The Effect of Soil Heterogeneity on the Vadose Zone Transport of Bacteria for Bioaugmentation

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ABSTRACT: Heterogeneity in hydraulic, physical and chemical properties of porous media can not only limit microbial dispersion but may also complicate the quantification of microbial transport processes and resultant microbial activities. The objectives of this research were to determine the potential for bacterial transport through an unsaturated soil block under transient flow conditions and to determine the influences of soil properties and phosphate additions. Despite the block consisting of >99% sand and appearing to be completely homogeneous, i.e., structure-less, flow was extremely heterogeneous as only 6-16% of the cross-sectional area exhibited flow with 88% of this flow occurring through just 4% of the area. The preferential flow paths exhibiting high and moderate flow rates were spatially consistent among rainfall events suggesting that soil properties caused the heterogeneity in flow rather than unstable wetting fronts. Transport of the GFP bacteria was extremely rapid with breakthrough occurring at the initiation of flow (0.1 h). Bimodal breakthroughs of GFP bacteria were observed for fast flow areas. The soil texture, rather than porosity, was the most significant property controlling the microbial transport as areas dominated by fine sand trapped the GFP bacteria. These findings demonstrate how apparent homogeneity in media properties does not equate with homogeneity in flow or transport of solutes and colloids. While bioremedial feasibility studies often center on soil chemical properties, this study indicates that consideration should be given to the physical and hydraulic properties of the soil as well.

INTRODUCTION

Most bioremedial practives involve the utilization of indigenous populations to degrade existing contaminants, however, recent studies have suggested that genetically engineered microorganisms (GEM's) could increase the efficiency of bioremediation. Substrate concentrations and cell density are important factors that determine the kinetics of contaminant biodegradation (Alexander and Scow, 1989). To insure contaminant degradation, bacteria and bio-stimulators (i.e. substrates) are often required to be transported throughout the contaminated area. Sediment heterogeneity (i.e. physical, chemical, and hydraulic properties variation) plays a significant role in determining the fate of microbial populations during transport. Water movement through soils with preferential flow pathways or immobile-regions could significantly decrease bioremediation efficiency by preventing contaminant degrading bacteria from reaching the contaminants that are dispersed within the soil matrix.

Objective. The objectives of this research were to determine the potential for bacterial transport through an unsaturated soil block under transient conditions and to determine the influences of soil properties and phosphate additions on bacterial transport and retention under unsaturated conditions.

Site Description. An undisturbed block was obtained from the 5.9 m depth, i.e the top of the groundwater table, at a DOE research site approximately 4 miles east of the township of Cheriton in Northhampton County, Virginia on the Chesapeake Bay peninsula. The soil at the sampling location was the Molena series (Sandy, mixed, thermic Psammentic Hapludults) and consists of coarse texture sediments. This soil is a strong brown loamy sand (0.71 m thick), with a substratum of strong brown sand to a depth of 1.8 m or more (USDA, 1989).

MATERIALS AND METHODS

The lack of soil structure in the underlying sand allowed a 35 cm x 35 cm x 70 cm pedon to be easily shaped in the face of a borrow pit at the site. A stainless steel box (32 cm x 32 cm x 50 cm), with open ends, was constructed to encase the undisturbed block. The stainless steel box was hydraulically pressed into the exposed pedon with negligible soil disturbance (Kinsall et al., 1997). A stainless steel plate with a cutting edge was driven horizontally across the bottom to sever the bottom of the encased block from the soil pedestal.

An acrylic grid of 64 flow collection chambers (3.75 cm x 3.75 cm) inside a 1 cm wide border flow collection area was fitted and sealed to the bottom of the stainless steel block by insetting it 1.5 cm into the soil. Each collection chamber had been partially filled with sterilized coarse sand to establish a hydrologic continuity between the soil and collection chambers. Two holes were drilled within the 1.0 cm annulus between the steel box and the 30 x 30 cm flow collection chambers to allow for collection of drainage from the border of the encased block. A rain simulator positioned above the soil block consisted of 64 application drippers spaced in a grid pattern 3.75 cm. Each dripper was controlled by a valve, which allowed for rainfall application rates to be easily adjusted as needed.

Simulated Rain Events and Effluent Sampling. A 0.001 M $CaCl_2$ solution, similar to the ionic strength of rainfall was applied to the surface of the blocks at a continuous rate of 5400 $cm^3\ h^{-1}$ for a duration of one hour, thereby simulating a 60 mm h^{-1} rainfall. The soil block received four rainfall applications, at least 2 days apart, prior to the start of the experiment to wet the soil to field capacity. Collection grids, containing Nalgene polypropylene (autoclavable) bottles, were used to collect effluent from the soil into each respective collection chamber. When the first bottle was filled to capacity, the entire collection grid of 64 bottles was removed and replaced with another grid containing 64 empty bottles, and the time recorded. Effluent was continually collected over a period of two days after each rainfall application until free drainage ceased. Each bottle containing effluent was weighed to obtain the flow rate.

A total of two rainfall applications were made on the block. During the first rainfall application, a solution containing a fluorescing bacteria, Pseudomonas putida (1×10^9 CFU L^{-1}), was applied to the surface of the block. This particular P. putida strain has been modified by the addition of the Green Fluorescent Protein (GFP) gene, which allows detection by fluorescent signal (Burlage et al., 1996). The bacterial solution was followed by two additional applications of 0.001 M $CaCl_2$, which was used to determine the flushing of phosphate and P. putida. Effluent samples from a fast, moderate, and a slow collection cell were analyzed with respect to time for microbial and chemical constituents

Soil Sampling and Analysis. Following the completion of the rainfall applications, the soil block was dissected into 5 cm depth increments with 64 individual samples (3.75 cm x 3.75 cm cell area) per depth increment. Horizontal slits, spaced at 5 cm increments, were made in the front face of the steel box at construction. These slits allowed the encased block to be sectioned into 5 cm depth increments through the use of a 3.2 mm thick stainless-steel plate. After the sheet had been pressed through the entire width of the block, the encased 5 cm layer was carefully lifted out of the steel box. The layer was then sectioned into 64, 3.75 cm x 3.75 cm area increments in a grid pattern that was aligned with the grid of 64 effluent collection cells. Samples from the 3-8 cm, 23-28 cm, and 43-48 cm depths were utilized for complete analysis. P. putida analysis for effluent and soil-extraction samples was conducted by the fluorescent signal given off by the GFP bacteria. A fluorescence spectrometer was used to determine the transmittance at a wavelength of 509 nm, with an excitable wavelength of 395 nm. The colony forming units (CFU) was determined by direct count of colonies growing on Luria-Bertani selective media which contained 50 mg tetracycline L^{-1}. The correlation between microbial concentration, i.e., CFU cm^{-3} of effluent, and the fluorescent signal was used to estimate the cells cm^{-3} of each sample. The lower limit detection in this analysis was approximately 10^1 cells cm^{-3}.

After subsamples of wet soil had been removed for microbial analysis, all samples were analyzed for bulk density(ρ_b) and gravimetric (θ_g) water content. To determine (θ_g), a 10g subsample was removed, oven dried at 105 °C and reweighed. The remaining sample was weighed wet, air-dried for two days and then reweighed. The (θ_g) of the air dried sample was also determined from a 10 g subsample. Bulk densities, volumetric and gravimetric water contents were calculated by using the samples cube volume of 3.75cm x 3.75cm x 5cm, and the wet and oven-dried weights as described by Gardner (1986). Percent porosity was calculated in each sample by using an expanded version of Danielson and Sutherland's method (1986), which involved the use bulk density and an estimated particle density of 2.65 g cm^{-3}. This method can be better described in the equation of:

$$\% \text{ Porosity} = (1 - \rho_b / 2.65 \text{ g cm}^{-3}) \times 100 \quad (1)$$

Particle size analysis of the sand block samples was conducted by using the Dry Sieving Method for fractionation of sand particles (Gee and Bauder, 1986), based on the

USDA scheme for particle sizes. Specific Surface area's were calculated by using the summation equation of**:**

$$\text{Specific surface } (a_m) = (6/p_s) \sum(c_i/d_i)(2) \qquad (2)$$

where c_i is the mass fraction of particles of average diameter d_i, and p_s is a particle density of approximately 2.65 g cm^{-3} (Hillel, 1980).

Soil chemistry was determined after soil-water extraction. Three grams of soil were combined with 27 ml of deionized water and placed on a shaker for 20 minutes. Soil solutions were thoroughly mixed and then filtered through a 0.45 µm Acrodisc filter. Effluent samples (10 ml) were analyzed for anions on the Ion Chromatograph (IC), and cations on the ICAP. A 1:9 ratio of soil/water was used for soil extractions to determine soil pH. Both soil and effluent pH was determined on an expandable ion analyzer, which is discussed by McLean (1982). Total phosphate concentrations were determined from soil extractions on the ICP and Ion Chromatograph (IC) respectively.

Pearson correlation analysis was conducted using SAS on all variables for effluent according to rain event, and for all soil property variables according to layer. Cumulative flow and effluent bacteria concentrations were also analyzed for correlation, by layer and rain event, to all soil properties of their vertically aligned samples.

RESULTS AND DISCUSSION

Spatial Distribution of Flow. During the 4 rain events, only 6-16% of the 64 collection cells exhibited outflow. The numbers of collection cells where flow was observed included 3 fast flowing cells(>400 cm/rain event), 1 medium flowing cell (200-400 cm/rain event) and 5-7 slow flowing cells (<200 cm/rain event). Spatial distributions of flow among the collection cells were consistent for all rain events, which indicates that the preferential flow paths were controlled by soil properties. The funnel flow concept, which is the occurrence of preferential flow paths through structure-less media, appears to be supported by these findings (Selker et al., 1992). Flow volumes during the first rainfall were highest of the four rainfall events (Figure 1). Approximately 98% of the applied solution was collected from the base of the block indicating that the block was at field capacity before the event. The second rainfall event, however, produced the lowest effluent volumes, with 88% of the applied solution collected from the base of the block. The two remaining events produced effluent volumes similar to the first event with

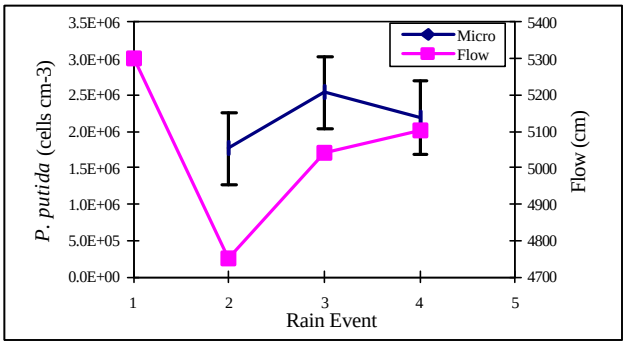

Figure 1.Total Flow vs. Averaged Effluent Microbial

collection of 94-95% of the applied solution. The first occurrence of flow was approximately 12 minutes after initiation of rainfall for all of the rain events, and occurred in the same collection cell each time.

***Pseudomonas putida (GFP)* Populations in Effluent.** GFP concentrations in collected effluent were found to vary greatly both spatially and temporally within each rainfall event. Effluent samples revealed that only 4% of the applied P. putida populations were accounted for in the collection chambers, leaving 96% remaining in the soil block. The second rainfall event produced the lowest GFP numbers(average 1.76 x 10^6 cells cm^{-3}) while the highest populations were detected during the third and fourth rainfall events (Figure 1). This increase was due to the flushing of GFP from the matrix into preferential flowpaths. A slight decrease in GFP numbers was noted during the fourth rainfall and was probably due to adsorption or depletion of populations by previous rainfall events. At any particular location, the indigenous microbial populations generally exhibited the highest concentration at the initiation of flow and as flow continued, indigenous populations decreased. Secondary peaks in GFP concentrations were seen during the recession limits of the hydrograph (Figure 2A). Such bimodal breakthrough curves are typically explained as proof of a dual pore system, in which rapid breakthrough occurs from the preferential flow paths followed by a delayed breakthrough from matrix pores. This was attributed to the flushing of previously immobile cells from low porosity areas into preferential flow pathways. However, flow occurring in areas where slower flow rates were observed produced the highest variability (Figure 2B). This fluctuation in effluent microbial concentrations was attributed to the hydrodynamic dispersion effects of a greater contribution of flow through the soil matrix regions. As flow continues, diffusion from the less mobile pore regions into the preferential flow paths is not able to maintain this initial high concentration so populations decrease.

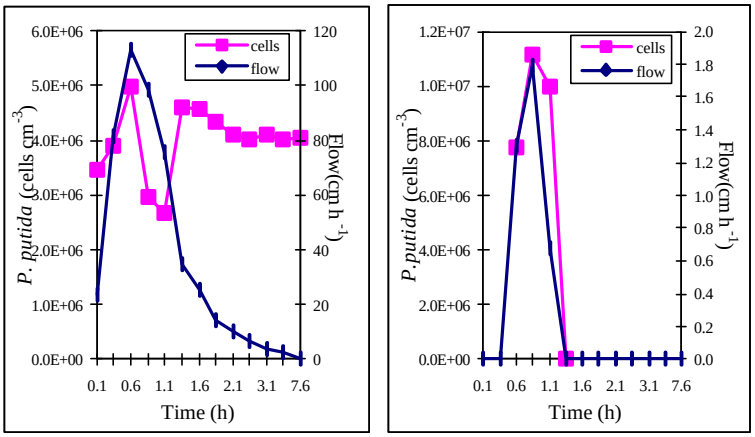

Figure 2A Figure 2B

Figure 2A. Effluent *Pseudomonas putida* Concentrations vs Flow in (A) Fast Flow Cells and (B) Medium Flow Cells.

Soil Physical Properties. In our studies, the soil particle sizes, rather than soil porosity, determined the mobility of colloids (bacteria)(Table 1). Soil porosity is based on the total porosity of a volume of soil, rather than individual pore size. Clay has a higher porosity

than that of sand, however individual pore diameters are larger in sands due to the large size of sand particles. Thus, in terms of microbial transport, bacteria could move farther in sandy soils even with a lower porosity than that of clay. While there is no correlation between porosity and flow or porosity and microbial transport, an indirect relationship does exist between soil texture and flow due to the arrangement of soil particles (soil structure). Fine sand within the 3-8cm layer of the block produced a filtering effect, which significantly lowered the mobility of GFP bacteria. The small particle sizes, such as fine sands, trapped microbes while regions with coarse sand particles allowed the microbes to be readily flushed through the soil block. The highest gravimetric water contents were detected in the samples that had the highest percentages of fine sand particles. Gravimetric water contents in both blocks were found to be relatively low, which was attributed to evaporation losses. The highest microbial concentrations were observed in the top layer (3-8cm) and middle (23-28cm) layers of the block, which consisted primarily of smaller sand particles.

Physical Property	3-8 cm	23-28 cm	43-48 cm
Porosity (%)	44.63 ± 5.38	44.88 ± 3.81	36.12 ± 3.75
Grav. Water (g g^{-1})	0.07 ± 0.08	0.20 ± 0.01	0.14 ± 0.03
Sand (%)	99.82 ± 0.40	99.84 ± 0.04	99.51 ± 0.002
* Very Coarse (%)	0.18 ± 0.19	0.52 ± 0.44	27.15 ± 7.30
* Coarse (%)	8.60 ±1.9	1.16 ± 0.57	35.4 ± 5.12
* Medium (%)	4.60 ± 1.1	2.34 ± 0.47	1.9 ± 0.70
* Fine (%)	85.1 ± 2.0	94.7 ± 0.61	34.03 ± 7.77
* Very Fine (%)	1.43 ± 0.09	1.12 ± 0.18	1.04 ± 0.33
* Residual Silt (%)	0.09 ± 0.03	0.16 ± 0.04	0.22 ± 0.13
Surface Area (cm^2 g^{-1})	57.70 ± 0.24	57.76 ± .003	57.60 ± 0.08
Bacteria (cells kg^{-1})	$1.10 \times 10^{12} \pm 5.79 \times 10^{12}$	$1.07 \times 10^{12} \pm 1.50 \times 10^{11}$	$5.26 \times 10^{11} \pm 2.26 \times 10^{11}$

Table 1: Mean Values and Standard Deviations for Soil Physical Properties and *Pseudomonas putida* Concentrations for the Soil Block (*Size fractions defined by the USDA scheme.)

Soil Chemical Properties. Soil chemistry was found to be less influential on the distribution of microbial populations than the physical properties of the soil. Phosphate concentrations were highly correlated with microbial distributions within the layers. It was expected that retention to iron and magnesium oxides greatly inhibited phosphate transport. While phosphate distributions were essentially limited to the surface layers of both blocks, the highest concentrations were detected in the lower porosity areas. Limited phosphorous mobility was also reported by Jones and Lee (1977). They observed that phosphorus movement was greater in coarse sands, while effective P removal occurred in soils composed primarily of medium and fine sands. Which also agrees with findings from Rubaek et al., (1999). They reported that total and organic phosphorus contents in soil samples increased as soil particle sizes decreased. Correlations between phosphate contents and soil pH were difficult to interpret in the soil layers, since most of the samples from both blocks had a slightly acidic to neutral

pH, which is the conditions in which phosphate is most available to microbial populations. Soil pH did affect microbial distributions, with the highest populations existing in the samples with a pH of 6.0-7.0, and the lowest numbers in the samples that were > 7.0. The limited correlation between microbial populations and chemical properties is likely due to the fact that these blocks were essentially 100% sand. Chemistry could play a more significant role if there was an appreciable clay content.

Distribution of *Pseudomonas putida* populations. *Pseudomonas putida* populations were highest in the 3-8 cm and 23-28 cm layers of the soil block. These were the layers that were predominately composed of fine sand particles. In general, as the particle size decreases so does the average pore entrance size, thereby restricting microbial penetration. This lack of penetration inhibited the transportability of microorganisms through the block, and limited the movement of microbes to the 43-48 cm layer. Microbial filtration or adsorption to the fine sand particles could have affected flow movement by reducing the dimensions of the pores by aggregation of the filtered bacteria cells. This would decrease the transportability of the microbes with time and possibly reroute flow, as well as prevent further bacterial transport. Vandevivere and Baveye (1992) report that microbial transport through porous media was significantly decreased due to clogging of pore throats by large aggregations of the applied bacteria. Particle-size distributions also likely affected spatial heterogeneity of soil water and phosphates, which were found to be highly correlated to distributions of microbial populations. Phelps et al. (1994) reported similar results. They found that additions of several growth stimulants resulted in the increased stimulation, however additions of phosphate and water produced the greatest stimulation.

While many manipulative practices can be utilized to increase the efficiency of bioremediation, i.e. nutrient additions and GEM's, bioremedial success will be predominately governed by the spatial variability of soil properties existing at the contaminated site. Variability in soil conditions can greatly vary within a small distance and must be taken into account when determining if bioremedial practices are practical for contaminate cleanup. While the general practice of most environmental companies is to limit the number of soil samples analyzed for bioremediation feasibility studies, decisions made from these samples may prove to lessen bioremedial efficiency, since the samples analyzed may not fully represent existing soil conditions. Increasing the number of samples for analysis will not only assist the researcher in determining the spatial variability of soil properties, but will also help determine what practices need to be applied for microbial proliferation, which in turn will increase the success of bioremediation. While bioremedial feasibility studies often center on soil chemistry and nutrient concentrations, our studies indicate that equal consideration should be given to the physical properties of the soil.

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