FACTORS AFFECTING BACTERIAL TRANSPORT
THROUGH AQUIFER MATERIAL FOR THE
BIOREMEDIATION OF HAZARDOUS WASTES

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Abstract

Bacterial affinity for soil and sediments is determined by an assortment of interactive physical, chemical and biological factors. The MARK column reactor and associated test procedure was used to establish the affinity of bacterial strain Cd-1, a shallow groundwater isolate, for Fe-oxide-coated and uncoated quartz sand. On the uncoated sand, the average biocolloid collector affinity decreased by an order of magnitude over the 1-cm MARK reactor. On this basis, it is suggested that there is a distribution of surface properties within even monoclonal bacterial populations. The standard MARK procedure indicated that a precipitated Fe-oxide coating increased the average affinity of the bacterium for the sand collector by an order of magnitude or more. However, preequilibration of the coated quartz surface with either artificial groundwater or a phosphate-buffered mineral salts solution reversed the effect of the Fe-oxide on bacterial affinity. Results can be explained in terms of heterogeneous equilibrium considerations and the probable nature of the bacterial surface. The effect of iron coatings on bacterial transport through saturated sediments cannot be anticipated without detailed information regarding the nature of heterogeneous equilibria that affect the net collector surface charge.

Background

Microbial transport through porous media has been diligently examined from the perspective of public health protection for several decades. Recently the number of investigators in this area increased substantially as the motives for biocolloid transport research expanded to encompass promotion of bacterial transport for bioremediation and oil-field repressurization. Microbial transport is also a prominent area of exploration for those probing the origin of deep subsurface bacteria. In light of the number of recent investigations and multipurpose efforts,
it may be surprising that so much remains to be resolved with respect to the physical, chemical and biological factors that affect microbial transport through porous media.

The most obvious determinants of bacterial sorption or, conversely, mobility in saturated porous media are related to colloid-collector electrostatic interactions. Under conditions that are suitable for growth, many bacteria are negatively charged. These should sorb more readily to iron oxides, which are positively charged in the neutral pH range, than to quartz, which carries a net negative surface charge under similar conditions. This has, in fact, been established experimentally. Van Loosdrecht et al. (1990) suggested that hydrophobicity plays a more substantial role in surface attachment when bacterial characteristics or chemical conditions produce a bacterial surface with only a modest charge density. In their view, the independent variables that control cell-surface affinity can be lumped into electrostatic and hydrophobic effects.

The complexity of sorting through cause-effect relationships increases rapidly, however, if one considers the potential roles of cell exudates, appendages, mobility, chemotaxis, and probably other bacterial factors. Were this not enough, the response of the collector surface may be influenced by groundwater chemistry in the forms of pH, ionic strength, concentrations of potential-determining ions, organics, etc. In systems comprised of real sediments, physical and chemical heterogeneities in the collector matrix play roles in colloid transport that have been speculated upon but seldom analyzed. When the great number of potential determinants of bacterial transport/attachment in porous media are considered, residual uncertainties in this field become more understandable.

The collection of data to study factors affecting cell adhesion can itself be problematic. Most of the data that led to currently held theories regarding the physical, chemical and biological determinants of cell-surface adhesion were obtained in column-transport or batch sorption experiments. In this context, difficulties frequently attend the measurement of suspended cell densities. Plate counts are very sensitive but carry unavoidable uncertainties; they cannot be effectively used to estimate cell-surface affinity unless experiments are designed to produce substantial time- (batch reactor) or depth-dependent (column reactor) differences in suspended cell concentrations. In general, standard column experiments require incredible effort and diligence; they are not likely to produce the body of evidence that is necessary to differentiate effects attributable to each of the potentially relevant determinants of cell adhesion in the near future.

To circumvent the problems imposed by standard column investigations, we investigated a number of surrogate procedures with which to rapidly estimate bacterial affinity for well characterized collector materials. In the first of these methods, mono-disperse cell suspensions were passed through large-bore filters, and the attenuation of radiolabeled cells was measured by comparing label densities in the pre- and post-filtration suspensions (Logan et al., 1993). Results correlated well with those obtained using better characterized column materials and responded qualitatively to changes in solution ionic strength as predicted based on theoretical considerations (Jewett et al., in press).
Difficulties that were inherent in the application of commercial filters for this purpose -- primarily unavoidable uncertainties in collector geometries and the limited selection or inappropriate nature of commercially available collector materials -- led to development of a mini-column reactor (MARK). The MARK reactor proved more suitable for rapid, reliable estimation of cell-surface affinity (Gross et al., 1995). As originally configured, MARK columns consisted of 3-cc syringe barrels that were packed with acid-washed, 40-mm borosilicate glass spheres. Column experiments benefitted immensely from the small size of the glass beads, which permitted numerous collisions between suspended cells and the collector surface in the short columns. The narrow size range and sphericity of commercially available beads facilitated mathematical treatment of collision and collector efficiencies. MARK column materials could be conveniently extruded and sectioned for determination of depth-dependent affinities of microbes for the collector material. Finally, a variety of collector surfaces could be investigated by chemically modifying the bead surfaces prior to use. The convenience afforded by radiolabel measurements of cell numbers and the short columns makes it possible to run several replicate column experiments in hours. Procedure accuracy and reliability support meaningful measurement of low-end cell-surface affinities that were previously inaccessible.

Here we describe experiments in modified MARK columns that show the effects of Fe(III)-oxide coatings on bacterial affinity for silica sand. The bacterial isolate selected for study was native to the U.S. Department of Energy field transport study site in Oyster, Virginia. The increase in cell affinity due to the Fe-oxide coating was reversed by preequilibrating the coated sand with aqueous-phase phosphate ion. Results highlight the importance of surface chemical effects on biocolloid sorption. The speed and convenience offered by the procedure makes it possible to investigate the numerous potential determinants of cell-surface interactions.

**Modeling -- Filtration Theory**

The affinity of biocolloids for collector surfaces has been represented by a single parameter, $\alpha$, which is defined as the collision efficiency or fraction of collisions between cells and collectors that result in cell retention on the collector surface. Although $\alpha$ has, in general, not been successfully predicted on theoretical grounds, under specific experimental conditions it is possible to estimate $\alpha$ based on colloid retention data. Per the theoretical treatment of Yao et al. (1971) and the semiempirical approach of Rajagopalan and Tien (1976) and Rajagopalan et al. (1982), colloid transport through a column containing porous media can be represented by the one-dimensional transport equation:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial z^2} - U \frac{\partial C}{\partial z} - kC + R_d$$  \hspace{1cm} (1)

where:

- $C =$ Concentration of particles (bacteria) in the bulk fluid
\[ D = \text{Dispersion coefficient} \]

\[ z = \text{position coordinate relative to the direction of transport} \]

\[ U = \text{interstitial velocity} \]

\[ k = \text{adsorption rate coefficient} \]

\[ R_d = \text{desorption rate from the collector} \]

Assuming that colloid concentrations are time invariant and neglecting dispersive transport and desorption, equation (1) simplifies to:

\[ U \frac{\partial C}{\partial z} = -kC \quad (2) \]

The adsorption rate coefficient is obtained from filtration theory (O’Melia and Tobiason, 1988). For a bed containing uniform spherical collectors:

\[ k = -\frac{3}{2} \frac{(1-\theta)}{d_c} U \eta \alpha \quad (3) \]

where:

\[ \theta = \text{porosity of the filter or column} \]

\[ d_c = \text{collector (glass bead) diameter} \]

\[ \alpha = \text{Rate that particles attach to a collector surface} \]

\[ \text{Rate that particles collide with a collector surface} \]

\[ \eta = \text{Rate that particles collide with a collector surface} \]

\[ \text{Rate that particles approach a collector surface} \]

Using clean-bed particle trajectory methods (Rajagopalan and Tien, 1976; Rajagopalan et al., 1982), \( \eta \) can be calculated as:

\[ \eta = 1.0A_p N_{LO}^{1/8} N_R^{15/8} + 0.00338A_S N_G^{1.2} N_R^{0.4} + 4A_i^{1/3} N_{Pe}^{-2/3} \quad (4) \]
where:

\[ N_R = \frac{d_p}{d_c} \]  
(effects of interception)

\[ N_0 = 2d_p^2(\rho_p - \rho)g/(9\mu U) \]  
(effects of sedimentation)

\[ N_{LO} = \frac{H}{(9\pi \mu d_p^2 U)} \]  
(London van der Waals interactions)

\[ N_{pe} = \frac{2Ud_c}{D_p} \]  
/effects of Brownian diffusion)

\[ A_s = \frac{2(1 - p^5)/(2 - 3p + 3p^5 - 2p^6)\text{ (effects of adjacent collectors)}}{\text{and } d_p \text{ is the suspended particle (bacteria) diameter; } d_c \text{ is the collector (borosilicate glass bead) diameter; } \rho_p \text{ is the density of the suspended particles; } \rho \text{ the fluid density; } g \text{ is the gravitational constant; } \mu \text{ is the absolute viscosity of the fluid; } U \text{ is the approach velocity of the fluid; } H \text{ is the Hamaker constant; } D_p \text{ is the Brownian diffusion coefficient; } \theta \text{ is the porosity of the porous media (column of beads); and } p = (1 - \theta)\text{.}}

Substituting for \( k \) in equation (2):

\[ \frac{\partial C}{\partial z} = -\frac{3(1-\theta)}{2} \frac{\eta \alpha}{d_c} C \]  
(5)

Integrating equation (5) over the length of the column yields:

\[ \frac{C_f}{C_o} = \exp \left[ -\frac{3}{2} \frac{(1-\theta)}{d_c} \eta \alpha L \right] \]  
(6)

where \( L \) is the length of the column, \( C_o \) is the influent concentration of bacteria, and \( C_e \) is the effluent concentration.

The fraction of particles retained within a length of column is:

\[ F = 1 - \left( \frac{C_e}{C_o} \right) \]  
(7a)

or alternatively
\[ F = 1 - \left( \frac{N_e}{N_o} \right) \]  \hspace{1cm} (7b)

where:

\( N_e \) is the number of particles leaving the column (C_e x V) \( N_o \) the number of particles entering the column (C_o x V), and \( V \) is the volume of the suspension applied to the column. Collision efficiency is calculated from fraction retained using:

\[ \alpha = -\frac{2d_e}{3(1-\theta)\eta L} \ln(1 - F) \]  \hspace{1cm} (8)

It is apparent from equation (8) that if \( \eta \) can be adequately estimated based on the physical properties of the bed, colloid and suspending fluid, then colloid-collector affinity can be estimated based on fractional retention in a filter bed of depth \( L \).

**Methods**

The MARK procedure and associated methods for measuring bacterial retention are summarized schematically in Figure 1. Procedural details are as summarized by Gross et al. (1995). In essence, suspended cells are radiolabeled with \( ^3 \)H-leucine, and fractional retention during passage through a short reactor containing porous media is obtained by comparing label counts in the filter bed with those from cells removed from a similar suspension volume using a standard 0.22 \( \mu \)m filter. Both of these counts (the bed and filter counts) are corrected for unassimilated \( ^3 \)H-leucine that is retained during the two filtration procedures. It is possible to extend the basic procedure by extruding the contents of the MARK reactor following the filtration step and sectioning the cylindrical core to measure label retention as a function of bed depth. Using equation (8), \( \alpha \) can be estimated for each bed section. The precision of the sectioning procedure and related estimates of cell affinity was improved by weighing core sections rather than measuring section thickness.

The effect of Fe(III)-oxide coatings on cell-surface affinity was investigated by coating size-sorted quartz sand before it was added to the MARK reactor. Coatings were applied by pH-dependent precipitation of Fe(III)-oxide from a concentrated FeCl\(_3\) solution. Photomicrographs of coated materials (not shown) suggested that the coating covered a significant portion of the grain. Cell retention was compared in MARK columns containing coated and uncoated quartz sand.

The importance of heterogeneous equilibria as a determinant of collector surface characteristics, and thus bacterial affinity for the surface, was investigated by varying the composition and volume of the prerinse water that was applied to the MARK columns. Collector
Figure 1. MARK procedure summary. Cells were grown to early stationary phase, labeled with $^{3}\text{H}$-leucine under starvation conditions, and diluted to a density of 1E6 cells/ml. Two mls of the resultant test suspension were passed through 1 cm of 40-µm borosilicate glass beads packed into a syringe barrel. Retained label was measured as scintillation count A (total bead count). The test solution was also passed through a 0.2-µm Nucleopore filter, and retained label was used to generate the total filter count (C). Two mls of the cell-free filtrate was passed through a second MARK column, and retained label provided the control bead count (B). An identical 2-ml aliquot was filtered through a second 0.2-µm Nucleopore filter to produce the filter background measurement (D). The fraction of cells retained by the MARK reactor $[FR = (C_o - C_t)/C_o]$ was calculated by $FR = (A-B)/(C-D)$. 

$$FR = \frac{A - B}{C - D}$$

A = total bead count
B = control bead count
C = total filter count
D = filter background
Materials and water recipes used in experiments that form the basis of this report are summarized in Table 1.

Results and Discussion

Results of MARK experiments are generally represented in terms of (i) depthwise or (ii) average collision efficiency ($\alpha$) over the 1- or 2-cm lengths of the MARK columns. The potential effect of Fe-oxide coatings on quartz sand is well illustrated via comparison of Figures 2 and 3. The effectiveness of the precipitation technique used in these experiments was investigated visually via scanning electron microscopy (micrographs not shown) and by X-Ray Photoelectron Spectroscopy, which identifies the average elemental composition in about the top 50 angstroms of a surface. Results of the elemental analysis, summarized in Table 2, reinforce the general picture provided by electron micrographs -- that the Fe(III)-oxide covered a substantial portion of the quartz sand. The uncoated quartz was essentially iron-free.

The affinity of starved, radiolabeled Cd-1 cells, originally from the Department of Energy field site in Oyster, Virginia, was about 10-fold greater for the Fe-oxide coated material than for the (otherwise identical) uncoated quartz sand. It is evident that the average microbial affinity for the uncoated quartz decreased monotonically with distance traveled, even over the very short length of the MARK reactor. Such a result would be expected if bacterial affinity for the ostensibly uniform quartz surface were a distributed property within the starved bacterial population -- that is, if $\alpha$ values were not equal among the microbes that comprised the population. Preferential removal of the stickier elements would yield a residual bacterial population that grew, on average, less and less sticky as it progressed through the porous media. A more complete discussion of the source and implication of variable cell-collector affinity is provided in Albinger et al. (1994).

The increase in average population $\alpha$ that occurred with depth in reactors containing Fe(III)-oxide coated quartz (Figure 3) is more difficult to interpret. Desorption studies in which the volume of post-sorption rinse water was systemically varied from 0.2 to 40 mls (~1 to 200 pore volumes) indicated that sorbed cells did not desorb to any substantial degree under the conditions of the study (results not shown). Furthermore, operations that comprise the MARK procedure did not solubilize or redistribute Fe(III) within the short columns (Fe analytical results not shown). It is possible, however, that surface properties of the bed material were affected by the composition of the prerinse solution. In these experiments, the prerinse, cell-suspension, and post-sorption rinse solutions were all developed from the artificial groundwater (AGW -- Table 1). Sorption of potential-determining ions, e.g. phosphate ion, to the Fe-modified sand surface during the prerinse procedure would have affected the top layers of the MARK column contents first. Under these circumstances, application of progressively larger prerinse volumes should push the zone of very high cell-collector affinity further into the bed, eventually eliminating the highest $\alpha$ values upon breakthrough. Prerinse (AGW) volume was varied from 2 mls (standard MARK volume) to 50 mls in order to test this hypothesis. Results (Figure 4) suggest that cell affinity for the iron-coated surface is in fact highly dependent on groundwater composition. Preequilibration of the coated surfaces with the AGW, which is assumed to have occurred during the 50-ml
Table 1. Compositions of rinse waters used in MARK column preparatory steps.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Component</th>
<th>Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial Groundwater</td>
<td>NaCl</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>KCl</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>CaSO₄</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>CaCO₃</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>Ca(NO₃)₂</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>KNO₃</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>K₂HPO₄</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MgSO₄</td>
<td>0.065</td>
</tr>
<tr>
<td>KNO₃</td>
<td>KNO₃</td>
<td>0.190</td>
</tr>
<tr>
<td>KNO₃/H₂PO₄</td>
<td>KNO₃</td>
<td>0.190</td>
</tr>
<tr>
<td></td>
<td>H₂PO₄</td>
<td>0.001</td>
</tr>
</tbody>
</table>

- Quartz grains used as collector material were acquired from Unimin Corp. Grains were separated into a uniform size range via wet sedimentation and cleaned via an acid wash and muffling technique outlined by Litton and Olson (1993).

- Iron oxide coatings were applied by chemical precipitation of a FeCl₃ solution following the procedure utilized by Mills et al., (1994).

Table 2. Properties of quartz sand following precipitations of Fe-oxide coatings.
Batch #1 was used exclusively in the experiments described here.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Composition (XPS)</th>
<th>Total Iron Content (mg-Fe/g-digested particles)*</th>
<th>Amorphous Iron Content (mg-Fe/g-digested particles)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitated quartz Batch #1</td>
<td>Fe₂O₃</td>
<td>0.590</td>
<td>0.173</td>
</tr>
<tr>
<td>Precipitated quartz Batch #2</td>
<td>Fe₂O₃</td>
<td>0.315</td>
<td>0.086</td>
</tr>
</tbody>
</table>

* Sample digestion techniques outlined by Chao, et al. (1983) for the selective extraction of amorphous iron oxides.
Figure 2. Affinity of bacterial strain Cd-1 for uncoated quartz sand. Alpha is defined as the fraction of cell-collector collisions that result in bacterial sorption. Affinity is sensitive to depth in the quartz sand column. Rinses were with artificial groundwater (Table 1). Separate lines represent replicate experiments.

Figure 3. Affinity of bacterial strain Cd-1 for quartz sand coated with Fe(III) oxide. Alpha is defined as the fraction of cell-collector collisions that result in bacterial sorption. Depth refers to depth in the MARK columns at which the measurement applies. Prerinse volume was 2 mls of artificial groundwater. Separate lines represent replicate experiments.
Figure 4. Affinity of bacterial strain Cd-1 for an Fe-oxide quartz sand. Alpha is defined as the fraction of cell-collector collisions that result in bacterial sorption. Affinity is a function of depth in the sand column and prerinse volume. Rinses were with artificial groundwater.

Figure 5. Affinity (α -- defined as the fraction of cell-collector collisions that result in cell sorption) of bacterial strain Cd-1 for Fe-oxide-coated quartz sand. Prerinses were with KNO₃ solution -- same pH and ionic strength as artificial groundwater. Affinity was sensitive to depth in the MARK reactor but relatively insensitive to prerinse volume.
prerinse, completed reversed the effect of the Fe(III)-oxide coating. The process produced a surface that, in terms of cell-collector affinity, was identical to that of uncoated quartz.

To verify that the ionic composition of AGW that comprised the prerinse and microbe-suspension volumes was responsible for observed changes in cell retention, MARK columns containing Fe-oxide coated quartz were prerinised with a KNO₃ solution (same ionic strength and pH as AGW). Neither potassium nor nitrate ions were expected to interact strongly with the oxide surface. Experimental results (Figure 5) indicate that KNO₃ prerinse volumes in the range 0.5 to 10 ml had little effect on microbe-surface affinity, again implicating AGW components as a source of the prerinse-dependent differences that are illustrated in Figure 4.

Our initial hypothesis relative to the source(s) of prerinse effects was that phosphate was among the responsible chemical species. In related experiments, a prerinse solution was developed that contained 5.75 μM total phosphate (pH 6.0). The ionic strength of this solution was adjusted to that of AGW with KNO₃. Phosphate prerinse volumes from 10 to 50 mls dramatically lowered the affinity of the Fe-oxide coated surface for strain Cd-1, suggesting that phosphate ion and/or related protonated species are among the relevant potential-determining ions in this application (Figure 6). Comparison of MARK results after the phosphate-solution prerinse with those generated using AGW suggests that preequilibration with the phosphate solution had a somewhat stronger effect on subsequent cell attachment to Fe-oxide-coated quartz than did AGW. The phosphate content of the latter solution may have been lower than the target concentration due to removal of a phosphate precipitate (via filtration) during the preparation of AGW.

The theoretical basis for the interaction of Fe(III) oxides with aqueous-phase phosphate species was summarized by Stumm (1992). Phosphate ion acts as a bidentate ligand, forming a mononuclear or binuclear chelate at the oxide surface (Figure 7). This is a ligand-exchange reaction that lowers the net surface charge on the oxide surface. Sigg and Stumm (1981) suggested that the capacity of goethite (α-FeOOH) to bind phosphate in the neutral pH range was considerable.

The zero point of charge of α-FeOOH and other Fe(III) oxides is greater than 7.0, indicating that hydrated surfaces of these minerals are positively charged in the neutral pH range. Under the same circumstances, the surfaces of silica sands are negatively charged. Since bacterial surfaces are also negative at neutral pH, electrostatic considerations should inhibit bacterial sorption on silica surfaces. On the other hand, precipitation of iron oxide on silica sand could raise or reverse the net mineral surface charge at the pH of the experiments described here and promote bacterial sorption. Subsequent modification of the Fe-oxide surface with phosphate could restore the surface charge to something like its original value. A more rigorous characterization of the original silica and Fe-oxide-coated surfaces may support a priori prediction of pH and phosphate-dependent changes in the character and net surface charge of collector material used in the experiments.
Figure 6. Affinity ($\alpha$ -- defined as the fraction of cell-collector collisions that result in cell sorption of bacterial strain Cd-1 for Fe-oxide-coated quartz sand. Prerinses were with a KH$_2$PO$_4$ solution or a KN0$_3$ solution -- same pH and ionic strength as the artificial groundwater. Affinity was very sensitive to the ionic composition of the prerinse solution.
Figure 7. After Stumm (1992). Part A represents the dissociative chemisorption of water molecules to the surface of a metal oxide. Small, filled circles are metal atoms; larger empty circles represent oxygen. Part B illustrates the mechanism by which surface complexes are formed on hydrated iron oxide. Notice how complexation changes the net surface charge of the mineral surface.
Summary

The following conclusions are supported entirely or in part by experimental findings:

1. The affinity of bacterial strain Cd-1 for the uncoated quartz sand collector decreased monotonically with depth in the MARK reactor. It is probable that depth-dependent variation in $\alpha$ resulted from the nonhomogeneous nature of surface characteristics in the monoclonal suspension of starved bacteria.

2. Precipitation of an Fe-oxide coating on the surface of the silica quartz dramatically increased the affinity of Cd-1 for the collector surface, as measured in the standard MARK procedure. It is probable that the Fe-oxide coating increased the net surface charge on the quartz surface, decreasing or reversing the repulsive force between the bacterium and the unmodified collector.

3. Preequilibration with the chemical constituents of the artificial groundwater recipe used in these experiments reversed the effect of the Fe-oxide coating on bacterial affinity for the collector. That is, depth-dependent $\alpha$ values representing the affinity of strain Cd-1 for (i) uncoated quartz sand and (ii) coated quartz that was preequilibrated with AGW were indistinguishable.

4. Phosphate may be the most important determinant of collector surface potential and bacterial affinity for the Fe-coated collector. Preequilibration of the coated quartz with an aqueous, phosphate-buffered solution reversed the effect of the Fe-oxide coating on bacterial affinity and attachment in MARK tests.

5. Careful consideration of heterogeneous reactions involving the collector surface and aqueous-phase chemical constituents is required to make a priori decisions regarding the probable effect of iron-oxide coatings on bacterial transport through porous media.

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References


