Journal of Contaminant Hydrology, 6 (1990) 321–336
Elsevier Science Publishers B.V., Amsterdam

THE INFLUENCE OF MINERALOGY AND SOLUTION CHEMISTRY ON THE ATTACHMENT OF BACTERIA TO REPRESENTATIVE AQUIFER MATERIALS

MARTHA A. SCHOLL*, AARON L. MILLS, JANET S. HERMAN and GEORGE M. HORNBERGER

Department of Environmental Sciences, University of Virginia, Charlottesville, VA 22903 (U.S.A.)

(Received February 1, 1990; accepted for publication August 14, 1990)

ABSTRACT

Scholl, M.A., Mills, A.L., Herman, J.S. and Hornberger, G.M., 1990. The influence of mineralogy and solution chemistry on the attachment of bacteria to representative aquifer materials. *J. Contam Hydrol.*, 6: 321–336.

The rate and extent of bacterial attachment to mineral surfaces (chips of quartz, muscovite, limestone, and Fe-hydroxide-coated quartz and muscovite) was investigated by counting the numbers of bacterial cells (Lula-D, an indigenous groundwater organism) associated with each surface over time. The degree of attachment of cells to mineral surfaces was correlated with the sign of the surface charge as estimated from literature values for the isoelectric point; attachment of the negatively charged bacteria was much greater to the positively charged surfaces of limestone, Fe-hydroxide-coated quartz, and Fe-hydroxide-coated muscovite than to the negatively charged surfaces of clean quartz and clean muscovite. Batch experiments determined that the numbers of bacteria attached to clean muscovite increased with increasing ionic strength of the solution, and the numbers attached to clean quartz were greater at pH 5 than at pH 7. In columns of clean quartz sand under saturated flow conditions, bacteria initially broke through at 1 pore volume but continued to elute for at least 7 pore volumes. Columns of Fe-hydroxide-coated sand retained more of the bacteria added to the columns (99.9% vs. 97.4%), and elution of cells ceased after the primary breakthrough. The results indicate that surface interactions between the mineral grains in an aquifer and the bacterial cells must play an essential role in determining the movement of bacteria through saturated porous media.

INTRODUCTION

Understanding the movement of bacteria in porous media is an essential component of understanding the fate of much groundwater contamination. Pollution of groundwater supplies by hydrocarbons, solvents and other organic contaminants is a matter of increasing concern, and pollution by micro-organisms is of major significance to public health. Existing methods for the reclamation of chemically contaminated aquifers are expensive and complete

* Present address: U.S. Geological Survey, Water Resources Division, 345 Middlefield Road, Menlo Park, CA 94025, U.S.A.

0169-7722/90/$03.50

restoration is often not achieved. In situ biodegradation of contaminants, either by stimulation of indigenous microbes upon nutrient addition or by injection of genetically engineered or pollutant-adapted microorganisms, is being explored as an efficient mitigation option for many polluted aquifers (Lee et al., 1988; Thomas and Ward, 1989). Introduction of microorganisms to an aquifer cannot guarantee appropriate dispersal of the microbial cells throughout the zone of contamination. To understand, and perhaps control that dispersal, requires knowledge of the mechanisms that promote or retard microbial movement through porous media.

Quantitative information on advective transport of colloid-sized particles (including bacteria) through porous media is limited (McDowell-Boyer et al., 1986; McCarthy and Zachara, 1989), but field and laboratory studies of bacterial retention in soils or other natural sediments have yielded some observations concerning transport of microbes. Keswick et al. (1982) measured bacterial movement through the subsurface of up to 900 m in 2.5 days. Harvey et al. (1989) injected several sizes of synthetic microspheres having different surface functional groups and also indigenous bacteria labeled with fluorescent stain into sandy aquifer sediments on Cape Cod, Massachusetts, U.S.A. Both particle size and surface chemistry affected transport of microspheres, but for spheres in the size range of bacteria (0.2–1.4 μm diameter), surface chemical properties (including charge) had a greater effect on retention than did size. In a separate experimental injection, dye-labeled bacteria traveled faster than a bromide tracer, suggesting that bacteria are transported predominantly through networks of larger pores. The importance of transport of bacteria through macropores was also supported by Smith et al. (1985) who found that at least 93% of bacteria added to repacked soil cores were retained, while only 21% to 78% were retained in structurally intact cores. In addition, they found that the fraction of injected bacteria in the effluent increased with increasing flow rate in the intact cores, suggesting that groundwater flow rate may be an important factor in the dispersal of bacteria in the subsurface. Under static conditions, motility and growth of bacteria have been shown to account for transport rates of up to 0.4 cm h^{-1} in nutrient-saturated sand and sandstone (Jenneman et al., 1985; Reynolds et al., 1989).

Because bacteria are particles, physicochemical colloid filtration theory (Yao et al., 1971; McDowell-Boyer et al., 1986) provides a conceptual basis for studies of the advective transport process. Filtration theory contains both physical and chemical components to describe retention of colloids in porous media. The physical controls on filtration are based upon particle size characteristics of the porous media and of the advected colloid. Chemical filtration is described by the Derjaguin–Landau and Verwey–Overbeek (DLVO) theory (Shaw, 1976), which states that initial contact of colloidal particles with surfaces is determined by the additive effect of the attractive and repulsive forces at the interface (van der Waals and electrostatic forces). The balance of these forces may result in adhesion of particles at some distance (a

few nm) from the surface. DLVO theory has been used as a basis to describe initial adhesion of bacteria from a fluid phase to a solid surface (Marshall et al., 1971). In addition to the forces mentioned above, hydrophobic and steric forces can also contribute to binding of cells to solid surfaces (Rutter and Vincent, 1984).

Unlike nonliving particles, bacteria are capable of growth, which may change both their size and the chemical characteristics of their cell walls (McEldowney and Fletcher, 1986; van Loosdrecht et al., 1987a). Bacterial adhesion may be reversible or, if the cells are capable of producing adhesive substances to anchor themselves to surfaces, it may be permanent (Marshall et al., 1971). Theoretically, particulate matter can only move a finite distance from a source before being completely removed from suspension. Because bacteria can multiply, a single cell (in favorable growth conditions) could give rise to a large number of cells that could be transported further.

Studies of bacterial attachment in groundwater systems can contribute to prediction of the extent of bacterial transport in aquifers and to development of biorestoration strategies. A large body of literature has identified a number of factors which potentially influence adhesion of bacteria in groundwater systems, including bacterial species, nutritional status, growth stage, concentration of cells in suspension, the chemistry and surface area of the geological materials, and the chemistry, flow rate and temperature of the pore water. While the DLVO theory of colloid aggregation has been applied with some success to explain physicochemical aspects of initial bacterial adhesion to solid surfaces (Marshall et al., 1971; van Loosdrecht et al., 1989), information on which of the above factors control bacterial attachment in the environment is lacking.

The present work examined the effects of mineralogy, ionic strength and pH on initial bacterial adhesion to aquifer materials. The primary objective of the study was to determine whether aquifer mineralogy might be a predictable influence on bacterial transport in groundwater. Experiments were designed to investigate whether different minerals collected significantly different numbers of bacteria, and whether the presence of a surface coating on the minerals homogenized the chemical characteristics of the surface with respect to bacterial attachment. Additional experiments examined whether solution chemistry could control initial bacterial attachment in the system studied. The results indicated that solution pH and ionic strength affected bacterial attachment to the minerals. Bacterial adhesion to quartz, muscovite and limestone differed according to the sign of the surface charge of the materials as estimated by literature values for isoelectric points. An Fe-hydroxide coating on quartz and muscovite appeared to reverse their surface charge from negative to positive. Although coated with the same material, the coated minerals did not exhibit the same affinity for bacteria. Results of an experiment with sand columns showed that the differences in attachment to clean and Fe-hydroxide-coated quartz were present under flow conditions.

MATERIALS AND METHODS

Bacteria

Resting cells of a pure culture of bacteria (referred to as Lula-D), were used in the experiments. Lula-D is an *Arthrobacter* sp. collected by Balkwill and Ghiorse (1985) during a study of a pristine aquifer at Lula, Oklahoma, U.S.A. Lula-D cells in resting stage were nonmotile, coccoidal ($\sim 0.9\,\mu$m diameter), and relatively slow to utilize growth media. Immediately upon receipt of the isolate from D. Balkwill, cultures were grown in peptone–yeast extract (PYE) broth and portions were frozen at -70°C in glass vials to preclude any changes in the isolate that could alter the surface or metabolic characteristics of the cells between experiments.

An artificial groundwater (AGW) solution (6 mg KNO_3, 138 mg $MgSO_4 \cdot 7H_2O$, 48 mg $CaSO_4 \cdot 2H_2O$, 19 mg NaCl, 47 mg $NaHCO_3$, 4 L filtered deionized water) was used to wash cells and as the fluid medium in all experiments. The unadjusted solution pH was 6.7 and the specific conductivity was 70 μS cm^{-1}. Prior to use the AGW was filtered through a 0.2-μm pore-diameter membrane.

For each experiment, a vial containing Lula-D cells was thawed, and the cells inoculated into dilute PYE medium (0.25 g peptone, 0.25 g yeast extract, 0.03 g $MgSO_4 \cdot 7H_2O$, 0.0035 g $CaCl_2 \cdot 2H_2O$, 2 L deionized water). After 48 h of growth, the cells were harvested by centrifugation, rinsed once in AGW, then resuspended in AGW and allowed to sit for 15–17 h. (A test indicated that after removal from medium and resuspension in AGW, the cells did not grow or change in size or shape over the next 72 h.) At the beginning of each experiment, the cell suspension was diluted with AGW to the desired concentration measured turbidimetrically using a standard cure of absorbance at 360 nm vs. Lula-D concentration as determined by acridine orange direct counts (AODC; Hobbie et al., 1977). At the end of each experiment, the suspension absorbance was measured again to make sure no growth had occurred.

Mineral substrata

The geological materials used in the batch experiments were chips $\sim 1.5 \times 2$ cm $\times < 3$ mm thick from single crystals of quartz and muscovite, or microcrystalline (lithographic) limestone. Quartz chips were cut on a diamond saw, polished with 120 then 600 grit, and cleaned in detergent solution then hexane to remove all traces of cutting oil. The chips were placed in an ultrasonic bath in 1 *N* NaOH followed by 5% HCl (30 min each), rinsed in filtered (0.2-μm pore diameter) deionized water (FDIW), and air-dried. Muscovite chips were cut with a razor, then peeled to obtain a fresh surface. They were combusted at 500°C for 22 h to destroy organic matter and placed in an ultrasonic bath for 30 min in 5% HCl followed by 30 min in the bath in 0.04 *N* KOH. The chips were then rinsed in FDIW, and air-dried. Limestone was cut

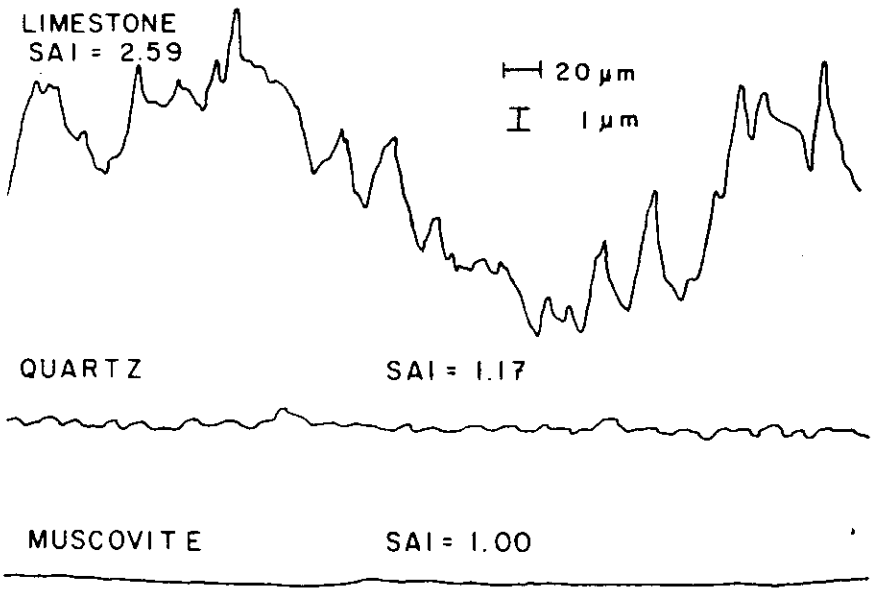

Fig. 1. Examples of sections of surface profiles of the limestone, quartz and muscovite chips used in the experiments. Profiles were obtained through the use of a stylus instrument.

on a water-cooled saw, polished with 120 grit, then rinsed several times in FDIW in an ultrasonic bath.

Because differences in surface micromorphology could lead to large differences in surface area exposed to the bacterial suspension, an estimate of the relative surface area of the different mineral chips was obtained using surface profiles produced with a stylus instrument (Dektak®). Three chips of each mineral type were randomly chosen for measurement. Surface sections 1–3 mm in length were measured in two perpendicular directions, generating profiles of ~6 mm of the surface of each chip. Profile charts were divided into sections representing 0.24 mm of actual surface, and 6 such sections were randomly chosen for measurement with an opisometer. The average profile length was computed for quartz, muscovite and limestone. Each value was then normalized to yield a surface area index (SAI) by dividing each value by the value for muscovite, i.e. SAI for muscovite = 1 (Fig. 1).

For column experiments, crushed quartzite (Pennsylvania Glass Sand Corp.), 0.25-mm-diameter average grain size, was used as the packing material. The sand was rinsed repeatedly in deionized water to remove fine particles then cleaned as described above for the quartz chips.

For experiments examining the effect of iron coatings on the minerals, quartz and muscovite chips were coated with $Fe(OH)_3$ by immersing them in a stirred 0.162 *M* solution of $FeCl_3$ and titrating slowly over a 60-h period with 0.25 *N* NaOH. The sand for the column experiment was coated similarly except that the coating took place in a culture flask on a rotary shaker.

Adhesion of bacteria to mineral chip surfaces

Attachment of bacteria to mineral chips was examined by a method adapted

from the approaches of Fletcher and Loeb (1979) and Mills and Maubrey (1981). Twenty mL of the diluted bacterial suspension were aseptically pipetted into acid-washed, autoclaved, 30-mL glass beakers. Randomly selected mineral chip replicates were hung vertically in the bacterial suspension, about two-thirds immersed. At the end of the prescribed attachment period, the chips were lifted from the beakers and immediately rinsed gently by dipping each chip three times into a separate beaker of filter-sterilized AGW of the same pH and ionic strength as the treatment (fresh AGW for each chip). The rinsed chips were hung to dry, then placed in a disposable Petri dish. The surface of each chip was flooded with a 0.02% (w/v) acridine orange, 3.7% formaldehyde dye-fixative solution for 2–5 min. The chips were then gently tipped, the stain was blotted off the end, and the chips were rehung to dry. Two replicate samples of the bacterial suspension were fixed with filtered (0.2-μm pore diameter) formaldehyde (2% final concentration) at the end of each experiment and counted using the standard AODC technique.

Bacteria on the chips were counted under epifluorescent illumination at 1000 ×. Counting proceeded as follows: 25 fields per replicate were counted when there were < 10 cells per field, otherwise counting was stopped at 15 fields or 200 individuals, whichever occurred first. Data were reported as cells mm^{-2} after adjusting for differences in surface area by dividing by the SAI.

Bacterial transport through sand columns

Glass chromatography columns (20 cm × 2 cm) containing ~66 g of clean or Fe-hydroxide-coated quartz sand were used in the column experiment. The columns had a saturated pore volume of ~20 mL and the porosity was estimated to be 0.44 (based on the water volume at saturation and assuming a particle density of 2.65 $g\,cm^{-3}$). The flow system consisted of a solution reservoir above the columns, and 60-mL plastic syringes below the columns for sample collection (Fig. 2). The flow system was driven by a mechanical vacuum extractor (Concept Engineering®), which pulls the syringe plungers apart creating a steady flow through the columns. The experiment was run at a flow rate of 21 $mL\,h^{-1}$ until seven pore volumes had passed through the columns.

Acid-washed columns with coarse-porosity glass frits were packed with sand, followed by a screen to keep the sand in place, and autoclaved immersed in FDIW in 1000-mL cylinders. Immediately after sterilization, the screen was removed and the columns sealed at top and bottom with sterile serum bottle stoppers. Autoclaving the saturated columns degassed the water in the columns. The columns were connected to the AGW reservoir and to the collection syringes using sterile blood collection sets with Luer-lock® fittings on one end, 30 cm of tubing, and needles on the other end to penetrate the serum stoppers. Approximately 2 pore volumes of AGW were passed through the columns to flush out debris and to replace the FDIW. Columns without any sand packing were tested to determine the retention of bacteria by the tubing, column walls, frits, etc. Recovery of added bacteria always exceeded 99%.

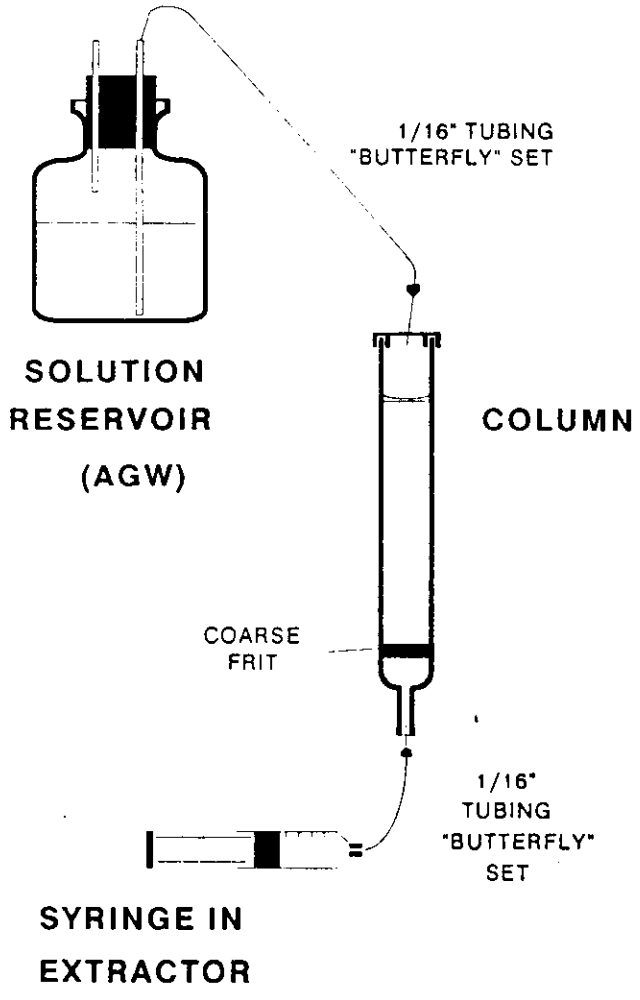

Fig. 2. Apparatus for passing artificial groundwater (AGW) through sand columns ($\frac{1}{16}'' \approx 1.6$ mm). AGW was fed to the column by siphon action; the syringe controlled flow by acting as a flow restrictor.

The bacterial culture was prepared as described for the batch experiment, but the cells were allowed to sit in AGW for 48 h prior to use. One mL of bacterial suspension ($1.77 \cdot 10^9$ cells mL^{-1}) was injected with a syringe into each column through the top stopper. Two 1-mL portions of the bacterial suspension were fixed and counted by AODC for determination of the initial bacterial concentration. Effluent samples were collected from the syringes, fixed, and counted using AODC techniques as previously described.

Data analysis

Experiments with mineral chips were arranged in a block factorial design. Data collected were adjusted to take into account the different surface areas of the minerals, using the estimates obtained from the surface profiles which were normalized to the surface area estimate for muscovite (Fig. 1). The data were then analyzed by the combined probabilities method (Fisher, 1970) which

was utilized in place of a two-way ANOVA. *t*-Tests were used for the batch experiments that examined ionic strength and pH. For column experiments, graphical comparisons were made of the bacterial breakthrough curves.

RESULTS

Surface profiles of the mineral chips (Fig. 1) were used to determine surface area indices for each type of mineral or rock chip used. The limestone had more than twice the estimated surface area of the other two minerals examined due to the low degree of polishing attainable for this soft material. The highly polished quartz and cleavage face of the muscovite were much closer in surface texture.

Solution chemistry and bacterial adhesion

Bacterial adhesion to muscovite chips increased as the ionic strength of the AGW solution increased over the range of specific conductivities tested. The number of bacteria observed on the chips were 148 ± 57.1, 299 ± 8.2 and 584 ± 148.4 mm^{-2} for specific conductivities of 17, 132 and 610 $\mu S\,cm^{-1}$, respectively (data are mean ± 1 standard error of the mean, $n = 3$). When quartz chips were incubated in a suspension of Lula-D in AGW, $7.5 \cdot 10^2$ and $2.6 \cdot 10^2$ cells mm^{-2} were attached to chips in suspensions adjusted to pH 5.04 and 7.52, respectively. A *t*-test confirmed that the effect of pH was statistically significant ($p = 0.015$, $n = 4$).

Mineral identity and coating and bacterial attachment to chips

Quartz, muscovite, limestone, Fe-hydroxide-coated quartz and Fe-hydroxide-coated muscovite chips were incubated for periods of 2–16 h in suspensions of Lula-D cells in AGW at concentrations of $\sim 2.5 \cdot 10^8$ cells mL^{-1}. For all of the substrata tested, the number of bacteria attached increased with time for periods up to 8 h (Fig. 3). After 8 h, attachment leveled off on quartz and muscovite, but continued to increase on limestone and the Fe-hydroxide-coated quartz and muscovite.

Bacterial attachment to the uncoated quartz and muscovite surfaces reached a maximum of $\sim 3.1 \cdot 10^3$ — $3.9 \cdot 10^3$ cells mm^{-2} for immersion periods of $\geqslant 8$ h. In contrast, for limestone, Fe-hyroxide-coated quartz and Fe-hydroxide-coated muscovite, the number of attached bacteria after 8 h was $11 \cdot 10^3$ — $18 \cdot 10^3$ cells mm^{-2}, and at 16 h, the numbers had reached $25 \cdot 10^3$ — $40 \cdot 10^3$ cells mm^{-2}. This bacterial coverage of the chip surfaces ranged from $\sim 0.2\%$ to a maximum of 2.9% of the measured surface area, given the 0.9 μm diameter for each spherical bacterial cell. In most cases, coverage was $\lesssim 1\%$. Except for occasional concentration of cells near the edges of chips or edges of overlapping sheets on the muscovite, the distribution of cells on the chip surfces appeared to be random.

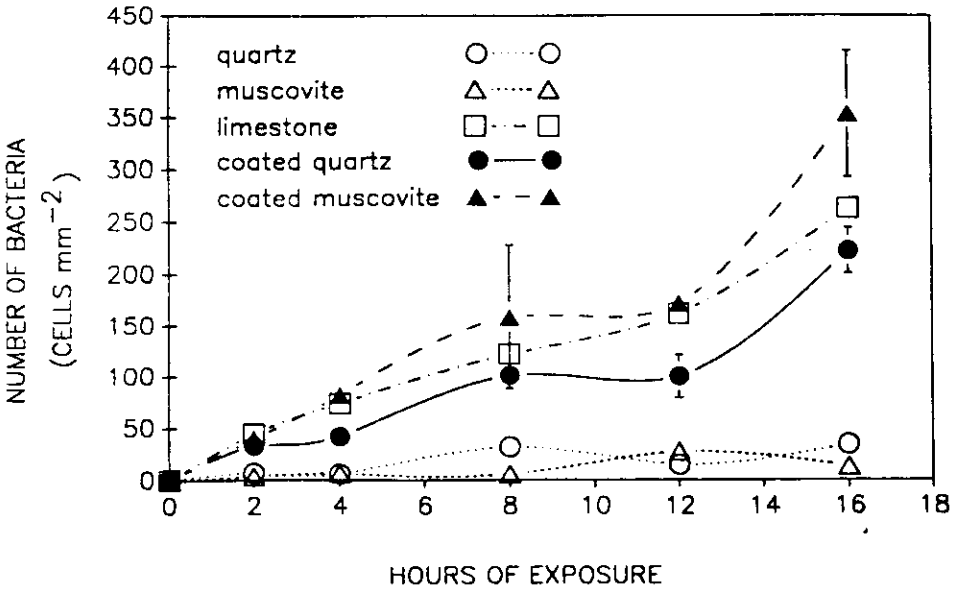

Fig. 3. Attachment of Lula-D to uncoated chips of quartz, muscovite and limestone and to quartz and muscovite chips coated with Fe-hydroxide. *Error bars* represent 1 standard error, some fell within the size of the point as drawn.

The hypothesis that different minerals have different affinities for bacteria held true for the limestone vs. quartz and muscovite, but there was no significant difference in bacterial attachment to quartz and muscovite (Fig. 3). Unlike the uncoated quartz and muscovite, the Fe-hydroxide-coated quartz and muscovite showed distinctly different affinities for bacteria, with attachment to the coated quartz consistently lower than to the coated muscovite ($p < 0.10$).

Surface coating and bacterial attachment and transport under flow conditions

Bacterial breakthrough curves from the column experiments showed that the procedure used provided good replication between similar columns (Fig. 4). The two columns containing Fe-hydroxide-coated material discharged virtually the same number of bacteria; however, there was a "delayed breakthrough" in one of the columns, comprised of $\sim 3.2 \cdot 10^2$ and $4.9 \cdot 10^1$ cells mL^{-1} at pore volumes 5.3 and 6.3, respectively. After this unexplained small peak, the bacterial counts for that column returned to zero (below detection). It should be noted that the secondary peak in this column was several orders of magnitude smaller than the primary peak, and was not quantitatively important in total bacterial breakthrough.

The peak bacterial breakthrough from the columns packed with Fe-hydroxide-coated quartz sand was more than an order of magnitude smaller than the peak breakthrough from the columns packed with clean sand. Peak breakthrough occurred at ~ 1 pore volume for both treatments. After 7 pore volumes, bacteria were still eluting from the columns containing clean quartz whereas bacteria stopped eluting from the columns containing Fe-hydroxide-

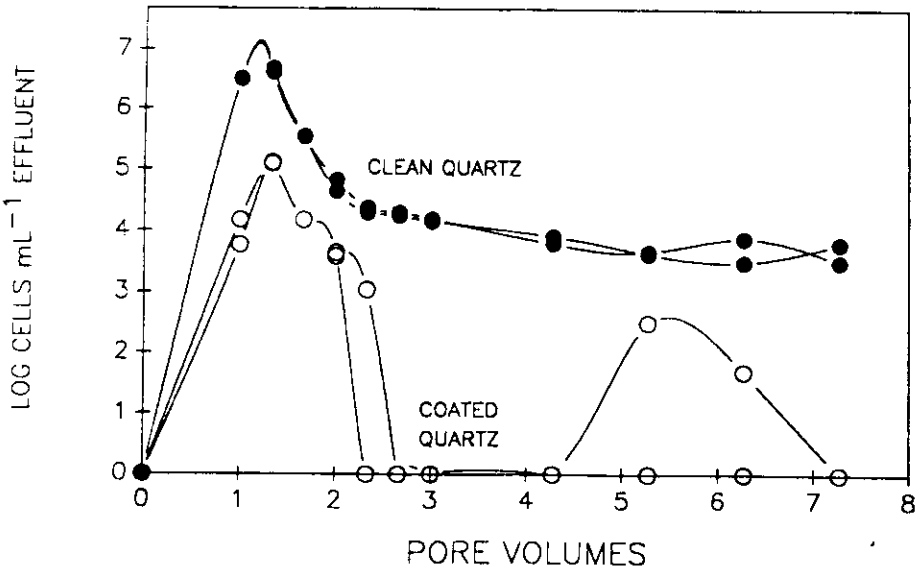

Fig. 4. Breakthrough of Lula-D cells in columns of uncoated quartz sand or quartz sand that was coated with Fe-hydroxide. *Symbols* and *lines* are for individual columns (2 per treatment).

coated quartz after ~2.3 pore volumes (except for the small breakthrough observed in one replicate). 97.4% and 99.9% of the bacteria injected were retained in the columns containing quartz and Fe-hydroxide-coated quartz, respectively.

DISCUSSION

The surface texture of a mineral grain determines the amount of surface area to which cells can attach and can also affect eventual colonization of grain surfaces (DeFlaun and Mayer, 1983). If surface irregularities are on a scale similar to bacterial size, a larger area of the cell wall may be able to interact with the surface during initial adhesion. Larger irregularities may provide protection from shear forces associated with water flow, grazing by protozoa, etc. In the present work, the microtopography of the mineral surfaces was measured to factor out, but not study, textural effects on bacterial attachment to the chips. It was assumed that the iron coating did not substantially alter surface texture. Muscovite and quartz were quite smooth compared to limestone. Given that the maximum vertical slope measured was ~1 μm in 5 μm (see Fig. 1), the scale of vertical inhomogenity on the limestone surface would not be likely to create any microhydraulic effects in these experiments, although textural differences did account for an increase in the total surface area by a factor of 2.6 over that of muscovite (see Fig. 1).

The sign and magnitude of the surface charge on a mineral or rock particle can also control attachment of bacterial cells which come into contact with the surface. Bacterial adhesion would be expected to be greatest to mineral surfaces having the greatest difference in surface charge (in both sign and magnitude) from the bacterial cells. Literature values for the pH_{zpc} (pH at zero

TABLE 1

Estimates of pH_{zpc}-values (isoelectric points) for the minerals and bacteria

Material	pH_{zpc}	Charge at experimental pH
Quartz	2.0*[1]	–
Muscovite	3.5*[2]	–
Calcite	8.0–9.5*[3]	+
$Fe(OH)_3$	8.5*[1]	+
Gram positive bacteria	1.8–4.1*[4]	–

*[1] Stumm and Morgan (1981).
*[2] Hendershot and Lavkulich (1983).
*[3] Somasundaran and Agar (1967).
*[4] Harden and Harris (1953).

point of charge) of the minerals and bacteria were the basis for estimating the sign and relative magnitude of surface charge. At solution pH values equal to the pH_{zpc}, the surface has no net electrostatic charge. At solution pH-values lower then the pH_{zpc}, the surface is positively charged and becomes increasingly positive at lower and lower pH-values. At solution pH-values higher than the pH_{zpc}, the surface is negatively charged and likewise becomes more negative at higher pH-values. The largest number of bacteria would be expected to adhere to limestone and the Fe-hydroxide-coated minerals, followed by muscovite and then quartz (Table 1). Results from the present experiments were consistent with predictions based on pH_{zpc}, except for muscovite which was expected to collect a larger number of cells than quartz. This discrepancy is most likely due to the fact that the pH_{zpc} was determined on crushed mineral particles. Muscovite layers possess a negatively charged cleavage face and positively charged edges. The area of edges exposed for a crushed mineral sample is much greater than for a 1.5 × 2-cm mineral chip. Therefore, the reported pH_{zpc}-value could have been higher than that of the muscovite used in the present study, but it might be accurate for muscovite grains in aquifer sediments.

Two possibilities regarding the effect of the Fe-hydroxide treatment were considered — either the coating would cause both the quartz and muscovite to take on the charge characteristics of Fe-hydroxide, or each coated mineral would possess a net charge resulting from the interaction of the underlying material with the Fe-hydroxide coating. The experimental results showed a significant difference between bacterial adhesion to the coated quartz and the coated muscovite. The most likely explanation is that the muscovite took up more of the Fe-hydroxide than the quartz as a result of coating of internal (interlayer) surfaces. As a result, the overall charge of the coated muscovite surface became more positive than that of the coated quartz. Such a differential coating may also occur in the environment, and this explanation lends support to the concept of a unique surface charge arising from the combination of the

underlying mineral and the coating material (Hendershot and Lavkulich, 1983).

Greater numbers of cells attached to quartz at pH 5.04 than at pH 7.52. This observation is consistent with the concept that oxide surfaces become more protonated as pH decreases, leading to a greater number of positively charged sites and fewer negatively charged sites at the solid–liquid interface. Therefore, more of the negatively charged bacterial cells would be expected to encounter favorable attachment sites on the quartz surface at lower pH and to remain attached through the rinsing and staining procedures (carried out at the experimental pH). McEldowney and Fletcher (1988) also observed attachment of reversibly adhering bacteria to increase with decreasing pH. The solution pH may also affect surface groups on the bacterial cell wall, but the effect of pH on the surface properties of Lula-D is unknown.

Adhesion of Lula-D cells to muscovite increased with increases in ionic strength of the AGW over a specific conductivity range of 10–600 $\mu S\,m^{-1}$. Several studies have shown that bacterial adhesion increases with increasing ionic strength of the solution for various strains of bacteria and on various surfaces: glass (Marshall et al., 1971), quartz sand (Sharma et al., 1985), polystyrene (van Loosdrecht et al., 1989) and hydroxyapatite (Gordon and Millero, 1984). The theoretical explanation for such observations is that greater adhesion of bacteria occurs as the electrical double layer on both the mineral surface and the cells becomes more compact, allowing the cell to approach the surface to a distance at which van der Waals forces exceed electrostatic repulsion.

The results of the present experiments suggest that in dilute circumneutral pH solutions, like those often found in unpolluted aquifers, bacteria in solution maybe able to travel farther without becoming attached to the mineral substrate than in contaminated aquifers having higher total dissolved solids or lower pH where attachment of suspended cells may be comparatively more rapid. It is evident that development of techniques for enhancement of bacterial transport through aquifers would benefit from investigations into modifying the chemistry of the fluid phase or of bacterial surfaces.

Published studies of adhesion of various soil and enteric organisms to polystyrene surfaces (van Loosdrecht et al., 1987a, b) and of enteric bacteria to mineral surfaces (Stenstrom, 1989) suggest that hydrophobic interactions comprise the major control on bacterial attachment to those surfaces. The present work differed from the aforementioned studies in that the substrata tested were hydrophilic minerals and the bacterial strain was an indigenous groundwater species with a relatively hydrophilic cell wall. [Determination of the contact angle of Lula-D cells by the method of van Loosdrecht et al. (1987a) yielded a value of 30°, indicating that the cell wall contains both hydrophobic and hydrophilic surface groups, but is predominantly hydrophilic under the experimental conditions.] Stenstrom (1989) concluded that the attachment of enteric organisms to mineral surfaces was governed by hydrophobicity of the cells; however, his experiments were done in 0.1 *M* physiological saline and

buffer, a concentration ~100 times that used in the present study. Under the higher ionic strength conditions, the electrical double layer might be compressed enough to allow hydrophobic bonding to take place. Conversely, at low ionic strengths, electrostatic effects may play a role in the reversible adhesion of hydrophobic cells to hydrophobic surfaces (van Loosdrecht et al., 1989). Fletcher and Loeb (1979) tested attachment of a marine organism to both hydrophilic and hydrophobic materials in seawater, and concluded that the degree of hydrophobicity of the solid controlled adhesion to the hydrophobic plastics tested (Teflon®, polyethylene, polystyrene, etc.), whereas surface charge controlled adhesion to the hydrophilic substrata tested (glass, Ge, Pt, mica). Evidence from the present experiments suggests that in systems where hydrophobicity of solid surfaces is not a factor, as may be the case in aquifers with low levels of organic carbon, the surface charge of the minerals in the aquifer will be the major control on initial adhesion of bacteria.

Extrapolation of these laboratory results to the environment is premature without further experimentation on the effects of organic matter on bacterial adhesion. Organic carbon levels in most groundwater environments are very low, but it is known that the fraction of organic carbon in sediments partly controls sorption of hydrophobic organic contaminants in aquifers (Curtis et al., 1986). Organic matter could play a role in attachment of bacteria with hydrophobic cell walls to sediment particles. Whether most groundwater organisms under natural conditions have predominantly hydrophilic or hydrophobic surfaces is unknown at this time. Dissolved organic matter may also alter the composition and charge of Fe-hydroxide coatings on mineral particles (Davis, 1982).

Retention of cells in the columns was very high; ~97.4% and ~99.9% of the cells in the original injection were retained in the columns containing quartz and Fe-hydroxide-coated quartz, respectively, after 7 pore volumes had passed through. The high retention in both types of columns suggests that most of the bacteria are removed by physical filtration, i.e. pore clogging. The high retention is similar to that obtained by Smith et al. (1985) in repacked soil columns, and Harvey et al. (1989) in the field. All these studies suggest that extensive advective transport to depth in the subsurface may be limited to macropores or fractures. Similarly, the results imply that in zones of low-permeability, non-advective processes such as growth or motility may play the most important role in widespread dispersal of bacteria (Jenneman et al., 1985; Reynolds et al., 1989).

Filtration is comprised of both physical (pore clogging) and chemical (surface–surface) interactions. While the percentage of cells retained in the columns by each mechanism is unknown, the difference in the numbers of cells collected from the columns (2.6% vs. 0.1% of the bacteria added) indicates different degrees of bacterial attachment to the porous media in the two treatments. That difference can be attributed to electrostatic effects on adhesion of Lula-D cells to the coated and uncoated mineral surfaces based on the results of the trials with coated and uncoated chips.

If different surface charges associated with mineral particles can cause differential retention of monospecific bacterial cells, it seems only logical that a single surface charge derived from one mineral or one dominant mineral in a mixture of substrata could explain differential retention of several bacteria with differing surface charge characteristics. How surface charge characteristics of mixtures of mineral particles and bacterial types interact in natural aquifer material can presently only be guessed at.

The bacteria eluting from the quartz columns at 7 pore volumes could have been cells that traveled through the columns by slower, less direct flow paths than the majority of the breakthrough; alternatively, the tail on the curve could also be due to reversibly adhered bacteria that became detached from quartz grains and resumed transport through the columns. While the overall effect of differences in surface chemistry on the retention of bacteria by the columns may seem to be overwhelmed by the effect of filtration, the mere presence of the tail is important. Bacteria are not inert particles and can multiply when placed in appropriate conditions; even small differences in retention among different media types may have large impacts at some later time or at some distance down gradient. If bacteria were inert particles, a reasonable extinction distance, (a distance in which all particles would be expected to be removed) could be determined given the filtration properties of a specific length of aquifer material. Movement of even a single viable cell into an area suitable for growth could greatly extend the range of movement beyond that predicted by the extinction distance.

CONCLUSIONS

Initial bacterial adhesion in the systems studied was sensitive to changes in ionic strength and pH of the artificial groundwater, mineralogy and time allowed for contact with a surface. The sign and magnitude of charge at the solid–liquid interface determined the extent of initial bacterial adhesion to the mineral surfaces tested. This suggests that surface charge may control adhesion in dilute aqueous environments with low organic carbon levels.

The results support the idea of a unique surface charge arising from each solid–coating combination. The effect of charge sign changes on a mineral surface caused by a coating outweighs the relatively minor differences between surfaces having the same charge. The column experiment confirmed that the surface charge effect on bacterial adhesion was important under flow conditions as well as in the quiescent batch system.

The results of the present work are supported by those of Marshall et al. (1971), Rutter and Vincent (1984), and others in the application of DLVO theory to describe initial bacterial adhesion to surfaces. The sensitivity of the adhesion process to environmental changes indicates the need for further laboratory experimentation in conjunction with field testing.

ACKNOWLEDGEMENTS

This work was supported by a Moore Research Award from the Department of Environmental Sciences and by a grant from the Jeffress Memorial Trust.

1985. Characterization of subsurface bacteria associated with
ma. Appl. Environ. Microbiol., 50: 580–588.
erts, P.V., 1986. Sorption of hydrophobic organic compounds by
F. Hayes (Editors), Geochemical Processes at Mineral Surfaces.
, Am. Chem. Soc., Washington, DC, pp. 191–216.
atural dissolved organic matter at the oxide/water interface.
: 2381–2393.
, 1983. Relationships between bacteria and grain surfaces in
Oceanogr., 28: 873–881.
hods for Research Workers. Hafner, New York, NY, 14th ed., 326

Influence of substratum characteristics on the attachment of a
surfaces. Appl. Environ. Microbiol., 37: 67–72.
984. Electrolyte effects on attachment of an estuarine bacterium.
: 495–499.
1953. The isoelectric point of bacterial cells. J. Bacteriol., 65:

ith, R.L. and LeBlanc, D.R., 1989. Transport of microspheres and
a sandy aquifer: Results of natural- and forced-gradient tracer
echnol., 23: 51–56.
h, L.M., 1983. Effect of sesquioxide coatings on surface charge of
mples. Soil Sci. Soc. Am. J., 47: 1252–1260.
asper, S., 1977. Use of Nuclepore filters for counting bacteria by
pl. Environ. Microbiol., 33: 1225–1228.
J. and Knapp, R.M. 1985. Microbial penetration through nutrient-
Appl. Environ. Microbiol., 50: 383–391.
erba, C.P., 1982. The use of microorganisms as groundwater tracers:
0: 142–149.
len, R.C., Bedient, P.B., Ward, C.H. and Wilson, F.T., 1988. Biores-
inated with organic compounds. CRC (Chem. Rubber Co.) Crit. Rev.

tchell, R., 1971. Mechanism of the initial events in the sorption of
J. Gen. Microbiol., 68: 337–348.
M., 1989. Subsurface transport of contaminants. Environ. Sci.

R. and Sitar, N., 1986. Particle transport through porous media.
901–1921.
r, M., 1986. Effect of growth conditions and surface characteristics of
attachment to solid surfaces. J. Gen. Microbiol., 132: 513–523.
er, M., 1988. Effect of pH, temperature, and growth conditions on the
terium and three nongliding bacteria to polystyrene. Microbiol. Ecol.,

R., 1981. Effect of mineral composition on bacterial attachment to
s. Microbiol. Ecol., 7: 315–322.
Jenneman, G.E. and McInerney, M.J., 1989. Mechanisms of microbial
e materials. Appl. Environ. Microbiol., 55: 2280–2286.
B., 1984. Physicochemical interactions of the substratum, micro-
l phase. In: K.C. Marshall (Editor), Microbial Adhesion and Aggrega-
rk, NY, pp. 21–38.
and Yen, T.F., 1985. Reversible and irreversible surface charge modifi-
r facilitating transport through porous media. Colloids Surfaces, 16:

Shaw, D.J., 1976. Introduction to Colloid and Surface Chemistry. Butterworth, Boston, MA, 2nd ed., pp. 168–177.

Smith, M.S., Thomas, G.W., White, R.E. and Ritonga, D., 1985. Transport of *Escherichia coli* through intact and disturbed soil columns. J. Environ. Qual., 14: 87–91.

Somasundaran, P. and Agar, G.E., 1967. The zero point of charge of calcite. J. Colloid Interface Sci., 24: 142–147.

Stenstrom, T.A., 1989. Bacterial hydrophobicity, an overall parameter for the measurement of adhesion potential to soil particles. Appl. Environ. Microbiol., 551: 142–147.

Stumm, W. and Morgan, J.J., 1981. Aquatic Chemistry, An Introduction Emphasizing Chemical Equilibria in Natural Waters. Wiley, New York, NY, 2nd ed., 780 pp.

Thomas, J.M. and Ward, C.H., 1989. In situ biorestoration of organic contaminants in the subsurface. Eniron. Sci. Technol., 23: 760–766.

van Loosdrecht, M.C.M., Lyklema, J., Norde, W., Schraa, G. and Zehnder, A.J.B., 1987a. The role of bacterial cell wall hydrophobicity in adhesion. Appl. Environ. Microbiol., 53: 1893–1897.

van Loosdrecht, M.C.M., Lyklema, J., Norde, W., Schraa, G. and Zehnder, A.J.B., 1987b. Electrophoretic mobility and hydrophobicity as a measure to predict the initial steps of bacterial adhesion. Appl. Environ. Microbiol., 53: 1898–1901.

van Loosdrecht, M.C.M., Lyklema, J., Norde, W. and Zehnder, A.J.B., 1989. Bacterial adhesion: a physicochemical approach. Microbiol. Ecol., 17: 1–15.

Yao, K., Habibian, M.T. and O'Melia, C.R., 1971. Water and waste water filtration: concepts and applications. Environ. Sci. Technol., 5: 1105–1112.