Environmental Science & Technology

Article

pubs.acs.org/est

Deposition of *Cryptosporidium parvum* Oocysts in Porous Media: A Synthesis of Attachment Efficiencies Measured under Varying Environmental Conditions

Yeonjeong Park,[†,‡] E. Robert Atwill,[§] Lingling Hou,[∥] Aaron I. Packman,[⊥] and Thomas Harter*[,†]

[†]Land, Air, and Water Resources, University of California, One Shields Ave., Davis, California 95616-8628, United States

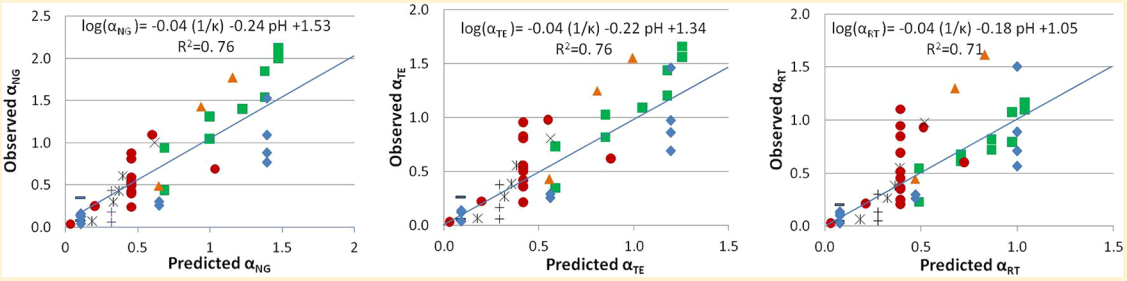
[‡]Han-River Basin Environmental Office, Ministry of Environment, 231, Mangwol-Dong, Hanam-si, Gyeonggi-do 465-731, Republic of Korea

[§]School of Veterinary Medicine, University of California, One Shields Ave., Davis, California 95616-8734, United States

[∥]Kearney Agricultural Center, 9240 S. Riverbend Ave., Parlier, California 93648, United States

[⊥]Civil and Environmental Engineering, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3109, United States

Supporting Information



ABSTRACT: An extensive set of column experiments was performed with freshly harvested *Cryptosporidium parvum* oocysts to evaluate the effects of solution chemistry, surface coatings, interactions with other suspended particles, and pore fluid velocity on the fate and transport of this widely occurring waterborne pathogen in sandy porous media. We synthesized our data set with a comprehensive literature survey of similar experiments, to compute attachment (collision) efficiencies (α) used in colloid filtration theory (CFT) using three models for the single collector efficiency (η) across a wide range of experimental conditions. Most prior experiments have observed the transport of surface-treated, sterile *C. parvum* oocyst in porous media. Our column data confirm for freshly harvested oocysts that the presence of iron coatings on the sand medium and the presence of suspended illite clay drastically enhance oocyst deposition. Increasing ionic strength and decreasing pH also systematically enhance the attachment efficiency. Attachment efficiency decreases only at a very high ionic strength, most likely as a result of steric repulsion and possibly other changes in oocyst surface properties. Attachment efficiencies vary with fluid flow rate but without showing specific trends. We found that the computed attachment efficiency across all reported experiments could be reliably estimated using a regression model based on parameters related to ionic strength and pH. The regression model performed better with the Nelson-Ginn η model and Tufenkji-Elimelech η model than with the Rajagopalan-Tien η model. When CFT is used in environmental assessments, the proposed regression model provides a practical estimator for attachment efficiencies of *C. parvum* oocyst deposition in porous media for a variety of environmental conditions unfavorable to attachment.

INTRODUCTION

Cryptosporidium is one of the most common enteric parasites in humans and domestic animals, and outbreaks of *Cryptosporidiosis* frequently occur worldwide.[1−3] The primary species that infect humans are *C. hominis* and *C. parvum*. The former is primarily shed by infected humans, whereas the latter is shed by infected humans, livestock, and some wildlife species. The majority of outbreak incidents have been attributed to primary infection from consumption of contaminated drinking water or recreational water.[4,5] One of the main sources of drinking water contamination is agricultural runoff and drainage that has come into contact with feces from infected livestock, especially young calves, and other animals.[2,6]

Because of the protective thick wall of the environmentally transmissive stage − the oocyst − *Cryptosporidium* can remain infective under cool moist conditions for several months.[5,7] Filtration techniques are commonly used to remove *C. parvum* oocyst from drinking water.[8,9] Natural porous media (soils and aquifers) also effectively filter *C. parvum* oocyst.[10,11] Hence, many groundwater sources are better protected from *C. parvum* oocyst contamination than surface water sources, although this

Received: February 13, 2012
Revised: June 5, 2012
Accepted: August 3, 2012
Published: August 3, 2012

protection is not complete.[5] Filtration in natural porous media is also exploited in bank filtration, for which EPA provides *C. parvum* oocyst attenuation credits.[12−14]

Most reports on the fate of *C. parvum* oocysts in the environment use epidemiological risk models and their transmission rates are generally qualitative.[15,16] In contrast, quantitative physicochemical analysis of *C. parvum* oocyst fate and transport in porous media is most commonly based on colloid filtration theory (CFT),[17] which explicitly accounts for the processes leading to deposition of colloids on collector particles (e.g., sand grains). The single collector efficiency, η, of the CFT model accounts for the physical mechanisms of colloidal transport to collector surfaces, that is, advection, diffusion, gravitational sedimentation, and interception. Several approaches have been proposed to estimate η in idealized porous media based on pore-scale flow field representations and surface−surface interactions.[17−20] Individual models for η differ in their representation and mathematical treatment of the various processes that lead to attachment, for example, hydrodynamic retardation, diffusion, particle rotation, wedging of oocysts between collector surfaces, and the inclusion of attractive van der Waals forces between surfaces.[20]

Oocyst-collector interactions include electrical double layer forces (repulsive or attractive), van der Waals forces (attractive), hydration effects (repulsive), hydrophobic forces (attractive), steric interaction of adsorbed layers (repulsive), and polymer bridging (attractive).[21−23] Electrical double layer and van der Waals interactions between surfaces are explicitly considered in the Derjaguin, Landau, Verwey, and Overbeek theory of colloid interaction (DLVO theory[24,25]). Within CFT, the interactions and forces not explained by η are implicitly incorporated into a dimensionless parameter referred to as attachment efficiency (α). The attachment efficiency, near unity for favorable deposition, represents the fraction of collisions that lead to permanent attachment under steady state flow. For porous media transport of *C. parvum* oocysts, the magnitude of α has not been systematically quantified and catalogued.

Elimelech[26] showed that the attachment efficiency (α) is proportional to the exponential form of the dimensionless total interaction energy, which is determined by the solution chemistry based on DLVO theory (Supporting Information). Solution chemistry-based equations can be an efficient tool to estimate attachment efficiency when DLVO theory holds. In practice, theoretical determination of α based on DLVO theory has thus far not confirmed experimental observations.[27] Typically, α is estimated from experimental observations, for example, laboratory column or field breakthrough curves[28,29] specifically under controlled unfavorable conditions.[22,27] When α is obtained directly from observations, the magnitude of α is also affected by the choice of the η model.[20] For *C. parvum* oocysts, no systematic comparison of α has been performed for different η models.

Oocyst treatment prior to experimentation is a potentially important experimental variable in the determination of α because surface properties have possibly significant effect on attachment. For laboratory and field experiments, oocysts are typically inactivated by heat,[30] formalin,[31,32] or UV radiation.[33] Inactivation of the oocysts with formaline or heat is known to alter the structure of surface proteins and reduce steric repulsion, which results in increased adhesion to collectors.[34] Only few transport experiments have used freshly harvested oocysts with minimal treatment.[35]

This article has three objectives: (1) measure the deposition and evaluate α of freshly harvested, unsterilized *C. parvum* oocysts in sand column experiments under a wide range of systematically varying environmental conditions including ionic strength (IS), pH, cosuspended sediments, fluid velocity, and pore grain surface characteristics; (2) compile literature-reported *C. parvum* oocyst breakthrough curve data to determine whether oocyst attachment efficiency systematically varies as a function of environmental conditions, especially pH and IS, two key variables controlling DLVO interactions; (3) compare experiment-based α values obtained from three key models of the single collector efficiency including the classic model developed by Rajagopalan and Tien[18] and two models that have recently been proposed by Tufenkji and Elimelech[19] and by Nelson and Ginn,[20] here referred to as RT, TE, and NG models, respectively. We are particularly interested in determining whether the choice of η model systematically affects the relationship between α and solution chemistry . The overarching objective of this article is to provide a comprehensive, empirically based resource for selecting appropriate α values in *C. parvum* oocyst transport models of streambeds, filters, and hydrogeologic materials under typical environmental conditions.

MATERIALS AND METHODS

Collection and Purification of *C. parvum* Oocysts. Wild-type *C. parvum* oocysts were used in all experiments. Fresh feces were collected from naturally infected calves at 9 to 21 days of age from three commercial dairies in Tulare, California. Oocysts were then used within 2 to 7 days of fecal collection. In general, over 90% of these oocysts are infectious when freshly harvested from young dairy calves, as shown in Hou et al.[36] These oocysts were confirmed as *C. parvum*, using the genotyping scheme described by Xiao et al.[37] Using an acid fast protocol to detect oocysts,[38] samples having more than 25 oocysts per 400 microscopic field on fecal smears were washed through a series of 40, 100, 200, and 270 mesh sieves with Tween water (0.2% Tween 20 in deionized water [vol/vol]). The resulting suspension was centrifuged at 1500*g* for 20 min in a 250 mL centrifuge tube, supernatant discarded and the pellet diluted in Tween water. Discontinuous sucrose gradients were used to purify *C. parvum* oocysts from fecal suspensions.[39] Purified oocysts were stored in a solution containing 0.001% amphotericin, 0.006% penicillin G, 0.01% streptomycin sulfate, and 0.01% Tween 20.[36] Using a phase contrast hemacytometer (bright line hemacytometer: Hausser Scientific, Horsham, Pennsylvania), concentrations of purified oocysts were determined as the arithmetic mean of eight independent counts. It is likely that there are small random errors associated with individual subsamples of a large volume of oocysts but that the error is unbiased due to random sampling.

Column Experiments. Twelve experiments were conducted in two Plexiglas columns, 5.1 cm in inner diameter and 20 cm in length. The porous medium was Accusand (Mesh 30/40, UNIMIN Corporation, Ottawa, Minnesota). Columns were wet-packed firmly in degassed deionized water (DIW) and then rinsed by flow-through of 10 pore volumes (p.v.) of degassed DIW. The pore volume was computed based on the total dry weight of sand added to each column, the total column volume, and the specific density of the Ottawa sand used in the experiments (2.65 g/cm^3). Experimental solutions were prepared by dissolving NaCl or $MgCl_2$ in DIW to the desired ionic strength followed by degassing. The solution pH was

Table 1. Summary of *C. parvum* Transport Experiments in Porous Media: Parameter Values, Special Features on Each Article, Peak Value (C/C_0) of *Cryptosporidium* Breakthrough Curve Data, Calculated Collector Efficiencies, and Calculated Attachment Efficiencies; Subscripts RT, TE, NG Represent Models Developed by Rajagopalan and Tien,[18] Tufenkji and Elimelech,[19] and Nelson and Ginn; "-", Not Specified in the Article; Other Parameter Values Are Reported in Table S4 of the Supporting Information

citation	feature		column length [m]	TDS [mM]	pH	C/C_0	approach velocity [m/s]	porosity	collector diameter [m]	η_{TE}	η_{RT}	η_{NG}	α_{TE}	α_{RT}	α_{NG}	λ [1/m]
Brush et al. 1999 [45]	glassbeads		0.109	-	-	0.585	2.94×10^{-4}	0.32	2×10^{-3}	0.0019	0.0025	0.0016	3.95	2.94	4.55	4.92
	sand			-	-	0.484		0.33	8.5×10^{-4}	0.0029	0.0029	0.0025	1.49	1.48	1.72	6.66
	shale aggregate			-	-	0.563		0.36	5.9×10^{-4}	0.0035	0.0032	0.0031	0.67	0.74	0.77	5.27
Harter et al. 2000 [35]	CS fast		0.1	3 (conductivity: 220 μS/cm)	7.9	0.738	8.22×10^{-5}	0.42	1.4×10^{-3}	0.0041	0.0036	0.0036	0.83	0.95	0.94	3.03
	CS slow					0.104	8.22×10^{-6}	0.37	1.4×10^{-3}	0.049	0.067	0.034	0.51	0.37	0.73	22.64
	MS fast					0.2	8.22×10^{-5}	0.44	4.25×10^{-4}	0.0058	0.005	0.0055	0.96	1.1	1.0	16.09
	MS slow					0.0022	8.22×10^{-6}	0.45	4.25×10^{-4}	0.038	0.031	0.032	0.56	0.69	0.66	61.19
	FS fast					0.0052	8.22×10^{-5}	0.51	1.8×10^{-4}	0.0099	0.0094	0.01	0.81	0.85	0.8	52.59
Hsu et al. 2001 [57]	glassbeads		0.1	10 as $NaClO_4$	2.4	0.15	2.53×10^{-5}	0.39	2×10^{-3}	0.023	0.031	0.018	1.29	0.96	1.65	18.97
					3.4	0.1							1.56	1.17	2.0	23.03
					5.6	0.21							1.06	0.79	1.36	15.61
					8.7	0.22							1.03	0.77	1.32	15.14
					10.2	0.32							0.77	0.58	0.99	11.39
					11.2	0.41							0.61	0.45	0.78	8.92
	polystyrene				2.4	0.1		0.37		0.024	0.036	0.019	1.51	1.0	1.94	23.03
					3.4	0.18							1.13	0.74	1.44	17.15
					5.6	0.2							1.06	0.7	1.35	16.09
					8.7	0.16							1.2	0.8	1.54	18.33
					10.2	0.3							0.79	0.52	1.01	12.04
					11.2	0.28							0.84	0.55	1.07	12.73
	glassbeads			1 as $NaClO_4$	5.6	0.34		0.39		0.023	0.031	0.018	0.73	0.55	0.94	10.79
				3.2		0.3							0.82	0.61	1.05	12.04
				10		0.2							1.09	0.82	1.4	16.09
				32		0.12							1.44	1.08	1.85	21.2
				100		0.1							1.56	1.17	2.0	23.03
	polystyrene			1 as $NaClO_4$		0.59		0.37		0.024	0.036	0.019	0.35	0.23	0.44	5.28
				3.2		0.21							1.03	0.68	1.31	15.61
				10		0.19							1.09	0.72	1.4	16.61
				32		0.16							1.2	0.8	1.54	18.33
				100		0.08							1.66	1.1	2.12	25.26
Logan et al. 2001 [11a]	coarse	wastewater	0.6	-	-	0.004	1.16×10^{-6}	0.48	1.4×10^{-3}	0.26	0.22	0.13	0.041	0.05	0.084	9.22
		wastewater		-	-	0.0015	4.63×10^{-7}			0.72	0.64	0.25	0.017	0.02	0.051	10.8
		tap water		187 μS	7	0.0002	4.63×10^{-7}			0.72	0.64	0.25	0.024	0.027	0.069	14.63
		tap water				0.0062	1.16×10^{-6}			0.26	0.22	0.13	0.038	0.046	0.077	8.48
	fine	tap water				0.0004	1.16×10^{-6}	0.46	3.1×10^{-5}	0.47	0.56	0.54	0.0007	0.0006	0.00062	13.1
		tap water				0.0002	4.63×10^{-7}			0.75	0.81	0.7	0.0005	0.0005	0.00053	14.63
		wastewater		-	-	0.0002	4.63×10^{-7}			0.75	0.81	0.7	0.0005	0.0005	0.00053	14.63
		wastewater		-	-	0.0004	1.16×10^{-6}			0.47	0.56	0.54	0.0007	0.0006	0.00061	12.98
Dai and Hozalski 2002 [63]	none	none	0.25	10 as $CaCl_2$	6.85	0.49	1.39×10^{-5}	0.4	5.5×10^{-4}	0.0015	0.0013	0.0012	0.81	0.98	1.0	2.85
	biofilm	none				0.77							0.3	0.36	0.37	1.05
	none	NOM[b]				0.86							0.17	0.21	0.21	0.6

dx.doi.org/10.1021/es300564w | Environ. Sci. Technol. 2012, 46, 9491–9500

Table 1. continued

citation	feature	column length [m]	TDS [mM]	pH	C/C_0	approach velocity [m/s]	porosity	collector diameter [m]	η_{TE}	η_{RT}	η_{NG}	α_{TE}	α_{RT}	α_{NG}	λ [1/m]
	biofilm				0.85							0.18	0.22	0.23	0.65
	none		chlorine		0.83							0.21	0.26	0.26	0.75
	none		75 μM as Al_3^+		0.27							1.48	1.8	1.84	5.24
Tufenkji et al. 2004 [30]	clean quartz sand	0.071	1 as KCl	5.7	0.41	4.2×10^{-4}	0.43	2.1×10^{-4}	0.0049	0.0047	0.0043	0.43	0.45	0.49	12.56
			3.16		0.075							1.25	1.3	1.43	36.48
			10		0.04							1.55	1.62	1.77	45.34
Tufenkji and Elimelech 2005 [64]	glassbeads	0.126	1 as KCl	8	0.78	8.3×10^{-5}	0.37	3.3×10^{-4}	0.0077	0.0078	0.007	0.065	0.065	0.072	1.97
			10		0.36							0.27	0.27	0.3	8.1
			30		0.23							0.39	0.38	0.43	11.66
			100		0.12							0.56	0.55	0.61	16.83
Abudalo et al. 2005 [31]	λ^c: 0	0.1	0.1 as NaCl	5.7	0.7650	8.8×10^{-6}	0.38	9.2×10^{-4}	0.033	0.041	0.025	0.059	0.047	0.078	2.68
	λ: 0.32			5.7	0.0330							0.75	0.6	1.0	34.11
	λ: 0.04			8	0.2350							0.32	0.25	0.42	14.48
	λ: 0.04			10	0.6000							0.11	0.089	0.15	5.11
Bradford and Bettahar 2005 [48]	ottawa aquifer sand	0.13	1 as NaCl	7	0.31	1.83×10^{-5}	0.36	7.1×10^{-4}	0.013	0.016	0.011	0.38	0.3	0.43	9
		0.126			0.24	1.67×10^{-5}	0.34	3.6×10^{-4}	0.019	0.023	0.017	0.17	0.13	0.18	11.33
		0.127			0.11	2.17×10^{-5}	0.35	1.5×10^{-4}	0.034	0.038	0.033	0.059	0.053	0.06	17.38
Hijnen et al. 2005[61,62]	castricum 0.5	0.5	760 μS/cm	8	0.0001	5.32×10^{-6}	0.36	1.8×10^{-4}	0.066	0.077	0.063	0.038	0.033	0.039	17.96
	castricum 0.9				0.0005	1.06×10^{-5}			0.043	0.048	0.043	0.049	0.044	0.05	15.2
	roosteren 0.9		560 μS/cm	8.2	2×10^{-7}	1.04×10^{-5}	0.32	5×10^{-4}	0.029	0.043	0.026	0.4	0.27	0.45	30.85
	roosteren 2.5				6.3×10^{-8}	2.88×10^{-5}			0.013	0.017	0.012	0.95	0.76	1.04	33.16
Hijnen et al. 2007 [33]	source water from river Rhine	1.5	turbidity of 0.1 FTU	8	1.8×10^{-5}	8.3×10^{-5}	0.41	2.8×10^{-4}	0.0079	0.0075	0.0074	0.21	0.22	0.22	7.28
Abudalo et al. 2010 [32]	DOM[d] 0 mg/L	0.1	0.1 as NaCl	5.7	0.3	8.8×10^{-6}	0.38	9.2×10^{-4}	0.033	0.042	0.025	0.26	0.21	0.35	12.04
	DOM 20 mg/L				0.57							0.12	0.096	0.16	5.62
this study		0.2	0.075 as NaCl	7	0.81	8.22×10^{-5}	0.32	4.25×10^{-4}	0.0135	0.0143	0.0120	0.032	0.031	0.036	1.05
			3 as NaCl		0.274		0.33		0.0127	0.0131	0.0114	0.217	0.209	0.241	6.47
			9 as $MgCl_2$		0.5		0.32		0.0133	0.014	0.0119	0.109	0.103	0.122	3.47
			15 as NaCl		0.002		0.32		0.0132	0.0139	0.0118	0.979	0.930	1.095	31.07
			100 as NaCl		0.77		0.32		0.0128	0.0133	0.0114	0.043	0.041	0.048	1.31
	iron coating in grains		3 as NaCl		0.128		0.34		0.0122	0.0125	0.0110	0.362	0.353	0.400	10.28
	oocysts with illite clay				0.045		0.34		0.0124	0.0128	0.0112	0.534	0.518	0.592	15.51
	oocysts with Illinois River SS				0.004		0.34		0.0123	0.0126	0.0111	0.957	0.932	1.060	27.48
				5.5	0.027		0.34		0.0124	0.0127	0.0112	0.622	0.604	0.690	18.06
				8.5	0.26		0.33		0.0127	0.0132	0.0114	0.224	0.216	0.250	6.74
				7	1.4×10^{-5}	8.22×10^{-6}	0.32		0.0647	0.0934	0.0544	0.361	0.250	0.429	55.88
					0.17	2.47×10^{-4}	0.32		0.0088	0.0082	0.0075	0.420	0.454	0.493	8.86

[a]For comparison only. This experiment was performed under pulsed, unsaturated condition, which leads to significantly higher attenuation. Not included in the regression analysis. [b]NOM, natural organic matter. [c]λ, fraction of positively charged surface on the grain. [d]DOM, dissolved organic matter.

dx.doi.org/10.1021/es300564w | Environ. Sci. Technol. 2012, 46, 9491–9500

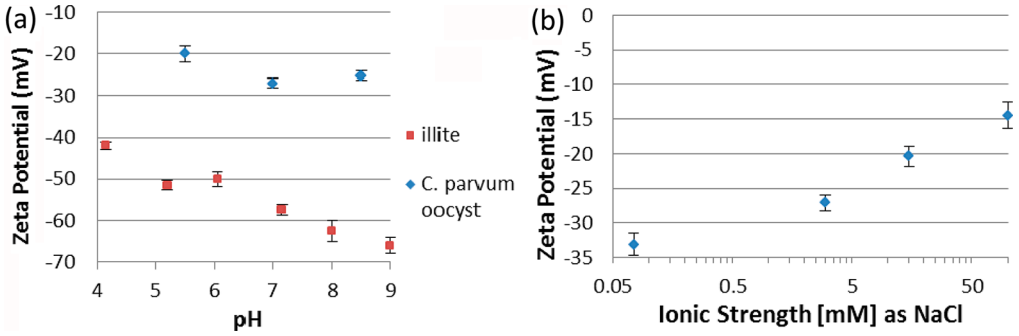


Figure 1. (a) Zeta potential of *C. parvum* oocyst and illite at 3 mM NaCl as a function of pH, (b) zeta potential of *C. parvum* oocyst at pH 7 with respect to ionic strength. Error bars represent one standard deviation of multiple measurements (10 runs for oocyst and 3 runs for illite).

adjusted with 1 M HCl or 1 M $CaHCO_3$. Oocyst suspensions were prepared by diluting the stock suspension with the selected experimental solution to a concentration of 10^6 oocysts/mL. Columns were rinsed with the desired experimental solution for at least 10 p.v. before oocyst injection. The oocyst injection occurred over 1.5 p.v., without flow interruption, and was followed by 1600 to 2400 p.v. of oocyst-free solution. Results on oocyst transport are reported in terms of the number of pore volumes flushed through the column. Outflow was continually collected at one minute intervals (about 0.08 p.v.) for the first 60 min, then 10 mL aliquots were collected at 60 min (about 5 p.v.), 180 min (about 15 p.v.), and 720 min (about 60 p.v.) intervals. In this article, we focus on the analysis of the breakthrough curve during the oocyst injection and steady-state breakthrough (first 3.5 p.v. of the breakthrough curve). Attached *C. parvum* oocyst profiles were also determined from the retained *C. parvum* oocysts in soils after the completion of experiments (data not shown in this article). Fifteen grams of sample was added in extraction solution and mixed on a wrist action shaker. Representative 10 μL aliquots of the supernatant were smeared onto a glass slide and the direct immunofluorescent assay was performed.

Enumeration of *C. parvum* Oocysts in Aliquots of Column Water. Tween 80 (110 μL) was added to each column water sample resulting in a 0.1% Tween 80 solution. To enumerate oocyst samples from the peak phase of the breakthrough curve, samples were diluted 1:100. Samples from other periods of the breakthrough curve were concentrated 100× by centrifuging for 10 min at 1000*g*, discarding the supernatant and resuspending the pellet in 0.1% Tween water. Ten microliters of the resulting suspension were smeared onto a commercially prepared glass slide and air-dried overnight. A direct immunofluorescent assay was performed using a commercial kit (*Cryptosporidium*/*Giardia* direct immunofluorescent detection kit A 400 FL CryptoaGloTM, WaterborneTM, Inc., New Orleans, LA) with an Olympus BX60 microscope at 400× magnification. All oocysts were enumerated in each 10 μL subsample. Positive and negative controls were analyzed with each set of slides. Each oocyst concentration measurement was adjusted for the percent recovery of the direct immunofluorescent assay based on control measurements, which were performed in different solutions, boiled distilled water and 15 mM NaCl, with purified *C. parvum* oocysts to a final concentration of 10^5 oocysts/mL. Each control suspension was passed through the column apparatus without sand and processed as described above. Four replicate enumerations were performed for each control suspension. The percent recovery for this assay was estimated as described previously.[15]

Preparation of Suspended Sediments. The association of *C. parvum* oocysts with suspended particles was investigated using illite clay. Illite clay was obtained from Ward's Natural Scientific (Rochester, N.Y.) and prepared to yield stable fine colloidal suspensions following the methods of Packman et al.[40] Solid blocks of clay were ground with a mortar and pestle and then placed in a rolling-ball mill with alumina balls for 24 h. The milled clay was wet sieved through a nylon mesh with a pore size of 50 μm. The sediments that passed through the mesh were concentrated via sedimentation, and the resulting sediment suspension was stored at 4 °C. For column experiment, a solution was prepared with 200 mg/L of illite sediment in 3 mM NaCl solution mixed with 10^6 oocysts/mL of *C. parvum* oocysts. Oocysts and illite was mixed prior to adding to the column to associate the oocysts with illite first.

Zeta Potential of *C. parvum* Oocyst and Illite Clay. The zeta potentials of *C. parvum* oocysts and illite clay were determined using a Brookhaven Instruments Corp. (Holtsville, N.Y.) Zeta PALS particle analyzer. This instrument subjects the particle to an alternating current and measures the resulting velocity of the particles in the suspension. The electrophoretic mobility is converted by the instrument to a zeta potential using the Smoluchowski approximation. Oocyst suspensions were prepared in the desired background solution at concentrations of 10^6 oocysts/ml. Zeta potential of *C. parvum* oocyst was measured at different pH (5.5, 7, 8.5 at 3 mM NaCl) and ionic strength (0.075, 3, 15, 100 mM as NaCl at pH 7). The pH of the NaCl solution was adjusted from 5.5 to 8.5 using 1 M HCl or 1 M $CaHCO_3$. For illite clay, sediment suspensions were prepared in the desired background solution at concentrations of 200 mg/L. The pH of the illite suspensions was adjusted from 4 to 10 using 5 mM HCl to 5 mM NaOH.

Iron-Oxyhydroxide Coating Procedure. Accusand (200 g) and $FeCl_3$ (20 g) were weighed in a clean 1000 mL glass flask and mixed with 400 mL DI water. Aliquots (10 mL) of 2 M NaOH were added until the pH reached a value between 4.5−5.0. DI water was then added until the total volume was 800 mL. HCl and NaOH were used to adjust the pH until it was stable at pH 4.5−5.0. The flask with the mixture was shaken vigorously for 24−30 h on an orbital shaker (Fisher Model 361) at speed 350 rpm. The coated sand was rinsed repeatedly with DI water and dried at 90 °C. The rinsing and drying procedure was repeated twice.

Computation of η and α. Collector efficiency (η) was computed using the RT, TE, and NG models (Table 1). Attachment efficiency was then estimated using computed η

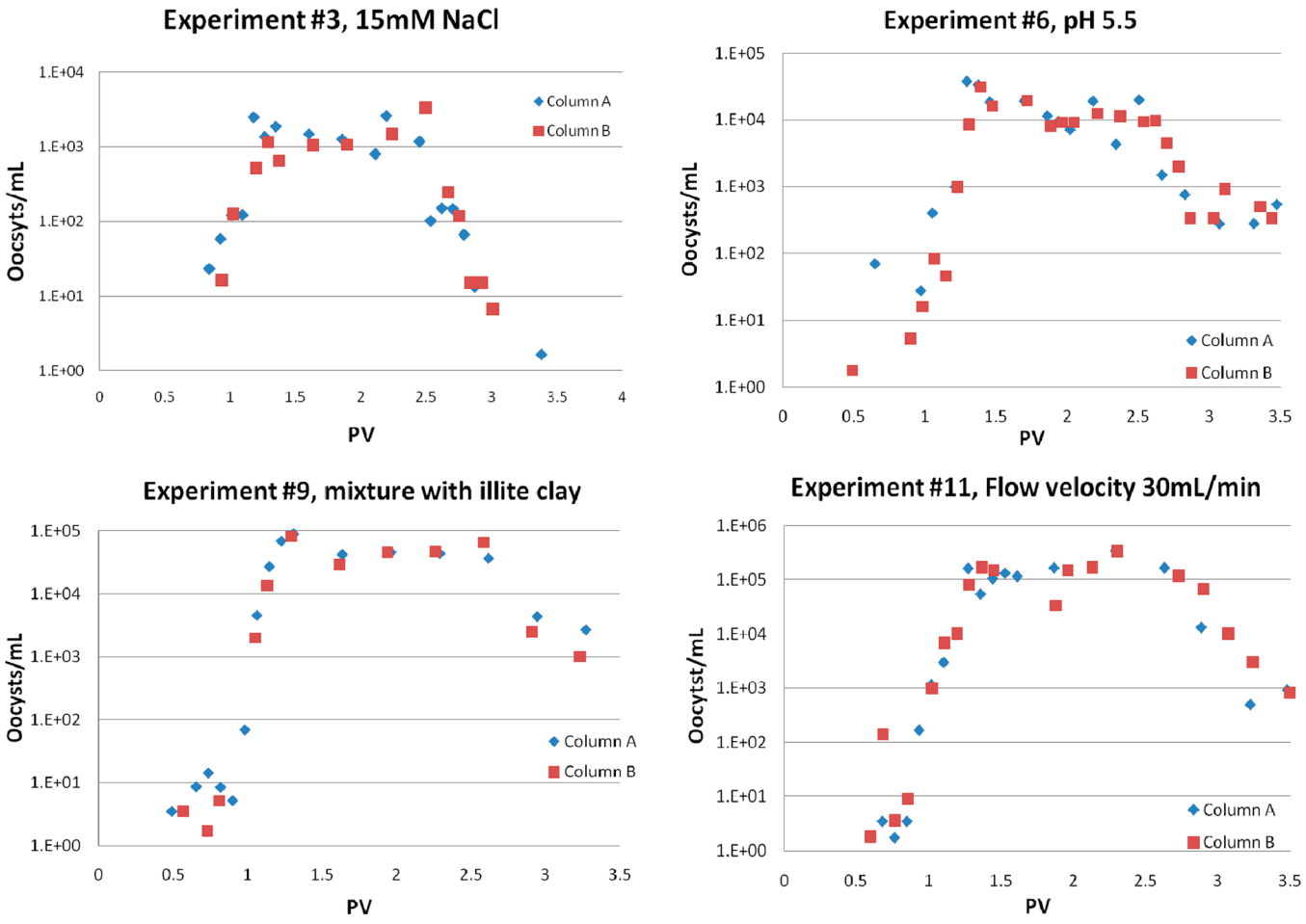


Figure 2. Representative, semilogarithmic *C. parvum* oocyst breakthrough curves (experiment #3, 6, 9, 11). Input concentration in all experiments was 10^6 oocysts/mL. Unless indicated otherwise, background solution is 3mN NaCl without suspended sediments, pH 7, flow rate is 10 mL/min, and the porous medium is uncoated quartz sand.

and maximum observed peak breakthrough concentrations. Statistical hypothesis testing (two-tailed *t* test) [41] was performed for experiments #1–#9 to test whether sample means of α_{RT} and α_{NG} (lowest and highest α values, respectively) were significantly different considering intrinsic experimental variability. The 20–40 *C. parvum* oocyst observations from two replicate experiments during the peak breakthrough period (between 1.75 and 2.75 p.v. based on the length of the peak for each experimental condition) were each considered to generate α values for the hypothesis testing. We also compiled a comprehensive set of laboratory *C. parvum* oocyst BTC data from existing literature and computed RT-, TE-, and NG-based η and α values.

RESULTS AND DISCUSSION

Throughout the ambient conditions of the experiments, the oocysts are negatively charged. At ionic strength of 3 mM, zeta potentials of fresh oocysts were highest at pH 5.5 (−20 mV), decreased to −27 mV at pH 7, consistent with previous observations,[42,43] and then became slightly less negative at pH 8.5. In contrast, the zeta potentials of illite clay decreased linearly with pH from −42 mV at pH 4 to −66 mV at pH 9 (Figure 1). At neutral pH, a log–linear change of the zeta potential, from −33 mV to −15 mV, was observed across a more than 3 orders of magnitude increase in ionic strength. Unfavorable deposition is therefore expected in the negatively charged quartz sand.[44] Standard deviations of oocyst zeta potentials were small at ±2 mV (Figure 1).

Replicate experiments produced fairly consistent BTC peak concentrations (Figure 2) with minor variability and differences that are typical for studies involving fresh, viable biocolloids.[45–47] Statistical analysis of α values across the peak period data shows that the mean α estimates for each of the three η models are not significantly different given the measurement variability, except in cases where the deposition of *C. parvum* oocyst was relatively high (Supporting Information). Where the deposition was high, variations in peak values were low (#3, #6, #9 in Table S2 of the Supporting Information) and, hence, differences between α_{RT} (lowest among three models) and α_{NG} (highest among three models) become significant. Therefore, the choice of the η model for predictive purposes is particularly relevant for large α, i.e., under favorable deposition conditions.

Tufenkji et al.[30] and Bradford and Bettahar [48] observed physical straining of *C. parvum* oocyst in porous media, which is expressed by hyper-exponential column profiles and high colloid retention near the inlet.[48] In the experiments reported here, straining processes appeared negligible because, first, column profiles did not show evidence of physical straining such as hyper-exponential profiles near the inlet, and, second, a first-order mathematical model for attachment could describe both, the oocyst breakthrough curves and the profiles (results not shown). Calculated *C. parvum* oocyst collector efficiency

dx.doi.org/10.1021/es300564w | *Environ. Sci. Technol.* 2012, 46, 9491–9500

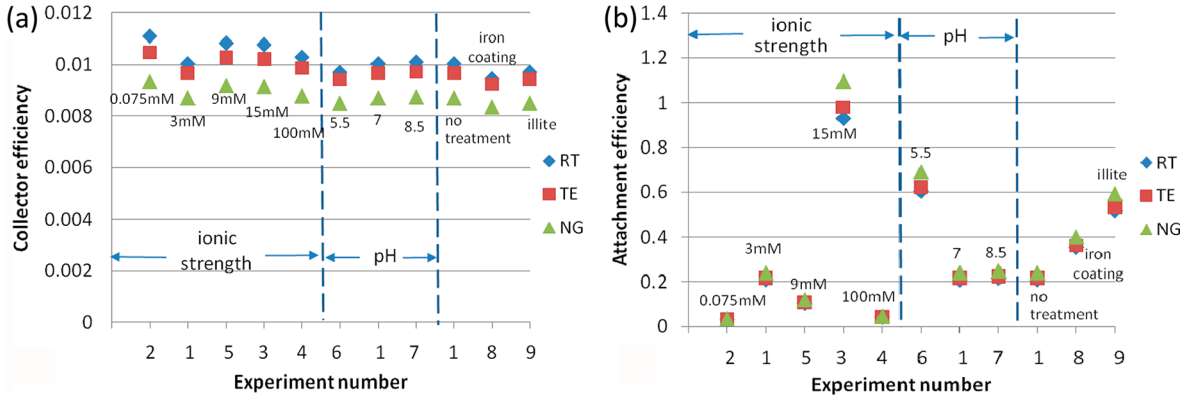


Figure 3. Collector efficiencies (a) and attachment efficiencies (b) of *C. parvum* oocyst calculated using the RT, TE, and NG models and breakthrough curve data. Three sets of experiments with different chemical properties are plotted for comparison and separated by vertical dashed lines. Particle diameter: 5×10^{-6} [m], collector diameter: 4.25×10^{-4} [m], fluid approach velocity: 8.22×10^{-5} [m/s], Hamaker constant: 1×10^{-20} [kg m^2/s^2], particle density: 1050 [kg/m^3], fluid temperature: 296 [°K]. The pH and ionic strength were 7 and 3 mM respectively unless otherwise specified. Collector efficiencies in (a) vary solely due to varying experimental porosities (as shown in Table 1).

varied among RT, TE, and NG models by as much as 18.7% (relative to η_{NG}) with an average difference of 14.6% (Figure 3). The largest difference (between η_{RT} and η_{NG}) stems primarily from the varying diffusion models because η_D (diffusion) exhibits the largest difference relative to η_I (interception) or η_G (gravity) given the size and density of *Cryptosporidium* (diameter ~5 μm and specific gravity ~1050 kg/m^3) (Figure S2 of the Supporting Information). The RT equation assumes that the collector efficiency from diffusion is additive to the collector efficiency resulting from numerical trajectory analysis on interception and sedimentation processes. The TE and NG models consider all mechanisms simultaneously and additionally incorporate hydrodynamic retardation in the diffusion coefficient. The NG model further introduces a correction factor to the diffusion term to cause the collector efficiency to asymptotically approach unity, which affects the magnitude of η_D.[20]

Effect of Solution Chemistry. Attachment efficiencies of freshly harvested *C. parvum* oocysts increased in direct proportion to IS for NaCl electrolyte concentration varying from 0.075 mM to 15 mM (part b of Figure 3). These results are consistent with previously observed colloid deposition behavior.[49,50] At the highest IS of 100 mM NaCl, however, α decreased to a value comparable to that observed under 3 mM NaCl. This is inconsistent with the zeta potential at high ionic strength (Figure 1) and resulting electrical double layer forces (Supporting Information) indicating the presence of strong non-DLVO forces at high IS, such as steric repulsion.[51] A similar reversed trend at high IS was previously reported in Kim et al.[52] The experiment with a divalent electrolyte (experiment #5, 9 mM of $MgCl_2$) did not result in the expected higher attachment efficiency relative to the experiments observed from other divalent ions (e.g., Ca^{2+}). Mg^{2+} weakly associates with carboxyl groups in the oocyst wall and tends to yield charge neutralization while Ca^{2+} forms cation bridging with carboxyl groups and leads to deposition enhancement.[53] These results underscore the fact that electrokinetic interactions have a limited influence on attachment at higher IS. For example, the experiment by Butkus et al. [54] showed no difference between the aggregation of *C. parvum* oocysts suspended in distilled water and in 500 mM NaCl. However, it appeared that non-DLVO forces in our experiments only dominated at the highest IS (100 mM NaCl).

Experiments performed at pH 5.5 and 7 at 3 mM ionic strength (experiments #6 and #1) are consistent with oocysts having less negative zeta potential at higher pH. The isoelectric pH of oocysts has been found to be ~3.3[43,55,56] and oocyst attachment efficiency has been found to decrease as ambient pH increases.[31,57] However, the attachment efficiency at pH 8.5 was slightly smaller than pH 7 corresponding to a slightly more negative zeta potential of *C. parvum* oocysts at pH 8.5.

Effect of Mineral Coatings and Clay on Collectors. Iron oxyhydroxide coated sand (exp. #8) led to higher deposition (87%) of fresh oocysts than the uncoated sand media (experiment #1, 73%) due to their charge heterogeneity and the presence of positive surface charges.[31,58] Attachment efficiency, α_{NG}, increased from 0.24 to 0.4 with the iron coating. While quartz surfaces are strongly negatively charged, regardless of pH or IS, *C. parvum* oocysts, with their negative surface charge, attach to oxyhydroxide mineral coating more strongly than to clean sand. The increase in deposition is not as strong as that induced by variations in IS (Figure 1). This is attributed to the limited density of the oxyhydroxide coatings on the quartz sand achieved with the coating protocol used. We followed the iron-oxyhydroxide coating procedure in Abudalo et al.[31] They showed that 75 ± 5% of the grain surfaces were covered by a relatively uniform layer of iron oxyhydroxide.

C. parvum oocysts showed very high removal efficiency in the presence of suspended illite clay (95.5% in exp. #9). Given the exponential deposition profiles observed within the porous medium, this is not considered to be due to straining.[48] Instead, association of oocysts with inorganic suspended sediments favors attachment to stationary collector surfaces because the illite particles are denser than the oocysts, favoring deposition by sedimentation.[59] Hence, the presence of suspended sediments significantly enhanced *C. parvum* oocyst deposition by way of attachment to illite and subsequent illite particle filtration, with α_{NG} more than doubling from 0.24 (without illite) to 0.59. *C. parvum* oocyst association with suspended sediments has also been found to increase the rate of removal from open water bodies through a combination of enhanced sedimentation and filtration.[60]

Effect of Fluid Velocity. When chemical properties do not change, conventional CFT predicts that α will be constant, as the collector efficiency model is presumed to entirely represent the effects of pore fluid flow in the colloid approach velocity.

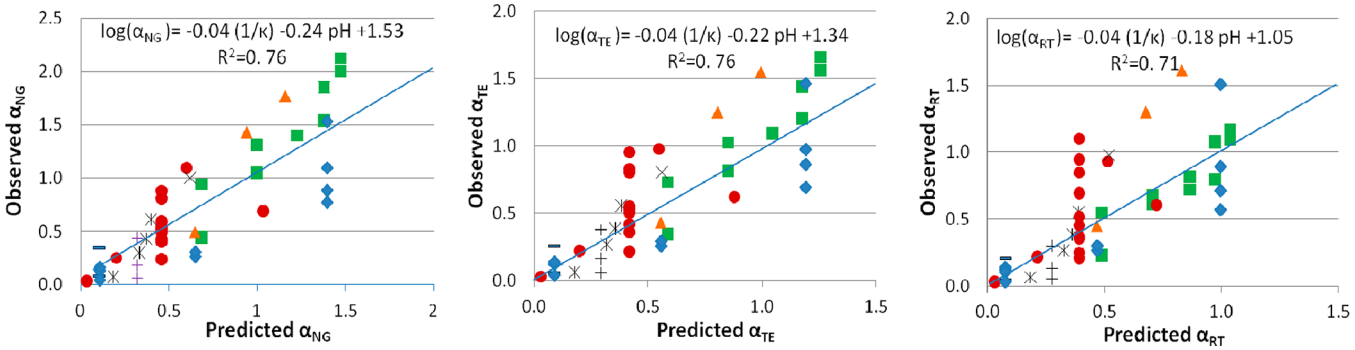


Figure 4. Multiple regression prediction (and associated equations) and experimentally estimated α using BTC data reported in the literature and the results from our experiments. Experimental data were selected where IS (molar concentration) and pH were reported. Experimental data represent a range of chemical solutions, collector grain size, approach velocity, temperature, oocyst preparations, and oocyst sources. One to one line is drawn for reference. (□: Hsu et al. (2001),[57] ○: our experiments and Harter et al. (2000),[35] Δ: Tufenkji et al. (2004),[30] X: Tufenkji and Elimelech (2005),[64] -: Abudalo et al.(2005;2010),[31,32] x: Dai and Hozalski (2002),[63] +: Bradford and Bettahar (2005),[48] ◇: Kim et al. (2010)[52]).

Hydrodynamic forces also mediate the local (microscale) attachment process, especially for complex and reversibly bound particles such as *C. parvum* oocysts. The attachment efficiency of the experiment performed at high velocity was twice as high as that in the lower-velocity control experiment having identical chemical conditions (experiment #1). However, the α value of the experiment conducted with an order of magnitude slower velocity than in #1 was also twice as high as the one observed in experiment #1, except for α_{RT}. Logan et al.[11] and Hijnen et al.[61,62] similarly found that α increases with fluid velocity (Table 1). In contrast, Harter et al.[35] found that α (calculated using the RT model) decreased with increasing velocity when other conditions were identical. Across multiple experiments and investigators, there is no observable consistent trend or linear relationship between approach velocity and α.

Synopsis of Attachment Efficiency Across Multiple Investigations. The analysis of α values obtained from our experiments and those computed based on experimental data reported by Harter et al.,[35] Hsu et al.,[57] Dai and Hozalski,[63] Tufenkji et al.,[30] Tufenkji and Elimelich,[64] Abudalo et al.,[31] Bradford and Bettahar,[48] Abudalo et al.,[32] and Kim et al.[52] shows that increasing pH from 5.6 to 8.5 and changing the IS from 0.075 mM to 100 mM systematically changes α. When the effect of IS was expressed in the form of the inverse Debye length $(1/\kappa)$[65,66] (Supporting Information), we found strong linear correlation between attachment efficiency and solution chemistry. A multiple linear regression analysis of log α as a function of $(1/\kappa)$ and pH yielded coefficients of determination (R^2) of 76% for α_{NG}, 76% for α_{TE}, and 71% for α_{RT} (Figure 4). The remaining, unexplained variability of measured α may be due to variations in organic matter/mineral content in porous media, grain size distribution, packing, pore water velocity, and oocyst properties in each experiment. The fairly high R^2 for the α_{NG} and α_{TE} indicates that a physicochemically consistent mathematical treatment of the approach process[20] significantly improves the predictability of α from DLVO-related, easily measurable experimental variables (pH and IS). For validation of the approach, a multiple regression model was developed using all of the above data except Dai and Hozalski,[63] Abudalo et al.,[31] and Abudalo et al. [32] (which were randomly selected for validation purpose). Validation results demonstrated that the multiple regression model can predict α sufficiently well using independently obtained experimental data (Supporting Information).

The experimental α values range over approximately 2 orders of magnitude, from well below 0.1 to over 1. Predicted attachment efficiencies fall within half an order of magnitude or better of the measured value, regardless of velocity, suspended sediment, collector surface properties, and other variations across experiments. Results suggest that the latter all affect collision efficiency but not as strongly as IS and pH across the observed range. The proposed empirical relationship therefore provides a practical tool to predict changes in collision efficiency across wide range of environmental conditions. Effects of environmental controls other than pH and IS on attachment add uncertainty to this predictive model. As an engineering tool, however, the empirical model provides a significant improvement over using $\alpha = 1$ as the naïve model alternative (Table 1). The synthesis of results in Table 1 provide further guidance on the range of α values that can be used in predictive models of *C. parvum* oocyst transport.

Overall, the transport of freshly harvested, minimally treated, viable *C. parvum* oocysts in porous media follows similar filtration patterns as that observed in experiments with inactivated oocysts. Even though comprehensive process-based models for *C. parvum* oocysts deposition are not yet available, our analysis, which combines new experiments and an exhaustive analysis of reported data, exhibit a consistent pattern of *C. parvum* oocyst deposition that can be used to support environmental modeling of oocyst transport in porous media (sand filters, streambeds, hydrogeologic media). For deposition to relatively homogeneous sand and glass beads, the DLVO-based, independently developed regression model (Figure 4) based on variations in IS (captured in form of the inverse of Debye length) and pH provides a practical tool for estimating α in environmental fate and transport models.

Additional systematic research efforts are needed to improve understanding of attachment efficiency of *C. parvum* oocysts by investigating the role of non-DLVO forces in oocyst attachment to surfaces, and the role of interactions with other suspended matter in mediating oocyst deposition. To the degree that CFT is applicable to field and larger scale transport — an open scientific question — the regression equations (Figure 4) provide an empirical tool to adjust attachment efficiency according to pH and ionic strength of groundwater.

■ ASSOCIATED CONTENT

Supporting Information

Additional background on CFT, α, η, and DLVO theory; calculation of α using interaction energy, hypothesis testing results of the significance in difference among three α values computed by three models (NG, TE, and RT), verification of multiple regression model for α, and additional parameter values for experiments in Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: thharter@ucdavis.edu, tel: 530-752-2709, fax: 530-752-5262.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to acknowledge the generous support provided by USDA CSREES Awards 2004-00854 and 2008-35102-18733. We also thank Jennifer Drummond at Northwestern University for analyzing the zeta potential of *C. parvum* oocysts.

■ REFERENCES

(1) Griffiths, J. K. *Human Cryptosporidiosis: Epidemiology, Transmission, Clinical Disease, Treatment, and Diagnosis*; Academic Press Limited: San Diego, CA, 1998; Vol. *40*.

(2) Fayer, R. Cryptosporidium: a water-borne zoonotic parasite. *Vet. Parasitol.* **2004**, *126* (1−2), 37−56.

(3) Appelbee, A. J.; Thompson, R. C. A.; Olson, M. E. Giardia and Cryptosporidium in mammalian wildlife - current status and future needs. *Trends Parasitol.* **2005**, *21* (8), 370−376.

(4) Fricker, C. R.; Crabb, J. H. *Water-Borne Cryptosporidiosis: Detection Methods and Treatment Options*; Academic Press Limited: San Diego, CA, 1998; Vol. *40*.

(5) Rose, J. B.; Lisle, J. T.; LeChevallier, M. *Waterborne Cryptosporidiosis: Incidence Outbreaks, and Treatment Strategies*; CRC Press: Boca Raton, FL, 1997.

(6) Ferguson, C.; Husman, A. M. D.; Altavilla, N.; Deere, D.; Ashbolt, N. Fate and transport of surface water pathogens in watersheds. *Crit. Rev. Environ. Sci. Technol.* **2003**, *33* (3), 299−361.

(7) Jenkins, M.; Trout, J. M.; Higgins, J.; Dorsch, M.; Veal, D.; Fayer, R. Comparison of tests for viable and infectious Cryptosporidium parvum oocysts. *Parasitol. Res.* **2003**, *89* (1), 1−5.

(8) Fogel, D.; Isaacrenton, J.; Guasparini, R.; Moorehead, W.; Ongerth, J. Removing Giardia and Cryptosporidium by slow sand filtration. *J. Am. Water Work Assoc.* **1993**, *85* (11), 77−84.

(9) Edzwald, J. K.; Kelley, M. B. Control of Cryptosporidium: From reservoirs to clarifiers to filters. *Water Sci. Technol.* **1998**, *37* (2), 1−8.

(10) Mawdsley, J. L.; Brooks, A. E.; Merry, R. J. Movement of the protozoan pathogen Cryptosporidium parvum through three contrasting soil types. *Biol. Fert. Soils* **1996**, *21*, 30−36.

(11) Logan, A. J.; Stevik, T. K.; Siegrist, R. L.; Ronn, R. M. Transport and fate of Cryptosporidium parvum oocysts in intermittent sand filters. *Water Res.* **2001**, *35* (18), 4359−4369.

(12) EPA, Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule) In *Safe Drinking Water Act*: **2006**; Vol. *71*, pp 653-786.

(13) Tufenkji, N.; Ryan, J. N.; Elimelech, M. The promise of bank filtration. *Environ. Sci. Technol.* **2002**, *36* (21), 422a−428a.

(14) Weiss, W. J.; Bouwer, E. J.; Aboytes, R.; LeChevallier, M. W.; O'Melia, C. R.; Le, B. T.; Schwab, K. J. River filtration for control of microorganisms results from field monitoring. *Water Res.* **2005**, *39* (10), 1990−2001.

(15) Atwill, E. R.; Hoar, B.; Pereira, M. D. C.; Tate, K. W.; Rulofson, F.; Nader, G. Improved quantitative estimates of low environmental loading and sporadic periparturient shedding of Cryptosporidium parvum in adult beef cattle. *Appl. Environ. Microbiol.* **2003**, *69* (8), 4604−4610.

(16) Cortis, A.; Harter, T.; Hou, L. L.; Atwill, E. R.; Packman, A. I.; Green, P. G. Transport of Cryptosporidium parvum in porous media: Long-term elution experiments and continuous time random walk filtration modeling. *Water Resour. Res.* **2006**, *42*, (W12S13), doi:10.1029/2006WR004897.

(17) Yao, K. M.; Habibian, M. M.; Omelia, C. R. Water and waste water filtration - concepts and applications. *Environ. Sci. Technol.* **1971**, *5* (11), 1105−&.

(18) Rajagopalan, R.; Tien, C. Trajectory analysis of deep-bed filtration with sphere-in-cell porous-media model. *AIChE J.* **1976**, *22* (3), 523−533.

(19) Tufenkji, N.; Elimelech, M. Correlation equation for predicting single-collector efficiency in physicochemical filtration in saturated porous media. *Environ. Sci. Technol.* **2004**, *38* (2), 529−536.

(20) Nelson, K. E.; Ginn, T. R. New collector efficiency equation for colloid filtration in both natural and engineered flow conditions. *Water Resour. Res.* **2011**, *47*, (W05543), doi:10.1029/2010WR009587.

(21) Elimelech, M.; Gregory, J.; Jia, X.; Williams, R. A. *Particle Deposition and Aggregation: measurement, modeling and simulation*. Butterworth-Heinemann: Oxford, England, 1995.

(22) Elimelech, M.; Omelia, C. R. Kinetics of deposition of colloidal particles in porous-media. *Environ. Sci. Technol.* **1990**, *24* (10), 1528−1536.

(23) Gregory, J. *Particles in Water; Properties and Processes*. IWA Pub.Taylor & Francis: Boca Raton, FL, 2005; p 180.

(24) Derjaguin, B.; Landau, L. Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. *Acta Physico Chemica USSR* **1941**, *14*, 633−662.

(25) Verwey, E. J. W.; Overbeek, J. T. G. *Theory of the Stability of Lyophobic Colloids*; Elsevier: Amsterdam, 1948.

(26) Elimelech, M. *The Effect of Particle Size on the Kinetics of Deposition of Brownian Particles in Porous Media*; Johns Hopkins University, Baltimore, MD, 1989.

(27) Elimelech, M.; Omelia, C. R. Effect of Particle-Size on Collision Efficiency in the Deposition of Brownian Particles with Electrostatic Energy Barriers. *Langmuir* **1990**, *6* (6), 1153−1163.

(28) Ginn, T. R.; Wood, B. D.; Nelson, K. E.; Scheibe, T. D.; Murphy, E. M.; Clement, T. P. Processes in microbial transport in the natural subsurface. *Adv. Water Resour.* **2002**, *25* (8−12), 1017−1042.

(29) Schijven, J. F.; Hassanizadeh, S. M. Removal of viruses by soil passage: Overview of modeling, processes, and parameters. *Crit. Rev. Env. Sci. Tec.* **2000**, *30* (1), 49−127.

(30) Tufenkji, N.; Miller, G. F.; Ryan, J. N.; Harvey, R. W.; Elimelech, M. Transport of Cryptosporidium oocysts in porous media: Role of straining and physicochemical filtration. *Environ. Sci. Technol.* **2004**, *38* (22), 5932−5938.

(31) Abudalo, R. A.; Bogatsu, Y. G.; Ryan, J. N.; Harvey, R. W.; Metge, D. W.; Elimelech, M. Effect of ferric oxyhydroxide grain coatings on the transport of bacteriophage PRD1 and Cryptosporidium parvum oocysts in saturated porous media. *Environ. Sci. Technol.* **2005**, *39* (17), 6412−6419.

(32) Abudalo, R. A.; Ryan, J. N.; Harvey, R. W.; Metge, D. W.; Landkamer, L. Influence of organic matter on the transport of Cryptosporidium parvum oocysts in a ferric oxyhydroxide-coated quartz sand saturated porous medium. *Water Res.* **2010**, *44* (4), 1104−1113.

(33) Hijnen, W. A. M.; Dullemont, Y. J.; Schijven, J. F.; Hanzens-Brower, A. J.; Rosielle, M.; Medema, G. Removal and fate of Cryptosporidium paruum, Clostridium perfringens and small-sized centric diatoms (Stephanodiscus hantzschii) in slow sand filters. *Water Res.* **2007**, *41* (10), 2151−2162.

(34) Kuznar, Z. A.; Elimelech, M. Role of surface proteins in the deposition kinetics of Cryptosporidium parvum oocysts. *Langmuir* **2005**, *21* (2), 710−716.

(35) Harter, T.; Wagner, S.; Atwill, E. R. Colloid transport and filtration of Cryptosporidium parvum in sandy soils and aquifer sediments. *Environ. Sci. Technol.* **2000**, *34* (1), 62−70.
(36) Hou, L.; Li, X.; Dunbar, L.; Moeller, R.; Palermo, B.; Atwill, E. R. Neonatal-mouse infectivity of intact Cryptosporidium parvum oocysts isolated after optimized in vitro excystation. *Appl. Environ. Microbiol.* **2004**, *70* (1), 642−646.
(37) Xiao, L. H.; Escalante, L.; Yang, C. F.; Sulaiman, I.; Escalante, A. A.; Montali, R. J.; Fayer, R.; Lal, A. A. Phylogenetic analysis of Cryptosporidium parasites based on the small-subunit rRNA gene locus. *Appl. Environ. Microbiol.* **1999**, *65* (4), 1578−1583.
(38) Harp, J. A.; Jardon, P.; Atwill, E. R.; Zylstra, M.; Checel, S.; Goff, J. P.; DeSimone, C. Field testing of prophylactic measures against Cryptosporidium parvum infection in carves in a California dairy herd. *Am. J. Vet. Res.* **1996**, *57* (11), 1586−1588.
(39) Arrowood, M. J.; Sterling, C. R. Isolation of Cryptosporidium Oocysts and Sporozoites Using Discontinuous Sucrose and Isopycnic Percoll Gradients. *J. Parasitol.* **1987**, *73* (2), 314−319.
(40) Packman, A. I.; Brooks, N. H.; Morgan, J. J. Kaolinite exchange between a stream and streambed: Laboratory experiments and validation of a colloid transport model. *Water Resour. Res.* **2000**, *36* (8), 2363−2372.
(41) Fisher, R. A. *Statistical Methods for Research Workers*; Oliver and Boyd: Edinburgh, London,, 1925; p ix p., 1 l.,.
(42) Drozd, C.; Schwartzbrod, J. Hydrophobic and electrostatic cell surface properties of Cryptosporidium parvum. *Appl. Environ. Microbiol.* **1996**, *62* (4), 1227−1232.
(43) Ongerth, J. E.; Pecoraro, J. P. Electrophoretic mobility of Cryptosporidium oocysts and Giardia cysts. *J. Environ. Eng.-Asce* **1996**, *122* (3), 228−231.
(44) Attinti, R.; Wei, J.; Kniel, K.; Sims, J. T.; Jin, Y. Virus' (MS2, phi X174, and Aichi) Attachment on sand measured by atomic force microscopy and their transport through sand columns. *Environ. Sci. Technol.* **2010**, *44* (7), 2426−2432.
(45) Brush, C. F.; Ghiorse, W. C.; Anguish, L. J.; Parlange, J. Y.; Grimes, H. G. Transport of Cryptosporidium parvum oocysts through saturated columns. *J. Environ. Qual.* **1999**, *28* (3), 809−815.
(46) Huang, C. P.; Pan, J. R. S.; Huang, S. H. Collision efficiencies of algae and kaolin in depth filter: The effect of surface properties of particles. *Water Res.* **1999**, *33* (5), 1278−1286.
(47) Lutterodt, G.; Basnet, M.; Foppen, J. W. A.; Uhlenbrook, S. The effect of surface characteristics on the transport of multiple Escherichia coli isolates in large scale columns of quartz sand. *Water Res.* **2009**, *43* (3), 595−604.
(48) Bradford, S. A.; Bettahar, M. Straining, attachment, and detachment of Cryptosporidium oocysts in saturated porous media. *J. Environ. Qual.* **2005**, *34* (2), 469−478.
(49) Ryan, J. N.; Elimelech, M. Colloid mobilization and transport in groundwater. *Colloids Surf., A* **1996**, *107*, 1−56.
(50) Huber, N.; Baumann, T.; Niessner, R. Assessment of colloid filtration in natural porous media by filtration theory. *Environ. Sci. Technol.* **2000**, *34* (17), 3774−3779.
(51) Kuznar, Z. A.; Elimelech, M. Adhesion kinetics of viable Cryptosporidium parvum oocysts to quartz surfaces. *Environ. Sci. Technol.* **2004**, *38* (24), 6839−6845.
(52) Kim, H. N.; Walker, S. L.; Bradford, S. A. Coupled factors influencing the transport and retention of Cryptosporidium parvum oocysts in saturated porous media. *Water Res.* **2010**, *44* (4), 1213−1223.
(53) Janjaroen, D.; Liu, Y.; Kuhlenschmidt, M. S.; Kuhlenschmidt, T. B.; Nguyen, T. H. Role of divalent cations on deposition of Cryptosporidium parvum oocysts on natural organic matter surfaces. *Environ. Sci. Technol.* **2010**, *44* (12), 4519−4524.
(54) Butkus, M. A.; Bays, J. T.; Labare, M. P. Influence of surface characteristics on the stability of Cryptosporidium parvum oocysts. *Appl. Environ. Microbiol.* **2003**, *69* (7), 3819−3825.
(55) Considine, R. F.; Drummond, C. J. Surface roughness and surface force measurement: A comparison of electrostatic potentials derived from atomic force microscopy and electrophoretic mobility measurements. *Langmuir* **2001**, *17* (25), 7777−7783.
(56) Hsu, B. M.; Huang, C. P. Influence of ionic strength and pH on hydrophobicity and zeta potential of Giardia and Cryptosporidium. *Colloids Surf., A* **2002**, *201* (1−3), 201−206.
(57) Hsu, B. M.; Huang, C. P.; Pan, J. R. Filtration behaviors of Giardia and Cryptosporidium - Ionic strength and pH effects. *Water Res.* **2001**, *35* (16), 3777−3782.
(58) Ryan, J. N.; Gschwend, P. M. Effect of iron diagenesis on the transport of colloidal clay in an unconfined sand aquifer. *Geochim. Cosmochim. Acta* **1992**, *56* (4), 1507−1521.
(59) Searcy, K. E.; Packman, A. I.; Atwill, E. R.; Harter, T. Association of Cryptosporidium parvum with suspended particles: Impact on oocyst sedimentation. *Appl. Environ. Microbiol.* **2005**, *71* (2), 1072−1078.
(60) Searcy, K. E.; Packman, A. L.; Atwill, E. R.; Harter, T. Deposition of Cryptosporidium oocysts in streambeds. *Appl. Environ. Microbiol.* **2006**, *72* (3), 1810−1816.
(61) Hijnen, W. A. M.; Brouwer-Hanzens, A. J.; Charles, K. J.; Medema, G. J. Transport of MS2 phage, Escherichia coli, Clostridium perfringens, Cryptosporidium parvum and Giardia intestinalis in a gravel and a sandy soil. *Environ. Sci. Technol.* **2005**, *39* (20), 7860−7868.
(62) Hijnen, W. A. M.; Brouwer-Hanzens, A. J.; Charles, K. J.; Medema, G. Transport of MS2 phage, Escherichia coli, Clostridium perfringens, Cryptosporidium parvum, and Giardia intestinalis in a gravel and a sandy soil. (vol 39, pg 7860, 2005). *Environ. Sci. Technol.* **2006**, *40* (4), 1371−1371.
(63) Dai, X. J.; Hozalski, R. M. Effect of NOM and biofilm on the removal of Cryptosporidium parvum oocysts in rapid filters. *Water Res.* **2002**, *36* (14), 3523−3532.
(64) Tufenkji, N.; Elimelech, M. Spatial distributions of Cryptosporidium oocysts in porous media: Evidence for dual mode deposition. *Environ. Sci. Technol.* **2005**, *39* (10), 3620−3629.
(65) Matthess, G.; Bedbur, E.; Gundermann, K. O.; Loof, M.; Peters, D. Investigation on filtration mechanisms of bacteria and organic particles in porous-media 0.2. Hydraulic, hydrochemical and sedimentological system properties controlling the filter factor. *Zentralblatt Fur Hygiene Und Umweltmedizin* **1991**, *191* (4), 347−395.
(66) Debye, P.; Huckel, E. The theory of electrolytes I. The lowering of the freezing point and related occurrences. *Physikalische Zeitschrift* **1923**, *24*, 185−206.