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 mini-column experiments to affect the transport of bacteria within porous media. Attachment efficiencies of bacteria were estimated from their retention on quartz, iron oxide coated quartz (Fe-quartz), and Fe-quartz coated with SOM (SOM-Fe-quartz). Suwannee River Humic Acid (SRHA) and Soil Humic Acid (SHA) were used to represent organic matter (SOM and DOM), and were added to radiolabeled bacterial suspensions (10^6 cells/ml, pH = 7.7) prior to transport. Coating quartz with iron oxide increased bacterial retention 160% relative to uncoated quartz. Coating Fe-quartz with SOM lowered bacterial retention, resulting in a fraction retained only 33% greater than retained on uncoated quartz. Compared to 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 quartz, bacterial retention in the presence of DOM was reduced by 20%. However, when DOM adsorption to the porous media was increased by coating the quartz with iron oxide, 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 effects of DOM were the same for both types of humic acids (SHA or SRHA). 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 words—facilitated transport, enhanced transport, bacteria, colloid transport, natural organic matter, groundwater, transport, DOM, NOM

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 useful 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 substratum. Experiments have shown that manipulating solution chemistry causes the most dramatic changes in bacterial transport (Gannon *et al.*, 1991; 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 lesser effects on bacterial transport than changes in ionic strength (Scholl and Harvey, 1992; Jewett *et al.*, 1995). 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; McEldowney and Fletcher, 1986; Herald and Zottola, 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 lipophilic and ionic groups, the latter being mostly carboxyl, hydroxyl, amino, sulfate, and phosphate groups (Sharma *et al.*, 1985). Dissolved organic matter (DOM) has been shown to facilitate the transport of a number of inorganic and organic contaminants in groundwater (McCarthy and Zachara, 1989; Magee *et al.*, 1991; Abdul *et al.*, 1990; Mantoura *et al.*, 1978; Odem *et al.*, 1993; Johnson and Amy, 1995), and to increase the electrophoretic mobility, and hence stability, of particles and bacteria in lakes and streams (Gerritson and Bradley, 1987). Dissolved

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polyelectrolytes such as heparin and sodium pyrophosphate, have been shown to facilitate bacterial transport through aquifer sediment (negatively charged) by sorbing onto bacterial cell walls and increasing 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; Powelson *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 sediment sorption sites (Scholl and Harvey, 1992). This is plausible considering that DOM, bacteria, and sediment surfaces contain many of the same 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 and modification of the bacterial surface charge (Sharma *et al.*, 1985), it 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 lowering 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 A1264 (Balkwill, 1993) was used in our studies to represent bacteria which tend to adhere to aquifer material. Strain A1264 was collected at a depth of 203 m in the Middendorf formation at the DOE Savannah River Site. Oxygen levels at the site of origin were measured to be extremely low but not necessarily anoxic. The cell is rod shaped and is gram-negative. Positive response has been determined for the following tests: nitrate reductase, Arginine dihydrolase, Gelatinase, and Oxidase, whereas negative responses have been determined for Tryptophanase, Glucose fermentation, Urease, Esculin hydrolysis, and PNPG beta-galactosidase. The general physiological characteristics of strain A1264 are consistent with the pseudomonads, but the comparison is not yet definitive (Dr David Balkwill, pers. commun.). A1264 was grown aerobically in a 5% PTYG (peptone, tryptone, yeast extract, glucose medium) batch culture to a stationary phase cell density of about 10^9 ml^{-1}. Growth was monodisperse with a doubling time of 2 h. Suspensions were diluted to a cell concentration of 10^6 ml^{-1} with 3-(N-morpholino)-propanesulfonic acid, sodium salt (MOPS) buffer (4.264 g/l) amended with $MgSO_4 7H_2O$ (0.05 g/l), NH_4Cl (1.0 g/l),

K_2HPO_4 (0.0174 g/l), $CaCl_2$ (0.0885 g/l), $FeCl_3 6H_2O$ (0.0666 g/l). The latter two salts were sterile-filtered ($< 0.2\,\mu m$) and added after autoclaving to avoid their precipitation. ^3H-leucine (0.04 mCi per 100 ml), and, in some cases, DOM (5 mg/l), were added to the buffered suspension (pH 7.7), and shaken at 150–200 rpm for 18 h to permit label uptake. During the labeling periods, bacteria doubled 2–4 times, re-entering stationary phase prior to their use in transport experiments.

Sediment materials

Quartz (40-μm mean dia., ranging from 10–50 μm) was separated from bulk ground silica (mesh size 140) from Unimin (Troy Grove, Ill), using a wet sedimentation technique. Quartz was prepared by a method modified from Litton and Olson (1993), in which quartz was soaked for 24 h in 12 N HCl, and then rinsed in a column (plug flow rinsing) with ultrapure water until conductivity and pH were stabilized (usually about 2 h). This was followed by drying at 100°C and heating for 8 h in a muffle furnace at 800°C in a covered porcelain crucible. The quartz was re-hydrated before use by boiling in ultrapure water for 4 h. A portion of the quartz was coated with iron-oxide (Fe-quartz) using a method modified from Edwards and Benjamin (1989). Quartz (40 g) was added to ultrapure water (100 ml) and 0.1 M $Fe(NO_3)_3$ (1 ml) and stirred constantly during slow (~ 15 min) addition of 0.1 M NaOH (3 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 iron concentration of about 0.3 mg Fe per g quartz, as determined by A_{510} measurement (Schilt, 1969).

Sediment-sorbed DOM is tightly held, desorbs very slowly (Dunnivant *et al.*, 1992; Gu *et al.*, 1994), and is considered to reflect sediment organic matter (SOM), (Murphy *et al.*, 1990; Murphy *et al.*, 1994). A portion of the Fe-quartz media was coated with dissolved humic substances to reflect aquifer sediment coated with SOM. This SOM-coated Fe-quartz (SOM-Fe-quartz) was prepared by mixing Fe-quartz with a DOM solution (5 mg/l, 3 min) in a graduated cylinder, and repeating this process until the measured u.v.$_{254}$ absorbance of DOM mixed with the modified Fe-quartz was $\geqslant 90\%$ of the initial 5 mg/l solution. DOM sorption to Fe-quartz was monitored by difference by u.v. measurement of aqueous humic concentration at 254 nm (Shimadzu UV-160A). Desorption of SOM from SOM-Fe-quartz during the mini-column experiments was assumed to be negligible due to slow DOM desorption kinetics (Dunnivant *et al.*, 1992; Gu *et al.*, 1994; Johnson and Amy, 1995). For the purposes of this paper, sediment-sorbed DOM is herein referred to as sediment organic matter (SOM). Column experiments were performed using all three porous media, one of which did not sorb DOM. Thus facilitated bacterial transport observed in the absence of DOM-sediment interaction (i.e. during transport through quartz) was attributed to modification of the bacterial surface, whereas 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)

Suwannee River Humic Acid (SRHA) and Soil Humic Acid (SHA), obtained from the International Humic Substances Society, were used as representative types of organic matter. Both humic acids were used to produce SOM 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 vs DOM on bacterial retention. SHA was extracted from a mollisol in Illinois and SRHA was obtained from Suwannee River water. Both humic acids are comprised of molecules of a spectrum of molecular weights, but SHA has

a higher average molecular weight than SRHA (Johnson and Amy, 1995), in agreement with the general observation that humic substances of terrestrial origin have higher molecular weights than those of aquatic origin (Thurman, 1985). 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 DOMs to quartz. Liu and Amy (1993) similarly reported no interaction of SHA with quartz at pH 6 and an ionic strength of 10^{-4} M.

Sorption of DOM to Fe-quartz to produce SOM-Fe-quartz was monitored by u.v. measurement of aqueous DOM concentration equilibrated with Fe-quartz (Fig. 1). Repeated equilibration of DOM with Fe-quartz was required in order to reach $\geqslant 90\%$ of the equilibrium DOM concentration, or $C/C_0 \geqslant 0.9$. SHA sorbed more strongly than SRHA to Fe-quartz, resulting in 2.37 mg SHA and 0.1 mg SRHA sorbed to 1 g Fe-quartz; once adsorbed, these humic acids are referred to as SOM. Stronger sorption to Fe-quartz of SHA relative to SRHA corroborated the results of previous research performed on oxide-coated minerals (Liu and Amy, 1993; Johnson and Amy, 1995), 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 Gloor, 1982; Murphy et al., 1990).

Mini-column (MARK) procedure

Bacterial transport through the porous media was examined using the MARK (microbe and radiolabel kinesis) method (Gross et al., 1995). In this method, bacterial retention was examined in miniature columns (3 cc polypropylene LeurLok syringe, 0.8 cm i.d.) packed with 40 μm quartz or modified quartz (1.5 g, introduced as a slurry) supported by a 0.8 cm dia. GF/D filter (Whatman). Syringe columns were placed on a vacuum manifold (Alltech) equipped with LeurLok connections. Vacuum pressure was adjusted to produce a fluid approach velocity of 0.09 cm/s. Column media were pre-rinsed with either 2 ml of the MOPS buffer, or in DOM experiments, buffer plus DOM (5 mg/l), followed by 2 ml of the labeled bacterial suspension. The buffer solution was identical to the labeled bacterial suspension except for an absence of bacteria and radiolabel. After introduction of the labeled bacterial suspension, the column was rinsed with 4 ml of the appropriate buffer solution to minimize sorption of unassimilated radiolabel. The mini-column 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 (Ecolite) and 1 ml of 10% HCl to promote cell dissolution. Vials were rotated end-over-end on a Cole Parmer tube rotator (~ 10 r.p.m.) for >18 h and analyzed on a Beckman LS 3801 scintillation counter with quench correction. Sorption of unassimilated label was monitored in a parallel experiments by addition of a cell-free suspension prepared by 0.2-μm filtering of the labeled bacterial suspension. Adsorbed unassimilated radiolabel measured in the parallel column was subtracted from total adsorbed radiolabel measured in the experimental columns. Adsorbed unassimilated radiolabel was only 10% or less of total adsorbed radiolabel. Total assimilated radiolabel was determined by passing 2 ml of the dilute, labeled suspension through 0.2 μm polycarbonate filters (Poretics) with correction for sorption of unassimilated radiolabel to filters. Filter-sorbed unassimilated radiolabel was less than 1% of total assimilated radiolabel. Overall mass balance could not be performed in our experimental system due to inability to recover effluent radiolabel retained in the GF/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_r, was calculated from the ratio of the total assimilated radiolabel retained in the column to the total assimilated radiolabel retained on the filter. Cell concentrations in labeled suspensions were determined using standard acridine orange direct count (AODC) procedures (Hobbie et al., 1977) with black 0.2 μm pore diameter polycarbonate (Poretics) filters backed with 5 μm cellulose acetate (Millpore) backing filters (25 mm dia.).

Experiments using DOM were run simultaneously with DOM-free experiments to allow direct comparison and elucidation of the effects of DOM on a specific bacterial population. Simultaneous experiments were run for both DOM sources on all three substrates: quartz, Fe-quartz, SOM-Fe-quartz. An additional set of experiments was performed simultaneously to compare the relative effects of SRHA versus SHA on bacterial transport.

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 mini-columns (triplicates) 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-quartz7-15 and Fe-quartz9-3. 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-quartz was always greater than that in quartz and SOM-Fe-quartz ($P < 0.001$; Fig. 2, Table 1). Furthermore, bacterial retention depended more upon the surface chemistry 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 triplets of bars, grouped according to type of porous media. The first bar of each triplet shows grouped results (mean and standard deviation)
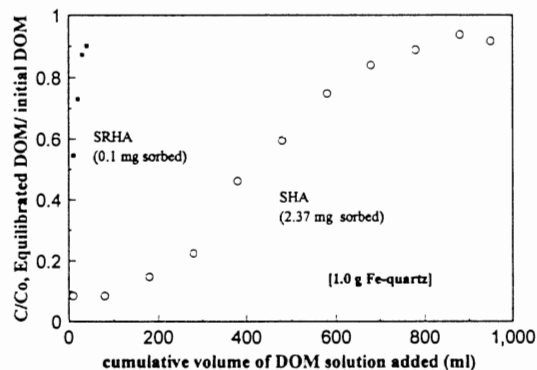

Fig. 1. Equilibration of DOM with Fe-quartz. Cumulative amount of DOM equilibrated with Fe-quartz vs relative concentration of DOM in the aqueous phase (aqueous DOM concentration divided by initial DOM concentration). Sorbed masses estimated from integration of area above curves to $C/C_0 = 1.0$.
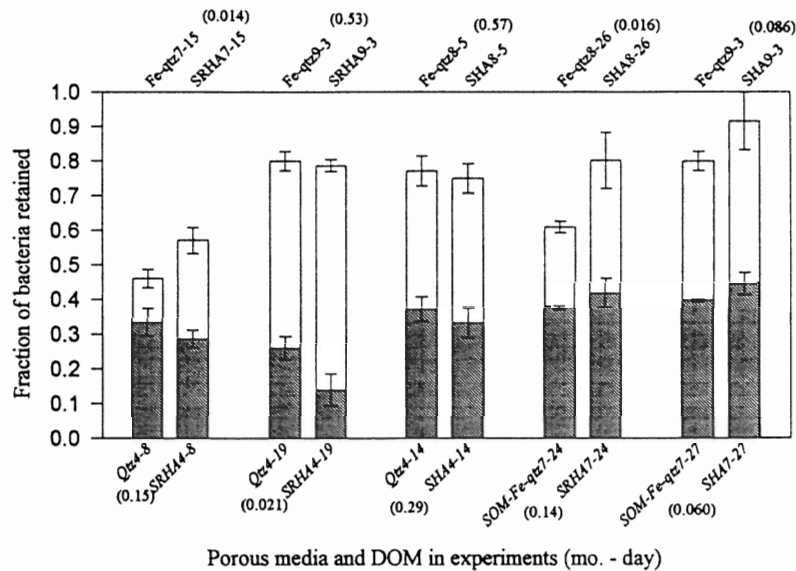
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Fig. 2. Fraction of bacteria retained for all experiments performed. Each bar represents triplicate mini-columns (mean and standard deviation). Pairs of bars represent same-day experiments performed in the absence of DOM (left side of pair), and presence of DOM (right side of pair). Notation on the left side of the pair states the porous media used, whereas notation on the right side of the pair states the DOM used in the experiment. Both sides state the date of the experiment. Three sets of same-day experiments were performed on quartz (left bottom portion of graph), two on SOM-Fe-quartz (right bottom), and five on Fe-quartz (top). Probability of difference between the pair members is shown in parentheses. "Qtz" = quartz.

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 triplets 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

| Hypothesis	Comparison	P value	Conclusion
Identical experiments performed on different days yield different F_rs	Same experiment performed on separate days		True
	Fe-quartz: 7–15 vs 9–3	<0.001	
	Fe-quartz + SRHA: 7–15 vs 9–3	<0.001	
	quartz: 4–8 vs 4–19	0.06	
	quartz + SRHA: 4–8 vs 4–19	0.008	
	Fe-quartz: 8–5 vs 8–26	0.004	
	Fe-quartz + SHA: 8–5 vs 9–3	0.04	
Iron-oxide coating of quartz alters F_r	All Fe-quartz F_rs vs all quartz F_rs	<0.001	True
SOM coating of Fe-quartz alters F_r	All SOM-Fe-quartz F_rs vs all Fe-quartz F_rs	<0.001	True
SOM coating of Fe-quartz yields F_r different from that on quartz	All SOM-Fe-quartz F_rs vs all quartz F_rs	<0.001	True
DOM presence alters F_r on quartz	DOM presence/absence ratios for quartz vs unity	<0.001	True
DOM presence alters F_r on Fe-quartz	DOM presence/absence ratios for Fe-quartz vs unity	0.12	Possibly
DOM presence alters F_r on SOM-Fe-quartz	DOM presence/absence ratios for SOM-Fe-quartz vs unity	0.06	Possibly
DOM presence affects F_r differently on quartz vs Fe-quartz and SOM-Fe-quartz	DOM presence/absence ratios for: quartz vs Fe-quartz quartz vs SOM-Fe-quartz	<0.001 0.003	True
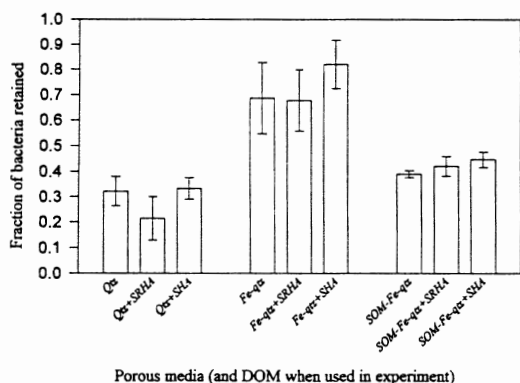
Fig. 3. Fraction of bacteria retained on quartz, Fe-quartz and SOM-Fe-quartz, in the absence and presence of DOM. Mean and standard deviation shown for each sediment type. "F_r" = fraction retained, "Qtz" = quartz, and " + " refers to presence of DOM.

Fig. 5. Same-day comparison of SRHA vs SHA in DOM-induced effects on A1264 transport. Each bar represents triplicate mini-columns (mean and standard deviation). Probability of difference for same-day pairs is given in parentheses.

(0.41 ± 0.03). 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 P value, or level of significance that bacterial retention was significantly different in the presence of DOM (t-test), is shown in parenthesis between pairs in Fig. 2. Not all results were statistically significant (P ranged from 0.014 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-quartz and SOM-Fe-quartz. To achieve statistically significant comparisons, ratios of bacterial retention obtained in the
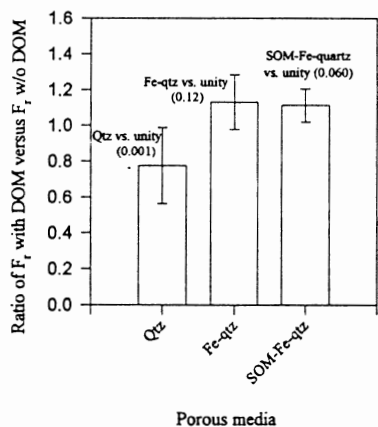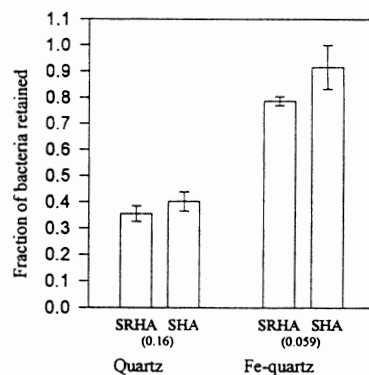

Fig. 4. Ratios of fraction retained for DOM vs DOM-free experiments on quartz, Fe-quartz, and SOM-Fe-quartz. Ratios less than unity indicate DOM facilitation of A1264, ratios greater than unity indicate DOM retardation of A1264. Mean and standard deviation shown for each sediment type. "F_r" = fraction retained, "Qtz" = quartz, "w/o" = without.

presence and absence of DOM were made for the same bacterial culture (same day) (Fig. 4, Table 1). Ratios for quartz were significantly different ($P = 0.001$, t-test) from ratios for Fe-quartz as were the ratios obtained for quartz vs those of SOM-Fe-quartz ($P = 0.003$, Mann–Whitney Rank Sum Test). The mean ratios were 0.77 ± 0.21 in quartz, 1.13 ± 0.15 in Fe-quartz, and 1.11 ± 0.09 in SOM-Fe-quartz. The quartz ratios were significantly lower than those from Fe-quartz and SOM-Fe-quartz, indicating that DOM effected bacterial retention differently in quartz relative to the other two porous media.

Comparison of quartz ratios to unity showed significant difference ($P < 0.001$, Mann–Whitney Rank Sum Test), whereas the ratios from Fe-quartz and SOM-Fe-quartz were shown to differ from unity to a less significant level ($P = 0.12$, and $P = 0.06$, 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-quartz and SOM-Fe-quartz were greater than unity.

Possible differences in the effects of SRHA vs SHA as DOM on bacterial retention, were investigated in same-day experiments in quartz and Fe-quartz (Fig. 5). Consistent with previous results, bacterial retention on quartz was lower than bacterial retention on Fe-quartz ($P < 0.001$, t-test). Bacterial retention tended to be higher in the presence of SHA vs SRHA, but the difference was not statistically significant ($P = 0.16$ in quartz, and 0.06 in Fe-quartz, t-tests).

DISCUSSION

Effect of iron oxide and SOM coatings on bacterial retention

Experiments which examined bacterial transport through quartz, Fe-quartz, 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, due in 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, 1980). Bacterial retention to hydrophillic surfaces such as glass is dominated by electrostatic interactions, whereas adhesion to hydrophobic surfaces (such as sulfonated polystyrene) is driven by both hydrophobic and electrostatic interactions (van Loosdrecht et al., 1989; van Loosdrecht et al., 1990). Humic acid structure is also complicated, as humic acids themselves are mixtures of molecules of varying molecular weights, acidities, and elemental compositions (Thurman, 1985). DOM is negatively charged in natural waters and is known to confer its negative charge to DOM-coated materials (Tipping and Cooke, 1982; Davis, 1982; Jardine 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 (pH_{zpc} of quartz is ~ 2) can be assumed to be negatively charged (Gross and Logan, 1992), making the bacteria–quartz interaction electrostatically unfavorable. The fact that iron-oxide presence increases bacterial retention in our experiments suggests a negative bacterial surface charge, consistent with the results of other experiments involving negatively charged bacteria (Mills et al., 1994; Scholl and Harvey, 1992).

Iron-oxide ($pH_{zpc} \sim 8$) 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 observed 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 repulsion, and resulted in the observed diminished bacterial retention on SOM-Fe-quartz relative to Fe-quartz. This result agreed with previous observations 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; Thorn 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 of 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 substratum surface, since the largest changes in bacterial retention were associated with grain surface differences (quartz, Fe-quartz, and SOM-Fe-quartz), and this was true despite the presence or absence 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 fact 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 bacteria–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 polyelectrolytes which sorbed irreverisbly 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 positively 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
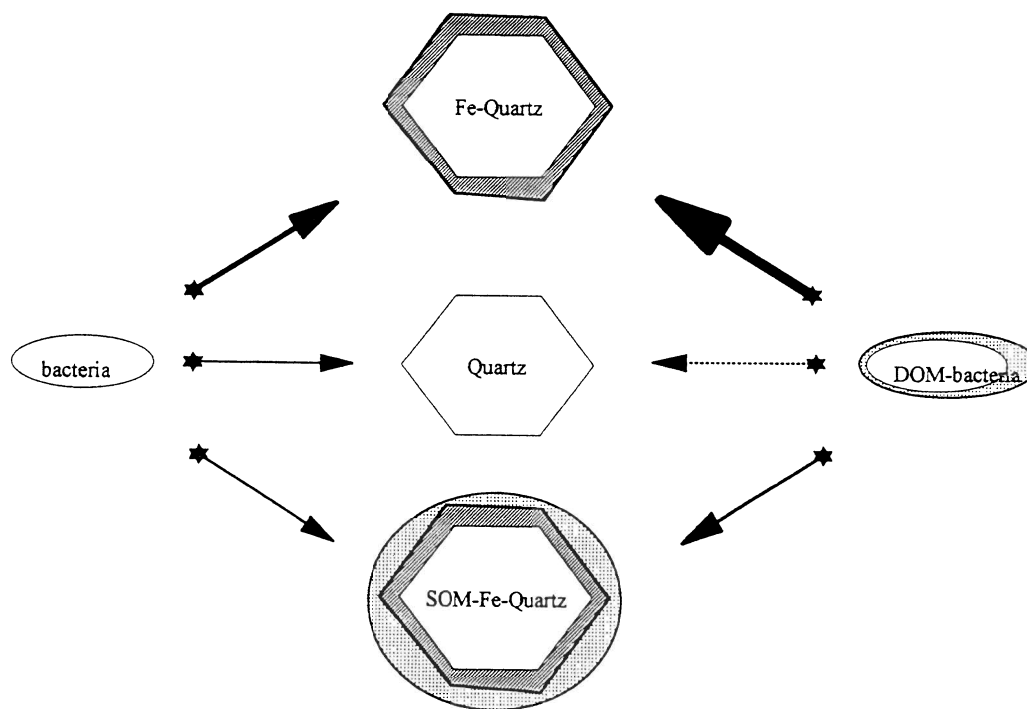
Fig. 6. Schematic diagram of relative effects of DOM and porous media (including SOM) on bacterial retention. Arrow thickness increases with increasing retention.

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 substrata, 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)

$$F_r = 1 - \exp\left[-\frac{3}{2} \frac{(1 - \theta)}{d_c} \alpha \eta L \right], \qquad (1)$$

where F_r is fraction retention, θ is the column porosity, d_c the diameter of the porous media particle, α is the bacterial attachment efficiency, η 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

Table 2. Values of experimental sediment parameters

Parameter	Symbol	Value	Units
Column length	L	0.01	m
Column porosity	θ	0.04	Dimensionless
Collector diameter	d_c	40	μm
Approach velocity	v	0.09	cm/s
Collector efficiency	η	0.0314	Dimensionless

Table 3. Bacterial attachment coefficients and associated travel distances (meters) for a two-log reduction in cell concentration. DOM presence was simulated using a 20% reduction in F_r in quartz, and a 10% increase in F_r in Fe-quartz and SOM-Fe-quartz

Porous media	DOM present?	F_r	Attachment efficiency	Travel distance (m)
Quartz	No	0.32	0.055	0.12
	Yes	0.26	0.042	0.15
Fe-quartz	No	0.69	0.16	0.039
	Yes	0.76	0.20	0.032
SOM-Fe-quartz	No	0.39	0.070	0.093
	Yes	0.43	0.079	0.082

delivery to a point far removed from the injection well for the purpose of bioremediation, bacterial transport prior to a two-log reduction in concentration should achieve distances of > 10 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 coarser-grained aquifer sediments, the increase in travel distance for bacterial transport through an aquifer sediment ($500\,\mu$m) under typical flow conditions (1 m/d), 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 0.60 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 0.91 to 2.1 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-altered bacterial retention was increased on Fe-quartz and SOM-Fe-quartz, but decreased on quartz. Increased and decreased bac-

terial 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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