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# Influence of Different Chemical Treatments on Transport of *Alcaligenes paradoxus* in Porous Media

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**Seven chemicals, three buffers, and a salt solution known to affect bacterial attachment were tested to quantify their abilities to enhance the penetration of *Alcaligenes paradoxus* in porous media. Chemical treatments included Tween 20 (a nonionic surfactant that affects hydrophobic interactions), sodium dodecyl sulfate (an anionic surfactant), EDTA (a cell membrane permeabilizer that removes outer membrane lipopolysaccharides), sodium $PP_i$ (a surface charge modifier), sodium periodate (an oxidizer that cleaves surface polysaccharides), lysozyme (an enzyme that cleaves cell wall components), and proteinase K (a nonspecific protease that cleaves peptide bonds). Buffers included MOPS [3-(*N*-morpholino)propanesulfonic acid], Tris, phosphate, and an unbuffered solution containing only NaCl. Transport characteristics in the porous media were compared by using a sticking coefficient, α, defined as the rate at which particles stick to a grain of medium divided by the rate at which they strike the grain. Tween 20 reduced α by 2.5 orders of magnitude, to α = 0.0016, and was the most effective chemical treatment for decreasing bacterial attachment to glass beads in buffered solutions. Similar reductions in α were achieved in unbuffered solutions by reducing the solution ionic strength to 0.01 mM. EDTA, protease, and other treatments designed to alter cell structures did not reduce α by more than an order of magnitude. The number of bacteria retained by the porous media was decreased by treatments that made *A. paradoxus* more hydrophobic and less electrostatically charged, although α was poorly correlated with electrophoretic mobility and hydrophobicity index measurements at lower α values. Filtration model calculations indicate that α values of <0.01 are necessary to achieve bacterial transport over distances large enough to facilitate bioremediation of contaminated groundwater aquifers. Chemical treatments that reduce α from 0.61 (1 mM MOPS buffer; ionic strength, 70 mM) to 0.0016 would increase bacterial penetration from 0.16 to 60 m, with only a 2-log reduction in cell concentration under typical groundwater and soil conditions (assuming a water velocity of 1 m $day^{-1}$ and an average soil grain diameter of 500 μm).**

Research on bacterial attachment has been directed towards either promoting attachment, as in preventing pathogen mobility in potable aquifers (1, 4, 16, 20), or preventing attachment to a diverse array of surfaces such as teeth, prosthetic implants, and ship hulls (21). The potential for using bacteria in subsurface bioremediation treatment processes has stimulated research on reducing bacterial attachment to soil particles. Current bioremediation strategies frequently involve stimulation of indigenous microbial populations through in situ nutrient addition or injection of microbial species that have been selected (or engineered) to degrade specific chemical pollutants in the subsurface, thereby infusing specific traits into the subsurface microbial community (6, 11, 20). The success of bacterial injection, however, depends on the ability of the bacteria to be transported to the subsurface pollutants (2). Both bacterial injection and nutrient addition can increase bacterial numbers at the well head and cause soil plugging (24, 25).

Bacteria are removed from water flowing through porous media by collisions and adhesion to soil particle surfaces. Collisions are generated by Brownian diffusion, London-van der Waals attractive forces, interception, and gravitational settling (26, 36). Bacterial adsorption and attachment to a surface occur in two steps. The first step, reversible adsorption, occurs when a bacterium overcomes secondary repulsive forces between itself and a surface and adsorbs to the surface (21). Reversibly attached bacteria can still exhibit Brownian motion, and their removal can be promoted by increasing fluid shear or by dramatically changing the chemistry of the bulk solution (3). Irreversible attachment is a time-dependent process and occurs when the bacterium synthesizes extracellular adhesive materials (8, 21). Since adding chemicals such as chlorine to promote bacterial detachment after irreversible attachment can damage cell integrity (3), increased bacterial transport is best achieved by methods that promote reversible detachment, limiting the potential for irreversible attachment.

When desorption and dispersion are neglected, the concentration of bacteria in water injected into porous media ($C_o$) will decrease exponentially with distance, $L$, from the point of injection according to the following equation:

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

where θ is the column porosity, $d_c$ is the diameter of the porous medium particle, and η is the collector efficiency. The fraction of bacteria retained, $F_r$, after traveling a distance $L$ is $F_r = (1 - C/C_o)$. All chemical interactions are incorporated into α, the sticking coefficient (36). Under typical groundwater conditions, α must be <0.01 to permit bacterial transport over distances on the order of 100 m with less than a 2-log reduction in cell concentration. Since collisions between bacteria and soil particles are dominated by diffusion, all variables in equation 1 except α are defined by the physical system and cannot be sufficiently altered to achieve bacterial penetration over distances of 10 to 100 m. The only mechanisms for enhancing penetration involve modification of α through alteration of the chemistry of the solution, the collector, or the bacterial surface.

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Chemical treatments have previously been used to modify bacterial surfaces and decrease attachment. Except in a few instances (22, 28), the experimental conditions either have not been representative of flow through porous media (e.g., rotating disks [23], flow cells [3], and flat surfaces [12]) or have produced only qualitative results (19, 21, 34).

In order to quantify the effectiveness of chemical treatments that are known to promote bacterial penetration in porous media, we measured the sticking coefficients of bacteria as a function of salt concentration, buffer, and chemical treatment of the bacterial surface. Seven different chemical treatments were selected on the basis of their previously demonstrated abilities to prevent attachment to or promote detachment from surfaces. Ionic strengths and pHs of the test solutions used were chosen to represent common groundwater conditions (pH of approximately 7 and ionic strength of 0.1 to 10 mM). We used a novel minicolumn method of calculating sticking coefficients on the basis of retention, and not breakthrough, of bacteria in porous medium columns. The method permitted a more precise calculation of $\alpha$ for bacteria with low attachment probabilities (10, 14). Electrophoretic mobility (EM) and relative hydrophobicity were measured after each chemical treatment since both electrostatic and hydrophobic interactions govern interactions between bacteria and surfaces (31, 33).

## MATERIALS AND METHODS

**Organism and growth conditions.** *Alcaligenes paradoxus* was isolated from pesticide-contaminated soil at Hill Air Force Base, Utah. This bacterium can degrade several aromatic compounds of environmental concern, including (2,4-dichlorophenoxy)acetic acid (35). *A. paradoxus* is gram negative and rod shaped and was nonmotile under the experimental conditions in this study.

Cultures were prepared from inocula previously frozen (−4°C) in microcentrifuge tubes in mineral salts medium containing (per liter) $MgSO_4 \cdot 7H_2O$, 112 mg; $ZnSO_4 \cdot 7H_2O$, 5.00 mg; $NaMoO_4 \cdot 2H_2O$, 2.50 mg; $KH_2PO_4$, 1.50 μg; $Na_2HPO_4 \cdot 7H_2O$, 3.74 μg; $NH_4Cl$, 0.50 μg; $CaCl_2$, 14.0 mg; and $FeCl_3 \cdot 6H_2O$, 0.22 mg. The latter two components were added separately by sterile filtration after autoclaving. Cultures were grown to a cell density of $\sim 10^9$ ml$^{-1}$ in tryptic soy broth on a tube rotator. Three-milliliter volumes were centrifuged (6,000 × *g*) for 5 min, washed three times in mineral salts medium, diluted to $0.5 \times 10^7$ to $1.0 \times 10^7$ cells ml$^{-1}$, and grown to a final cell density of $2 \times 10^8$ ml$^{-1}$ on (2,4-dichlorophenoxy)acetic acid in mineral salts medium supplemented with yeast extract (100 mg liter$^{-1}$). Growth on (2,4-dichlorophenoxy)acetic acid (100 mg liter$^{-1}$ was carried out in 125-ml Erlenmeyer flasks (50-ml liquid volume) at 22 to 24°C with continuous shaking. Cell concentrations were estimated from light scattering with a spectrophotometer (Shimadzu UV-160A) set at 600 nm.

Cells were radiolabeled by transferring 2 to 3 ml of the cell suspension to a sterile test tube containing 10 to 30 μl of [$^3$H]leucine (79 Ci mmol$^{-1}$, 1 mCi ml$^{-1}$; ICN) and incubating the suspension on a tube rotator for 0.5 h. Labeled cells (~0.15 dpm cell$^{-1}$) were not washed to remove unassimilated label since centrifugation has been found to alter sticking coefficients (unpublished results).

**Bacterial retention test procedure.** Sticking coefficients were calculated by the microbe and radiolabel kinesis (MARK) method (10). In this method, $\alpha$ is calculated from the retention of radiolabeled bacteria in miniature columns (3-ml plastic LuerLok syringes, 0.8-cm inner diameter) packed with 40-μm-diameter borosilicate beads (Whatman) supported by a 0.8-cm-diameter GF/D filter (Whatman). Beads were cleaned by soaking (with agitation) in 10% $H_2SO_4$ for 3 h and rinsing with ultrapure water (Milli-Q; Millipore Corp.). A bead slurry (1.5 g [dry weight]) was transferred to each syringe barrel and stirred with a Pasteur pipette to remove trapped air, ensure homogeneity, and provide a level bead surface. Syringe columns were mated to an Alltech vacuum manifold equipped with LuerLok connections.

Six milliliters of the bacterium-free test solution (test solution with chemical treatment) was run through the reactor to acclimate glass bead surfaces. Vacuum pressure was manipulated to produce a fluid approach velocity of 0.09 cm s$^{-1}$. Radiolabeled cells (2 ml, $10^6$ ml$^{-1}$) were pipetted onto the top of the column and drawn through the column by a vacuum, and the column was rinsed with 4 ml of solution to wash out unattached bacteria and liquid-phase radiolabel.

Bacterial retention was calculated by direct measurement of label retained in the top 1 cm of the column rather than in the column effluent, since cell retention in the large-pore GF/D filter (18) supporting the porous medium altered total column bacterial retention. The minicolumn was removed from the manifold, its lower end was cut off, and a syringe plunger was used to extrude a damp, 1-cm-long cylindrical aggregate of beads from the column. This top centimeter was transferred to a scintillation vial containing 5 ml of cocktail (Budget-Solve; RPIC), and the remainder of the column was discarded. The vials were shaken for ≥24 h and analyzed on a Beckman LS 3801 scintillation counter with quench correction. All MARK column experiments were run in triplicate. The $F_r$ for the top 1 cm of the column was calculated from the total radiolabel retained in the column, which was corrected for adsorption of unassimilated label, as previously described (10). Standard deviations on $F_r$ were calculated by the method of Skoog et al. (29). Sticking coefficients were calculated by using equation 1, with column conditions of $L$ = 1 cm, $d_c$ = 40 μm, and $\theta$ = 0.40 and $\eta$ = 0.025, calculated by using the filtration equation of Rajagopalan and Tien (22).

TABLE 1. Buffer types, concentrations, and ionic strengths used

| Expt no.[a] | Buffer type | Buffer concn (mM) | Solution ionic strength (mM)[b] |
|---|---|---|---|
| 1 | Phosphate | 6.7 | 13 |
| 2 | Tris | 10 | 10 |
| 3 | Phosphate | 1 | 14 |
| 4 | MOPS | 1 | 70 |
| 5 | Tris | 10 | 9 |
| 6 | Phosphate | 4 | 9 |
| 7 | MOPS | 1 | 10 |
| 8 | MOPS | 0.1 | 0.9 |
| 9 | Tris | 10 | 7 |
| 10 | Phosphate | 0.4 | 0.85 |
| 11 | None | | 1 |
| 12 | None | | 0.01 |

[a] Experiment numbers correspond to those in Table 2.
[b] NaCl was added to produce the indicated ionic strengths except in experiment 12.

Cell concentrations were derived from total counts determined by standard acridine orange direct-count procedures on black 0.2-μm-pore-size polycarbonate filters (Poretics) backed during filtration with 5-μm-pore-size cellulose acetate filters (Millipore) (25-mm diameter). Projected areas of stained cells were measured with an epifluorescence microscope (Olympus BH-2) and an image analysis system (Cue-2; Olympus) at a magnification of ×1,000. The average cell diameter was calculated from projected areas as 1.04 μm (±0.08 μm; $n$ = 500).

**Test chemicals.** Seven chemicals in one of three buffers or in unbuffered NaCl, all prepared in ultrapure water (Milli-Q), were used to treat *A. paradoxus* suspensions and minicolumns. Chemicals were added to cell suspensions ($10^6$ ml$^{-1}$) and agitated on a shaker table for 45 min to allow the chemicals to react with the cell surfaces. A broad range of chemical treatments was selected to investigate the contributions of several chemical mechanisms that are potentially responsible for bacterial adsorbance to surfaces.

Buffer-chemical treatment combinations were chosen on the basis of previously demonstrated or predicted abilities to alter the attachment of bacteria to surfaces. The buffers used were phosphate ($KH_2PO_4$ and $K_2HPO_4$; Fisher), Tris-HCl, and MOPS [3-(*N*-morpholino)propanesulfonic acid, $C_7H_{15}NO_4S$, $pK_a$ = 7.2; Calbiochem]). Different buffers were necessary to achieve a range of ionic strengths while avoiding treatment-specific precipitation reactions involving buffer constituents. Therefore, a complete experimental matrix of each buffer and chemical treatment was not tested.

Because of the known influence of ionic strength on bacterial penetration in porous media (14, 15, 22), NaCl was tested both as a treatment chemical and as a method for achieving a constant ionic strength during chemical treatments. The pHs of NaCl test solutions were not adjusted since they remained in the range of 7.0 to 7.2. The pHs of other solutions were adjusted by adding HCl or NaOH. The ionic strength within each treatment series was held constant at the highest ionic strength produced by a chemical dose by varying the amount of NaCl used in each experiment. The ionic strength was determined by measuring solution conductivity with an Orion model 122 conductivity meter and by converting conductivity to ionic strength on the basis of calibration with NaCl solutions.

The two surfactants Tween 20 and sodium dodecyl sulfate (SDS) were chosen on the basis of experiments by others (13, 37) showing decreased attachment of bacteria to various surfaces. Tween 20 (polyoxyethylene 20 sorbitan mono-laurate; Calbiochem) was prepared (by volume) in a phosphate buffer solution containing 0.51 g of $KH_2PO_4$ per liter, 0.52 g of $K_2HPO_4$ per liter, and 0.147 g of $CaCl_2$ (pH 7.0) per liter. SDS (Bio-Rad) crystals were added to a 0.01 M Tris-HCl ($C_4H_{11}NO_3$, HCl, $pK_a$ = 7.2; Sigma) buffer. Other chemicals used in this study that were known to affect bacterial attachment were EDTA (7) (tetrasodium salt EDTA; Fisher), sodium periodate (12) (*meta*-$NaIO_4$; Fisher), sodium $PP_i$ (28) ($Na_4P_2O_7$; Fisher), proteinase K (Sigma), and lysozyme (5) (Sigma). The types and concentrations of buffers and the ionic strengths of solutions used in chemical treatment experiments are listed in Table 1.

**Cell viability.** In each experiment the viability of nonradiolabeled cells was tested by plate counts after exposure to test chemicals for 1 h. Dilutions in 0.1% peptone (Difco) were used for plate counts on R2A agar (Difco).

**Analyses of protein and dissolved organic carbon.** The effectiveness of treatment with protease or lysozyme was evaluated by monitoring concentrations of protein or dissolved organic carbon, respectively, in cell-free liquid samples prepared by filtration through polycarbonate filters (0.2-μm pore size; Poretics Corp.). Dissolved-protein concentrations in protease-treated samples were measured by using a colorimetric assay with the bicinchoninic acid protein assay reagent (Pierce). Lysozyme hydrolyzes the β(1,4) glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine in bacterial cell walls. The effectiveness of lysozyme in dissolving cell wall components was confirmed by measuring increases in dissolved organic carbon with a TOC-5000 carbon analyzer (Shimadzu) following enzyme treatment.

**EM and hydrophobicity measurements.** The EMs of *A. paradoxus* and glass beads were measured with a Rank Brothers Mark II EM machine equipped with a 1.9-mm-inner-diameter cylindrical glass electrophoresis cell and platinum-coated electrodes. Bacterial samples were grown as described above (without radiolabel) and suspended in chemical solutions for EM measurements. Acid-washed and rinsed glass beads were crushed in order to minimize their rate of sedimentation in the glass electrophoresis cell. Diameters of the fractured particles ranged from less than 0.1 μm to greater than 20 μm; approximately 75% of the particles were between 2 and 5 μm in diameter (determined by optical microscopy).

A modified version of the bacterial-adherence-to-hydrocarbons test developed by Rosenberg (27) was used to measure changes in the hydrophobicity of *A. paradoxus* due to chemical treatments. Cells were grown and harvested as previously described and diluted to $10^6$ ml$^{-1}$. One milliliter of the bacterial suspension containing a test chemical was transferred to a 10-mm-inner-diameter glass test tube containing 100 μl of *n*-hexadecane (Fisher). The mixture was vortexed for 1 min and then allowed to stand undisturbed for 30 min, although the aqueous and hexadecane phases typically separated within seconds. Cell concentrations in the water phase ($C_{WHC}$) were measured with a Multisizer II (Coulter Corp.) and compared with cell concentrations in control tubes (no test chemical) ($C_{WH}$). In order to account for cell adhesion to the glass, parallel tests were run on cell suspensions in tubes without hexane to determine cell concentrations in solution with a chemical ($C_{WC}$) and without a chemical ($C_W$) after 30 min. All tests were run in triplicate. The index of relative hydrophobicity (Δ) was defined by using the following equation:

$$\Delta \equiv \frac{C_{WHC} - (C_{WC} - C_W)}{C_{WH}} \quad (2)$$

If Δ was >1, then the chemical treatment decreased the hydrophobicity of the bacterium, while if Δ was <1, the chemical treatment increased hydrophobicity. The standard deviation for Δ was calculated by the method of Skoog et al. (29). Cell concentrations in the aqueous phase were measured twice for each sample.

## RESULTS

A maximum of 99.7% of the bacteria could be retained in the top 1 cm of the minicolumn if all collisions between bacteria and the porous medium resulted in attachment. This upper limit of removal is calculated by using the column characteristics (medium particle diameter and porosity) and the experimental conditions (flow velocity, etc.) in equation 1 and by assuming $\alpha = 1$. Cell retention in the column is equal to or less than this maximum, depending on the specific chemical solution, since each chemical solution can produce a different probability of attachment between the bacteria and the porous medium surface. By measuring $F_r$ in the top of the column it was possible to quantify the effect of these different chemical solutions on the efficiency of bacterial collisions, α, with the porous medium particles. For example, the retention of 1.1% of bacteria ($F_r = 0.011$) in the top 1 cm of the column indicates that only 1 in every 50 collisions ($\alpha = 0.02$) resulted in attachment.

**Effects of ionic strength and buffer.** Chemicals that modify cell surfaces ($PP_i$, SDS, etc.) were added at concentrations that produced different ionic strengths. The ionic strength was held constant at different doses of a chemical by the addition of NaCl to maintain a constant solution conductivity. Ionic strength has been previously shown to affect the magnitude of α (14, 15, 22), which is in agreement with findings in this study. The influence of solution ionic strength on bacterial attachment can be seen by analyzing data from the chemical treatment control experiments (no chemicals added other than buffers and NaCl [Table 2]). Ionic strength explained 80% of the variation in α in a double-log regression analysis (Fig. 1). At the lowest ionic strength (0.01 mM, no buffer), α was 0.0016 and only 0.90% ± 0.11% of bacteria were retained, while at the highest ionic strength (70 mM), 97% ± 14% of bacteria were retained in the column. The highest sticking coefficient in a buffered solution was relatively close to unity ($\alpha = 0.61$), indicating that at a high ionic strength nearly all bacterial collisions result in removal.

Previous studies have shown that the solution pH is not a significant factor in bacterial attachment to glass surfaces (15). The solution pH in the present study was held constant, but different buffers were used in conjunction with chemical treatments. Although we were unable to systematically evaluate the effects of different chemical buffers (because of precipitation reactions with some chemical-buffer combinations), we did not find any statistically significant effect of buffer type or concentration on α. The results of a regression analysis of buffer concentration and α were insignificant ($r^2 = 0.13$). The type of buffer had only slight effects on α. At similar ionic strengths MOPS buffers generally produced the highest α values and Tris buffers produced the lowest α values. In phosphate-buffered solutions there was some indication that α decreased in proportion to phosphate concentration (Table 2). However, the importance of the buffer type was not further examined since more significant changes in sticking coefficients could be obtained through manipulation of ionic strength and the addition of other chemicals.

**Effects of chemical treatments on cell retention.** The most effective treatment for reducing α in unbuffered solutions was to reduce the ionic strength of the solution to 0.01 mM; in buffered solutions, the lowest α values were obtained by adding surfactants (Tween 20 or SDS [Table 2]). These three conditions reduced α by more than 1 order of magnitude. Tween 20 reduced α by approximately 2 orders of magnitude, from 0.38 (no Tween 20) to 0.0016 (Tween 20 concentration of 0.1%, vol/vol). SDS reduced α by an order of magnitude, from 0.064 (no SDS) to 0.0067 (SDS concentration of 0.01% [wt/vol]). Proteinase K, EDTA, and $PP_i$ reduced α by less than an order of magnitude at all concentrations tested. The small reduction in attachment after proteinase K treatment may have been due to insufficient release of protein, since no soluble protein was detected after enzyme treatment.

Three treatments increased attachment efficiencies. Lysozyme (100 μg ml$^{-1}$) affected cell wall components, as indicated by a 50% increase in dissolved organic carbon after treatment, but increased α by 2 orders of magnitude, from 0.0048 to 0.74. In MOPS buffer, EDTA reduced α by 44%, but in Tris buffer, EDTA increased α from 0.084 to 0.17. Sodium periodate treatment (1 mM) increased α from 0.052 to 0.10.

**Cell survival during chemical treatments.** Most chemical treatments did not adversely affect bacterial viability (Table 2). SDS at a concentration of 0.1% was lethal to *A. paradoxus* but did not affect cell viability at the lowest concentration (0.01%). Sodium periodate decreased bacterial viability by 71 to 92%, probably as a result of oxidation of the cell surface.

**EM and hydrophobicity.** The EMs of *A. paradoxus* and glass beads were negative, indicating a net repulsive force between bacteria and collector surfaces for all experimental conditions (Table 3). The magnitudes of the EMs varied from −1.9 to −3.0 μm V$^{-1}$ cm s$^{-1}$ for *A. paradoxus* and from −2.0 to −5.6 μm V$^{-1}$ cm s$^{-1}$ for the crushed glass beads.

The hydrophobicity index for bacteria was unity (Table 3) in a $10^{-4}$ M (NaCl) solution. Higher concentrations of NaCl increased the relative hydrophobicity, but most chemical treatments made bacterial surfaces less hydrophobic (Δ >1). The total range in Δ values was ≥0.60 to ≤1.7, with the surfactant

TABLE 2. Effects of different chemical treatments on bacterial retention in minicolumns

| Expt no.[a] | Chemical | Concn of chemical | Survival (%) | Mean $F_r$ ± SD[b] | α |
|---|---|---|---|---|---|
| 1 | Tween 20 | 0% | 100 | 0.89 ± 0.05 | 0.38 |
| | | 0.01% | 100 | 0.011 ± 0.002 | 0.0019 |
| | | 0.1% | 100 | 0.0090 ± 0.0017 | 0.0016 |
| 2 | SDS | 0% | 100 | 0.31 ± 0.01 | 0.064 |
| | | 0.01% | 100 | 0.037 ± 0.008 | 0.0067 |
| | | 0.1% | 0 | NM[c] | NM |
| 3 | $NaIO_4$ | 0 mM | 100 | 0.26 ± 0.02 | 0.052 |
| | | 0.5 mM | 29 | 0.32 ± 0.05 | 0.067 |
| | | 10 mM | 8 | 0.43 ± 0.07 | 0.10 |
| 4 | EDTA | 0 mM | 100 | 0.97 ± 0.14 | 0.61 |
| | | 0.1 mM | 100 | 0.93 ± 0.09 | 0.47 |
| | | 0.5 mM | 100 | 0.92 ± 0.12 | 0.46 |
| | | 1 mM | 100 | 0.85 ± 0.11 | 0.34 |
| 5 | EDTA | 0 mM | 100 | 0.38 ± 0.04 | 0.084 |
| | | 0.1 mM | 100 | 0.41 ± 0.04 | 0.092 |
| | | 1 mM | 100 | 0.59 ± 0.04 | 0.17 |
| 6 | $Na_4P_2O_7$ | 0 mM | 100 | 0.79 ± 0.04 | 0.27 |
| | | 0.1 mM | 100 | 0.79 ± 0.09 | 0.27 |
| | | 1 mM | 100 | 0.72 ± 0.10 | 0.22 |
| | | 10 mM | 100 | 0.47 ± 0.02 | 0.11 |
| 7 | $Na_4P_2O_7$ | 0 mM | 100 | 0.68 ± 0.12 | 0.20 |
| | | 0.1 mM | 100 | 0.68 ± 0.06 | 0.20 |
| | | 1 mM | 100 | 0.53 ± 0.15 | 0.13 |
| | | 10 mM | 100 | 0.39 ± 0.05 | 0.086 |
| 8 | $Na_4P_2O_7$ | 0 mM | 100 | 0.18 ± 0.02 | 0.034 |
| | | 0.01 mM | 100 | 0.13 ± 0.05 | 0.025 |
| | | 0.1 mM | 100 | 0.13 ± 0.01 | 0.025 |
| | | 1 mM | 100 | 0.28 ± 0.01 | 0.057 |
| 9 | Proteinase | 0 mg/ml | 100 | 0.27 ± 0.04 | 0.055 |
| | | 0.1 mg/ml | 100 | 0.22 ± 0.02 | 0.044 |
| 10 | Lysozyme | 0 mg/ml | 100 | 0.027 ± 0.003 | 0.0048 |
| | | 0.1 mg/ml | 100 | 0.97 ± 0.10 | 0.61 |
| | | 0.1 mg/ml | 100 | 0.98 ± 0.14 | 0.74 |
| 11 | NaCl | 1 mM | 100 | 0.082 ± 0.010 | 0.014 |
| 12 | None | 0 mM | 100 | 0.0090 ± 0.0011 | 0.0016 |

[a] Buffer type, buffer concentration, and solution ionic strength were fixed within each experiment series at the values indicated in Table 1.
[b] $F_r$ indicates the fraction of bacteria retained in top 1 cm of the column.
[c] NM, not measured.

SDS producing the largest decrease in cell surface hydrophobicity.

The overall relation between attachment and bacterial EM and hydrophobicity (Fig. 2) is consistent with previous results obtained by others using different techniques (32). The fraction of bacteria retained in the top 1 cm of the column was lowest under chemical conditions producing a low EM but was relatively unaffected by bacterial hydrophobicity. A multiple linear regression of EM and Δ was significant ($P < 0.05$) on the basis of either fractional retention or α ($r^2 = 0.60$), but it was not significant on the basis of log α ($r^2 = 0.45$, $P = 0.12$). Consequently, EM and Δ were not consistent predictors of α over several orders of magnitude or when α was ≪1. The lack of a significant predictor of log α supports the need for determination of α values on a case-by-case basis.

## DISCUSSION

In the absence of chemical treatments, 80% of the variation in α was due to solution ionic strength. Correlation between ionic strength and bacterial attachment was expected from previous studies (9, 15, 22), and different types of buffers made no significant contributions to variations in this overall trend. At higher ionic strengths (>0.1 mM), Tween 20 was the most effective chemical for reducing α and not decreasing cell viability. Surfactants lower the interfacial tension between surfaces and liquids. The measured hydrophobicity of *A. paradoxus* was reduced by Tween 20, as were the EMs of both the collectors and the bacteria. Changes in EMs were relatively small but were consistent with colloid chemistry theory, which predicts that adsorbed nonionic polymers can reduce surface

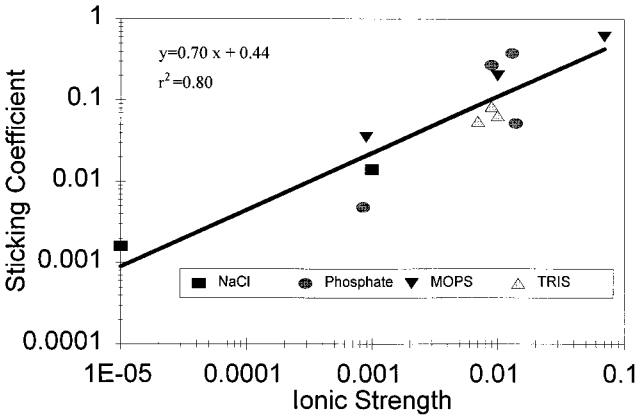


FIG. 1. Efficiencies of bacterial attachment (α) to glass beads in MARK columns in chemical treatment control experiments (no chemicals added other than buffers and NaCl) as a function of ionic strength (molar) of the solution.

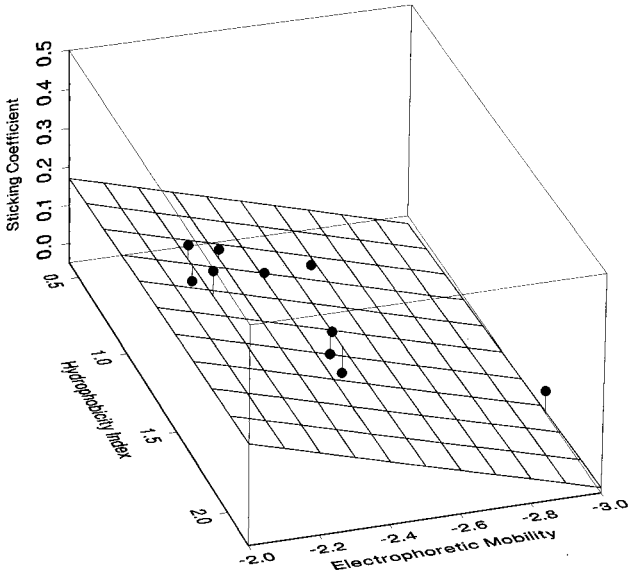


FIG. 2. Efficiencies of bacterial attachment to glass beads after chemical treatment as a function of EM and hydrophobicity index.

charges by obstructing the electrokinetic movement of the diffuse double layer (17). It is doubtful that Tween 20 caused stearic interference between the collector surface and the bacteria, because its molecular weight is low relative to those of bacterial surface proteins and polysaccharides.

Of all the chemical treatments, SDS decreased the hydrophobicity of *A. paradoxus* by the largest margin, but it was lethal to cells at the higher concentration (0.1%). SDS made the EMs of the collectors more negative but only slightly increased the bacterial EM. This increase indicates that adsorbed SDS molecules made only a small contribution to the bacterial surface charge. Both SDS and Tween 20 significantly reduced bacterial attachment, even though the test solutions were at relatively high ionic strengths that would have otherwise destabilized the colloids.

The chemical treatments that had the largest effects on α (surfactants and NaCl) were those treatments that did not alter cell surface structures. Enzymes, periodate, and EDTA were used to alter specific types of surface structures, but these treatments did not decrease α by more than 50%. This suggests that the reversible attachment of hydrophobic bacteria such as *A. paradoxus* to glass surfaces is primarily controlled by chemical interactions and not by disruption or removal of cell surface structures.

Our finding that attachment is positively correlated with EM and hydrophobicity is consistent with previous studies (32, 33), but we found that this relationship was not significant ($r^2$ = 0.45, $P$ = 0.12) when we considered the full range of sticking coefficients by using log α (and not $F_r$). Attachment was primarily controlled by EM ($r^2$ = 0.59), since a linear regression with only bacterial hydrophobicity ($r^2$ = 0.29) did not significantly predict attachment. Bacterial attachment to glass is pri-

TABLE 3. Effects of chemical treatments on EM and hydrophobicity of *A. paradoxus* and glass beads

| Chemical type (buffer) | Chemical concn | EM (μm $V^{-1}$ cm $s^{-1}$) of: | | Bacterial hydrophobicity (mean Δ ± SD) |
|---|---|---|---|---|
| | | Bacterium | Glass beads | |
| NaCl (none) | 10 mM | −1.9 | −3.9 | 0.60 ± 0.026 |
| | 1 mM | −2.6 | −3.6 | 0.76 ± 0.020 |
| | 0.1 mM | −2.7 | −2.8 | 1.0 ± 0.047 |
| | 0.01 mM | −2.4 | −2.6 | NM[a] |
| Tween 20 ($6.7 \times 10^{-3}$ M $PO_4^{-2}$) | 0% | −2.8 | −3.0 | NM |
| | 0.01% | −2.5 | −2.0 | 1.3 ± 0.080 |
| | 0.1% | −2.5 | −2.0 | 1.4 ± 0.11 |
| SDS ($10^{-2}$ M Tris) | 0% | −2.9 | −3.8 | NM |
| | 0.001% | −2.9 | −3.6 | 1.3 ± 0.30 |
| | 0.01% | −3.0 | −3.6 | 1.7 ± 0.087 |
| Periodate ($10^{-3}$ M $PO_4^{-2}$) | 0 mM | −2.3 | −5.1 | NM |
| | 0.5 mM | −2.5 | −2.5 | 1.3 ± 0.30 |
| | 1 mM | −2.4 | −2.5 | 0.97 ± 0.17 |
| EDTA ($10^{-2}$ M Tris) | 0 mM | −2.2 | −4.6 | NM |
| | 0.1 mM | −2.2 | −5.3 | 0.94 ± 0.12 |
| $PP_i$ ($10^{-3}$ M MOPS) | 1 mM | −2.2 | −5.6 | 0.91 ± 0.089 |
| | 0 mM | −2.0 | −4.0 | NM |
| | 0.1 mM | −2.2 | −4.5 | 1.2 ± 0.052 |
| | 1 mM | −2.3 | −5.2 | 0.87 ± 0.041 |

[a] NM, not measured.

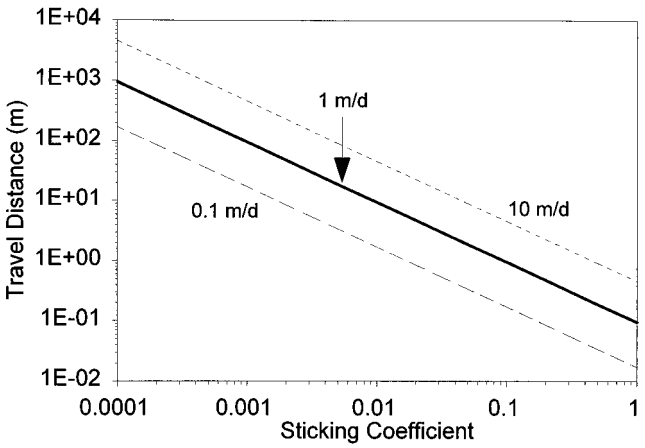


FIG. 3. Predicted travel distances of bacteria in a groundwater aquifer. Distances for groundwater superficial velocities of 0.1, 1, and 10 m day$^{-1}$ are indicated. (Calculations are based on 99% bacterial removal. $d_c$ = 500 μm, $T$ (temperature) = 296 K, $\rho_p$ = 1,080 kg m$^{-3}$, $\theta$ = 0.40).

marily controlled by electrostatic interactions since clean glass surfaces are highly charged and relatively hydrophilic at neutral pHs because of surface hydroxyl groups (33). Apparently the main function of the chemical treatments on attachment at low $\alpha$ values ($<0.01$) was to alter the solution chemistry in some manner not adequately predicted by measurement of hydrophobicity or bacterial EM.

For colloids with relatively low (negative) surface charge densities, hydrophobic forces may dominate. van Loosdrecht et al. (31) demonstrated that more-hydrophobic bacteria adhered in larger amounts to hydrophobic (polystyrene) surfaces than to hydrophilic surfaces. They also observed that decreasing the hydrophobicity of a hydrophobic (polystyrene) surface with protein coatings (to model natural organically coated collectors) decreased the adhesion of hydrophobic bacteria. Their findings for hydrophobic surfaces were presented as a three-dimensional surface by using a nonlinear regression of percent attachment of 23 different strains of bacteria as a function of hydrophobicity, determined by contact angle measurements, and EM (31). Since they did not provide statistical analysis of the three-dimensional surface, we reanalyzed their data with a multiple linear regression and confirmed that their finding for bacterial attachment to the polystyrene surface was significant ($r^2 = 0.73, P < 0.05$). As expected for the hydrophobic surface, the majority of the variation in percent attachment was explainable by cell hydrophobicity ($r^2 = 0.60$) and was only weakly correlated to EM ($r^2 = 0.04$). We were unable to similarly analyze their data from a separate study for glass surfaces (32), but in this latter study they found that EM significantly affected the percent attachment of bacteria to glass surfaces.

While van Loosdrecht et al. (30–33) measured the relative affinities of bacteria over a relatively small range of attachment, the MARK method permits quantitative prediction of bacterial transport properties in porous media over several orders of magnitude. Equation 1 can be used in conjunction with typical groundwater and soil conditions (velocity, 1 m day$^{-1}$; $d_c$, 500 μm) to predict attenuation of biocolloids in soil aquifers (Fig. 3). Without any chemical treatment, *A. paradoxus* in a buffered medium (1 mM MOPS; ionic strength, 70 mM; $\alpha = 0.61$) would travel only 0.16 m before cell numbers were reduced by 99%. Although it is possible to reduce the local ionic strength of groundwater in situations in which cells are introduced as a suspension, it may not be feasible to produce widespread changes in ionic strength due to complicating factors such as redissolution of minerals. However, by adding surfactants such as Tween 20 (0.1% [Table 3]) to water and bacterial suspensions pumped into aquifers, a reduction in $\alpha$ to 0.0016 should permit bacteria to travel up to 16 m prior to similar reductions in cell numbers (99%). At higher groundwater velocities of 10 m day$^{-1}$, typical of pumped aquifers during soil remediation, bacteria at this low $\alpha$ of 0.0016 would be capable of traveling 290 m prior to a 2-log attenuation.

This calculation points out the utility of chemical treatments in promoting bacterial dispersal in contaminated aquifers. While the magnitude of changes in transport distances in this study is due to the specific chemical treatments examined here, and while cases in this study are limited to those in which bacterial interactions with soil particles are similar to those with glass surfaces, the techniques and calculations used in this study should apply to other types of porous media as well. Additional experiments with site-specific soils and bacterial strains are being conducted in our laboratory to see if our findings can be extended to soils. Even if chemical treatments need to be evaluated on a site-by-site basis, the ability to rapidly screen soil conditions in the laboratory should make it possible to optimize conditions for bacterial-injection field studies and bioremediation treatment schemes.

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