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Influence of ionic strength and pH on hydrophobicity and zeta potential of *Giardia* and *Cryptosporidium*

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Abstract

Surface charge and hydrophobicity of microorganisms are involved in interfacial interaction of cells, such as flocculation and adhesion. The calculated zeta potentials of *Giardia* cysts and *Cryptosporidium* oocysts in waters at neutral pH were on average −17 and −38 mV, respectively. The isoelectric points of cysts and oocysts were estimated as pH 2.2 and pH 3.3, respectively. The hydrophobicities of cysts and oocysts, obtained from the microbial adherence to hydrocarbons (MATH) test, were determined by initial removal rate. Although the initial removal rates of oocysts were larger than those of cysts in all trials, both demonstrated marked hydrophobicity. The hydrophobicities were significantly increased with decreasing pH for both protozoa.

Keywords: *Giardia*; *Cryptosporidium*; Zeta potential; Microbial adherence to hydrocarbons (MATH)

1. Introduction

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 [1,2]. Water is the major source for massive outbreaks of infection, as a result of contamination of source water [3]. Frequent outbreaks have urged the authorities to explore solutions for removing *Giardia* cysts and *Cryptosporidium* oocysts from water supplies.

Surface charge of the microorganism is a key index in the evaluation of its coagulation behavior in water treatment process. It was suggested that the hydrophobicity of the microorganism could mediate the microbial adhesion to suspended particles or filtering devices [4]. Reaction conditions, such as pH, ionic strength, temperature, and solution components could influence the surface characteristics of the cells [5], e.g. surface charge and hydrophobicity. Understanding the surface charge and hydrophobicity of *Giardia* cysts and *Cryptosporidium* oocysts and their responses to reaction conditions will help clarify the processes involved in sorption of (oo)cysts onto particle surfaces. Therefore, it will aid in the development

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of filtration media, the determination of coagulant dosing, as well as the selection of cleaning agents during analysis of (oo)cyst.

There are different ways to determine the electrical potential at the microorganism surface. van Loosdrecht et al. [4] indicated that zeta potential could be regarded as the electrical potential with some assumptions. Five methods, namely, microbial adherence to hydrocarbons (MATH), salt aggregation, hydrophobic chromatography, adhesion to polystyrene and latex particle agglutination are commonly used to detect the hydrophobic properties of microorganisms [6]. Among them, the MATH is the most practical and frequently adopted method [7]. The MATH was developed by Rosenberg et al. [9]. Its measurement is based on the degree of adherence of cells to various hydrocarbons following a brief period of mixing. The decrease in adsorption in the lower aqueous phase is used to measure the hydrophobicity of the cell surface, from which a hydrophobicity index is generated ($A\%$ = percentage of adhesion) [10]. Recent observation by van der Mei et al. [8] further indicated that MATH could measure the complicated interplay of long-range van der Waals and electrostatic forces and of various short-range interactions.

The measurement of electrical potentials and hydrophobicities for cysts and oocysts have been reported by Ongerth and Pecoraro [11], Brush et al. [12], Adin et al. [13], and Karaman et al. [14]. However, their results varied widely and presented little information about the influence of pH and ionic strength.

The aim of this work was to estimate the hydrophobic and surface electrostatic properties of *Giardia* cysts and *Cryptosporidium* oocysts. The measurements of hydrophobicity and zeta potential were performed at various pH values and ionic strengths in order to investigate the effects of environmental factors on the intricate surface characteristics of the parasites.

2. Materials and methods

2.1. Preparation of cysts and oocysts

Cysts and oocysts used in this study were obtained from Waterborne, Inc. (Louisiana, USA) and the Pleasant Hill Farms (Idaho, USA), respectively. These protozoan parasites were purified by the Percoll purification method, followed by the addition of 500 μg ml^{-1} penicillin, 100 μg ml^{-1} gentamicin, 500 μg ml^{-1} streptomycin, and 2% formalin. The (oo)cysts were diluted with a 0.1% phosphate buffered saline (PBS) solution to desired concentrations as the stock solutions, which were stored at 4 °C and were thoroughly mixed before each use. The numbers of cysts and oocysts in the stock solution were counted using the immunofluorescence assay technique. To do this, samples were mixed thoroughly and pipetted directly from the stock preparation vial and then onto the glass slides (Dynal® Spot-On), stained with fluorescent-labeled antibodies (Hydrofluor™ Combo *Giardia*/*Cryptosporidium*; Ensys, Inc., NC, USA), washed with 0.1% PBS solution, and then counted.

2.2. Measurement of zeta potential

Zeta potentials of *Giardia* cysts and *Cryptosporidium* oocysts were measured with a zeta meter (System 3.0, Zeta-Meter, USA). Water sample (25 ml) was transferred to a 40 ml volumetric flask, then aliquot of the (oo)cyst stock solution, containing approximately 10^7 (oo)cysts, was added to give sufficient (oo)cysts density in the field of view. Zeta potential was obtained from the (oo)cysts speed for a given applied electric field using the Smoluchowiski equation. In this study, $NaClO_4$ was used as the background electrolyte. The pH value of the water sample was adjusted by using 0.1 M HCl and 0.1 M NaOH. The pH and conductivity were monitored before each measurement of zeta potential.

2.3. Measurement of hydrophobicity

The hydrophobicities of cysts and oocysts were determined with the MATH test. A so-called kinetic MATH test, modified by Lichtenberg et al. [15], was employed to investigate the effect of pH and ionic strength on hydrophobicities by means of a complete fraction design. The pH values of water samples were adjusted from 2.4 to 11.1 with 0.1 N $HClO_4$ and 0.01 N NaOH with 1.0×10^{-2} M

$NaClO_4$ as background electrolyte. Water samples in various strengths of background electrolyte, from $10^{-1.0}$ M to $10^{-3.0}$ M, were also prepared when the pH was controlled at 5.6. Water sample (1 ml) was transferred into the eppendorf and then seeded with 50 μl aliquot of the (oo)cyst stock solution.

The number of (oo)cysts in the water sample (A_0) was counted, followed by the addition of 200 μl octane. The two-phase system was vortexed for 30 s. After standing for 5 min, three distinct phases appeared in the water sample: the top layer consisted of the octane, the middle layer of a emulsion of (oo)cysts and water, and the bottom layer of the aqueous (oo)cyst suspension. The (oo)cyst concentration in the bottom layer (A_t) was measured. Concentrations A_t and A_0 were determined using the indirect fluorescent antibody (IFA) staining procedure. To do this, 50 μl of water samples before adding octane and those collected from the lower aqueous phase were respectively pipetted onto the glass slides (Dynal® Spot-On), and stained with fluorescent-labeled antibodies (Hydrofluor™ Combo *Giardia*/*Cryptosporidium*; Ensys, Inc., NC, USA). The numbers of (oo)cysts on slides were counted and then transferred to units of concentration, A_t and A_0. The value of $\log(1 - A_t/A_0) \times 100$ was plotted against the mixing time. The slope was taken as the initial removal rate, R_0 (min^{-1}), and was obtained as a measure for hydrophobicity.

3. Results and discussion

3.1. Zeta potential of cysts and oocysts

The measurement of zeta potential is sensitive to the change in ionic strength (conductivity). Since the conductivity varied with the pH, it is impossible to keep all the samples at one value. Therefore, zeta potentials of *Giardia* cysts and

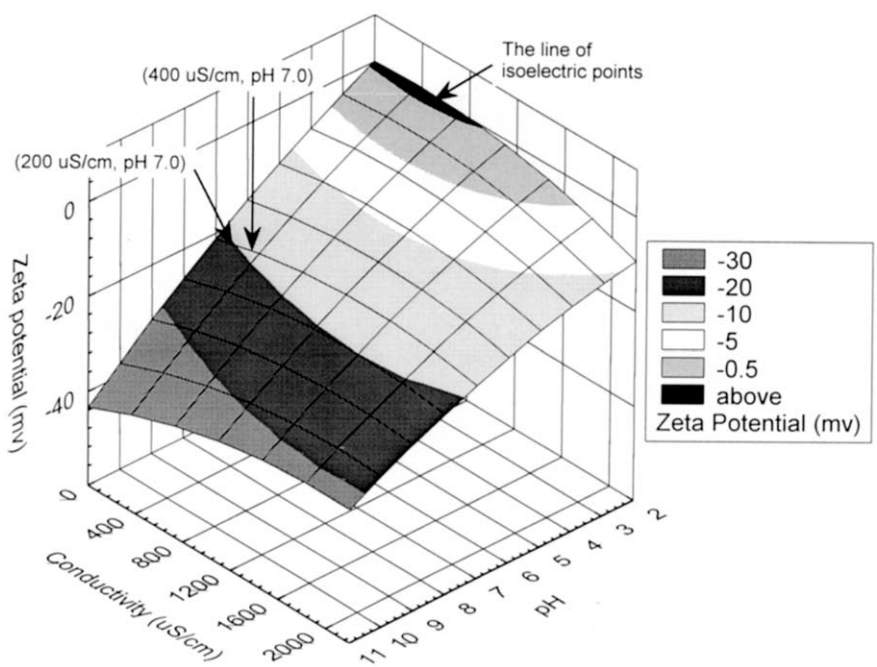

Fig. 1. Relationship between zeta potential and interaction factors as determined by conductivity and pH for *Giardia* cysts.

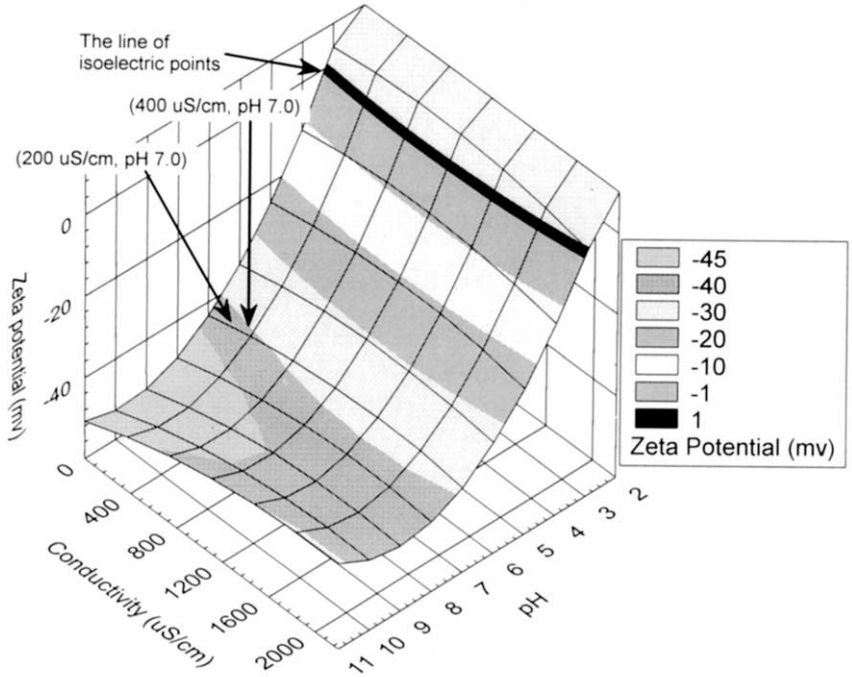

Fig. 2. Relationship between zeta potential and interaction factors as determined by conductivity and pH for *Cryptosporidium* oocysts.

Cryptosporidium oocysts in different conductivities and pH values are presented in three-dimensional graphs, as shown in Figs. 1 and 2. It appears that the absolute values of zeta potential for *Giardia* cysts and *Cryptosporidium* oocysts decrease with increasing ionic strength, as predicted by the theory of electrical-double layer compression. The zeta potentials of cysts and oocysts also increase negatively with increasing pH. The calculated zeta potentials for (oo)cysts indicate that they are strongly negatively charged at neutral pH (from -13 to -20 mV for cysts; from -37 to -42 mV for oocysts); in this respect, they may be expected to behave like other colloidal particles in raw waters. The reduction in zeta potential with increasing $[H^+]$ suggests that the use of conventional coagulation and flocculation by metal salt should work for (oo)cysts removal.

The respective zeta potentials of *Giardia* cysts and *Cryptosporidium* oocysts in raw water at pH 7.0 were -17 and -38 mV by assuming its conductivity of 400 μS cm^{-1}. Similarly, the zeta potentials of cysts and oocysts in tap water were determined as -18 and -40 mV, respectively, by assuming a conductivity of 200 μS cm^{-1} at pH 7.0. Zeta potentials of tap and raw waters thus determined are more negative than those reported previously [11,13].

In Figs. 1 and 2, the isoelectric points of cysts and oocysts at various conductivities were located in the darkened area as pointed by the line of isoelectric points. At a conductivity of 400 μS cm^{-1}, they were pH 2.2 and pH 3.3 for cysts and oocysts, respectively. Various isoelectric points have been reported. Previous works by Ongerth and Pecoraro [11], Drozd and Schwartzbrod [7], and Brush et al. [12] have reported the isoelectric points of oocysts as pH 3.9, pH 2.18 and pH 2.37, respectively. The study of Ongerth and Pecoraro [11] showed that the isoelectric point of cysts was pH 3.6. In addition, different sources of (oo)cysts, purification methods, storage solutions, and media would influence the surface properties of (oo)cysts.

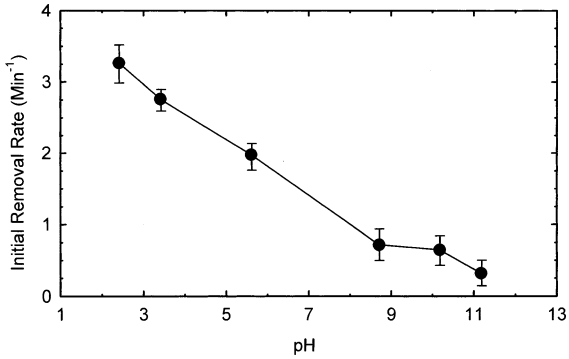

Fig. 3. Initial removal rates of cysts with various pH values in the presence of 0.01 M $NaClO_4$.

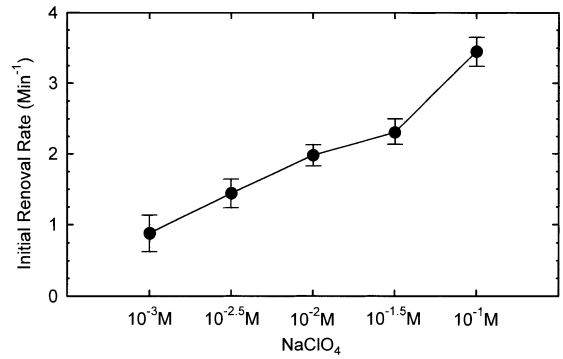

Fig. 5. Initial removal rates of cysts with various concentrations of $NaClO_4$ at pH 5.6.

3.2. Hydrophobicity of cysts and oocysts

In this study, six trial suspensions were experimented to estimate variations in hydrophobicities of (oo)cysts with respect to pH in the presence of 1.0×10^{-2} M $NaClO_4$. The results for cysts and oocysts were displayed in Figs. 3 and 4, respectively. Figs. 3 and 4 also show that the increase in initial removal rates of cysts and oocysts directly parallel the decrease in negative surface charge. It appeared that the hydrophobicities of (oo)cysts strongly increase towards acidic pH values. The initial removal rates of oocysts were generally higher than cysts, while a much wider variation was observed in cysts. The initial removal rate for *Giardia* cysts was ranged from 0.32 to 3.27 min^{-1}, and that of *Cryptosporidium* oocysts was ranged from 2.43 to 3.97 min^{-1}.

To study the relationship between the hydrophobicity of (oo)cysts and the background ionic strength, the initial removal rates at various concentrations of $NaClO_4$ at pH 5.6 were calculated. These graphs (Figs. 5 and 6) show that the initial removal rates for oocysts were relatively higher than the cysts in all trials, and the adhesions of cysts and oocysts to octane were moderately dependent on the ionic strength. When the ionic strength was increased from 10^{-3} to 10^{-1} M $NaClO_4$, the initial removal rates for cysts increased from 0.88 to 3.45 min^{-1}. For *Cryptosporidium* oocysts, the rate increased from 2.41 to 3.61 min^{-1} when the ionic strength was increased from $10^{-2.5}$ to 10^{-1} M $NaClO_4$. The ionic strength in the medium affects the adhesion of (oo)cysts to octane through the change on the surface hydrophobicity of (oo)cysts.

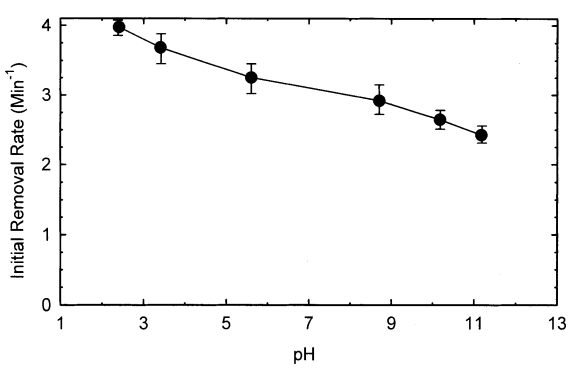

Fig. 4. Initial removal rates of oocysts with various pH values in the presence of 0.01 M $NaClO_4$.

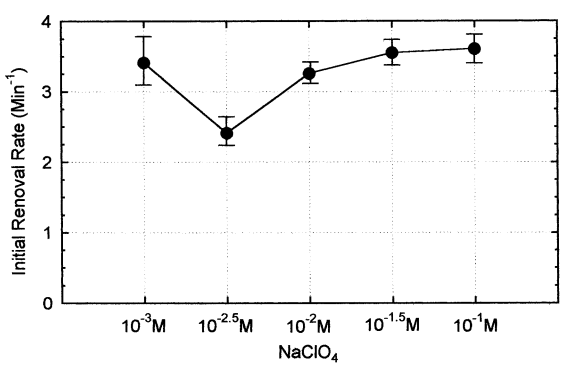

Fig. 6. Initial removal rates of oocysts with various concentrations of $NaClO_4$ at pH 5.6.

4. Conclusion

Giardia cysts and *Cryptosporidium* oocysts showed a pH-dependent surface charge, with zeta potentials becoming less negative as pH was reduced. Zero zeta potential was reached at pH 2.2 for cysts and pH 3.3 for oocysts. The absolute values of zeta potential for *Giardia* cysts and *Cryptosporidium* oocysts decreased with the increase of the suspension at a small scale. The determination of hydrophobicity through the MATH test demonstrated that the initial removal rates of cysts were lower than oocysts in all trials. The initial removal rates of (oo)cysts were also affected by the pH. The ionic strength of the medium had a moderate influence on the adhesion of cysts and oocysts onto octane. The initial removal rate was raised when the ionic strength was increased.

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