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# FILTRATION BEHAVIORS OF *GIARDIA* AND *CRYPTOSPORIDIUM*—IONIC STRENGTH AND PH EFFECTS

BING-MU HSU[1], CHIHPIN HUANG* and JILL RUHSING PAN
Institute of Environmental Engineering, National Chiao Tung University, Hsinchu, Taiwan, R.O.C.

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**Abstract**—The laboratory-scale filtration tests of *Giardia* cysts and *Cryptosporidium* oocysts in both 2 mm-Φglass beads and 2 mm-Φ polystyrene beads filters were conducted to investigate their filtration behaviors. The protozoan parasites were used as target particles, while the chemical system altered by changing the electrolyte concentration and pH. The results significantly indicate that ionic strength have a positive effect on the removal efficiencies for *Giardia* cysts and *Cryptosporidium* oocysts. The removal efficiency of two filters for *Giardia* cysts slightly decreased from pH 2.4 to 8.7 and decreased significantly in pH as pH up to 8.7, while that for *Cryptosporidium* slightly rippled beyond pH 8.7, and with the decrease in pH up to pH 8.7. The experimental collision efficiencies from the interactions between colloids and the filter media were calculated with a semi-empirical approach of the single sphere model and clean-bed filtration theory. The results also indicated that experimental collision efficiencies for (oo)cysts corresponded to the (oo)cysts removal efficiencies in all trials, and oocysts exhibits higher collision efficiencies than cysts.


*Key words*—filtration, *Giardia* cysts, *Cryptosporidium* oocysts, collision efficiency

## INTRODUCTION

The protozoan parasites *Giardia* and *Cryptosporidium* have been recognized as the common pathogenic protozoa of the gastrointestinal tract. Many outbreaks of giardiasis and cryptosporidiosis have been reported in the last few decades (Frost *et al.*, 1996; SoloGabriele and Neumeister, 1996). Human infection with these pathogens is usually through either direct contact or ingestion of contaminated food and/or water (Donnelly and Stentiford, 1997; Pell, 1997). Water is perhaps the major source for massive outbreaks of infection, as a result of contamination of source water. Investigation has shown that commonly used disinfectant in the water treatment plants, such as chlorine, is virtually useless in inactivating such kinds of parasites (Haas *et al.*, 1994). Filtration, on the other hand, is likely to be the most practical treatment technology used for *Giardia* cysts and *Cryptosporidium* oocysts removal (Swertfeger *et al.*, 1999; Fogel *et al.*, 1993).

Some surface properties of the *Giardia* cysts and *Cryptosporidium* oocysts, i.e. electrophoretic mobility and hydrophobicities, have been investigated by Ongerth and Pecoraro (1996) and Brush *et al.* (1998). Adin *et al.* (1999) have conducted a bench scale filtration column to study the removal efficiencies of *Cryptosporidium* oocysts. However, the interactions of *Giardia* cysts and *Cryptosporidium* oocysts with filter media while changing ionic strength and pH of water samples were not realized. The filtration system of actual water treatment process is hard to simulate for their complex components and mechanisms. Many papers determined the overall (oo)cysts removal efficiency of the filtration by evaluating the number difference of (oo)cysts between the inlet and outlet. However, information about the contribution of simplex mechanism for (oo)cyst removal in the filtration system is very limited. Therefore, it is important to discover the simplex mechanism for (oo)cyst removal in the deep-bed filtration system.

Our group has investigated the differences of collision efficiency between organic (algae) and inorganic (kaoline) particles as well as the effects of ionic strength and pH on them (Huang *et al.*, 1999). This approach showed some interesting results including different filtration behavior between inorganic and organic particles and the significant effects of their surface properties on the filtration, which deserved to go further. In this study, therefore, we investigated the filtration behaviors of *Giardia* cysts and *Cryptosporidium* oocysts at various pH values and ionic strengths. Beside that, two filtration media, glass beads and polystyrene beads, were tested in

*Author to whom all correspondence should be addressed. Tel.: +886-3-572-6463; fax: +886-3-572-5958; e-mail: cphuang@green.ev.nctu.edu.tw
[1]Present address: Department of Medical Research, Chiayi Christian Hospital, Chiayi, Taiwan, R.O.C.

parallel to realize the effect of different media on the (oo)cyst filtration. The overall removal percentages of (oo)cysts is not the point of this study, but the (oo)cyst collision efficiency during the deep-bed filtration. In other words, we only investigated the microscopic function of (oo)cysts filtration, not macroscopic. Through the investigation of the (oo)cysts filtration in a lab-scale packed column, the single-collector efficiency ($\eta$) was calculated, and the experimental collision (or attachment) efficiency ($\alpha_{exp}$) with the observed removal efficiency were determined.

## MATERIALS AND METHODS

### *Preparation and Use of the Column Filters*

The experimental apparatus of this study is shown in Fig. 1. The column for the filter consisted of a 48 mm i.d. × 15 cm acrylic column with two adjustable bed supports. Glass beads and polystyrene beads were the two chosen media. They were first cleaned by soaking in the hot 10 g/l Alconox detergent solution for at least 2 h, followed by rinsing a few times with deionized water. They were then soaked in 5 N nitric acid for at least 12 h, and rinsed with deionized water again. All deionized water used for glassware washing and solution preparation was obtained from a Milli-Q system (Millipore Corp.). The porosity of the glass beads and polystyrene beads were measured to be 0.39 and 0.37, respectively, by a volumetric method.

The tracer study was first performed to determine the duration of complete breakthrough. The column was filled with clean media, and distilled water was pumped into the column to saturate the system. A 10 g $NaNO_3$/l solution was introduced into the column as a step input, and the nitrate concentration in the effluent was monitored with UV absorption measurements (wavelength was 302 nm) at 1-min interval. The $NaNO_3$ concentration was found to be directly proportional to UV absorption in the range of interest (0–10 g/l). The breakthrough results were indicative of a plug flow system and complete mix system in series.

### *Preparation of Protozoa-contained sample and calculation for (Oo)cysts*

The cysts and oocysts were obtained from Waterborne, Inc. (Louisiana, USA) and the Pleasant Hill Farms (Idaho, USA), respectively, and were diluted to desired concentrations by 0.1% PBS (phosphate buffered saline) as the stock solution. The numbers of cysts and oocysts in the stock solution were counted before seeded experiment using the indirect fluorescent antibody (IFA) staining procedure.

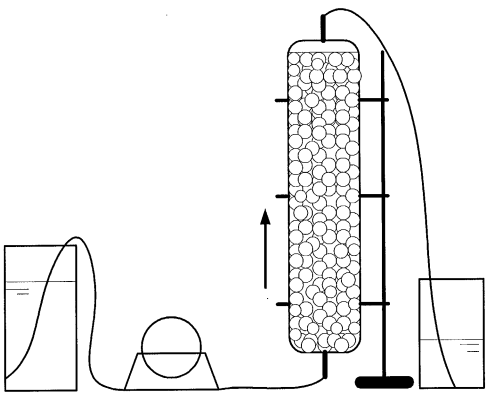

Fig. 1. The experimental apparatus of deep-bed filtration in this study.

Five hundred milliliters of the deionized water were collected in a plastic 1-l container. The pHs of water samples were adjusted from 2.4 to 11.2 with 0.1 N $HClO_4$ and 0.01 N NaOH in the presence of $1.0 \times 10^{-2}$ M $NaClO_4$ as background electrolyte. Water samples with various background electrolyte (eg, $10^{-1.0}$ M, $10^{-1.5}$ M, $10^{-2.0}$ M, $10^{-2.5}$ M, and $10^{-3.0}$ M) at pH 5.6 were also prepared. Water samples of given background electrolyte and pH level were then seeded with $1.0 \times 10^4$ (oo)cysts by taking a specific volume from the stock solution. Prior to each experimental run, the entire system was washed with the ionic solution of the designated $NaClO_4$ and pH, free of protozoa, with an upflow mode for at least 20 min. Experiments were carried out at room temperature (24–25°C) with downward vertical flow at 10.0 ml/min (approach velocity = $2.53 \times 10^{-5}$ m/s). Forty milliliters of filtrate were collected 4 min after the time of complete breakthrough.

To investigate the removal efficiencies for *Giardia* cysts and *Cryptosporidium* oocysts, we counted the concentration of cysts and oocysts in the effluent and influent by IFA staining procedure described in the instruction provided by the company (Hydrofluor™ Combo *Giardia*/*Cryptosporidium*; Ensys, Inc., NC, USA). The collected suspensions and filtrate were applied to each 25-mm diameter cellulose-acetate membrane, stained with fluorescent-labeled antibodies (Hydrofluor™-Combo *Giardia*/*Cryptosporidium*; Ensys, Inc., NC, USA) and examined with epifluorescent microscope at 200 × magnification (Olympus, Japan).

### *Theoretical approach*

*Giardia* cysts and *Cryptosporidium* oocysts were the colloidal particles in our column filtration experiments. The rationale of $\eta$ involves the physical aspect of a better understood filtration model, while collision efficiency accounts for the less important factors in the chemical aspect (Elimelech, 1992). With the combination of trajectory and dimensional analysis, the process of filtration can be formulated. The calculation of the single-collector efficiency has been derived by Rajagopalan and Tien (1976) as following:

$$\eta = 4A_s^{1/3}P_e^{-2/3} + 0.72A_sN_{LO}^{1.8}N_R^{15.8} + 0.0024A_sN_G^{1.2}N_R^{-0.4} \quad (1)$$

where $A_s$, defined as $2(1-p^5)/w$, accounts for the effect of adjacent collectors on the collector efficiency, $p = (1-\varepsilon)^{1/3}$, $w = 2 - 3p + 3p^5 - 2p^6$, and $\varepsilon$ the porosity of the porous medium. $N_R$, indicating the importance of interception, is the ratio of the size of suspended particle to the size of collector $(a_p/a_c)\cdot P_e$ represents the role of Brownian diffusion ($2a_cU/D_{BM}$), where $U$ is the approach velocity to a collector (m/s). The term $D_{BM}$, defined as $kT/(6\pi\mu a_p)$, is the Brownian diffusion coefficient, where $\mu$ is the dynamic viscosity of fluid density, $k$ the Boltzmann's constant, and $T$ the absolute temperature. $N_{L0}$ reflects van der Waals interaction ($H/9\pi\mu a_p^2U$), where $H$ is the Hamaker constant (J). $N_G$ accounts for gravity effect ($2a_p^2(\rho_p-\rho)g/9\mu U$), where $\rho_p$ is the density of the suspended particles, $\rho$ the fluid density, and $g$ the gravitational constant.

By applying the clean-bed filtration model and a mass balance of particles over a differential packed-bed volume, the experimental collision efficiency ($\alpha_{exp}$) can be related to the initial (clean-bed) removal, $(C/C_0)_0$, as follows (Elimelech and O'Melia, 1990)

$$\alpha_{exp} = -\ln\left(\frac{C}{C_0}\right)_0\left(\frac{4a_c}{3(1-\varepsilon)L\eta}\right) \quad (2)$$

where $a_c$ is the radius of the collector, $\varepsilon$ the porosity of the filter bed, $L$ the column depth, and $\eta$ the single-collector efficiency. The initial clean bed removal efficiency, $(C/C_0)_0$, is the value taken when a complete breakthrough of an inert tracer occurs.

## RESULTS AND DISCUSSION

### *Breakthrough curves*

The breakthrough curve of column filtration is presented as $C/C_0$ with respect to time. $C$ and $C_0$ are the effluent and influent concentrations of $NaNO_3$, respectively. The duration of complete breakthrough described in Fig. 2 for glass beads and in Fig. 3 for polystyrene beads was about 14 min and 13 min, respectively. The porosity ($\varepsilon$) of the glass beads, 0.39, was higher than that of the polystyrene beads, 0.37. Therefore, the duration of complete breakthrough for glass beads was longer than that for polystyrene beads. In this study, 14 min were designed as duration of complete breakthrough.

### *Removal efficiency*

The removal efficiencies, $1 - C/C_0$, for both *Giardia* cysts and *Cryptosporidium* oocysts with respect to different media, $NaClO_4$ concentrations, and pH levels were presented from Figs 4–7. Results showed that removals of cysts were lower than oocysts in all trials. Because of the size of the filter media used in this study, the removal mechanism of these protozoa with respect to the intercept can be neglected. The removal ratio of (oo)cysts was, therefore, influenced mostly by the collision. The removal ratio was determined by the surface characteristics of (oo)cyst, especially the hydrophobicity and surface charge. In a separate study of ours, the hydrophobicity and surface charge of oocysts were larger than those of cysts, which are in agreement with the determined result of (oo)cyst removal efficiencies.

Figures 4 and 5 show the removals of (oo)cysts in two kinds of medium with various background electrolyte at pH 5.6. The removal of *Giardia* cysts was ranged from 0.21 to 0.80 and that of *Cryptosporidium* oocysts from 0.41 to 0.92, and the variation in removal efficiencies by the polystyrene beads was larger than that by the glass beads for both parasites. Removals of both parasites increased with the $NaClO_4$ concentration. This finding agreed with the DLVO theory, that the total interaction energy and

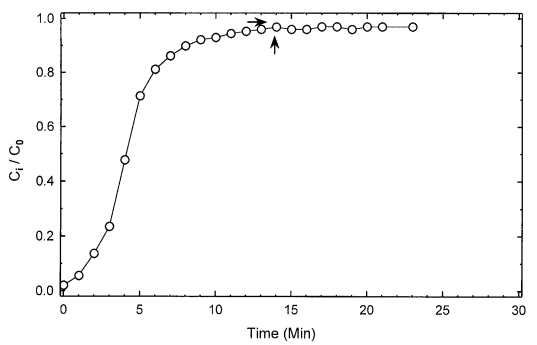


Fig. 2. Breakthrough curve of an inner tracer $NaNO_3$ in the 0.1 m bed depth column packed with 2 mm-Φglass beads. The volumetric flow rate is 10.0 ml/min.

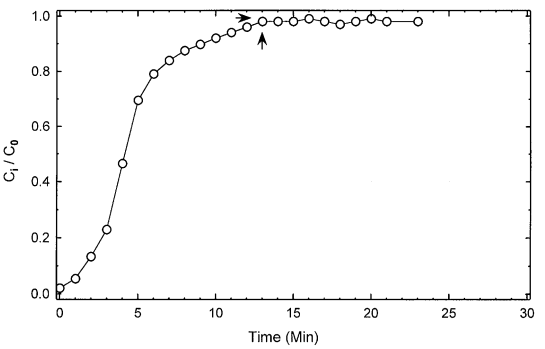


Fig. 3. Breakthrough curve of an inner tracer $NaNO_3$ in the 0.1 m bed depth column packed with 2 mm-Φpolystyrene beads. The volumetric flow rate is 10.0 ml/min.

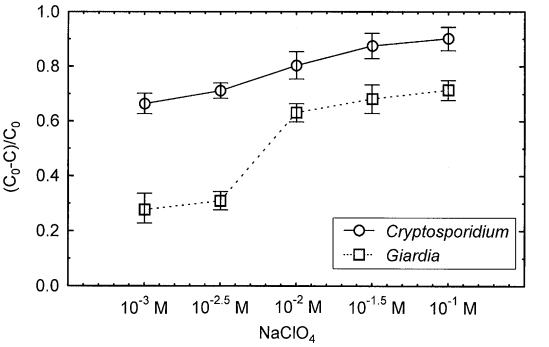


Fig. 4. Removal efficiencies of (oo)cysts in packed glass beads column with various concentrations of $NaClO_4$ at pH 5.6.

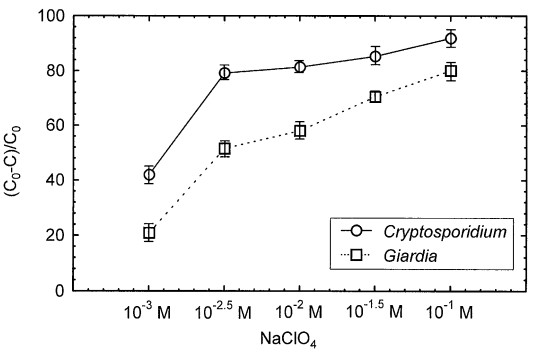


Fig. 5. Removal efficiencies of (oo)cysts in packed polystyrene beads column with various concentrations of $NaClO_4$ at pH 5.6.

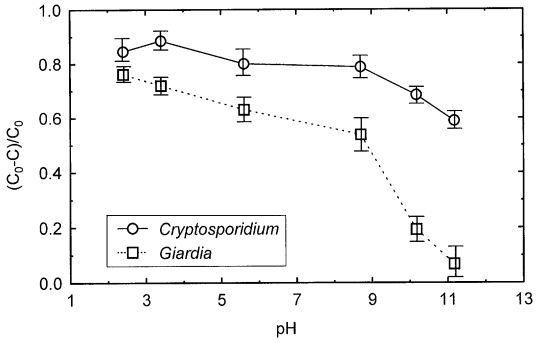


Fig. 6. Removal efficiencies of (oo)cysts in packed glass beads column with various pH in the presence of 0.01 M $NaClO_4$.

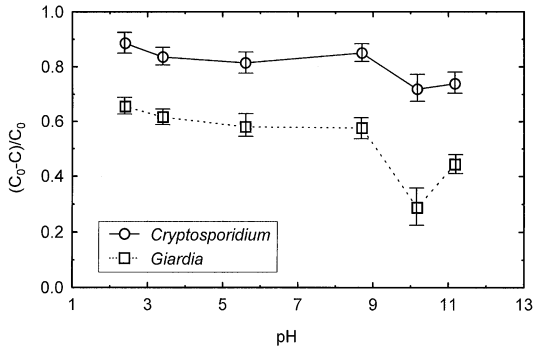


Fig. 7. Removal efficiencies of (oo)cysts in packed polystyrene beads column with various pH in the presence of 0.01 M $NaClO_4$.

the height of the energy barrier of the reaction decrease as the concentration of $NaClO_4$ in the suspension increases, and that the van der Waals interaction is independent of the solution chemistry.

The effects of pH, with $1.0 \times 10^{-2}$ M $NaClO_4$ as the background electrolyte, on (oo)cysts removals from different filtration media were illustrated in Figs 6 and 7. The variation of parasite removal efficiencies on pH values from different packed column was much greater by glass beads (Fig. 6), than by polystyrene beads (Fig. 7). The removal of *Giardia* cysts was ranged from 0.07 to 0.76 and that of *Cryptosporidium* oocysts was ranged from 0.59 to 0.92. The removal of both parasites decreased with the pH value. This can be explained by the pH effect on zeta potentials (Ongerth and Pecoraro, 1996). The glass and polystyrene beads carried negative charge at their surfaces, which increased with the pH. Therefore, the repulsive force between particles and collectors were increased. Because the complicated process of life cycles for both protozoa entail the coordinated production, processing, and transport of wall constituents for assembly into a protective (oo)cyst wall, little is known about this process and the identity of (oo)cyst wall constituents. In this study, we extrapolate the surface contained protein of (oo)cyst by observing the variations on the (oo)cyst removal efficiency at different pH values. Figures 6 and 7 also showed that the removal efficiencies of two filters for *Giardia* cysts slightly decreased from pH 2.4 to 8.7 and decreased significantly in pH as pH up to 8.7, while that for *Cryptosporidium* slightly rippled beyond pH 8.7, and with the decreased in pH up to pH 8.7. This phenomenon can be explained by the ionization of amino acids on the (oo)cysts surface. All amino acids have a basic skeleton consisting of a α-carbon linked to an amino group ($-NH_2$), a carboxyl group (COOH), a hydrocarbon atom (H), and R group. The variations among the amino acids occur at the R group, which is different in each amino acid and imparts the unique characteristics to the molecule and to the proteins that contain it (Talaro and Talaro, 1999). Protein molecules, which consist essentially of α-amino acids linked by peptide (–CONH–) linkages, are the major surface components of the (oo)cysts. The surface charge of (oo)cysts originates from the ionization of R groups in the amino acids. Most of the R groups in amino acids are not ionized at pH = 7, and the $pK_a$ values of amino acids are from 1.71 to 3.0 (Smith and Martell, 1976). However, the R groups for monocarboxy diamino acids — lysine and arginine are positively charged at pH = 7, and their $pK_a$ values are 10.5 and 12.5, respectively. About 99% of the $-NH_2$ groups would ionize to $-NH_3^+$ groups when $pH = pK_a - 2$ (i.e. pH = 8.5–10.5), and thus infer the dominant components of proteins in (oo)cysts surfaces.

In the study of Adin *et al.* (1999), the removal efficiencies of *Cryptosporidium* oocysts were respectively 0.09 and 0.10 for fined sand and coarse sand at $2.5 \times 10^{-2}$ m/min. Although the removal efficiency was influenced by the approach velocity, the removal of oocysts in their study were still lower than ours. In the study of Huang *et al.* (1999), the reported removal for algae was also much lower than the removals of cyst and oocyst in this study.

### *Experimental collision efficiencies*

The values used for the parameters in equations (2) and (3) are: fluid density ($\rho$) is 998 kg/m$^3$, fluid viscosity ($\mu$) $8.94 \times 10^{-4}$ N s/m$^2$,. packed column length ($L$) 0.1 m, (oo)cysts densities ($\rho_p$) 1080 kg/m$^3$, the diameters ($d_p$) of cysts and oocysts respectively $10.1 \times 10^{-6}$ and $5.0 \times 10^{-6}$ m, approach velocity ($U$) $2.53 \times 10^{-5}$ m/s, absolute temperature ($T$) 298 K, and gravitational acceleration ($g$) 9.81 m/s$^2$. The Hamaker constants ($H$) were determined to be $1.20 \times 10^{-21}$ and $1.15 \times 10^{-21}$ J for both parasites in glass beads media and polystyrene beads media, respectively. The Boltzman constant ($k$) is $1.38 \times 10^{-23}$ J/K. The theoretical porosity ($\varepsilon$) was 0.39 for glass beads and was 0.37 for polystyrene beads and their sizes ($d_c$) were both $2 \times 10^{-3}$ m.

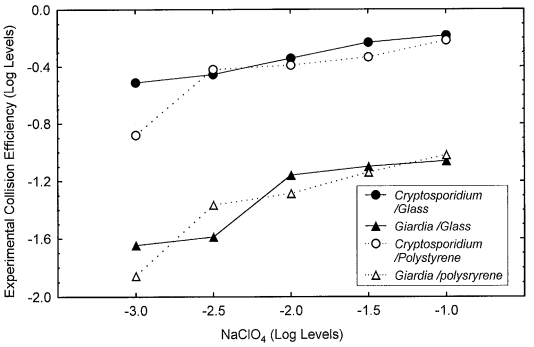


Fig. 8. Experimental collision efficiency of (oo)cysts with various concentrations of $NaClO_4$ at pH 5.6.

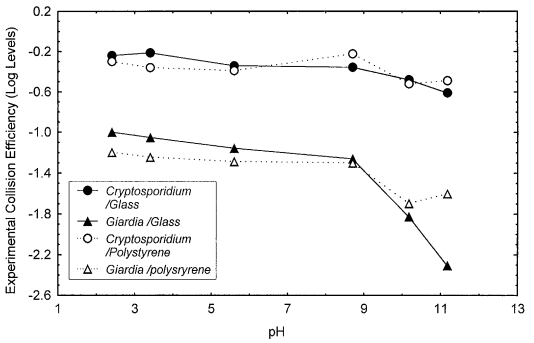


Fig. 9. Experimental collision efficiency of (oo)cysts with various pH in the presence of 0.01 M $NaClO_4$.

In this study, experimental collision efficiencies were calculated from the results of column filtration experiments. The logarithm of experimental collision efficiency for (oo)cysts under various background electrolyte at pH 5.6 was presented in Fig. 8. The experimental collision efficiency for (oo)cysts gradually increased with the increasing $NaClO_4$ concentration. As the electrolyte concentration increases, the diffuse layer is compressed, which facilitate the successful attachment of the colloidal particles. Figure 8 also points out that at various background electrolyte, the variation of collision efficiencies between (oo)cyst suspension and polystyrene beads was higher than that between (oo)cyst suspension and glass beads.

Figure 9 shows the experimental stability curves of both parasites at various pHs. The experimental collision efficiency decreased with increasing pH, which is similar to the removal efficiency of parasite filtration in different media, where Fig. 9 also displayed that at various pH values, the variation of collision efficiencies of the glass beads was higher than that of the polystyrene beads for both parasites.

When the particle size is smaller than a Brownian particle, 1 μm in diameter, the mechanism of particle transport is dominated by diffusion. Therefore, a particle smaller than a Brownian particle will have better single-collector efficiency in the deep filter. As for non-Brownian particles, transport is controlled by gravity, fluid drag and interception in the deep filter. In this study, the average diameters of *Cryptosporidium* oocysts and *Giardia* cysts were measured to be 5.0 and 10.1 μm. Therefore, *Giardia* cysts resulted in better single-collector efficiency than the *Cryptosporidium* oocysts. Both Figs 8 and 9 pointed out that the experimental collision efficiencies of *Cryptosporidium* oocysts were higher than those of *Giardia* cysts. The experimental collision efficiencies of *Giardia* cysts were ranged from 0.02 to 0.10 and those of *Cryptosporidium* oocysts were ranged from 0.13 to 0.65. This is because of the higher removal efficiency and the lower single-collector efficiency ($\eta$) of *Cryptosporidium* oocysts. According to the study of Huang *et al.* (1999), the experimental collision efficiencies of algae ($d_p = 5.0 \times 10^{-6}$ m) in all trials were higher than cysts but lower than oocysts.

## CONCLUSIONS

A semi-empirical approach for predicting collision efficiencies of *Giardia* cysts and *Cryptosporidium* oocysts for the colloid deposition in porous media was presented. The chemical composition of the suspension was changed by adjusting the electrolyte concentrations and pH. The results revealed that the experimental collision efficiencies and removal efficiencies of both protozoan parasites had similar trends in all trials. The experimental collision efficiencies of oocysts were higher than cysts. The experimental collision efficiencies of both parasites appeared to be increased with the electrolyte concentrations, and higher experimental collision efficiency of (oo)cysts filtration was observed at lower operating-pH. By adjusting the electrolyte concentrations, the variation in removal efficiencies and experimental collision efficiencies of both parasites by the glass beads can be less significant than by the polystyrene beads. However, the contrary results between glass beads and polystyrene beads were obtained by adjusting the pH values.

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