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Effect of Sodium Chloride on Transport of Bacteria in a Saturated Aquifer Material

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Determinations were made of the influence of NaCl concentration, cell density, and flow velocity on the transport of *Pseudomonas* sp. strain KL2 through columns of aquifer sand under saturated conditions. A pulse-type boundary condition was used. The experiments were conducted by using 0.3-m-long Plexiglas columns with an internal diameter of 0.05 m. When a 1-h pulse of a 0.01 M NaCl solution containing 10^8 cells per ml was added at a flow rate of 10^{-4} m s^{-1}, the bacterial density in the effluent never exceeded 2.2% of the density of cells added, and only 1.5% of the bacteria passed through the aquifer material. In contrast, when the bacteria were applied in distilled water, the relative cell density in the effluent approached 100%, and 60% of the bacteria were transported through the aquifer solids. Under these conditions, the breakthrough of *Pseudomonas* sp. strain KL2 was slower than chloride. When the flow rate was 2.0×10^{-4} m s^{-1}, the cell density in the effluent reached 7.3% of that added in 0.01 M NaCl solution, but only 3.9% of the bacteria were transported through the aquifer particles. On the other hand, the density in the effluent approached 100% of that added in deionized water, and 77% of the added bacteria were recovered. When the density of added cells was 10^9 cells per ml at a flow rate of 10^{-4} m s^{-1}, the densities in the effluent reached 70 and 100% of those added in salt solution and deionized water, respectively, and 44 and 57% of the bacteria were transported through the aquifer solids. Replacement of the NaCl solution with deionized water caused some of the retained cells to be carried through the column. We suggest that the movement of bacteria added to sandy aquifers for bioremediation of contaminated sites may be promoted by modifying the chemical composition of the carrying solution.

Many toxic organic chemicals persist at underground waste sites despite being readily biodegradable under laboratory conditions. When this occurs, bacteria selected for their capacity to degrade the contaminants and to proliferate after injection in the aquifer might be added to enhance biodegradation. However, for such an approach to bioremediation to be successful, the introduced species must be able to reach the contaminated zone and to move through the porous material along a possible contaminant plume.

Considerable research has been devoted to the movement of microorganisms in soil and subsurface materials because of concern with the dissemination of pathogens from groundwater recharge, land spreading operations, and the disposal of manure, municipal sludge, or wastewaters (1, 3, 10). The reported distances of bacterial transport in soils and aquifers vary from centimeters to kilometers (4, 8, 22). Gerba et al. (8) observed that bacteria added to soil are largely removed at or near the surface. Wollum and Cassel (21) reported that only 0.2% of the added bacterial cells and 6% of the streptomycete conidia were recovered in the effluent after passage of 4 pore volumes of water through a sand column; >90% of the organisms were recovered within 3 cm of the surface. Similarly, <1% of the bacteria injected into a sandy aquifer were recovered at a sampling well 1.7 m from the site of injection (11). However, it has been reported that infiltrating solutions with low ionic concentration will decrease retention of bacteria by sand (12, 19). The retention efficiency of coliforms by acid-treated sand was higher when bacteria were suspended in tap than in distilled water, and cells suspended in triply distilled water were not retained (9). Furthermore, the application of rainwater or distilled water has been noted to result in the desorption of viruses from soil particles (6, 7, 18).

Because of the importance of bacterial movement to bioremediation with introduced microorganisms and the paucity of information on the effect of ionic concentration on the movement of bacteria through subsurface earth materials, a study was initiated of the breakthrough of bacteria through columns of saturated aquifer sand. The influences of the presence or absence of an electrolyte (NaCl) in the carrying fluid, bacterial density, and flow velocity on the transport of the cells were evaluated.

MATERIALS AND METHODS

Pseudomonas sp. strain KL2 was originally isolated from Kendaia loam. This strain was selected because, of the 19 bacteria tested by Gannon et al. (5), it showed good mobility in homogeneous columns of disturbed Kendaia loam. The bacterium was grown for 24 h at 30°C in 250-ml Erlenmeyer flasks containing 200 ml of Trypticase soy broth. The cultures were incubated on a rotary shaker (model 3528-1; Lab. Line Instruments, Inc., Menrose Park, Ill.) at 100 rpm. The cells were collected by centrifugation, washed twice in deionized water with a resistivity of 18.3 megohms cm, and resuspended in either deionized water with the same resistivity or a 0.01 M NaCl solution. The pH of the suspensions was 7 ± 0.2. Ten minutes before initiating a test of breakthrough, possible cell aggregation was assessed by examining wet mounts and by measuring the turbidity at 550 nm of

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the bacterial suspension at 0 and 5 min by the procedure described by Calleja (2). Bacterial aggregation was not observed in either deionized water or the NaCl solution by these methods.

Sandy aquifer material from Eastport, N.Y., was collected with auger drillers from a depth of 11 m, special precautions being taken not to mix it with surface soil. Analysis of the solids after they were air-dried and passed through a 2-mm sieve showed that they contained 90.4% coarse sand (0.5 to 2 mm), 3.1% medium sand (0.25 to 0.5 mm), 4.6% fine sand (50 μm to 0.25 mm), 1.9% silt (2 to 50 μm), and 0.07% clay (<2 μm).

The experiments were conducted by using 0.3-m-long Plexiglas columns (Soil Measurement Systems, Tucson, Ariz.) with an internal diameter of 0.05 m. The aquifer sand was constantly stirred while it was poured into the column to prevent layering of separate particle sizes. The sand was packed uniformly to a bulk density of 1.75 ± 0.012 Mg/m^3 by gently tapping the walls of the column. Bottles containing 0.01 M NaCl solution, deionized water, and a suspension of *Pseudomonas* sp. were connected to the column, and a peristaltic pump was used to supply these liquid solutions at constant flow velocities. All tubing, glassware, and valves were autoclaved to avoid contamination, and the aquifer solids were sterilized by irradiation with ^{60}Co (2.5 Mrads). The columns were set up vertically and saturated from below with sterile deionized water. A constant upward-flow velocity was maintained thereafter with the peristaltic pump.

Two types of boundary conditions could in principle be adopted in tests of column breakthrough, a step increase in concentration or a pulse-type boundary condition. The latter was chosen largely to limit the risk of clogging at the inlet end of the column, but also because the resulting breakthrough curves usually provide more information on the mechanisms affecting transport than the curves obtained with a step increase in concentration. To obtain a pulse-type boundary condition, the sterile deionized water percolating through the column was replaced for 1 h by the bacterial suspension. In experiments in which bacteria were suspended in an NaCl solution, the bacterial pulse was followed by a sterile 0.01 M NaCl solution. Otherwise, the flow of deionized water was resumed. In a study to determine the effect of a sudden reduction in the ionic concentration of the solution, a pulse of bacteria suspended in the NaCl solution was followed by 0.01 M NaCl solution for 1 h and then by deionized water for 2 h.

In all cases, the effluent from the columns was collected in a fraction collector and its pH was continuously monitored. The bacterial concentration in each fraction was determined by the drop plate method (16) on Trypticase soy agar, using triplicate samples for each dilution. All experiments were carried out in duplicate, and the temperature was maintained at 3 ± 1.5°C to limit bacterial growth and death.

Numerical integration of the untransformed data, using the trapezoidal rule (15), yielded the percentage of bacteria recovered in the effluent after a given period of time.

The breakthrough of a tracer (chloride) was determined separately under the same conditions by applying a 0.01 M NaCl solution for 1 h followed by deionized water. The chloride concentration in successive fractions of the effluent was measured with a chloridometer (Haake Buchler Instruments, Saddlebrook, N.J.). In all cases, perfectly symmetrical breakthrough curves showed that chloride behaved as a tracer and that preferential pathways were absent from the column.

RESULTS

Experiments were first conducted with a flow rate of 10^{-4} m s^{-1} and with 10^8 cells per ml. This flow rate was equivalent to 3.5 pore volumes per h. The data show a distinct effect of the NaCl concentration of the carrying liquid phase on the extent of bacterial breakthrough. When *Pseudomonas* sp. strain KL2 was suspended in 0.01 NaCl solution, the cell density in the effluent, C, never exceeded 2.2% of the input density of cells, C_0; i.e., the ratio C/C_0 did not exceed 0.022 (Fig. 1a). In contrast, the values of C/C_0 approached 1.0 after approximately 70 min when the bacteria were suspended in deionized water (Fig. 1b). The breakthrough of bacteria suspended in deionized water was slower than that of chloride. The times for measurable appearance of chloride and bacteria were 12 and 15 min, values that are quite similar, but the relative concentration (C/C_0) of chloride in the effluent increased rapidly thereafter and reached unity 30 min after application of the pulse, whereas the ratio for the bacteria increased more gradually. The tail ends of the breakthrough curves for chloride and bacteria suspended in deionized water appear to coincide, a sharp drop occurring in both cases at approximately 75 min. The precise time of the fall in value of C/C_0 for cells suspended in 0.01 M NaCl solution is not certain because of the variations in measured cell densities.

Within 2 h after application of the pulses, 100% of the chloride and 60% of the bacteria suspended in deionized water passed through the column, whereas only 1.5% of the cells suspended in 0.01 M NaCl solution was collected in the effluent. Although some scatter was evident in the data, the differences in recovery were consistently pronounced.

In the first experiment, as in all of the following ones, the pH of the effluent was within measurement error of that of the applied bacterial suspension (7 ± 0.2), in spite of the fact that the carrying fluid contained no buffer.

A marked influence of NaCl concentration was also evident at a flow rate of 2.0×10^{-4} m s^{-1}, which is equivalent to 6.9 pore volumes per h. With *Pseudomonas* sp. strain KL2 suspended in 0.01 M NaCl solution, the relative concentration (C/C_0) in the effluent reached 0.073 (Fig. 1c). In this instance, 3.9% of the cells were transported through the column. Both of these values are higher than those noted previously at the lower flow rate. With cells suspended in deionized water, in contrast, the value of C/C_0 reached 1.0 approximately 30 min after application of the pulse, and breakthrough was evident sooner (Fig. 1d). The behavior of the bacteria suspended in deionized water coincided with that of chloride after the initial breakthrough, the same drop in C/C_0 being observed between 65 and 70 min. At the end of the test period, 77% of the cells and 99% of the chloride were recovered in the effluent. The times for first breakthrough of cells and chloride were shorter at the higher flow rate than at the lower flow rates.

Similar experiments were conducted with 10^9 cells per ml and a flow rate of 10^{-4} m s^{-1}. At these bacterial densities, the patterns of breakthrough were similar for the 0.01 M NaCl solution and deionized water (Fig. 2). However, the values of C/C_0 differed, reaching 1.0 for cells suspended in distilled water and 0.7 for bacteria suspended in NaCl solution. A precipitous drop in C/C_0 occurred at between 70 and 80 min for cells suspended in NaCl solution or distilled water, a time similar to that associated with the tail ends of the breakthrough curves for chloride and bacteria noted previously under the same flow conditions. In columns

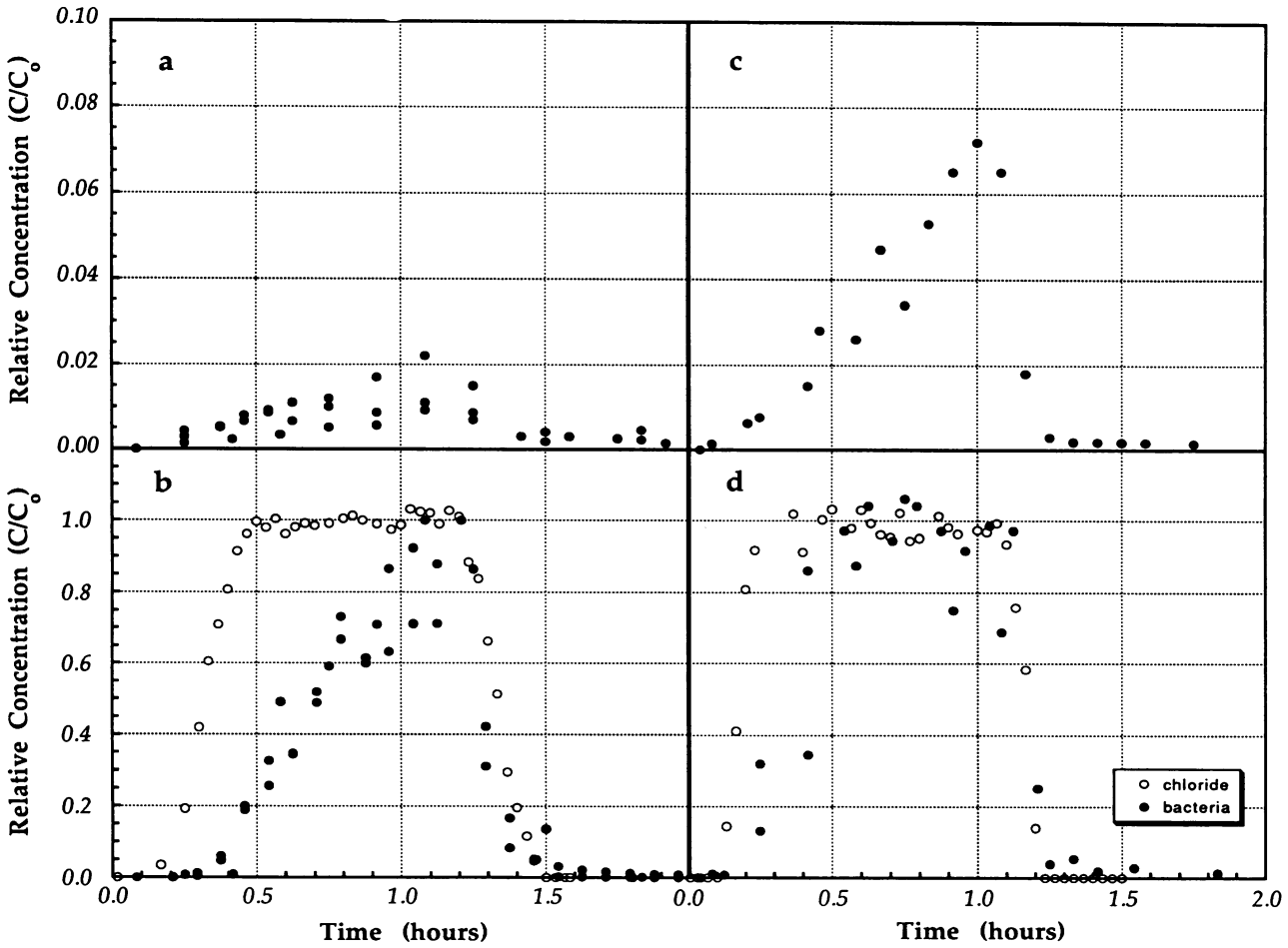

FIG. 1. Breakthrough of bacteria added at 10^8 cells per ml and flow rates of 10^{-4} (left two panels) or 2.0×10^{-4} (right two panels) m s^{-1}. The cells were suspended in 0.01 M NaCl solution (a and c) or deionized water (b and d). The chloride breakthrough is shown for comparison in the bottom panels for the two flow rates.

receiving NaCl and deionized water, respectively, 44 and 57% of the cells were transported through the column.

In the last series of experiments, the flow rate was 10^{-4} m s^{-1} and the bacterial density was 10^8 cells per ml. The conditions were therefore identical to those under which the results depicted in Fig. 1a and b were obtained. The continuous application of the NaCl solution did not displace the bacteria that were retained in the column. Indeed, when only the NaCl solution was used, a single peak of bacterial breakthrough was observed (Fig. 3). The highest C/C_0 ratio was 0.02. However, when the NaCl solution was replaced by deionized water, a second peak of bacterial breakthrough was noted. The relative bacterial concentration at the second peak reached 0.12.

DISCUSSION

The retention of bacteria in porous media has been attributed to several causes (20): straining or filtration at pore constrictions, sedimentation in the pores, diffusion in pores not contributing actively to the transport of water (e.g., dead-end pores and micropores), and adsorption. These various mechanisms probably interact with each other and may therefore, to a large extent, be operationally indistinguishable. For example, adsorption of bacteria on solid surfaces in the porous medium may reduce the dimensions of the pore constrictions and thereby enhance straining.

When the inflowing solution was deionized water, the bacteria readily moved through the sand column after an initial stage of retention. This high mobility of bacteria may be attributable to the low adsorptive capacity and large pores of the sandy aquifer material. Because of the very low clay content (0.07%) of this material, it is unlikely that the markedly reduced bacterial breakthrough observed when the NaCl solution was used may have resulted from a direct effect of the electrolyte on the hydraulic properties of the solid matrix. Far more probable is that the bacterial cells were removed from the bulk liquid phase by interactions among themselves or with the solid surfaces, interactions promoted by an increase in NaCl concentration.

Solid surfaces in aquifer materials generally carry electrostatic charges. These charges may be permanent (not pH dependent), in which case they are generally negative, or they may depend on the pH of the surrounding electrolyte solution. Similarly, the surfaces of bacterial cells have pH-dependent charges. The need to maintain electroneutrality gives rise to a particular distribution of ions, termed the diffuse layer, in the electrolyte solution in the immediate vicinity of these charged surfaces. An increase in the ionic concentration of the electrolyte solution leads to a decrease

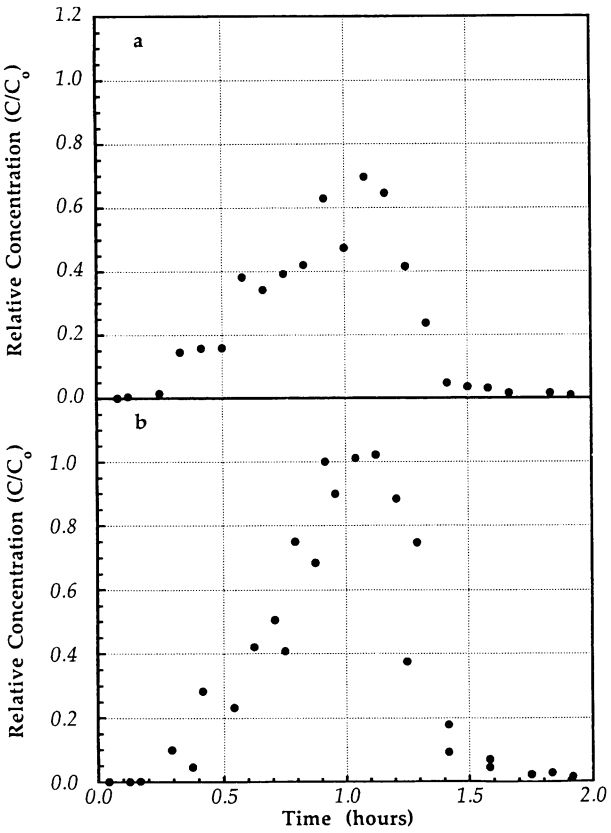

FIG. 2. Breakthrough of bacteria in tests with 10^9 cells per ml and a flow rate of 10^{-4} m s^{-1}. Bacteria were suspended in (a) 0.01 M NaCl solution or (b) deionized water.

in the thickness of the diffuse layers and in the average distance between surfaces carrying similar charges. As a result, cell aggregation and cell adsorption on charge-bearing solid surfaces may be promoted (13, 22).

Since bacterial aggregation was not observed in either deionized water or NaCl solution, the enhanced retardation of bacterial transport observed in the presence of NaCl may have resulted from a direct effect of the ionic concentration on the adsorption of cells.

The differences in bacterial transport with 0.01 M NaCl and deionized water were greater at a density of 10^8 cells than at 10^9 cells per ml. This observation may be accounted for if it is assumed that electrostatic interaction was the key mechanism of cell adsorption, with hydrophobic interactions and polymer bridging (14) playing only minor roles. Under these conditions, a smaller percentage of the larger than of the smaller population would be retained by the limited number of adsorption sites. Also, the finite adsorption capacity of the aquifer solids would be filled progressively as a pulse advances in a column, resulting in a retardation of the bacterial breakthrough. Once the adsorption capacity of the aquifer material is filled, bacteria would be able to move freely or to replace adsorbed bacteria. The tracerlike behavior that would then result may explain the coincidence of the tail ends of the breakthrough curves of bacteria and chloride in Fig. 1b and d.

Appreciable differences between the two experiments involving different cell concentrations were not evident when deionized water was used (cf. Fig. 1b and 2b), possibly

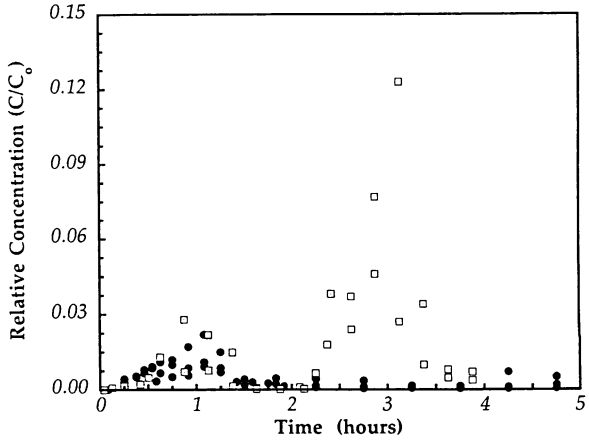

FIG. 3. Breakthrough of bacteria in tests with 10^8 cells per ml and a flow rate of 10^{-4} m s^{-1}. The bacterial pulse was followed by 0.01 M NaCl solution for 1 h and then deionized water (open squares) or by 0.01 M NaCl solution for 4 h (closed circles).

because of the low adsorption capacity of the aquifer solids under these conditions.

The higher flow velocity resulted in a higher percentage of the bacterial cells passing through the columns. However, it also led to a marked increase in the number of cells being retained by the aquifer material. This increase is equal to 152% in the case of deionized water and to 420% when the NaCl solution was used. It may have resulted from somewhat faster adsorption under the higher flow velocity regime. Smith et al. (17) also concluded that velocity of water flow is an important factor in bacterial movement through soil.

The replacement of the NaCl solution by deionized water resulted in a second peak of bacterial breakthrough. This probably is not the result of an effect on straining, since retention of bacteria by this means would not likely be reversible unless the aquifer solids were disturbed. Instead, reducing the ionic concentration of the aqueous phase probably reversed the retention resulting from adsorption.

The data suggest that, under appropriate conditions, bacteria are readily transported through sandy aquifer materials. Hence, for bioremediation of sandy aquifers contaminated with organic pollutants, the use of solutions with low ionic concentration may markedly enhance the movement of introduced bacteria to sites of contamination. However, it may be possible to achieve the same result by other means that would be easier to implement in practice. Because the surface charge of bacteria is pH dependent, it may be possible to increase the repulsion between bacterial cells and solid surfaces by modifying the pH of the carrying fluid within the limits of viability of the organism. Further research is needed to investigate this possibility.

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