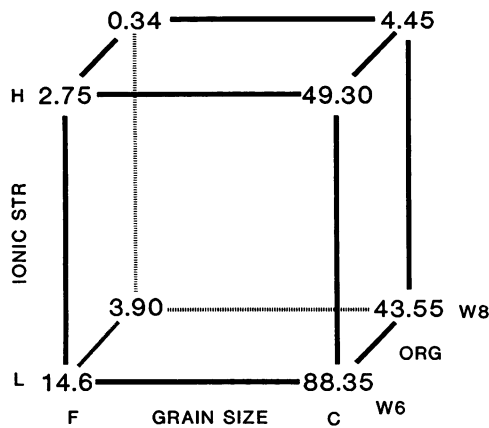
FIG. 6. Graphic representation of the strength-of-effect analysis as described by Box and Draper (4). The following values represent the differences in the average effect for each level of the three factors examined: grain size, 41.02; ionic strength, −25.69 bacterial size (ORG), −23.39.

TABLE 4. Treatment combinations ranked by percent recovery

Organism[a]	Ionic strength	Mineral grain size[b]	%R
W6	Low	CHO	88.35
W6	Low	HET	79.15
W6	High	CHO	49.30
W8	Low	CHO	43.55
W8	Low	HET	39.05
W6	High	HET	19.70
W6	Low	FHO	14.50
W8	High	HET	11.70
W8	High	CHO	4.45
W8	Low	FHO	3.90
W6	High	FHO	2.75
W8	High	FHO	0.34

[a] W6, coccus; W8, the larger rod.
[b] FHO, fine-grained, homogeneously packed sand; CHO, coarse-grained, homogeneously packed sand; HET, heterogeneous.

results are consistent with the observation for the homogeneous cases that dispersion is greatest in the larger cells (W8). If dispersive processes are responsible for delivery of the cells to the central coarse-grained tubule, then W8 should have shown more cells in the second peak relative to the first peak (as compared with W6), and, in fact, it did.

Ranking the strength of effects. Insight into the relative importance to transport efficiency of each of the variables examined can be gained by ranking all of the treatments in order of percent recovery (see Table 4). Experimental runs involving low ionic strength, coarse sand (including that contained in the structured heterogeneity), and/or small cells tended to be grouped towards the top of the listing (i.e., higher recovery), while those involving high ionic strength, fine sand, and/or large cells were grouped lower on the list (lower recovery). The clearest division appears to be between fine and coarse sands, leading to the conclusion that, among the variables tested and at the range of values tested, grain size of the porous media may be the dominant factor in determining overall transport efficiency; this would certainly be consistent with filtration theory. The ranking can be further broken into only coarse-grained columns and only fine-grained columns to determine what might be the next most important factor. In coarse-sand experiments, there was a marked division between runs involving the different strains of bacteria sizes, while in the fine-sand experiments (as well as those with structured heterogeneities), differing ionic strength appeared to be more important than the bacterial strain used in determining recovery.

A more quantitative assessment of the strengths of effect of the various treatments can be accomplished by following the computations outlined in Box and Draper (4). This procedure is designed to produce a response surface with the resultant strength of effect. The values obtained for each treatment were as follows: grain size, 41.02; ionic strength, −25.69; and organism, −23.39 (Fig. 6). The effect of grain size was the largest in magnitude, in agreement with the ranking observations (Table 4). The effects of differing the ionic strength or the organism (W6 or W8) were nearly equal to one another (the negative sign indicates that the percent recovery increases as the size of the bacterial cells or ionic strength decreases) and were smaller than that of the grain size effect. The similarity in magnitude of the strengths of the

organism and ionic strength effects is consistent with the qualitative ranking assessment in which these two effects seemed to exchange importance with change in grain size.

The order of importance can only be truly applied to the variables considered over the range tested; we might reasonably state that, for sandy aquifers, a tripling in average grain size would have a more pronounced effect on transport efficiency than would an order of magnitude decrease in ionic strength from around 0.01 to 0.001. The same may or may not be true for an aquifer of finer texture or for a different range of ionic strengths. Nevertheless, the ranges examined are common in soils and aquifers, and a knowledge of the strengths of effects at these ranges can enhance theoretical understanding of the transport properties of bacteria in porous media. Such information can now be used to test model approaches (21) that will ultimately be used to predict the potential for transport of bacteria as pathogens, facilitators of pollutant transport, or mediators of pollutant destruction. Continued refinement of these approaches along with the addition of the biological variables such as growth, motility, etc., will ultimately provide a comprehensive theory of bacterial transport for all applications.

ACKNOWLEDGMENTS

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