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Laboratory Investigations on the Role of Sediment Surface and Groundwater Chemistry in Transport of Bacteria through a Contaminated Sandy Aquifer

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N The effects of pH and sediment surface characteristics on sorption of indigenous groundwater bacteria were determined using contaminated and uncontaminated aquifer material from Cape Cod, MA. Over the pH range of the aquifer (5-7), the extent of bacterial sorption onto sediment in uncontaminated groundwater was strongly pH-dependent, but relatively pH-insensitive in contaminated groundwater from the site. Bacterial sorption was also affected by the presence of oxyhydroxide coatings (iron, aluminum, and manganese). Surface coating effects were most pronounced in uncontaminated groundwater (pH 6.4 at 10 ºC. Desorption of attached bacteria (up to 14% of the total number of labeled cells added) occurred in both field and laboratory experiments upon adjustment of groundwater to pH 8. The dependence of bacterial sorption upon environmental conditions suggests that bacterial immobilization could change substantially over relatively short distances in contaminated, sandy aquifers and that effects caused by changes in groundwater geochemistry can be significant.

Introduction

The transport of bacteria through porous media is a subject of current interest, having application in the fields of bioremediation and public health. Strategies for in-situ remediation of contaminated aquifers require information on the transport of pollutant-adapted or genetically engineered microbes. Where drinking water supplies are derived from groundwater, transport of pathogenic bacteria from sewage and solid waste disposal sites is also of great concern. In sandy aquifers, the degree of bacterial transport can be governed largely by sorptive interactions with stationary grain surfaces. An important aspect of modeling microbial transport in porous media is how best to account for sorption processes (1-8). The DLVO (Derjaguin-Landau and Verwey-Overbeek) theory of colloid stability (9) describes the interaction of electrostatic and van der Waals forces in flocculation of colloidal suspensions and is often used, in part, to describe bacterial sorption in aqueous systems (10-13). Colloid filtration theory has been invoked in models describing experimental observations of bacterial transport within a sandy aquifer and in sand columns (2, 4). Colloid filtration models quantify the physical mechanisms of particle contact with surfaces but use empirical coefficients to describe the effects of aqueous chemistry (14). Tobiason and O'Melia (15) modified the colloid filtration model to account for electrical double-layer (EDL) interaction forces; however,

the modified approach could not predict particle deposition under some experimental conditions. The complex interactions of chemical and physical forces in colloid deposition in porous media have not yet been integrated into a predictive model (16), but existing colloid filtration theory is useful in understanding physical aspects of bacterial transport in porous media. The present study focuses on the chemical influences on bacterial attachment as they are transported through sandy aquifer sediments of Cape Cod, MA. Disposal of treated sewage in infiltration beds since 1936 has resulted in a plume of water with elevated concentrations of total dissolved solids, chloride, dissolved organic carbon (DOC), and detergents that is over 4 km long and 1 km wide (17). Physical and chemical conditions in the heterogeneous, contaminated aquifer may change substantively within 1 m or less (18).

Although there have been many studies involving bacterial adhesion onto solid surfaces, most have involved laboratory-grown bacteria, well-defined (model) surfaces, and physicochemical conditions that differ from those present in the subsurface. Our approach to the laboratory study was to test bacterial sorption under more natural conditions in order to assess how chemical heterogeneity may affect transport of bacteria in a contaminated sandy aquifer. In this study, we investigated sorption of indigenous aquifer bacteria onto core material from an oxic, uncontaminated zone of the aquifer and from a suboxic area within the contaminant plume, using groundwaters collected near the source of the contaminant plume and outside the boundary of the plume.

The experiments tested the effect of three nonspecific variables (presence of oxyhydroxide coatings on sand grains, presence of organic matter in water and on sand grains, and water composition) and one specific variable (pH) on bacterial sorption to the aquifer material. Desorption of bacteria in response to slightly alkaline conditions was also measured. The experiments showed that the geochemistry of the groundwater environment has a strong influence on bacterial sorption and desorption in aquifer sediments and will therefore have an effect on bacterial transport in sandy aquifers.

Experimental Section

Core Material and Groundwater. The mineralogy and organic content of the aquifer material have been characterized by Barber (19). The aquifer consists of glacial outwash deposits, predominantly medium to coarse sand, with median particle size range of 0.25-1-mm diameter. There is very little silt or clay-sized material. The mineralogy and organic content vary with particle size fraction, with most of the magnetic and heavy minerals and organic matter in the smaller particle size fractions. The coarser sand fraction is predominantly (-90%) quartz and feldspar, with -10 % lithic fragments (mostly granite), goethite, limonite, and glauconite pellets. Oxyhydroxide and organic coatings on grain surfaces are very important in sorptive processes (20, 21) and vary depending on location of the core sample with respect to the source area of the contaminant plume. Core material was collected from an uncontaminated zone above the plume and from a contaminated zone directly under the sewage infiltration beds. Cores were taken from the field site on Cape Cod and stored frozen, air-dried, and sieved prior to use. All experiments were performed using uncontaminated core material with the exception of the investigation involving surface coating effects, in which organically contaminated core material was used. Groundwater and sediment chemistries are listed in Table L To preclude straining of bacteria (bacteria becoming trapped in intergranular pore spaces too small for them to pass), the 0.5-1-mm grain size fraction (estimated minimum pore aperture of 77-jum diameter) was used in all packed-sand columns.

Table 1. Chemistry of Groundwater and Aquifer Sediment Used in the Bacterial Sorption experiments

Groundwater

source	org contain well S318-36	uncontam. well S315-61	artificial
depth (m)	11.8	20.0	n/a
Ca	20.4	2.5	20.4
Mg	3.4	1.6	3.4
Na	50.6	9.2	50.6
K Cl	8.1	1.1	8.1
SO,	36.9	12.9	63.2
NO, alk (HCO3)	26.6	7.1	26.6
DOC ionic strength	10.6	1.8	10.6
spec cond (uS/cm) av	42.5	8.3	73.0
PH (autoclaved)	4.4	0.4	<0.4
	4.01 x 10-3	8.83 x 10-4	4.63 x 10`
	410	96	419
	7.3	6.4	7.2

Core Material

source	org contain well site S318	uncontam well site F350
depth (in)	11.1-11.9	6.7-8.2
spec condb (,uS/cm)	409	50-86 4.0-6.8
dissolved 02 b	70 ppb	mg/L 5.76-5.84
PH b	5.80	below detectn
sed-assoc organic C' (%)	0.01 ± 0.0003	

Extractable Metal'

	site 318			site 350
	U	N	O	U
extracted Fe	0.261		0.271	0.168
extracted Al	0.067			0.128
extracted Mn	0.134		0.077	0.038

'All chemical constituents in milligrams per liter, unless otherwise noted. 'In-situ parameters. 'Oxyhydroxide coatings on grain surfaces as determined by oxalate extraction. Numbers are in milligrams per gram of sediment. Standard errors are :520%. Treatments: U, untreated; N, NaOH-treated, 0, oxalate-treated.

The effect of organic and oxyhydroxide coatings on mineral grains on bacterial sorption was tested by removing the coatings from contaminated core material with sequential NaOH and oxalate leaches. These treated sediments were used in bacterial sorption experiments with contaminated, uncontaminated, and artificial groundwater. Organic coatings were removed by leaching 150 g of sieved core material in 500-mL Nalgene bottles with 400 mL of 0.1 M NaOH for 3 h on a shaker. The sand was rinsed with 0.05 M HCl briefly, then rinsed repeatedly with distilled deionized water (MilliQ system, Millipore Corp.), and dried at 85 'C. One portion of the treated sand was further leached with 350 mL of oxalic acid (3%) at 70 'C for 1 h (adapted from ref 22), rinsed briefly in 0.05 M NaOH, then rinsed repeatedly with MilliQ water, and dried. Organic content of treated and untreated sediment fractions was determined on a Leco carbon analyzer. All sediment fractions had organic carbon content below detection except the untreated contaminated sediment. The oxalate leachate was analyzed for Fe, Al, and Mn by inductively coupled argon plasma analysis (ICAP) (Table I).

Both contaminated and uncontaminated groundwater was used in the experiment (Table I). The contaminated water was collected from 11-m depth underneath the sewage infiltration beds. The uncontaminated water was

collected at 18-m depth upgradient of the sewage treatment plant. Water samples were collected at the field site, shipped overnight, and stored at aquifer temperature (10 oQ. One day prior to an experiment, an appropriate amount of the water was filtered (0.2)um) to remove bacteria and then autoclaved. For the experiment that assessed pH dependency of sorption, the pH was adjusted with NaOH or HCl immediately before use. Artificial groundwater (AGW) chemically similar to the contaminated groundwater but without appreciable dissolved organic carbon (DOC) was used to determine the effect of DOC upon bacterial sorptive behavior. However, since the charge balance on the contaminated water depends, in part, upon the presence of dissolved organic compounds which contribute to anionic charge, it was difficult to make up an AGW with the exact ionic strength and pH of the contaminated water.

Bacteria. Bacteria used in the experiments were concentrated from the "contaminated" water sample by filtration of 1.1 L of water onto a 0.2-Am pore size membrane filter (Nuclepore Corp.). Filtration was used rather than centrifugation in order to collect all sizes of bacteria present in the water sample. The water sample collected was large (40 Q so that all the experiments could be run using the same population of bacteria. Bacterial numbers in the sample did not change appreciably during the time the experiments were run. The sampling method would seem to have selectively collected unattached groundwater bacteria, although bacteria collected under the infiltration beds may not have had sufficient time to sorb to aquifer sediments. This morphologically diverse population has been used in previous subsurface transport experiments performed at the site (2, 23). The collected bacteria were stained with 4',6-diamidino-2-phenylindole (DAPI; a DNA-specific fluorochrome) at 5 ug/ml, final concentration for 1 h, then refiltered, rinsed free of DAPI solution, resuspended in 1:1 MilliQ water and filtered groundwater, and stored at 4 'C. Immediately before addition to the experimental columns, the bacterial concentrate was placed in a sonication bath for 120 s to disperse any aggregated bacteria. The DAPI metabolically impairs the bacteria, resulting in minimal to no observed growth under experimental conditions.

Batch/Column Experiments. The columns were glass 20-mL syringe barrels (Popper & Sons) placed vertically in a rack. Columns were acid-washed and baked for 1.5 h at 400 'C prior to use. Glass beads, 3 min in diameter, were used as support for the sand in the columns so that sand grains did not clog the outlet. The beads were treated with dimethyldichlorosilane (Glass-Treat, Alltech Inc.), which was found to be the best treatment to minimize bacterial interaction with the glass beads at the relatively low ionic strength of the groundwaters used. The tips of the syringes were extended with 4 cm. of 1/8-in.-diameter latex tubing, with a tubing clamp to control outflow.

Columns were prepared by placing 2.5 g of glass beads in the bottom of the column. Sand (10 g) was poured into each column containing the beads and 10 mL of the appropriate groundwater. There were no visible air bubbles in the sand pack, and the sand did not fractionate into layers by size. Columns and groundwater solutions were placed in a constant-temperature room at 10 'C, and the columns were allowed to equilibrate with repeated rinses of groundwater before addition of the bacteria. The aquifer material was found to have acid- and base-neutralizing capacity. Therefore, for the pH-dependent sorption experiments with pH substantially higher or lower than the natural groundwater, 50 AL of 0.25 N HCl or NaOH was added to 5 mL of water and allowed to react within the sand for 15 min, followed by three 5-mL rinses of the appropriate pH groundwater at 20-min intervals. This procedure served to stabilize the pH in the experiments.

After equilibration, each column contained 1 mL of water above the sandpack to which 1 mL of bacterial concentrate was added. The columns were allowed to flow briefly into sample vials in order to draw the bacterial solution into the pore space of the sand pack. Columns were left in the dark to minimize photoinactivation of the DAPI stain. After 5 h, the unattached bacteria were rinsed out of the aquifer material by pipeting 20 mL of water (>6 pore volumes) through the columns into sample vials; previous tests determined that >90% percent of the unattached cells came out in the first 10 mL of rinse water. Experimental pH was determined by taking the average of two measurements-one from a control column which contained only groundwater for the duration of the experiment, the other from eluent pH (filtered prior to measurement). Bacteria were enumerated using epifluorescent microscopy (23). Sorbed bacteria were determined by difference between the number of bacteria added and the number of unattached bacteria in the eluent. The relative flow rates of the columns were monitored qualitatively for most of the experiments. However, there was no correlation between bacterial abundance in the eluent and the flow rate out of the columns (average 0.26 mL/s).

Field Experiment. Experiments to study the transport of indigenous bacteria through sandy aquifer sediments have been performed at the USGS groundwater study site at Cape Cod, MA, and are described in detail by Harvey et al. (2, 23). Briefly, indigenous bacteria were concentrated from groundwater collected from below the sewage infiltration beds, stained with DAPI, and reintroduced along with various tracers to an area of the aquifer within the contaminant plume. In the May 1990 tracer test discussed herein, concentrations of the stained bacteria and a bromide tracer were monitored in samplers up to 12.8 m downgradient for up to 30 days. A desorption phase involved injection of groundwater adjusted to pH 8 with 0.05 M phosphate buffer 20 days after the initial injection of bacteria.

Results

Time, Concentration, and pH Dependency. Bacterial sorption was tested for 10 g of uncontaminated aquifer material, for times from 1 to 24 h. The rate of sorption in the experimental system was highest within the first 2 h. Little additional sorption occurred after 5 h (Figure 1A). Subsequent experiments were performed with a sorption equilibration time of 5 h. Although growth of stained cells was unlikely within the time frame of the experiment (2), it was desirable to keep the experiments brief.

Bacterial sorption onto uncontaminated aquifer material was tested at concentrations of added bacteria that ranged from 4.31×10^4 to 3.95×10^7 cells/mL (Figure 1B). For 10 g of aquifer material and 5-h sorption time at 10 *C, sorption of DAPI-stained cells was linear over the range of concentrations tested. Bacterial concentrations used in all subsequent experiments ranged from 2 x 101 to 4×10^7 cells/mL. Comparison of results between experiments on a percentage-sorbed basis is possible because the ratio of sorbed to unattached cells varied only slightly (-2%) over this range.

The pH dependency of bacterial sorption onto uncontaminated sediments in contaminated groundwater differed markedly from that in uncontaminated groundwater

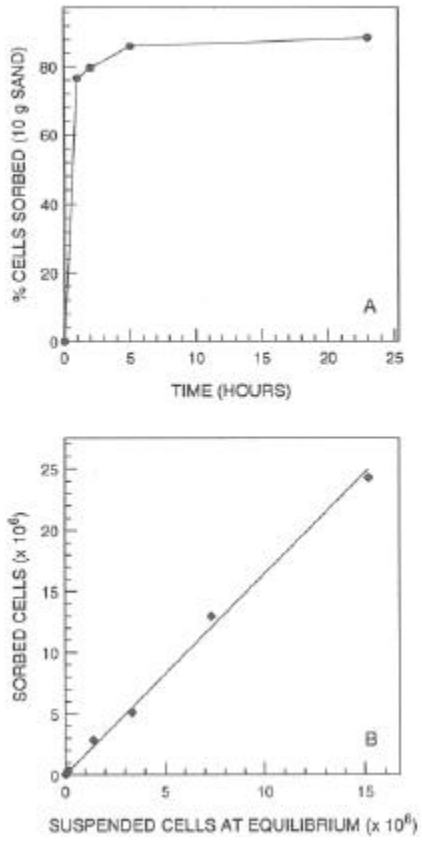

Figure 1. Bacterial sorption with time and added bacterial concentration. Experiments had two replicates per treatment. In)k, error bars are within the size of points as shown.

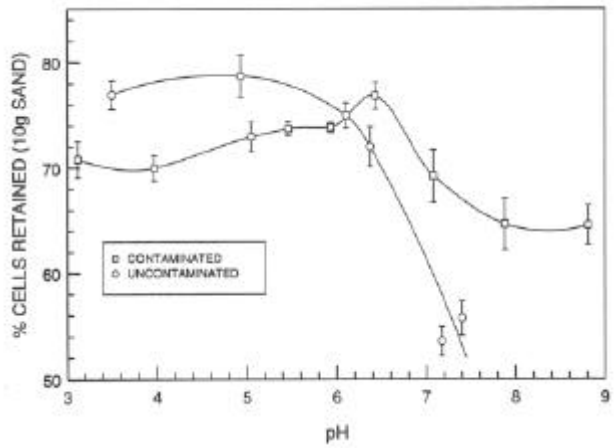

Figure 2. Effect of pH on bacterial sorption to uncontaminated aquifer material in contaminated and uncontaminated groundwater. Experiments had three replicates per treatment. Error bars are one standard error.

(Figure 2). In uncontaminated water, sorption was highest at low pH and decreased significantly (as an "adsorption edge") in response to increasing pH from pH -6 to 7. However, bacterial sorption in contaminated groundwater varied less systematically in response to changes in pH. The largest response was a decrease of 12% between pH 6.5 and 8 compared with a difference of 21 % between pH 6 and 7 in the uncontaminated water.

Sediment Surface Effects. Untreated contaminated and uncontaminated core materials varied in the amount and type of oxyhydroxide coatings. The untreated contaminated-zone sand yielded a slightly higher concentration of iron and aluminum in the leaching procedure but had 7 times less surface-associated Mn than the uncon

Table IL Bacterial Sorption to Aquifer Material Treated To Remove Organic Coatings and/or Oxyhydroxide Coatings in Contaminated and Uncontaminated Groundwater,

	groundwater		
treatment	uncontam	contam	artificial
untreated	43.5 =L 2.8	42.4 ± 1.6	50.6 ± 1.7
NaOH-treated	40.0 ± 3.5	49.8 + 2.2	53.1 ± 0.9
oxalate-treated	26.7 ± 4.5	48.1 ± 2.6	46.8 :E 2.4
exptl pH	6.4	7.0	7.25

'Experiments had three replicates and were performed at 10 *C. Numbers are the percentages of added bacteria that were retained in the sand columns, ±1 standard error.

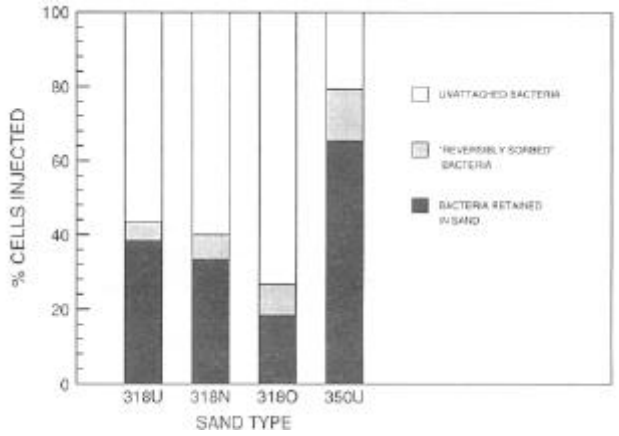

Figure 3. Initial sorption (black and medium shading) followed by desorption (medium shading) of bacteria in response to groundwater adjusted to pH 8. Sediment types are contaminated untreated (318U), contaminated NaOH-treated (318N), contaminated oxalate-treated (3180) and uncontaminated untreated (350U) aquifer material from the Cape Cod study site. Experiments used three replicates per treatment.

taminated aquifer material (Table I). The NaOH treatment, which was intended to remove organic coatings associated with grain surfaces, also appeared to leach some of the Al (Table 1). The 1.5-h oxalic acid leach to remove oxyhydroxide coatings from the sand compared favorably with the hydroxylamine hydrochloride leaching method recommended by Chao and Zhou (22), indicating dissolution of the primary minerals did not occur to any great extent.

The degree of bacterial sorption in response to the different grain-surface treatments varied with groundwater type (Table II). The least bacterial sorption occurred in uncontaminated groundwater in oxyhydroxide-stripped (oxalate-treated) sand. The most sorption occurred in organic-leached (NaOH-treated) sand and AGW. In uncontaminated water, the presence of oxyhydroxide coatings on the sand grains made a significant difference in the degree of bacterial sorption. In artificial or contaminated groundwater, differences between sand types were less obvious. There was less sorption to the untreated sand in contaminated water than to the same material in AGW.

Desorption. Desorption of bacteria was observed in a laboratory experiment (Figure 3) and in a field test (Figure 4). In the laboratory experiment, uncontaminated groundwater and contaminated, NaOH- and oxalate-treated, and uncontaminated sands were used. The first 20-niL rinse of groundwater was followed by 15 mL (5 pore volumes) of groundwater adjusted to pH 8. The number of cells that eluted from a control column rinsed with unamended groundwater was negligible. The bacteria that were rinsed out of the columns after 5-h equilibration with the aquifer material were defined as unattached, and those

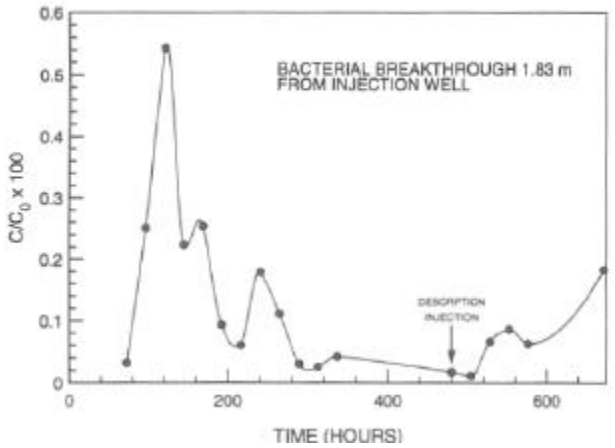

Figure 4. Breakthrough of DAPI-stained bacteria in the field experiment. Bacterial breakthrough was calculated relative to a conservative tracer (bromide).

that eluted from the columns with pH-adjusted groundwater were defined as desorbed. A total of 5-8% of the sorbed bacteria in untreated and treated contaminated aquifer material were desorbed, whereas 14% of the sorbed bacteria were removed from uncontaminated material (which also collected the largest number of bacteria initially).

In the field experiment, desorption occurred in response to a 98-L injection of water adjusted to pH 8 and buffered with 0.05 M phosphate, which was injected 20 days after the initial injection of bacteria. An approximate determination of the degree of sorption and an estimate of desorption during the time frame of the experiment was made for the breakthrough in the multilevel sampler closest to the injection point at 1.83 m downgradient (Figure 3). The bacterial breakthrough was adjusted by comparison to the breakthrough of a conservative tracer to reflect the percentage of the injection traveling past that sampler. The estimate of desorption is low because the monitoring ended before the entire breakthrough was recorded.

Discussion

pH. Bacteria have an isoelectric point ranging from about 2 to 4, depending on species and growth stage (24), giving them a negative surface charge at the pH of most natural waters. The ionizable functional groups on bacterial cell walls that are thought to contribute most to surface charge are carboxyl, hydroxyl, and amino groups (25, 26). Oxides, which include many aquifer minerals, have a pH-dependent surface charge due to the silanol and aluminol groups at the solid/liquid interface (20). Therefore, pH has a significant effect on the character of the EDL for these materials and on the sorption of colloidal particles. In our experiments with groundwater bacteria, maximum sorption to the aquifer material occurred at pH -6.5 for the contaminated water and pH -5 for the uncontaminated water. The shape of the sorption curve in the two groundwater types, however, is very different (Figure 2).

Although there have been studies of adhesion to plastics, studies of pH effects on bacterial sorption in natural materials are few. Scholl et al. (27) found that adhesion of a groundwater organism to quartz was 3 times greater at pH 5 than at pH 7.5. Goldschmid et al. (28) tested bacterial filtration in sand columns at pH 3.9, 5.2, and 9.3. Bacterial retention in the columns was greatest at pH 3.9 and decreased with increasing pH.

Studies of sorption of dissolved organic matter and of proteins to natural materials are useful for comparison; these substances possess some of the same functional groups thought to be important in bacterial sorption. In a study of natural organic matter sorption to various oxides, Davis (29) found that organic matter adsorbed to silica in measurable amounts only at a pH less than 3. Organic matter adsorbed to kaolinite, rutile (Ti02), and alumina (-y-A1203) to a greater degree; the peak in adsorption occurred around pH 3 for kaolinite, 4 for rutile, and 5 for alumina, and sorption decreased with increasing pH. Stuart et al. (30) presented results of protein adsorption to various surfaces. All proteins tested adsorbed to hydrophobic surfaces. Adsorption to hydrophilic hematite was dominated by electrostatic interactions, and some of the proteins did not sorb.

In the present study, the pH dependency of bacterial sorption onto aquifer sediments in uncontaminated water is similar to that of many negatively charged sorbates [e.g., organic matter and inorganic anions (20)] in the presence of oxyhydroxides. However, the degree of bacterial sorption in contaminated groundwater was lower at low pH and higher at high pH than that observed for uncontaminated groundwater. Competitive sorption by di- or trivalent anions such as sulfate, carbonate, or phosphate may be occurring in this system, making the surface less favorable for bacterial sorption in the lower pH range. Dissolved organic matter has been shown to alter the surface charge on suspended particulate matter (29, 31, 32). In our experiments, the 4 mg/L DOC in the contaminated groundwater may also compete with the labeled bacteria for surface sites. Goldschmid et al. (28) noted that addition of sewage pond effluent to the fluid in their column experiments decreased bacterial filtration efficiency and postulated that the decrease was due to polyanionic organic compounds in the effluent. Tipping and Heaton (33) found that adsorption of humic substances to manganese oxides decreased with increasing pH from 4 to 10. In the presence of 10-4 M CaC12, however, organic matter adsorption stayed constant with increasing pH, and at 10-3 M CaC12, the trend was reversed and sorption increased with increasing pH. The authors thought that the enhanced adsorption in the presence of calcium was due to the calcium acting as a bridge between oxide [0]- groups on the mineral surface and anionic groups on the humic substances, either by specific complexation or by nonspecific accumulation of the cations in the interfacial area. In the present study, Ca ions (5 x 10-4 M Ca 21 , Table 1) may enhance sorption of the bacteria in the contaminated water at higher pH. Goldschmid et al. (28) noted that an increase in ionic strength caused an increase in filtration efficiency of bacteria at high pH and a decrease in efficiency at low pH, also in agreement with our results. Ionic strength, pH, and the presence of divalent cations and anions all may affect the magnitude of the electrostatic attraction or repulsion between cell and surface.

Aquifer pH ranges from -4.5 in the zone of recharge near the water table to - 7 in portions of the plume. Within the suboxic zone of the contaminant plume, pH is generally about 5.5-6.5. Our results suggest optimal bacterial mobility at pH 8 and above with little variation in the extent of sorption over the pH range observed for the core of the contaminant plume. In the uncontaminated area of aquifer surrounding the plume, however, there appears to be greater potential for bacterial sorption to be affected by changes in pH. These findings may be applicable in biorestoration schemes in which the downgradient fringes of a contaminant plume are seeded with

bacteria adapted to contaminant degradation in order to prevent further contaminant migration. However, the importance of pH in dispersal of genetically engineered or waste-adapted indigenous bacteria in an aquifer would depend strongly upon the degree of contamination and chemistry of the aquifer.

Sediment Surface and Groundwater Chemistry. Several studies of bacterial attachment to natural materials under static conditions have been published (10, 27, 34, 35), as well as studies of bacterial transport through soil or sand columns (27, 28, 36-39). Enhanced bacterial attachment to oxyhydroxide-coated surfaces was expected from results of earlier investigations (27, 35, 40) that indicated greater bacterial adhesion to positively charged surfaces. Dissolved organic matter also sorbs more readily to iron and aluminum oxides than to silica (29). Over the pH range 5.4-7.2, 53-79% of the bacteria added to our sand columns sorbed to uncontaminated aquifer material (total concentration of leachable Fe, Al, and Mn was 0.33 mg/g), whereas only 40-51% sorbed to contaminated aquifer material (total leachable Fe, Al, and Mn was 0.40 mg/g). Although the total leachable Fe, Al, and Mn for both materials are similar, the uncontaminated sediments had a much greater amount of surface-associated Mn.

In accordance with the DLVO theory, bacterial sorption to positively charged surfaces should occur at all levels of ionic strength but decrease with increasing ionic strength. For negatively charged surfaces, bacterial sorption will occur at higher ionic strength, at the point where van der Waals attractive forces overcome the repulsion due to the overlapping EDL of the bacterium and the mineral surface (13). The inorganic constituents in the contaminated groundwater are approximately 5 times more concentrated than in the uncontaminated groundwater. The two waters are not identical in composition; however, the results from this study agree with the above predictions. Sorption to the oxyhydroxide-coated (NaOH-treated and untreated) sand was much greater than to oxalate-treated material in uncontaminated water, but not proportionately higher in the higher ionic strength contaminated and artificial groundwaters. On the oxalate-treated sand (presumably mostly quartz and feldspar with negatively charged surface), bacterial sorption was much lower in uncontaminated, lower ionic strength water than in either contaminated or artificial groundwaters (Table II).

One of our questions about bacterial sorption in aquifers was whether hydrophobic effects were a factor in the aquifer environment; i.e., does organic matter on grain surfaces act as a separate phase for "partitioning" of hydrophobic cells onto aquifer material? Van Loosdrecht et al. (10, 41, 42) discussed the relative importance of hydrophobic and electrostatic interactions in determining bacterial sorption to surfaces. Most published studies agree that when a hydrophobic interaction is present, cells are firmly adhered to the surface. However, Gannon et al. (38) found that cell hydrophobicity differed according to the assay used and was not a predictor of the extent of transport through soil. Reversible sorption seems to occur when the bacteria/surface association is governed largely by electrostatic interactions (10, 35, 43). The contaminated aquifer material used in this test had very little organic carbon (-0.01%, Table I). The results of the experiment did not clearly show whether either the DOC or the organic surface coatings on untreated sediment had an effect on bacterial sorption. The untreated sand from the contaminated zone sorbed significantly fewer bacteria in contaminated water than in the AGW. If the 4 mg/L DOC in the contaminated groundwater acted to inhibit bacterial sorption by competing for positively charged surface sites on the sand, sorption to the NaOH-treated (oxyhydroxide-coated) sand would be expected to be inhibited also, but it was not to any great extent. Experiments with better defined organic materials and sorbents are needed to answer these questions.

The pH dependence and higher degree of bacterial sorption to oxyhydroxide-coated sand in uncontaminated groundwater were similar to what is typically observed for inorganic anions and suggest that electrostatic interactions contributed to bacterial sorption behavior. In the contaminated water and sediment, the results were less clear and hydrophobic interaction between bacteria and surfaces cannot be ruled out without further experimentation. We may predict that the suboxic core of the plume, where oxyhydroxide coatings on sand presumably occur to a lesser degree due to the reducing conditions, may be more favorable for bacterial transport than the surrounding aquifer. However, the difference in groundwater composition between the contaminant plume and surrounding aquifer complicates this prediction. The effect of organic matter, either dissolved or as grain coatings, is not clearly defined. Further experimentation on the effects of DOC and divalent cations and anions is needed, as these may have a significant effect on bacterial sorption in the contaminated groundwater.

Desorption. 'Mere is evidence that initial (electrostatic) attachment of bacteria to surfaces can be reversed in response to chemical changes, motility, or shear forces in fluid (10, 12, 40, 43, 46). Prediction of desorption in a groundwater environment is difficult, as there are likely to be different types of bacteria/surface associations. There is evidence that enteric: bacteria in soils near septic systems are reversibly sorbed to soil surfaces and become remobilized due to an influx of low ionic strength rainwater to the soil (44, 45). Sharma et al. (37) showed that chemical modification of bacterial surfaces significantly affected transport of the cells through sand columns. There is also much evidence to suggest that certain species of bacteria attach permanently to surfaces; other species may attach after some time period of reversible attachment in which they manufacture extracellular polymers (e.g., ref 11).

Although some desorption occurred at pH 8, most of the bacteria in the columns were not dislodged. Bales et al. (47) reported similar results for virus desorption studies. It was expected that a larger percentage of bacteria would desorb from the oxalate-treated sand than from any of the oxyhydroxide-coated sands. There was slightly greater desorption from oxalate-leached sand, but not to the extent expected. Instead, the highest proportion of desorption was seen on the untreated uncontaminated material, indicating that a larger proportion of the bacteria/surface associations on that type of sand were affected by the change in water chemistry (Figure 3).

Comparison of the laboratory and field experiments is of interest, but the experimental conditions were dissimilar so a direct comparison is not possible. The bacteria traveled much further from the injection point to the collection point in the field experiment. The laboratory experiments used the 0.5-1-min size fraction of aquifer material. The same volume of material in most parts of the aquifer would have more surface area, owing to the influence of the smaller grains size particles. Therefore, in the field experiment, a cell in transport was more likely to encounter an unoccupied surface site to which it became attached. In the field, 0.8% of the sorbed, labeled bacteria desorbed in response to the pH 8 injection and were transported downgradient to the observation well. Un

doubtedly, many more labeled bacteria desorbed during the alkaline injection but resorbed during transport downgradient (as the pH 8 injection became neutralized by the aquifer sediments). However, it is clear that at least a portion of the initial labeled population injected into the aquifer had reversibly sorbed within the first 2 m of transport.

The propensity for desorption of bacteria associated with a surface depends on the nature of the cell/surface bond; if cells are electrostatically attracted to a surface, a change in chemistry may cause desorption; if the bond is more specific, the bond may not be easily broken. There is probably more than one mechanism for sorption of the diverse bacterial species in these heterogeneous aquifer sediments, which would lead to a variety of bacteria/ surface associations that have different susceptibility to desorption.

In summary, though several theories appear to explain certain aspects of bacterial sorption to natural materials, it is difficult to predict bacterial transport under conditions present in an aquifer until experiments with materials from the aquifer are performed. Our results show that there are significant differences in sorption of bacteria in the contaminated and uncontaminated sediments and groundwater from the Cape Cod, MA, study site. Bacterial sorption in the uncontaminated groundwater was qualitatively predictable on the basis of electrostatic interactions between cell and surfaces. Sorption in the contaminated groundwater was more difficult to interpret. With more experimental data it should be possible to find correlations between commonly measured field parameters such as specific conductance, dissolved oxygen, and DOC and the extent of bacterial transport at different sites, as long as the interactions between these parameters can be determined. With the pH 8 phosphate-buffered desorbent used in our experiments, reversibly sorbed bacteria represented no more than 14% of the sorbed bacteria. Other desorbents need to be tested before any conclusion can be reached on the numbers of reversibly sorbed bacteria in aquifer systems.

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