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# Effects of altered groundwater chemistry upon the pH-dependency and magnitude of bacterial attachment during transport within an organically contaminated sandy aquifer

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## ABSTRACT

The effects of a dilute (ionic strength =  $5 \times 10^{-3}$  M) plume of treated sewage, with elevated levels (3.9 mg/L) of dissolved organic carbon (DOC), upon the pH-dependency and magnitude of bacterial transport through an iron-laden, quartz sand aquifer (Cape Cod, MA) were evaluated using sets of replicate, static minicolumns. Compared with uncontaminated groundwater, the plume chemistry diminished bacterial attachment under mildly acidic (pH 5.0–6.5) in-situ conditions, in spite of the 5-fold increase in ionic strength and substantively enhanced attachment under more alkaline conditions. The effects of the hydrophobic neutral and total fractions of the plume DOC; modest concentrations of fulvic and humic acids (1.5 mg/L); linear alkyl benzene sulfonate (LAS) (25 mg/L); Imbentin (200  $\mu$ g/L), a model nonionic surfactant; sulfate (28 mg/L); and calcium (20 mg/L) varied sharply in response to relatively small changes in pH, although the plume constituents collectively decreased the pH-dependency of bacterial attachment. LAS and other hydrophobic neutrals (collectively representing only ~3% of the plume DOC) had a disproportionately large effect upon bacterial attachment, as did the elevated concentrations of sulfate within the plume. The findings further suggest that the roles of organic plume constituents in transport or bacteria through acidic aquifer sediments can be very different than would be predicted from column studies performed at circumneutral pH and that the inorganic constituents within the plume cannot be ignored.

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## 1. Introduction

Increasing attention is being focused on the role of DOC on bacterial attachment in granular media. This is largely because of the important role of subsurface bacterial transport in transmission of waterborne disease and the fact that sources of bacterial pathogens in groundwater are often sources of organic contaminants (You et al., 2005). There is growing evidence that the attachment behavior of bacteria in porous media is affected by factors not considered in classical DLVO theory (Foppen et al., 2007; Tufenkji, 2006). For example, hydrophobic forces, steric repulsion, and straining can lead to transport observations that are not well described by DLVO-based, colloid-filtration models. Although it was earlier established that DOC can enhance microbial transport through granular media (Gross and Logan, 1995; Johnson and Logan, 1996), more recent findings suggest that this is not always the case (Cheng et al., 2007). Subsurface plumes of organic contaminants are typically complex organic mixtures (Lyngkilde and Christensen, 1992) characterized by sharp geochemical gradients and inorganic chemistries that can be quite different from the rest of the aquifer (Repert et al., 2006). Consequently, better information regarding the effect of plume constituents at different physicochemical conditions may be needed in order to predict more accurately bacterial transport behavior in organically contaminated groundwater.

It is well established that DOC can affect bacterial attachment in granular media. Although some studies on this topic suggest that the presence of DOC leads to a decreased attachment of bacteria (Amirbahman and Olson, 1995) and microspheres (Franchi and O'Melia, 2003), other studies indicate that this is not always the case. It was reported that although SRHA reduced bacterial attachment to quartz sand at pH 7.7 by ~20%, it caused an increase in bacterial retention to Fe-coated quartz by ~10% (Johnson and Logan, 1996). However, a more recent study found that DOC consisting of biosolids extract had no measurable effect upon the transport of the bacteriophage MS-2 in sandy soil at pH 7 (Cheng et al., 2007). Clearly, the issue of whether or not DOC promotes or inhibits microbial transport in granular media is complex and depends upon experimental conditions, aqueous chemistry, and the nature of the organic matter, bacteria, and grain surfaces.

Since the mid 1980s, injection and recovery tests have been conducted in a sandy (iron oxide-coated quartz and feldspar), sole-source aquifer on Cape Cod, MA in order to gain a better understanding of the processes controlling subsurface microbial transport. The aquifer has been impacted by an extensive (6 km-long), but rather dilute (<500  $\mu\text{S}/\text{cm}$  specific conductance,  $5 \times 10^{-3}$  M ionic strength, 3.9 mg/L DOC) organic plume resulting from a 60 year discharge of treated sewage onto the aquifer (LeBlanc, 1984). Because of anaerobic microbial metabolic processes (Abrams et al., 1998) and lime application, the contaminant plume is less acidic (pH 5.8–6.5) than uncontaminated zones within the aquifer (pH 5.0–5.8) (Table 1). In spite of the ~5-fold higher ionic strength (Scholl and Harvey, 1992), microbial transport occurs to a much greater extent within the organic contaminant plume (Blanford et al., 2005; Pieper et al., 1997). In earlier studies, static minicolumns packed with

**Table 1 – Values for chemical constituents and physical parameters for the uncontaminated aquifer and contaminant plume.**

Constituent	Uncontaminated aquifer	Contaminant plume
Calcium <sup>a</sup>	2.5 mg/L	20.4 mg/L
Magnesium <sup>a</sup>	1.6 mg/L	3.4 mg/L
Chloride	11.8 mg/L	12.3 mg/L
Nitrate <sup>a</sup>	1.8 mg/L	10.6 mg/L
Sodium	8.4 mg/L	47.3 mg/L
Sulfate	7.1 mg/L	28.2 mg/L
DOC	0.4 mg/L	3.9 mg/L
Specific conductance	62 $\mu\text{S}/\text{cm}$	488 $\mu\text{S}/\text{cm}$
Ionic strength <sup>a</sup>	0.8 mM	4.5 mM
pH	5.0–5.8 <sup>b</sup>	5.8–6.5 <sup>c</sup>

a Scholl and Harvey (1992).

b (Hess et al., 2001).

c (Davis et al., 2001).

aquifer sediments were used to compare the attachment behaviors of native aquifer bacteria and protozoa (Harvey et al., 2002) and to study the overall effect of the contaminant plume upon the pH-dependency of bacterial attachment (Scholl and Harvey, 1992). Static minicolumns were used to approximate flow-through conditions in the aquifer under steady-state geochemical conditions. The static columns facilitated studying microbial attachment behavior in a controlled, reproducible manner using a large number of replicated systems. Although results of the latter study indicated that the contaminant plume had a considerable effect upon bacterial attachment, it was not clear which plume constituents were most important and how their relative contributions varied with pH.

This report describes static minicolumn studies designed to better assess the roles of environmentally relevant concentrations of individual plume constituents upon bacterial attachment. Specifically, separate series of static minicolumns were used to assess relative effects of the hydrophobic and total fractions of the plume DOC, anionic (LAS) and nonionic (Imbentin) surfactants, humic and fulvic acids, sulfate, and calcium upon the magnitude and pH-dependency of bacterial attachment. The study involved both uncontaminated groundwater that was amended with organic and inorganic plume constituents and contaminant plume groundwater that was chemically altered by partial and complete organics removal without impacting the inorganic composition. Our initial hypothesis was that the manner in which a number of individual organic and inorganic constituents affect bacterial attachment can be pH dependent. Consequently, the overall effect of the plume upon bacterial transport would be expected to change across pH gradients that characterize the contaminant-impacted aquifer. However, the overarching goal of the study was to gather information that would facilitate a better prediction of changes in bacteria transport behavior with temporal and spatial changes in plume chemistry. Because the physical and chemical properties of the study site are typical of many other glacial outwash aquifers characterized by iron oxide-coated quartz sand, information collected in our study may facilitate

a better understanding of bacterial transport at a number of other organically contaminated sites.

## 2. Methods

### 2.1. Groundwater and sediment

The study site is a sand and gravel aquifer located on Cape Cod, MA. Contaminated groundwater (3.9 mg/L DOC, 490  $\mu$ S/cm specific conductance, pH 6.5) was collected from a well (S314-51) located in the core of the plume ~30 m downgradient from the sewage disposal beds. Water samples from this well were collected at a depth of 15.5 m below land surface (BLS) and an elevation of 8.17 m above sea level (ASL), which is ~6.4 m below the water table. Uncontaminated groundwater (<1 mg/L DOC, 60  $\mu$ S/cm, pH 5.0) and sediments were collected from well F350-13, 3 km downgradient from the sewage infiltration beds at a depth of 3.9 m BLS (~7.67 m ASL and 2.7 m below the water table), but well above the contaminant plume. Uncontaminated aquifer sediments were collected with a piston-type core barrel using a hollow-stem auger (Zapico et al., 1987) at the F350-13 well site. The sediments were homogenized and sieved (0.5–1.0 mm grain size) in order to preclude the possibility of bacterial losses due to straining during pore water sampling of the static columns. Samples were maintained at 4 °C until processed. Groundwater inorganic chemistries were assessed according to published methods (Garbarino and Taylor, 1979). Calcium and sodium were assayed by inductively-coupled atomic-emission spectroscopy (ICP-AES), whereas chloride and sulfate were determined by ion chromatography. Summaries of the chemical data for contaminated and uncontaminated groundwater are provided in Table 1.

### 2.2. Static column tests

The pH dependencies of attachment for the community of free-living groundwater bacteria were assessed using static minicolumns (Scholl and Harvey, 1992; Harvey et al., 2007), which were designed to simulate the physicochemical conditions present in aquifer sediments. Static columns consisted of 20 mL glass syringes (Popper & Sons) that were dry-packed with 15 g of sieved and homogenized aquifer sediment. The columns were subsequently conditioned with approximately 4–6 pore volumes (~2.5 mL) of pH-adjusted, filter-sterilized (0.2  $\mu$ m pore size) groundwater prior to bacteria application to columns. Separate series of static minicolumns were set up to span a pH range of ~3–9 using unaltered or chemically altered groundwater collected from the contaminant plume or the uncontaminated zone of the Cape Cod aquifer. Three replicate minicolumns were used for each individual pH value. The final pH of each static minicolumn was measured to within 0.1 pH units with a Beckman model phi-12 pH meter & electrode. A consistent number of DAPI-labeled bacteria were loaded into each static minicolumn by drawing an appropriately diluted seed solution (1.0 mL) into saturated, pre-equilibrated sand pack in order to achieve a loading of ~4.6  $\times 10^6$  bacteria/g. After 4-hour incubations, the pore water within each column was drained by gravity and added to a subsequent “chaser” volume of filtered

(0.2  $\mu$ m pore size) rinse water put through the column in order to collect residual unattached bacteria. Fractional attachment was determined from the differences between the total number of bacteria that were added to the column and the total number subsequently recovered. Where necessary, the ionic strength (IS) of the pore water was appropriately adjusted with NaCl to give a final ionic strength similar to that within the contaminant plume sampled at well S314-51 ( $4 \times 10^{-3}$  M). The static minicolumns were held in a refrigerated incubator at  $10 \pm 1$  °C in order to match the ambient temperature of the aquifer (LeBlanc, 1984).

### 2.3. Bacteria

Water samples were obtained from well S314-51 and kept at 4 °C until used in experiments. The community of unattached bacteria were concentrated on 0.45  $\mu$ m (pore size) Supor cellulose acetate filters (Gelman), stained with the DNA-specific dye 4',6-diamino-2-phenylindole dihydrochloride (DAPI) at a final concentration of 5  $\mu$ g/mL, and resuspended in filter-sterilized uncontaminated or contaminated groundwater as described in an earlier study (Scholl and Harvey, 1992). Bacterial concentrations in the feed were diluted appropriately so that the loading to the columns was consistently ~4.6  $\times 10^6$  bacteria per gram of sieved aquifer sediment. Enumerations of DAPI-labeled bacteria were performed using epifluorescence microscopy (Harvey et al., 1984).

### 2.4. Dissolved organic matter

Two methods were employed to assess the effects of selected components of the plume DOC on the magnitude and pH-dependency of bacterial attachment. First, DOC was removed from samples of contaminated groundwater before using it as a suspending medium for bacteria added to the static minicolumns. Second, specific compounds (LAS, representative of the anionic surfactants within the plume; Imbentin, a model nonionic surfactant; and Suwannee River humic, SRHA and fulvic acids, SRFA, representative of the natural organic matter, NOM, in the aquifer) were amended to uncontaminated groundwater and subsequently used to make up the pore water within replicate series of static minicolumns. Although humic and fulvic acid are naturally present within uncontaminated zones of the aquifer, the low concentration of DOC in these zones (0.4 mg/L; Scholl and Harvey, 1992) made isolation of humic material from the aquifer impractical. Consequently, for the DOC amendment studies, SRHA and SRFA, obtained from the International Humic Substances Society, were added to uncontaminated groundwater. For the surfactant amendment studies, LAS (C10-14; Conda Vista, Austin, TX), or Imbentin (Imbentin-N/7A, nonylphenol mono-tri ethoxylate; AG Kolb, Hedingen, Switzerland) was added. In each case, the DOC was well mixed with the groundwater. Final concentrations of the amended test constituent are listed in Table 2. DOC concentrations were measured by an OI organic carbon analyzer (Aiken, 1992).

Two experiments involving removal of organic matter from the contaminated groundwater were conducted to examine how the type of plume DOC affects bacterial attachment to aquifer sediments. In the first experiment,

**Table 2 – Test constituents, applied concentrations, and pH ranges for static column series.**

Test Series	Test Constituent	Concentration (final)	Groundwater type used	Amendment	Specific Alterations	pH tested
1 <sup>a</sup>	None added	0.9 mg/L DOC	Uncontaminated	None	None	3.3–8
2	Collective chemistry	3.9 mg/L DOC	Contaminant plume	None	None	3.5–8.8
3	Plume DOC	0.1 mg/L DOC	Contaminant plume	None	DOC removed by UV oxidation	3.6–7.9
4	Hydrophobic neutrals	0 mg/L	Contaminant plume	None	HbN <sup>b</sup> removed by XAD8 resin	3.5–6.8
5	Anionic surfactants	25 mg/L	Uncontaminated	LAS <sup>c</sup>	Added LAS homologs (C10–C14)	3.6–8.3
6	Nonionic surfactant	200 µg/L	Uncontaminated	Imbentin <sup>c</sup>	Added Imbentin N/A7,	3.6–7.7
7	Humic acids	1.5 mg/L	Uncontaminated	SRHA <sup>c</sup>	Added SRHA (G. Aiken)	4.1–8.7
8	Fulvic acids	1.5 mg/L	Uncontaminated	SRFA <sup>c</sup>	Added IAHS SRFA	3.7–8.5
9	Divalent anions	28 mg/L	Uncontaminated	Sulfate <sup>c</sup>	Added as Na <sub>2</sub> SO <sub>4</sub>	3.6–7.6
10	Divalent cations	20 mg/L	Uncontaminated	Calcium <sup>c</sup>	Added as CaCl <sub>2</sub> ·H <sub>2</sub> O	3.6–8.7

a Data from Harvey et al., 2002.

b Hydrophobic neutral fraction of plume DOC.

c Also amended with NaCl in order to achieve an ionic strength of the contaminant plume, i.e.,  $\sim 5 \times 10^{-3}$  M.

contaminated ground water, adjusted to pH 7.0, was passed through a column packed with XAD8 resin to remove the hydrophobic neutral fraction of contaminant plume DOC. The XAD8 resin was prepared according to published procedures (Aiken et al., 1992), rinsed with  $\sim 1$  L distilled water, then rinsed alternatively with 50 mL each of 0.1 N NaOH and 0.1 N HCl. The rinse was changed when eluent pH changed to above 8.0 or below 5.0. In the other experiment, the DOC within the contaminated groundwater sampled from the organic plume was removed by ultraviolet (UV)-oxidation. This involved recirculating the contaminant plume groundwater through a quartz flow-through cell placed in a UV (185 nm) light source (Model SP-2 UV oxidation unit, Aquafine Corp., Valencia, CA) for 16 hours at 10 °C. Approximately 97% of the plume DOC was removed in this manner.

### 2.5. Calcium and sulfate

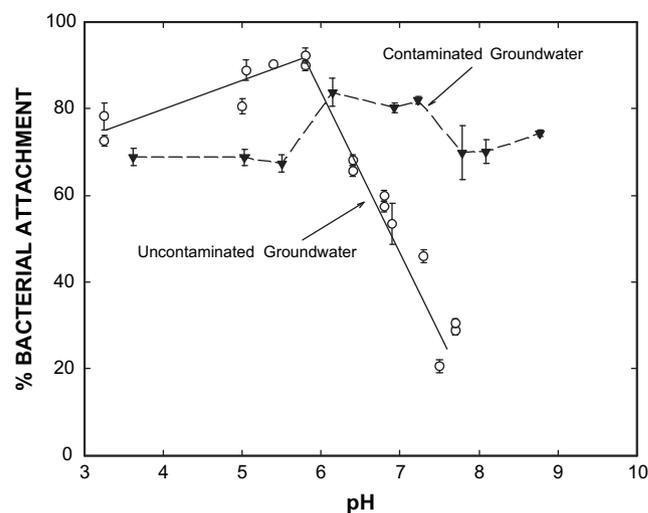
In a separate series of columns, calcium (added as CaCl<sub>2</sub>·H<sub>2</sub>O) and sulfate (added as Na<sub>2</sub>SO<sub>4</sub>) were amended to uncontaminated groundwater. The final concentrations of sulfate and calcium (28 and 20 mg/L respectively; Table 2) were chosen to be consistent with the elevated concentrations of these constituents in the contaminant plume (Table 1).

## 4. Results

The collective effect of elevated organic and inorganic constituents within the contaminant plume upon the pH-dependency of bacterial attachment is depicted in Fig. 1. Bacterial attachment in the presence of uncontaminated groundwater was strongly pH dependent, judging from a 70 % drop in fractional attachment (from 92 to 21 percent) that accompanied a 2-unit increase in pH (from pH 5.8 to 7.9). In contrast, bacterial attachment in the presence of groundwater obtained from the contaminant plume was considerably less pH dependent and varied only 16% (from 67 to 84 percent) over the tested pH range of 3.5–8.8 (Fig. 1). The plume chemistry caused a decrease in bacterial attachment at pH values below 6 relative to the ambient groundwater, in spite of the 5-fold higher ionic strength. However, fractional attachments at pH

values above 6 were always elevated relative to the ambient groundwater control.

The effects of individual plume constituents upon bacterial attachment within the aquifer sediments are summarized in Table 3. Removal of the hydrophobic neutral fraction ( $\sim 3\%$  of the plume DOC) by passage through XAD8 resin altered the pH-dependency of bacterial attachment (Fig. 2). This removal also resulted in a substantive increase in fractional attachment relative to the unaltered plume water under acidic conditions, but had little effect at pH 7. However, removal of  $\sim 97\%$  of the plume DOC by UV oxidation resulted in a sharp decrease in bacterial attachment relative to that observed for



**Fig. 1 – Variation in fractional bacterial attachment as a function of pH for static minicolumns packed with Cape Cod aquifer sediments. Triangles indicate fractional attachment in the presence of groundwater collected from the organic contaminant plume. Circles indicate fractional attachment in the presence of uncontaminated groundwater (reproduced from Harvey et al., 2002 with permission of the publisher and shown for comparative purposes). All data points represent the average for three replicate columns. Error bars represent the standard error around the mean.**

**Table 3 – Effect of dissolved organic and inorganic constituents on transport of bacteria in Cape Cod Aquifer sediments at different pH conditions.**

Constituent	Relative effect			Comments
	pH 5–6	pH 6–7	pH 7–8	
<b>Organic</b>				
Sewage plume hydrophobic neutrals	++	+		Fractionated on XAD8 resins
LAS (anionic surfactant)	++	+	–	Mixture of C10–C14 homologs
Imbentin (nonionic surfactant)	+/-	+/-	–	Model surfactant
Humic acid	++	+/-	–	Isolated from the Suwannee River, GA
Fulvic acid	+/-	–	–	Isolated from the Suwannee River, GA
<b>Inorganic</b>				
Sulfate	++	+	+/-	
Calcium	+	–	–	
Plume composite	++	+/-	–	Organic and inorganic constituents

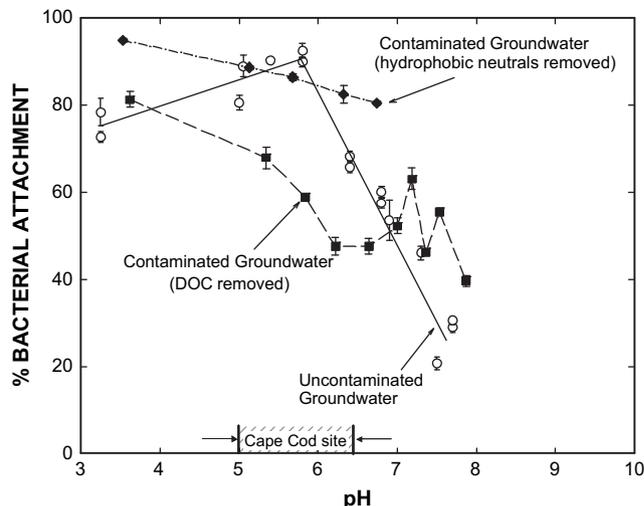
++ indicates a strong role in promoting bacterial transport, + a moderate role in promoting bacterial transport, +/- a weak or indeterminate role in promoting bacterial transport, – a moderate antagonistic role in promoting bacterial transport, and – a strong antagonistic role in promoting bacterial transport through Cape Cod aquifer sediments, as evidenced by changes in fractional bacterial attachment in static minicolumns.

the unaltered plume under mildly acidic to mildly alkaline conditions.

Amendment of ambient groundwater with anionic LAS surfactants at environmentally relevant concentrations (25 mg/L) resulted in a substantive decrease in fractional attachment, but only under acidic conditions (Fig. 3). Little difference in fractional attachment in the presence of LAS was observed at pH values above 7. In contrast, amendment of ambient groundwater with 200 µg/L of the model nonionic surfactant, Imbentin, had little effect upon bacterial attachment under the acidic conditions representative of the Cape Cod aquifer, but substantively enhanced bacterial attachment under more alkaline conditions.

Addition of humic and fulvic acids into uncontaminated groundwater decreased the pH-dependency of bacterial attachment and also resulted in greater attachment under alkaline conditions (Fig. 4) relative to that for the unamended, uncontaminated groundwater. The decreased bacterial attachment under acidic conditions (pH 5–5.8) representative of the uncontaminated aquifer was more pronounced in the presence of humic acid as compared with fulvic acid.

The effect of amendments of a divalent anion and cation are depicted in Figs. 5 and 6, respectively. Increasing the sulfate concentration in the uncontaminated groundwater to levels consistent with the plume chemistries caused a considerable drop in bacterial attachment under acidic conditions (Fig. 5). At pH 6, fractional attachment in the presence of the added sulfate was only about half of what it was in the presence of unamended groundwater, in spite of



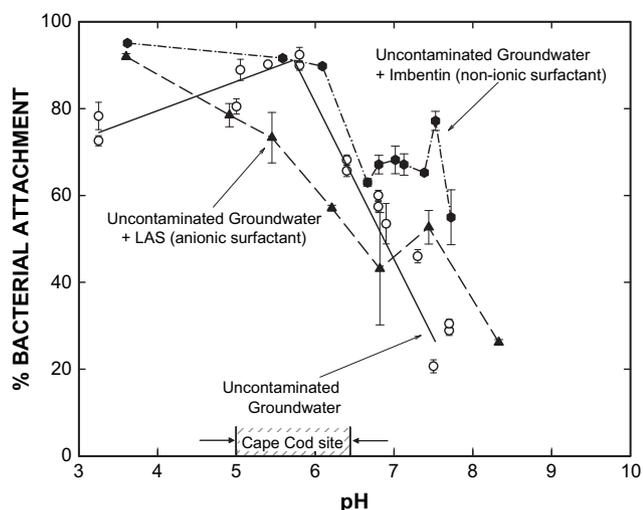
**Fig. 2 – Variation in fractional bacterial attachment as a function of pH for static minicolumns packed with Cape Cod aquifer sediments. Squares indicate attachment in the presence of organic contaminant plume after removal of the DOC by UV oxidation. Diamonds indicate attachment in the presence of organic contaminant plume after removal of the hydrophobic neutral fraction of the DOC by XAD8 resin. Circles indicate fractional attachment in the presence of uncontaminated groundwater (reproduced from Harvey et al., 2002 with permission of the publisher and shown for comparative purposes). All data points represent the average for three replicate columns. Error bars represent the standard error around the mean.**

the higher ionic strength. However, at pH 7.5 there was little effect on bacterial attachment. The addition of calcium to a level representative of what is present in the plume had the effect of increasing bacterial attachment at pH values above 6 (Fig. 6). However, this effect was most pronounced at pH values greater than 7.5.

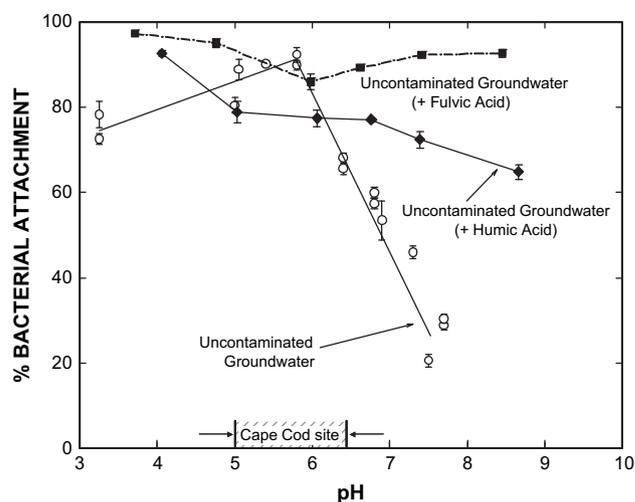
## 5. Discussion

### 5.1. Alterations in bacterial attachment caused by removal of hydrophobic neutral and total DOC

Bacterial attachment in uncontaminated aquifer sediments at the Cape Cod site has been shown to be strongly pH dependent (Harvey et al., 2002). Although the organic contaminant plume enhanced bacterial transport under in-situ (acidic) conditions in spite of the ~5-fold increase in ionic strength (Scholl and Harvey, 1992), this was not the case under neutral or alkaline conditions (Fig. 1). Consequently, the roles that dissolved natural and contaminant organic carbon have in enhancing or inhibiting bacterial transport through the aquifer may also be pH dependent. The issue of the pH-dependency of bacterial attachment is relevant in this study for several reasons. First, contaminated aquifers can exhibit substantial gradients in pH. Second, most of the detailed column studies examining the effects of DOC upon bacterial



**Fig. 3** – Variation in fractional bacterial attachment as a function of pH for static minicolumns packed with Cape Cod aquifer sediments. Triangles indicate attachment in the presence of uncontaminated groundwater amended with LAS surfactants. Hexagons indicate attachment in the presence of uncontaminated groundwater amended with nonionic surfactant Imbentim N/A7. Circles indicate fractional attachment in the presence of uncontaminated groundwater (reproduced from Harvey et al., 2002 with permission of the publisher and shown for comparative purposes). All data points represent the average for three replicate columns. Error bars represent the standard error around the mean.



**Fig. 4** – Variation in fractional bacterial attachment as a function of pH for static minicolumns packed with Cape Cod aquifer sediments. Diamonds indicate attachment in the presence of uncontaminated groundwater amended with Suwannee River humic acid. Squares indicate attachment in the presence of uncontaminated groundwater amended with Suwannee River fulvic acid. Circles indicate fractional attachment in the presence of uncontaminated groundwater (reproduced from Harvey et al., 2002 with permission of the publisher and shown for comparative purposes). All data points represent the average for three replicate columns. Error bars represent the standard error around the mean.

attachment in granular media have been performed at higher pH than is typically found in drinking water aquifers. Finally, knowing the pH-dependency of a given solute upon bacterial attachment behavior can provide important clues as to the mechanism involved in the effect.

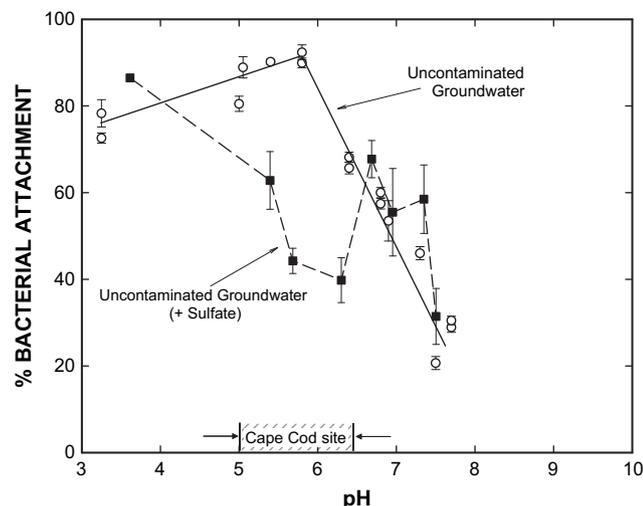
Removal of plume DOC by UV oxidation caused a substantive change in the pH-dependency of bacterial attachment, particularly under the acidic conditions of the aquifer (Fig. 2). However, the contaminant plume also had elevated concentrations of inorganic constituents known to affect bacterial attachment in granular media. Consequently, in order to better understand the role of the plume DOC in bacterial transport it becomes necessary to examine the effects of the other plume constituents, including sulfate and calcium, over the same pH range.

### 5.2. Alterations in bacterial attachment caused by surfactants

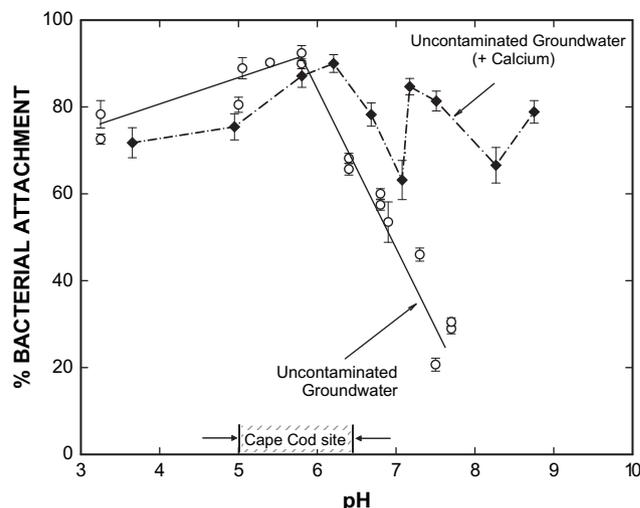
Although the small amount of DOC in uncontaminated areas of the aquifer are predominantly (~90%) hydrophilic, the elevated (3.9 mg/L) DOC immediately downgradient from the loading beds is almost evenly divided between hydrophobic and hydrophilic moieties (Barber, 1992). Removal of the hydrophobic neutral fraction of the plume DOC, which includes the nonionic and anionic surfactants (Field et al.,

1992b), caused an increase in bacterial attachment over much of the aquifer pH range (Fig. 2) as compared with attachment in the presence of unaltered plume groundwater (Fig. 1). This suggests that the presence of the hydrophobic neutral fraction, which constituted only ~3% (~0.1 mg/L) of the plume DOC, has a disproportionately large role in facilitating the advective transport of bacteria within the contaminant plume. Further downgradient in the plume where the hydrophobic neutral concentrations are much higher because of residual concentrations of refractory alkylbenzene sulfonates (1–2 mg/L) (Barber, 1992), the role of hydrophobic DOC in promoting bacterial transport should be correspondingly greater.

Not surprisingly, an amendment of LAS surfactants, which would also be represented in the hydrophobic neutral fraction of the DOC, to uncontaminated groundwater resulted in an 11–33% decrease in bacterial attachment across the 5.0–6.5 pH range of the aquifer sediments (Fig. 3). In an unrelated study involving microbial transport within the Cape Cod plume, an injection of 25 mg/L LAS into the contaminant plume resulted in a detachment of 87% of PRD-1 (bacteriophage) that had sorbed in a previous injection (Pieper et al., 1997). It has been reported that the presence of sodium dodecyl sulfate caused a log unit decrease in attachment efficiency ( $\alpha$ ) of the bacterium *Alcaligenes paradoxus* in the presence of glass beads (Gross and Logan, 1995), although the concentration used to achieve that effect (0.01–0.1%) would be high for LAS-impacted



**Fig. 5 – Variation in fractional bacterial attachment as a function of pH for static minicolumns packed with Cape Cod aquifer sediments. Squares indicate attachment in the presence of uncontaminated groundwater amended with sulfate. Circles indicate fractional attachment in the presence of uncontaminated groundwater (reproduced from Harvey et al., 2002 with permission of the publisher and shown for comparative purposes). All data points represent the average for three replicate columns. Error bars represent the standard error around the mean.**



**Fig. 6 – Variation in fractional bacterial attachment as a function of pH for static minicolumns packed with Cape Cod aquifer sediments. Diamonds indicate attachment in the presence of uncontaminated groundwater amended with calcium. Circles indicate fractional attachment in the presence of uncontaminated groundwater (reproduced from Harvey et al., 2002 with permission of the publisher and shown for comparative purposes). All data points represent the average for three replicate columns. Error bars represent the standard error around the mean.**

groundwater. In the latter study, the decrease in collision efficiency was accompanied by an increase in the bacterium's hydrophobicity. In the present study, LAS amendment only reduced bacterial attachment to aquifer sediment grains under acidic conditions, where the iron oxyhydroxides that characterize the Cape Cod aquifer sediments (Ryan et al., 1999) exhibit a strong net positive charge. Changes in the net charge caused by a sorbing anionic surfactant should lead to a decreased propensity of negatively charged bacteria for attachment.

Nonionic surfactants are also environmentally important constituents in sewage-derived subsurface contaminant plumes (Swartz et al., 2006). The effects of an amendment of the nonionic surfactant, Imbentin, upon the attachment behavior of bacteria suspended in uncontaminated Cape Cod groundwater were fundamentally different than those caused by the LAS amendment (Fig. 3). This was evidenced by the disparate pH dependencies of bacterial attachment for the two types of surfactants. Unlike LAS, Imbentin clearly increased bacterial attachment under alkaline conditions, where grains within the quartz-dominated sediments and suspended bacteria would both exhibit a strong net-negative surface charge. The effect of the Imbentin amendment was manifest at a very low concentration (200  $\mu\text{g/L}$ ), suggesting that under neutral to slightly alkaline conditions, certain nonionic surfactants may affect bacterial transport behavior at sub part-per-million levels. This has direct relevance to the Cape Cod aquifer plume, because of the low concentrations of nonionic surfactants that have been detected therein (Barber et al., 1992). Also, in contrast to results of the LAS amendment,

addition of Imbentin did not produce a decreased bacterial attachment under the acidic conditions found in the Cape Cod aquifer. Because Imbentin ( $\text{pK}_a \sim 10.3$ ) is uncharged over the pH range tested, increases in bacterial attachment under alkaline conditions likely result, at least in part, from increases in hydrophobic interactions between the bacterial and grain surfaces. In a recent study (Brown and Jaffe, 2006), linear polyoxyethylene alcohol surfactants were shown to increase the hydrophobicity of *Sphingomonas* sp., although it was earlier observed that enhanced transport of this bacterium through aquifer sediments at pH 7 was also due to an expansion of the electrical double layer (EDL) by displaced counter ions caused by the sorbing surfactant (Brown and Jaffe, 2001). Furthermore, the increase in bacterial transport was found to increase with ethoxy chain length from 4 to 23. Because Imbentin has an average ethoxy chain length of only 1–2, its effect on EDL expansion would be considerably less. It is likely that any effects of Imbentin upon grain surface hydrophobicity under alkaline conditions would likely be due to its partitioning into the small amount of organic matter (0.0001  $f_{oc}$ ; Barber, 1994) associated with the grain surfaces, although there would also be polar interactions between the oxygen in the ethoxy group of the Imbentin and the Si–OH groups on the quartz surface.

### 5.3. Alterations in bacterial attachment caused by amendments of natural organic matter

Groundwater amended with SRHA or with SRFA altered the pH-dependency of bacterial attachment compared to

uncontaminated groundwater (Fig. 4). Although an amendment of humic acid (3.5 mg/L final concentration) decreased bacterial attachment in the pH range of the uncontaminated aquifer (5.0–5.8), the concentration of humic material within the Cape Cod aquifer is considerably lower (Barber, 1992) and has a less-aromatic structure. The lower bacterial attachment in the presence of SRHA as compared with that observed for the lower molecular weight SRFA is consistent with that reported elsewhere (Amirbahman and Olson, 1995). The interaction of DOC with grain surfaces within the Cape Cod contaminant plume should largely involve the positively charged patches of iron oxides (Barber et al., 1992), because sorption of SRHA onto pure quartz does not readily occur (Johnson et al., 1996).

The presence of DOC has been reported to enhance the electrostatic repulsion between the oocysts of protozoan parasite *Cryptosporidium parvum* and glass beads, thus leading to decreased attachment (Dai and Hozalski, 2002). It is reported that negatively charged humic acid adsorbed to both the colloid (latex microspheres) and the silica collectors, resulting in additional electrostatic and steric contributions to the repulsive energy barrier for attachment (Franchi and O'Melia, 2003). Based upon charge considerations, it is expected that humic acids would reduce bacterial attachment to iron-laden sediments under acidic conditions due to partial charge neutralization of the iron oxide surfaces. It was reported that addition of soil humic acid to iron-coated quartz reduced bacterial attachment to a level that was only 33% higher than attachment to uncoated quartz (Johnson and Logan, 1996). In the present study, it is likely that the decrease in bacterial attachment in aquifer sediments within the pH range of the aquifer results primarily from alteration in the surface charge of the iron-coated quartz grains. However, the increased attachment under more alkaline conditions is more likely due to a combination of effects, including the increased ionic strength in the amendment relative to unaltered, uncontaminated groundwater and increased hydrophobicity of the bacterial and collector surfaces caused by sorption of humic acid. It should be noted that although the DOC in uncontaminated groundwater in the Cape Cod consists largely of what is defined operationally as humic substances (Barber, 1992), the total amount of DOC is only 0.4 mg/L (Scholl and Harvey, 1992). Consequently, the effect of humic substances on bacterial transport in the Cape Cod aquifer is likely to be modest.

#### 5.4. Alterations in bacterial attachment caused by calcium and sulfate

Addition of sulfate to uncontaminated groundwater caused the largest decline in bacterial attachment over the pH range investigated for any of the amendments (Fig. 5). However, sulfate had little effect upon bacterial attachment at pH-neutral to slightly alkaline conditions. Little is known about the effect of sulfate upon bacterial attachment in model or natural granular media, although it is known that sulfate binds strongly to iron oxides. It was reported that adsorption of sulfate onto a mixture of 5 mM Fe(OH)<sub>3</sub> and 17 mM SiO<sub>2</sub> was ~100% at pH 5, but near zero at pH 7 (Meng and Letterman, 1996). In the absence of iron, sulfate did not sorb to SiO<sub>2</sub> at

a pH above 4. Consequently, sulfate would not be expected to sorb to uncoated quartz grain surfaces over the pH range tested in our static column tests. However, it is likely that under acidic conditions of the aquifer sulfate would strongly compete with bacteria for binding sites on the iron oxyhydroxides that characterize the surfaces of the quartz grains within the Cape Cod aquifer sediments (Ryan et al., 1999). Stollenwerk (1995) showed experimentally that sulfate readily sorbs to Cape Cod aquifer sediments at a pH values of 6 or less. The pH-dependency pattern for bacterial attachment in sulfate-amended uncontaminated groundwater was remarkably similar to that exhibited in the system where the suspending medium was plume groundwater in which the DOC had been removed by UV oxidation (Fig. 2). Because the contaminated groundwater contains a concentration of sulfate similar to that of the sulfate-amended groundwater, it is likely that sulfate substantively enhances bacterial transport within the contaminant plume.

It is well known that dissolved calcium can result in increased microbial attachment in granular media. However, the effect of calcium amendment upon bacterial attachment (Fig. 6) was less than expected. Calcium enhanced bacterial attachment under alkaline additions, but resulted in only modest decreases in bacterial attachment at pH values below 5.8. The role of Ca<sup>+2</sup> in bacterial transport within the contaminant plume may also involve the enhanced partitioning of DOC onto the solid phase, thereby facilitating changes in the character of the grain surfaces. For example, recent studies have shown that calcium also alters the effect of humic acid on colloidal attachment in granular media (Dai and Hozalski, 2002; Davis et al., 2002) and it has been shown that sorption of fulvic acids onto FeOOH can be increased by calcium amendment (Teermann and Jekel, 1999). Calcium is also known to render bacterial attachment to surfaces less reversible (Hassan and Frank, 2003). In our study, the modest effect of calcium upon bacterial attachment under acidic conditions may, in part, result from the very low concentration of humic substances in the uncontaminated groundwater used in the calcium amendment experiment.

## 6. Summary and conclusions

The plethora of column studies has been useful in providing a better understanding of many factors governing bacterial attachment behavior in granular media (Harvey et al., 2007). However, because organic contaminant plumes are characterized by complex mixtures of DOC, altered inorganic chemistries, and steep geochemical gradients, additional information is needed in order to transfer the results of flow-through column studies typically run at circumneutral pH to microbial transport in the field. This is because bacterial attachment to aquifer sediments can be highly pH dependent and because many shallow, drinking water aquifers are acidic due to the presence of humic and fulvic acids and acidic recharge. For the constituents tested in our study, their individual and collective effects upon bacterial transport were generally quite different under the acidic conditions of the aquifer relative to the circumneutral pH conditions at which most column tests are run. Although wastewater

contaminant plumes may contain a number of organic compounds that can affect bacterial attachment, our study suggests that the small hydrophobic neutral fraction of the contaminant DOC is particularly important in governing bacterial transport within the contaminant plume at the Cape Cod site. Also, it is clear that elevated concentrations of inorganic divalent ions, such as sulfate and calcium, cannot be overlooked. Although not considered in our study, it is worth mentioning that phosphate sorbs to the aquifer sediments even more extensively than sulfate (Stollenwerk, 1995) and the large quantity of sediment-bound phosphorus in the upgradient portion of the contaminant plume (Stollenwerk, 1996) predictably occupies ~10–20% of the adsorption sites (Kent et al., 2007). Consequently, the role of phosphate in promoting bacterial transport within the plume should not be overlooked.

In the absence of the DOC and elevated sulfate, the five-fold increase in ionic strength between the plume and uncontaminated groundwater (Scholl and Harvey, 1992) would predictably decrease bacterial transport by at least several fold, judging from results of other bacterial attachment studies conducted at different ionic strengths, e.g., (Gross and Logan, 1995). The current study indicates that the presence of anionic surfactants at environmentally significant concentrations (Field et al., 1992a) would greatly facilitate bacterial transport in an aquifer such as the one we studied. More research is needed on the role of anionic surfactants in the transport of bacteria through aquifers, because of their use in surfactant-enhanced remediation (Sabatini et al., 1995) and their prevalence in subsurface domestic-wastewater plumes (Swartz et al., 2006). The model nonionic surfactant tested was remarkably effective at altering bacterial attachment behavior at minute concentrations under alkaline conditions, but had little effect at the pH of the contaminant plume, suggesting the effects of anionic and nonionic surfactants upon bacterial transport may involve very different mechanisms. Although DOC has been shown to increase microbial transport through granular media at high concentrations (Dai and Hozalski, 2002), results from our study indicate that the very low levels of humic substances within the contaminant plume (Barber, 1992) would be much less effective in promoting transport than the hydrophobic neutral fraction of the plume DOC.

In conclusion, the elevated concentrations of organic and inorganic compounds within plumes of contaminated groundwater can have a substantial effect on transport of bacteria therein. The altered chemistry within the dilute ( $5 \times 10^{-3}$  M ionic strength; 3.9 mg/L DOC), 6 km-long plume of treated sewage effluent may control transport of bacteria because of: (1) the increase in pH, (2) the hydrophobic neutral DOC, particularly the LAS surfactants, and (3) elevated concentrations of sulfate. In general, the chemistry within the plume had the effect of making bacterial attachment onto grain surfaces much less pH dependent.

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