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Microcosm Experiments and Modeling of Microbial Movement Under Unsaturated Conditions

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Abstract

Colonization of bacteria in porous media has been studied primarily in saturated systems. In this study we examine how microbial colonization in *unsaturated* porous media is controlled by water content and particle size. This is important for understanding the feasibility and success of bioremediation via nutrient delivery when contaminant degraders are at low densities and when total microbial populations are sparse and spatially discontinuous. The study design used 4 different sand sizes, each at 4 different water contents; experiments were run with and without acetate as the sole carbon source. All experiments were run in duplicate columns and used the motile organism *Pseudomonas stutzeri* strain KC, a carbon tetrachloride degrader. At a given sand size, bacteria traveled further with increasing volumetric water content. At a given volumetric water content, bacteria generally traveled further with increasing sand size.

Water redistribution, solute transport, gas diffusion, and bacterial colonization dynamics were simulated using a numerical finite-difference model. Solute and bacterial transport were modeled using advection-dispersion equations, with reaction rate source/sink terms to account for bacterial growth and substrate utilization, represented using dual Monod-type kinetics. Oxygen transport and diffusion was modeled accounting for equilibrium partitioning between the aqueous and gas phases. The movement of bacteria in the aqueous phase was modeled using a linear impedence model in which the term D_m is a coefficient, as used by Barton and Ford (1995), representing random motility. The *unsaturated* random motility coefficients we obtained (1.4 x 10^{-6} to 2. 8 x 10^{-5} cm^2/sec) are in the same range as those found by others for *saturated* systems (3.5 x 10^{-6} to 3.5 x 10^{-5} cm^2/sec). The results show that some bacteria can rapidly migrate in well sorted unsaturated sands (and perhaps in relatively high porosity, poorly sorted unsaturated sands – see Figure 5) at volumetric water contents that naturally occur in many sandy vadose zones.

Background

In situ bioremediation of contaminants can offer advantages in cost, speed, public acceptance, and final cleanup levels achieved relative to physical removal methods. However, microbial populations in the unsaturated (i.e., vadose) zone are sparse and spatially discontinuous, especially in deep vadose zones and in arid climates with very low moisture and nutrient flux. Delivery of aqueous or vapor phase nutrients to increase microbial populations and activity in the vadose zone is possible. However, for the vadose zone, there is a lack of knowledge on:

(1) The ability of microbes to colonize, via motility and growth processes, uninhabited sediment volumes in response to nutrient delivery, and
(2) How microbial colonization is controlled by water content and particle size.

The objectives of this research were to address these knowledge gaps in static unsaturated column experiments using well sorted, near-spherical quartz sand.

Approach

Washed, grade 20/30 (0.71 mm diameter), 30/40 (0.53 mm diameter), 40/50 (0.36 mm diameter) and 50/70 (0.21 mm diameter) Accusands were used. Volumetric water contents were selected to provide for different degrees of pore-scale water connectivity and water film thicknesses.

Columns were constructed by removing the tip end of a plastic syringe, adding 1.0 cm^3 oven-dry sand to the bottom of the column (inoculation zone), adding 2 x10^7 washed cells to the dry sand in the appropriate volume of mineral salts to achieve the desired water content, adding pre-wetted sand in small increments (prepared with mineral salts and centrifugation to achieve the desired water content) to the remainder of the column, removing air voids as sand increments were added by applying minimal pressure with a packing implement, and completing the column by sealing the syringe with a rubber stopper (Figure 1).

Paired treatments (each run in duplicate) included mineral salts:
- lacking acetate in the inoculation and colonization zones
- containing acetate (3.5 mM) in the colonization zone

Columns were stored at room temperature in racks in the position depicted in Figure 2 for various lengths of time, the porous media extruded and sampled in 1.0 cm^3 (0.6 linear cm) increments, and plate counts performed.

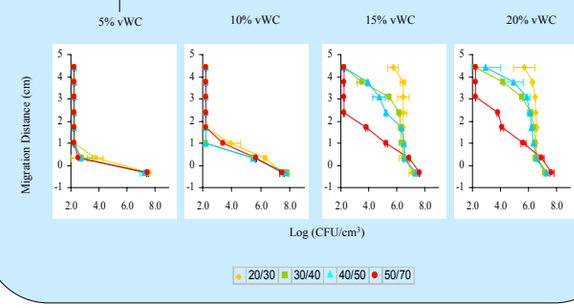
To enable modeling of microbial movement, water distribution profiles were determined in parallel replicate columns (at all volumetric water contents in all sands) at various points in time. In addition, growth kinetics were determined in batch cultures under the nutrient conditions that existed in the column studies.

An overview of the modeling approach is described in the abstract. Using the approach of Barton and Ford (1995, *Appl. Environ. Microbiol.*), equations developed for modeling molecular diffusion were applied to describe bacterial movement in fully saturated cases using a 'random motility coefficient'. The term 'random' is used to distinguish this motility from experiments designed to possess a gradient in an attractant to induce chemotactic motility. For application to unsaturated cases, we used an equation for modeling molecular diffusion in unsaturated conditions (Olesen et al. 1996, *Soil Science*) and incorporated the random motility coefficient. Effective diffusion coefficients, D_{eff}, were calculated for each experimental condition using the equation,

$$D_{eff} = D_m 0.45\theta\left(\frac{\theta-\theta_c}{\theta_s-\theta_c}\right)$$

where D_m is the random motility coefficient, θ is the volumetric water content, θ_c is a critical water content for transition from connected to non-connected water films, and θ_s is the saturated water content or porosity. The random motility coefficient was estimated by optimization to experimental data. The flow and transport equations used in this work are based on a continuum assumption. Hence the parameters in these equations represent effective properties at a macroscopic scale, rather than microscopic (e.g. pore or molecular) scales.

Figure 2. Effect of sand size and vWC on bacterial movement after 24 hr in the absence of acetate as carbon source. Each panel shows bacterial profiles for the 4 sands. At a given volumetric water content, bacteria generally traveled further with increasing sand size.



Nearly all of the bacterial movement observed at 24 hr after inoculation in both the absence and presence of acetate was found to be the result of a very rapid physical process during the first 10 *minutes* post-inoculation. This was demonstrated with experiments in which both colored microspheres of differing charge and bacteria were added to columns. The movement was not due to extrusion of the sand during sectioning, because use of an alternative method (a heated razor to quickly slice/melt through the plastic and section the column) did not change the resulting bacterial distribution. Water distribution curves at 10 minutes and 24 hours demonstrated that macroscopic water redistribution (i.e., partial gravity-driven drainage) was complete after 10 minutes. Thus, we believe that the rapid and extensive movement of bacteria resulted from a Marangoni-like effect (Adamson and Gast, 1997, Physical Chemistry of Surfaces, John Wiley and Sons) caused by microscopic chemical or hydrological turbulence at gas-liquid interfaces that results in rapid spreading and rafting of bacteria by convective flow along these surfaces.

Figure 3. Effect of vWC and sand size on colonization after 24 hr in the presence of acetate as carbon source. Each panel shows CFU bacterial profiles for the 4 vWC's. At a given sand size, bacteria traveled further with increasing volumetric water content.

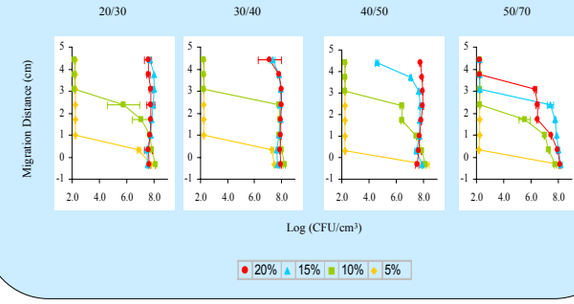


Figure 1. Experimental system in 10 cm^3 plastic syringe for determining colonization profiles.

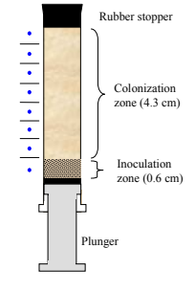


Figure 4. Rates of acetate-driven colonization observed in 24 hr experiments (Fig. 3) were maintained over a 7 day period. Curves are for 40/50 sand at 3 different vWCs. Note the columns are 6x longer than in Figures 2 and 3.

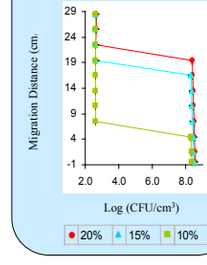
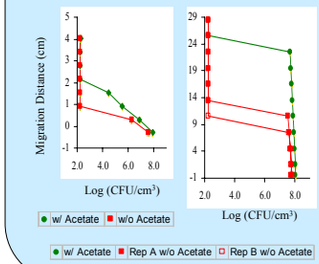
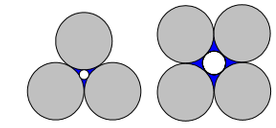


Figure 5. Effect of acetate on microbial distribution in a poorly-sorted non-spherical sand at 20% vWC. Panel A, 24 hr; panel B, 7 days. Rates of acetate-driven colonization were maintained over a 7 day period. Although porosity was higher in the repacked column compared to in situ, colonization in poorly-sorted non-spherical sand was similar to that in well-sorted highly spherical sand.



Calculation of the theoretical water content at which bacteria would not be expected to move from pore to pore in our experiments. The critical water content, θ_c, below which diffusive transport at the macroscopic scale is effectively zero, was calculated from the geometries and volumes of pendular rings, depicted here in blue. This figure shows pore scale cross sections through hypothetical porous media consisting of uniform, spherical particles (similar to the uniform grain size, highly spherical quartz sands we used) for two different types of packing: close pack (on left) and cubic pack (on right).



Close pack (θ_s = 0.2596, θ_c = 0.0631)

Cubic pack (θ_s = 0.4764, θ_c = 0.0867)

The average porosity of our packed columns was 0.426, which is in between the theoretical values for the ideal packings shown in the Figure. The critical water content, θ_c, represents the volumetric water content at which water transitions from a funicular (connected) to a pendular (disconnected) state. This water content was calculated for the porosity of our sand packs by interpolation of the critical water contents determined for the two ideal packings. This critical value was θ_c = 0.081. Thus the 10% vWC columns would contain connected water while the 5% vWC columns would not.

Results of Model Simulations

Figure 6. Example of observed and simulated bacterial distributions in the presence of acetate in 40/50 sand at 15% vWC. The 10 minute curve was used as the initial distribution, and the model was used to simulate the growth of bacteria while fitting the observed points to determine the random motility coefficient. Simulations were run for 4 sands x 4 vWC's with and without acetate; random motility coefficients are plotted in Figure 7.

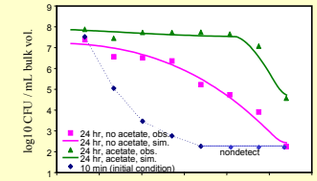
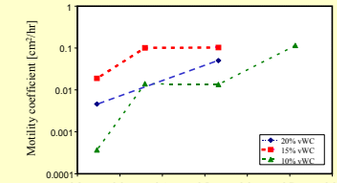


Figure 7. Random motility coefficients as a function of vWC and sand size in the presence of acetate. Values are not shown for cases where bacterial profiles were flat or pore water became unconnected; in both cases the model is unable to solve the equations. Decreasing values at smaller grain diameters are due to decreasing minimum water film thickness in the connected pore water, which restrict bacterial motility. The same pattern and range of values was found for the no acetate data sets.



Conclusions

- The random motility coefficient becomes effectively zero at the vWC where water in pores transition from a fenicular (connected water film) regime to a pendular (disconnected) regime. Our experimental data matched theoretical predictions.
- The random motility coefficient decreases with smaller median grain size
- These reductions in the random motility coefficient are due to shorter mean free path lengths available for flagella-mediated swimming. Barton and Ford showed this effect in saturated porous media. We show the same effect for the first time in unsaturated porous media, where water films interrupt the length of runs by motile bacteria.
- The random motility coefficients we obtained (0.005 to 0.1 cm^2/hr, equal to 1.4 x 10^{-6} to 2. 8 x 10^{-5} cm^2/sec) are in the same range as those found for saturated systems (3.5 x 10^{-6} to 3.5 x 10^{-5} cm^2/sec). This indicates *Pseudomonas stutzeri* KC does not significantly partition to air-water interfaces, some researchers have suggested these interfaces attract and 'trap' bacteria via surface tension effects. Alternatively, such trapping may occur to some extent but be counteracted by a portion of the population able to avoid shorter mean free path lengths and accelerate migration by passively or actively avoiding interfaces.
- The results show that some bacteria can rapidly migrate in well sorted unsaturated sands (and perhaps in relatively high porosity unsaturated poorly sorted sands – see Figure 5) at volumetric water contents that naturally occur in many sandy vadose zones.
- Addition of gaseous or soluble nutrients to contaminated sandy vadose zones appears to be a feasible technology for nutrient-driven colonization of low biomass and/or heterogeneously colonized vadose zone environments.

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