Effect of Solution Ionic Strength and Iron Coatings on Mineral Grains on the Sorption of Bacterial Cells to Quartz Sand

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Understanding the interaction between bacterial cells and solid surfaces is essential to our attempts to quantify and predict the transport of microbes in groundwater aquifers, whether from the point of view of contamination or from that of bioremediation. The sorption of bacterial cells suspended in groundwater to porous medium grains was examined in batch studies. Bacterial sorption to clean quartz sand yielded equilibrium, linear, adsorption isotherms that varied with the bacterial strain used and the ionic strength of the aqueous solution. Values of $K_D$ (the slope of the linear sorption isotherm) ranged from 0.55 to 6.11 ml g$^{-1}$, with the greatest sorption observed for the highest groundwater ionic strength. These findings are consistent with the interpretation that an increasingly compressed electrical double layer results in stronger adsorption between the like-charged mineral surface and the bacterial cells. When iron-oxihydroxide-coated sand was used, however, all of the added bacteria were adsorbed up to a threshold of 6.93 $\times 10^{9}$ cells g$^{-1}$ of coated sand$^{-1}$, beyond which no further adsorption occurred. The irreversible, threshold adsorption is the result of a strong electrostatic attraction between the sesquioxide coating and the bacterial cells. Experimental results of adsorption in mixtures of quartz and Fe(III)-coated sand were successfully predicted by a simple additive model for sorption by the two substrate phases. Even small amounts of Fe(III)-coated sand in a mixture influenced the extent of adsorption of bacterial cells. A quantitative description of adsorption in the mixtures can be realized by using a linear isotherm for reversible adsorption to the quartz grains with a $y$ intercept that represents the number of cells irreversibly adsorbed to the Fe(III)-coated sand.

The study of the contamination of groundwater and aquifers has recently broadened to include questions about the presence and movement of bacteria because they may be pollutants (13, 45) or they may be agents of biorestitution in aquifers contaminated by organic compounds (37). In either case, a better understanding of the mechanisms that promote or retard transport of microbial cells through porous media is needed.

Interaction between bacteria and solid surfaces is a subject of importance in many scientific disciplines; the adhesion of bacteria to surfaces has been investigated with a number of artificial and natural materials (3). Of most importance to issues related to bacteria in groundwater are results that show strong bacterial adhesion to natural mineral surfaces (31, 32). These studies point to the importance of sorption, or nonspecific association between the microorganisms and solid mineral surfaces, in the retention of bacteria in aquifers.

One approach taken to interpret the sorption event is the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, in which the bacteria are treated as colloids and the changes in free energy between charged cell surfaces and a solid particle surface are evaluated as a function of separation distance (25, 26, 29, 40). Bacteria first approaching the solid surface experience generic physicochemical forces that have traditionally been quantified in adsorption theory (38, 43). Models developed for chemical compounds to characterize the distribution of mass between aqueous and solid phases have been applied to bacterial sorption (29, 41) and typically relate concentration of sorbed bacteria to concentration of suspended bacteria through a sorption isotherm.

The three common equilibrium isotherms used in solute adsorption have been used to describe the adsorption of bacteria to various substrates (5, 45). In a theoretical development, Hendricks et al. (19) expected the physicochemically based, nonlinear Langmuir isotherm to describe adsorption of bacteria on soils. Fitting experimental data derived from batch experiments with kaolinite clay, silt loam, and silica sand, they reported the $K_{max}$ value (the maximum number of cells sorbed per gram of mineral matter) for the isotherm, although little resolution of the low-concentration, linear portion of the isotherm was possible with their data. Rather than assuming an isotherm model outright, other investigators have attempted to fit various isotherms to batch adsorption data. Lindqvist and Bengtsson (23) reported linear isotherms (i.e., $K_D$ values, linear slopes of the regression lines of the number of cells sorbed against the number of cells in suspension at equilibrium) for batch experiments in which bacteria partitioned between soil and ground- or lake water. In later work, Lindqvist and Enfield (24) suspected that the positive intercepts obtained in fitting a linear isotherm to their data suggested that a nonlinear isotherm would be a more accurate description of their data. Fitting a Freundlich isotherm, however, did not significantly improve the description of the data. Bales et al. (1) fitted a Freundlich isotherm to experimental data on bacteriophage adsorption to silica beads but, after finding that the exponent was 1.0, inferred that the adsorption isotherm was linear.

The fact that data are fit well by an isotherm model does not provide definitive information about the mechanisms involved (6, 9). Indeed, mechanisms of adsorption for bacteria to soil are incompletely understood. Some studies have been specifically undertaken to address the role of electrostatic forces (26, 40). The interaction between clays and bacteria in soils,
although thought to be influenced by a number of environmental factors, is usually dominated by electrostatic interactions owing to the surface-active nature of the particulates involved (35).

Although an equilibrium sorption mechanism is implied in the success of these isotherms in describing the partitioning of bacteria between aqueous and solid phases, little work has been done to elucidate the influence of important factors on said isotherms. A quantitative description of the retention of bacteria through sorption to geological materials has not been exhaustively explored, so neither the type of isotherm appropriate to describe equilibrium with natural materials nor the effect of substrate characteristics or solution chemistry has been adequately evaluated.

**Factors influencing sorption.** The factors that may influence the attraction of the cell surface for the mineral surface include the electrostatic charge on each (5), the hydrophobic or hydrophilic nature of each (10, 27, 39), and the chemical composition of the conducting fluid (16, 41) as some balance of attractive and repulsive forces is achieved.

The effect of the chemical composition of the aqueous solution is expected from theory (26, 42) and has been demonstrated in the laboratory. Increasing the ionic strength of the aqueous solution has been shown experimentally to increase the extent of bacterial sorption to a variety of natural (14, 33, 45) and artificial (26, 41) surfaces, as well as to enhance retention in sand columns during transport experiments (11, 12). In the DLVO theory, it is expected that surfaces of like charge can attract one another at high electrolyte concentrations when the electrical double layer is compressed due to the increased concentration of electrolytes (12, 16, 29, 41). Although conceptually sound, the effect on isotherm models remains unquantified.

The effect of substrate mineralogy, beyond a simple surface area dependence, has been demonstrated, and the results can be interpreted as a charge effect. The similar negative charges of quartz (36) and bacterial cells (15, 41) lead to lesser sorption than the opposing charges of calcite or ferric oxyhydroxide-coated quartz (32, 36). The effect of sesquioxide coatings has been shown to be more important in establishing the charge characteristics of the surface than the effect of the underlying bulk mineral (18).

**Interaction between bacterial cells and mineral surfaces in aquifers.** Transport of bacteria through sandy aquifers has been investigated in several field settings; notable are the experiments of Harvey and others in a glacial outwash aquifer on Cape Cod (17). The prevalence of quartz sand partially coated by ferric oxyhydroxides has been established at a variety of sites, including aquifers composed of glacial outwash sand (2) and Coastal Plain sand (30). With the exception of the work by Scholl and Harvey (31), the effect on bacterial sorption of coatings of ferric oxyhydroxides on sand grains has not previously been investigated experimentally.

**Objectives.** The goals of the present research were to identify and quantify factors important in the sorption of bacteria to mineral surfaces in the natural hydrogeological environment. The work was carried out in controlled and replicated batch experiments involving indigenous groundwater bacteria, an artificially prepared groundwater, and quartz and iron-oxyhydroxide-coated quartz grains. The effects of bacterial suspension concentration, solution composition, and mineral surface coatings were evaluated for their impact on attachment of bacteria.

**MATERIALS AND METHODS**

**Geologic media.** A clean, rounded, quartz sand (glassmaking sand; Unimix Corp., New Canaan, Conn.) was used in this study. The sand was sieved to yield the size fraction (0.36 to 0.42 mm in diameter) used for the experiments. The sand was combined with 10% HNO₃ in an Erlenmeyer flask and placed on a rotary shaker for 2 h, the supernatant fluid was decanted, deionized water (DIW) was added to the flask, the flask was shaken briefly, and then the supernatant was removed again. The DIW rinse was repeated five times, by which point the supernatant no longer looked cloudy. Then 0.5 N NaOH was combined with the sand, and the flask was shaken for 2 h and rinsed five times with DIW. Finally, the sand was spread on a tray and placed overnight in an oven at 90°C. Batches of sand treated in this manner were combined in a single container and mixed well to provide a source of clean sand from which portions were removed as needed for the experiments.

**Iron-oxyhydroxide coating procedure.** Quartz grains with Fe(III)-oxyhydroxide coating were used for many of the experiments. The coating process involved adding approximately 200 g of sand to a flask containing 20 g of FeCl₃·6H₂O dissolved in 400 ml of DIW. The initial solution pH was approximately 1.9. This mixture was placed on a shaker table, and portions of about 30 ml of 0.5 M NaOH were added periodically until the pH reached between 3.5 and 4.0. Smaller portions (approximately 1 ml) of NaOH were then added until the pH was in the 4.5 to 5.0 range. Measurements of pH were taken a few minutes after each NaOH portion was added; a total of approximately 400 to 450 ml of NaOH was added for each batch of sand coated. Once the pH was between 4.5 and 5.0, the solution was allowed to shake vigorously for 24 to 36 h to ensure complete coating. The sand was then rinsed repeatedly in DIW, dried at 90°C, rinsed, and dried again before use in the experiments. One large batch of coated sand was prepared from several smaller batches and drawn from for all of the experiments described.

**AGW.** The solution used for sorption experiments was an artificial groundwater (AGW) similar to that used by Fontes et al. (11). The formulation for the AGW used in this study was as follows: KNO₃, 0.75 mM; MgSO₄, 7.00 mM; CaSO₄, 5.10 mM; NaCl, 1.70 mM; NaHCO₃, 7.00 mM. The total ionic strength of the AGW as mixed was 57.9 mm (millimolality). Other ionic strengths were obtained by dilution; those concentrations correspond to total ionic strengths of 1.16, 5.79, and 11.6 mm.

**Bacteria and growth conditions.** The bacterial strains used were taken from the groundwater culture collection of the Laboratory of Microbial Ecology at the University of Virginia (11). Strains W8 and S1 were selected for use in this study on the basis of their differences in cell surface hydrophobicities. The hydrophobicity of each strain was determined as the water contact angle (5); strains W8 and S1 had contact angles of 22°C and 15°C, respectively (28). Strain W8 is a non-spore-forming, nonmotile, weakly gram-positive rod (note that the original reference [11] incorrectly reported this strain as gram negative), with a median width of approximately 0.45 μm and a median length of approximately 1.2 μm. Strain S1 is a non-spore-forming, nonmotile, gram-negative rod with a median width of approximately 0.58 μm and a median length of 1.3 μm (44). Dimensions were obtained from photomicrographs of the cells as described in Blum and Mills (4).

The strains were grown in half-strength peptone-yeast extract broth at 21°C and aerated by gentle swirling on a rotary shaker (125 rpm) for 1 (S1) or 2 (W8) days, the length of time required to attain stationary phase for these organisms under...
the culture conditions imposed. The cells were then removed from suspension by centrifugal sedimentation at 40,000 × g for 10 min. The pellet was resuspended in filtered AGW. The suspended cells were placed on a rotary shaker for a period of 24 to 36 h to ensure that they had entered a resting stage where growth ceased. Counts taken at the beginning and end of the experiments indicated that there was no change in cell numbers during the period of the experiment (3 h). For each suite of experiments, unincubated DIW and AGW blanks were counted to check for background cells. Counts never exceeded 10^4 cells ml⁻¹, which was insignificant with respect to the bacterial concentrations used for all of the batch experiments.

A series of batch experiments compared attachment of bacterial cells to clean quartz sand with attachment to the same sand that had been coated with Fe(III)-oxyhydroxide as described below. The experiments were generally conducted by adding 26 ml of a bacterial stock suspension to each of three Erlenmeyer flasks. Immediately, 1 ml of suspension from each flask was extracted with a pipette and counted by using the aerolime orange direct count method (20). Counting was done under epifluorescent illumination at ×1,000 magnification. Either 200 cells or 10 microscope fields were counted, whichever level was attained first. It was impossible to prepare three replicates of identical cell concentrations, but the number of cells in each of the three flasks in any experiment was close (e.g., standard errors of the mean were occasionally as high as 25% but were usually in the range of 5 to 6%). Unincubated blanks were counted during each day of sampling to check for background cells. Sand (12.5 g) was then added to each flask to yield an initial solid/solution ratio of 1:2 (wt/vol). The flasks were allowed to swirl gently on a rotary shaker (125 rpm) for approximately 3 h. The time of 3 h was determined in an initial experiment in which the time course approach to maximum sorbed concentration was monitored; 3 h was chosen to ensure equilibrium between cells and substrate (7). The number of bacteria sorbed to the sand grains was obtained by counting the number of bacteria in the initial sand-free bacterial suspensions and in the final (equilibrium) suspensions. The number of cells sorbed to the mineral surfaces was determined by subtracting the number of cells in the final suspension from the number of cells in the initial suspension. Similar samples of suspension from control flasks, containing no mineral substrate, were also counted during each experiment to ensure that any loss of cells other than by sorption to the sand could be accounted for. In no experiment did we measure any sorption to the blanks. This basic experiment was repeated to examine the sorption of bacteria to both uncoated and coated sand under a variety of conditions.

Cell desorption, a condition necessary to allow use of equilibrium assumptions, was examined in these experiments by setting up a series of flasks under the same conditions described for the adsorption experiments but incorporating the lowest-ionic-strength AGW, bacterial strain W8, and an initial bacterial suspension concentration of about 2 × 10^5 cells ml⁻¹. Bacterial counts were made on 1.0 ml of suspension withdrawn from the replicate (n = 3) flasks at 3, 24, 48, and 72 h. After each sampling, the suspension was decanted and AGW was added to return the liquid volume to 25 ml again. Any carryover of cells was not accounted for in the calculations.

**Sorption isotherms.** Sorption isotherms were determined for strains W8 and S1 with both uncoated and coated sand and a variety of ionic strengths of the AGW. For the uncoated, clean quartz sand, sorption was determined after a 3-h incubation. Each experiment was done in a 50-ml Erlenmeyer flask containing 25 ml of AGW of 1.16-, 5.79-, 11.6-, or 57.9-mm ionic strength. Bacteria were added to the flasks to achieve initial concentrations within a range of approximately 1 × 10^6 to 2 × 10^6 cells ml⁻¹. After the incubation, the number of cells remaining in suspension was determined by making one aerolime orange direct count for each of the three replicates for any treatment (i.e., combination of bacterial strain, ionic strength, or initial cell concentration). Control flasks containing cells suspended in AGW were counted at the beginning and end of the incubation to determine if cell growth had occurred. In no experiment did the change in cell numbers exceed the counting error for these types of experiments. Similar isotherms were not done for Fe(III)-coated sand, because at the cell concentrations used, all cells were adsorbed by the coated sand.

**Saturation.** To determine if an upper limit of bacterial sorption to Fe(III)-coated sand existed, an experiment with Fe(III)-coated sand in AGW (1.16 mm) was conducted at initial bacterial concentrations ranging from 5 × 10^6 to 4.2 × 10^7 cells ml⁻¹. The number of cells remaining in suspension was counted, and the number sorbed was determined as the difference between the initial and final counts expressed as the number of cells sorbed per gram of sand. The resulting data were then fitted to a Langmuir isotherm by curve-fitting routines available in SigmaPlot version 5.01 (22). Fitting the data to the mathematical formulation of the Langmuir adsorption isotherm implies nothing about mechanism of adsorption; it was simply convenient to describe the adsorption threshold with the S_max value (S_max = the maximum amount of bacteria able to be sorbed to the sand; analogous to the K_max of Hendricks et al. [19]).

**Mixed medium experiment.** The results of the saturation experiment showed that Fe(III)-coated quartz sand used in this study had a very high sorption capacity (about 6.93 × 10^7 cells g of sand⁻¹). Below the initial concentration of 5 × 10^6 suspended cells g of sand⁻¹ added, nearly all cells were sorbed to the sand. Therefore, a set of experiments was performed to determine the number of cells sorbed to mixtures of less than 100% Fe(III)-coated and clean quartz sand. Again, the same general experimental setup was utilized, with AGW at the lowest and highest ionic strengths and bacterial strain W8. Three experiments with different ratios of coated to uncoated sand (1, 5, and 10%, by weight) were performed with three replicates of each treatment. The following equation was derived to examine the theoretical amount of adsorption to the mixed sand:

\[
S_{\text{mixed}} = x S_{\text{max}} + (1 - x) K_d \left[ \frac{C_{\text{added}} - XS_{\text{max}} M/V}{1 + K_d M/V} \right]
\]

where \(S_{\text{mixed}}\) is the number of cells adsorbed to the sand mixture in cells per gram; \(x\) is the fraction of Fe(III)-coated sand; \(S_{\text{max}}\) is the maximum number of bacteria that sorb to Fe(III)-coated sand in cells per gram (6.93 × 10^7; from Fig. 3); \(K_d\) is the slope of the isotherm for the corresponding organism-ionic strength combination (6.11; from Fig. 1); \(C_{\text{added}}\) is the initial bacterial concentration in cells per milliliter; \(M\) is the mass of sand added (12.5 g); and \(V\) is the volume of liquid in the experimental flask (25 ml).

The equation represents a stepwise approach to solution of the problem. First, the number of cells available for sorption to the uncoated sand is determined as the cell concentration remaining after the Fe(III)-coated sand has removed, irreversibly, all of the cells it can hold. The concentration of cells remaining in suspension was then used to determine the number of cells removed from suspension by the uncoated sand at equilibrium by applying the isotherms displayed in Fig. 1. Finally, the total concentration of cells (per gram of mixed sand) is determined as the sum of the concentration of cells...
sorbed to the Fe(III)-coated sand and the equilibrium concentration of cells (per gram) sorbed to the uncoated sand.

**RESULTS**

**Adsorption isotherms.** The adsorption isotherms in Fig. 1 show a clear effect of ionic strength on sorption of bacteria to clean quartz sand and an additional effect of bacterial strain as well. The isotherm slopes ($K_d$) increased with increasing ionic strength over the range of 1.16 to 57.9 mm. An effect of bacterial strain was also seen. At the lowest ionic strength, the $K_d$ values were similar for both strains, i.e., 0.55 and 0.56 ml g$^{-1}$ for strains S1 and W8, respectively. At the highest ionic strength, the difference in $K_d$ values had increased substantially, with strain W8 being the more strongly sorbed ($K_d = 6.11$) as opposed to S1 ($K_d = 3.72$); however, the ionic strength effect within each strain appeared to reach a plateau with a smaller increment of adsorption for additional salt added to the bulk solution (Fig. 2).

Experiments were run with either uncoated or Fe(III)-coated quartz sand grains to determine the effect of surface coating on adsorption. The effect of mineral surface coatings (surface charge) was apparent. With the negatively charged uncoated quartz, the maximum amount of cells adsorbed for the most sorption-favorable conditions of highest-ionic-strength AGW and strain W8 was 75% of the initial bacterial suspension concentration. The least amount of adsorption was approximately 28% with strain S1 and lowest-ionic-strength AGW. When coated quartz was used, practically all of the cells adsorbed to the positively charged iron-coated quartz; i.e., counts taken from the final suspensions did not differ significantly from background counts obtained from AGW or DIW blanks.

Isotherms were not generated for the batch experiments involving coated quartz and the experimental bacterial concentrations. When the general batch experimental procedure was carried out for strain W8, almost all of the cells sorbed to the coated sand grains regardless of cell suspension concentration, ionic strength of the AGW, or cell surface hydrophobicity. Batch experiments performed on strain S1 using the lowest-ionic-strength AGW resulted in the same “total adsorption” effect; thus, further study with higher ionic strengths would not have been productive.

Additional batch experiments were performed with Fe(III)-coated sand, using higher initial cell suspension concentrations to determine an isotherm appropriate for the coated sand grains. Apparent saturation of the sorption sites on the sand was reached at a sorbed concentration of $6.93 \times 10^8$ cells g$^{-1}$. Once that level was reached, no additional cells sorbed (Fig. 3). When samples of the sand were placed in AGW of the same
ionic strength for 48 h, no desorption of cells above the background could be detected.

**Mixed medium experiment.** When different amounts of Fe(III)-coated sand were added to uncoated sand to determine the relative sorption due to each sand fraction, the equation given above was used to predict values of bacterial sorption in mixed media. Parameter values required in that equation were obtained with results from the nonmixed media. The predicted results agreed well with the observations (Fig. 4). The experimental data suggested that bacterial sorption increased with increasing percentages of the Fe(III)-coated sand. The predicted values of sorption, on the other hand, decreased slightly before increasing over the range of mixture composition tested. The predicted values also have a distinct deviation from a smooth relationship (viz., the parabola shown in Fig. 5). The reason that the predicted values drop before they begin to increase with increased amounts of Fe(III) coating is the balance between reversible sorption to clean sand and irreversible threshold sorption to Fe(III)-coated sand for the solid/solution ratio used.

**Discussion**

Application of an equilibrium isotherm is supported by the observation of both adsorption and desorption occurring for suspensions in contact with uncoated sand grains. Both time-course sorption and desorption experiments were run (data not shown), and the curves appeared to reach constant levels early within the experimental period allotted; this rapid equilibration has been observed in other studies as well (41). The isotherms are linear and, with zero intercepts, satisfy the model for \( K_d \) (1, 23, 24). The actual values obtained here (0.55 to 6.11 \( \text{ml g}^{-1} \); Fig. 1) are not dissimilar to those reported in the literature for bacterial sorption to clay, silica sand, and bulk soil (6.4 and 12.6 \( \text{ml g}^{-1} \) in Lindqvist and Bengtsson [22]; 0.4 to 120 \( \text{ml g}^{-1} \) in Lindqvist and Enfield [24]).

Although the transport experiments of Fontes et al. (11) and Gannon et al. (12) demonstrated an effect of ionic strength on cell retention, and the laboratory work of Gordon and Millero (14) and Sharma et al. (33) showed increased adsorption to minerals with greater ionic strengths, the present work defines the relationship between ionic strength and bacterial sorption isotherms (Fig. 2). The range of ionic strengths used (ca. 1 to ca. 58 mm) is reasonable in terms of dilute groundwater, and the impact of solution composition is significant. For strain S1, the \( K_d \) at the highest ionic strength (57.9 mm) is 6.76 times greater than it is at the lowest ionic strength (1.16 mm). For strain W8, this factor is a 10.9-fold increase in the value of \( K_d \).

The mechanistic basis of the observed ionic strength effect is related to the shrinking of the double layer as the ionic strength increases (34). According to the DLVO theory, as the concentration of countercations increases, the secondary free energy minimum, i.e., the location at which reversible sorption occurs as a result of balanced attractive and repulsive forces between particles, is forced closer to the actual mineral surface (26). The potential energy barrier to the secondary minimum in the DLVO theory shrinks, and the bacterial cells more readily occupy reversible adsorption sites with increasing ionic strength of the aqueous solution (16, 41). Thus, in quantified isotherms of bacterial adsorption, the \( K_d \) value is higher at higher ionic strengths (Fig. 2). There is a limit, however, to the compression of the double layer; beyond a certain level, continued addition of electrolyte compresses it no further (14, 33). The asymptotic limit represents the condition for which the electrostatic energy barrier near the surface ceases to exist (16). Within the range of ionic strength typically encountered in groundwater, the thickness of the electrical double layer is inversely proportional to the square root of ionic strength (8, 41). The implicit relationship between \( K_d \) and the thickness of the electrical double layer can be seen in the increasingly positive \( K_d \) values with increased ionic strength (Fig. 2). A linear regression of \( K_d \) on square root of ionic strength yields a good fit \( (r^2 = 0.95 \text{ for W8}; r^2 = 0.99 \text{ for S1}) \), giving support to the expectations suggested by van Loosdrecht et al. (41).

While a number of cell surface-related variables might contribute to the differences seen between the strains of bacteria used in this study, the strong effect of ionic strength on sorption suggests that the sorptive processes are dominated by electrostatic mechanisms as opposed to cell surface hydrophobicity, specific chemical interactions between the cells and the mineral surfaces, or other such variables. The data suggest that the strains may vary in terms of their total charge density, their zero point of charge, or any other electrostatic variable controlled by the specific chemical makeup of the cell surface that is related to electrostatic charge on the cell surface.

The sorption of bacteria to Fe(III)-coated sand is not reversible. Nearly complete uptake of cells to the surface...
occurs up to a threshold beyond which no further cells are attracted to the mineral surface (Fig. 3). Subsequent exposure of the bacterium-laden Fe(III)-coated sand grains to bacterium-free, dilute AGW does not promote desorption. The sorption of negatively charged bacterial cells (15, 41, 42) to positively charged Fe(III)-oxihydroxide coating (18, 32, 36) is clearly favored by electrostatic attraction. Long-range electrostatic interactions are key to the early stages of irreversible bacterial adhesion (16, 26, 41). Subsequent to electrostatic attraction establishing reversible adsorption of the cell, specific surface structures of the cell, i.e., appendages, may be used to secure a more permanent attachment to the mineral surface (41). It is not known for the strains used in the present study whether adhesion with specific surface structures is actually occurring and is thus the cause for irreversible adhesion to Fe(III)-coated sand. In the DLVO theory, it is also possible for strongly electroattractive particles to overcome any potential energy barrier and enter the primary minimum (41). For bacteria, attraction to a positively charged surface has been great enough to place the cells into the primary energy minimum adjacent to the mineral surface, i.e., into irreversible adsorption (41). So, for the positively charged surface of Fe(III)-coated sand used in this study, electrostatic attraction may be adequate to drive irreversible adhesion without the need for specific surface structures being formed. The only other research focusing on the effect of Fe(III) coatings on sand grains in bacterial sorption (31) found partial desorption from natural sand amounting to only 5 to 14% of the initially sorbed cells. In using natural sands, the confounding effects of organic matter and anions, both of which may adsorb to the Fe(III) coating and alter the net positive charge for attraction of bacteria, may cause partial desorption, whereas in the present experiment, in the absence of competing anionic adsorbates, desorption did not occur.

The theory of heterogeneous mixtures of sorbates is not well developed (6). Nevertheless, our results suggest that a simple linear superposition of effects suffices to describe sorption in mixtures of clean quartz sand and Fe(III)-coated sand. This, in fact, may explain some isotherms observed for natural sands. Our results imply that the isotherm for a heterogeneous mixture would be linear but with an offset on the y axis (Fig. 6). The offset represents bacteria sorbed to Fe(III)-coated grains. For clean sand, with increasing suspension concentration, we observe more sorption to the sand, and the relationship is linear. If this were not recognized, one could fit a nonlinear isotherm, probably incorrectly, to sorption data. Although the authors fit the data to a nonlinear Langmuir adsorption isotherm, the results presented for bacterial sorption to a silt loam in Hendricks et al. (19) could be very well fit by a linear isotherm with a non-zero intercept. Lindqvist and Enfield (24) recognized that their observations of bacterial sorption to bulk sandy soils appeared linear, but the non-zero intercept caused them to believe a nonlinear Freundlich isotherm might be more appropriate. Our results indicate that the correct approach may be, in fact, the linear isotherm with an intercept. The indication is that geochemical heterogeneity must be characterized in field materials to provide a sufficient basis for describing bacterial partitioning between groundwater and aquifer solids.

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