ENHANCED TRANSPORT OF BACTERIA IN POROUS MEDIA BY SEDIMENT-PHASE AND AQUEOUS-PHASE NATURAL ORGANIC MATTER

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Abstract—Aqueous-phase dissolved natural organic matter (DOM) and sediment organic matter (SOM) were shown in laboratory microirrigation experiments to affect the transport of bacteria within porous media. Attachment efficiencies of bacteria were estimated from their retention on quartz, iron oxidized quartz (Fe-quartz), and Fe-quartz coated with SOM (SOM-Fe-quartz). Sawtelle River Humic Acid (SRA) and Soil Humic Acid (SHA) were used to represent organic matter (SOM and DOM) and were added to sand columns to enhance bacterial retention. Fe-quartz retention of bacteria increased with iron oxide concentration in bacterial retention 160% relative to uncoated quartz. Coating Fe-quartz with SOM lowered bacterial retention, resulting in a fraction retained only 31% greater than retained on uncoated quartz. Combined with these effects, the effect of DOM on bacterial retention was secondary, and reflected the extent of DOM adsorption to the porous media. When DOM did not interact with the porous media, as in the case of SHA, bacterial retention in the presence of DOM was reduced by 20%. However, when DOM adsorption to the porous media was increased by coating the iron oxide with bacterial retention on the Fe-quartz increased by 10%. When Fe-quartz surfaces were loaded with DOM to equilibrium conditions to produce SOM-Fe-quartz, the presence of DOM in the applied solution also increased bacterial retention by 10%. The effect of DOM were the same for both types of humic acids (SRA or SHA). These results suggest that SOM and DOM affect bacterial transport by increasing the negative surface charge of the Fe-quartz and bacteria, respectively. The largest decrease in bacterial retention (60%) was associated with coating of Fe-quartz by SOM in the absence of DOM.

Key word—facilitated transport, enhanced transport, bacteria, collision transport, natural organic matter, groundwater, transport, DOM, NON

INTRODUCTION

A major obstacle to successful bioremediation of subsurface contaminants is the inability to deliver microbes to the contaminated area of an aquifer. Bacteria can attach to aquifer material and plug areas proximal to injection wells, necessitating facilitation of bacterial transport in the subsurface. Greater than order-of-magnitude changes in bacterial attachment efficiencies are required in order to facilitate their transport over distances critical for bioremediation (Gross and Logan, 1995). Facilitation of bacterial transport may be brought about by changes in either solution chemistry or by changes in the surface properties of the bacteria or substrate. Experiments have shown that manipulating solution chemistry causes the most dramatic changes in bacterial transport (Gannon et al., 1997; Martin et al., 1991; Jewett et al., 1995). Reduction of ionic strength or addition of surfactants (Jackson et al., 1994) can decrease bacterial sticking coefficients by more than an order of magnitude (Gross and Logan, 1995). Increases in pH have produced large effects on bacterial transport than changes in ionic strength (Scholl and Harvey, 1992; Jewett et al., 1993). Modification of bacterial surface properties by oxidation and cleavage of surface polysaccharides and proteins also decrease attachment less than an order of magnitude (Fletcher, 1976; McElroy and Fletcher, 1986; Hiralal and Zostola, 1989; Vaara, 1992), and in some cases reduce bacterial viability. The bacterial surface is capable of participating in complex sorption phenomena (Daniels, 1980) due to the presence of a variety of surface lipopolysaccharide and ionic groups, the latter being mostly carboxyl, hydroxy, amino, sulfate, and phosphate groups (Sharma et al., 1983). Dissolved organic matter (DOM) has been shown to facilitate the transport of a number of inorganic and organic contaminants in groundwater (McCarthy and Zachary, 1989; Magee et al., 1991; Abdul et al., 1990; Mantovani et al., 1978; Oden et al., 1993; Johnson and Amy, 1995), and to increase the electrophoretic mobility, and hence the mobility, of particles and bacteria in lakes and streams (Gerritsen and Bradley, 1987). Dissolved

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polyelectrolytes such as heparin and sodium pyrophosphate, have been shown to facilitate bacterial transport through aqueous sediment (negatively charged) by sorbing onto bacterial cell walls and reversing the negative charge of the bacterial surface (Sharma et al., 1985).

The presence of iron oxide in sediment has been shown to increase bacterial retention (Mills et al., 1994). In contrast, the presence of sediment organic matter (SOM) has been shown to increase the groundwater transport of bacteria and viruses (Harvey et al., 1989; Powell et al., 1991; Scholl and Harvey, 1992). The mechanism of DOM facilitation of bacterial transport was presumed to be due to competition between DOM and bacteria for adsorption sites (Scholl and Harvey, 1992). This is plausible considering that DOM, bacteria, and sediment surfaces contain many of the ionic functional groups considered to be important in sorption (Daniels, 1980). However, because dissolved polyelectrolytes have been observed to facilitate bacterial transport by sorption to the cell surface, we believe that sorption of the cell surface and modification of the bacterial surface charge (Sharma et al., 1985) is possible that DOM enhances bacterial transport by sorbing to the bacterial surface and altering cell surface properties.

This paper examines the importance of alteration of the bacterial surface versus modification of the sediment surface in organic matter facilitated bacterial transport. The major goal of this paper is to quantitatively determine the importance of organic matter facilitated bacterial transport relative to enhancement of bacterial transport by other means such as low-swing solution ionic strength. We will compare the resulting decreases in bacterial attachment efficiency to the order-of-magnitude decreases considered necessary to achieve increases in transport distance useful for bioremediation.

METHODS

Bacterial suspensions

Savannah River strain A124 (Ball, 1985) was used in this study to represent bacterial which tend to adhere to the sediment material. Strain A124 was collected at a depth of 203 m in the Middle River basin at the DSM Savannah River Site. Oxygen levels at the site of origin were measured to be extremely low but not anaerobic. The cell is rod-shaped and strain A124 is gram-negative. Positive reaction has been determined for the following tests: nitrate reduction, Agarone diphosphate, Glutamate, and Oxidase. External negative reactions have been determined for Trypan blue, Glucose fermentation, Urease, Fucnan hydrolysis, and NPNK beta-galactosidase. The physiological characteristics of strain A124 are consistent with the pseudomonad, but the companion is not yet definitive (Dr. David F. Ball, pers. comm.). A124 was grown autotrophically in 3% PTH/peptone, yeast extract, yeast extract, glucose (medium) batch culture in a stationary phase cell density of about 10^11 mL^-1. Growth was monitored with a doubling time of 2% suspensions were diluted to a cell concentration of 10^7 mL^-1 with 3-MON-methyl)-propanesulfonic acid, sodium salt (NPSF) buffer (0.264 g/L) amended with MgSO4.7H2O (0.15 g), NaCl (0.1 g), K2HPO4 (0.0174 g), CaC2O4, (0.0083 g), FeC2O4.4H2O (0.0046 g). The two sets were sterilized (c.02 μm) and added after autoclaving to avoid their precipitation. Both sets (0.462 g/L) 100 mL, and, in some cases, DOM (3 mg/mL) were added to the buffered suspension (pH 7.7), and shaked at 120 rpm for 18 h to permit label uptake. During the labeling period, bacteria doubled 2-4 times, re-instituting phase prior to their use in transport experiments.

Sediment materials

Quartz (40 μm mean diam. range) from 10-20 μm was separated from bulk ground/drilled rock core taken from Union (Troy, Georgia, Ill) using a wet sedimentation technique. Quartz was prepared by a method modified from Cline and Ollson (1993), in which quartz was washed 24 h in 5% HCl, and then rinsed in a column (35 cm flow) with ultrapure water until conductivity and pH were stabilized (usually around 2 h). This was followed by drying at 100°C and being for 8 h in a muffle furnace at 800°C in a covered porcelain crucible. The quartz was hydrated before use by boiling in ultrapure water for 4 h. A portion of the quartz was coated with hexamet (Fe-quartz) using a method modified from Edwards and Benjamin (1989). Quartz (40 μg) was added to ultrapure water (100 mL) and 0.1 M NaF (1 mL) and stirred constantly during slow (~13 mm) addition of 0.1 M NaOH (1 mL). After settling the quartz, the supernatant was decanted and the quartz rinsed three times with ultrapure water. The entire procedure was repeated four times to result in an ion concentration of 0.5 mg Fe per 100 mL, as determined by AAS analysis (Scholl, 1969).

Sediment/dissolved DOM is tightly held, despite very low (Dunham et al., 1992; Gu et al., 1994) and is considered to affect sediment organic matter (SOM) (Murphy et al., 1991). A portion of the Fe-quartz media was coated with dissolved humic substances to reflect quifer solution coated with SOM. The SOM-coated Fe-quartz (SOM-Fe-quartz) was prepared by mixing Fe-quartz with 100 mL solution (3 mg/mL) 3 mm in a graduated cylinder, and repeatedly this process used the measured water exchanged. After coating, the modified Fe-quartz was 2% of the initial 3 mg/mL solution. DOM sorption to Fe-quartz was demonstrated by in vitro measurement of anion exchange concentration at 254 nm (Shimadzu UV 160A). Desorption of DOM from SOM-Fe-quartz during the micro-column experiments was assessed to be negligible due to use DOM description spectroscopy (Dunham et al., 1992; Gu et al., 1994). Johnson and Asey, 1992). For the purposes of this paper, sediment/dissolved DOM is considered to affect sediment organic matter (SOM). Column experiments were performed using all three porous materials, and each experiment was performed with each DOM. This facilitated bacterial transport observed in the absence of DOM/fundamental DOM transport through quartz was attributed to modification of the bacterial surface, while the effect of DOM/sediment interaction could be observed in the facilitated transport of bacteria in the other sediments.

Dissolved Organic Matter (DOM) and Sediment Organic Matter (SOM)

Savannah River Ethylene Alcohol (SRAHA) and Salt Humic Acid (SHA), obtained from the Long Island Humic Substances Society, were used as representative types of organic matter. Both humic acids were used in sediment and DOM. In experiments where both SOM and DOM were present, the same humic acid was used for both SOM and DOM. This allowed comparison of the effect of SOM on DOM transport. SRAHA was extracted from a molten in Illinois and SRAHA was obtained from Savannah River water. Both humic acids are comprised of extractions of a spectrum of molecular weights, but SRAHA has...
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a higher average molecular weight than SRHA (Johnson and Amy, 1993), in agreement with the general observation that humic substances of terrestrial origin have higher molecular weights than those of aquatic origin (Thurman, 1983). SHA and SRHA solutions used in column experiments were applied at a concentration of 5 mg/l (= 2.5 mg/l DOC). Measurement of DOM concentration after passage through quartz mini-columns indicated no measurable sorption of DOM to quartz. Liu and Amy (1993) similarly reported no interaction of SHA with quartz at pH 6 and anionic strength of 10^{-5}.M.

Sorption of DOM to Fe-oxide to produce SOM-Fe(III) was monitored by u.v. measurement of supernatant DOM concentration equilibrated with Fe-oxide (Fig. 1). Removal of DOM with Fe-oxide was required in order to reach a90% of the equilibrium DOM concentration, i.e. C_{DOM}/C_{DOM, max}. SHA sorbed more strongly than SRHA to Fe-oxide, resulting in 2.37 mg SHA and 0.1 mg SRHA sorbed in 1 g Fe-oxide. Once adsorbed, these humic acids are referred to as SOM. Stronger sorption to Fe-oxide of SHA relative to SRHA corroborated the results of previous research performed with oxide-coated minerals (Liu and Amy, 1993; Johnson and Amy, 1990), and other research which has shown that sorption of DOM to sediment (to become SOM) increases with increasing molecular weight of the organic matter (Davis and Gloire, 1982; Murphy et al., 1990).

**Mini-columns (MARK) procedure**

Bacterial transport through the porous media was examined using the MARK (microbe and radiolabel known) method (Grins et al., 1993). In this method, bacterial retention was examined in microscale columns (Nc polypropylene Luer-Lok syringes, 0.8 cm i.d.) packed with 10-15 nL quartz or modified quartz (1.5, introduced as a slurry) supported by a 0.3 cm dia. G/F D filter (Whamain). Syringe columns were packed in a vacuum manifold (Alltech) equipped with Luer-Lok connections. Vacuum pressure was used to create a fluid-flow velocity of 0.09 cm/s. Column media were pre-irradiated with either 2 ml of the MOPS buffer, or in DOM experiments buffer plus DOM (3 mg/l), followed by 2 ml of the labeled bacterial suspension. The buffer solution was identical to the bacterial suspensions except it was filtered with 0.45 μm of the appropriate buffer solution to minimize sorption of unassimilated radiolabeled bacteria. After introduction of the labeled bacterial suspensions, buffer solution was removed from the manifold, its lower end cut off, and a syringe plunger used to extract a 1-cm-long slice of the influent-end of the column material. The 1-cm slice was transferred to a scintillation vial containing 5 ml of cocktail (Ecoluent I and 1 ml of 10^{4} SC) to promote cell disassociation. Vials were rotated end-over-end on a Cole Parmer tube rotator (10 r.p.m.) for >15 min and analyzed on a Beckman LS 5801 scintillation counter with quench correction. Sorption of unassimilated label was monitored in parallel experiments by addition of a cell-free suspension prepared by 0.5 μm filtering of the labeled bacterial suspension. Adsorbed unassimilated radiolabeled in the parallel column was subtracted from total adsorbed radiolabel measured in the experimental columns. Adsorbed unassimilated radiolabeled was only 10% or less of total adsorbed radiolabel. Total assimilated radiolabel was determined by passing 2 ml of the 10-μL labeled suspension through 0.2 μm polyethersulfone filters (Pallflex) with permission for sorption of unassimilated radiolabeled β filters. Filter-attached unassimilated radiolabeled was less than 1% of total radiolabeled transported. Overall mass balance could not be performed in our experimental system due to inability to recover effluent radiolabeled retained in the G/F D depth filter used to retain the porous media in the mini-column. All MARK column experiments were run in triplicate. The fraction of cells retained in the top of the column, F_{ret}, was calculated from the ratio of the total assimilated radiolabeled retained in the column to the total assimilated radiolabeled measured on the filter. Cell concentrations in labeled suspensions were determined using standard acetone (trotting direct count (ADCC) procedure (Wolfe et al., 1977) with black 0.2 μm pore diameter polycarbonate (Pallflex) filters backed with 3 μL cellulose acetate (Millipore) cutting filters (23 mm dia.).

**RESULTS**

Bacterial retention differed significantly among bacterial cultures grown on different days, presumably due to slight differences in growth histories. Mean values and standard deviations of fraction retained for min-columns (triplicate) are shown in Fig. 2. The variation in bacterial retention for identical experiments performed on different days can be seen, for example, by comparing experiments Fe-oxide-7 vs 15 and Fe-oxide-9. Statistical comparison of retention values obtained on different days for equivalent experiments showed significant differences (P ranging from <0.001 to 0.06, Table 1), which were attributed to variations in bacterial cultures.

Despite day-to-day variations in bacterial cultures, bacterial retention by Fe-oxide was always greater than that in quartz and SOM-Fe-oxide (P < 0.001, Fig. 2, Table 1). Furthermore, bacterial retention depended more upon the surface characteristics of the porous media than the presence or absence of DOM. Mean values of fraction bacteria retained for triplicate mini-columns are shown in Fig. 3, with three replicates of bars, grouped according to the porous media. The mean values at each day shows grouped results (mean and standard deviation).
for experiments performed in the absence DOM. The second and third bars show grouped results for experiments performed in the presence of DOM (SRHA or SHA). Comparison between triplicates was made using the t-test for quartz vs Fe-quartz and the Mann-Whitney Rank Sum Test for comparisons which failed normality or equal variance tests (quartz vs SOM-Fe-quartz, and Fe-quartz vs SOM-Fe-quartz). Comparisons showed that differences in bacterial retention due to the porous media were statistically different (P < 0.001, see also Table 1). Mean values of fraction of bacteria retained were lowest in quartz (0.28 ± 0.10), highest in Fe-quartz (0.73 ± 0.14), and intermediate in SOM-Fe-quartz.

Table 1. Hypotheses tested using column retention data

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Comparison</th>
<th>P value</th>
<th>Conclusion</th>
</tr>
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<tbody>
<tr>
<td>Identical experiments performed on</td>
<td>Same treatment performed</td>
<td>&lt;0.001</td>
<td>True</td>
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<tr>
<td>different days yield different F3</td>
<td>on separate days</td>
<td></td>
<td></td>
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<tr>
<td>Fe-quartz: 7-15 vs 9-3</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>quartz: 7-15 vs 9-3</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>quartz: 8-19 vs 4-19</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartz: 8-19 vs 8-5 vs 9-3</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron-oxide coating of quartz filters F3</td>
<td>All Fe-quartz F3 vs all quartz F3</td>
<td>&lt;0.001</td>
<td>True</td>
</tr>
<tr>
<td>SOM coating of Fe-quartz filters F3</td>
<td>All SOM-Fe-quartz F3 vs all Fe-quartz F3</td>
<td>&lt;0.001</td>
<td>True</td>
</tr>
<tr>
<td>SOM coating of quartz yields F3</td>
<td>All SOM-Fe-quartz F3 vs all quartz F3</td>
<td>&lt;0.001</td>
<td>True</td>
</tr>
<tr>
<td>DOM presence filters F3, on quartz</td>
<td>DOM presence/absence ratio for quartz vs unity</td>
<td>&lt;0.001</td>
<td>True</td>
</tr>
<tr>
<td>DOM presence filters F3, on Fe-quartz</td>
<td>DOM presence/absence ratio for quartz vs unity</td>
<td>0.12</td>
<td>Possibly</td>
</tr>
<tr>
<td>DOM presence filters F3, on SOM-Fe-quartz</td>
<td>DOM presence/absence ratio for quartz vs SOM-Fe-quartz</td>
<td>0.06</td>
<td>Possibly</td>
</tr>
<tr>
<td>DOM presence effects F3, differently on</td>
<td>DOM presence/absence ratio for quartz vs SOM-Fe-quartz</td>
<td>&lt;0.001</td>
<td>True</td>
</tr>
<tr>
<td>quartz vs Fe-quartz and SOM-Fe-quartz</td>
<td>DOM presence/absence ratio for quartz vs SOM-Fe-quartz</td>
<td>0.003</td>
<td>True</td>
</tr>
</tbody>
</table>
Comparison tests performed within triplets were inconclusive, indicating that day-to-day variations were too great to determine the effect of the presence or absence of DOM using this data analysis procedure. The $F$ value, or level of significance that bacterial retention was significantly different in the presence of DOM ($t$-test), is shown in parentheses between pairs in Fig. 2. Not all results were statistically significant ($P$ ranged from 0.04 to 0.57), indicating that while DOM tended to affect bacterial retention, comparison between experiments performed on different days was inconclusive. By examining the data in a format which accounted for day-to-day variations it was shown that DOM reduced bacterial retention on quartz, and that DOM increased bacterial retention on Fe-oxide and SOM-Fe-quartz. To achieve statistically significant comparisons, ratios of bacterial retention obtained in the presence and absence of DOM were made for the same bacterial culture (same day) (Fig. 4, Table 1). Ratios for quartz were significantly different ($t = 0.00$, $t$-test) from ratios for Fe-oxide as were the ratios obtained for quartz vs those of SOM-Fe-quartz ($P = 0.03$, Mann-Whitney Rank Sum Test). The mean ratios were $0.77 \pm 0.21$ in quartz, $1.17 \pm 0.15$ in Fe-oxide, and $1.17 \pm 0.09$ in SOM-Fe-quartz. The quartz ratios were significantly lower than those from Fe-oxide and SOM-Fe-quartz, indicating that DOM affected bacterial retention differently in quartz relative to the other two porous media.

Comparison of quartz ratios to unity showed significant differences ($P < 0.001$, Mann-Whitney Rank Sum Test), whereas the ratios from Fe-oxide and SOM-Fe-quartz were shown to differ from unity to a less significant level ($P = 0.12$, and $P = 0.05$, respectively). Although the differences between the ratios and unity were not all statistically significant, the data indicate that the ratios from quartz were less than unity, whereas those from Fe-oxide and SOM-Fe-oxide were greater than unity.

Possible differences in the effects of SRHA vs SHA as DOM on bacterial retention, were investigated in similar experiments in quartz and Fe-oxide (Fig. 3). Consistent with previous results, bacterial retention on quartz was lower than bacterial retention on Fe-oxide ($P < 0.001$, $t$-test). Bacterial retention tended to be higher in the presence of SHA vs SRHA, but the difference was not statistically significant ($P = 0.14$ in quartz, and $P = 0.06$ in Fe-oxide, $t$-tests).

**Fig. 4. Ratios of fraction retained for DOM vs DOM-free experiments on quartz, Fe-oxide, and SOM-Fe-quartz. Ratios less than unity indicate DOM (calculation of AI234; ratios greater than unity indicate DOM retardation of AI234. Mean and standard deviation shown for each sediment type. $F_{0}^{0} = $ fraction retained. **

**Fig. 5. Same-day comparison of SRHA vs SHA in DOM-induced effects on AI234 transport. Each bar represents triplicate mini-columns (mean and standard deviation). Probability of difference for same-day pairs is given in parentheses.**

**Fig. 3. Fraction of bacteria retained on quartz, Fe-oxide, and SOM-Fe-quartz, in the absence and presence of DOM. Mean and standard deviation shown for each sediment type. $F_{0}^{0} = $ fraction retained. **

**Fig. 6. Effect of iron oxide and SOM on bacterial retention.**

Experiments which examined bacterial transport through quartz, Fe-oxide, and SOM-Fe-quartz, in the absence of DOM, allowed determination of the
effect of SOM (sediment-sorbed DOM) on bacterial transport. Bacterial retention differed between the three porous media (quartz, Fe-quartz, and SOM-Fe-
quartz) to a statistically significant extent (Fig. 3), and retention was higher on Fe-quartz than either quartz or SOM-Fe-quartz. To further explore the reasons for these differences, we ran one case to the effect of iron oxide, and in the other case to the effect of SOM, it is appropriate to review the nature of the different surfaces involved.

Bacteria have complex surfaces with a charge and hydrophobicity dependent on strain and solution chemistry (Daniels, 1983). Bacterial retention to hydrophobic surfaces such as glass is dominated by electrostatic interactions, whereas adhesion to hydrophobic surface (such as sulfonated poly-

styrene) is driven by both hydrophobic and electro-

static interactions (van Loosdrecht et al., 1989; van Loosdrecht et al., 1990). Hydroxyl acid structure is also complicated, as humic acids themselves are mixtures of molecules of varying molecular weights, acidic, and elemental compositions (Thurman, 1981). DOM is negatively charged in natural waters and is known to counter its negative charge to DOM-coated materials (Tipping and Cooke, 1982; Davis, 1982; Jørgensen et al., 1989). DOM sorption increases grain surface hydrophobicity and thus increases sorption of hydrophobic entities (Murphy et al., 1990). Under the conditions of our experiments, both bacteria and quartz surfaces (pH9 of quartz is ~2) can be assumed to be negatively charged (Gross and Logan, 1997), making the bacteria–quartz interaction electrostatically unfavorable. The fact that iron-oxide presence increases bacterial retention in our exper-

iments suggests a negative bacterial surface charge, consistent with the results of other experiments in-

volving negatively charged bacteria (Mills et al., 1994; Scholl and Harvey, 1992).

Iron-oxide (pHpZC ~ 3) coating of quartz altered the surface charge of the quartz grains, resulting in a bacteria–grain interaction that was more favorable. Iron oxide sorption to quartz reduced the negative surface charge of the quartz grain, resulting in the observed increased bacterial retention on Fe-quartz relative to quartz. This result is consistent with the work of others (Scholl and Harvey, 1992) who ob-

served increased bacterial retention on sediments to be associated with the presence of positively charged oxides.

SOM coating of Fe-quartz established a negative grain surface charge, increased bacteria–grain repul-

sion, and resulted in the observed diminished bac-

terial retention on SOM-Fe-quartz relative to Fe-quartz. This result agreed with previous obser-

vations of decreased bacterial retention in sediments coated with SOM (Harvey et al., 1989; Scholl and Harvey, 1992).

The decreases in bacterial retention due to SRHA and SHA coating of Fe-quartz were equivalent (Fig. 3). SHA was present on SOM-Fe-quartz at a much higher concentration than SRHA (by a factor of 24). A conclusive explanation why both humic coatings would result in the same bacterial retention cannot be presented based on our results. However, it is plausible that the similar retention displayed by the two humic acid coatings is related to the respective abilities of the humic acids to place negatively charged functional groups across the surface of the grain and to increase the overall negative surface charge of the grain. Given the higher aliphaticity and higher charge density of SRHA relative to SHA (Thurman, 1985; Thom et al., 1989), it is possible that a lower mass of SRHA was necessary to achieve a similar overall negative grain surface charge as a greater mass of sorbed SHA. Regardless of the mechanism by which the SOM types attained equivalent bacterial retention, it is clear that the primary effect of SOM coating or Fe-quartz was to reduce bacterial retention by increasing electrostatic repulsion between the bacteria and the porous media.

Effect of DOM on bacterial retention

Past investigations have presumed that organic matter facilitates bacterial transport by competing with bacteria for sediment sorption sites (Harvey et al., 1989; Scholl and Harvey, 1992). We also conclude that organic matter affected bacterial transport mainly through modification of the substra-
tum surface, since the largest changes in bacterial retention were associated with grain surface differ-

ences (quartz, Fe-quartz, and SOM-Fe-quartz), and this was true despite the presence of DOM (Fig. 3). The presence of DOM did however affect bacterial retention slightly (Fig. 4), but this effect on retention was secondary to the larger effect of SOM on bacterial attachment.

Since DOM does not interact with quartz (Liu and Amy, 1993; Johnson and Amy, 1995) under the conditions of these experiments, the data that DOM altered bacterial retention on quartz suggests that DOM modified the bacterial surface. This observed slight facilitation of bacteria by DOM is consistent with increased bacterial–quartz repulsion. Increased bacteria–quartz repulsion would be promoted by an increase in the negative bacterial surface charge due to sorption of DOM. This result has also been observed in experiments performed using polyethylene terephthalate which sorbed irreversibly to bacterial surfaces, increased their negative surface charge, and facilitated their transport (Sharma et al., 1985). This hypothesis is further strengthened by the fact that DOM slightly increased bacterial retention on Fe-

quartz (Fig. 4), a result which would be expected if the negative charge of the bacterial surface had been increased, thereby increasing attraction to the posi-

tively charged iron oxide surface. It is unlikely that the observed changes in bacterial transport can be explained by changes in grain surface or cell surface hydrophobicity. If the effect of DOM sorption were to increase bacterial surface or grain surface
hydrophobicity, bacterial retention would have increased in the presence of DOM on all porous media, including quartz. Since DOM did not produce increased bacterial retention for all substrates, and because the observed effects of DOM were consistent with electrostatic considerations (for an inferred negative bacterial surface charge), we conclude that DOM sorbed to the bacterial surface, and increased the negative bacterial surface charge.

Extent of SOM and DOM facilitation of A1264 transport:

The relative effects of DOM on bacterial retention on the three substrates is summarized qualitatively in Fig. 6. Bacterial retention to the three porous media (quartz, Fe-quartz, and SOM-Fe-quartz) is represented by arrows increasing in thickness with increased retention. Retention is shown for both the presence and absence of DOM. The lowest retention was in quartz in the presence of DOM (dashed arrow), whereas the highest retention was in Fe-quartz in the presence of DOM (thickest arrow).

Predicting the magnitude of changes in bacterial transport:

To understand their effect on long distance transport of bacteria, values for the bacterial attachment efficiency were estimated from the experimental data, and from measured system parameters (Table 2), according to the filtration equation (Yao et al., 1971)

\[ F_r = 1 - \exp \left( -\frac{\pi L}{2 \mu} \right) \theta (1 - \theta) \]

where \( F_r \) is fraction retention, \( \theta \) is the column porosity, \( d \) is the diameter of the porous media particle, \( \eta \) is the bacterial attachment efficiency, \( \eta \) is the collector efficiency, and \( L \) is the length of the column. The collector efficiency is a parameter which determines the frequency with which bacteria will strike the porous media, and is calculated from the physical properties of the porous media. The estimated bacterial attachment efficiencies, which determined whether a bacteria sorbs upon striking the porous media, are given for A1264 in each of the experimental porous media (Table 3). Travel distances \( L \) associated with a 99% reduction in bacterial concentration \((C/C_0 = 0.01)\), were calculated using the equation (1) and the estimated attachment efficiencies. At steady state \( C/C_0 = 1 - F_r \).

Coating of Fe-quartz with SOM in our experiments achieved a reduction in fraction bacteria retained from 0.69 in Fe-quartz to 0.39 in SOM-Fe-quartz, this translated to a change in the bacterial attachment coefficient from 0.16 to 0.070. For SOM and DOM effects to be important in facilitating bacterial transport.

<table>
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<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
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<td>Column length</td>
<td>( L )</td>
<td>0.01</td>
<td>m</td>
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<tr>
<td>Column porosity</td>
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<td>Dimensionless</td>
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<td>( \mu m )</td>
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<td>Approach velocity</td>
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</tr>
<tr>
<td>Collector efficiency</td>
<td>( \eta )</td>
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</tbody>
</table>
delivery to a point for removed from the injection well for the purpose of bioremediation, bacterial transport prior to a two-log reduction in concentration should achieve distances of >13 m (Gross and Logan, 1995). Assuming that the estimated SOM-induced changes in attachment efficiency can be used to estimate the effect on bacterial transport in column-grown aquifer sediments, the increase in travel distance for bacterial transport through an aquifer sediment (300 μm) under typical flow conditions (1 μl h⁻¹) can be extrapolated. Given the above sediment and flow conditions and the experimentally determined SOM-induced decrease in the bacterial attachment coefficient (from 0.16 to 0.07), the increase in the travel distance associated with a 99% decrease in cell concentration is from 0.50 to 1.4 m. If a three-log reduction (99.9%) in bacterial concentration is used to define a removal limit, the associated increase in travel distance is from 0.41 to 4.1 m. These increases in travel distance (1 m) indicate that the effect of SOM on bacterial transport, while significant, is relatively unimportant to the enhancement considered necessary for bioremediation purposes. The effect of DOM on bacterial retention was smaller, and therefore is also minor compared to changes needed to facilitate transport. The ubiquitous presence of SOM and DOM in groundwater aquifers suggests that they do have an impact on bacterial retention in the subsurface. However, manipulation of DOM and SOM content in the subsurface is unlikely to enhance bacterial transport sufficiently for the purposes of bioremediation.

CONCLUSIONS

Iron-oxide coating of quartz increased bacterial fraction retained by 160% due to lowering or reversal of negative surface charge by zero to positively charged iron oxide. Loading SOM onto iron-oxide coated quartz lowered bacterial retention by 44%, presumably due to increased negative sediment surface charge. Bacterial retention was additionally, but to a lesser extent, altered by the presence of DOM, due to alteration of the bacterial surface by DOM. DOM and bacterial retention was increased on Fe-quartz and SOM-Fe-quartz, but decreased on quartz. Increased and decreased bacterial retention by DOM were represented by changes in the fraction retained of about 10 and 20, respectively. The largest decrease in fraction retained translated to a 60% decrease in the bacterial attachment efficiency, more than doubling the travel distance required for a two-log reduction in concentration. This magnitude of facilitated transport however, is considered to be insufficient for the purpose of enhancing subsurface delivery for bioremediation.

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REFERENCES


Enhanced transport of bacteria


