

# The effect of distribution of iron-oxyhydroxide grain coatings on the transport of bacterial cells in porous media

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**Abstract** Among the demonstrated processes influencing the transport of bacteria through aquifers, the deposition of cells on mineral surfaces is one of the most important. For example, understanding the transport of introduced bacteria through aquifers is essential to designing some in situ bioremediation schemes. The impact of the presence and distribution of Fe(III)-oxyhydroxide-coated sand grains on bacterial transport through porous media was evaluated in column experiments in which bacteria (short rods; 1.2  $\mu\text{m}$  length) were eluted through columns of quartz sand (0.5–0.6 mm in diameter) for several conditions of chemical heterogeneity of mineral substrate. Fe(III)-oxyhydroxide-coated sand was present as 10% of the mass, and it was arranged in three treatments: (1) homogeneously distributed, and present as a discrete layer (2) at the top and (3) at the bottom of 14-cm-long sand columns. A pulse input of  $10^8$  cells  $\text{ml}^{-1}$  was introduced in an artificial groundwater solution flowing at 14  $\text{cm h}^{-1}$  through the column, and eluted cells were counted. Peak breakthrough occurred at 1.0 pore volume. A large proportion of cells were retained; 14.7–15.8% of the cells were recovered after three pore volumes of solution had eluted through clean quartz sand, and only 2.1–4.0% were recovered from the Fe(III)-oxyhydroxide-coated sand mixtures. The three physical arrangements of the chemical heterogeneity resulted in essentially the same breakthrough of cells, indicating that the spatial distribution of iron coating does not affect the transport of bacteria. The results of the column transport experiments, which mimic hydrogeological conditions encountered in

field problems, are consistent with our mechanistic understanding of bacterial sorption.

**Key words** Transport · Bacteria · Iron · Groundwater

## Introduction

Hydrologists and geoscientists have become increasingly aware of the need to understand the occurrence and migration of microorganisms in groundwater. Interest in the transport of bacteria in soils, sediments, and bedrock is motivated by concerns about bacteria as contaminants, as in the degradation of drinking-water quality by sewage or septic waste (Gerba and Bitton 1984; Yates and Yates 1988), the fate of genetically manipulated microbes released to the environment either intentionally or inadvertently (Sayler 1986), and the role of bacteria in the bioremediation of contaminated aquifers (Thomas and Ward 1989). The in situ bioremediation of aquifers contaminated by recalcitrant organic compounds may be accomplished by the introduction of selected or genetically altered strains of microorganisms capable of carrying out biodegradation reactions. In this remediation concept, the transport of the bacterial cells from an injection site through the aquifer to the location of contamination is a paramount factor in determining the ultimate success of the cleanup scheme (Wilson and others 1986; Thomas and Ward 1989; National Research Council 1993). Interest has been further piqued by the recognition of abundant, active, and diverse microbial communities deep in the saturated sediments that make up some aquifers (Balkwill and Ghiorse 1985; Fliermans and Balkwill 1989; Ghiorse and Wilson 1988). Despite the importance of understanding the movement of bacteria in aquifers, available theories are incomplete (Elimelech and O Melia 1990; Harvey and Garabedian 1991); elucidation of factors influencing the transport of bacteria is needed (Fontes and others 1991; Harvey 1991), and understanding the processes involved in bacterial migration is especially critical for conditions appropriate to the hydrogeological environment. Among the demonstrated processes influencing the transport of bacteria through aquifers, the deposition of cells

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on mineral surfaces is one of the most important. A variety of complex biological, physical, and chemical phenomena are involved in determining bacterial attachment to mineral surfaces, including electrostatic adsorption (Daniels 1980), hydrophobic interaction (van Loosdrecht and others 1987), and attachment by cellular excretions (van Loosdrecht and others 1990). The general physico-chemical concept used to describe the adsorption of charged solutes has been found to successfully describe the partitioning of bacterial cells between an aqueous suspension and solid phase (Daniels 1980; Marshall 1980; Rutter and Vincent 1984). Long-range electrostatic interactions are critical elements of bacterial adhesion to soil mineral surfaces (Stotzky 1985; van Loosdrecht and others 1989; Harvey 1991). The sign and magnitude of the surface charge of the minerals have been found to control the attachment of bacteria to grain surfaces (van Loosdrecht and others 1989; Scholl and others 1990; Mills and others 1994). Bacteria, usually negatively charged (Harden and Harris 1953), interact weakly with quartz which normally has a negative surface charge in contact with natural waters ( $\text{pH}_{zpc}$ , zero point of charge, is 2.0) but adsorb strongly to  $\text{Fe}(\text{OH})_3$  which normally has a positive surface charge ( $\text{pH}_{zpc}$  is 8.5; Stumm and Morgan 1981). In soils and sediments, Fe(III)-oxyhydroxide coatings often form the interface between mineral grains and groundwater (Hendershot and Lavkulich 1983) and effectively promote bacterial attachment to the aquifer solids (Scholl and others 1990).

Sedimentary and diagenetic processes lead to chemical and physical heterogeneity in sedimentary geological formations at a variety of spatial scales. The impact of mineralogy and grain size of aquifer solids on the extent of adsorption of solutes in the field recently has been highlighted (Robin and others 1991; Barber and others 1992; Fuller and others 1996). Although sorption processes are also important in limiting the transport of bacteria through aquifers (Yates and Yates 1988; Harvey 1991), the impact of natural heterogeneity of material properties on this process has received scant attention. This is especially troublesome as the community of hydrogeologists is currently giving significant attention to the transport of bacteria in the subsurface.

Distributions of Fe(III)-oxyhydroxide-coated minerals in certain sandy aquifers on the Coastal Plain (Ryan and Gschwend 1990, 1992) and in glaciofluvial aquifers (Barber and others 1992; Scholl and Harvey 1992) are heterogeneous. The heterogeneity may result from sedimentary processes and exist at the microscale (multimineralic mixtures of grains within a facies), at the macroscale (local stratigraphic features), and at the megascale (major geochemical facies changes; Barber and others 1992). Diagenetic processes can also result in heterogeneous distributions of Fe(III)-oxyhydroxide-coated mineral grains. Fe(III)-oxyhydroxides can accumulate as physically discrete bodies such as bands and mottles (Schulze 1988) that often result from periodic flooding or fluctuating groundwater levels and attendant alternating redox conditions (van Breemen 1988). The existence of chemical

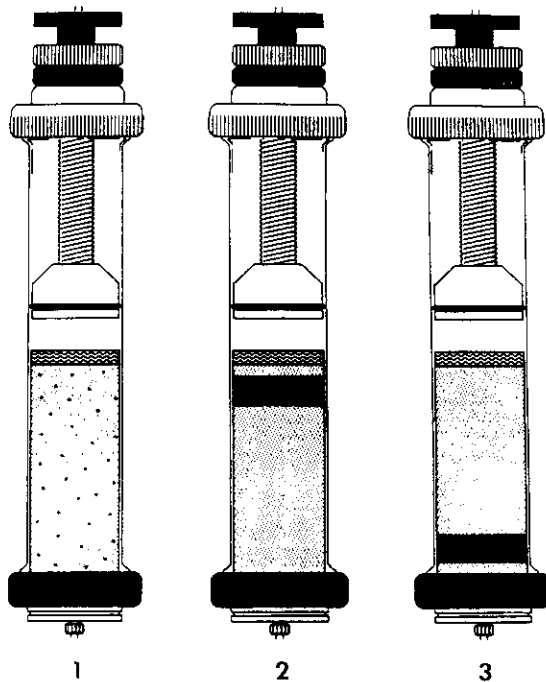
heterogeneity at the field scale has been established in the literature; however, the effect of Fe(III)-oxyhydroxide distribution on the transport of bacteria has not been thoroughly elucidated.

It is not clear whether the distribution of the chemical heterogeneity through which the bacterial suspension flows should have any effect in bacterial mobility. Mills and others (1994) described bacterial sorption on quartz sand observed in batch experiments by equilibrium, linear adsorption isotherms and to Fe(III)-oxyhydroxide-coated sand as irreversible, threshold adsorption. If the mechanisms of bacterial attachment to aquifer solids, either in the field or in laboratory column simulations, are adequately described by the batch data, different distributions of chemical heterogeneity would not affect the transport of bacteria. For instance, in a one-dimensional flow system, consider bacteria contacting the Fe(III)-oxyhydroxide-coated sand first. A certain portion of cells will be removed irreversibly from suspension according to the capacity of the Fe(III)-oxyhydroxide material. The remaining cells, coming in contact with clean quartz sand farther along the flowpath, will equilibrate according to a linear isotherm. If the order of contact is reversed, following initial linear sorption on quartz, a fraction of the remaining cells, again according to a capacity threshold, will be sorbed onto the Fe(III)-oxyhydroxide. The amount collected at the end of the flow path will be the same in the two cases. Yet, the progression from an understanding of sorption mechanisms based on batch studies to transport experiments is confounded by the introduction of new variables: solid-to-solution ratio, flowing groundwater, and spatially heterogeneous distribution of sorption substrates; and, each new factor may bring with it an effect (Jackson and others 1984; Miller and others 1989; Wise 1993). Verification of batch-based mechanistic understanding in column transport experiments is rarely achieved and therefore represents a significant advancement toward understanding bacterial transport in the field.

The present study sought to demonstrate the importance of Fe(III)-oxyhydroxide coatings present in abundance comparable to natural deposits on bacterial transport, and to determine whether the sequence in which the cells contact a heterogeneity, as a result of the distribution of Fe(III)-oxyhydroxide in the matrix, affects the transport of bacteria.

## Materials and methods

Column experiments were conducted in which bacterial cells were eluted through quartz sand for several conditions of chemical heterogeneity of mineral substrate (Fig. 1). We used mixtures of 90% clean quartz sand [no Fe(III)-oxyhydroxide coating] and 10% Fe(III)-oxyhydroxide-coated sand. The three treatments performed in duplicate were homogeneously distributed 10% Fe(III)-oxyhydroxide-coated grains and 10% Fe(III)-oxyhydrox-



**Fig. 1** Schematic diagram showing the experimental columns. Three different distributions of 10% Fe(III)-coated sand grains were used in bacterial transport experiments: a homogeneously distributed and present as a discrete band **b** at the top and **c** at the bottom of the column. The light gray areas indicate clean quartz sand in the column, and dark bands or distributed dots indicate the location of Fe(III)-coated grains. All grains were the same size (0.5–0.6 mm diameter)

ide-coated grains present as a discrete layer at either the top or the bottom of the sand column.

#### Preparation of bacteria

The bacterium used, strain W8, was isolated from pore water in an unconsolidated sand aquifer on the Eastern Shore of Virginia. Strain W8 is an indigenous species and was collected from an area unaffected by contamination. It is maintained as part of the culture collection at the University of Virginia and has been used in previous studies in our research group (Fontes and others 1991; Hornberger and others 1992; Mills and others 1994). The bacteria, weakly gram-positive rods (approximately 1.2 by 0.45  $\mu\text{m}$ ), were grown in half-strength peptone-yeast extract broth for 2–3 days. The cells were centrifuged to remove them from suspension, and the pellet was resuspended in a dilute artificial groundwater solution (AGW) of ionic strength 0.00089 m and pH 6.9 (Mills and others 1994). The cells remained in the AGW for 24 h to ensure that they had entered a resting state (Fontes and others 1991).

#### Preparation of sand

Fine quartz sand (0.5–0.6 mm diameter; Unimin Corporation) was cleaned by rinsing sequentially in 10%  $\text{HNO}_3$ , deionized water (DIW), 0.5 N NaOH, and DIW again (Mills and others 1994). The sand was then dried in an

oven at 90 °C. Some of the clean quartz sand was then coated by adding sand to a flask containing an acidic solution prepared from  $\text{FeCl}_3 \cdot \text{H}_2\text{O}$  in DIW (Mills and others 1994). The flask was placed on a shaker table, and its contents were slowly titrated with variably sized increments of 0.5 N NaOH. The slurry at final pH between 4.5 and 5.0 was then allowed to shake for 24–36 h to ensure complete coating of the quartz sand by Fe(III)-oxyhydroxide precipitate. The newly coated sand was rinsed repeatedly with DIW, dried in an oven at 90 °C, rinsed, and dried again.

#### Column transport experiments

The transport experiments were conducted in acid-washed, autoclaved, glass chromatography columns (4.8-cm inside diameter; Kontes). A total of 175 ml of water was poured into each column. In addition, 440 g of sand, comprised of 90% “clean” quartz sand and 10% Fe(III)-oxyhydroxide-coated sand, was then poured into the column. The water was drained from each column until it reached the top of the sand. The amount of water removed was subtracted from the input (175 ml) to determine the amount of water residing in the pores of the sand column. The final length of the sand column was 14 cm. The pore volume of the columns was 105 ml, and the porosity was 0.35. A standing pool of AGW was maintained above the sand to ensure an equal distribution of solution entering the column (Fontes and others 1991; Hornberger and others 1992). Flow of AGW was regulated by a variable-flow peristaltic pump placed at the outlet of the column. The flow rate used was 1.0 pore volume  $\text{h}^{-1}$  (14 cm  $\text{h}^{-1}$ ). This rate is faster than many natural groundwater flow rates in sandy aquifers (e.g., 0.4 cm  $\text{h}^{-1}$  in an Atlantic Coastal Plain sandy aquifer; Freeze and Cherry 1979) but was used to ensure a reasonable timescale under which the experiments could be conducted. Once a constant flow rate was established in the columns, a 5-ml pulse of  $10^8$  bacterial cells  $\text{ml}^{-1}$  was introduced to the top of the columns. When the pulse had drained to the top of the sand, the flow of AGW was restarted. All of the eluted AGW was collected in 0.1 pore volume (approximately 10 ml) fractions, and the cell concentrations determined from each fraction using the acridine orange direct count method of Hobbie and others (1977). Duplicate columns for each of the treatments were run. The results were compared to an experimental control consisting of a set of transport experiments identical to those described here, but in which 100% clean quartz sand was used (Morley 1995).

#### Iron determination

The amount of iron deposited on the sand by our synthesis procedure was determined by extraction using an ammonium oxalate method adapted from Lovely and Phillips (1986). The extractions were performed on 100% clean sand and 100% Fe(III)-oxyhydroxide-coated sand. The extraction solution was made up of 28 g ammonium oxalate and 15 g oxalic acid in 1 l DIW. A 500 ml portion of the solution was added to 3 g of the sand for each test.

The vials were covered to exclude light and were stirred on a magnetic plate for 24 h. The supernatant was sampled, filtered, and analyzed by ion chromatography for total iron (Moses and others 1988).

## Results

The amounts iron extracted from the Fe(III)-oxyhydroxide-coated sand and the clean quartz sand were  $35.8 \mu\text{mol g}^{-1}$  and  $0.36 \mu\text{mol g}^{-1}$ , respectively. The iron content of the 10% Fe(III)-oxyhydroxide-coated sand (90% clean quartz sand) mixture, calculated by linear interpolation from extraction data on the end-members, was  $3.9 \mu\text{mol g}^{-1}$ .

Breakthrough curves for bacteria for all the columns [plot of the reduced concentration of bacteria ( $C/C_0$ ) versus the dimensionless time, i.e., pore volume] were compared by looking at the timing of the peak breakthrough, the timing of the center of "mass" of the breakthrough (for our purposes, abundance and mass are used interchangeably), the shape of the curve, and the percentage recovery of bacteria over three pore volumes (the total length of the experiment; Fig. 2 and Table 1). The peak of the bacterial breakthrough for each of the columns in the experiment was at approximately one pore volume. The concentration of cells at the peak breakthrough (Fig. 2) from the clean quartz columns was considerably greater than from the columns with a 10% mixture of Fe(III)-oxyhydroxide-coated sand as was total mass recovery over three pore volumes (Table 1). The average mass recovery for the clean columns was 15.25%, and the average for all the Fe(III) columns was 3.0%, with the percentage recovery of bacteria through each of the three physical arrangements of chemical heterogeneity being similar.

The timing of the center of mass of the breakthrough (uncorrected for background mass of bacterial cells) was similar among all the columns (Table 1). Including the clean column, there was no significant overall effect of the presence or location of Fe(III) on the center of mass (ANOVA,  $P=0.066$ ). The agreement across the three Fe(III) treatments in the timing and magnitude of the peaks along with the similarity of the overall shapes of the curves indicated that there was no significant difference among them.

Using the ANOVA model to examine the contrast between all the Fe(III) columns and the clean columns showed that there was also no significant delay in breakthrough due simply to the presence of the iron ( $P=0.244$ ). Although the average center of mass for all six Fe(III) columns, 1.25, was higher than that of the average of the clean columns, 1.055, the suggestion of a small amount of retardation was not statistically significant.

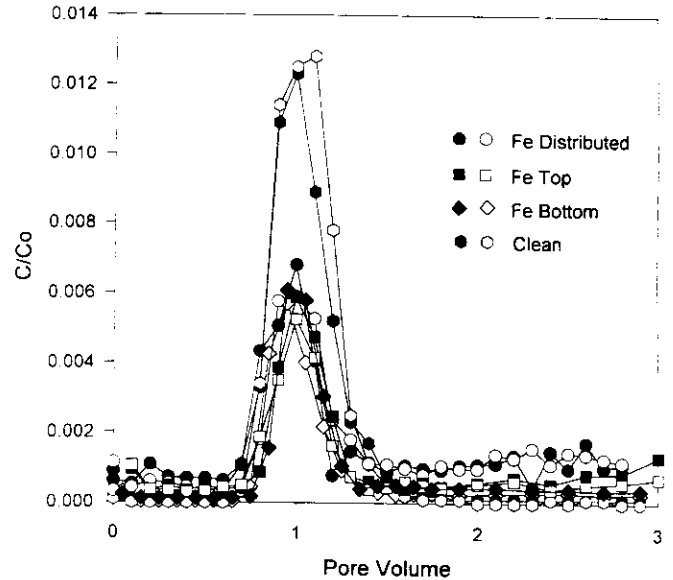


Fig. 2 The breakthrough of bacteria for each of the experimental treatments. The plot shows the reduced concentration of bacteria ( $C/C_0$ ) vs. number of pore volumes eluted. The closed (Column A) and open (Column B) symbols represent duplicate columns run for each treatment

Table 1

The center of mass and the percentage recovery for each of the experimental treatments. Columns A and B were duplicate columns run for each experiment. The center of mass is reported as the pore volume (normalized time) at which time half of the total number of bacterial cells eluted in the entire experiment (three pore volumes) had exited the column. No background correction was applied to remove a background level of cells from the calculation. The percentage recovery for each of the columns is the abundance of bacterial cells recovered expressed as a percentage of the input abundance. The averages of all six Fe(III)-containing columns and of the two clean columns are also reported with standard deviations in parentheses

	Center of mass		Percentage recovery	
	Column A	Column B	Column A	Column B
Fe distributed	1.29	1.38	4.0	2.1
Fe top	1.36	1.18	3.4	2.8
Fe bottom	1.18	1.11	2.1	3.6
Average	1.25 ( $\pm 0.10$ )		3.0 ( $\pm 0.73$ )	
Clean	1.05	1.06	14.7	15.8
Average	1.055 ( $\pm 0.005$ )		15.25 ( $\pm 0.55$ )	

## Discussion

The presence of relatively small amounts of Fe(III)-oxyhydroxide coatings on sand grains has a profound effect on the transport of bacteria. Our mass-retention results (Table 1) show a five-fold decrease in percentage of eluted cells when only 10% of the aquifer sand was coated with Fe(III)-oxyhydroxide as compared to clean

quartz sand. The large magnitude of the overall effect is consistent with the observations made for batch systems in which Fe(III)-oxyhydroxide coatings on mineral grains sorbed large numbers of bacteria (Scholl and others 1990; Mills and others 1994).

The breakthrough curves (Fig. 2) and the number of cells recovered (Table 1) for each of the three distributions of chemical heterogeneity are similar. These results indicate that distribution of Fe(III)-oxyhydroxide-coated sand within the flow path does not affect the transport of bacteria. These findings from the column experiments, although not quantitatively predictable from our batch experiments, are consistent with the mechanistic interpretation of the results of batch experiments conducted by Mills and others (1994). In that study, we concluded that bacteria sorb onto quartz sand according to a linear, reversible isotherm and onto Fe(III)-oxyhydroxide-coated sand irreversibly according to the capacity of the coating. The results of the present study show that given a certain amount of Fe(III) admixed with the clean quartz sand, regardless of the details of the spatial distribution, the same number of cells will be sorbed.

Quantifying the bulk amount of Fe(III)-oxyhydroxide present in the flow path should be adequate to describe the retention of bacteria in simple hydrogeological scenarios simulated by these column experiments. Although bacterial attachment is the result of a variety of complex biological, physical, and chemical phenomena (Harvey 1991), many of these factors are not explored in the present work while one factor is emphasized. In our investigation of the impact of Fe(III)-oxyhydroxide grain coatings, the experimental material used was similar in bulk iron content ( $3.9 \mu\text{mol g}^{-1}$ ) to some natural materials that compose important water supply aquifers in coastal and glaciated environments. Using ammonium-oxalate extraction methods, Scholl and Harvey (1992) reported the iron content of a glaciofluvial outwash sand from Cape Cod, Massachusetts, to be  $4.48 \mu\text{mol g}^{-1}$ . Similarly, extractions performed by Zachara and Smith (1993) on Holocene Coastal Plain sand from Oyster, Virginia, produced a range of iron content from 1.07 to  $17.3 \mu\text{mol g}^{-1}$ . Although it is clear that the Fe(III)-oxyhydroxide content of natural sands can vary widely, the material we selected for experimentation is within a range of conditions reported in the literature from hydrogeological research sites. Thus, we expect the phenomenological observations made here to be applicable to many natural systems. The simple, one-dimensional flow system that was employed in the experiments can be an analog for a number of important field situations as well. Vertical infiltration of bacteria from organic-rich surface layers through the unconsolidated vadose zone, transport in horizontally layered sedimentary aquifers at discharge and recharge zones where a large vertical component of groundwater movement exists, and transport through large-scale crossbeds where the groundwater flows across the crossbedding, all create opportunities for bacteria in groundwater to contact Fe(III)-oxyhydroxide-rich layers oriented perpendicularly or obliquely to flow.

Our conclusions may not be valid for hydrogeological conditions of preferred flow paths arising from physical (i.e., grain size) heterogeneity or for flow parallel to layers of chemical heterogeneity, because in these conditions the bacteria may not contact all the aquifer solids equally. In any case, it is clear that the presence of Fe(III)-oxyhydroxide-coated sand increases the retention of bacterial cells relative to an Fe(III)-free system. In cases where the bacteria contact all mineral phases equally, such as in the experiments performed here, the nature of the spatial distribution of the Fe(III)-oxyhydroxide in the flow path does not affect transport. Thus, the magnitude of the impact of a chemical heterogeneity influencing sorption is likely to be linked to the distribution of physical heterogeneities.

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