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Transport of pathogens in water saturated sand columns

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Abstract Groundwater protection from microbial contamination necessitates a solid understanding of the factors controlling the migration and retention of pathogenic organisms (biocolloids) in the subsurface. Although coliform bacteria and coliphages are used worldwide to indicate fecal pollution of groundwater, their transport behavior is not fully understood. This study focuses on the transport behavior of three waterborne pathogens (*Escherichia coli*, MS2, and ΦX174) in laboratory-scale columns packed with clean quartz sand. Three different grain sizes and three pore water velocities were examined. The attachment behavior of *Escherichia coli*, MS2, and ΦX174 onto quartz sand was evaluated. The mass recoveries of the biocolloids examined were shown to be proportional to the sand size, and they were shown to be highest for *Escherichia coli* and lowest for MS2. The single collector removal and collision efficiencies were quantified using the classical colloid filtration theory.

1 Introduction

Groundwater may be accidentally contaminated with infective human enteric viruses from human and animal sewage through wastewater discharges, sanitary landfills, septic tanks, and agricultural practices or by artificial groundwater recharge, which is often used to reverse the rapid depletion of aquifers (Anders and Chrysikopoulos 2005; Masciopinto et al. 2008). To predict the presence of pathogens in water and wastewater, microorganisms known as indicator organisms (e.g. bacteria *Escherichia coli*, and coliphages MS2 and ΦX174), which are commonly associated with fecal contamination, are monitored.

Many studies examining the interaction of microorganisms with soil, sand, gravel or other model granular materials have been conducted using laboratory-scale columns under well-controlled environmental conditions. Theoretical and experimental studies have examined the effect of pore water solution chemistry (Bolster et al. 2001), fluid velocity (Chrysikopoulos and Sim 1996), matrix moisture content, temperature, grain size (Anders and Chrysikopoulos 2006, 2009) and presence of surface coatings (Bolster et al. 2001) on microbial transport and retention

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in porous media. Quartz sand, either clean or coated, as well as glass beads have all been employed as model granular materials in such studies. Although a large number of studies on microbial transport have been published over the past two decades, our ability to predict the migration of bacteria, viruses or protozoa in natural subsurface environments remains limited.

The objectives of this study were to characterize the transport and attenuation of *E. coli*, MS2, and ΦX174 in 'clean' saturated quartz sand laboratory columns, and to examine the influence of grain size and pore water velocity on their transport and attenuation. The collision efficiencies of the three biocolloids examined were estimated and the factors that control biocolloid deposition were discussed.

2 Materials and methods

2.1 Bacterial and bacteriophage suspensions

The bacterial strain *Escherichia* (*E.*) *coli* (ATCC 13706-B1) is a well-characterized, Gram-negative, typical representative of the coliform bacteria. *E. coli* cells are motile rod shaped with approximate dimensions of 0.6 μm in width and 2 μm in length, or an equivalent spherical diameter of 1.21 μm. To provide a uniform inoculum for each experiment, a stock culture was cultivated in 50 mL of tryptic soy broth medium for 6 h to early stationary phase, harvested by centrifugation for 10 min at 5000×g and washed twice with sterile phosphate buffered saline (PBS) solution (1.2 mM NaCl, 0.027 mM KCl, and 0.10 mM Na_2HPO_4) to remove nutrients. Finally, the pellet was suspended in PBS solution and stored at 4 °C until application. Aliquots were taken to count the bacteria. Bacteria were suspended and diluted in PBS solution at pH=7 to concentrations of $1.94\pm0.06\times10^8$ colony-forming units per milliter (CFU/mL). The *E. coli* concentration was determined using optical density measurements (at 410 nm) with a UV-visible spectrophotometer (UV-1100, Hitachi). The bacterial cell concentrations (plate colonies) were determined using a standard spectrophotometer calibration curve.

The bacteriophage MS2 is a F-specific, single-stranded RNA phage with 31% nucleic acid content, whose host bacterium is *E. coli* (ATTC 15597-B1); whereas, the bacteriophage ΦX174 is an icosahedral, single-stranded DNA phage with 26% nucleic acid content, whose host bacterium is *E. coli* (ATTC 13706-B1). The MS2 particle diameter ranges from 24 to 26 nm; whereas, the ΦX174 particle diameter ranges from 25 to 27 nm. MS2 has hydrophobic protein coat; whereas, ΦX174 has hydrophilic protein coat. Both bacteriophage were assayed by the double-layer overlay method (Adams 1959), where 0.1 mL of the appropriate host bacterium and 0.1 mL of a diluted virus sample solution were mixed in a centrifuge tube. The mixture was combined with molten soft-agar medium (4.5 mL) maintained at

45 °C in a tube and poured onto a petri dish containing solid agar medium. The plates were solidified for 10 min and incubated overnight at 37 °C. Viable virus concentrations were determined by counting the number of plaques in each host lawn and reported as plaque-forming units per milliter (PFU/mL). Only dilutions that resulted in the range of 20-300 plaques per plate were accepted for quantification. All virus concentrations reported represent the average of two replicate plates. Bacteriophages, MS2 and ΦX174, were suspended and diluted in PBS solution at pH=7 to concentrations of 10^3-10^6 PFU/mL.

2.2 Chloride Analysis

Chloride, in the form of potassium chloride, was chosen as the nonreactive tracer for the transport column experiments. The nonreactive tracer solution was prepared with 0.01M KCl in PBS solution. It should be noted that alkali halides are the most commonly used salts for subsurface fluid tracing owing to a minimal effect on solution ionic strength (IS) (Chrysikopoulos 1993). Chloride concentrations were measured using ion chromatography (ICS-1500, Dionex Corp., Sunnyvale, CA).

2.3 Column packing material

Quartz sand of various sizes was used as packing material in the experimental columns. The sand was purchased directly from the manufacturer (Filcom Filterzand & Grind) and sieved into various size distributions. In this study three size distributions were used: (a) coarse (1.18-1.7 mm or sieve No 16), (b) medium (0.425-0.600 mm or sieve No 40), and (c) fine (0.150-0.212 mm or sieve No 100). The coefficient of uniformity, $C_u=d_{60}/d_{10}$, for each sand fraction were estimated to be C_u=1.19, 1.21, 1.2 for fine, medium, coarse sand, respectively. The chemical composition of the sand reported by the manufacturer was: 96.2% SiO_2, 0.15% Na_2O, 0.11% CaO, 0.02% MgO, 1.75% Al_2O_3, 0.78% K_2O, 0.06% SO_3 and 0.46% Fe_2O_3, 0.03% P_2O_5, 0.02% BaO, and 0.01% Mn_3O_4. The total organic carbon (% TOC) content, measured by the Walkley-Black method (i.e., chemical oxidation of the organic fraction) (Black, 1965), was found equal to 0.08±0.04% for the coarse, and 0.1±0.1% for both medium and fine sand fractions. Prior to the experiments, the sand fractions were cleaned with 0.1 M HNO_3 (70%) for a 3 h time period to remove surface impurities (e.g., iron hydroxide and organic coatings) that could promote physicochemical deposition of the biocolloids, rinsed with deionized water, then soaked in 0.1 M NaOH for a 3 h time period, and rinsed again with deionized water. After the cleaning steps, the sand was dried in an oven at 105 °C, and then stored in screw cap sterile beakers until use in the column experiments.

2.4 Column experiments (PBS experiments)

The glass columns (2.5 cm diameter and 30 cm length) were packed wet with sand under vibration to minimize any layering or air entrapment. The porosity of the sand column was determined by standard procedures and it was ranged from 0.36 to 0.43. The estimated bulk density was ranged from 1.65 to 1.72 g/cm^3. Prior to each experiment, the packed column was equilibrated by pumping 10 pore volumes of the background PBS solution through the column at a constant discharge rate of Q=2.5, 1.5 and 0.8 mL/min, corresponding to specific discharge or approach velocities of q=0.51, 0.31 and 0.16 cm/min, respectively. A suspension of each biocolloid of the same background PBS solution was pumped for 3 pore volumes at the same discharge rate followed by 5 pore volumes of biocolloid-free PBS solution. The apparatus used for the biocolloid transport experiments are shown in Figure 1.

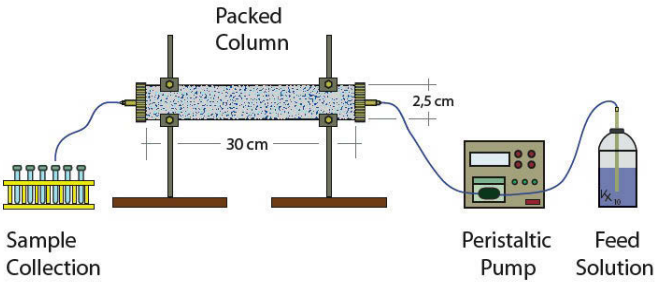

Fig. 1. Schematic illustration of the experimental apparatus.

3 Theoretical developments

3.1 Moments

The biocolloid concentration breakthrough data obtained at location x=L were analyzed by the absolute temporal moments:

$$m_n(x) = \int_0^\infty t^n C_i(x,t)\, dt \tag{1}$$

where the subscript n=0, 1, 2, ... indicates the order of the moment, and subscript i indicates *E.coli*, MS2, and ΦX174. The zeroth absolute temporal moment, m0,

quantifies the total mass in the concentration breakthrough curve; the first absolute moment, m1, describes the mean residence time; and second absolute temporal moment, m2, describes the degree of spreading of the concentration breakthrough curve. Also, the normalized temporal moments are defined as:

$$M_n(x) = \frac{m_n(x)}{m_0(x)} = \frac{\int_0^\infty t^n C_i(x,t)dt}{\int_0^\infty C_i(x,t)dt} \tag{2}$$

The first normalized temporal moment, M_1, characterizes the center of mass of the concentration breakthrough curve and defines the mean breakthrough time or average velocity. The second normalized temporal moment, M_2, characterizes the spreading of the breakthrough curve. Worthy to note is that that the ratio $M_{1(i)}/M_{1(t)}$ indicates the degree of velocity enhancement of biocolloid i relative to the conservative tracer. If this ratio is less than one, there exists velocity enhancement of biocolloid transport. If this ratio is greater than one there exists biocolloid retardation. Furthermore, the mass recovery, M_r, of the tracer or the suspended particles is quantified by the following expression:

$$M_r(L) = \frac{m_0(L)}{C_{i_0} t_p} = \frac{\int_0^\infty C_i(L,t)dt}{\int_0^{t_p} C_i(0,t)dt} \tag{3}$$

where L is the porous medium length.

3.2 Colloid filtration theory

Classical colloid filtration theory (CFT) was used to quantitatively compare the microbial and viral attachment onto quartz sand. CFT assumes that the removal of particles is described by first-order kinetics with a spatially and temporally constant rate of particle deposition, and the concentrations of suspended and retained particles decrease log-linearly with distance. However, recent studies (Tufenkji and Elimelech, 2004) have suggested that colloid retention decreased hyper-exponentially with distance, suggesting that the attachment rate coefficient is not constant. In the absence of straining, which is defined as the trapping of particles in pores that are too small to pass through, this hyper-exponential deviation from CFT could be attributed to the concurrent existence of both favorable and unfavorable colloidal interactions with collector surfaces (Tufenkji and Elimelech, 2004). The dimensionless collision efficiency, α (the ratio of the collisions resulting in attachment to the total number of collisions between particles and collector grains) and the dimensionless single-collector removal efficiency for favorable deposition, η_0 were calculated from each biocolloid breakthrough curve.

4 Results

The normalized chloride breakthrough data for the coarse sand and three different specific discharge velocities are presented in Figure 2. Similar results were observed (not shown) for the medium and fine sand. The normalized *E. coli* breakthrough data are presented in Figure 3 together with the fitted model predictions. For all cases considered the ratio $M_{1(i)}/M_{1(t)}$ was smaller than one, which indicated that the velocity of *E. coli* is enhanced by 7%–15%. The M_r values, ranged from 94.48 to 100%, indicate that there was no significant *E. coli* retention by the packed column. Slight attachment of *E. coli* onto the quartz sand was observed at the lowest q. However, no distinct relationships between M_r or $M_{1(i)}/M_{1(t)}$ and q or d_c could be established from the experimental results.

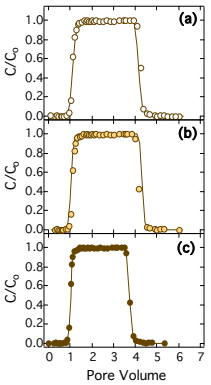

Fig. 2. Tracer breakthrough data (symbols) and fitted mathematical model predictions (solid curves) for specific discharge velocities of (a) 0.16, (b) 0.31, and (c) 0.51 cm/min in water-saturated columns packed with coarse sand.

Figure 4 presents the normalized MS2 breakthrough data together with the fitted model predictions. With the exception of the case of fine sand at q=0.51 cm/min, all estimated M_r values were quite low (ranged from 43.47 to 96.49%), suggesting that the MS2 particles retained in the packed column were either irreversibly attached onto the sand grains or inactivated. For the slowest specific discharge velocity (q=0.16 cm/min) M_r decreased with decreasing sand size; however, for the other two velocities employed there was no clear trend. Hence, MS2 attachment cannot be attributed to d_c variations, but to possible sand grain physicochemical heterogeneities. With the exception of the case of medium sand with q=0.31 cm/min, where slight retardation was observed ($M_{1(i)}/M_{1(t)}$=1.05>1), all calculated $M_{1(i)}/M_{1(t)}$ ratios were smaller than one, suggesting that the velocity of MS2 is enhanced by 2%–19% compared to the tracer.

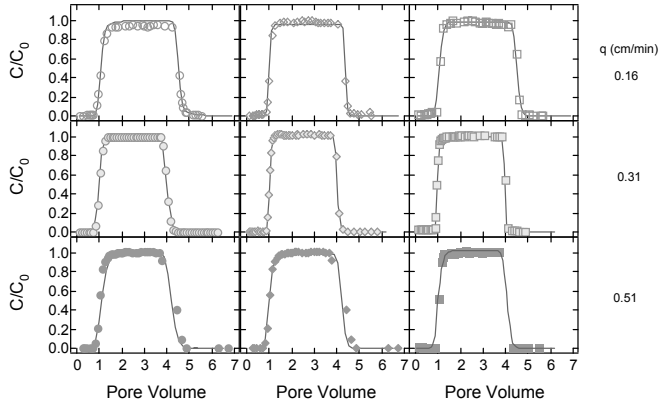

Fig. 3. Experimental *E. coli* CN13 breakthrough data (symbols) and fitted mathematical model predictions (solid curves) for volumetric flow rates of 0.16 cm/min (open symbols), 0.31 cm/min (filed symbols), and 0.51 cm/min (solid symbols) in water-saturated columns packed with coarse (circles), medium (diamonds) and fine (squares) sand.

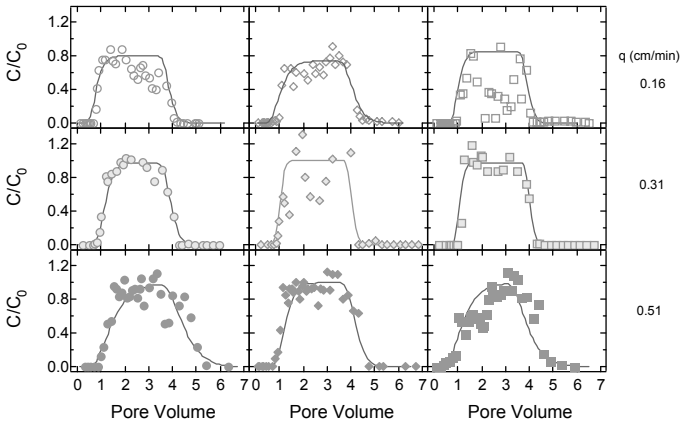

Fig. 4. Experimental MS2 breakthrough data (symbols) and fitted mathematical model predictions (solid curves) for volumetric flow rates of 0.16 cm/min (open symbols), 0.31 cm/min (filed symbols), and 0.51 cm/min (solid symbols) in water-saturated columns packed with coarse (circles), medium (diamonds) and fine (squares) sand.

Figure 5 shows the normalized ΦX174 breakthrough data together with the fitted model predictions. The calculated M_r values, ranged from 82.65 to 100%, indicated that there was no significant ΦX174 retention in the packed column. Furthermore, all calculated $M_{1(i)}/M_{1(t)}$ ratios were smaller than one, suggesting that the velocity of ΦX174 is enhanced by 4%–18% compared to the tracer. However, it should be noted that no clear trends between M_r or $M_{1(i)}/M_{1(t)}$ and q or d_c could be determined from the experimental results.

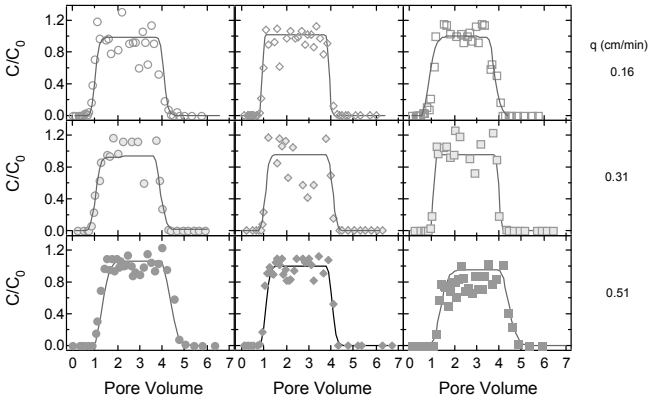

Fig. 5. Experimental ΦX174 breakthrough data (symbols) and fitted mathematical model predictions (solid curves) for volumetric flow rates of 0.16 cm/min (open symbols), 0.31 cm/min (filed symbols), and 0.51 cm/min (solid symbols) in water-saturated columns packed with coarse (circles), medium (diamonds) and fine (squares) sand.

The single collector removal efficiency under favorable deposition conditions, η_0, was calculated for MS2, ΦX174 and *E. coli*, three sand sizes (coarse, medium and fine), and three specific discharge velocities. Collision efficiencies, α, for MS2, ΦX174 and *E. coli* for the experimental conditions of this study, using the previously calculated η_0 values, are similar to values reported in the literature for MS2 (α=0.00045-0.0422 (Chu et al., 2003)), for ΦX174 (α=0.00077-0.0162 (Chu et al., 2003)), and for *E. coli* (α=0.008-0.875 (Foppen et al. 2007)).

5 Summary and Conclusions

The results of this study indicated that although the biocolloid mass recovery and degree of velocity enhancement were affected by the interstitial water velocity and sand grain size, no clear trends could be determined. Worthy to note is that the M_r of ΦX174 was higher than that of MS2 for all cases examined in this study. It was found that the dispersivity values for MS2 were higher than those obtained for ΦX174, which could be attributed to the higher charge repulsion between MS2 and sand grains and to the hydrophobic protein coat of MS2. Moreover, as it was expected, the dispersivity values for the smaller bacteriophages were found to be greater than that of the larger bacteria. The single collector removal efficiency was shown to be affected by the sand grain size and water velocity. The experimental collision efficiencies suggest more favorable attachment conditions for bacteriophage MS2 than for ΦX174. However no significant effect of sand grain size and interstitial velocity on the collision efficiency was observed. It is possible that factors as grain surface area, angularity and roughness may have contributed to physicochemical filtration and biocolloid retention.

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