Journal of Environmental Quality

TECHNICAL REPORTS

GROUNDWATER QUALITY

Transport and Retention of *Cryptosporidium Parvum* Oocysts in Sandy Soils

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A series of miscible-displacement experiments was conducted to examine the retention and transport behavior of *Cryptosporidium parvum* oocysts in natural porous media. Three soils and a model sand were used that differed in physical and geochemical properties. Transport behavior was examined under various treatment conditions to help evaluate retention mechanisms. Significant retention of *Cryptosporidium* oocysts was observed for all media despite the fact that conditions were unfavorable for physicochemical interactions with respect to DLVO theory. The magnitude of *Cryptosporidium* retention was not influenced significantly by alterations in solution chemistry (reduction in ionic strength) or soil surface properties (removal of soil organic matter and metal oxides). On the basis of the observed results, it appears that retention by secondary energy minima or geochemical microdomains was minimal for these systems. The porous media used for the experiments exhibited large magnitudes of surface roughness, and it is suggested that this surface roughness contributed significantly to oocyst retention.

J. Environ. Qual. 41
doi:10.2134/jeq2011.0414
Received 31 Oct. 2011.
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Waterborne diseases caused by pathogen-contaminated groundwater continue to be of concern. Groundwater contamination potential is associated with land disposal practices that favor the entry of pathogenic microorganisms into the subsurface environment, including municipal solid waste disposal in landfills, leaking septic systems, feedlots, the use of animal excreta as manure, and the inadequate disposal of human excreta in national parks and other areas where toilets are not provided (Santamaría and Toranzos, 2003). The fate of pathogenic microorganisms in the subsurface and their potential impact on groundwater quality is not fully understood.

The enteric protozoa *Cryptosporidium parvum* is among the most important microbial contaminants associated with a high risk of waterborne illness. Field surveys of *Cryptosporidium* spp. show that groundwater contamination with low concentrations of *Cryptosporidium* oocysts is frequent (Lisle and Rose, 1995; Hancock et al., 1997). These low levels of contamination are of great concern because human infections can be produced by ingestion of only a few oocysts. This pathogen has been the cause of several outbreak events in the United States and Europe (D'Antonio et al., 1985; Bridgman et al., 1995; O'Donoghue, 1995; Casemore et al., 1997; Craun et al., 1998). As a result of these and related issues, the transport, retention, and filtration of *Cryptosporidium* in porous media have begun to receive significant attention (Mawdsley et al., 1996; Brush et al., 1999; Harter et al., 2000; Hsu et al., 2001; Logan et al., 2001; Darnault et al., 2003; Tufenkji et al., 2004; Bradford and Bettahar, 2005; Hijnen et al., 2005; Tufenkji and Elimelech, 2005; Cortis et al., 2006; Tufenkji et al., 2006; Boyer et al., 2009; Abudalo et al., 2010; Kim et al., 2010; Mohanram et al., 2010). Although this and related research have contributed greatly to our understanding of biocolloid transport in porous media, there remains significant uncertainty regarding operative retention mechanisms, particularly for natural geomedia (Tufenkji et al., 2006; Bradford and Torkzaban, 2008; Johnson et al., 2010).

The majority of prior transport experiments for colloids in general, and for *Cryptosporidium* specifically, have been conducted with model porous media (e.g., glass beads, acid-treated

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Abbreviations: DLVO, Derjaguin–Landau–Verwey–Overbeek; PFBA, pentafluorobenzoic acid.

sand), whereas few have used natural soils or sediments. In addition, there has been minimal direct comparison of *Cryptosporidium* transport behavior observed for experiments conducted with model media to those for natural geomedia. The objective of this study was to investigate the transport behavior of *C. parvum* oocysts in natural soils. Miscible-displacement experiments were conducted for *C. parvum* oocysts using three soils and a model sand that differed in physical and geochemical properties. In addition, transport behavior was examined under various treatment conditions and was compared with that of two other protozoa (*Giardia lambia* and *Microsporidium Encephalitozoon intestinales*) to help evaluate potential retention mechanisms.

Materials and Methods

Porous Media and Aqueous Solutions

Four porous media were used for the experiments. The first is a soil, Eustis fine sand (siliceous, thermic Psammentic Paleudults), collected from the Ap horizon in Gainesville, Florida. The second is a soil, Vinton sand (sandy, mixed, thermic Typic Torrifluvent), collected from the Ap horizon at the West Campus Agricultural Center in Tucson, Arizona. The third is a coarse sieved fraction of the Vinton soil. The fourth is a commercially available, well sorted (20/30 mesh) natural quartz sand (Accusand). The soils were air-dried and sieved to remove the fraction >2 mm in diameter.

Pertinent properties of these porous media are listed in Table 1. The Eustis soil has a relatively high organic carbon content and a relatively low metal-oxide content, whereas the Vinton soil has the reverse. The Accusand serves as a model sand representative of the type of porous media used in a majority of prior colloid transport studies reported in the literature.

The specific solid surface areas of the porous media were measured using the N_2/BET method (SSSA, 2002). Geometric-based solid surface areas (S_{ga}) were calculated using an assumption of spherical particles with smooth surfaces: $S_{ga} = 6(1 - n)/d$, where n is porosity and d is median grain diameter. Quantification of surface roughness was conducted for the Vinton and treated Vinton media via atomic force microscopy using standard procedures by the University of Arizona Center for Surface and Interface Imaging. The zeta potentials of the porous media were measured in 10 mmol L^{-1} NaCl solution using laser Doppler electrophoresis (Zetasizer Nano, Malvern, Inc.). Samples of the media were crushed following standard procedures before measurement (Johnson et al., 2010). The measured values were −51.2 ± 12, −43.0 ± 8, and −38.9 ± 6 mV for Accusand, Eustis, and Vinton, respectively. Capillary-pressure/saturation characteristic curves were measured under primary drainage conditions, and these data were used to estimate pore-size distributions using $r = 2\sigma/P_c$, where r is pore radius, σ is surface tension, P_c is capillary pressure, and it is assumed that the media are water wetting with a contact angle of zero.

Sodium chloride (10 mmol L^{-1}) was used to create the electrolyte solution for the majority of the experiments. The electrical conductivity of the solution in contact with the porous media ranged from 1.25 to 1.3 mS cm^{-1}. Selected additional experiments were conducted using deionized, distilled water. The pH of the column effluent was monitored for the transport experiments and exhibited minimal change from the influent pH (pH 7).

Pathogens

Cryptosporidium parvum oocysts were obtained from Waterborne, Inc. The supplier purified the oocysts from calf feces by sucrose and Percoll gradient centrifugation after initial extraction of feces with diethyl ether. They were treated with a 1% formalin solution to render them nonviable. The microorganism stocks were refrigerated at 4°C in the dark until use. Suspensions were prepared at approximately 10^5 oocysts mL^{-1} for the miscible-displacement experiments (Table 2).

Cryptosporidium oocysts are spherical to elliptical in shape and generally range between 4 and 5 μm in diameter. The diameters of the oocysts used in this study were measured using digital images collected with a camera (Nikon D70) attached to a calibrated fluorescent microscope. The images were processed with ImageJ software (National Institutes of Health), which was used to determine mean lengths of the major and minor axes. These were 4.8 ± 0.5 and 4.4 ± 0.5 μm, respectively. The zeta potentials for *Cryptosporidium* oocysts range from approximately −10 to −40 mV (Kuznar and Elimelech, 2005; Byrd and Walz, 2007; Abudalo et al., 2010).

Nonviable *G. lambia* cysts were obtained from Waterborne, Inc. The supplier purified the cysts from gerbil's feces by sucrose and Percoll gradient centrifugation. The stock solutions were refrigerated at 4°C in the dark until use, wherein suspensions were prepared at approximately 10^5 oocysts mL^{-1} for the miscible-displacement experiments (Table 2). The diameters of the cysts used in this study were measured as described above, resulting in mean lengths of the major and minor axes of 12.5 ± 0.8 and 7.5 ± 0.7 μm, respectively.

Microsporidium Encephalitozoon intestinales spores were obtained from the American Type Culture Collection (ATCC). They were grown on RK13 rabbit kidney cells (line number CC1–37, ATCC) and Vero (EG) green monkey kidney cells

Table 1. Properties of porous media.

Porous medium	Sand	Silt	Clay	TOC†	Median grain diameter d_{50}	Uniformity coefficient U_c	Pore diameter‡			Particle density	SiO_2	Fe§	Al§	Mn§
							>100	100–10	<10					
	%				mm	d_{60}/d_{10}	μm			g cm^{-3}	%	μg g^{-1}		
Eustis	95	<1	4.4	0.38	0.27	2.3	8	81	11	2.64	97.7	310	690	19
Vinton	97	1.8	1.2	0. 1	0.26	2.0	21	61	18	2.69	54.4	1700	1400	130
Coarse Vinton	100	0	0	0	3	1.4	100	0	0	2.69	71.5	–	–	–
Accusand	100	0	0	0.04	0.71	1.2	98	1	1	2.66	99.8	14	12	2.5

† Total organic carbon.

‡ Percent of pores with designated size.

§ Elements dissolved from soils in 5 mol L^{-1} HNO_3 extractions.

(CCL-81, ATCC). The spores were withdrawn from the cell-culture media and concentrated by centrifugation. Percoll (Sigma-Aldrich) was added to promote purification by separation. The final pellet was washed with 0.01 mol L^{-1} phosphate buffer solution (0.54 g L^{-1} $NaHPO_4$ and 0.88 g L^{-1} KH_2PO_4; pH 7) and centrifuged for 15 min at 1500 × *g*. Suspensions were prepared at approximately 10^5 oocysts mL^{-1} for the miscible-displacement experiments (Table 2). The diameters of the spores were measured as described above, resulting in mean lengths of the major and minor axes of 2.4 ± 0.6 and 1.2 ± 0.5 μm, respectively.

Column Experiments

Transport experiments were conducted using a stainless steel column (7.05 cm long and 2.1 cm inner diameter; Alltech). The conditions for the various column experiments are provided in Table 2. The transport experiments were conducted in triplicate for most of the experiments. The columns were packed with dry porous media, and then electrolyte solution was injected at a low flow rate into the bottom of the vertically oriented column to achieve uniform saturation. Solution was injected at a flow rate equal to that used for the transport experiment for approximately 2 d before the start of the experiment to equilibrate the porous media to the electrolyte solution. The column preparation procedures used have been shown to produce uniformly packed, fully saturated conditions, as confirmed via X-ray microtomographic imaging (Brusseau et al., 2008).

An experiment was initiated by injecting into the column three to four pore volumes of solution containing the protozoan, followed by several pore volumes of solution devoid of protozoa. A single-piston, high-pressure liquid chromatography pump (Accuflow, Fisher Scientific Inc.) was used to maintain constant flow rates (equivalent to mean pore-water velocity of approximately 10 cm h^{-1}; see Table 2) throughout the experiments. Effluent samples (2 mL) were collected continuously in 20-mL polypropylene tubes using a fraction collector (RediFrac, Farmacia LKB). Pentafluorobenzoic acid (PFBA) was used as a conservative, nonreactive tracer to characterize the hydrodynamic properties of the packed columns. The PFBA samples were analyzed using an ultraviolet-visible spectrophotometer (Shimadzu UV-1601) at λ = 243 nm.

Additional experiments beyond those conducted under standard conditions (10 mmol L^{-1} NaCl) were conducted for *Cryptosporidium* with the Vinton and coarse Vinton media to further investigate retention mechanisms (Table 2). These experiments were designed to alter conditions such that physicochemical attachment via electrostatic and van der Waals interactions would be minimized. The two treatments were: (i) the ionic strength of the solution was reduced by using deionized water, and (ii) the porous media were treated before the experiment to remove potential surface attachment sites associated with organic matter and metal oxides/hydroxides. The operative hypothesis was that the altered conditions would result in higher effluent recoveries than those observed for the baseline experiments if physicochemical attachment interactions were a significant retention mechanism for the Vinton soil. For the second treatment, soil samples were extracted sequentially with 3 mol L^{-1} NaOH and 5 mol L^{-1} HNO_3 solutions. The samples were bathed in each solution for approximately 48 h while placed on a shaker table. After treatment, the samples were washed multiple times with deionized water.

A final set of experiments was conducted for the Vinton soil with two other protozoa, *G. lambia* and *E. intestinales*. These two biocolloids are larger and smaller than *Cryptosporidium*, respectively. Single-collector efficiency coefficients (η_0) can be calculated to examine the expected impact of biocolloid size differences on retention following standard colloid filtration theory. For this approach, the collector efficiency is considered to be comprised of three transport components: diffusion, advection (interception), and sedimentation. Using measured and literature data, η_0 values of 0.3, 1.3, and 8.5 were calculated for the conditions of our experiments for *Microsporidium*, *Cryptosporidium*, and *Giardia*, respectively. The sedimentation component was the predominant

Table 2. Experiment conditions and recoveries.

Porous media	*n*	Ionic strength	Porosity	q	Bulk density	C_o	Effluent recovery†	Total recovery‡
		mmol L^{-1}	–	cm h^{-1}	g cm^{-3}	oocysts mL^{-1}	%	
Baseline experiments								
Eustis	4	10	0.39 (0.011)§	3.3	1.60	3.0E+05	0.1 (0.03)	101 (37)
Vinton	3	10	0.46 (0.002)	3.3	1.50	2.0E+05	0.5 (0.6)	71 (24)
Coarse Vinton	2	10	0.47	3.3	1.50	1.0E+05	33	94
Accusand	3	10	0.34 (0.011)	3.3	1.80	2.6E+05	9 (0.8)	–
Additional experiments								
Vinton¶	1	0	0.46	3.3	1.50	2.8E+05	0.2	78
Vinton#	1	10	0.46	3.3	1.50	2.4E+05	0.1	58
Coarse Vinton¶	1	0	0.46	3.3	1.43	3.0+05	22	80
Coarse Vinton#	3	10	0.48	3.3	1.40	4.3E+05	19 (8)	65 (29)
Giardia††	1	10	0.46	3.3	1.50	4.1E+05	0.6	–
Microsporidia††	2	10	0.46	3.3	1.50	4.6E+05	27	–

† The effluent recovery is obtained by dividing the total numbers eluted by the total numbers injected.

‡ Total recovery is the sum of effluent recovery and soil-phase recovery.

§ Values in parentheses represent the SD.

¶ In these experiments, deionized water was used as the flushing solution.

Soil washed with 3 mol L^{-1} sodium hydroxide and 5 mol L^{-1} nitric acid.

†† These experiments were conducted with untreated Vinton soil.

contributor (>94%) to the predicted collector efficiencies for all three biocolloids, which is to be expected given that all three are in the μm size range. Given the following equalities (e.g., Tufenkji and Elimelech, 2005):

$$\eta = \alpha\eta_0$$

and

$$\eta = -\frac{2d_c}{3(1-\theta)L}\ln(C/C_0)$$

where α is the attachment efficiency coefficient, d_c is the collector (porous media) mean diameter, θ is porosity, L is column length, C is the effluent concentration, and C_0 is the influent concentration, the effluent recoveries (C/C_0) can be calculated for the three biocolloids with a known value for α. Based on the η_0 values, recoveries are predicted to be approximately an order of magnitude larger for *Microsporidium* versus *Cryptosporidium* and several orders of magnitude larger for *Cryptosporidium* versus *Giardia*, given the range of attachment coefficients reported in the literature. In addition, essentially complete retention is predicted for *Giardia*, the largest biocolloid. The measured recoveries reported below will be compared with these predicted values.

For all *Cryptosporidium* experiments except those conducted with Accusand, the porous media was removed from the column at the completion of the experiment to determine the total number of *Cryptosporidium* oocysts associated with the soil and their spatial distribution. The bottom end piece of the column was removed without disturbing the packed bed, and the porous media was pushed out and separated into three 1-cm-wide and one 3-cm-wide sections. Each of the sections was placed in a 50-mL centrifuge tube with 10 mL of deionized water. This solution was mixed for 30 min on a shaker. The supernatant was decanted in a 50-mL tube, and the number of *Cyptosporidium* oocysts in 10 μL of this solution was determined as described below. The total number of oocysts per dry weight of each section was then calculated.

Sample and Data Analysis

Analysis of the biocolloids was conducted using methods and procedures developed and used previously in our research (Rose et al., 1989; Watt et al., 2002). Oocysts in the effluent samples were concentrated by direct vacuum filtration through cellulose acetate membranes (0.22 μm pore-diameter; Sartorious Ag) previously treated with PBS (0.54 g L^{-1} $NaHPO_4$ and 0.88 g L^{-1} KH_2PO_4). The oocysts were then stained with 0.5 mL of 6 Crypto-a-glo antibody staining solution (Waterborne, Inc.) for 40 min. This solution contains a fluorescein-labeled mouse monoclonal antibody specific for *C. parvum*. Slides were prepared with DABCO-Glycerol Mounting Medium (2% 1,4 diazabicyclo(2.2.2) octane). The filters were washed twice with PBS and placed on the DABCO-coated slide, covered with more DABCO, and incubated. The cover slip was then placed on the slide and secured using clear nail polish (USEPA, 1996).

The number of *Cryptosporidium* oocysts in the effluent samples was determined directly on the membranes using an epifluorescent microscope (Olympus BH2-RFL; excitation 495 nm, emission 315 nm). Fifty randomly selected fields were counted at 1000× magnification, and the mean count determined for those fields was multiplied by the total number of fields to determine total numbers. The effluent concentrations were normalized by the injection concentration and plotted against pore volumes eluted to produce breakthrough curves. The breakthrough curves were subjected to standard temporal moment analysis to calculate recovery and mean travel time.

The *Giardia* cysts in the effluent samples were enumerated using the same methods as described above for *Cryptosporidium* using Giardi-a-glo antibody staining solution (Waterborne, Inc.). This solution contains a fluorescein-labeled mouse monoclonal antibody specific for *G. lambia*. The *Microsporidium* spores in the effluent samples were enumerated using the same methods as described above for *Cryptosporidium* using Microspor-a-glo antibody staining solution (Waterborne, Inc.). This solution contains monoclonal antibodies specific for *Enteromorpha intestinalis*.

Results

The breakthrough curves for the nonreactive tracer PFBA, which was used to characterize the hydrodynamic properties of the packed columns, were sharp and symmetrical and appeared at approximately one pore volume (data not shown). Mass recoveries of PFBA, determined by calculating the zeroth temporal moments, were >97% for all experiments. Péclet numbers were obtained by calibrating a solution for the one-dimensional advective-dispersive transport equation to the measured data; the values were >50 for all media. These results indicate relatively ideal hydrodynamic behavior (i.e., no preferential flow) for the packed-column systems.

Breakthrough curves for *Cryptosporidium* transport in the various porous media are shown in Fig. 1. The arrival wave of *Cryptosporidium* occurred at approximately one pore volume, similarly to PFBA. This is supported by the results of the moment analyses, which produced equivalent travel times.

The effluent recoveries of *Cryptosporidium* for all experiments are reported in Table 2. Recovery of oocysts in the column effluent was greater for the two porous media with larger median grain sizes (Table 2). Specifically, mean recoveries for the experiments conducted with Accusand and the coarse-sieved Vinton were 9 and 33%, respectively. In contrast, the mean effluent recoveries for Eustis and Vinton soils were significantly lower (<1%). The Vinton and Eustis soils had statistically similar recoveries despite significant differences in metal oxide and organic matter content (Table 1). The effluent recoveries observed for the model porous medium (Accusand) are within the range of those reported for similar sandy media under similar experimental conditions (Harter et al., 2000; Bradford and Bettahar, 2005; Cortis et al., 2006; Abudalo et al., 2010; Kim et al., 2010).

The soil-phase concentrations of *Cryptosporidium* as a function of column length are presented in Fig. 2. The majority of the distributions deviate somewhat from log-linear behavior. This behavior is inconsistent with standard colloid filtration theory, for which log-linear distributions are observed, and has been observed in a number of studies examining colloid transport (Tufenkji et al., 2003; Li et al., 2004; Blanford et al., 2005; Foppen et al., 2007; Pang, 2009).

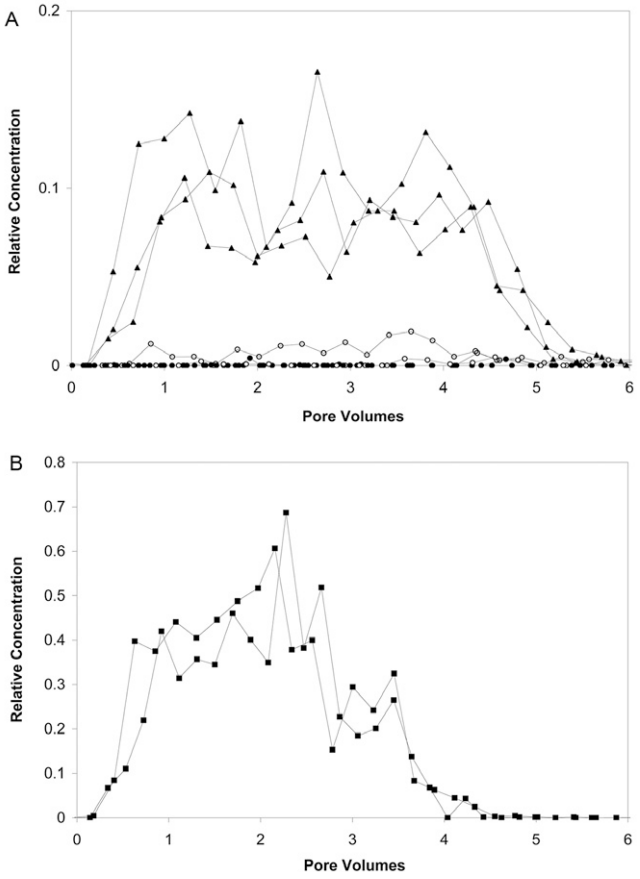

Fig. 1. Breakthrough curves for *Cryptosporidium* oocysts in 10 mmol L^{-1} NaCL solution. (A) Closed circles, Eustis; open circles, Vinton; closed triangles, Accusand. (B) Closed squares, coarse Vinton. The results of replicate experiments are shown. Relative concentration (C/C_0) and pore volumes (Qt/V_w) are in nondimensional form, where Q is flow rate, t is time, and V_w is the volume of water retained by the packed column.

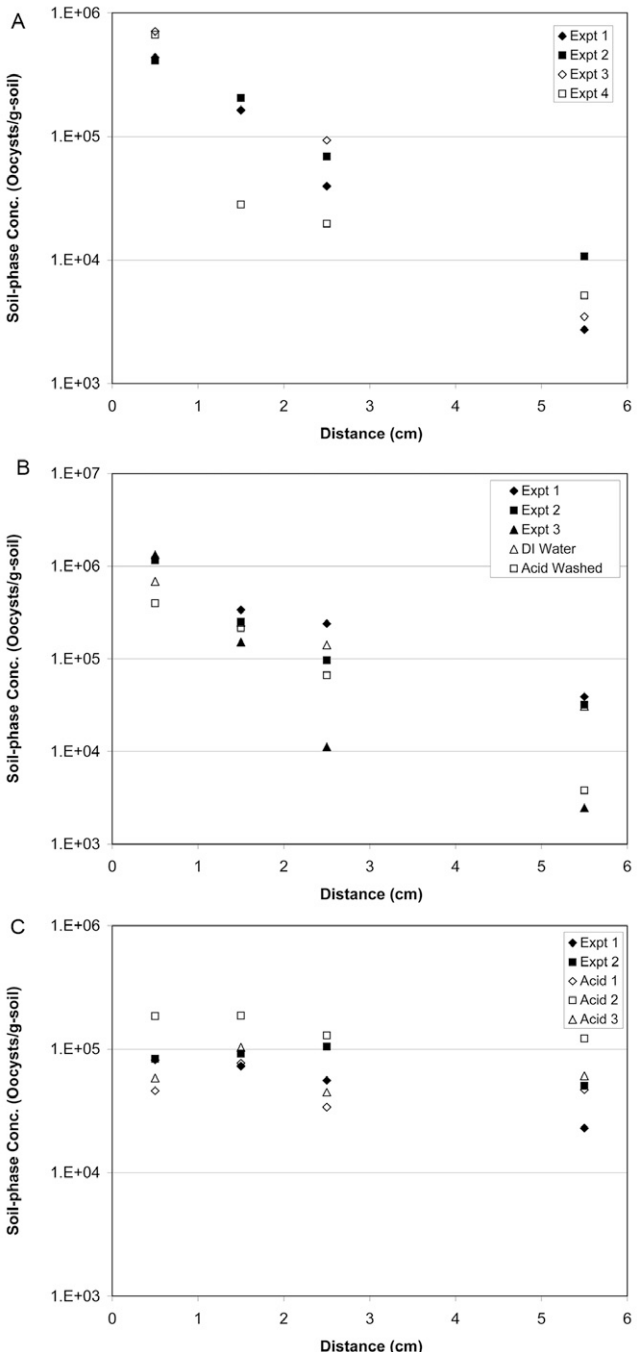

Fig. 2. Measured soil-phase distributions of *Cryptosporidium* oocysts for (A) Eustis soil, (B) Vinton soil, and (C) coarse-sieved Vinton soil. The data denoted as "Acid" and "DI water" represent the results obtained from the experiments conducted with the treatments noted in Table 2 and discussed in the text.

The total recoveries, determined as the sum of oocysts recovered in the column effluent and those recovered from the porous media, are reported in Table 2. The total recoveries averaged 101, 71, and 94% for the Eustis, Vinton, and coarse Vinton media, respectively, demonstrating excellent mass-balance closure for Eustis and coarse Vinton. Although total recoveries are not routinely reported for *Cryptosporidium* transport studies, the few prior measurements ranged from 41 to 130% (Harter et al., 2000; Bradford and Bettahar, 2005; Kim et al., 2010).

The impact of a reduction in solution ionic strength on *Cryptosporidium* retention and transport was investigated for the Vinton and coarse-Vinton media. For both media, the effluent recoveries for these experiments are similar to the recoveries obtained for the experiments conducted with the 10 mmol L^{-1} solution (Table 2). The impact of removing soil organic matter and metal oxides from the porous media was also examined for the Vinton and coarse-Vinton media. The effluent recoveries for both treated media are similar to the recoveries obtained for the experiments conducted with the respective untreated media (Table 2).

The transport of *Cryptosporidium* in the Vinton soil was compared with that of two other protozoa, *Giardia* and *Microsporidium*. Effluent recoveries were 0.6 and 27% for *Giardia* and *Microsporidium*, respectively, compared with 0.5% for *Cryptosporidium*. Recoveries were predicted based on standard colloid filtration theory, and the calculated values were approximately an order of magnitude larger for *Microsporidium* versus *Cryptosporidium* and several orders of magnitude larger for *Cryptosporidium* versus *Giardia*. In addition, essentially complete retention was predicted for *Giardia*. In contrast to the predicted recoveries, measurable recovery was observed for *Giardia*, and this recovery was similar to that observed for *Cryptosporidium*. This observed behavior is inconsistent with that predicted based on colloid filtration theory.

Discussion

Given that the zeta potentials for the oocysts and the soils are negative, the conditions for retention of the *Cryptosporidium* oocysts in the experiments presented above are considered to be unfavorable with respect to the DLVO (Derjaguin–Landau–Verwey–Overbeek) theory (Israelachvili, 1992), which is typically used to describe colloid attachment via physicochemical (electrostatic and van der Waals) interactions. Thus, retention should be minimal for the conditions of the experiments following classic DLVO theory. Several mechanisms have been proposed to explain colloid retention under unfavorable conditions, including straining by physical trapping in pores (McDowell-Boyer et al., 1986; Tufenkji et al., 2004; Bradford and Bettahar, 2005; Tufenkji and Elimelech, 2005), capture at certain phase interfaces such as at grain–grain contacts (Li et al., 2006; Xu et al., 2006; Johnson et al., 2010; Kim et al., 2010), association with secondary electrostatic energy minima (McDowell-Boyer, 1992; Hahn and O'Melia, 2004; Redman et al., 2004), and retention by locally favorable microdomains associated with geochemical surface heterogeneity or surface roughness (Johnson et al., 1996; Bhattacharjee et al., 1998; Shellenberger and Logan, 2002). In addition, steric interactions between surface components of *Cryptosporidium* oocysts and geomedia surfaces have been observed to influence retention (Considine et al., 2000; Byrd and Walz, 2005, 2007; Kuznar and Elimelech, 2005; Gao and Chorover, 2009).

The reduction in solution ionic strength had minimal impact on measured retention, which suggests that retention by a secondary energy minimum was not significant for these systems. Similarly, treatments to remove metal oxides and organic matter from the porous media had minimal impact on measured retention. This suggests that physicochemical attachment to locally favorable microdomains associated with geochemical heterogeneity was relatively insignificant for these systems. Significant retention of oocysts was observed for the Accusand (~90%) and coarse Vinton media (~70–80%), which have larger median grain diameters and minimal pore space with estimated diameters <100 μm (Table 1). Straining and capture at grain–grain contacts are expected to have minimal impact for these two porous media given the relative sizes of the oocysts, media grains, and pores, based on the results of prior research (McDowell-Boyer et al., 1986; Tufenkji et al., 2004; Bradford and Bettahar, 2005; Tufenkji and Elimelech, 2005; Xu et al., 2006; Johnson et al., 2010).

Given the apparent inconsequence of straining, capture at grain–grain contacts, secondary energy minima, and geochemical microdomains, the significant oocyst retention observed for the coarse Vinton and Accusand media and for the soils may be related in part to the impact of surface roughness associated with the porous media. Porous media surface roughness has been shown to enhance colloid retention by two means: (i) localized reductions in electrostatic energy barriers and (ii) the development of low-velocity microdomains (Johnson et al., 1996; Bhattacharjee et al., 1998; Shellenberger and Logan, 2002). Qualitative examination of the porous media using scanning electron microscopy reveals that the media exhibit significant surface roughness. Additionally, quantification of the surface roughness via atomic force microscopy provided a root mean square roughness of 133 and 110 nm for the untreated and treated coarse Vinton, respectively. Another means by which to assess the degree of surface roughness is to compare the calculated geometric (smooth surface) specific soil surface areas to the measured surface areas obtained via N_2/BET analysis. The geometric specific surface areas, which exclude the contribution of surface roughness, are 51, 135, and 145 $cm^2\ cm^{-3}$ for Accusand, Eustis, and Vinton, respectively. The specific soil surface areas measured with N_2/BET are 1500, 11,000, and 53,000 $cm^2\ cm^{-3}$ for those three media. The surface areas measured with N_2/BET, which include the contribution of surface roughness to surface area, are much larger than the geometric areas, indicating that these media have significant surface roughness.

Straining and capture at grain–grain contacts are expected to have minimal impact for the Accusand and coarse Vinton media. Conversely, it is anticipated that they may be significant retention mechanisms for the Vinton and Eustis media, given that these media have a sizeable fraction of pores with estimated diameters <10 μm (Table 1). Finally, steric interactions between porous medium surfaces and macromolecules comprising the oocyst surface have been shown to influence retention of *Cryptosporidium* oocysts. In addition, prior studies have shown that treatment of *Cryptosporidium* oocysts with formalin can alter surface properties and, for example, enhance attachment (Kuznar and Elimelech, 2005; Byrd and Walz, 2007; Gao and Chorover, 2009). It is likely that such interactions influenced retention in our systems.

Conclusions

A series of miscible-displacement experiments was conducted to examine the transport of *Cryptosporidium* oocysts in two natural soils, a coarse-sieved fraction of one of the soils, and a model sand. Significant retention of *Cryptosporidium* oocysts was observed for all media despite the fact that conditions were unfavorable for physicochemical interactions with respect to DLVO theory. Additional experiments were conducted with solution or soil conditions altered such that physicochemical attachment via electrostatic and van der Waals interactions would be minimized. On the basis of the observed results, it appears that retention by secondary energy minima or geochemical microdomains was insignificant. The porous media used for the experiments exhibited large magnitudes of surface roughness, and it is suggested that this surface roughness contributed significantly to oocyst retention. Surface roughness has been reported to contribute to colloid retention for systems comprised of model porous media, and the associated impacts are anticipated to be of even greater significance for natural soils given the latter's greater complexity and heterogeneity.

Acknowledgments

We thank the reviewers, technical editor, and editor for constructive comments and Ann Russo for assistance. This research was funded by a grant received from the US Environmental Protection Agency STAR program, with additional support provided by the US Department of Agriculture National Research Initiative Program.

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