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Microbial transport through porous media: The effects of hydraulic conductivity and injection velocity*

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Abstract

The effects of hydraulic conductivity and injection velocity on microbial transport through porous media were investigated. Glass chromatography columns were packed separately with clean quartz sand of two diameters (0.368 mm or 0.240 mm) and two hydraulic conductivities (1.37×10^{-1} cm/s and 3.65×10^{-2} cm/s respectively). Three injection velocities, 1.18×10^{-3}, 2.35×10^{-3} and 4.73×10^{-3} cm/s were investigated. Microbial transport under the conditions tested was limited and could be predicted mathematically using a model for physicochemical filtration.

Introduction

The fate and transport of microorganisms in the subsurface environment has been the subject of research for decades [1–4]. Early efforts were mainly concerned with public health and the contamination of drinking water supplies by pathogenic microorganisms [5–8]. Later research was aimed at industrial applications such as inoculation of microorganisms to achieve microbial enhanced oil recovery [9] and hazardous waste site remediation [10]. This work was motivated in part by interest in the injection of microorganisms with novel metabolic capabilities to remediate hazardous waste sites as well as by the importance of assessing the transport of pathogenic microorganisms to infiltration galleries and wells used for drinking water supplies.

Seed microorganisms have been used for decades in the initiation of industrial fermentation and wastewater treatment operations. The success of inoculation depends on several criteria: organisms must retain their specialized metabolic capability; organisms must come into contact with the contaminants and nutrients; and environmental conditions must be conducive to contaminant biodegradation and microbial survival [11]. These criteria can be controlled in bioreactors, but may be difficult, if not impossible, to control in expansive natural ecosystems.

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Considerable attention and publicity have been given to the potential use of microorganisms to combat oil slicks in marine and freshwater environments. The technology of seeding selected bacteria and fungi to oil spills was patented by Azarowicz and Bioteknika International Inc. [12]. However, Gutnick and Rosenberg [13] and Atlas and Bartha [14] found inoculation to be ineffective in reducing oil contamination in marine environments. More optimism was expressed for seeding and nutrient supplementation in more contained environments [12,13,15].

Inoculation of microorganisms into the subsurface for enhanced bioresoration is an emerging technology that may have met with some success [10,16,17]. In the majority of cases, the role of the introduced organisms in degradation of contaminants cannot be determined because appropriate control plots were not incorporated into the experimental designs and results were not quantitatively measured throughout the course of the projects [18]. A detailed laboratory investigation performed under conditions approximating those *in situ* is needed to assess the kinetics of degradation, the potential for toxicity, the nutritional requirements of the organisms in the subsurface [19], and the factors affecting transport and attachment of organisms.

Microbial transport and attachment

Studies have shown a wide range of microbial mobility in the saturated subsurface and through porous media [20–23]. It is difficult to elucidate the factors that affect this transport because of the wide variety of experimental conditions, incompleteness of reports, and lack of appropriate controls in the published research. Jang et al. [24] suggested that the rate of bacterial spreading in a formation is affected by the following factors: the porous structure, mineral composition and the wettability of rock minerals; the hydrodynamics of the aquifer; surface physicochemical and microbiological properties of the system; and secondary effects, such as the straining and plugging of the smaller pores and the aggregation of bacterial cells. The transport of microorganisms through porous media may be predicted with mathematical models derived from physicochemical filtration theory [25].

Microorganisms can adhere to almost any surface in any environment, which affects both the distribution and activity of the organisms [26,27]. Microbial transport is dependent on the retention of the microorganisms through attachment to the surfaces of the media particles. However, the complexity and heterogeneity of aqueous and soil environments have made it extremely difficult to study events related to the attachment of microorganisms in the subsurface. ZoBell [28] first suggested that sorption occurred in two phases, an early, reversible sorption where bacteria are weakly held to the surface and a later irreversible sorption that involves a more permanent attachment of the organism to the surface. It is not known whether the primary factors controlling the degree of bacterial association with surfaces are related to the capacity of

the solid interfaces to support growth, purely to physical-chemical interactions, or to some combination of these factors. It is known that microbial attachment may be influenced by the nutrient concentration, ionic strength, production of biopolymers, presence of surface tension depressants or detergents, effects of velocity and shear forces, integrity of the cell wall, temperature [29], extracellular polysaccharides [30], and cellular appendages [31]. Martin [32] summarized a variety of investigations where electrostatic and hydrophobic forces emerged as potential determinants of initial, reversible microbial attachment while irreversible attachment was controlled by the formation of extracellular polysaccharides.

Models for particle deposition in porous media

Models based on a physicochemical description of particle deposition in porous media have been applied to water filtration and may also be useful in estimating the retention of microbes in subsurface environments. Particle deposition may be considered as a two-step process of transport and attachment. Important transport mechanisms for particles in the size range of 10^{-2} to 10^{2} μm include interception, gravity, and Brownian diffusion. A particle following a streamline within the porous medium that passes within a distance of one particle radius from the sand grain will be intercepted by the aquifer material. Alternatively, suspended particles, such as microbial organisms, may exit the fluid streamline and come in contact with sand grains as the result of Brownian or gravitational forces. In addition, London–Van der Waals and electrical double layer forces are likely to be important at small separation distances between the particle and the medium and must be considered in modeling the particle attachment step. In the absence of all forces other than interception, contact between suspended particles and the porous medium may still occur.

The collector efficiency of a grain of filter medium or aquifer material is defined as the rate at which particles strike the grain (collector) divided by the rate at which particles approach the collector. The collector efficiency of a filter grain can be modified to include a parameter, α, that describes the fraction of collisions between particles and filter grains resulting in particle attachment, yielding the single collector removal efficiency, η_r. The collision efficiency factor, α, is considered to be a function of surface interactions between the particle and the collector and, therefore, is affected by the solution chemistry.

The single collector removal efficiency, η_r, can be included in a mass balance on particles in an incremental slice of the porous medium and integrated to yield an expression for the fraction of particles remaining in suspension after passing a distance, L, through the porous medium:

$$n/n_o = \exp\left[\alpha 3L(1-f)\eta_r/2d_c\right] \quad (1)$$

where n and n_o are the effluent and influent concentrations of particles re-

spectively, f is the porosity of the medium, and d_c is the average diameter of the medium. Equation (1) may be useful in estimating the fraction of organisms remaining in suspension after vertical passage through aquifer media of homogeneous composition that is well described by an average diameter. Under these conditions, the potential for success in the inoculation of an aquifer for the purposes of bioremediation might be assessed. The effects of hydraulic conductivity on microbial transport are contained in the estimates of aquifer porosity and media size. The roles of injection velocity, microbial size, and density in the transport and retention of microorganisms are captured in the calculation of η_r, which is derived from a consideration of particle transport by Brownian diffusion, gravity and interception.

Experimental systems design

This research was designed to quantify the effects of injection velocity and hydraulic conductivity on the movement of microorganisms through the subsurface. *Rhodotorula* sp., a species of yeast found in ground water [33], was selected from a number of candidate organisms due to its shape and surface characteristics. A bench-scale physical model was used to collect data, which were evaluated using a model of physicochemical filtration. A similar approach has been used by others [25,32,34] in an attempt to describe the distance bacteria can travel in an idealized, one-dimensional porous medium. Details of the experimental design and variables for each of the experiments are shown in Table 1.

The average linear velocities (volumetric flux/porosity × cross sectional area, or Q/nA) investigated ranged from 3.00×10^{-3} to 1.20×10^{-2} cm/s, a factor of four. These injection velocity values corresponded to ground water velocities that range from 2.59 m/day to 10.36 m/day (8.55 ft/day to 34.21 ft/day), values reported for both natural ground water flow and forced gradient tracer studies [4,22,23]. The calculated hydraulic conductivity values were 1.37×10^{-1} and 5.59×10^{-2} cm/s respectively for 45 and 70 mesh sand. The lower value of hydraulic conductivity, 5.59×10^{-2} cm/s, corresponded to a value reported for the hydraulic conductivity at the site of an aviation gasoline spill in Traverse City, Michigan [35]. The higher value of hydraulic conductivity, 1.37×10^{-1}cm/s, corresponded to a value reported for the hydraulic conductivity at a site in Cape Cod, Massachusetts. This site, described by Harvey et al. [4], is the focus of an active research effort to describe the movement of microorganisms through the saturated subsurface.

The experimental apparatus consisted of a high capacity infusion/withdrawal syringe pump (Harvard Apparatus, South Natick, MA) connected with 1/16 in. i.d. Teflon tubing to duplicate chromatography columns (Spectrum® Medical Industries, Inc., Los Angeles, CA) filled with sand. The columns were connected to a fraction collector (Isco, Inc., Lincoln, NB) to collect the ef-

TABLE 1

Experimental design

Parameters	Grain size (mesh)-Flow rate (cm^3/min)			
	45-0.4	45-0.8	45-1.6	70-1.6
Fluid injection				
Specific discharge[a] (cm/s)	1.18×10^{-3}	2.36×10^{-3}	4.73×10^{-3}	4.73×10^{-3}
Average linear velocity[b] (cm/s)	3.00×10^{-3}	6.00×10^{-3}	1.20×10^{-2}	1.21×10^{-2}
Microorganisms				
Mean size of microorganisms (μm)[c,d]	4.35 (0.421)	3.63 (0.454)	3.63 (0.454)	4.35 (0.421)
Concentration of microorganisms in injection stream (CFU/ml)[e]	3.55×10^{5}	2.28×10^{5}	2.14×10^{5}	1.22×10^{5}
Sand				
Calculated hydraulic conductivity[f] (cm/s)	1.37×10^{-1}	1.37×10^{-1}	1.37×10^{-1}	5.59×10^{-2}
Mean sand diameter (mm)[d]	0.368 (7.64×10^{-3})	0.368 (7.64×10^{-3})	0.368 (7.64×10^{-3})	0.240 (7.51×10^{-3})
Bulk density (g/cm^3)	1.60	1.60	1.60	1.61
Particle density (g/cm^3)	2.64	2.64	2.64	2.64
Porosity (%)	39.39	39.39	39.39	39.02

[a]Volumetric flux/cross sectional area (Q/A).
[b]Specific discharge/porosity (Q/nA).
[c]Volume averaged diameter.
[d]Standard deviation.
[e]Colony forming units/ml.
[f]Calculated using Carmen-Kozeny equation.

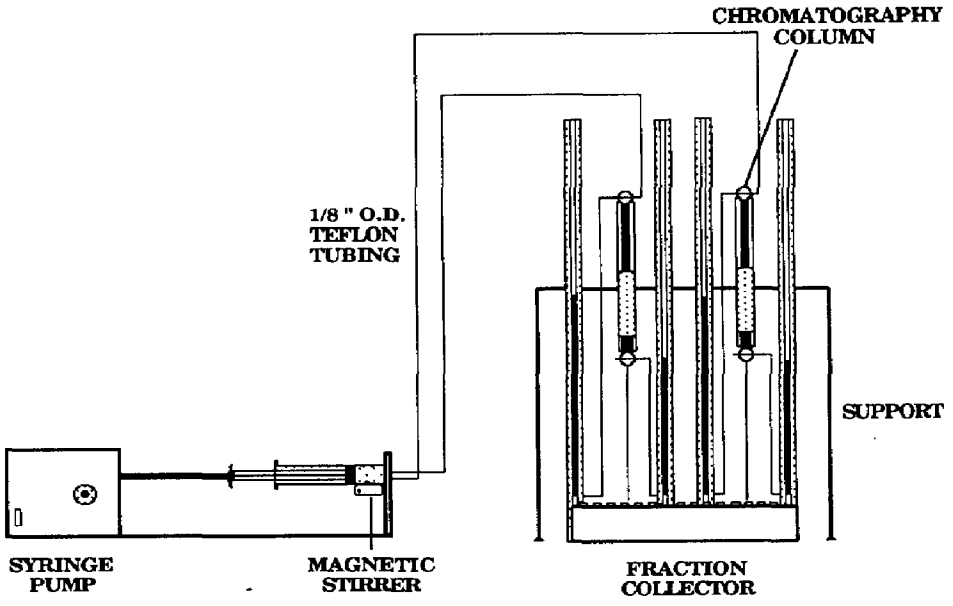

Fig. 1. Experimental system design.

fluent. A simple water manometer was connected to the inlet and outlet of each column to determine the pressure differential during the experiments (Fig. 1).

Results and discussion

Tritium breakthrough curves

The combined tritium breakthrough curves for all experiments are presented in Fig. 2. The tritium concentration measured in the effluents from the duplicate columns (identified as L and R for left and right) were expressed as fractions of the influent concentrations in each experiment and were plotted as a function of the number of pore volumes eluted from the columns. Inspection of the plot revealed a sigmoidal breakthrough curve with a midpoint ($n/n_o = 0.5$) equal to one pore volume, which is characteristic of uniform plug flow through homogenous packed porous media. The similarity in the curves indicated the reproducibility of flow characteristics and column packing between experiments.

Effect of injection velocity

The results from experiments examining the effect of flow rate on the transport of *Rhodotorula* sp. are summarized in Fig. 3. Data obtained from duplicate columns were not statistically different (at a 95% confidence interval). At a given filtration rate, microorganisms began to break through the columns after approximately 0.5 pore volumes were eluted, roughly corresponding to the breakthrough curves produced using tritiated water. At all three injection velocities, microbial numbers in the effluents continued to increase slowly after the passage of one pore volume and appeared to level off after 8 to 10 pore volumes. An increase in the injection velocity resulted in a decrease in the

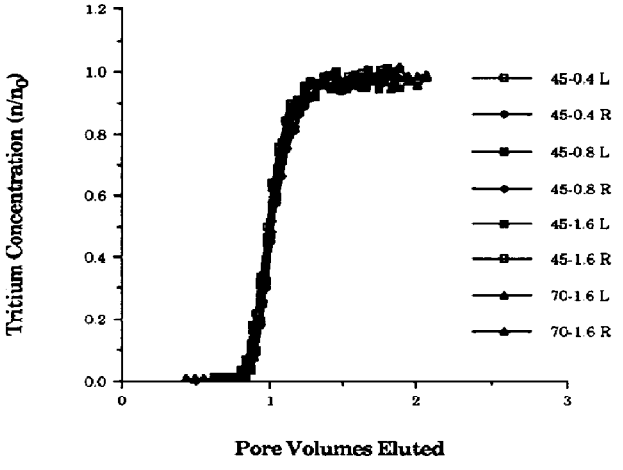

Fig. 2. Tritium breakthrough curves for all experiments.

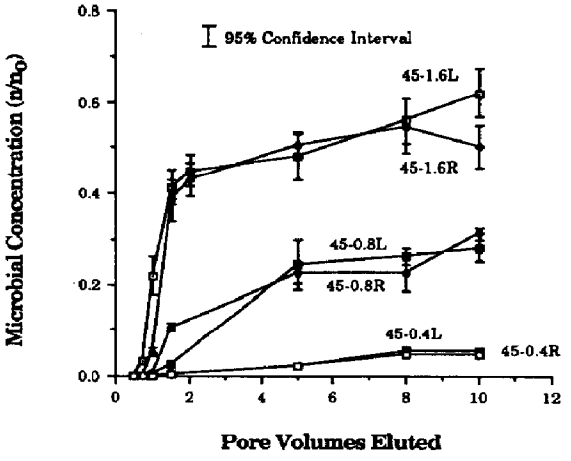

Fig. 3. Effect of injection velocity on the transport of microorganisms.

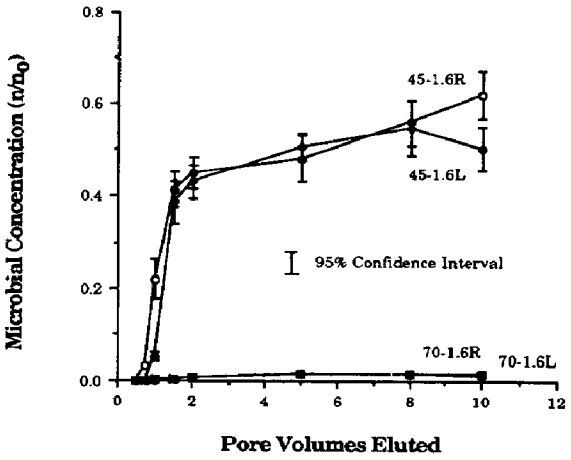

Fig. 4. Effect of hydraulic conductivity on the transport of microorganisms.

retention of microorganisms by the porous media. For the 45 mesh sand ($K = 1.37 \times 10^{-1}$ cm/s), a doubling of the flow rate nearly doubled the number of organisms transported through the column. The observed retention of *Rhodatorula* sp. as a function of flow rate corresponded well with the expected retention of microorganisms calculated from eq. (1).

Effect of hydraulic conductivity

The results from experiments examining the effect of hydraulic conductivity on the transport of *Rhodotorula* sp. are summarized in Fig. 4. At given hydraulic conductivity values, microbial breakthrough after approximately 0.5 pore volumes roughly corresponded to the breakthrough curves produced using tritiated water. The microbial numbers in the effluents continued to increase slowly and appeared to level off after 8 to 10 pore volumes. A reduction of the hydraulic conductivity by about a third resulted in a tenfold reduction in the

number of organisms transported. Again, experimental trends are predicted accurately by the physicochemical model for particle deposition (eq. 1).

Conclusions

The results of this research indicate that inoculation of an aquifer with microorganisms could be best performed by creating conditions of high injection velocity in a formation of great hydraulic conductivity. However, the most significant increases in the transport of microbial cells are likely to be brought about through manipulation of the collision efficiency, α. It may be possible to alter the surface properties of the organism by, among other things, controlling the ionic strength of the solution, by varying the nutritional status of the organism, and/or by introducing "stabilizing agents" to the injection mixture [32].

Under conditions likely to exist in most ground waters, retention of microorganisms should be high, and their transport should be limited in the absence of inhomogeneities in the aquifer material, such as cracks and channels. The majority of an aquifer may not be perfused by an injection of microorganisms when transport is controlled by flow through preferential paths. Therefore, the ability to successfully seed and bioremediate an aquifer may be limited by the "filtration" of microorganisms into relatively homogeneous portions of the aquifer. The *Rhodotorula* sp. was predicted to penetrate porous media (45 mesh-1.6 cm^3/min) approximately 80 cm with a decrease in microbial numbers of five orders of magnitude. Growth of the microorganism, which is likely to enhance microbial transport over longer periods of time, is not taken into account by this model. Harvey et al. [4] and Martin [32] have speculated on the importance of growth and shedding or sloughing of cells for the colonization of porous media by microorganisms.

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