Multi-Scale *Cryptosporidium*/Sand Interactions in Water Treatment: A Review

Water Research

Revised
July 20, 2006

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Abstract

Owing to its widespread occurrence in drinking water supplies and its significant resistance to environmental stresses, *Cryptosporidium parvum* is regarded as one of the most important waterborne microbial parasites. Accordingly, a substantial research effort has been aimed at elucidating the physical, chemical and biological factors controlling the transport and removal of *Cryptosporidium* oocysts in natural subsurface environments and drinking water treatment facilities. In this review, a multiscale approach is taken to discuss the current state-of-knowledge on *Cryptosporidium*-sand interactions at a nano-scale, bench-scale and field-scale relevant to water treatment operations. Studies conducted at the nano-scale and bench-scale illustrate how techniques based on the principles of colloid and surface chemistry are providing new insights about oocyst-sand interactions during transport of *Cryptosporidium* oocysts in granular porous media. Specifically, atomic force microscopy and impinging jet experiments reveal the importance of oocyst surface biomolecules in controlling *Cryptosporidium*/sand interactions by a mechanism of steric hindrance. Traditional bench-scale column transport studies conducted over a broad range of experimental conditions highlight the role of physicochemical filtration and physical straining in the removal of oocysts from the pore fluid. Such experiments have also been used to evaluate the influence of biofilms formed on grain surfaces and the presence of natural organic matter on oocyst-sand interactions. Whilst filtration studies conducted at the plant-scale have been useful for evaluating the effectiveness of various materials as surrogates for *Cryptosporidium* oocysts, at this macro-scale, little could be learnt about the fundamental mechanisms controlling oocyst-sand interactions. This review of the literature on *Cryptosporidium*-sand interactions at different length scales points to the importance of combining studies at the plant-scale with well-controlled investigations conducted at the nano- and bench-scales. Furthermore, because oocyst surface properties play an important role in controlling the extent of interaction with sand surfaces, a thorough discussion of *Cryptosporidium* oocyst characteristics and electrical properties is presented.

Keywords: *Cryptosporidium*, colloid filtration, drinking water, atomic force microscopy, steric interactions, porous media

1. Introduction

The protozoan parasite *Cryptosporidium parvum* (*C. parvum*) has emerged as one of the most important microbial pathogens for drinking water safety and is associated with a high risk of waterborne illness (Lederberg et al., 1992; Rose, 1997). *C. parvum* is commonly transmitted via the faeces of infected mammalian hosts in its environmentally resistant stage — the oocyst (Lederberg et al., 1992; Smith, 1992; Mawdsley et al., 1995). Outside the mammalian host, the oocyst is biologically dormant and incapable of replication (Fayer et al., 1997). However, once infected, wild and domestic mammals may shed a large number of oocysts into the environment (e.g., a calf may excrete up to 10 million oocysts/day) (Rose, 1997). Ingestion of a small number of viable oocysts (as little as 10) can lead to cryptosporidiosis, a diarrheal disease that is potentially lethal for immunosuppressed individuals (Wolfson et al., 1985; Lederberg et al., 1992; Casemore et al., 1997; Rose, 1997).

Because *C. parvum* has been detected in a wide range of wild and domestic animals — including cattle, deer, and pigs — the contamination of water supplies by mammalian faecal wastes is of serious concern (Rose, 1997). In fact, surveillance data for *C. parvum* indicates the prevalence of the parasite throughout the environment, and sources are probably present in every surface water catchment (Lisle and Rose, 1995; Ionas et al., 1998; Quintero-Betancourt and Botero De Ledesma, 2000). *C. parvum* has been found in rivers and streams, lakes and reservoirs, raw and treated sewage, and treated surface waters. The parasite is increasingly regarded as an important cause of enteric disease and several waterborne outbreaks of cryptosporidiosis have been documented (Casemore et al., 1997). Outbreaks have been reported in the United Kingdom (Lisle and Rose, 1995) as well as the United States (Lisle and Rose, 1995; SoloGabriele and Neumeister, 1996) most notable of which was the outbreak in Milwaukee where up to 400,000 people were infected and 87 deaths of immunocompromised patients were attributed to the disease (Smith and Perdek, 2004). Occasional outbreaks have occurred with no measured changes in source water quality or

treatment processes (Roefer et al., 1996). Such episodes, combined with the serious consequences of cryptosporidiosis outbreaks, have necessitated research on reliable processes to defend drinking water supplies from contamination with *Cryptosporidium.*

Resistance of the *C. parvum* oocyst to several environmental stresses including chlorination during water treatment poses a significant challenge to the protection of drinking water supplies from contamination (Campbell et al., 1982; Hayes et al., 1989; West, 1991; Rose, 1997). As a result, water utilities are showing increased interest in oocyst removal in granular porous media, using approaches such as deep-bed (granular) filtration, riverbank filtration and slow-sand filtration, to ensure the safety of drinking water (Timms et al., 1995; Huck et al., 2002; Tufenkji et al., 2002).

To better understand and predict the removal of *C. parvum* oocysts in these settings, a growing research effort has been directed at elucidating the factors controlling oocyst-sand interactions as well as the fundamental mechanisms governing the filtration of oocysts. Studies related to the general behaviour of *Cryptosporidium* oocysts in granular porous media have been carried out at various length scales, from the microscale to the field scale. The use of atomic force microscopy (AFM) has made possible the characterization of nanoscale interaction forces between oocysts of *C. parvum* and model sand surfaces (Considine et al., 2000; Considine et al., 2001; Considine et al., 2002; Byrd and Walz, 2005). Measurements of oocyst deposition onto flat quartz surfaces in a radial stagnation point flow (RSPF) cell have provided further insight into the factors that influence *Cryptosporidium*-sand interactions at the microscale (Kuznar and Elimelech, 2004, 2005). Several researchers have investigated the filtration behaviour of *Cryptosporidium* at the bench-scale, using laboratory columns packed with a wide range of different sediments or model granular materials (Mawdsley et al., 1996; Brush et al., 1999; Harter et al., 2000; Hsu et al., 2001; Logan et al., 2001; Dai and Hozalski, 2002, 2003; Tufenkji et al., 2004; Abudalo et al., 2005; Bradford and Bettahar, 2005; Hijnen et al., 2005; Tufenkji and Elimelech, 2005b). In these studies, various aspects of *C. parvum* transport and removal in granular porous media have been examined, including the influence of solution chemistry, fluid flow rate, and sediment grain size. Column transport experiments have also provided considerable insight into the relevance of proposed surrogates for *C. parvum* and the relative importance of the different oocyst

removal mechanisms (e.g., physicochemical filtration and physical straining). Finally, evaluation of process performance at drinking water treatment facilities can be used to understand *Cryptosporidium* filtration at a larger scale. A thorough review of such studies completed at the pilot and full-plant scale was recently presented by Emelko et al (Emelko et al., 2005). Their work emphasizes the need to better understand the effects of various operating conditions on the removal of oocysts in different treatment schemes.

As always, plant personnel are focused on the provision of safe drinking water of the highest possible quality. The series of cryptosporidiosis-related incidents worldwide, their regulatory aftermaths and their economic consequences have seriously challenged the faith that operators and customers have in the clarification technologies that are available. It is the lack of knowledge about what happens to these protozoa in coagulation, chlorination and filtration that causes the greatest lack of confidence. In spite of the recent upsurge in related research projects in both the laboratory and the field, the manner in which the oocysts interact with flocs and flocculants and membranes and filter media remains largely unknown. Our objective in preparing this paper is not only to highlight the advantages of using the technologies of colloid science to provide some of the answers by looking at what is happening to the oocysts at the various interfaces present in the drinking water system but also to critically evaluate the resultant data and to point out the remaining gaps in the collective data base. First, the physical, chemical and electrical properties of *Cryptosporidium parvum* oocysts are described. This is followed by a discussion of studies probing oocyst-surface interactions at the nano-scale (i.e., examining the role of oocyst surface biomolecules in oocyst attachment). From the nano-scale, we move on to discuss intermediate, bench-scale studies of *Cryptosporidium* transport and removal in granular porous media. Studies of *Cryptosporidium* filtration at the field or plant scale and the relevance of surrogates for this parasite are discussed next. After completing a survey of the literature on *Cryptosporidium*-sand interactions at different length scales, we compare *Cryptosporidium* filtration behavior to that of other waterborne pathogens and discuss the importance of physical straining in *Cryptosporidium* filtration.

2. Characterization of the *Cryptosporidium* Oocyst

Removal of *C. parvum* oocysts in engineered water treatment processes or the natural subsurface environment usually involves direct contact of the oocyst wall with the granular (filter) medium (e.g., sand, biofilm-coated sand, anthracite, or garnet). It follows that oocyst surface properties should play an important role in controlling the strength of the abovementioned interactions between the protozoan parasite and the collector surface (filter medium). Structural surface features of the oocyst wall could give rise to steric interactions or even enhanced adhesion of the oocyst. The surface charge density of the *Cryptosporidium* oocyst and the nature and concentration of the background electrolyte will govern the extent of electrical double layer (EDL) interactions. Finally, genotyping is an important characterization tool for water treatment because it informs the risk assessment of water systems. A good understanding of *Cryptosporidium* oocyst properties is therefore important in the study of *Cryptosporidium*/sand interactions. Here, we examine the current state-of-knowledge of the oocyst wall structure as well as electrokinetic characterization and genotyping of the oocyst.

2.1. Structure of the Oocyst Wall

During maturation within the host organism, *C. parvum* oocysts can develop with either thin or thick walls. Thin-walled oocysts are believed to remain within the host and initiate auto-infection whereas thick-walled oocysts are released from the body to infect other hosts (Fayer et al., 1997). The thick-walled oocyst exhibits impressive hardiness in natural and engineered environments and is thus of greater interest to water treatment practitioners and will be the focus of this section.

C. parvum oocysts can survive a wide range of environmental stresses, including certain degrees of freezing (Robertson et al., 1992), common disinfectants (Campbell et al., 1982; Chauret et al., 2001), exposure to seawater (Robertson et al., 1992; Fayer et al., 1998) and various exposure levels to conventional water treatment processes (e.g., ozonation) (Carey et al., 2004). The resistance of *C. parvum* to environmental pressures and its ability to retain infectivity have been attributed to the trilayered oocyst wall. This tough wall provides protection to the four infective sporozoites found within the oocyst

(Fayer et al., 1997; Tilley and Upton, 1997). A unique feature of the *C. parvum* oocyst wall is the presence of a suture at one end along which the oocyst splits open during excystation, enabling release of the sporozoites (Harris and Petry, 1999; Petry, 2004). Thin sections of *Cryptosporidium* oocysts examined by transmission electron microscopy (TEM) reveal the oocyst wall to be about 40 nm thick (Harris and Petry, 1999; Petry, 2004). The outer layer of the wall is relatively thin (~ 5 nm) and believed to be composed of acidic glycoprotein. The thicker (~ 20 nm) inner layer of the oocyst wall is thought to be a filamentous glycoprotein (Bonnin et al., 1991; Harris and Petry, 1999) and is strongly immunogenic (i.e., capable of inducing an immune response) (Tilley and Upton, 1997). At least three antigens are associated with this surface (Tilley and Upton, 1997; Petry, 2004), one of which is a molecule rich in cysteine, proline, and histidine (Ranucci et al., 1993; Tilley and Upton, 1997). This high cysteine content may allow the formation of a large number of disulfide bonds thereby conferring structural integrity to the oocyst wall (Ranucci et al., 1993). Sandwiched between the inner and outer layers of the oocyst wall is a ~ 5 nm electron-transparent space followed by a 10 nm thick central glycolipid/lipoprotein layer (Petry, 2004). This central layer may consist of two membranes (Fayer et al., 1997) and, along with the thick inner layer, is believed to provide rigidity and elasticity to the oocyst wall (Harris and Petry, 1999).

The stability of the *C. parvum* oocyst wall following exposure to various chemical treatments has been examined by Harris and Petry (Harris and Petry, 1999). Isolated oocyst walls exhibit a surprising level of resistance to prolonged contact with three different proteases (pepsin, trypsin, and proteinase K). Treatment of *C. parvum* oocyst walls with a neutral surfactant (Nonidet P40), chloroform, or phenol extraction solutions further failed to damage the oocyst wall (Harris and Petry, 1999; Petry, 2004). These researchers also evaluated mechanical means to disrupt the oocyst wall, namely, grinding using a mortar and pestle, and ultrasonication for up to 20 minutes. Failure of these methods to cause breakage of the oocyst wall underscores the stability and strength of this protective shell.

Representative AFM images of an oocyst are shown in Figure 1. The roughly spherical nature of the oocyst and inherent surface roughness is evident. The oocyst particles cannot be described as smooth. The largest asperities are on the order of 250

nm in diameter and up to approximately 55 nm high (Considine et al., 2000; Considine et al., 2001; Considine et al., 2002). The root mean square roughness over micron-sized areas has been found to be in the range from 5 to 20 nm (Considine et al., 2000; Considine et al., 2001; Considine et al., 2002). An AFM measurement of the mechanical properties of *C. parvum* oocysts indicates that oocyst wall surfaces are at least as hard as solid inorganic materials such as sand particles (Considine et al., 2001).

[FIGURE 1]

Application of advanced microscopy techniques has provided valuable information on the structural nature of the *C. parvum* oocyst wall (Fayer et al., 1997; Petry, 2004). Yet, our understanding of the oocyst wall composition remains quite limited. Molecular details regarding the makeup of the outer and inner layers of the oocyst wall are lacking. For instance, the structures of the glycoproteins which are believed to form these layers have not been identified. Knowledge of such details is of particular importance in attempts to better understand and characterize the physicochemical forces which control oocyst-surface interactions. Thus, there is clearly a need to develop further research studies in this area.

2.2. Electrical Properties

The surface charge of microorganisms is an important factor in determining their colloidal stability and removal in natural and technological aqueous environments (Elimelech et al., 1995; Lytle et al., 2002). Processes involving oocyst-oocyst interaction (i.e., aggregation) and oocyst-surface interaction (i.e., physicochemical filtration) are controlled in part by electrical properties of the oocyst and collector surface in question (e.g., sand grain). In fact, several researchers have found a direct relationship between the measured zeta potential of *C. parvum* oocysts and their transport potential in granular porous media (Hsu et al., 2001; Hsu and Huang, 2002; Tufenkji et al., 2004; Abudalo et al., 2005; Tufenkji and Elimelech, 2005b). In such studies, the interaction energy between oocysts and collector surfaces has been commonly described using the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Derjaguin and Landau, 1941;

Verwey and Overbeek, 1948) which requires knowledge of the electrical properties of both the oocysts and the collector surfaces. For instance, in calculating the electrostatic double layer (EDL) contribution to the overall interaction energy, colloid and collector surface potentials are commonly replaced with measured zeta potentials. Surface charge characterization of *C. parvum* oocysts is also important in obtaining general predictions of oocyst removal during treatment of drinking water or migration in the natural subsurface environment. Hence, a substantial research effort has been directed at evaluating the electrophoretic mobility (EPM) of *C. parvum* oocysts over a broad range of environmentally-relevant solution conditions (Drozd and Schwartzbrod, 1996; Ongerth and Pecoraro, 1996; Brush et al., 1998; Karaman et al., 1999; Considine et al., 2000, 2002; Hsu and Huang, 2002; Lytle et al., 2002; Butkus et al., 2003; Dai and Hozalski, 2003; Kuznar and Elimelech, 2005).

Whole particle microelectrophoresis is the traditional technique for characterizing surface charge of colloids or biocolloids such as *C. parvum* (Van der Mei and Busscher, 2001; Lytle et al., 2002). The zeta (ζ) potential of *C. parvum* oocysts is determined from measurements of the EPM under an applied electric field. The EPM of *C. parvum* oocysts as measured by Lytle et al (Lytle et al., 2002) is presented as a function of solution pH in Figure 2. Careful inspection of the figure reveals that the isoelectric point of *C. parvum* is approximately pH 2. As the solution pH increases, the absolute EPM of the oocysts increases (i.e., the oocyst surface charge becomes increasingly negative). Thus, under conditions commonly found in the natural environment, *C. parvum* is generally negatively charged. Also shown in Figure 2 is the role of solution ionic strength on the EPM of *C. parvum* oocysts. The data was measured using solutions of oocysts suspended in phosphate-buffered water. The measurements clearly show that the oocysts become more negatively charged with a decrease in solution ionic strength. These observations have important implications for the transport and removal of *C. parvum* oocysts in the natural subsurface or in water treatment facilities. This issue will be discussed in more detail in *Section 4*.

[FIGURE 2]

Dissolved divalent ions (e.g., calcium) and dissolved natural organic matter (NOM) are commonly found in natural surface and subsurface waters. The concentration of these components can vary widely in different geographical locations based on local environmental conditions. It is well known that NOM can adsorb onto the surfaces of suspended particles or sediment grains, thereby altering their surface characteristics (Kretzschmar et al., 1999; Franchi and O'Melia, 2003). Divalent cations in aqueous systems have also been shown to cause changes in the electrokinetic properties of various colloids and collectors (Kretzschmar et al., 1999). As a result, the influence of dissolved divalent cations and dissolved NOM on the electrophoretic mobility (or ζ-potential) of *C. parvum* oocysts has been examined by several researchers. Dai and Hozalski (Dai and Hozalski, 2002, 2003) reported the ζ-potential of "clean" and NOM-coated oocysts as a function of calcium concentration. Their results are shown in Figure 3, where the measurements obtained in the absence and presence of NOM are represented by the solid and open symbols, respectively. Adsorption of NOM onto the surface of the *C. parvum* oocysts causes a significant increase in the magnitude of the oocyst ζ-potential (i.e., the oocysts become more negatively charged). In contrast, an increase in Ca^{2+} concentration gives rise to a decrease in the magnitude of the oocyst surface charge (i.e., the oocysts become less negatively charged). Other researchers have observed similar effects of dissolved organic carbon and divalent cations on oocyst ζ-potential (Thomas et al., 2001; Considine et al., 2002; Kuznar and Elimelech, 2004). The influence of these solution components on the electrokinetic properties of *C. parvum* oocysts can play an important role in the migration behaviour of this parasite in saturated porous media. In fact, studies conducted under well-controlled laboratory conditions have demonstrated the effects of dissolved NOM and/or calcium on *C. parvum* adhesion to quartz substrates or glass beads (Considine et al., 2002; Dai and Hozalski, 2002, 2003; Kuznar and Elimelech, 2004). In general, adsorption of NOM onto the oocyst surface results in decreased attachment of *C. parvum* to silica surfaces (Dai and Hozalski, 2002, 2003). On the other hand, oocyst attachment to collector surfaces increases in the presence of divalent cations (Kuznar and Elimelech, 2004).

[FIGURE 3]

Electrokinetic characterization of *Cryptosporidium* is typically accomplished using oocysts obtained from calf feces, following a certain pre-treatment procedure. A broad range of different oocyst purification and preservation methods have been used prior to electrokinetic characterization. The most common approaches for oocyst purification involve sucrose or Percoll© centrifugation gradients (Brush et al., 1998; Karaman et al., 1999; Harter et al., 2000; Butkus et al., 2003), but some techniques also include the use of additional chemicals such as cesium chloride and ethyl acetate (Drozd and Schwartzbrod, 1996; Brush et al., 1998; Dai and Hozalski, 2002, 2003). Preservation techniques for purified oocysts also vary between different studies. Purified oocysts have been stored in the absence or presence of antibiotics and surfactants. Most studies of *C. parvum* electrophoretic mobility reported in the literature have involved the use of different oocyst sources, purification protocols, and storage methods. These inconsistencies between different investigations sometimes cause difficulties in interpretation and comparison of experimental findings. Several researchers (Brush et al., 1998; Considine et al., 2002; Butkus et al., 2003) have previously reported on the influence of pre-treatment and storage methods on measurements of oocyst electrophoretic mobility. Their findings show how the measured surface charge of *C. parvum* oocysts can vary considerably when different purification or pre-treatment approaches are used, even when the oocysts are obtained from the same source. It is further interesting to note that significant variations in measured electrophoretic mobility have been observed in samples of *C. parvum* oocysts obtained from the same source and handled in an identical manner (Kuznar and Elimelech, 2004). The influence of oocyst pre-treatment on measured electrophoretic mobility is shown in Figure 4. Brush et al (Brush et al., 1998) demonstrated how the electrophoretic mobility of oocysts purified with the EAPS (ethyl acetate-Percoll©-sucrose) method (solid symbols) is significantly different from that of oocysts purified using a more simple deionized water-sucrose (DIS) method.

[FIGURE 4]

2.3. Genotyping

The characterization of *Cryptosporidium* to the genotype and sub-genotype levels is important for water treatment because (1) it assists water suppliers to understand human health risks associated with water sources, and (2) it supports health authorities to identify potential causes of cryptosporidiosis outbreaks (Widmer, 1998; Morgan et al., 1999; Fayer et al., 2000; Xiao and Ryan, 2004). For example, the level of water treatment associated with the forested water catchments of Melbourne, Australia, is disinfection only (i.e., the supplies are unfiltered) and this situation has been supported by the molecular characterization of *Cryptosporidium* populations in the native fauna, which have been found to be genotypes that are non-infective to humans.

Of the 14 species of *Cryptosporidium* that have been documented (Fayer et al., 2000; Xiao and Ryan, 2004), it is the *C. hominis* (human specific) and *C. parvum* (not human specific) that have been associated with human infection. The different species are not sufficiently differentiated using conventional methodologies, which, combined with the non-specificity of *C. parvum* (O'Donoghue, 1995; Fayer et al., 2000), has necessitated a role for characterization of *Cryptosporidium* at the molecular level. The molecular techniques have therefore been the subject of extensive research and have been reviewed in detail elsewhere (Widmer, 1998; Fayer et al., 2000; Xiao and Ryan, 2004).

3. Oocyst-Surface Interactions at the Nano-Scale

Surface forces play a major role in governing colloidal interaction and adhesion in sand-*Cryptosporidium parvum* systems. In *Cryptosporidium*/sand studies a common approach has been to interpret system behaviour in terms of the DLVO theory of colloidal stability (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948). The original DLVO calculations were based on the interaction between incompressible and uniformly charged surfaces of ideal geometry (typically infinite flat plates) interacting in electrolytes consisting of point charges dispersed in a continuum phase (such as water).

Under certain frequently encountered conditions, the force of interaction ($F_{sphere\text{-}sphere}$) between two particles (of inter-surface separation H) is related to the interaction energy between two flat plates ($W_{flat\text{-}flat}$) by the Derjaguin approximation:

$$\frac{F_{sphere-sphere}}{R} = 2\pi W_{flat-flat} \quad (1)$$

where R is the harmonic mean of the radii of the two spheres.

van der Waals interaction originates from dipole interactions associated with the electric field produced by one dipole, acting on a second dipole, and generally, when water is the intervening medium, leads to an attraction between the interacting atoms or molecules. van der Waals interaction between spherical particles is generally described by:

$$\frac{F}{R} = \frac{-A_H}{6H^2} \quad (2)$$

where F, R, and H are defined as above, and A_H is the non-retarded Hamaker constant. van der Waals interactions can be computed using Hamaker constants determined from the bulk material dielectric data and Liftshitz theory according to the procedure outlined by Hough and White (Hough and White, 1980).

There are several studies of AFM measurement of van der Waals interaction indicating that very good agreement between experiment and theory can be obtained (Larson et al., 1993; Biggs and Mulvaney, 1994; Drummond et al., 1996). The Hamaker constant is generally relatively small ($< 1 \times 10^{-20}$ J) in aqueous biological systems. Consequently, van der Waals interaction is short-range and this, in combination with surface roughness, hydration and steric effects, means that although van der Waals interaction is always present it is frequently not a major contributor to surface interaction behavior in bio-systems.

The charging of sand and *C. parvum* oocyst surfaces in aqueous media can arise from dissociation of surface groups and/or the adsorption of ions onto the surface. When a charged surface exists in aqueous solution, a counter ion concentration profile will manifest near the surface and the ion density is usually described in terms of a Boltzmann distribution. An electrical double layer is represented as a layer of charge at the surface, and a diffuse layer of ions decaying away from the surface. The solution of the Poisson-Boltzmann equation is usually employed to calculate the force of interaction between opposing surfaces due to diffuse layer overlap. In systems where van der Waals and electrical double layer interactions dominate, AFM measurements generally agree with

calculated electrical double layer interaction at low ionic strengths (Johnson et al., 1995; Larson et al., 1995, 1997; Considine and Drummond, 2001). Surface roughness effects complicate AFM force curve analyses when the extent of the surface force is comparable to the degree of surface roughness (Johnson et al., 1995; Considine and Drummond, 2001).

Despite the fact that DLVO theory can be employed to interpret the interaction in many colloidal systems, the inherent assumptions of DLVO theory are potentially problematic for biocolloidal systems. Biocolloids can be soft, deformable, non-homogeneous (in terms of topography, geometry and surface chemistry) and dynamic. The scientific community's ability to describe the behaviour of these types of systems is still embryonic. Nevertheless, the AFM is proving to be an important tool by allowing the comparison of experiment and theory in biocolloidal systems.

The AFM has been used to measure the surface forces of interaction between *Cryptosporidea* and sand. Originally developed as a surface-imaging tool, the use of the AFM has been extended to include the measurement of surface forces of interaction between individual *Cryptosporidea* and model sand particles in aqueous solutions (Considine et al., 2002). Figure 5 shows a schematic representation, constructed from AFM soft contact mode images, of a model sand (silica) particle interacting with a *C. parvum* oocyst. AFM surface force measurements have been interpreted in terms of a model of the *Cryptosporidium* surface, which includes both physical and electrical components. The investigations demonstrate the importance of surface forces in *Cryptosporidium*/sand interactions.

[FIGURE 5]

The surface forces are measured as the separation between individual oocysts and silica surfaces are decreased. In the usual AFM set-up a silica particle is attached to an AFM cantilever (weak spring). An oocyst is attached to a flat plate residing on a piezoelectric ceramic tube that can be either expanded or contracted either to advance the oocyst closer to the particle or to retract it away from the particle. The force of interaction calculated from the cantilever deflection is expressed in milli-Newtons (mN),

and is normalised by the harmonic mean of the radii of the two surfaces (m^{-1}), in order to facilitate comparison between particles of different radii. The normalised force, as a function of separation (nm) for the surface approach of an oocyst and a silica particle is presented in Figure 6. The data consists of three distinct regions; at very long range the cantilever experiences zero force and so does not deflect with piezo movement, at intermediate range the cantilever experiences repulsive surface forces and deflects non-linearly with piezo movement, and after contact the cantilever deflects linearly with piezo movement. The calibration and interpretation of the force-separation data reported here has been described in detail elsewhere (Considine et al., 2001).

[FIGURE 6]

3.1 Role of Biomolecules in Oocyst-Surface Interactions

In dilute KNO_3 solutions where the pH was varied, all force-separation curves between oocysts and the model sand surface (silica) exhibited a repulsive force at separations prior to contact, on surface approach (Considine et al., 2001). In order to investigate the origin of the repulsive force, a conventional approach has been to evoke theoretical models and obtain fits with experimental data (Israelachvili, 1992). The surface force measurements have shown that the oocyst surface is not well approximated by DLVO theory alone and that a steric force coexists with the electrical double layer force. Therefore, the repulsive force has been modelled using DLVO theory combined with a model of a polylectrolyte brush based principally on the developments of Witten and Pincus (Witten and Pincus, 1987) and Pincus (Pincus, 1991).

Many theoretical models of the force of interaction between polymer-bearing surfaces have been proposed (Alexander, 1977; De Gennes, 1982; Witten and Pincus, 1987; Miklavic and Marcelja, 1988; Milner et al., 1989; Zhulina et al., 1992; Von Goeler and Muthukumar, 1995). Calculations for charged polymers adsorbed at interfaces reveal a brush-like conformation that extends further with increasing charge density or decreasing salt concentration (Miklavic and Marcelja, 1988; Milner et al., 1989). Neglecting chain stiffening and excluded volume effects, Witten and Pincus (Witten and

Pincus, 1987) and Pincus (Pincus, 1991) quantified the interaction forces in terms of the counter-ion osmotic pressure (Π) as a function of separation distance H to be:

$$\Pi \approx \frac{2fN_B k_B T}{d^2 H} \tag{3}$$

where f is the fraction of ionicity (or fraction of monomers carrying an ionic charge), N_B is the number of monomers within the hydrophilic block, d is the grafted interchain distance, k_B is the Boltzmann constant, and T is the temperature. It has been shown (Abraham et al., 2000) that the force of interaction between two polyelectrolyte brushes of individual width L_B can be given by:

$$F/R = \frac{4\pi f k_B T N_B}{d^2} \ln\left[\frac{2L_B}{H}\right] \tag{4}$$

The above equation corresponds to the interaction of two polymer-bearing surfaces. However, the experimental situation is that the silica surface is well described by electrical double layer theory in 1 mM KNO_3 (Considine et al., 2001) and the brush is logically located on the oocyst surface. Therefore, the parameter $2L_B$ has been taken to be the brush width on the oocyst surface. Although the grafted inter-chain distance d is unknown it has been fixed at 2.31 nm for all calculations. This value was chosen since the oocyst wall has been shown to consist of predominantly proline, cystine, and histidine (Ranucci et al., 1993); the largest of these amino acids is histidine, measuring 2.31 nm in the longest linear dimension predicted from the van der Waals radius of the constituent atoms. Although the value of d has been arbitrarily fixed, it is unlikely to vary as a function of pH, and does permit the investigation of other parameters that describe the steric force of interaction. The value of the temperature T has also been fixed in these calculations at 298 K. The remaining terms correspond to the fraction of ionicity, f, and the number of monomers within the hydrophilic block, N_B. Both of these dimensionless terms describe the chemical nature of the extended brush, and are expected to be related, that is, as f increases N_B will probably also increase, given the increase in charge on the polypeptide backbone. Therefore N_B and f have been combined to form $N_B f$, where large values correspond to a brush that is composed of molecules that are highly charged and predominantly hydrophilic.

The measured normalised force versus separation data for the interaction between an oocyst and a silica sphere, in 1 mM KNO_3 at pH = 8.9, has been presented in Figure 7. The DLVO fit has been presented as thin solid lines with the upper and lower limit both being shown. The calculation has been made for the interaction of two surfaces governed by a Hamaker constant of 1 x 10^{-20} J and diffuse layer potentials corresponding to the measured ζ-potentials, immersed in the bulk electrolyte concentration of 1 mM KNO_3. In order to obtain an order of magnitude fit, the origin of the plane of charge in the DLVO calculation has had to be shifted around 35 nm from the point of contact. The data is seen to be well described by DLVO theory at separations > 35 nm with close agreement with the predicted decay length (9.5 nm). At separations < 35 nm but > 10 nm, the force of interaction is well described by the theory of Pincus (thick line). The calculation has been based on a brush width ($2L_B$) of 50 nm with a $N_B f$ of around 0.3, and all other parameters fixed.

[FIGURE 7]

The overlay of the experimental data and theoretical curves presented in Figure 7 permit the diagnosis of the origin of the exponential repulsive force. At separations greater than 35 nm, the interaction is well described by electrical double layer theory. At separations between 10 and 35 nm the interaction is well described as the collapse of a polyelectrolyte brush of width 50 nm. The implication from the theoretical modelling is that protein 'hairs' extend into solution from the surface of *Cryptosporidium*, and act as a significant repulsive barrier. Not all oocysts exhibited force-separation curves that could be fit with DLVO and Pincus predictions in the same manner as for Figure 7. The inability to obtain agreement with DLVO theory is probably a combined result of the significant surface roughness and extension of the oocyst surface proteins into the electrical double layer. Indeed, it is somewhat surprising that frequent agreement with DLVO theory can be obtained at all, given the complexity of the surface.

Despite the fact that there is a relatively high repulsive interaction on approach, *Cryptosporidium parvum* oocysts and silica surfaces can adhere. Figure 8 displays both surface approach and surface retraction force curves. The retraction curves can be

explained in terms of inter-surface bridging of macromolecules that were originally on the surface of the oocyst. The series of jump-outs is most likely due to different tether lengths being released.

[FIGURE 8]

3.2 Supporting Evidence for the Presence of a Steric Hindrance

In addition to the AFM measurements discussed in the previous section, several other studies have provided evidence that an "electrosteric" repulsion mechanism governs *Cryptosporidium*/sand interactions in aqueous media (Kuznar and Elimelech, 2004; Byrd and Walz, 2005; Kuznar and Elimelech, 2005; Kuznar and Elimelech, 2006). Using an impinging jet apparatus which simulates the hydrodynamic conditions at the forward stagnation point of a spherical sand grain, Kuznar and Elimelech (Kuznar and Elimelech, 2004, 2005; Kuznar and Elimelech, 2006) demonstrated the important role of surface biomolecules in oocyst-sand interactions. The radial stagnation point flow (RSPF) technique used by these researchers allows for the real-time investigation of oocyst deposition onto quartz (silica) surfaces under well-controlled hydrodynamic and chemical conditions.

Initial experiments revealed that the rate of oocyst deposition onto a clean quartz surface was significantly limited (near zero) in monovalent salt solutions, even at high ionic strengths where DLVO theory predicts no repulsive electrostatic energy barrier (Kuznar and Elimelech, 2004). When divalent salt solutions ($CaCl_2$) were used, the researchers observed greater oocyst deposition rates which increased with ionic strength. Based on these observations, Kuznar and Elimelech suggested that oocyst interaction with the silica surface may be hindered by an "electrosteric" repulsion which can be reduced or eliminated in the presence of calcium ions. To further investigate this hypothesis, the researchers compared the deposition kinetics of viable *C. parvum* oocysts with those treated (inactivated) with either heat or formalin (Kuznar and Elimelech, 2005). Heat or formalin treatments are expected to alter the structure of *C. parvum* oocyst surface proteins and thus reduce the degree of steric repulsion. In effect, oocyst

deposition rates onto the quartz surface increased significantly for formalin and heat-treated oocysts in comparison to untreated oocysts.

In a more recent study, oocyst deposition kinetics of viable *C. parvum* oocysts were compared with those after treatment with a digestive enzyme (proteinase K) to cleave oocyst surface macromolecules (Kuznar and Elimelech, 2006). Low deposition rates were observed with untreated oocysts over a wide range of solution salt concentrations. However, when oocysts were treated with proteinase K to remove surface macromolecules, the "electrosteric" repulsive force was eliminated and oocyst deposition onto the quartz surface increased significantly (Kuznar and Elimelech, 2006). Taken together, the results of these RSPF and AFM measurements strongly suggest that biomolecules on the oocyst surface play an important role in *Cryptosporidium*/sand interactions by a mechanism of steric hindrance.

These findings highlight the need to better understand oocyst-surface interactions at the nano-scale in our effort to protect recreational and drinking water supplies from *C. parvum* contamination. For example, there is a gap in our understanding of the influence of other raw water components (i.e., environmental macromolecules, flocculants, coagulants, etc) on biomolecule-surface interactions. The results of nanoscale investigations of *Cryptosporidium*-surface interactions also underscore the need to better relate such findings to bench- and field scale oocyst transport behavior. Although the use of RSPF and AFM has demonstrated the importance of oocyst surface biomolecules in oocyst-surface interactions, the precise impact of "electrosteric" interactions at greater length scales and under conditions relevant to treatment facilities or natural environments is unknown.

4. Studies of *Cryptosporidium* Filtration at the Laboratory-Scale

As outlined in the previous section, AFM and RSPF experiments allow researchers to examine *C. parvum*–sand interactions at the nanoscale. To relate these findings to *Cryptosporidium* filtration behaviour in water treatment facilities or the natural subsurface environment, it is necessary to conduct further studies at an intermediate, or bench–scale.

Several researchers have studied the transport and removal of *Cryptosporidium* oocysts in granular porous media using classical laboratory–scale packed column experiments (Brush et al., 1999; Harter et al., 2000; Hsu et al., 2001; Logan et al., 2001; Dai and Hozalski, 2002, 2003; Tufenkji et al., 2004; Abudalo et al., 2005; Bradford and Bettahar, 2005; Hijnen et al., 2005; Tufenkji and Elimelech, 2005b). In these experiments, a cylindrical tube – usually a glass or plastic column – is first packed with the granular media of interest which can vary from pure quartz sand (Tufenkji et al., 2004) to natural soils (Harter et al., 2000; Hijnen et al., 2005) and uniform glass beads (Hsu et al., 2001; Dai and Hozalski, 2002, 2003). Prior to injecting a suspension of *C. parvum* oocysts, the packed column is flushed with a particle–free electrolyte solution of the same chemical composition as that of the microbial suspension. This procedure allows for physicochemical equilibration of the packed porous medium. A solution of *C. parvum* oocysts suspended in the same electrolyte solution is then injected at the column inlet and sometimes followed by a final flush with particle–free electrolyte solution. During the colloid transport experiment, the concentration of *C. parvum* oocysts in the column effluent can be monitored in real–time using a flow–through cell and spectrophotometer (Tufenkji and Elimelech, 2005b) or effluent samples can be collected for subsequent quantification by epifluorescence microscopy (Abudalo et al., 2005; Bradford and Bettahar, 2005). Results of column deposition studies can be compared quantitatively by considering the classical colloid filtration theory (CFT). In this model, the transport of oocysts to the grain surface is expressed in terms of a single-collector *contact* efficiency, η_0, whereas the contribution of colloidal (e.g., DLVO) forces to oocyst removal is included in an empirical attachment (collision) efficiency, α (Yao et al., 1971; Elimelech et al., 1995). Measurements of oocyst concentration in the column effluent can be used to determine the value of α under different experimental conditions:

$$\alpha = -\frac{2}{3}\frac{d_c}{(1-f)L\eta_0}\ln(RB) \tag{5}$$

Here, L is the filter medium length, f is the bed porosity, d_c is the average grain size and RB is the normalized oocyst concentration at the column outlet at the initial stages of the oocyst breakthrough curve or the cumulative fractional breakthrough in the case of a pulse injection (Elimelech et al., 1995). The value of η_0 in Equation 5 can be calculated

using a recently developed correlation equation (Tufenkji and Elimelech, 2004). Following completion of the colloid transport experiment, some researchers have dissected the porous matrix into sections to determine the spatial distribution of oocysts within the packed bed (Harter et al., 2000; Bradford and Bettahar, 2005; Tufenkji and Elimelech, 2005b). This additional step provides the data required to evaluate the overall mass balance of the experiment as well as the validity of CFT in describing oocyst transport (Tufenkji and Elimelech, 2005a; 2005b).

By modifying the composition of the background electrolyte solution, the size and nature of the granular media or other experimental conditions, researchers have examined various aspects of *Cryptosporidium* filtration behaviour using laboratory-scale packed column experiments. In general, the results of oocyst transport experiments demonstrate an increase in oocyst removal with increasing solution ionic strength or decreasing solution pH (Hsu et al., 2001; Tufenkji et al., 2004; Abudalo et al., 2005; Tufenkji and Elimelech, 2005b). As discussed above in *Section 2.2*, the zeta potential of *C. parvum* oocysts becomes less negative with increasing solution ionic strength. Similarly, the zeta potentials of *C. parvum* oocysts become less negative with decreasing solution pH, suggesting that to some degree, the oocysts behave as model colloids in response to changes in solution chemistry. This observed dependence of oocyst attachment with changes in solution salt concentration or pH confirms that physicochemical filtration plays an important role in oocyst removal in saturated porous media (Tufenkji et al., 2004).

Most of the studies mentioned above have been carried out using columns packed with model porous media (e.g., glass beads or pure quartz sand). To better understand the transport and removal of *C. parvum* oocysts in natural subsurface environments or engineered water treatment processes, laboratory experiments have also been conducted using natural aquifer materials (Brush et al., 1999; Harter et al., 2000; Bradford and Bettahar, 2005; Hijnen et al., 2005) or using biofilm- and NOM-coated granular media (Dai and Hozalski, 2002, 2003). The effect of NOM and biofilm coating on the removal of *Cryptosporidium* has been studied in columns packed with glass beads (Dai and Hozalski, 2002). Interestingly, these researchers found that the oocyst removal efficiency decreased from ~ 51% for the clean packed bed to ~ 23% in the biofilm-coated bed and to

a greater extent (~ 14% removal efficiency) in the presence of NOM (Dai and Hozalski, 2002). For an experiment where both biofilm-coated grains and NOM were considered, these researchers found that the degree of oocyst removal was similar to that for the experiment with NOM alone (~ 15%) (Dai and Hozalski, 2002). Clearly, these findings have important implications for water treatment facilities treating source waters containing NOM and/or using biological filters as both of these factors can reduce *C. parvum* removal efficiency.

In an effort to improve water treatment practitioners' ability to control process performance and protect drinking water quality, there is a need to identify reliable surrogate particles for viable *C. parvum* oocysts. Different types of particles have been proposed as potential surrogates (e.g., formalin-inactivated oocysts, heat-inactivated oocysts and latex microspheres) (Swertfeger et al., 1999; Edzwald et al., 2000; Emelko, 2003; Amburgey et al., 2005). However, few studies have actually compared the filtration behaviour of such surrogates with that of viable oocysts (Dai and Hozalski, 2003). The use of polystyrene latex microspheres as surrogate particles for *C. parvum* has been evaluated by comparing the removal efficiency of the microspheres with that of viable oocysts (Dai and Hozalski, 2003). Column transport experiments conducted in the presence of NOM or a commonly used coagulant in water treatment (alum) showed that the microspheres were generally conservative surrogates for the viable oocysts. Additional studies are required however to evaluate the reliability of these surrogate particles over a broader range of process operating conditions.

Experiments conducted with different-sized sands suggest that *C. parvum* oocysts may be subject to velocity enhancement (Brush et al., 1999; Harter et al., 2000). These types of experiments have also been used to show that physical straining of oocysts in pore throats that are too small to allow passage is an important mechanism of *C. parvum* removal in granular porous media (Tufenkji et al., 2004; Bradford and Bettahar, 2005; Hijnen et al., 2005). The role of straining in water treatment is discussed in detail in *Section 7*. Although several researchers have investigated the influence of physical heterogeneity of the porous matrix on *Cryptosporidium* filtration, few efforts have been directed at improving our understanding of how geochemical heterogeneity affects oocyst removal (Abudalo et al., 2005; Hijnen et al., 2005). Abudalo et al studied the removal of

C. parvum oocysts in columns of quartz sand coated with different amounts of a ferric oxyhydroxide (Abudalo et al., 2005). At the solution conditions of these experiments, the ferric oxyhydroxide coating is expected to provide *favourable* sites for oocyst deposition (i.e., the negatively charged oocysts will preferentially deposit onto the positively charged oxyhydroxide coatings). In effect, these researchers showed that the degree of oocyst attachment increases as the fraction of ferric oxyhydroxide coated sand in the packed column increased. However, their results obtained with *C. parvum* oocysts were not in very good agreement with theoretical expectations based on a patch-wise model of geochemical heterogeneity which predicts that the attachment efficiency, α, is equal to the fraction of ferric oxyhydroxide coated sand (Elimelech et al., 2000). One explanation for this unexpected behaviour is the possibility of oocyst retention in a shallow secondary energy minimum of the DLVO interaction energy profile (Abudalo et al., 2005). Because of its relatively large size, *C. parvum* should experience a fairly deep attractive energy well upon approach to a collector grain surface. Others have alluded to the importance of the secondary energy minimum in the retention of *C. parvum* oocysts (Tufenkji and Elimelech, 2005b) or bacterial cells (Redman et al., 2004). As discussed in Section 3 above, when oocysts are located close to sand surfaces, tethering can occur between oocyst surface macromolecules and the quartz surface. Further well-controlled laboratory experiments are needed to fully elucidate the influence of these alternative mechanisms on *Cryptosporidium*-sand interactions, in particular since these explanations do not seem to be supported by direct measurements using AFM.

5. Studies of *Cryptosporidium* Filtration at the Field or Plant Scale

Much of the early research effort at the plant scale was devoted to studies of the relative removal efficiencies of different treatment stages (Nieminski and Ongerth, 1995; Edzwald and Kelley, 1998), especially filtration, since this was the basis of the regulatory approach. Although the data collected was useful in an operational sense, it was hampered by inaccuracies of the available analytical methods, and little progress could be made to probe the mechanisms by which oocysts interacted with any of the chemical additives or surfaces present in the systems studied.

Despite recent advances in assaying technologies, it is still impossible to conduct a mass balance for oocysts in a field study. For this reason it is difficult to be certain of the fate of these protozoa in water treatment plants. It may be that as generally thought, the oocysts are concentrated by attachment to flocs and subsequently removed from the raw water before being transferred to the backwash liquor from the filters but that the detection limits in this complex liquor preclude confirmation. Alternatively, the stresses involved in attachment and detachment may render the cysts inactive and therefore not detectable. Or it may be that they remain on the sand filter, protected from the various backwashing regimes, slowly building up until a steady state is reached, at which stage there will be breakthrough into the product water almost regardless of the quality of the raw water. In this scenario, outbreaks will be the result of saturation of or competition for the adsorption sites on the filter media and would be considered eventually inevitable for every system.

The analytical limitations of current assay methods have also given rise to many studies using surrogate particles to determine removal efficiencies in pilot plant and plant trials. Materials used have ranged from purely synthetic colloids such as polystyrene beads (Baeza and Ducoste, 2004; Emelko and Huck, 2004), to biocolloids such as algae (Akiba et al., 2002), spores (Rice et al., 1996) and inactivated oocysts (Nieminski and Ongerth, 1995). Whilst the results have in most cases shown some correlation in terms of % removal for different treatment stages, the differences in surface properties between oocysts and surrogates were significant and little could be learnt about the mechanisms involved in the real systems under the range of conditions experienced in practice.

Until we have a better understanding of the forces of interaction between oocysts and flocs and filter media, it will not be possible to distinguish between the scenarios above and the fate of these pathogens and the factors which determine their outbreaks will remain a mystery. This has been the catalyst for our review of the information gleaned by the use of colloid and interfacial science techniques at a nano and macro scale.

6. Comparison of *Cryptosporidium* Behavior with Other Microorganisms of Environmental Interest

Water supplies contaminated with *Cryptosporidium* often also test positive for other pathogenic protozoa such as *Giardia* and Microsporidia or infectious bacteria (e.g., *Escherichia coli* and *Clostridium perfringens*) (Teunis et al., 1997; Payment et al., 2000). It is therefore of interest to understand and compare the filtration behaviour of such organisms to that of *C. parvum* oocysts. The transport behaviour of several bacterial species of environmental relevance has been examined using different laboratory techniques (e.g., packed columns or flow-cells of various configurations) (Bos et al., 1999; Harvey and Harms, 2001; Foppen and Schijven, 2006). However, it is difficult to draw general conclusions from these studies as they are often conducted using different culturing and handling techniques, solution chemistries and/or collector surfaces. Furthermore, few researchers have evaluated the transport and removal of bacterial cells in conjunction with *Cryptosporidium* or other protozoa (Hijnen et al., 2005). Such studies are particularly valuable as they allow for direct comparison of different pathogens and thus an improved understanding of the potential for water contamination.

Hijnen et al conducted column experiments using natural soil and water samples where they compared the filtration of *C. parvum* oocysts with that of *Giardia intestinalis* cysts, *Clostridium perfringens* spores, *Escherichia coli* cells and F-specific RNA bacteriophages (Hijnen et al., 2005). The degree of microbe removal was quite high (> 2 log for most organisms) in two different soils – a fine sandy soil and a gravel soil. However, the sequence of the microbe removal rate for the various organisms differed in the two soils suggesting that the ratio of particle to grain size was not the decisive factor of microbe removal. In the gravel soil, the order of microbe removal agreed with the size sequence of the microorganisms. A considerably different sequence of microbe removal was observed in the sandy soil revealing the influence of microbe and soil surface properties in the overall microbe transport behaviour (Hijnen et al., 2005).

In another study, the filtration behavior of *C. parvum* oocysts and *Giardia lamblia* cysts was compared using columns packed with glass or polystyrene beads (Hsu et al., 2001). *Cryptosporidium* oocysts consistently exhibited a greater degree of removal than *Giardia* cysts in both types of granular media over a broad range of ionic strength and pH conditions. Comparison of these results with those observed in similar experiments conducted with cells of a freshwater algae reveals that the sequence of microbe removal

in the glass bead system is *C. parvum* oocyst > algal cell > *Giardia* cyst (Huang et al., 1998). In contrast, the sequence of particle size is *Giardia* cyst > *C. parvum* oocyst ≈ algal cell. The findings of this research demonstrate the importance of evaluating the filtration of *C. parvum* oocysts and other microorganisms in parallel to better predict their transport potential in natural and engineered systems.

C. parvum oocysts are not unique as biocolloids possessing surface macromolecules that strongly influence their interaction with surfaces (Bremmell et al., in press). AFM force measurements have shown that steric interactions play an important role in the behaviour of many microorganisms. For example, a range of systems containing bacteria such as *Escherichia coli* (Ong et al., 1999; Velegol and Logan, 2002; Burks et al., 2003), *Shewanella oneidensis* (Lower et al., 2001), *Shewanella putrefaciens* (Gaboriaud et al., 2005), *Candida parapsilosis* (Emerson and Camesano, 2004) and *Pseudomonas aeruginosa* (Emerson and Camesano, 2004) strains have their behaviour with opposing surfaces governed at least in part by steric interaction. Some of the current authors have also found that the interaction of *Giardia lamblia* cysts with silica is dominated by the steric force originating from surface macromolecules (Ruohola et al., in preparation). These findings highlight the importance of evaluating well-known and emerging waterborne pathogens on a case-by-case basis when considering their removal potential in aqueous granular systems. Future research should be directed at characterizing the surface properties of microbial pathogens and parasites and attempting to relate this information to the key mechanisms governing microbe-surface interactions in different natural and engineered environments.

7. Importance of Physical Straining in *Cryptosporidium* Filtration

Physicochemical attachment has traditionally been regarded as the governing mechanism of colloid removal from the pore fluid in granular filtration. As described previously in *Section 4*, classic colloid filtration theory (CFT) considers the removal of colloids and biocolloids to be controlled mainly by the combined influence of electrical double-layer interactions and van der Waals forces. However, recent experimental studies conducted with model uniform colloids (Bradford et al., 2002; Bradford et al., 2004) and *C. parvum* oocysts (Tufenkji et al., 2004; Bradford and Bettahar, 2005; Hijnen

et al., 2005) reveal that a physical straining mechanism could play an important role in *Cryptosporidium* transport in granular porous media. Straining is the trapping of particles in pore throats that are too small to allow passage of particles (McDowell-Boyer et al., 1986), and is generally neglected in attempts to model or quantify (bio)colloid transport (e.g., straining is not considered in CFT).

Bradford and co-workers have been leading recent research efforts aimed at improving our understanding of physical straining in saturated porous media (Bradford et al., 2002; Bradford et al., 2003; Bradford and Bettahar, 2005; Bradford et al., 2005). Laboratory-scale experiments were conducted to evaluate the transport and deposition of model latex colloids in columns packed with different sands covering a wide range of grain sizes (Bradford et al., 2002). These researchers showed that the degree of colloid filtration (removal) increased with increasing colloid size or decreasing median sediment grain size. They also demonstrated how the spatial distribution of retained colloids in the packed column is not adequately described by a simple conceptual model of colloid transport which includes only colloid attachment and detachment (Bradford et al., 2003). Rather, the incorporation of a depth-dependent straining mechanism into their numerical model provides much better agreement with observed experimental results.

The role of straining in the transport of *C. parvum* oocysts has been examined more recently using the approach previously developed to evaluate straining of model colloids (Bradford and Bettahar, 2005). Both the fluid-phase oocyst concentrations at the column outlet and the spatial distribution of retained oocysts at the end of each packed column experiment were measured. Similar to the results obtained with the latex colloids, a decrease in the median grain diameter yielded a greater degree of oocyst removal. However, in contrast to those experiments conducted with latex microspheres, the *C. parvum* effluent concentration curves exhibited significantly delayed breakthrough with decreasing grain size (Bradford and Bettahar, 2005). Although straining played an important role in the filtration behaviour of *C. parvum*, the observed late breakthrough could not be attributed to this physical removal mechanism. In effect, the *C. parvum* data was best modelled by considering the contributions of physicochemical filtration (attachment), detachment and straining (Bradford and Bettahar, 2005). Based on mass balance considerations, these researchers also showed that a large percentage of oocysts

were retained by physical straining (from 68% for 710μm diameter sand, to 87% for 150 μm diameter sand).

Several researchers have considered straining of colloids in granular porous media as a purely geometric problem (McDowell-Boyer et al., 1986). Based on considerations of system geometry, it has been suggested that straining should not be important when the ratio of particle diameter to the median grain diameter (d_p/d_c) is greater than 0.154 for a single particle (or 0.082 for up to four particles) (Herzig et al., 1970). Similarly, Sakthivadivel proposed a limiting ratio of 0.05 for predicting straining of particles in pore throats (Sakthivadivel, 1966, 1969). Such geometric models used for predicting straining potential consider a porous medium consisting of uniform spherical grains (or collectors). In reality, however, sediment (sand) grains found in water treatment facilities or natural subsurface environments can be highly irregular in shape and may exhibit a broad distribution in grain diameter.

Comparison of *C. parvum* removal in columns packed with pure quartz sand versus glass beads of similar size has shown that the irregularity of sand grain shape contributes significantly to the straining potential of the porous medium (Tufenkji et al., 2004). Column transport experiments conducted using *C. parvum* oocysts suspended in KCl solutions of different ionic strength (1 to 10 mM) further demonstrated that physicochemical filtration contributes to oocyst removal in granular porous media (Tufenkji et al., 2004). In this study, researchers also presented experiments using model latex microspheres to illustrate the influence of grain shape in physical straining. The effluent breakthrough curves for 4.1 μm latex microspheres transported through columns packed with pure quartz sand or glass beads are shown in Figure 9a and 9b, respectively. In these experiments, the latex colloids were suspended in deionized water to minimize the degree of physicochemical filtration and the granular media were selected to be comparable in size. The significant differences observed in the degree of particle removal — nearly 40% in the quartz sand but close to nil in the glass beads — exemplifies the importance of considering grain shape in predictions of colloid straining potential (Tufenkji et al., 2004). Other findings (Hijnen et al., 2005) further suggest that simple extrapolation of grain size and microbe size to evaluate the potential for physical straining is most often inappropriate. Such considerations are particularly relevant in

attempts to predict oocyst removal capacity during riverbank filtration or *Cryptosporidium* transport in groundwater where sediment grains can be highly irregular and angular in shape (Tufenkji et al., 2002; Tufenkji et al., 2004).

[FIGURE 9]

8. Concluding Remarks

The research conducted thus far has demonstrated that techniques based on the principles of colloid and surface chemistry can provide new information about what is happening to oocysts in the filtration stage of a water treatment plant. The necessity of allowing for the presence of steric effects in any mechanistic consideration of the operating forces of interaction is now obvious from a number of studies and the need to incorporate steric interactions into any model of the sand-biocolloid system is quite clear.

What remains to be done is to investigate the impact of other factors (chemical and mechanical), to include other components of the system which may be present such as coagulants and flocculants and to examine the influence of shear during both filtration and backwashing. Beyond this the work must be extended to other microorganisms of interest in water clarification which may have different surface properties and which may interact with the filter media quite differently.

Ultimately the water industry needs to know what conditions are needed to maximise the attachment of these contaminants to the filter media during clarification and what conditions are required to detach the same organisms during backwashing. Regulatory pressure will dictate that the ultimate fate of these contaminants is also known. It seems likely that this information will be derived from a combination of studies which probe what is happening at both the nano- and macro- scales. We should be encouraged by the fact that our understanding of the important factors that govern the behaviour of *C. parvum* oocysts in water treatment processes is evolving very rapidly.

Acknowledgements

NT acknowledges the support of the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canada Research Chairs (CRC) Program and the Center for Host-Parasite Interactions at McGill University. DRD, RFC and CJD acknowledge the support of the Cooperative Research Centre for Water Quality and Treatment. CJD acknowledges the support of an Australian Research Council Federation Fellowship.

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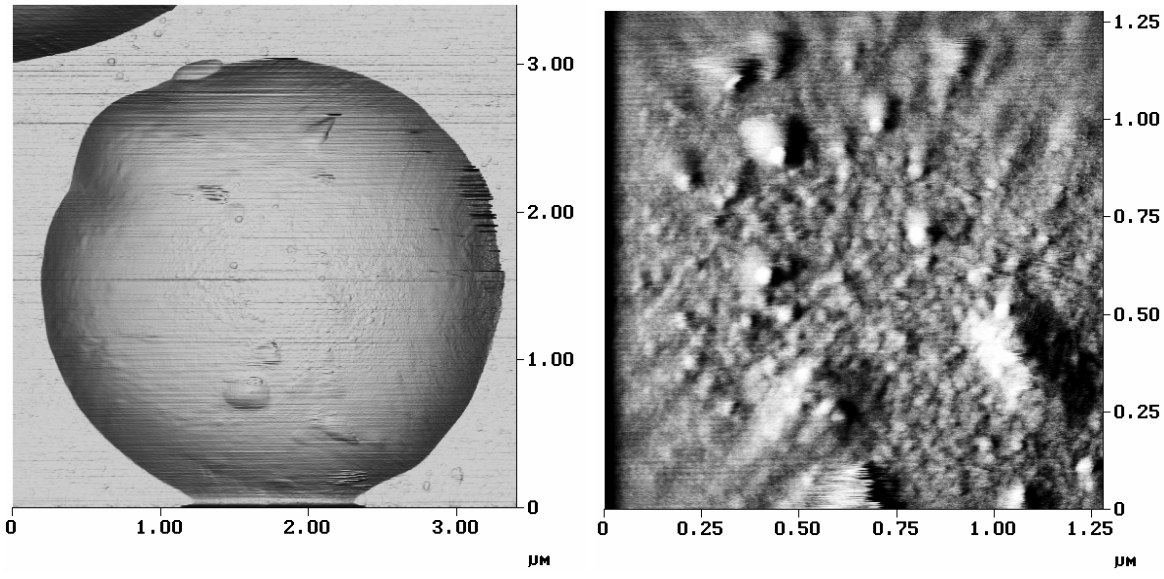
FIGURE 1. AFM image of a typical *Cryptosporidium parvum* oocyst obtained in soft contact (electrical double layer and steric force) mode. The image was obtained with a sharpened silicon nitride cantilever at pH 8.5 and at 1 mM KNO_3. (a) 3.4 μm scan, the total z-range of the figure is 1000 nm and the edge of a second oocyst can be observed in the top left corner of the image. (b) 1.25 μm scan showing the roughness of the oocyst surface.



(a) **(b)**

FIGURE 2. Electrophoretic mobility of viable *Cryptosporidium parvum* oocysts suspended in (△) 0.915, (□), 9.15, and (○) 91.5 mM phosphate-buffered water as a function of pH. Measurements were made at 25 ± 2 °C. Reprinted from (Lytle, et al., 2002), Copyright (2002), with permission from Elsevier.

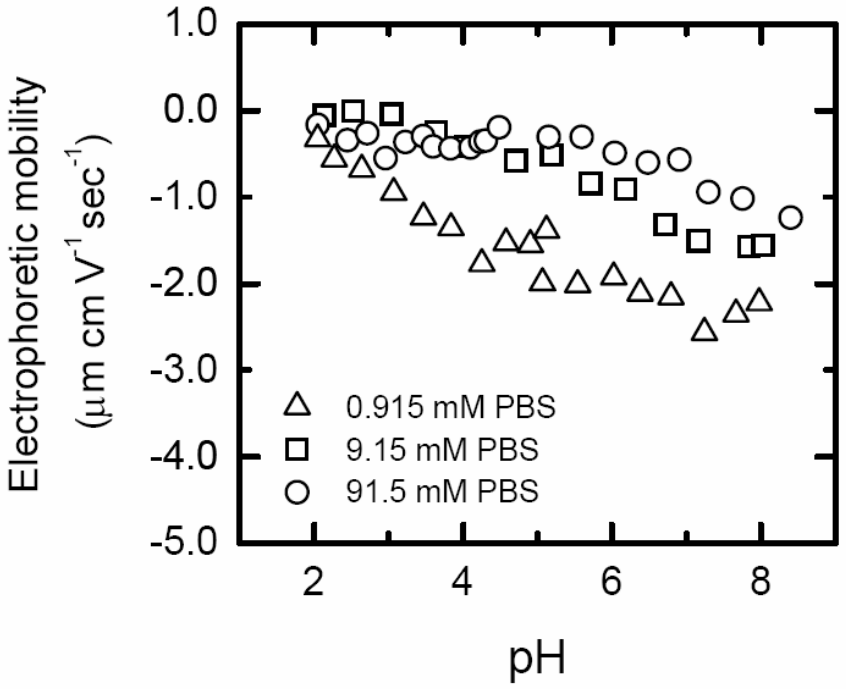


FIGURE 3. Comparison of the zeta potential of *Cryptosporidium parvum* oocysts in the (●) presence and (△) absence of NOM as a function of calcium concentration at pH 6.7. Reprinted from (Dai and Hozalski, 2002), Copyright (2002), with permission from Elsevier.

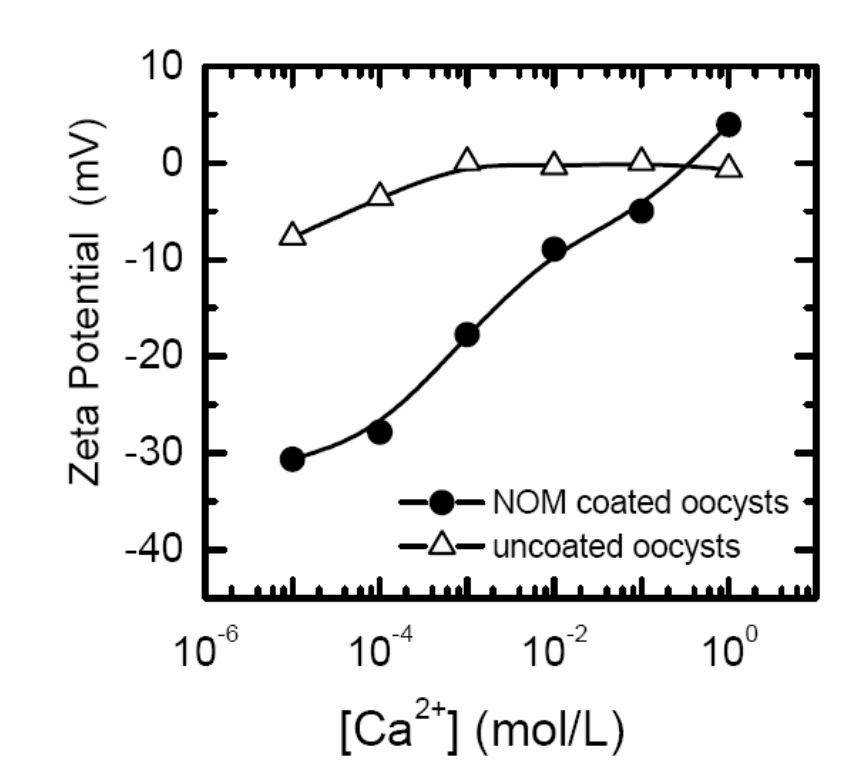


FIGURE 4. Electrophoretic mobility of *Cryptosporidium parvum* oocysts suspended in deionized water with added antibiotics. Oocysts were purified from calf feces using two different treatment approaches: (a) a deionized water-sucrose (DIS) method, and (b) an EAPS (ethyl acetate-Percoll©-sucrose) method. Reprinted with permission from (Brush, et al., 1998). Copyright (1998) American Society for Microbiology.

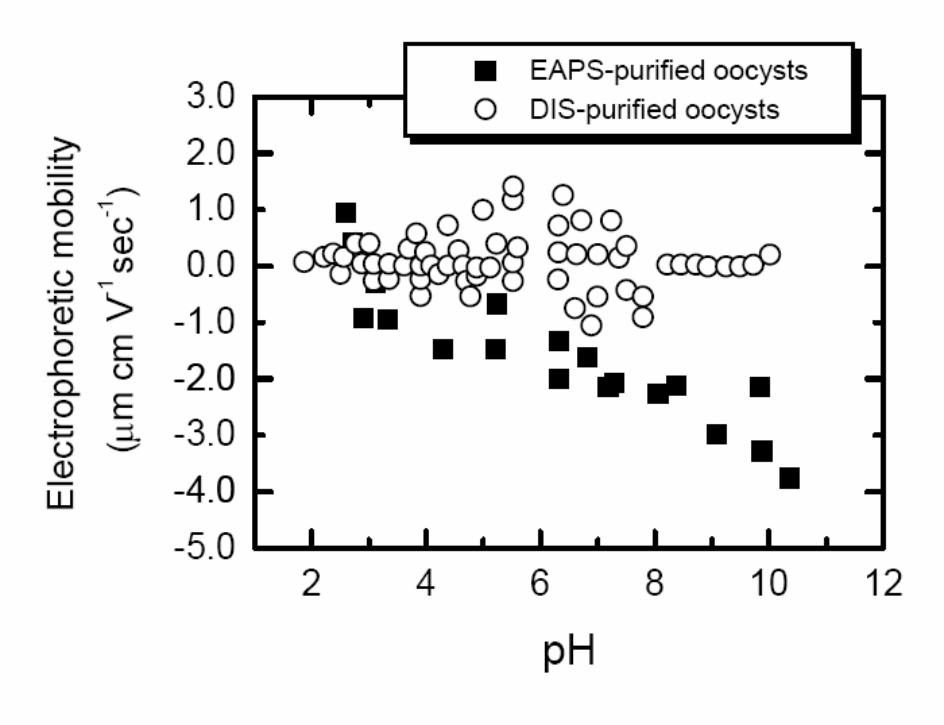


FIGURE 5. Schematic representation, constructed from AFM soft contact mode images, of a model sand (silica) particle (top image) interacting with a *Cryptosporidium parvum* oocyst (bottom image)**.**

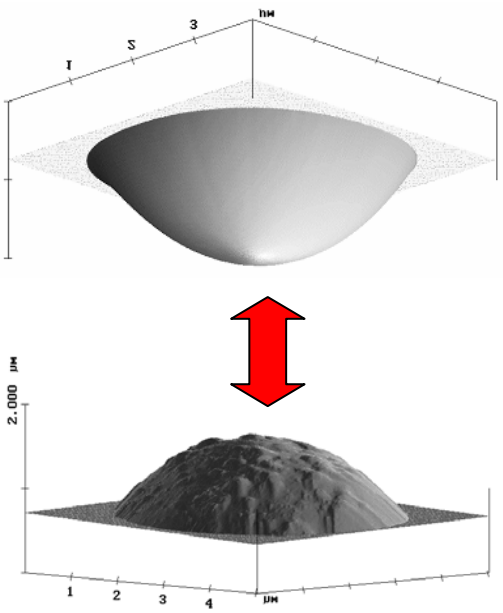


FIGURE 6. Typical force-separation data for the approach of an oocyst and a silica particle in 1 mM KNO_3 at pH = 8.9. Reprinted with permission from (Considine, et al., 2001). Copyright (2001) American Chemical Society.

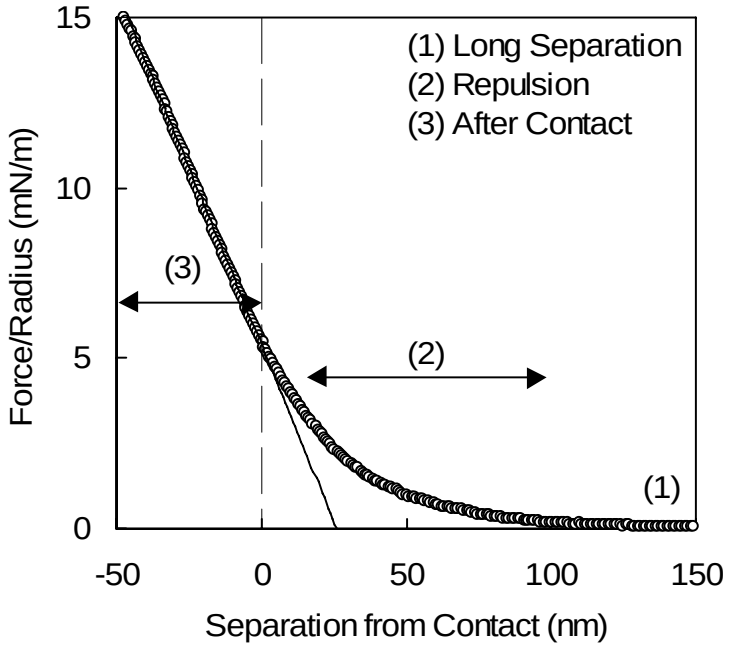


FIGURE 7. Normalised force of interaction between an oocyst and a silica sphere (open symbols) in 1 mM KNO_3 at pH 8.9. The thin solid lines correspond to the result of the DLVO calculation based on the corresponding ζ-potentials and the bulk electrolyte concentration, with the DLVO plane of charge origin being shifted around 35 nm to obtain an order of magnitude fit. The solid line corresponds to the result of the force predicted from a Pincus model of a polyelectrolyte brush of width 50 nm. Reprinted with permission from (Considine, et al., 2001). Copyright (2001) American Chemical Society.

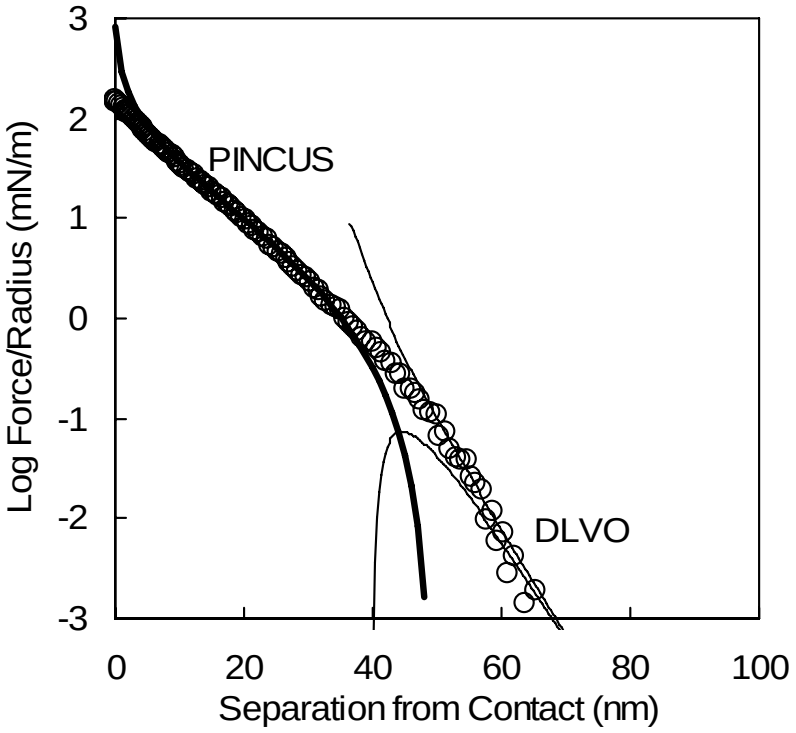


FIGURE 8. Interaction between an AFM silica tip and a *Cryptosporidium parvum* oocyst. Normalized force-separation curves are shown on surface approach (smooth curves showing an increase in repulsion as surface separation decreases close to contact) and on surface retraction (curves showing jump-outs) measured at pH 6.09 and 1mM KNO_3. The labels A4, D4, E2 and H7 correspond to different locations on the oocyst surface. For each of the four pairs of force curves, the retraction curves have been displaced one division on the force axis for clarity. Reprinted with permission from (Considine, et al., 2000). Copyright (2000) American Chemical Society.

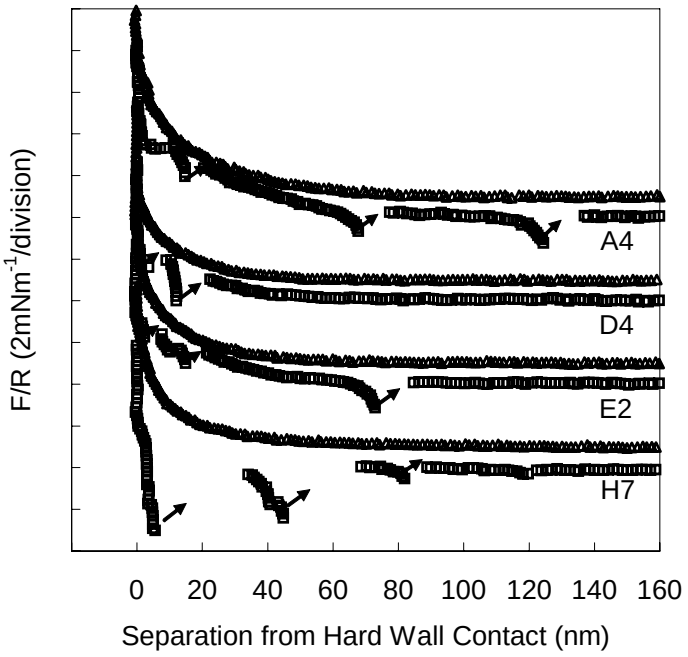


FIGURE 9. Comparison of microsphere removal in column packed with (a) high purity quartz sand and (b) soda-lime glass beads of similar diameter. Latex microspheres (4.1 μm diameter) suspended in deionized water were used as surrogate particles for *Cryptosporidium parvum* oocysts. Other experimental conditions were: approach velocity = 0.042 cm/s, mean glass bead diameter = 0.23 mm, mean quartz grain diameter = 0.21 mm, unadjusted pH (varied between 5.6 and 7.1), and temperature = 22-23 °C. Reprinted with permission from (Tufenkji, et al., 2004). Copyright (2004) American Chemical Society.