Transport and Deposition of Metabolically Active and Stationary Phase Deinococcus radiodurans in Unsaturated Porous Media

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Bioremediation is a cost-efficient cleanup technique that involves the use of metabolically active bacteria to degrade recalcitrant pollutants. To further develop this technique it is important to understand the migration and deposition behavior of metabolically active bacteria in unsaturated soils. Unsaturated transport experiments were therefore performed using Deinococcus radiodurans cells that were harvested during the log phase and continuously supplied with nutrients during the experiments. Additional experiments were conducted using this bacterium in the stationary phase. Different water saturations were considered in these studies, namely 100 (only stationary phase), 80, and 40%. Results from this study clearly indicated that the physiological state of the bacteria influenced its transport and deposition in sands. Metabolically active bacteria were more hydrophobic and exhibited greater deposition than bacteria in the stationary phase, especially at a water saturation of 40%. The breakthrough curves for active bacteria also had low concentration tailing as a result of cell growth. Collected breakthrough curves and deposition profiles were described using a model that simultaneously considers both chemical attachment and physical straining. New concepts and hypotheses were formulated in this model to include biological aspects associated with bacteria growth inside the porous media.

Introduction

Various contaminants have been improperly disposed of or inadvertently released into the vadose zone. Infiltrating water, which serves to recharge to groundwater supplies, may subsequently pass through these contaminated regions and leach contaminants to drinking water supplies. Bioremediation strategies such as bioaugmentation, biostimulation, and natural attenuation have been proposed as cost-effective means to clean up these contaminated sites (1, 2). These techniques rely on the use of metabolically active bacteria to degrade or immobilize recalcitrant pollutants (3–8). Bioaugmentation involves the introduction of cultured microorganisms that are able to metabolize and grow on the compounds of interest. In biostimulation the subsurface environment is modified by adding limiting nutrients to “stimulate” native bacteria that are capable of degrading contaminants. Natural attenuation uses the existing microbial community and subsurface conditions to contain the spread of contamination and to reduce concentrations.

Bioremediation has many advantages compared with other cleanup methods. For example, since the contamination can be treated in situ, the technology does not incur removal and disposal costs and thus post-cleanup costs can be substantially reduced. Although bioremediation holds great promise for dealing with intractable environmental problems, it is important to recognize that much of this promise has yet to be realized. This is due in part to our lack of understanding of microorganism interactions in different hydrologic environments and our inability to efficiently deliver microorganisms from an injection point to a contaminant plume or source zone. The design of efficient bioremediation strategies can be improved by a better understanding of the complex interplay of chemical, biological, and physical factors affecting bacteria transport and deposition.

One of the unresolved problems in bioaugmentation operations is our inability to predict the distribution of the inoculated bacteria throughout the soil profile. This information is needed to ensure adequate contact between specialized bacteria and target compounds. Early adsorption of bacteria can be detrimental to a successful remediation process. In particular, very little is known about transport and deposition of bacteria that are metabolically active. The majority of transport studies have been performed using bacteria cells that were unable to grow and reproduce, or were harvested in the late stationary phase of growth (4, 7, 9, 10).

In practical bioremediation applications, the bacteria cells must be metabolically active and be able to reproduce. In this case, the bacteria must be supplied with nutrients or be able to use components of the liquid or solid phases as a nutrient source. The status of bacteria needs to be considered since the transport behavior of metabolically active and nonactive bacteria cells may exhibit significant differences. Microbiological effects associated with the activity of the cells have typically been neglected in transport studies, but may prove to be significant for many practical bioremediation applications.

Differences in transport and deposition behavior can be caused by bacteria surface properties (6, 8, 11) that will influence interactions with the surfaces of solid soil particles (8). The nature of bacteria cell surfaces is a function of their physiological state (3, 12). It has been reported in the literature that the growth phase influences the cell surface hydrophobicity (13–15) and electrophoretic mobility (16). The hydrophobicity of bacteria surfaces has an important influence on their attachment to a solid matrix (3, 11, 13). Since hydrophobicity also plays a role in the initial adhesion of bacteria to the air–water interface (4, 17, 18), its influence on bacteria transport under unsaturated conditions can also be expected.

Incorporation of microbial processes into transport models has typically proceeded along two separate lines of investigation (19): bacteria transport has commonly been considered to be similar to that of inert colloids (4, 10); or the biodegradation of dissolved contaminants has been
modeled in the presence of a stationary bacteria that responds to various concentrations of substrates and electron acceptors (20—22). Since changes in metabolic activity may significantly alter the deposition rates of bacteria to solid surfaces, we have investigated how the physiological state of the bacteria impacts their movement through unsaturated systems and how these processes and factors can be mathematically modeled. An existing transport model that simultaneously accounts for attachment/detachment and time- and depth-dependent straining processes (23) has been expanded to consider possible changes due to cellular division and to take into account the more dynamic situations resulting from the metabolic activity of bacteria.

Materials and Methods

**Bacteria.** *Deinococcus radiodurans* (DSMZ 20539) is a Gram-positive, nonmotile, non-spor-forming, spherical (diameter 1.0—1.5 μm), obligate aerobic bacterium. Bacteria were grown on the agar plates consisting of ATCC Medium 220: CASO AGAR (Merck 105458), peptone from casein (15.0 g), peptone from soy meal (5.0 g), NaCl (5.0 g), agar (15.0 g), and distilled water (1000.0 mL). For column experiments the cells were cultivated at 30 °C in a nutrient broth that was agitated at 130 rpm using a thermostated shaker (Cortemax HK BRAUIN). The nutrient broth consisted of casein peptone, tryptic digest (10.0 g), yeast extract (5.0 g), glucose (5.0 g), NaCl (5.0 g), and distilled water (1000.0 mL). The bacteria cells were harvested from the nutrient broth during the log phase by gentle centrifugation (10 min, 7100 g) and resuspended in fresh media.

More detailed information on the surface characteristics and morphology of *Deinococcus radiodurans* has been previously reported (11). The low degree of hydrophobicity of this bacterium was quantified using the Microbial Adhesion to Hydrocarbon (MAH) approach (24).

**Sand.** Sterile fused silica sand (Teco-Sil, C-E Minerals Greenville, USA) was used as the porous medium in the column experiments. The median grain size and the coefficient of uniformity of this sand were determined to be 567 μm and 1.87, respectively. The van Genuchten (25) water retention curve and hydraulic conductivity function parameters were determined from multistep outflow data and inverse modeling (26). According to this approach, the porosity was 0.428, the saturated hydraulic conductivity was 0.576 cm min⁻¹, and the empirical parameters α, n, and l were 0.0234 cm⁻¹, 12.13, and 3.617, respectively (11).

**Column Experiments.** Only stationary phase bacteria could be used in transport experiments conducted at 100% water saturation because these bacteria are obligate aerobes. Both stationary-phase and log-phase bacteria were used in unsaturated transport experiments (80 and 40% water saturation). Description of the setup used in the packed column experiments has been previously reported (11). Only an abbreviated discussion is provided below.

The experimental column was constructed from Plexiglas and was 8 cm in diameter and 22.5 cm long. The air phase in the column was maintained at atmospheric pressure by means of two aeration openings. The column was equipped with tensiometers and pressure transducers near the top, in the middle, and at the bottom of the column to measure the matrix potential and to verify the uniformity of water saturation within the column under steady-state unit gradient (uniform matrix potential with depth) conditions. At the top of the column a sprinkling plate equipped with 80 stainless steel needles was used to evenly distribute the influent over the sand surface at a rate of 165—170 mL/h. The water and the solution were supplied to the sprinkling plate using a piston pump. The bottom was formed by a porous stainless-steel plate covered with a hydrophilic membrane (polyester fabric, mesh size 15 μm). The suction at the bottom of the column was adjusted to achieve unit gradient by changing the drip point elevation of a hanging water column. Steady-state water flow conditions were kept constant for the remainder of the transport experiment. The total column water content was continuously monitored gravimetrically using a digital balance. The column bottom was connected sequentially with PVC tubes to a conductivity probe (tracer), spectrophotometer, and UV detector (bacteria). Both the bacteria and the tracer outflow concentrations were monitored online.

For full water saturation (100%), the column was completely evacuated using a vacuum pump and subsequently flushed from the top with degassed buffer solution. In experiments conducted at 80% water saturation, water was introduced into the column by slowly pumping upward at a constant rate until a water saturation of approximately 80% was achieved. In contrast, water saturation of 40% was obtained by gradually reducing the inflow rate at the top while at the same time increasing the suction pressure at the bottom until unit gradient conditions were achieved (11). Before initiating the transport experiment with stationary phase cells, the sand pack was equilibrated by flushing it with several pore volumes of phosphate buffer solution (pH = 7 and 10⁻¹M). The solution used for the experiment with metabolically active bacteria was composed of peptone from casein (15.0 g), peptone from soy meal (5.0 g), NaCl (5.0 g), and distilled water (1000.0 mL) diluted 1000 times.

The final spatial distribution of retained bacteria was measured following each transport experiment according to the procedure described elsewhere (11). The deposition profile of retained cells per gram of dry sand was determined from measured values of the total organic carbon content (TOC) in the sand. Experimental mass balance was calculated independently for the cells in the effluent and retained cells and normalized by the total number injected into the column.

**Theoretical Basis.** The HYDRUS-1D code (27) was used to characterize the experimental data collected in this study. The bacteria transport was modeled using a modified form of the convection-dispersion equation, in which 2 kinetic adsorption sites were considered, and first-order kinetic growth was assumed on the solid and liquid phases. The mass balance equation is defined as follows:

\[
\frac{\partial \theta c}{\partial t} + \rho_b \frac{\partial s_1}{\partial t} + \rho_b \frac{\partial s_2}{\partial t} = \frac{\partial}{\partial x} \left( \theta b \frac{\partial c}{\partial x} \right) - \rho_b \frac{\partial c}{\partial t} + \mu_a \rho_b + \mu_b \rho_b
\]

\[s_1 + s_2 \]

where \(\theta\) is the volumetric water content; \(\rho_b\) [ML⁻³]; where M and L denote units of mass and length, respectively, is the soil bulk density; \(l\) [T]; \(T\) denotes units of time) is the time; \(q\) [LT⁻¹] is the flow rate; \(\alpha\) [L] is the spatial coordinate; \(D\) [LT⁻²] is the dispersion coefficient; \(C\) [N·L⁻⁴]; where \(N\) is the number of bacteria cells) is the bacteria concentration in the aqueous phase; \(s_i\) [N·L⁻³] and \(s_j\) [N·L⁻³] are the solid-phase concentrations associated with sites 1 and 2, respectively; and \(\mu_a\) [T⁻¹] and \(\mu_b\) [T⁻¹] are the liquid- and solid-phase growth coefficients, respectively.

Several studies (23, 28) divided sorption sites into two fractions \((s = s_1 + s_2)\) and assumed different rates or processes occurring at each sorption site. Bradford et al. (23) assumed that the first fraction of sorption sites, \(s_1\), represents processes of attachment/detachment and the second fraction, \(s_2\), represents irreversible straining. The mass balance equation for the solid phase is given as follows when growth on the solid phase is also considered:

\[
\rho_b \frac{\partial s_1}{\partial t} + \rho_b \frac{\partial (s_1 + s_2)}{\partial t} = \theta k_{s_1} C - k_{s_1} s_1 + \theta k_{s_2} C + \mu_b s_2
\]
where $k_a$ [T$^{-1}$] is the attachment coefficient; $k_d$ [T$^{-1}$] is the detachment coefficient; $k_{str}$ [T$^{-1}$] is the straining coefficient; and $\psi_1$ and $\psi_2$ are dimensionless colloid retention functions that account for time- and depth-dependent deposition on site 2, respectively. In this analysis, attachment to the solid phase and the air–water interface can be lumped in the $k_a$ term. Similarly, straining in pores and water films is also considered to be lumped using the $k_{str}$ term.

Bradford et al. (23) hypothesized that the influence of depth-dependent straining processes on colloid retention can be described using the following coefficient:

$$\psi_x = \left( \frac{d_c + x - x_0}{d_c} \right)^{-\beta}$$  (4)

where $d_c$ [L] is the median diameter of the sand grains, $x_0$ [L] is the coordinate of the location where the straining process starts (in this case the surface of the soil profile), and $\beta$ is an empirical factor controlling the shape of the spatial distribution.

The code allowed us to simultaneously fit the bacteria transport parameters ($k_a, k_d, k_{str}, \beta, \mu_1$ and $\mu_2$) while considering both the breakthrough curve and the retention profile in the nonlinear least-squares optimization routine. To minimize the potential for non-unique parameter fits, we used available experimental information to limit the number of parameters that were considered in these optimizations. Our selection procedure for parameter optimization will be discussed below in the Model Applications section. The mean pore water velocity and dispersivity that were used in simulations reported herein were obtained by fitting the solution of the convective dispersion equation for the tracer breakthrough curve.

Results and Discussion
Growth Curve and Hydrophobicity. Figure 1 shows the growth curve of *Deinococcus radiodurans* obtained by monitoring the cells suspension optical density (OD) and the bacteria surface hydrophobicity (measured using the MATH test) over the course of the bacterial growth curve. The first increase of the OD was detected between 6 and 7 h, with its value increasing from 0.06 to 0.08, indicating the end of the lag phase and the beginning of the log phase (bacteria binary division). After 28–29 h the OD stopped changing. This observation was clear evidence that the log phase was completed and the cells’ metabolic activity ended. After about 30 h the bacteria culture entered the stationary phase; i.e., only weak metabolic activity and no increase in the number of bacteria.

Inspection of Figure 1 demonstrates that the growth conditions influence the cell surface hydrophobicity of *Deinococcus radiodurans*. The initial hydrophobicity percentage was zero and nearly constant, with values scattered between 0 and 3%, during the lag phase. The hydrophobicity then increased during logarithmic growth, reaching a maximum value of almost 10% at the beginning of the stationary phase. As the stationary phase advanced, the hydrophobicity started to decrease and reached the initial value of 0% after about 100 h. In summary, during a period of high growth rates the bacteria were observed to become more hydrophobic, reaching a maximum value at the end of the log phase and the beginning of stationary phase, whereas lower hydrophobicity was measured during the subsequent phase of nutrient starvation, until it returned to its initial value.

These results are in agreement with other observations reported in the literature that indicate starvation decreased bacteria hydrophobicity and their adhesion to solid surfaces (13–15, 29, 30). Such changes in bacteria hydrophobicity have been attributed to changes in the surface molecular composition during the different life phases of the bacteria. Proteins and polysaccharides are molecules involved in interactions of bacteria cells with the solid interface (31). Since proteins and amino acids are hydrophobic components of extracellular polysaccharide, increased numbers of these molecules cause an increase in the surface bacteria hydrophobicity (4). Moreover, it was observed that bacteria starvation caused a decrease in the concentration of surface proteins (32). The bacteria hydrophobicity observed during the growth stage may be attributed to an initial increase in the amount of proteins on the bacteria surface during the log phase. A subsequent decrease in hydrophobicity may thus be caused by the decrease in the surface proteins during cell starvation.

Gargiulo et al. (11) reported on differences in shape and morphology of the log and stationary phase *Deinococcus radiodurans*. Log phase *Deinococcus radiodurans* tend to form pairs and tetracoccus-shaped aggregates of around 2–4 μm in diameter when grown in nutrient broth. Conversely, stationary phase *Deinococcus radiodurans* occurred primarily as single cells. Differences in the size of log and stationary phase *Deinococcus radiodurans* likely occurred as a result of differences in cell surface hydrophobicity (Figure 1). Colloid stability and aggregation is reported to be sensitive to the surface hydrophobicity (33, 34); i.e., hydrophobic colloids are less stable than hydrophilic colloids.

Decreasing hydrophobicity of soil bacteria during starvation has been recognized to be a result of a survival strategy (14). Under optimal growth (nutrient) conditions, it is beneficial for soil bacteria cells to be more hydrophobic and to firmly attach to solid surfaces and to each other. On the other hand, under unfavorable nutrient conditions, the hydrophilic microorganisms are smaller (less aggregation) and may more easily detach themselves from solid surfaces and be transported in the liquid phase to a new location in the environment that is richer in nutrients.

Transport and Deposition. Table 1 provides mass balance information for the bacteria transport experiments. Very good mass balance was obtained for the stationary phase bacteria (100–102%), and provides a high degree of confidence in our experimental procedures. Conversely, it was not possible

| TABLE 1. Percentage of Bacteria in the Outflow and Retained in the Column |
|-----------------------------|-----------------|----------------|-----------------|----------------|
| saturation                 | log phase       | stationary phase |
|                           | outflow         | tail       | retained        | outflow | retained |
| 80%                        | 75%             | 6%          | 45%             | 88%   | 14%      |
| 40%                        | 39%             | 8%          | 87%             | 47%   | 53%      |
TABLE 2. Model Parameters for Deinococcus radiodurans in Different Growth Phases and at Different Saturations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>100%</th>
<th>S.E.coef.</th>
<th>80%</th>
<th>S.E.coef.</th>
<th>40%</th>
<th>S.E.coef.</th>
</tr>
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<td></td>
<td>1.69 x 10^-4</td>
<td></td>
<td>2.38 x 10^-4</td>
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<td>k_d (min^-1)</td>
<td>2.27 x 10^-3</td>
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<td>2.13 x 10^-2</td>
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<td>2.78 x 10^-2</td>
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<td>k_m (min^-1)</td>
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<td>1.27 x 10^-3</td>
<td></td>
<td>1.73 x 10^-2</td>
<td></td>
</tr>
<tr>
<td>ß</td>
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<td></td>
<td>0.432</td>
<td></td>
<td>0.996</td>
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<tr>
<td>R^2 (btc^4)</td>
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<tr>
<td>R^2 (rp^4)</td>
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<td></td>
<td>0.980</td>
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</table>

**Deinococcus radiodurans (stationary phase)**

<table>
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<tr>
<th>Parameter</th>
<th>100%</th>
<th>S.E.coef.</th>
<th>80%</th>
<th>S.E.coef.</th>
<th>40%</th>
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<td>k_d (min^-1)</td>
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<td>N/A</td>
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<tr>
<td>ß</td>
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<td>N/A</td>
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<td>4.87 x 10^-4</td>
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<tr>
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<td>N/A</td>
<td></td>
<td>0.432</td>
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</tr>
<tr>
<td>R^2 (rp^4)</td>
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<td></td>
<td>N/A</td>
<td></td>
<td>0.957</td>
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</table>

**Deinococcus radiodurans (log phase)**

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<th>Parameter</th>
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<th>80%</th>
<th>S.E.coef.</th>
<th>40%</th>
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<td>ß</td>
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<td>N/A</td>
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<tr>
<td>R^2 (rp^4)</td>
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<td>N/A</td>
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</table>

* btc = breakthrough curve; rp = retention profile.
nomenon was attributed to cell growth. We hypothesize that the pronounced tailing was due to continuous bacteria multiplication in the solution and due to the mechanism called “cell division mediated transport” (37, 38). During cell division mediated transport the mother cells deposit on the mineral surface, grow, divide, and finally release the daughter cells into the aqueous phase.

The retention profiles at all saturations and for both active and nonactive cells were not exponential with depth as predicted by classical filtration theory (see values of $\beta$ in Table 2). The deposition profiles exhibited invariably greater retention in the section adjacent to the column inlet (Figures 3Band 4B). This incongruence with CFT has been reported by other researchers under both saturated (5, 23, 39-44) and unsaturated (10, 11, 36) conditions. The log-phase bacteria showed a more pronounced presence of bacteria at the column inlet than stationary bacteria at both saturations (80 and 40%). The log phase bacteria likely had enhanced cell–cell interactions and straining at the column inlet presumably due to the presence of more extensive protein molecules on the bacterium surface that caused “polymer bridging” and due to their larger cell/aggregate sizes. The relative number of bacteria present throughout the soil column is also higher in the case of log-phase cells compared with the stationary-phase cells. This observation can be attributed to enhanced attachment due to different surface characteristics of the metabolically active bacteria compared with the resting mode cells. In particular, proteins on the surface of active bacteria cells enhance the hydrophobicity and attachment along the soil packing.

Model Application. For stationary phase transport experiments the values of $\mu_w$ and $\mu_s$ were considered equal to zero because no growth occurred, and attachment ($k_a$ and $k_d$) and straining ($k_{str}$) model parameters were fit to breakthrough curves and deposition profiles. Model fits were more complicated for log phase transport experiments because growth also occurred in the liquid and solid phases. In this case, attachment ($k_a$ and $k_d$), straining ($k_{str}$), and growth ($\mu_w$ and $\mu_s$) model parameters were fit to breakthrough curves and deposition profiles. To minimize the number of fitting parameters that were optimized, the value of $\beta$ in eq 4 was set equal 0.432 based on previously published findings (23) when significant straining occurred. According to this modeling approach the influence of attachment and straining processes dominate different regions of the deposition profile (23); i.e., straining dominates near the sand surface and attachment dominates at greater transport distance.

Very good agreement was obtained between the observed and simulated data for the metabolically active and resting phases of the bacteria at both saturations (Figures 3A and 4B). Values of optimized parameters are summarized in Table 2. The attachment, $k_a$, detachment, $k_d$, and straining, $k_{str}$, coefficients increased as the saturation decreased (Table...
2) for both the resting mode cells and the log-phase bacteria. The attachment coefficient, which lumped attachment to the solid phase and the air-water interface, slightly increased as saturation decreased, probably due to the enhanced attachment to the air-water interface as the air-water interfacial area increased (11). Increasing values of $k_a$ with decreasing saturation, suggests that attachment to the air-water interface is approaching linear equilibrium conditions (45). Straining has been hypothesized to increase with decreasing water saturation because more of the water flux flows through small straining sites at lower saturations (11).

For a given water saturation, the value of $k_{str}$ was very similar for stationary- and log-phase cells. This finding suggests that straining is not much affected by the growth stage for these bacteria. In contrast, parameter values for $k_a$ and $k_{str}$ were very dependent on the growth stage of the cells. In particular, the values of $k_{str}$ were significantly lower for metabolically active cells. This observation indicates that stronger cell-cell interactions occurred for attached bacteria under active growth conditions. It is well-known that many bacteria are capable of forming a biofilm. Under certain conditions the bacteria cells communicate with each other by means of a process called quorum sensing and detect when they are assembled in large numbers as opposed to when they are essentially alone (46). According to the theory, bacteria in favorable growing conditions secrete and sense small signalling molecules to communicate and to promote biofilm formation. The metabolically active cells are not likely to reject each other and the deposition of one cell should not inhibit the deposition of the next cell. The cells try to get as close as possible to each other to form a more resistant structure. This effect may be further enhanced by increased hydrophobicity of metabolically active bacteria and the associated higher number and extension of proteins on the surface of these bacteria.

Mass balance information after recovery of the concentration plateau region of the breakthrough curve (before significant detachment occurred) also supports this enhanced cell-cell interaction hypothesis. Attachment for active and stationary cells accounted for 59.1 and 14.9% of the retained cell mass at a water saturation of 80%, respectively. Similarly, attachment for active and stationary cells accounted for 35.2 and 16.6% of the retained cell mass at a water saturation of 40%, respectively. Hence, increased deposition of log-phase compared to stationary-phase bacteria are attributed primarily to attachment. Straining was always the dominant mechanism of retention (>83.4%) for the stationary-phase bacteria. For log-phase cells, however, straining accounted for 40.9 and 64.9% of the retained cells at a water saturation of 80 and 40%, respectively.

The plateau region of the breakthrough curves shown in Figure 2 did not change much with time for the log-phase bacteria transport experiments, and suggests that $\mu_w$ was low and only accounted for a small fraction of $C_0$. Preliminary model fits to this data also indicated that the value of $\mu_w$ was very low ($\approx 0.46 \times 10^{-4}$ min$^{-1}$) and that the value of $\mu_w$ was significantly higher (1 order of magnitude) than that of $\mu_t$. This result indicates that the deposited cells have a higher division rate compared with the cell in the bulk solution. To minimize the potential for non-unique parameter fits, we therefore set the value of $\mu_t$ to zero in subsequent simulations with log-phase cells. Table 2 provides values of $\mu_w$ when $\mu_t$ was set equal to zero. The tailing of the bacteria outflow concentrations could be well described by this model formation at both saturations (80% and 40%) (Figures 3A and 4A). These observations support our hypotheses that deposited bacteria are particularly active in biofilm formation under favorable growth conditions and that the observed tailing in the breakthrough curves was primarily due to release of the daughter cells into the aqueous solution from attached mother cells (cell-division mediated transport).

Results from this study clearly indicate that the physiological state of the bacteria will influence its transport and deposition in soils producing a more dynamic system and resulting in different effluent breakthrough curves and deposition profiles for metabolically active bacteria. Additional studies are warranted to better understand bioremediation processes and to design more effective technologies. For example, further transport studies with different bacteria strains during their active states, and to better deduce mutual bacterial interactions, are desirable. The coupled influence of various pollutants, nutrients, substrates, and electron acceptors on the physiological state of the bacteria and their transport potential are topics of future studies.

Acknowledgments

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