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# Facilitation of bacterial transport through porous media by changes in solution and surface properties

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#### Abstract

Facilitation of bacterial transport for the purpose of bioaugmentation of contaminant degradation may be achieved by a number of methods typically involving changes in the properties of the groundwater or changes in the characteristics of the bacterial surface. Facilitated bacterial transport was investigated in laboratory experiments monitoring bacterial retention in quartz sand and glass bead mini-columns. Bacterial attachment efficiencies were estimated from bacterial retention using a steady-state filtration equation.

Decreased ionic strength resulted in decreased retention of *A. paradoxus*, with roughly order-of-magnitude decreases in retention accompanying order-of-magnitude decreases in ionic strength. Seven test chemicals were also examined in terms of their ability to modify bacterial surface properties and enhance bacterial transport. Of the seven test chemicals, the surfactants resulted in the most dramatic decreases in bacterial retention. The attachment efficiency of *A. paradoxus* on glass beads was lowered from 0.38 in the absence of Tween-20 to 0.0016 in the presence of 0.1 vol.% Tween-20, and from 0.064 in the absence of sodium dodecyl sulfate (SDS) to 0.0067 in the presence of 10 mg l<sup>-1</sup> SDS. Cell-surface modifying chemicals such as proteinase-k. EDTA, and pyrophosphate reduced bacterial attachment efficiencies. However, the reductions were less than an order of magnitude, even at the highest concentrations used. Bacterial attachment efficiencies were reduced from 0.055 in the absence of proteinase-k to 0.044 in the presence of 0.1 mg l<sup>-1</sup> proteinase-k, 0.61 in the absence of EDTA to 0.34 in the presence of 0.001 M EDTA, and 0.27 in the absence of pyrophosphate to 0.11 in the presence of 0.01 M pyrophosphate. Increased bacterial attachment efficiencies were observed for *A. paradoxus* on glass beads in the presence of 0.1 mg l<sup>-1</sup> lysozyme (0.74) vs. the absence of lysozyme (0.0048), and in the presence of 0.01 M periodate (0.10) vs. the absence of periodate (0.052).

Changes in porous media surface characteristics were also examined. Retention of Savannah River strain A1264 on quartz sand media was observed to be less than half that on iron oxide coated quartz sand media. Coating of iron oxide-quartz by sorbed humic acids resulted in a 44% decrease in bacterial retention, a value slightly greater than that on quartz porous media. In addition to the presence of sediment organic matter, the presence of dissolved organic matter (DOM) altered bacterial retention on the porous media. DOM decreased bacterial retention on quartz ( $\approx 20\%$ ), and increased bacterial retention on iron oxide-quartz ( $\approx 10\%$ ).

Keywords: Bacterial transport; Dissolved organic material; Porous media: Solution properties; Surface properties

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## 1. Introduction

Injection of contaminant-degrading microbes into the subsurface, a practice called bioaugmentation, is a promising low-cost method of aquifer remediation. However, bioaugmentation has achieved only limited success due to the tendency of bacteria to stick to aquifer sediments and to clog injection wells [1]. Hence, the ability to facilitate the transport of bacteria to a locus of aquifer contamination has been a subject of recent interest. This paper reviews several investigations performed in our laboratory which examined the effect of changing solution properties and bacterial surface properties on enhancing bacterial transport in porous media. An innovative experimental approach was used in our investigations. Bacterial retention on the porous media was monitored rather than bacterial concentration in the column effluent. The advantage of determining retention rather than breakthrough was that bacterial attachment efficiencies could be determined with greater resolution. Miniature (3 cc) porous media columns were used to determine retention, thereby allowing rapid determination of bacterial attachment efficiencies in a variety of solution and porous media conditions.

Bacterial attachment efficiencies can be determined empirically by comparison of experimental retention data to the filtration equation [2]:

$$\alpha = \frac{2}{3} \frac{d_{\rm c}}{\eta (1 - \theta) L} \ln \frac{C}{C_0} \tag{1}$$

where C is the effluent bacterial concentration,  $C_0$  the influent bacterial concentration. L the length of the porous media bed,  $d_c$  the collector (porous media grain) diameter.  $\theta$  is the porosity,  $\alpha$  the attachment efficiency, and  $\eta$  the collector efficiency, as calculated according to hydrodynamic models [3.4].

For non-sticky bacteria, the breakthrough concentration  $(C \cdot C_0)$  may approach unity in porous media laboratory columns of typical size. As  $C/C_0$  approaches unity, small errors in the measurement of C, and hence  $C/C_0$ , may produce large errors in  $\alpha$  [5], since the relationship between  $\alpha$  and  $C/C_0$  becomes asymptotic in the region where  $C/C_0$ 

approaches unity (Fig. 1). To avoid this difficulty, we developed a method to measure bacterial retention, rather than effluent concentration, on the porous media [6]. At steady state, fractional retention  $(F_r)$  equals  $1 - C_r C_0$ , and the resulting equation relating to  $F_r$  is:

$$\alpha = \frac{2}{3} \frac{d_{c}}{\eta (1 - \theta) L} \ln (1 - F_{r})$$
 (2)

The fact that a portion of the line in Fig. 1 rises above unity indicates that, given the calculated collision efficiency, an attachment efficiency greater than unity would be required to produce  $C/C_0$  less than about 0.75. Of course an attachment efficiency greater than unity is physically impossible, since a colloid cannot stick to a collector more frequently than it strikes a collector. However, attachment efficiencies greater than unity have been estimated from experimental results ( $\alpha = 4$ ; this paper), and indicate possible underestimation of collision efficiencies.

## 2. Objectives

The purpose of this paper is to review research investigating methods to reduce the attachment efficiency of bacteria during transport through aquifer sediments. The methods investigated included decreasing the solution ionic strength and introduction of a number of chemicals into solution to modify the bacterial surface and/or the porous media grain surface. First, we discuss the effects of

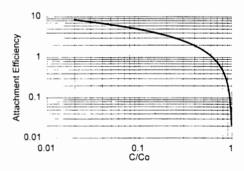


Fig. 1. Attachment efficiency vs. relative effluent concentration  $(C C_0)$  for the following conditions: 500  $\mu$ m sediment, porosity = 0.4, approach velocity = 1 m per day, collision efficiency = 0.026, which corresponds to bacterial diameter of 1.2  $\mu$ m.



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ine in Fig. 1 rises in the calculated efficiency greater produce  $C/C_0$  less chment efficiency appossible, since a remore frequently ever, attachment the been estimated this paper), and on of collision

review research the attachment ansport through ds investigated onic strength and cals into solution d or the porous uss the effects of



fluent concentration sediment, porosity = on efficiency = 0.026,  $1.2 \mu m$ .

changing ionic strength on bacterial retention. Second, we discuss the effects chemical modification of the bacterial and or grain surfaces using surfactants and a number of other cell-surface modifiers which have been shown to affect bacterial attachment efficiency. Third, we examine the effect of humic substance sorption to grain surfaces, and to bacterial surfaces, on the bacterial attachment efficiency. Finally, we compare the overall effectiveness of these treatments in relation to steadystate bacterial transport, and what can be expected in terms of additional transport distances gained through these treatments. In this review, we consider only the transportability of bacteria which have been enriched in a nutrient medium. Enriched bacteria have a larger size, a more hydrophobic surface, and hence a much stronger tendency for attachment, than do microbes which have been starved prior to introduction to sediment [7]. Although starved bacteria are more easily transported, nutrient-enriched bacteria may have greater ability to degrade contaminants if they have been acclimated to these contaminants prior to introduction to the subsurface [8].

## 3. Methods

All experiments described herein utilized the MARK (microbe and radiolabel kinesis) method [6] to examine bacterial retention in miniature porous media columns (3 cc plastic LeurLok syringe. 0.8 cm i.d.). The investigations described below were performed for a variety of bacteria, porous media, and solution conditions. The protocol of the mini-column experiments has been described elsewhere [6,9] but is briefly reviewed here. Prior to introduction of bacterial suspensions, column media were pre-rinsed with either 2 ml of the MOPS buffer, or in experiments which included chemical addition, various buffers containing different test chemicals [9–11]. 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 (2 ml), each column was again rinsed with 4 ml of the appropriate buffer solution. Total adsorbed radiolabel on the porous media was measured by scintillation counting. Sorption of unassimilated label was monitored in parallel experiments by addition of a cell-free suspension prepared by 0.2  $\mu$ m filtering of the labeled bacterial suspension. The extent of adsorption of unassimilated radiolabel to the column was subtracted from the total adsorbed radiolabel to result in total adsorbed assimilated radiolabel. Total assimilated radiolabel was determined by passing 2 ml of the dilute, labeled suspension through 0.2  $\mu$ m polycarbonate filters (Poretics) with correction for sorption of unassimilated radiolabel. The fraction retained,  $F_r$ , was determined by the ratio of total adsorbed assimilated radiolabel to total assimilated radiolabel according to the following equation:

$$F_{\rm r} = \frac{\rm ads_{\rm t} - ads_{\rm u}}{\rm filt_{\rm t} - filt_{\rm u}} \tag{3}$$

where ads, is the disintegrations per minute (DPM) measured from the porous media (1 cm) after introduction of buffer and bacterial suspension; ads<sub>u</sub> is the DPM measured from the porous media after introduction of buffer and bacteria-free suspension: filt, is the DPM measured from the filter after introduction of buffer and bacterial suspension: and filt, is the DPM measured from the filter after introduction of buffer and bacteria-free suspension. Cell concentrations in labeled suspensions were determined using standard acridine orange direct count (AODC) procedures [12]. Bacteria used our experiments included *Pseudomonas fluorescens* P17. Alcaligenes paradoxus, and Savannah River strain A1264. The properties of the bacteria used in these studies are described in the cited investigations.

# 4. Results and discussion

# 4.1. Effect of ionic strength on bacterial retention

A Decrease in ionic strength lowered bacterial retention of A. paradoxus on borosilicate glass beads (40  $\mu$ m) [9]. Experiments performed for bacterial suspensions with three different buffers are shown in Fig. 2. The effect of ionic strength on bacterial retention in the porous media occurred irrespective of the type of buffer. Order of magni-

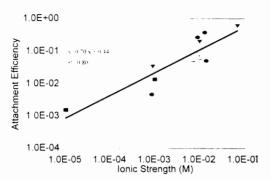


Fig. 2. Bacterial attachment efficiencies on borosilicate beads (40 µm) measured under a variety of ionic strengths in NaCl (■), and in three different solution buffers; phosphate (●), MOPS (▼), and TRIS (▲), as described in Ref. [9].

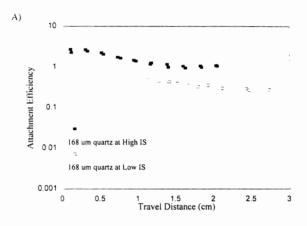
tude decreases in ionic strength produced order of magnitude decreases in attachment efficiency. At the highest ionic strength (0.07 M),  $F_r$  was equal to  $0.97 \pm 0.14$ , and the attachment efficiency approached unity. At the lowest ionic strength  $(10^{-5} \text{ M})$ ,  $F_r$  was  $0.009 \pm 0.001$  with the attachment efficiency equal to 0.0016. The surface charge of A. paradoxus under the conditions of these experiments was negative [9], as was the case for the glass beads (pH<sub>zpc</sub>  $\approx 2$ ). Our observations are consistent with other investigations in which bacterial retention [13-15] and colloid retention [16.17] increased with ionic strength under unfavorable conditions. Particle retention under unfavorable conditions is governed by the net result of electrostatic repulsion and van der Waals attraction [18]. The separation distance between the bacteria and the porous media surface at which van der Waals attraction dominates electrostatic repulsion is highly sensitive to the ionic strength of the solution. High ionic strengths compress the electrostatic repulsive layer (electrical double layer), and thereby increase particle retention [19].

Bacterial retention across the length of minicolumns was found to decrease with distance from the influent column end. This has been attributed to heterogeneity in the bacterial surfaces, even among a monoclonal population [20]. The effect of ionic strength on retention of bacterial strain *Pseudomonas fluorescens* P17 on quartz grains (168  $\mu$ m. range = 60-700  $\mu$ m) and borosilicate beads (40  $\mu$ m), was obvious [11] even in the

presence of heterogenous bacterial attachment efficiencies (Figs. 3a and b). The attachment efficiencies for the low ionic strength (0.004 M) are consistently below those at the higher ionic strength (0.6 M), even though both sets of data show changes in the attachment efficiency with increasing distance traveled.

# 4.2. Effect of surface modifying chemicals on bacterial retention

Chemical additions to the bacterial suspension can be used to modify either the bacterial or sediment grain surfaces, or both. A variety of



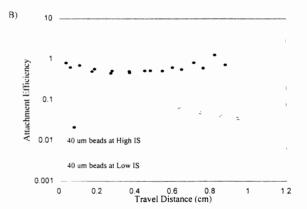


Fig. 3. Bacterial attachment efficiency on two porous media: A) borosilicate beads (40 µm) and B) quartz (168 µm); for two ionic strengths: 0.6 M (open symbols), and 0.004 M (closed symbols).

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o porous media: 168 μm); for two 0.004 M (closed chemical additives have been shown to modify bacterial retention on rotating disks [14], flow cells, and flat surfaces [21]. However, such surfaces may not be representative of the transport conditions in an aquifer. The use of experimental minicolumns provides an efficient means of determining bacterial attachment efficiencies in a system approximating aquifer conditions. Seven test chemicals previously shown in the literature to affect bacterial retention (two surfactants, Tween-20 and Sodium Dodecyl Sulfate (SDS): EDTA, sodium periodate, sodium pyrophosphate, proteinase-K, and lysozyme) were used in porous media minicolumns [9].

Table 1 shows results for chemical additions to bacterial suspensions applied to 40 µm borosilicate glass beads. Of the chemical treatments used, surfactant addition resulted in the largest decreases in bacterial retention. Tween-20 reduced the bacterial attachment efficiency by two orders of magnitude from 0.38 in the absence of Tween-20, to 0.0016 in the presence of 0.1 vol.% Tween-20. SDS lowered the bacterial attachment efficiency by one order of magnitude from 0.064 in the absence of SDS to 0.0067 in the presence of 10.0 mg l<sup>-1</sup> SDS. Among the other treatments used, proteinase-k, EDTA, and pyrophosphate reduced the attachment efficiency by less than an order of magnitude. Three of the treatments increased bacterial retention; lysozyme addition increased attachment efficiencies by two orders of magnitude, sodium periodate doubled the attachment efficiency, whereas EDTA addition resulted in a slight increase or decrease in attachment efficiency depending on the buffer

# 4.3. Effect of aqueous and sediment natural organic matter on bacterial retention

Bacterial retention was affected by both the presence of sediment-phase organic matter and the presence of dissolved organic matter [10]. Bacterial retention (strain A1264) on quartz was 38% of that on iron oxide coated quartz (Fe-quartz) (Fig. 4). This was consistent with the expectation that low-charged iron oxide (pH<sub>zpc</sub>  $\approx$  8) distributed on the quartz surface, would retain more bacteria than would negatively

charged quartz (pH<sub>zpc</sub>  $\approx 2$ ), under the conditions of these experiments (pH 8). The effect of natural organic matter (NOM) coatings on sediment was also examined. Retention of strain A1264 on iron oxide coated quartz pre-equilibrated with NOM (SOM-Fe-quartz) was 56% that on Fe-quartz. This was true for two sources of humic acid: Suwannee River Humic Acid (SRHA), and Soil Humic Acid (SHA). This result was consistent with the expected negative surface charge increase due to sorption of NOM on the sediment surface. Although steric interactions may play a role in reducing bacterial attachment efficiency, the main effect of NOM sorption on the surface characteristics of goethite has been shown to be alteration of surface charge [22]. Surface charge heterogeneity resulting from the presence of iron oxide on the quartz surface would be expected to increase bacterial deposition relative to that on quartz alone [23.24]. Sorption of NOM to Fe-quartz grain surfaces would be expected to mask underlying surface charge heterogeneity and reduce bacterial deposition [23].

Changes in bacterial retention due to the presence of aqueous-phase NOM are not shown in Fig. 4 because day-to-day bacterial culture differences caused changes in bacterial retention that exceeded the changes in bacterial retention due to aqueous NOM. Comparison of same-day results. shown as ratios of retention in the presence vs. absence of dissolved NOM, indicates a slight facilitation of bacteria by dissolved NOM during transport through quartz (Fig. 5). This decrease (20%) in bacterial retention on quartz in the presence of NOM indicated an increase in bacteria-quartz repulsion in the presence of dissolved organic matter. Because the dissolved humic substances have been shown to not sorb to quartz [25]. alteration of the bacterial surface by NOM was considered to be responsible for the decreased bacterial retention on quartz in the presence of dissolved NOM. Increased bacteria-quartz repulsion indicated an increased negative bacterial surface charge due sorption of NOM to the bacterial surface. This hypothesis was strengthened by the fact that dissolved NOM caused a 20% increase bacterial retention on Fe-quartz and SOM-

Survival

(%)

100

100

100

100

100

0

100

29

8

100

100

100

100

100

100

100

100

100

100

100

100

100

100

100

100

100

100

100

100

100

100

100

100

 $F_{R}(\pm SD)$ 

 $0.89 \pm 0.05$ 

 $0.011 \pm 0.002$ 

 $0.0090 \pm 0.0017$ 

 $0.31 \pm 0.01$ 

 $0.037 \pm 0.008$ 

 $0.26 \pm 0.02$ 

 $0.32 \pm 0.05$ 

 $0.43 \pm 0.07$ 

 $0.97 \pm 0.14$ 

 $0.93 \pm 0.09$ 

 $0.92 \pm 0.12$ 

 $0.85 \pm 0.11$ 

 $0.38 \pm 0.04$ 

 $0.41 \pm 0.04$ 

 $0.59 \pm 0.04$ 

 $0.79 \pm 0.04$ 

 $0.79 \pm 0.09$ 

 $0.72 \pm 0.10$ 

 $0.47 \pm 0.02$ 

 $0.62 \pm 0.12$ 

 $0.68 \pm 0.06$ 

 $0.53 \pm 0.15$ 

 $0.39 \pm 0.05$ 

 $0.18 \pm 0.02$ 

 $0.13 \pm 0.05$ 

 $0.13 \pm 0.01$ 

 $0.28 \pm 0.01$ 

 $0.27 \pm 0.04$ 

0.22 + 0.02

 $0.027 \pm 0.003$ 

 $0.98 \pm 0.14$ 

 $0.97 \pm 0.10$ 

sediment organic matter altering the sediment sur-

face. Coating quartz with iron oxide more than doubled bacterial retention. Coating Fe-quartz

with organic matter resulted in a decrease in

bacterial retention, or attachment efficiency, by about 50%. The effect of dissolved organic matter

was to decrease retention on quartz by about 20%.

and to increase retention on Fe-quartz and

SOM-Fe-quartz by about 10%.

nm

Table 1

Туре

SDSb

NaIO<sub>4</sub>a

**EDTA**<sup>c</sup>

EDTA<sup>b</sup>

Na<sub>4</sub>P<sub>2</sub>O-<sup>a</sup>

Na<sub>4</sub>P<sub>2</sub>O-c

Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>c

Proteinase<sup>b</sup>

Lysozyme<sup>a</sup>

nm = not measured. a Buffer used = phosphate. b Buffer used = TRIS. <sup>c</sup> Buffer used = MOPS.

Tween-20a

Chemical treatment

Effect of chemical treatments on bacterial retention in mini-columns

n

0.01%

 $10 \text{ mg } 1^{-1}$ 

100 mg l<sup>-1</sup>

 $10^{-2} M$ 

10<sup>-4</sup> M

 $10^{-3} M$ 

10<sup>-4</sup> M

 $10^{-3} M$ 

 $10^{-3} M$ 

 $10^{-2} M$ 

10<sup>-4</sup> M

 $10^{-3} M$ 

 $10^{-2} M$ 

10<sup>-4</sup> M

 $10^{-3} M$ 

 $0.1 \text{ mg ml}^{-1}$ 

 $0.1 \text{ mg ml}^{-1}$ 

 $5 \times 10^{-4} \text{ M}$ 

 $5 \times 10^{-4} \text{ M}$ 

0

0

0

0 10<sup>-4</sup> M

0

0 10<sup>-5</sup> M

0

Fe-quartz. This suggested that an increase in bac-

terial attraction for the positively charged iron

oxide was associated with the presence of dissolved organic matter. Increased bacterial attraction for

the sediment surface was consistent with increased

negative bacterial surface charge due to sorption

The effect of dissolved organic matter altering

the bacterial surface was secondary to the effect of

of NOM to the bacterial surface.

0.1%

Concentration

Fraction of bacteria retained (F.) on porous media

γ

0.38

0.019

0.0016

0.064

0.0067

0.052

0.067

0.10

0.61

0.47

0.46

0.34

0.084

0.092

0.17

0.27

0.27

0.22

0.11

0.20

0.20

0.13

0.086

0.034

0.025

0.025

0.057

0.055

0.044

0.0046

0.74

0.61

nm

r



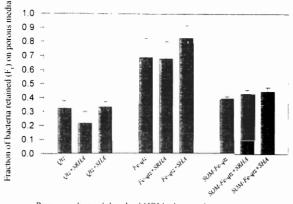
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0.38

0.019 0.0016 0.064 0.0067 nm 0.052 0.067 0.10 0.61 0.47 0.46 0.340.084 0.092 0.17 0.27 0.27 0.22 0.11 0.20 0.20 0.13 0.086 0.034 0.025 0.025 0.057 0.055 0.0440.0046 0.74

0.61

sediment surde more than ng Fe-quartz decrease in efficiency, by rganic matter by about 20%, -quartz and



Porous media (and dissolved NOM when used in experiment)

Fig. 4. Bacterial retention  $(F_r)$  measured on three porous media for three different conditions: 1) in the absence of dissolved NOM; 2) in the presence of dissolved SRHA (+SRHA); and 3) in the presence of dissolved SHA (+SHA).

# 4.4. Attachment efficiency and travel distance

Of the treatments discussed above, reducing solution ionic strength and addition of surfactants were most effective in facilitating bacterial transport. Transport distances resulting in a 100-fold reduction in cell concentration (Eq. 1) are shown in Fig. 6 for a typical bacteria-sediment system (porosity = 0.4, sediment grain diameter of 500  $\mu$ m, 1 m per day flow velocity, and collision efficiency = 0.026) for a continuum of attachment efficiencies. Considering a relatively low bacterial attachment efficiency of 0.07, only a 1.4 m distance would be traveled before a 100-fold reduction in cell concentration resulted. An order of magnitude decrease in the attachment efficiency to 0.007 results in a 14 m transport distance, an increase of 12.6 m. The transport distances for a 1000-fold reduction (99.9%) in cell concentration under the above conditions for attachment efficiencies of 0.07 and 0.007, would be 2.1 m and 21 m, respectively. Bacteria with modest attachment efficiencies of 0.05 therefore require a decrease in attachment efficiency of at least one order of magnitude in order to achieve transport distances desired for bioaugmentation purposes. Assuming a necessary reduction in attachment efficiency of one order of magnitude or greater, of the treatments described above, only reduction of solution ionic strength

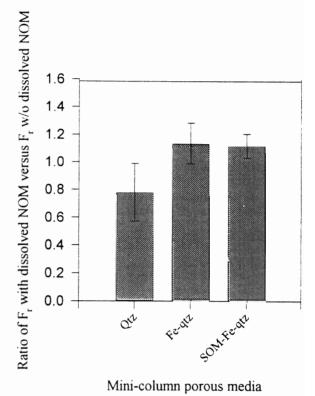


Fig. 5. Bacterial retention in the presence and absence of dissolved NOM, expressed as a ratio of the  $F_{\tau}$  measured in the presence absence, for three porous media, for same-day experiments (same bacterial culture). Values less than unity indicate decreased retention in the presence of dissolved NOM. Values greater than unity indicate increased retention in the presence of dissolved NOM.

and addition of surfactants appear to be useful for facilitating bacterial transport for the purposes of bioaugmentation. Ionic strength reductions in natural groundwater would increase bacteriasediment repulsion since bacteria and sediment surfaces are negatively charged at the pH of most groundwaters [26–29]. However, since bacterial surfaces are characterized by differing degrees of hydrophobicity and surface charge [30,31], the effects of the different treatments investigated, including ionic strength changes, may vary significantly for different bacterial strains.

The possible effects of surfactant addition to the subsurface on the contaminants themselves are worthy of discussion. Hydrophobic organic compounds may be strongly solubilized by surfactants.

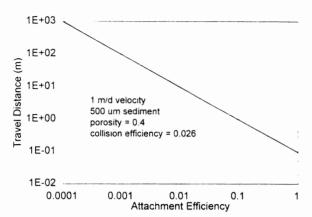


Fig. 6. Travel distance vs. attachment efficiency for hypothetical aquifer conditions:  $500 \, \mu m$  sediment, porosity = 0.4, approach velocity = 1 m per day, collision efficiency = 0.026, corresponding to a bacterial diameter of 1.2  $\mu m$ .

particularly if surfactant concentrations exceed the critical micelle level [32.33]. Solubilization of organic contaminants may result in contaminant mobilization [34–36] and spreading of contamination downgradient in an aquifer. Since contaminant concentrations would be lowered by the spreading process, the effect of spreading on contaminant biodegradation would depend on the resulting contaminant bioavailability and toxicity. In the case of some NAPLs, degradation is limited by available NAPL surface area as well as by NAPL dissolution rate [37,38], in which case solubilization mobilization might increase NAPL bioavailability by spreading the NAPL and increasing NAPL surface area. However, the effects of the solubilizing phase itself on contaminant bioavailability differs among different surfactants and contaminants. Emulsion treatment of crude oil has been shown to decrease degradation of aliphatic components and increase degradation of aromatic components [39]. Enhanced degradation of phenanthrene was observed in micellar solutions of alcohol ethoxylate [40], and degradation of ndecane and n-hexadecane was enhanced in aqueous solutions of linear alcohol ethoxylates [41]. Choice of a surfactant for use in facilitating bacterial transport must therefore be made with consideration of possible effects on contaminant bioavailability to avoid confounding of bioremediation

#### 5. Conclusions

A variety of means for facilitating bacterial transport in the subsurface were examined, including decreases in solution ionic strength, surfactant addition, addition of various cell-surface modifiers, and addition of natural organic matter. The greatest reductions (order of magnitude) in bacterial attachment efficiencies were achieved by decreasing solution ionic strength and by surfactant addition. The other treatments were observed to result in much lesser reductions in attachment efficiency. Because bacteria have complex surfaces characterized by different degrees of hydrophobicity and differing surface charges, the magnitude of the observed effects will vary between strains, and so our results should not be generalized to all bacteria. However, based on our results to date, ionic strength reduction and surfactant addition are most promising in terms of facilitating bacterial transport.

The MARK method allows efficient determination of bacterial attachment efficiencies in a system which approximates aquifer conditions. Because many experiments and replicates can be run under equivalent column conditions, determination of the effects of different solution conditions on bacterial transport can be performed efficiently. Since attachment efficiencies are determined from experimental conditions approximating subsurface transport, the results may be used to estimate expected travel distances for bacteria in aquifer sediments based on laboratory experiments performed under similar sediment and solution conditions..

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