MEASUREMENT OF BACTERIAL COLLISION EFFICIENCIES IN POROUS MEDIA

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Abstract—A new method, utilizing radiolabeled (14C-leucine) cells and 1-cm columns packed with 40-μm boro-silicate glass beads was used to estimate bacterial collision efficiency by directly measuring the retention of cells in porous media. At a fractional retention of 0.051 (n = 3), the coefficient of variation was 0.037, permitting meaningful estimation of collision efficiencies as low as 3 × 10^-4. Collision efficiency was a function of the ionic strength and species identity; x increased from 1.6 × 10^-4 to 1.4 × 10^-2 for A. paradoxus in 10^-4 and 10^-1 M NaCl solutions, respectively, and from 8.9 × 10^-4 to 6.2 × 10^-2 for P. fluorescents in the same solutions. Results were not sensitive to test parameters such as velocity, volume filtered and rinse volume. The new procedure provides a convenient, reliable, accurate method for estimating low-end biocolloid collision efficiencies in porous media. In the range x < 0.01, the method is economical, significantly faster, and much more reliable than other published procedures. Its application may accelerate efforts to establish functional relationships between biocolloid collision efficiency and governing physical-chemical variables.

Key words—bacteria, biocolloid, porous media, transport, filtration, radiolabel, collision efficiency

INTRODUCTION

Interest in bacterial adhesion at liquid/solid interfaces and transport through porous media is driven by a variety of practical considerations. These include prevention of biofouling (Marshall, 1992; Okuba and Matsumoto, 1983; Oberdorder and Peterson, 1985), pathogen mobility in potable aquifers (Alhajar et al., 1988, Corapcioglu and Haridas, 1985; Keswick, 1984), the dispersion of genetically engineered microorganisms following their hypothetical introduction into the environment (Harvey and Garabedian, 1991; Marlow et al., 1991) and the infusion of specific microbial strains into the subsurface environment for the destruction of hazardous organic compounds (Flahman et al., 1989; Harvey and Garabedian, 1991). The discovery of abundant, diverse microbial communities in the deep subsurface has motivated inquiry relative to their origin, including relationships among adhesion, transport and other processes requisite to their introduction into that environment.

Hydrosols are removed from water flowing through porous media by sorption to the fixed media surfaces. Transport of the hydrosols from the mobile fluid to the collectors can be driven by Brownian diffusion, interception and/or gravitational settling. The fraction of particles colliding with the collector that are actually retained is the collision efficiency (α). Collector efficiency (η), which is the fraction of particles that approach and strike a collector, can be adequately predicted from theory. Collision efficiency, however, has been successfully predicted using the electrostatic model of Derjaguin, Landau, Verwey, and Overbeek (Derjaguin and Landau, 1941)(DLVO) in only limited cases (Elimelech and O'Melia, 1990; Tobiason and O'Melia, 1989; Tobiason, 1989). More frequently α is empirically determined using the calculated collision frequency, a macrobalance across the porous medium, and measurements of collector diameter and media porosity (Rajaopalan and Tien, 1976; Yao et al., 1971). However, these approaches frequently suffer from the considerable effort that is associated with experimental design, particularly when the particles of interest are biocolloids with growth and die off capabilities. Procedures that depend upon contemporary measurements of column influent and effluent particle concentrations are of limited utility for estimation of low-end α values due to inherent limitations associated with comparison of large, similar numbers.

Martin et al. (1992) found that α's calculated from experiments in which bacteria were applied to columns packed with glass beads were in reasonable agreement with direct measurements on planar glass surfaces when an appreciable fraction of the bacteria was removed from solution. However, Jewett et al. (1993)
showed that small relative errors in enumerating particle concentrations produce large errors in \( x \) when fractional retention of particles on collectors is small. When investigating factors that enhance bacterial transport (i.e. create low \( x \)'s) in porous media, experiments must be designed for accurate, reproducible measurement of fractional retention. Column studies that use influent and breakthrough cell concentrations to calculate small collision efficiencies (\( x < 10^{-5} \)) are necessarily limited by the accuracy of cell counting techniques.

Our purpose was to develop a convenient, reliable mechanism for measuring bacterial \( x \)'s in the range \( 10^{-4} < x < 10^{-1} \). The procedure described here (microbe and radiolabel kinetics, or MARK method) relies on radiolabel incorporation to increase the accuracy of bacterial counts and avoids uncertainties relative to collector geometry and surface characteristics by using very small (40 \( \mu \)m) spherical glass beads as collector materials. We investigated test reproducibility in order to assign error to measurements of cell retention and estimates of collision efficiency. We establish the sensitivity of collision efficiency to reasonable variation in a number of test parameters. The low-end accuracy of estimated \( x \) values was greatly improved by directly measuring numbers of retained bacteria. The procedure avoids calculations based on differences between influent and effluent cell concentrations. The MARK method was then used to measure \( x \)'s for four different bacterial species under different chemical conditions.

### MATERIALS AND METHODS

**Bacterial strains**

The bacterial species used for these experiments were *Pseudomonas fluorescens* strain P17, *Aerobacter aerogenes* paradoxicus and two isolates from the Savannah River deep subsurface collection, strains A0500 and A1258. *P. fluorescens* strain P17 was isolated from a water distribution system in the Netherlands. Because P17 can use a variety of organic compounds at very low concentrations as sources of energy and carbon, it has been used as a test organism for measurement of assimilable organic carbon in drinking water (van der Kooij, 1979). Strain P17 readily assimilates radiolabeled \( \mathrm{H} \)-urea and grows reproducibly in defined media. *A. paradoxicus* was isolated from pesticide-contaminated surface soils at Hill A.F.B., Utah, and degrades 2,4-dichlorophenoxyacetic acid (2,4-D) and a variety of other halogenated aromatic compounds (Wu, 1993). Its transport/adhesion characteristics in porous media have not been previously investigated. Cells were monodisperse during growth in either 5 percent PTYG (described in the Bacterial Growth and Radiolabeling section) or a defined mineral salts medium with 2,4-D as the sole carbon source.

Strains A0500 and A1258 were obtained from the Savannah River collection (D.L. Balkwill, Florida State University) that was established within the U.S. Department of Energy Subsurface Science Program (DOE, 1990). A0500 was isolated from the Savannah River drilling site P28 at a depth of 180 m. Strain A1258 was isolated from the same well at a depth of 203 m. Growth of both strains was rapid and monodisperse in 5% PTYG.

**Bacterial growth and radiolabeling**

Three different media (Table 1) were used for bacterial growth and/or labeling in order to accommodate specific growth requirements or experimental objectives. In experiments to investigate reproducibility of MARK results and test their sensitivity to variations in fluid velocity, volume of

<table>
<thead>
<tr>
<th>Labeling period (h)</th>
<th>A paradoxicus</th>
<th>P fluorescens</th>
<th>A0500</th>
<th>A1258</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of label applied (( \mu ))</td>
<td>0.5</td>
<td>0.5</td>
<td>12-18</td>
<td>12-18</td>
</tr>
<tr>
<td>Suspension volume during labeling (ml)</td>
<td>10</td>
<td>10</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Total filter count (C)</td>
<td>17.5 ( \times 10^4 ) (n = 3)</td>
<td>27.1 ( \times 10^4 ) (n = 3)</td>
<td>157 ( \times 10^4 ) (n = 3)</td>
<td>114 ( \times 10^4 ) (n = 3)</td>
</tr>
<tr>
<td>措</td>
<td>3 ( \times 10^3 )</td>
<td>3 ( \times 10^3 )</td>
<td>100 ( \times 10^4 )</td>
<td>100</td>
</tr>
<tr>
<td>Label count background (D)</td>
<td>1.6 ( \times 10^4 ) (n = 3)</td>
<td>1.7 ( \times 10^4 ) (n = 3)</td>
<td>0.2 ( \times 10^4 ) (n = 3)</td>
<td>1.6 ( \times 10^4 ) (n = 3)</td>
</tr>
<tr>
<td>(DPM ( \times 10^4 ) filterate)</td>
<td>( \mathrm{NH}_4 \mathrm{Cl} )</td>
<td>( \mathrm{NH}_4 \mathrm{Cl} )</td>
<td>( \mathrm{NH}_4 \mathrm{Cl} )</td>
<td>( \mathrm{NH}_4 \mathrm{Cl} )</td>
</tr>
<tr>
<td>Label uptake (C - D)</td>
<td>15.9 ( \times 10^4 )</td>
<td>25.4 ( \times 10^4 )</td>
<td>157 ( \times 10^4 )</td>
<td>112 ( \times 10^4 )</td>
</tr>
<tr>
<td>(DPM ( \times 10^4 ) filtrate)</td>
<td>( \times 10^4 )</td>
<td>( \times 10^4 )</td>
<td>( \times 10^4 )</td>
<td>( \times 10^4 )</td>
</tr>
<tr>
<td>Fraction of label taken up</td>
<td>0.70</td>
<td>0.72</td>
<td>0.18</td>
<td>0.26</td>
</tr>
</tbody>
</table>

\( * \) = \( 10^7 \) cells ml\(^{-1}\).

\( \approx \) \( 10^9 \) cells ml\(^{-1}\).

This is also the concentration of label in \( 10^9 \) cells, assuming a suspension density of \( 10^9 \) cells ml\(^{-1}\). However, 2-3 doublings were observed during the 12-18 h period used to label A0500.

Fractional uptake is the ratio of label assimilated to total applied (per ml of culture). Both were measured values. Estimation of total applied was based on a single measurement, not shown.

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Table 1. Growth and labeling media component (per liter of deionized \( \mathrm{H}_2\mathrm{O} \))

<table>
<thead>
<tr>
<th>5% PTYG</th>
<th>Medium A</th>
<th>Medium B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl_2( \cdot )2H_2O</td>
<td>0.07 g</td>
<td>0.088 g</td>
</tr>
<tr>
<td>MgSO_4( \cdot )7H_2O</td>
<td>0.60 g</td>
<td>0.05 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.50 g</td>
<td>1.00 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.50 g</td>
<td>0.07 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.50 g</td>
<td>0.174 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>0.25 g</td>
<td>0.10 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.25 g</td>
<td>2.4-D</td>
</tr>
</tbody>
</table>

*Iron and calcium were added from a 1000X, filter-sterilized stock solution after other components were autoclaved (121°C, 30 min) and cooled to room temperature.

(N-morpholine)-propane-sodium-salt.

2,4-dichlorophenoxyacetic acid.
The test solution and rinse volume, *A. paradoxus* and Savannah River isolates A0500 and A1258 were grown in 5% PTYG. To investigate ionic strength effects, defined media were necessary; strain P17 and *A. paradoxus* were grown in Medium A and Medium B, respectively. Temperature was maintained at 22–23°C in all cases and pH was 7.2.

To label cells for the ionic strength experiment, 3 ml of *A. paradoxus* or P17 were harvested in late log phase (≈10^9 cells ml^-1), combined with 10 μl of 'H-(L)-leucine (ICN, 79 Ci mmol^-1, 1 mCi ml^-1) in 2% ethanol and incubated at room temperature for 30 min. One ml was then transferred to 100 ml of the test solution to produce a final cell number of approximately 10^6 cells ml^-1. Test solutions consisted of 10^-1 and 10^-3 M NaCl in unbuffered, deionized water. Because the Savannah River isolates, A0500 and A1258, assimilated radiolabeled substrates slowly, a modified procedure for label incorporation was developed. Cells grown in 5% PTYG were harvested during early-stationary phase and diluted to approximately 10^6 cells ml^-1 in mineral-salts solution (Medium A, without glucose) in order to produce oligotrophic conditions during the labeling period. After 40 μl of 'H-(L)-leucine was added, the suspension was incubated at room temperature for 12–18 h. Although cell numbers increased by a factor of 2–4, cultures were assumed to be stationary at the end of the radiolabeling procedure. On average, cell size decreased from an effective diameter of 1.5–1.2 μm during the incubation period. This method of radiolabel uptake was used in experiments designed to establish the reproducibility of test results and to explore variations attributable to rinse volume, fluid velocity, and test solution volume.

Both radiolabeling methods resulted in assimilation of sufficient 'H-leucine to permit accurate cell counting (Table 2). The longer exposure period of A0500 and A1258 (12–18 h) vs. 0.5 h) resulted in lower fractional uptake of applied label (0.2 vs. 0.7 for *A. paradoxus* and *P. fluorescens*).

However, 'H-leucine assimilated by the cells applied to the column was actually 5 times greater in the A0500 and A1258 suspensions.

Cell dimensions were measured via light microscopy and in an image analysis system (Cue2, Olympus). Effective diameter (d) was determined by estimating projected areas for individual cells, averaging results, and calculating the diameter of a circular equivalent (d = (4A/π)^0.5). The effective diameters for *A. paradoxus* and *P. fluorescens* were 1.0 and 1.1 μm, respectively.

Design and use of the mini-column reactors. The reactor for the MARK test was the bottom barrel of a 3-cc plastic, LuerLok syringe with 0.8-cc L.D. Syringes were prepared by inserting a 0.8-cm GF/D filter (Whatman, 2.7 μm nominal pore size) into the bottom of the syringe barrel. The barrel was then packed with 40-μm (Whatman) borosilicate glass beads. Beads were pretreated by soaking in a 10 percent sulfuric acid solution, with agitation, for ≥3 h and rinsing thoroughly in deionized water to remove the acid. About 1.5 ml of beads slurried in deionized water was transferred to each syringe barrel and stirred with a Pasteur pipette to remove trapped air, ensure homogeneity and level the bead surface. Syringe reactors were mated to an Alltech vacuum manifold equipped with LuerLok connections.

The beads were further rinsed with 6–10 ml of deionized water to achieve a final effluent pH of about 6.9. Approximately 6 ml (about 30 pore volumes) of the rinse solution (test solution without suspended microorganisms) was run through the reactor. At this point, reactor preparation was complete.

Microbe-retention experiments were initiated by adding 2 ml of the test solution containing radiolabeled microorganisms at the top of the column. Vacuum pressure was manipulated to produce a through-column flow rate of 4 ml min^-1 (approximate velocity of 0.125 cm s^-1). Two 2-ml rinse solutions, chemically identical to the test solution but without microorganisms, were then added to wash out unattached bacteria and liquid-phase radiolabel. After being sucked to apparent dryness, the syringe reactor was removed from the manifold, and its lower end was cut off. The syringe plunger was used to extrude beads from the column, which exited as a damp, cylindrical aggregate. All but the top 1.0 cm was discarded, and the top portion was transferred to a scintillation vial. Ten ml of scintillation cocktail (Budapest-Solvic; RPI) was added to complete the mixture. Cocktail and beads were shaken continuously for ≥18 h to separate radiolabel from the beads prior to measurement on a Beckman LS 3801 scintillation counter with quench correction. The resultant value was the "total bead count" (A).

Because cell washing altered experimental estimates of bacterial a's (data not shown), it was necessary to determine both the radiolabel content of the suspended bacteria before passage through the reactor and radiolabel retained on the beads due to sorption of liquid-phase, labeled organic compounds. Retained organic compounds consisted of bacterial exudates and unassimilated 'H-leucine. To estimate the total assimilated radiolabel per ml of the cell suspension (≈10^6 cells ml^-1), 2 ml of the test solution were removed and passed through a 0.2 μm polycarbonate filter (Poriloc). The filter and retained cells were transferred to a scintillation vial with 10 ml of scintillation cocktail. The resultant measurement was the "total filter count" (C). The filtrate was passed through a second mini-column reactor to determine the retention of liquid-phase radiolabel on the 30-μm beads or the "control bead count" (B). Finally, 10 ml of the cell-free, radiolabeled solution, obtained as filtrate, above was passed through a second 0.2 μm polycarbonate filter. The resultant background count (filter and retained label) was called the "filter background" (D).

Based on the procedures described, the fraction of cells retained (R) was calculated by

\[ R = \frac{R_{\text{total bead count}} - \text{control bead count}}{\text{total filter count} - \text{filter background}} = \frac{A - B}{C - D} \]  

**Modeling.** The transport of cells to collector surfaces was modeled using the clean-bed particle trajectory methods of Rajagopalan and Tien (1976) and Rajagopalan et al. (1982), which accounts for the contributions of Brownian diffusion, interception, and gravitational settling to the single-collector efficiency (η) using

\[ \eta = 1.0 \frac{A_{\text{w}}N_{\text{w}}L_{\text{w}}}{0.00338 A_{\text{w}}N_{\text{w}}L_{\text{w}} + 4 A_{\text{w}}N_{\text{w}}} \]  

where \( N_{\text{w}} \) is the ratio of the suspended particle and collector radii (\( a_{\text{p}}/a_{\text{c}} \)) and reflects the relative importance of interception of particle removal; \( N_{\text{w}} \) equals \( 2\pi L_{\text{w}} / g \left( \rho_{p} - \rho \right) \) and reflects the role of gravity effects or settling; \( N_{\text{w}} \) equals \( H / \left( \pi a_{\text{c}} U \right) \) and accounts for London van der Waals interactions in promoting particle/collection collisions; and \( \eta \) equals \( 2U_{\text{w}}/D_{\text{w}} \), representing the effects of Brownian
diffusion. The term \( A \) equals \( 2(1-\rho^2)/(2-3p+3p^2-2p^3) \), and accounts for the effect of adjacent collectors on the collector efficiency (Rajagopalan and Tien, 1976) where \( \rho = (1-\epsilon)^{1/3} \) and \( \epsilon \) is the porosity of the porous medium. The term \( D_\alpha \) is the Brownian diffusion coefficient \( (kT/(6\eta\mu)) \), where \( \mu \) is the dynamic viscosity of the fluid, \( \rho \) the fluid density, \( U \) the approach velocity, \( g \) the gravitational constant, \( \rho_0 \) the density of the suspended particle, \( H \) the Hamaker constant, \( k \) Boltzmann’s constant, and \( T \) the absolute temperature. Changes in collector geometry and surface availability due to bacterial attachment were neglected.

Collision efficiency \( (\epsilon) \) was accounted for in a liquid-phase mass balance over a differential element of the filter. Integrating the resultant balance, the steady-state, liquid-phase particle concentration is described over the length of a one-dimensional, clean-bed filter by

\[
N = N_0 \exp \left( -\frac{(1-\epsilon)x\eta \gamma}{2D_\alpha} \right) \tag{3}
\]

where \( x \) is the distance along the filter bed and \( N_0 \) is the influent particle concentration. The fractional retention \( (R) \) of particles on collector surfaces was used to calculate the fraction of unretained particles \( (N/N_0) \), using \( N/N_0 = 1 - R \).

The values used for the other parameters in equations (2-3) were: fluid density \( (\rho) \) equal to 998 kg m\(^{-3}\), absolute fluid viscosity \( (\mu/\rho) \) equal to 9.37 \times 10\(^{-4}\) (N s) m\(^{-1}\), \( \epsilon \) equal to 0.01, bacterial density \( (\rho_0) \) equal to 1080 kg m\(^{-3}\), approach velocity \( (U) \) equal to 0.125 \times 10\(^{-2}\) m s\(^{-1}\), \( T \) equal to 296 K, and gravitational acceleration equal to 9.81 m s\(^{-2}\). The Hamaker constant \( (H) \) was assumed to be 1.00 \times 10\(^{-19}\) J, and the Boltzmann constant \( (k) \) was 1.38 \times 10\(^{-23}\) J K\(^{-1}\). The theoretical porosity \( (\epsilon) \) of 0.4 for packed spheres was used for calculations of \( x \).

Standard errors for the estimated mean fraction retained \( (\bar{R}) \) were calculated after the method of Skoog et al. (1988). It was assumed that \( \bar{R} = (A-B)/(C-D) \) and that \( B, C, D, \) and corresponding standard deviations \( \sigma_B, \sigma_C, \) and \( \sigma_D \) were approximately constant, independent of the average total bead count \( (\bar{A}) \). The mean fraction retained and corresponding standard deviation \( (\sigma_A) \) were calculated using

Fig. 1. Sensitivity of fraction retained (A) and collision efficiency (B) to experimental approach velocities in the range 0.02-0.20 cm s\(^{-1}\). Sensitivity of fraction retained (C) and collision efficiency (D) to variation in MARK procedure test volume (2-15 ml). Sensitivity of fraction retained (E) and collision efficiency (F) to the volume of rinse solution applied (2-15 ml).
Table 4. Fractional retention and standard deviation ($\pm \sigma$) for Savannah river deep-subsurface isolates A1258 and A0500. In both cases $n = 3$.

<table>
<thead>
<tr>
<th></th>
<th>Total bead count ($\pm \sigma_a$)</th>
<th>Control bead count ($\pm \sigma_b$)</th>
<th>Total filter count ($\pm \sigma_c$)</th>
<th>Filter background ($\pm \sigma_d$)</th>
<th>Mean fraction retain$^a$ and standard deviation$^b$</th>
<th>Collision efficiency, $\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1258</td>
<td>167 $\pm 10.9$</td>
<td>15.1 $\pm 1.6$</td>
<td>228 $\pm 2.0$</td>
<td>3.2 $\pm 0.65$</td>
<td>0.68 $\pm 0.050$</td>
<td>0.27</td>
</tr>
<tr>
<td>A0500</td>
<td>167 $\pm 0.47$</td>
<td>0.63 $\pm 0.014$</td>
<td>314 $\pm 7.1$</td>
<td>0.39 $\pm 0.07$</td>
<td>0.051 $\pm 0.001$</td>
<td>0.0092</td>
</tr>
</tbody>
</table>

$R = \frac{a - b}{c - d}$

$^a$ Calculated using:

$$\sigma_a = \sqrt{\sigma_1^2 + \sigma_2^2 + \sigma_3^2}$$ for $y = a(\pm \sigma_a) + b(\pm \sigma_b) + c(\pm \sigma_c)$

and

$$\sigma_y = \sqrt{(\sigma_a/a)^2 + (\sigma_b/b)^2 + (\sigma_c/c)^2}$$ for $y = a(\pm \sigma_a) \cdot b(\pm \sigma_b) \cdot c(\pm \sigma_c)$.

$^b$ Calculated using mean fraction retained and equation (5).

Replicate data obtained for strain A0500. $\alpha$ was treated as an independent variable with a constant coefficient of variation. That is, $\alpha$ was assumed to be constant and estimated from experimental data. $\sigma_a$ was then defined as:

$$\sigma_a = R \left[ \frac{\sigma_1^2 + \sigma_2^2 + \sigma_3^2}{(A-B)^2 + (C-D)^2} \right]^{1/2}.$$  \hspace{1cm} (4)

Using $\gamma$'s calculated from equation (2), it was then possible to calculate $\alpha$ for $R$ by:

$$\alpha = -\frac{4a}{2(1-e^x)}.$$  \hspace{1cm} (5)

Similar formulations were used to calculate $\alpha$'s corresponding to $R - (\alpha \sigma_n^{-\alpha})$ and $R + (\alpha \sigma_n^{-\alpha})$, where $\sigma$ is based on the student's $t$ distribution, and $n$ is the number of replicates.

RESULTS

Effect of electrolyte concentration

Decreasing the salt concentration from $10^{-3}$ to $10^{-5}$ M decreased $\alpha$ from 0.062 to 0.0089 for *P. fluorescens* and from 0.014 to 0.0016 for *A. paradoxus* (Table 3). At both ionic strengths, *P. fluorescens* was about 5 times more adherent to the glass collectors than *A. paradoxus*.

Sensitivity to variation in test parameters

The sensitivity of MARK results to variation in approach velocity, the volume of test suspension applied to the reactor, and the volume of rinse solution applied to the reactor after filtration of the test suspension was evaluated using both A0500 and A. *paradoxus*. Independent variables were investigated individually; other test parameters were maintained at standard values noted in Fig. 1.

Results of velocity experiments in which approach velocity was systematically varied from 0.022 to 0.20 cm s$^{-1}$ are illustrated in Fig. 1. Although the fraction of *A. paradoxus* retained varied with approach velocity [Fig. 1(A)], calculated collision efficiencies were insensitive to variation in the range of velocities tested [Fig. 1(B)].

The volume of the test suspension applied to the MARK reactor was varied from 2 to 15 ml, other parameters constant. There was less than 25% difference in both fraction retained [Fig. 1(C)] and $\alpha$ [Fig. 1(D)] over the entire range tested and less than 10% variation between 2 and 10 ml. MARK results were insensitive to rinse-solution volume in the range 2–15 ml [Fig. 1(E) and 1(F)].

Reproducibility

Reproducibility of the MARK procedure was established by measuring cell retention in parallel tests involving deep subsurface strains A0500 and A1258. A0500 was considered representative of poorly retained, non-sticky microorganisms; A1258 was highly retained under appropriate chemical conditions. Replicate retention and background measurements were made independently (separate columns and polycarbonate filters) beginning with the same labeled suspension. The mean fraction retained was 0.68 for A1258 and 0.051 for A0500 corresponding to $\alpha$ values of 0.27 and 0.0092, respectively ($n = 3$, Table 4). Estimates of the standard deviation in fraction retained, calculated as shown in Table 4, were approximately 7 and 4% of the mean for A1258 and A0500, respectively. The latter value is especially significant because estimates of small $\alpha$'s are particularly sensitive to errors in the measurement of fraction retained.

Error analysis

Calculation of $\alpha$ using equations (1–2) from the fraction of bacteria retained requires estimation or measurement of filter porosity ($\epsilon$), size and density of particles to be removed ($d_p$, $\rho_p$), temperature and filter length. All are subject to error. The sensitivity of calculated $\alpha$ values to reasonable uncertainty in each of these parameters was examined in calculations that are summarized in Fig. 2. From the exercise, it was determined that $\alpha$ is insensitive to error in the estimation of particle density in the range 1.001–1.150 g-cm$^{-1}$ [Fig. 2(A)], temperature [288–303 K, Fig. 2(B)], and column length [0.9–1.1 cm, Fig. 2(C)]. Estimates of particle size or effective diameter and the porosity of the filter medium are more critical in terms of their effect on $\alpha$. Doubling the effective diameter from 1 to 2 $\mu$m caused a 60% decrease in the calculated
A 50% increase in the assumed porosity, from \( \varepsilon = 0.30 \) to 0.45, tripled \( \alpha \) for \( R \leq 0.1 \) (Fig. 2(E)).

**Discussion**

The MARK procedure offers several advantages over other methods for establishing colloid transport characteristics in porous media. In side-by-side tests and similar tests conducted at different points in time, the MARK procedure proved exceptionally reproducible, even when fractions retained were small. As discussed previously, this is a necessary characteristic for accuracy in the estimation of low-end \( \alpha \) values. In three tests involving strain A0500, conducted under what were ostensibly identical conditions, the mean fraction retained was 0.051 with a standard deviation of only 0.0019.

Sensitivity analyses (Fig. 2) suggest that the MARK method provides a robust estimate of microbial stickiness that is insensitive to variation in approach velocity, volume of test solution applied, and rinse volume at or near values selected for the standard test. Although the number of collisions between colloid and collector was a function of column hydraulics [Fig. 1(A)], collision efficiency was not [Fig. 1(B)].

Bead/filter affinities did not change during the course of a single test, i.e. filter-ripening did not affect the clean-bed status of the beads; \( \alpha \) was insensitive to the number of cells applied [Fig. 1(D)]. Retained cells covered a negligible fraction (<0.01%) of the overall population.

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**Fig. 2.** Sensitivity of collision-efficiency calculations to fraction of cells retained and input parameters: (A) microbe density (0.001–1.150 g cm\(^{-3}\)), (B) temperature (288–303 K), (C) column length (0.9–1.1 cm), (D) microbe diameter (0.50–2.00 \( \mu \)m), and (E) bed porosity (0.30–0.45). Note that all lines coincide in F(A) and (B), \( \alpha \)'s were calculated using equations (2) and (3).
Bacterial collision efficiencies

Fig. 3. Collision efficiencies corresponding to upper and lower limits to the 95% confidence interval on mean fraction retained. Fraction retained is the independent variable. Upper and lower limits of the 95% C.I. were calculated per procedures summarized in the Discussion section. Corresponding \( \alpha \)'s were obtained using a permutation of equation (3). Analysis indicates that MARK method results may be used to estimate bacterial \( \alpha \)'s \( \geq 3 \times 10^{-3} \) (horizontal line on graph).

bead surface area in each of the mini-column experiments described. Furthermore, the fractional retention of cells did not increase appreciably with increases in rinse volume [Fig. 1(E)], indicating that cell detachment was not an important determinant of fraction retained under the test conditions.

Increasing the ionic strength of solution increased the fractional retention of bacteria on the glass bead surfaces. The greater removal of bacteria at higher ionic strengths confirms the importance of electrostatic forces for bacterial attachment to hydrophilic surfaces that has been observed by others (e.g. Martin et al., 1992).

By counting retained, as opposed to unretained, cells in porous media, the MARK method is much better suited for estimating low-end collision efficiencies in porous media than are related procedures that rely on comparison of influent and effluent cell numbers. Estimates of \( \alpha \) based on the influent-to-effluent drop in cell counts are bound to fail unless the fraction retained is appreciable. Unfortunately, for a variety of practical reasons, the fraction of non-adsorbed, colloidal particles that is retained in laboratory columns is likely to be small. To increase the fraction retained, equation (3) suggests that one might increase column length and/or decrease the effective size of the collector. The glass beads used here are about as small as can reasonably be used without leading to appreciable colloid straining, and cost considerations prevent significant increase in column length when small beads are employed. Jewett et al. (1993) showed that columns on the order of 100 m in length would be necessary for acceptable estimates of low-end bacterial \( \alpha \) values when 3 mm beads are used as porous media. Cell sticking coefficients have also been estimated for adhesion to planar glass surfaces when \( \alpha \) values are small (Martin et al., 1991), but such systems necessarily suffer from geometric dissimilarities with porous media. Cell counts based on incorporated radiolabel are also more convenient than direct counts using fluorescent stains and more accurate than viable counts (plates or most probable number). Radiolabel can be incorporated into viruses, which are not normally counted using light microscopy.

It was suggested by Martin et al. (1992) that estimates of \( \alpha \) within an order to magnitude of the true sticking coefficient provide a reasonable basis for modeling bacterial colonization and biofilm development/activity in porous media. This criterion and statistics summarized in Table 4 were used to explore the limits of the MARK method for generating useful, low-end estimates of bacterial collision efficiencies. We calculated a minimum fraction retained \( (R) \) at which \( \alpha \) values corresponding to limits of the 95% confidence interval for \( R \) (the mean fraction retained in a parallel tests) differ by an order of magnitude (Fig. 3). As the empirically determined \( R \) decreases, measurement error and random variation increase the relative size of the 95% C.I. (Fig. 3). Eventually, the ratio of \( \alpha \)'s calculated at the upper and lower limits on that interval exceeds 10. From Fig. 3, the lower limit for reliable determinations of \( \alpha \) using the MARK method is \( \alpha = 3 \times 10^{-3} \).

In summary, the MARK method can be run in <2 days using materials that cost under $20 per test to obtain reliable estimates of \( \alpha \) in the range \( \alpha \geq 10^{-3} \). The method can readily be adapted to investigate the effects of more heterogenous sediments. In concert with other measurements of bacterial surface characteristics such as electrophoretic mobility, contact angle, hydrophobic interaction chromatography and various other partitioning techniques, it may be possible to use the MARK method to develop relationships between physical characteristics of the cell surface and bacterial transport in different types of porous media. The test should be useful to those concerned with pathogen attenuation in the subsurface, transport of genetically engineered microorganisms, and the introduction of microorganisms with novel metabolic capabilities for the bioremediation of subsurface contaminants.

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