Direct filtration of *Cryptosporidium* surrogates in drinking water treatment – a multiscale approach

by

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Abstract

Cryptosporidium, a waterborne protozoan pathogen that can cause gastrointestinal illness, is often found in surface waters that are used to supply drinking water. Filtration is a major barrier against *Cryptosporidium* in drinking water treatment processes. However, interactions between oocysts and filter media are still unclear and no satisfactory surrogates have been identified for quantifying their filtration removal in porous media. In the phase I of this study, polystyrene microsphere with a size, density, and shape similar to *Cryptosporidium* was modified with glycoprotein or synthesized biomolecules to mimic the surface properties of live *Cryptosporidium* oocyst. Interaction kinetics between live *Cryptosporidium*/modified microspheres and filter media were studied at the molecular-scale using a quartz crystal microbalance with dissipation monitoring (QCM-D) and at the laboratory-scale using sand-packed columns. Both QCM-D and column experiments underlined the importance of *Cryptosporidium* surface charge and hydrophobicity on their fate and transport in porous media. As compared to live *Cryptosporidium*, glycopolymer and zwitterionic polymer co-modified polystyrene microspheres (later called copolymers-modified microspheres) represent comparable surface properties, adsorption kinetics on filter surfaces, and transport and deposition behaviors in filter columns; hence were selected as appropriate *Cryptosporidium* surrogates for the lab-scale and pilot-scale filtration investigation.

Factors contributing to the solution chemistry were investigated using *Cryptosporidium* surrogates (copolymers-modified microspheres) established previously in the laboratory-scale filtration columns; they are ionic strength, pH, and DOC (dissolved organic matter)

of aqueous solution. Single-collector contact efficiency (η_0) and DLVO (Derjaguin-Landau-Verwey-Overbeek) interaction energy were calculated to facilitate the explanation of transport and retention of *Cryptosporidium* oocysts surrogates in the porous media. The value of single-collector contact efficiency (η_0) was determined using Tufenkji and Elimelech model (T-E model). In general, the results of surrogate transport experiments demonstrate an increase in surrogate removal with increasing solution ionic strength or decreasing solution pH. This observed dependence of surrogate attachment with changes in solution salt concentration and pH confirms that the significant role of physicochemical filtration in surrogate removal in the filtration columns when porous media are saturated with water. On the other hand, the natural organic matter (NOM) had negative effect on the retention of surrogates in the packed-bed column, which emphasizes the importance of optimal coagulation to remove NOM for the better filtration performance. This phase II study also validates the using of copolymers-modified microspheres as representative surrogates for *Cryptosporidium* oocysts in the condition that relevant to drinking water.

In the phase III pilot-scale study, the removal of *Cryptosporidium* oocyst surrogates was determined in a pilot-scale granular media filtration system operated in direct filtration mode at cold water temperature condition. The surface characteristics of these modified microspheres, including surface charge and hydrophobicity, resembled those of viable oocysts in drinking water conditions. Pilot-scale direct filtration challenge experiments were conducted to determine the impact of chemical pretreatments and filter design on the removal of *Cryptosporidium* surrogates dosed into the influent water. The operational parameters examined in the direct filtration mode included coagulant type (alum versus

PACl), filter aid polymer type (polyamine Magnafloc® LT-7981 versus polyDADMAC Magnafloc® LT-7995) and dose (0.5 versus and 2.0 mg/L), and filter configuration (i.e. regular versus and deep bed filters). The results indicated a higher *Cryptosporidium* surrogate removal was associated with higher polymer dose (2 mg/L), polyDADMAC polymer and the deep bed filter configuration. The chemical pretreatment conditions played important roles in the transport and removal of *Cryptosporidium* surrogates in the filter bed. Specifically, optimized doses of coagulants alum (0.454 mg/L as Al) determined from previous full-scale and pilot-scale investigation and filter aid polymer polyDADMAC (Magnafloc® LT-7995) together with a deep bed configuration achieved the highest log removal of *Cryptosporidium* surrogates.

This study improves our understanding on how surface characteristics impact *Cryptosporidium* transport behaviors in porous media and contributes to our capacity to evaluate the fate of *Cryptosporidium* in natural water purification systems and engineered aquatic environments. It demonstrates the effect of solution chemistry on the transport and deposition of surrogates. It also emphasizes the importance of optimizing chemical pretreatment and filter configuration for removing oocysts in cold-water conditions in granular media direct filtration.

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Chapter 1. Introduction

1.1 Background and motivation

The occurrence and removal of *Cryptosporidium* represents a topic of increasing concern among public health professionals, utility companies, and consumers of municipal drinking water. The emergence of this parasitic protozoa as an etiological agent of waterborne disease has prompted the promulgation of the *Surface Water Treatment Rule* (SWTR) in 1989 and the *Long Term* 2 *Enhanced Surface Water Treatment Rule* (LT2ESWTR) in 2006. Increasingly stringent regulations for drinking water quality require new evaluations that test the efficiency of water treatment processes. Although disinfection plays a key role in inactivating microbial contaminants from drinking water to protect public health, the extremely thick cell wall of *Cryptosporidium* oocysts renders conventional chlorine-based disinfection ineffective. Therefore, the creation of physicochemical barriers through coagulation and filtration, considered as the most effective way to remove pathogens before disinfection, will require optimization to maximize the removal of pathogens, especially *Cryptosporidium*.

The evaluation of the efficiency of water treatment processes, especially coagulation and filtration, inevitably involve spiking much higher concentrations (10^2-10^6 higher) of *Cryptosporidium* in the water matrix than those present in the natural environment. Since the advent of health concerns associated with the use of viable *Cryptosporidium* oocysts in pilot- and full-scale studies, many research groups and facilities have begun to use polystyrene microspheres as oocyst surrogates; such microspheres represent the size of oocysts in process optimization studies (Brush et al., 1998; Huck, 2001; Emelko and Huck, 2004). However, these investigations demonstrated different removal rates for *Cryptosporidium* oocysts and polystyrene microspheres, suggesting that polystyrene microspheres serve as a poor surrogate for *Cryptosporidium* oocysts and thus studies have failed to identify reliable surrogates for *Cryptosporidium*. These differences largely resulted from variations in filtration systems, different chemical solution conditions, and poorly characterized oocysts in the studies. The different surface properties, such as surface charge and hydrophobicity, which manifested between viable oocysts and microspheres,

may cause inaccurate predictions relating to the attenuation and transport of oocysts in porous media (Pang et al., 2012). These potential discrepancies limit the usage of unmodified microspheres for studying filtration characteristics. Therefore, a reliable, safe and non-expensive surrogate of *Cryptosporidium* is needed to quantify the filtration removal. Previous studies involved using various biological (Emelko, 2003; Emelko et al., 2005) and non-biological surrogates (Dai & Hozalski, 2003; Emelko & Huck, 2004; Pang et al., 2012; Stevenson et al., 2015) for *Cryptosporidium*. Biological surrogates such as yeast (*Saccharomyces Cerevusuae* Type II) may be considered as the surrogates for oocysts due to their low cost, surface charge and well aligned size range as compared to naturally occurring oocysts. However, the inherent variation of surface properties exists in yeast culture due to the variation in their growth phases, which limits its application as surrogates for *Cryptosporidium*. Studies of oocysts inactivated by heat or formalin have shown that the *Cryptosporidium* inactivation process can destroy the oocysts' surface macromolecules, reducing the deposition rate in a manner that does not occur with their live counterparts (Kuznar & Elimelech, 2006). Another limitation of using inactivated oocysts is the challenges post by their enumeration techniques, which involves complex purification, labelling and counting processes, making it time-consuming, labor-intensive and expensive.

Polystyrene microspheres that are similar to *Cryptosporidium* in size, aspect ratio, and buoyant density have been used as safe surrogates in a variety of bench-scale, pilot-scale, and groundwater field experiments (Amburgey et al., 2005; Emelko & Huck, 2004; Lu et al., 2017). These microspheres are chemically inert, negatively charged, and easy to detect (Behrens et al., 2001). However, previous studies have shown that these polystyrene microspheres exhibit significantly different deposition behaviors from live *Cryptosporidium* in porous media, which may be attributed to the different surface properties, such as surface charge and hydrophobicity, between viable oocysts and microspheres (Pang et al., 2012). A study performed by Pang et al. (2012) evaluated biotin- and glycoprotein-coated microspheres, and demonstrated that as compared to the unmodified microspheres, glycoprotein-coated microspheres better mimicked the macromolecular structure of *Cryptosporidium* oocysts. More recently, Stevenson et al.

(2015) and Zhang et al. (2017a) demonstrated that glycoprotein-coated microspheres share a good surface resemblance with viable *Cryptosporidium* oocysts. However, significant costs associated with glycoproteins hindered their application at the field scale. More importantly, the hydrophobicity of oocysts, which is known to impact the oocysts' deposition in porous media, cannot be represented by simply using glycoprotein modification of microspheres, which was not considered in these studies. Biomolecule-modified microspheres would potentially represent better surface resemblance to viable oocysts. Therefore, they would be used as representative surrogates for *Cryptosporidium* oocysts in treatment process study.

Direct filtration is often considered in drinking water treatment during conditions when source water turbidity and colour readings are low, i.e. Turbidity < 5 Nephelometric Turbidity Units (NTU) and color < 6 Total Colour Units (TCU). Direct filtration involves coagulation and flocculation directly followed by filtration, without a settling process, unlike conventional treatment where settling occurs before filtration. Direct filtration allows reduced chemical inputs, and residual solids production, and thus reduced operational costs (James et al., 2011). Although full-scale and pilot-scale studies on *Cryptosporidium* oocyst removal from water by conventional filtration systems have been evaluated previously, operational factors governing the removal of *Cryptosporidium* via direct filtration are not well studied. Previous conventional filtration studies at bench-, pilot- and full-scales showed that operational factors, such as influent water quality, coagulant condition, filter aid polymer type and dose, hydraulic loading rate, filter configuration, and temperature play significant roles in controlling *Cryptosporidium* removal. Such information helps to inform the operational strategies required for direct filtration. In particular, because in temperate climates direct filtration is often implemented in winter when the supply water has low turbidity, it is critical to evaluate the *Cryptosporidium* removal under low temperature conditions. Previous studies showed that alum is generally less effective at low temperatures, which was an observation that has been attributed to lower density flocs and aggregate size (Hanson and Cleasby, 1990). In comparison, alternative aluminum-based coagulants, such as polyaluminium chloride (PACl), have been shown to be effective under both low and normal water temperatures

(Gebbie, 2001). However, the performance of alum and PACl on *Cryptosporidium* removal under low temperature conditions has not been fully investigated. Further, it is important to optimize filter aid polymer (FP) under low temperature conditions for maximizing *Cryptosporidium* removal. FP works together with filter grains to collect destabilized particles from the coagulation process by promoting attachment to filter grains (Hendricks, 2006) and helping to form larger and stronger polymer-particle flocs (Zhu et al., 1996; Zhu et al., 2016), thus playing an important role in improving filter effluent quality, and reducing ripening duration and early breakthrough (Zhu et al., 1996). The types and dosages of FP are crucial to optimize effective particle removal during filtration. Further, previous studies demonstrated that deep bed filtration was an effective and economical treatment for low turbidity water with fine or colloidal size particles that are less than 30 μm in diameter (Swertfeger et al., 1999; Tien and Payatakes, 1979). However, no studies have been reported on the parallel filter performance comparisons for different filter configurations (regular bed vs. deep bed) under low water temperature conditions.

1.2 Research objectives

The overall goal of this research was to develop and select suitable *Cryptosporidium* surrogates and to examine *Cryptosporidium* surrogate removal in direct filtration using a multi-scale approach under Edmonton cold-water condition, as shown in Figure 1-1.

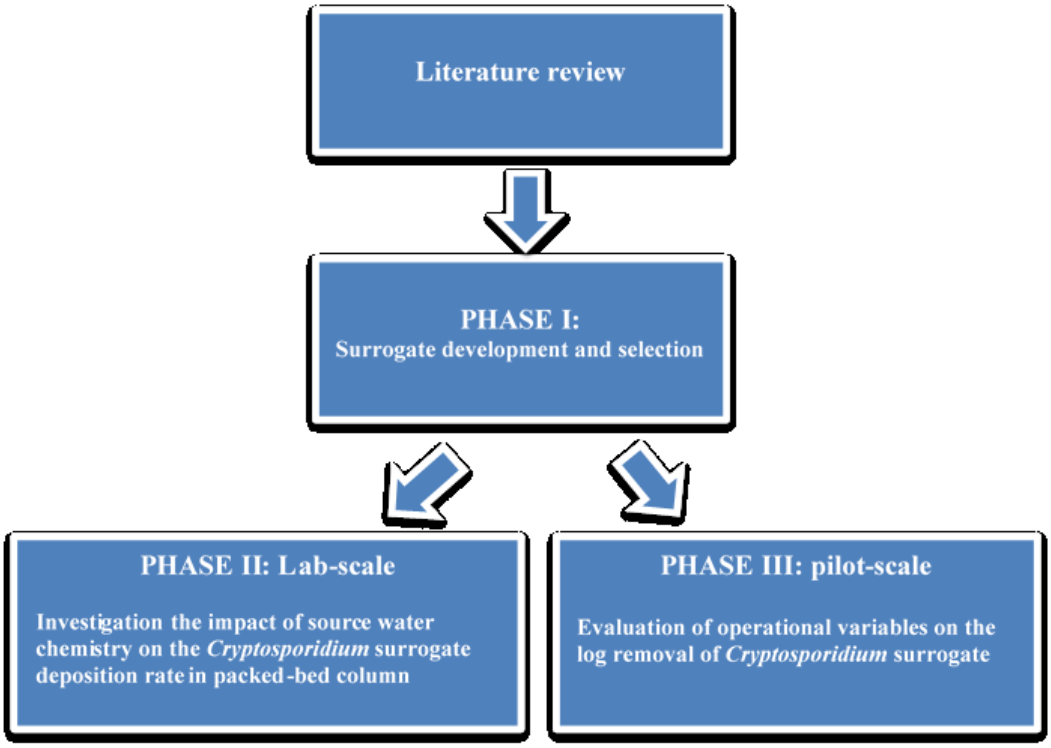

Figure 1-1. Research approach.

Specific objectives in pursuit of this goal were:

Phase I: Surrogates development and characterization

- Objective 1: Synthesis and development of representative *Cryptosporidium* surrogates
- Objective 2: The impact of surface charge and hydrophobicity on the deposition of *Cryptosporidium* oocysts with porous media
- Objective 3: Characterization of oocysts and their potential surrogates used in filtration experiment
- Objective 4: The comparison of the transport and deposition behaviors of oocysts and their potential surrogates

Phase II: Bench-scale experiments

- Objective 5: Factors controlling the transport and deposition of biomolecule modified microspheres as *Cryptosporidium* surrogates in filtration
- Objective 6: The impact of source water chemistry on the deposition kinetics of *Cryptosporidium* oocysts onto quartz surfaces

Phase III: Pilot-scale experiments

- Objective 7: The impact of operational variables on surrogate removal in pilot-scale direct filtration

To accomplish the objectives in pilot-scale study, the following were addressed:

- Characterize the surface properties of *Cryptosporidium* and potential surrogates
- Evaluate the used of modified fluorescence microspheres as surrogates for *Cryptosporidium* oocysts in laboratory packed-bed column
- Evaluate the effect of ionic strength, pH and dissolved organic carbon from the source water on the surrogate deposition rate and attachment efficiency
- Determine the impact of coagulant type variations on surrogate log removal
- Determine the impact of filter aid polymer type and dose on surrogate log removal
- Evaluate the influence of filter configuration (regular bed filters vs. deep bed filters) on the surrogate log removal
- Evaluate the correlation between on-line operation parameters (turbidity and particle counts) and surrogates log removal

1.3 Participants

This project was conducted jointly by the University of Alberta and the EPCOR Water Canada. The process development team (PDT) from EPCOR Water Canada was actively associated with the project planning, experiment design, and sample collection and analysis. The pilot experiments were conducted at E.L. Smith water treatment plant and the sample enumeration were done at the University of Alberta. The biomolecules that used to modify the surface of microspheres were prepared by Dr. Yinan Wang in the lab of Professor Ravin Narain in the Department of Chemical and Materials Engineering at the University of Alberta.

1.4 Research approach

This project accomplished into three phases, as shown in Figure 1-1. In phase I, the biomolecules representing the surface properties of oocysts were synthesized and conjugated onto surface of the traditionally used polystyrene microspheres. The surface charge and hydrophobicity of potential surrogates were compared with viable oocysts. Pack-bed column and QCM-D were used to verify the selection of surrogates for *Cryptosporidium*. In phase II, factors controlling the surrogates transport and deposition in granular bed media were investigated by laboratory-scale column study. During phase III in pilot-scale study, the performance of deep bed filters relative to regular bed filters during direct filtration were investigated to provide recommendations regarding potential conversion of some of the full-scale filtration filters and optimization of chemical pretreatment conditions in drinking water treatment plant.

1.5 Thesis organization

This thesis is divided into six chapters. **Chapter 1** consist of general induction and research motivation, research objective, participants, and research approach. **Chapter 2** includes a thorough review of the literature. **Chapter 3** describes the development of the potential *Cryptosporidium* surrogates and surface properties characterization between those proposed surrogates and viable oocysts. Molecular-scale QCM-D and laboratory-scale packed-bed column study were performed to compare the transport and deposition behavior of oocysts and their potential surrogates. **Chapter 4** presents the impact the source water chemistry on the deposition rate of surrogates by using laboratory-scale pack-bed columns. **Chapter 5** examines the impact of operational variables from chemical pretreatment processes and filter configuration on the removal of surrogates in pilot-scale filtration columns. The on-line real-time water quality parameters, such as turbidity and particle counts, to indicate the removal of surrogates, were also investigated in this chapter. The conclusion drawn from this research project and recommendations summarizes in **Chapter 6**.

References

Amburgey, J. E., Amirtharajah, A., York, M. T., Brouckaert, B. M., & et.al. (2005). Comparison of Conventional and Biological Filter Performance for Cryptosporidium and microsphere removal. *American Water Works Association. Journal; Denver*, *97*(12), 77–91,10.

Behrens, H., Beims, U., Dieter, H., Dietze, G., Eikmann, T., Grummt, T., … Tilkes, F. (2014). Toxicological and ecotoxicological assessment of water tracers. *Hydrogeology Journal*, *9*(3), 321–325. https://doi.org/10.1007/s100400100126

Brush, C. F., Walter, M. F., Anguish, L. J., & Ghiorse, W. C. (1998). Influence of Pretreatment and Experimental Conditions on Electrophoretic Mobility and Hydrophobicity of Cryptosporidium parvum Oocysts. *Applied and Environmental Microbiology*, *64*(11), 4439–4445.

Dai, X., & Hozalski, R. M. (2003). Evaluation of Microspheres as Surrogates for Cryptosporidium parvum Oocysts in Filtration Experiments. *Environmental Science & Technology*, *37*(5), 1037–1042. https://doi.org/10.1021/es025521w

Emelko, M. B. (2003). Removal of viable and inactivated Cryptosporidium by dual- and tri-media filtration. *Water Research*, *37*(12), 2998–3008.

Emelko, M. B., & Huck, P. M. (2004). Microspheres as Surrogates for "Cryptosporidium" Filtration. *Journal (American Water Works Association)*, *96*(3), 94–105.

Emelko, M. B., Huck, P. M., & Coffey, B. M. (2005). A review of Cryptosporidium removal by granular media filtration. *Journal (American Water Works Association)*, *97*(12), 101–115.

Gebbie, P. (2001). Using polyaluminium coagulants in water treatment. In *Proc. 64th AWIEO Conference*. Citeseer. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.517.5307&rep=rep1&type=pdf

Hanson, A. T., & Cleasby, J. L. (1990). The Effects of Temperature on Turbulent Flocculation: Fluid Dynamics and Chemistry. *Journal (American Water Works Association)*, *82*(11), 56–73. https://doi.org/10.2307/41293075

Hendricks, D. W. (2006). *Water Treatment Unit Processes: Physical and Chemical*. CRC Press.

Huck, P. M. (2001). Filter operation effects on pathogen passage / prepared by Peter M. Huck ... [et al.]; sponsored by Microbial/Disinfection By-Products Research Council. Denver, CO: AWWA Research Foundation and American Water Works Association, c2001.

Zhang, H., Seaman, J., Wang, Y., Zeng, H., Narain, R., Ulrich, A, & Liu., Y (2017a). Filtration of Glycoprotein-Modified Carboxylated Polystyrene Microspheres as Cryptosporidium Oocysts Surrogates: Effects of Flow Rate, Alum, and Humic Acid. Journal of Environmental Engineering, 143(8). https://doi.org/10.1061/(ASCE)EE.1943-7870.0001201

Kuznar, Z. A., & Elimelech, M. (2006). Cryptosporidium Oocyst Surface Macromolecules Significantly Hinder Oocyst Attachment. *Environmental Science & Technology*, *40*(6), 1837–1842. https://doi.org/10.1021/es051859p

Lu, P., Amburgey, J. E., Hill, V. R., Murphy, J. L., Schneeberger, C. L., Arrowood, M. J., & Yuan, T. (2017). Removals of cryptosporidium parvum oocysts and cryptosporidium-sized polystyrene microspheres from swimming pool water by

diatomaceous earth filtration and perlite-sand filtration. *Journal of Water and Health*, wh2017221. https://doi.org/10.2166/wh.2017.221

Pang, L., Nowostawska, U., Weaver, L., Hoffman, G., Karmacharya, A., Skinner, A., & Karki, N. (2012). Biotin- and Glycoprotein-Coated Microspheres: Potential Surrogates for Studying Filtration of *Cryptosporidium parvum* in Porous Media. *Environmental Science & Technology*, *46*(21), 11779–11787. https://doi.org/10.1021/es302555n

Stevenson, M. E., Blaschke, A. P., Toze, S., Sidhu, J. P. S., Ahmed, W., van Driezum, I. H., … Pang, L. (2015). Biotin- and Glycoprotein-Coated Microspheres as Surrogates for Studying Filtration Removal of Cryptosporidium parvum in a Granular Limestone Aquifer Medium. *Applied and Environmental Microbiology*, *81*(13), 4277–4283. https://doi.org/10.1128/AEM.00885-15

Swertfeger, J., Metz, D. H., DeMarco, J., Braghetta, A., & Jacangelo, J. G. (1999). Effect of filter media on cyst and oocyst removal. *American Water Works Association. Journal; Denver*, *91*(9), 90.

Tien, C., & Payatakes, A. C. (1979). Advances in deep bed filtration. *AIChE Journal*, *25*(5), 737–759. https://doi.org/10.1002/aic.690250502

Zhu, H., Smith, D. W., Zhou, H., & Stanley, S. J. (1996). Improving removal of turbidity causing materials by using polymers as a filter aid. *Water Research*, *30*(1), 103–114.

Zhu, T., Lawler, D. F., Chen, Y., & Lau, B. L. T. (2016). Effects of natural organic matter and sulfidation on the flocculation and filtration of silver nanoparticles. *Environ. Sci.: Nano*, *3*(6), 1436–1446. https://doi.org/10.1039/C6EN00266H

Chapter 2. Literature review

2.1 *Cryptosporidium* in drinking water

2.1.1 Importance, epidemiology and life cycle

Cryptosporidium was first recognized as a waterborne pathogen during an outbreak in Braun Station, Texas, where more than 2,000 individuals were afflicted with cryptosporidiosis (D'Antonio R.G. et al., 1985). Since that time, outbreaks affecting over a million individuals have been documented throughout North America and Europe, with the single largest epidemic occurring in Milwaukee, Wisconsin, in 1993 (Mac Kenzie et al., 1994). The USEPA estimated in 1993s that approximately 155 million people may be exposed to *Cryptosporidium* in contaminated water every year (USEPA, 2001). It is recognized as a major worldwide health problem in 1976 (Meisel et al., 1976), and it is the third or fourth most common cause of diarrhea worldwide (Neill et al., 1996).

Cryptosporidium has a complex life cycle and is present in Figure 2-1. It is completed in one to eight days and replicates in the body of the host, either from human or animal. Their life cycle is summarized based on the studies by Fayer & Ungar, 1986 and cited by Chung, 2012 as follows:

1. In the host gastrointestinal tract (usually the ileum or the jejunum) or the respiratory tract (which appears a more common site of infection in birds), up to four sporozoites excyst from an oocyst and enter the microvillus of an epithelial cell, where they differentiate into trophozoites.
2. Trophozoites undergo nuclear proliferation to form Type I meronts. Type I merozoites leave the meront to form either a Type I or Type II meront. Type II merozoites leave the meront to form microgametes or a macrogamont.
3. The microgamete fertilizes the macrogamont, which then develops into an oocyst. Oocysts sporulate in situ and either release sporozoites for autoinfection or pass from the body in the feces.

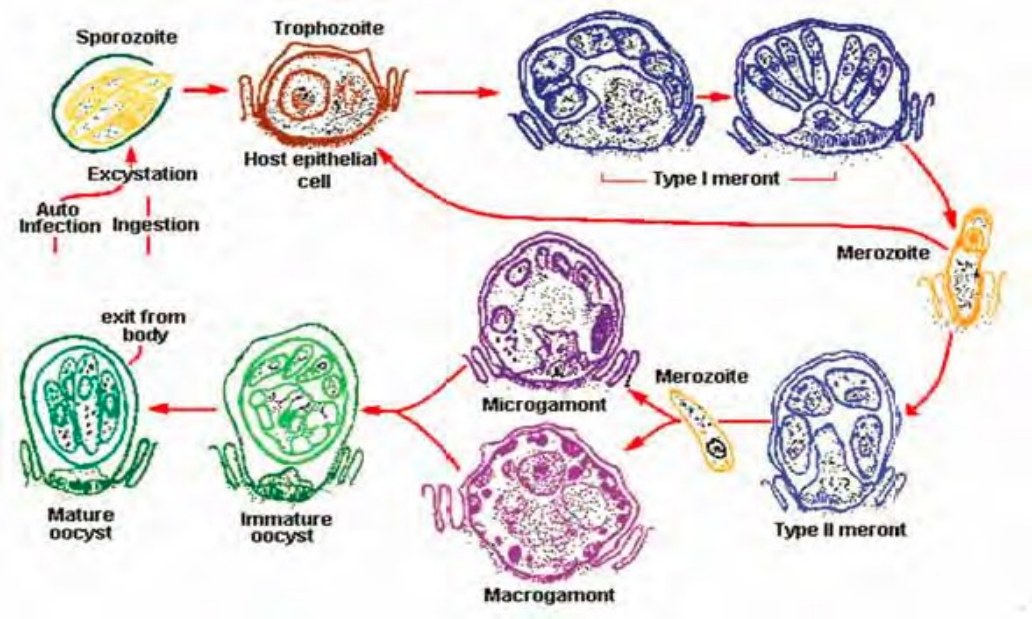

Figure 2-1. Life-cycle of *Cryptosporidium* (Source: Fayer & Ungar, 1986.)

Like other sporozoans, *Cryptosporidium* exhibits alternating cycles of sexual and asexual reproduction that are completed within the gastrointestinal tract of a single host (Xiao & Ryan, 2004). The ingested oocysts then release four infective sporozoites that attach to epithelial cells of the gastrointestinal tract and transform into trophozoites that finally undergo asexual reproduction. Multiple fission of the sporozoites occurs in the reproduction process, which including forming schizonts (known as Type I merozoites) containing eight daughter cells. When the daughter cells are released from the schizont and attach to further epithelial cells, the second generation of Type I merozoites is produced and schizogony repeats (Fayer & Ungar, 1986).

Typically, oocysts undergo one cycle of asexual reproduction and induce a self-limiting diarrhea (Schaechter et al., 1993). After asexual reproduction, schizonts containing four Type II merozoites are formed, which undergo sexual reproduction. Oocysts are then developed from zygotes after fertilization and subsequently excreted (Ryan et al., 2004). Approximately 80% of the oocysts formed from zygotes undergo the life cycle as describe above, which are resilient in the environment. The remaining 20% of oocysts fail to form an outer wall, only have a thin wall, which excyst within the gut and initiate an autoinfective cycle (Fayer et al., 1997; Emelko, 2001).

2.1.2 Transmission and occurrence

Cryptosporidium has worldwide distribution and occurs in several host species including mammals, birds, and fish (Emelko, 2001). Species infect humans includes *Cryptosporidium parvum* and *Cryptosporidium hominis* (Carey, Lee, & Trevors, 2004). *C. parvum* is associated with most human infections and is also common in livestock (Xiao et al., 2004, Rose, 1988). Transmission of *Cryptosporidium* mainly occurs by ingestion of contaminated water (e.g., surface, drinking or recreational water), food sources (e.g., chicken salad, fruits, vegetables) or by person-to-person contact (community and hospital infections). Zoonotic transmission of *C. parvum* occurs through exposure to infected animals (person-to-animal contact) or exposure to water (reservoir) contaminated by feces of infected animals. This parasite can be spread in several diverse ways. *Cryptosporidium* is more common in surface water than ground water because surface waters are more vulnerable to direct contamination from sewage discharges and runoff (USEPA, 2001). Although *Cryptosporidium* oocysts exist in various sources including tap, surface, and wastewater, they cannot reproduce, multiply or be cultured as bacteria (Efstratiou, Ongerth, & Karanis, 2017).

C. parvum oocysts have been detected in surface waters in concentrations as high as 10^4 /100 L and as low as 0.3/100 L (Smith et al., 1991; LeChevallier Mark W. & Norton William D., 1995; Lisle & Rose, 1995) regardless of whether the waters are pristine or impacted by human and animal activity (Emelko, 2001). *Cryptosporidium* oocysts have been found in more than 50% of raw sewage samples, 4.5% of raw water samples, and 3.5% of treated water samples (Wallis et al., 1996; Bukhari et al., 1997). Treated wastewater effluents and agricultural runoff are often significant sources of oocysts in surface waters (Rose, 1988). Several studies have indicated that watershed character and protection influence parasite contamination (Hansen & Ongerth, 1991; Ong et al., 1996). In the study of Ong et al. (1996), they found that water from rivers flowing through cattle pastures in British Columbia exhibited higher *Cryptosporidium* counts than water in a protected watershed. Although oocysts are found less frequently in ground water, Hancock et al. (1998) found that 5% of vertical wells, 20% of springs, 50% of infiltration galleries, and 45% of horizontal wells present *Cryptosporidium* oocysts. Source water contamination

by transporting of oocysts from feces-contaminated soil during weather events has been suggested to be another occurrence pathway in waters (Kramer et al., 1996).

2.1.3 Waterborne outbreaks of Cryptosporidiosis

According to the reported 199 waterborne disease outbreaks from 2004 to 2010, *Cryptosporidium* was one of the most common etiological agents (Balderrama-Carmona et al., 2014). Some of water-associated outbreaks were due to parasitic protozoan disease, with 60% from North American and 33% from Europe, in which about 24% of reported outbreaks were associated with contaminated drinking water systems with *Cryptosporidium* passing through filtered or unfiltered drinking water systems (Karanis, Kourenti, & Smith, 2007). In Karanis's review report, recreational water accounts for over 50% outbreaks, followed by contaminated drinking tap water (10.9%), mixed aetiology (10.9%) (such as contaminated water supply reservoirs, infected animals within the watershed, surface water with chlorination as only treatment, etc.) and contaminated water source such as lake, river, well etc. (10.3%). From these outbreaks, *C. parvum* and *C. hominis* are the two main causes for human cryptosporidiosis, with only one drinking-waterborne outbreak caused by *C. cuniculus* reported in England in 2008 (Cacciò et al., 2005; Chalmers & Giles, 2010; Pillai et al., 2009). Prior to *C. hominis* denoting the human genotype (Morgan-Ryan et al., 2002) , all cases were reported as *C. parvum* as the species of concern to humans (Carey et al., 2004). Human cryptosporidiosis is believed to involve both anthroponotic and zoonotic cycles of transmission (Casemore et al., 1997). Since 1982, human cryptosporidiosis has been documented in 95 countries on every continent except Antarctica (Fayer, 1997). It occurs in developed and developing countries, urban and rural areas, and in temperate as well as tropical climates (Ronald Fayer et al., 1997; O'Donoghue, 1995). Cryptosporidiosis is characterized by a self-limiting diarrhea and abdominal cramping. Diarrhea from *Cryptosporidium* infection can last 2 to 10 days (average 7 days) after becoming infected with the parasite in persons with health immune systems. People with weakened immune systems may develop serious, chronic, and sometimes fatal illness. The other symptoms, such as nausea, low-grade fever, malaise and occasional vomiting may also occur (WHO, 2016). Occasionally, people may experience

a recurrence of symptoms after a brief period of recovery before the illness ends. Table 2-1 summarized some of recently documented waterborne outbreaks of cryptosporidiosis.

Table 2-1. *Cryptosporidium* waterborne outbreaks

Location	Year	Health Impacts	Source	Deficiency
Braun Station, Texas	1984	2,006 cases	Ground water	Sewage contamination
Milwaukee, Wisconsin	1993	50 deaths, 400,000 cases	Lake	Treatment deficiency (Inadequate removal by the coagulation (recent change from PACl to alum) and filtration process of oocysts; recycling of filter backwash water)
North Battleford, Saskatchewan	2001	>6,000 cases	River (North Saskatchewan River)	Treatment deficiency (sedimentation contact unit did not satisfactorily remove suspended solids from the source water and result in filter breakthrough)
Northampton, England	2008	422 cases	Source water	Treatment deficiency (rabbit contaminated water supply in water treatment plant)
Ostersund, Sweden	2010	27,000 cases	Lake	Sewage contamination
Philadelphia, Pennsylvania	2015	6 cases	Direct contact with infected calves	Poor hygiene and animal cadaver handling
Coleg Gwent, Wales	2016	14 cases	Direct contact with infected farm animals	Close contact with infected animals or people
Central Ohio, Ohio	2016	209 cases	Swimming pool or water playground	Treatment deficiency

2.1.4 Treatment options for Cryptosporidium

Many outbreaks of cryptosporidiosis are associated with treatment deficiency during the water treatment operations due to:

- The prevalence and persistence of oocysts in the environment;
- The resistance to conventional chemical treatment or disinfection;
- The high infectivity of *C. parvum* oocyst.

In drinking water, multiple barriers are employed to reduce pathogens from source water, including the protection of the catchment area, filtration of surface water supplies, and disinfection by free chlorine, monocloramine, ozone or ultraviolet irradiation (Chung, 2012). The protection of the catchment area involves vegetation management, erosion and sediment control, storm water and wastewater management, and management of potentially contaminating activities, materials or goods (Hipsey and Brookes, 2017). Physicochemical barrier by coagulation and sand filtration is the most effective way against oocysts and has been applied by water utilities treating surface waters (Hijnen and Medema, 2010). Tfaily et al. (2015) examined 17 Canadian Water Treatment facilities and their results showed that log removals for *Cryptosporidium* can range from 1.11-7.24 with mean log removal of oocyst equals to 4.27. Generally, the first barrier against *Cryptosporidium* in drinking water is raw water storage reservoirs. Study by Bertolucci et al., (1998) showed that about 0.7-log removal of oocysts in a reservoir with a theoretical detention time of 18 days is achieved. Another study by van Breemen et al., (1998) demonstrated that approximately 1.3-log and 1.7-log removal of oocysts are achieved when stored for 10 and 24 weeks, respectively. *Cryptosporidium* can survive in different environmental conditions, which results in a much lower log removal in a reservoir. The second physicochemical barrier against *Cryptosporidium* are suggested to be coagulation, flocculation, sedimentation and filtration, which normally termed as conventional water treatment used to remove particles, organics, and chemicals. Many pilot- and full-scale studies demonstrated that conventional coagulation and sedimentation processes can achieve total about 0.5- to 2-log removal of oocysts. Study of full-scale oocyst removal by Nieminski & Ongerth (1995) demonstrated more than 4-log removal of oocyst can be achieved by optimized coagulation and filtration. The most effective component of conventional water treatment operation against oocyst is rapid sand filtration due to its low operational cost and the high treatment efficiency. Chlorine based disinfection (free and combined chlorine) has been shown to be ineffective at concentrations typically applied to drinking water (2-6 mg/L). UV disinfection has demonstrated over 3-log inactivation of oocysts (Clancy et al., 2000); However, it only effective in very clean waters with very low turbidity since the turbidity can dampen the treatment efficiency dramatically. *Cryptosporidium* oocysts have shown sensitivity to ozone, but a substantial cost is

associated with ozone treatment. The comparison of inactivation of *Cryptosporidium* oocysts and *Giardia* cysts indicated that oocysts were thirty times more resistant to ozone (Korich et al., 1990). Despite of the progress in the development of disinfection technologies, filtration used in drinking water treatment remain critical to achieving desirable levels of *Cryptosporidium* removal (Emelko, 2001).

In summary, the multiple barrier approach, including protection of water sources and treatment of water by flocculation, filtration and disinfection, will continue to be the most applicable method for the reduction and prevention of waterborne disease transmission (Karanis, Schoenen, & Seitz, 1998; Efstratiou, Ongerth, & Karanis, 2017).

2.2 *Cryptosporidium* Surrogate

Why do we need surrogate to study the removal of *Cryptosporidium* in the water treatment process? The reasons to this question are listed in the following：

- Difficulty in accurately detecting and enumerating *Cryptosporidium* has made it impractical to suggest regulatory guidelines for this pathogen;
- High cost and many efforts involved in routinely enumerating oocysts in environmental samples;
- Health risk was associated with working with live oocysts;
- Oocysts have relatively small numbers in most waters, particularly treated potable water (<10/10L), which make the examination of treatment process unavailable;
- Different concentrations of surrogates can be used for treatment efficiency study;
- Surrogate also bring the benefits, such as easy to detect, and no hazard involved.

A wide range of microbiological species (aerobic spores, anaerobic spores, algae, Virus/coliphage, *Giardia*, Heterotrophic plate counts), operational parameters (turbidity and particle counts) and synthetic materials (polystyrene or latex microspheres) have been used as surrogate for *Cryptosporidium* to assess the performance of water treatment processes and summarized by Chung (2012). Jakubowski et al. (1996) had proposed some criteria for the selection of suitable surrogates for *Cryptosporidium*, which are listed here:

- Should be at least as abundant as the *Cryptosporidium* (different concentrations of polystyrene microspheres can be prepared);
- Should be present whenever *Cryptosporidium* is present;
- Should not be reproducible in the environment;
- Should be easy to enumerate by methods that are specific and sensitive;
- Should be at least as resistant to the treatment under study; and
- Should survive slightly longer than *Cryptosporidium*.

To select the most representative surrogate for *Cryptosporidium*, a better understanding of *Cryptosporidium* characterization is critical and is reviewed in the below section.

2.2.1. Characterization of *Cryptosporidium*

Removal of *Cryptosporidium* oocysts in engineered water treatment processes usually involves direct contact of the oocysts wall with the granular filter media. Therefore, the surface properties of oocysts, as well as chemical composition and structure of the oocyst wall play an important role in controlling the strength of interaction with collector surface. This section of review examines the current state-of-knowledge of the oocyst wall structure and electrokinetic characterization of oocysts, which may confer environmental resistance on oocysts and help understand the key physical and chemical properties of the oocyst wall that affect the transport in filtration.

2.2.1.1 Structure of the oocyst wall

Jenkins et al. (2010) proposed four-layered structure in the oocyst wall by conventional thin-section electron microscopy analysis. The four layers includes three electron-dense layers (8.5±0.6nm; 13.0±0.5nm and 28.6±1.6nm, respectively) and an intermedia thin electron-translucent layer (4.0±0.2nm) as delineated in Figure 2-2. The total average thickness of the oocyst wall is measured as 54.1±4.1nm in study of Jenkins et al. (2010). Brush et al. (1998) using thin sectioning with a transmission electron microscope observed that oocysts cell wall is composed by three distinct layers: acidic glycoprotein outer layer, lipid central layer and filamentous glycoprotein inner layer, which is in agreement with the study by Dumètre et al., (2012); however they proposed that the total thickness of oocyst

wall is about 80 nm. Considine et al. (2001) using atomic force microscope observed oocyst cell and found that oocyst surface appeared molecularly "hairy" or as having a polyelectrolyte brush, which agrees with the notion that charged surface polymeric material extended into solution from the oocyst surface (Chung, 2012). Also, Considine et al. (2002) demonstrated that the net negative charge on the oocyst surface would lie at the edge or within the hairy layer of oocysts and would come from the ionizable surface groups along the polypeptide backbone. Another study done by Considine et al. (2001) indicate that the oocyst surface is predominantly hydrophilic, which is in agreement with the study by Dai and Hozalski (2003). They examined the surface hydrophobicity by measuring the hexadecane-water partitioning of oocysts, which indicated that the oocysts preferentially partitioned into the water phase and were relatively hydrophilic. Kuznar and Elimelech (2005) also observed the relatively hydrophilic properties of oocysts in their partition experiment with dodecane-water system.

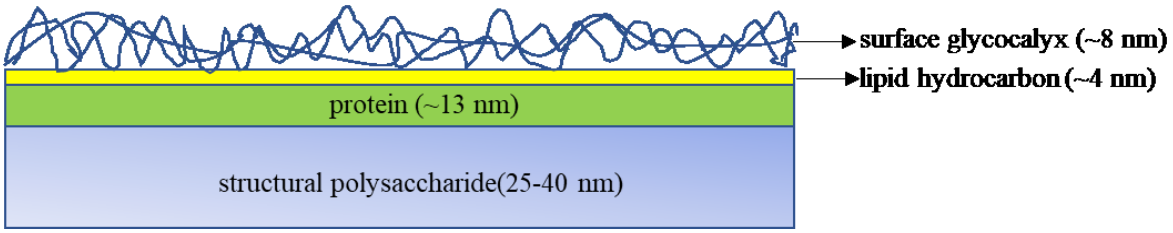

Figure 2-2. Proposed model for the *C. parvum* oocyst wall (modified from Jenkins et al., 2011).

2.2.2.2 Biochemical component of the oocyst wall

The biochemical components of oocyst wall still have not been determined precisely because of the variations in (1) isolation and purification procedures, (2) labelling and staining procedures, (3) the presence of sporozoites proteins in different research studies (Chung, 2012). The existence of a glycocalyx on the outer surface of the wall has been noted previously by many researches and various cell wall proteins with a wide range of molecular weights are discovered located in the inner layer of the oocyst wall (Spano et al., 1997; Chung, 2012).

Protein

Jenkins et al. (2010) determined the total protein content of purified oocyst walls by the Lowry protein assay and the proteins were identified by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). They concluded that purified oocyst wall contained 7.5% total protein. As described previously, the net negative charge of oocyst cell is due to the ionized or deprotonated carboxyl groups from protein (Dai and Hozalski, 2003). Kuznan and Elimelech (2005) examined the influence of surface proteins onto a quartz surface under well-controlled chemical and hydrodynamic conditions and they found that an electrosteric repulsion between oocyst and quartz subtract exists. The repulsion is caused by the presence of proteins on the oocysts surface. They surmised that these macromolecules are stretched into the solution due to electrostatic repulsion of surface ionizable groups distributed along the polypeptide backbone and the brushlike structure of surface proteins imparts steric repulsion significantly hindering oocyst deposition.

Carbohydrates

Jenkins et al. (2010) also determine the presence and content of carbohydrates in oocysts walls; glucose and galactose confirmed in their study, which is consistent with studies by Moore et al. (1998). Also, small amount of mannose, talose, glucofuranose, D-glucopyranose, and D-mammopyranose were detected in oocyst walls (Jenkins et al. 2010). Also, Nanduri et al. (1999) reported that the oocyst glycocalyx is composed mainly of glucose, with galactose, mannose, xylose, and ribose being the next most abundant components.

Fatty acid, hydrocarbons, and alcohols

Jenkins et al. (2010) identified saturated fatty acid using GC-MS (Gas chromatography-mass spectrometry) analysis for the total lipid extract from purified oocyst walls. They are comprised of hexadecanoic acid, octadecanoic acid, 11,14-Eicosadienoic acid, straight chain aliphatic hydrocarbons ranging from 11to 34 carbons in length, and several long-chain alcohols, including 2-decanoal, tridecanol, and 1-tetracosanal.

Lipids

The central layer of oocyst wall contains glycolipid/lipoprotein (Fayer et al., 1991) and the inner filamentous layer is believed to provide rigidity and elasticity to the oocyst wall (Harris & Petry, 1999). To further characterize the lipid present in the oocyst wall, Jenkins et al. (2010) using multiple methods proved the presence of glycolipids, phospholipids, amino compounds and choline-containing compounds. The most abundant lipids in oocyst wall was sphingolipids, followed by phospholipids and cholesterol (Chatterjee et al., 2010).

2.2.2.3 Electrical properties

Surface charge

Surface charge measurement for oocysts have been reported by Ongerth & Pecoraro (1996) and Rice et al. (1996). Wide variations were observed due to the differences in oocysts sources used, purification methods, storage solutions, and suspending media. Brush et al. (1998) measured the effects of purification method and presence of antibiotics on the surface charge. The results clearly showed that the oocysts purified by EAPS (ethyl acetate and Percoll-sucrose) method had significant different surface charge than the oocysts purified by the DIS (deionized water washes) method. In addition, the presence of antibiotics had negligible effect on electrophoretic mobility. Rice et al. (1996) used filtered natural lake water as their suspending medium and they claimed that the complex ionic makeup, the presence of unknown chemical species and buffering capacity, or the presence of chemically reactive ionic species can all affect electrophoretic mobility measurement. Moreover, the chemical makeup in natural waters may change over time, especially for river water. Therefore, specifying the above-mentioned determination details will aid in the accurate surface charge measurements.

Cryptosporidium oocysts have been reported to have a negative charge in the pH range of natural waters (pH 4-10), with the isoelectric point between 2-3 (Considine et al., 2002; Kuznar & Elimelech, 2004; Gao & Chorover, 2009). The negative zeta potential of *Cryptosporidium* oocysts rapidly increases until pH 4.5 (Bustamante et al., 2001) because the oocyst surface is more negatively charged with increasing pH above the isoelectric point (Gao & Chorover, 2009). However, the negative zeta potential of *Cryptosporidium* oocysts is almost constant at pH values between 4.5 and 8.5 (Bustamante et al., 2001).

Hydrophobicity

Net surface charge and hydrophobicity are key factors mediating microbial adhesion to solid surfaces (Brush et al., 1998). Brush et al. (1998) measured the effect of ionic strength on the hydrophobicity of oocysts and how it changes with aging. The results suggested that the hydrophobicity of the oocyst surface changes with the oocysts age after they excrete, and fresh oocysts were more hydrophilic than aged oocysts. For ionic strength of less than about 38 mM, the increasing in ionic strength was associated with the increasing hydrophilicity of fresh oocyst surface. For aged oocysts, the direct proportional relationship was only observed when ionic strength is less than 20 mM. Furthermore, hydrophobicity of fresh oocysts (2-week-old) were strongly dependent on the ionic strength of the suspending solution whereas a less dependence on the ionic strength of the suspending solution was observed for aging oocyst (2-month-old). Dai et al. (2004) examined the role of surface charge and hydrophobicity on the adhesion of *C. parvum* to solid surface. They concluded that surface charge was the more principal factor for *C. parvum*, dominating the hydrophobicity effects. Under different environmental conditions, the governing forces of interaction between oocysts and adhesion media can change between electrostatic and hydrophobic.

2.2.2 Microbiological surrogates: Spores, yeast, and inactivated oocysts

2.2.2.1 Yeast

So far, no quantitative surrogates for oocysts removal during water treatment have been identified. Baker's yeast cells (*Saccharomyces cerevisiae*) are either spherical or ellipsoidal in shape, with diameter typically 5-8 μm (Chung, 2012). Yeast is used as biological surrogate for *Cryptosporidium* due to their similarity in shape and size, resistance to chlorination, and stability in environment. The yeast cell wall is approximately 25 nm (Bowen et al., 1992) and their structure is complex. The outer layer is thought to be composed mainly by phosphomannan with phosphate groups that provide a net negative charge (Bowen et al., 1992). The rest of the cell wall is composed of a mammoprotein (Mustranta, Pere, & Poutanen, 1987), which contains both positively charged amino groups and negatively charged carboxyl groups, as well as chitin (uncharged) and lipid (Thonart, Custinne, & Paquot, 1982; Bowen et al., 2004; Dengis, Nelissen, & Rouxhet, 1995). In

summary, Dengis et al. (1995) claimed that the composition of yeast cell surface is mainly protein, polysaccharide, and hydrocarbon-like compounds and the hydrophobicity of cell surface is related to the type and concentration of proteins in the cell wall. Therefore, the hydrophobicity of yeast cell was significantly different to oocysts (predominantly hydrophilic) by using Microbial adhesion to hydrocarbon (MATH) assay and electrostatic interaction assay (Chung, 2012). Although yeast has surface groups that are negatively charged in the pH range of interest in drinking water (from 7 to 8) that are similar to oocyst cell, it seems to lack of the fine surface hairs in their cell wall structure (Chung, 2012). Also, yeast does not seem to have been widely tested as a surrogate for *Cryptosporidium*.

2.2.2.2 Spores

Bacterial spores are resistant to conventional water treatment processes but not as resistant to disinfection as *C. parvum* oocysts (Chauret et al., 2001). Nevertheless, spores have been widely used as surrogates for evaluating the treatment processes to removal of oocysts. Aerobic bacterial, such as *Bacillus* spores, most commonly used in the water treatment studies, are present in most surface waters and may grow in filtration system without posing hazards to public health. Swertfeger et al. (1999) stated that spores were a conservative indicator of oocyst removal but others (Huck, 2001; Emelko, Huck, & Coffey, 2005) did not observe direct relationship between spores' removal and oocyst removal. Anaerobic bacterial, such as *Clostridium perfringens* or sulphate-reducing clostridia, have also been used to monitor water quality (Hijnen et al., 2000; Hijnen et al., 2007). These spores can withstand extreme temperature and environmental stress compared to faecal indicator bacteria (Hijnen, Beerendonk, & Medema, 2006). It is mostly applied as an indicator for faecal contamination of raw waters in Europe (Chung, 2012).

2.2.2.3 Inactivated oocysts

Emelko et al. (2003) preformed a bench-scale research to examine if formalin-inactivated oocysts are reliable surrogates for viable oocysts during filter operation. The study showed the promise of inactivated oocysts, even though the surface charges of oocysts largely changed by the chemical inactivation process. Although the use of formalin-inactivated oocysts as surrogates for viable oocysts is desirable from a health and safety perspective,

they remain non-ideal because of the prohibitive cost and same analytical uncertainty as live oocysts (Clancy et al., 2000; Nieminski & Ongerth, 1995). Kuznar and Elimelech (2005) suggested that inactivation by either formalin or heat treatments altered the structure of surface proteins and thus reduced the steric repulsion, resulting in a higher deposition rate for inactivated oocysts than for viable oocysts. They also found that formalin treatment imparts an increased hydrophobicity to the oocysts surface and thus promotes the enhancement in oocysts deposition kinetics compared to heat treatment due to the cross-links between amino groups created by formalin. Because less sites are available on the oocysts surface (specifically on the amino groups) for water to form hydrogen bonds, the surface of the oocysts become more hydrophobic. In further study by Kuznar and Elimelech (2006), the results imply that biomolecules on the oocysts surface play important roles in controlling oocysts adhesion kinetics to quartz surfaces. These findings highlight the need to select a reprehensive and quantitative *Cryptosporidium* surrogate for drinking water treatment processes.

2.2.3. Non-microbiological surrogates: turbidity and particle counting

2.2.3.1 Turbidity

Traditionally, turbidity has been the main water quality parameter for assessing particle removal in water treatment. The guideline from Health Canada (2012) states that: "For conventional and direct filtration, less than or equal to 0.3 NTU in at least 95% of measurements either per filter cycle or per month and never exceed 1.0 NTU". It also states (2017) that the design and operation of filtration systems should be optimized to reduce turbidity to as low as possible, with a goal of less than 0.1 NTU in treated water at all times from individual filters. Although it is difficult to establish a direct relationship between turbidity and oocyst removal, turbidity has still been used as an indicator for filter performance (Bastos, Viana, & Bevilacqua, 2013). Many pilot- and full-scale studies have demonstrated that turbidity is approximate indicators of pathogen removal by drinking water treatment process but is not reliable quantitative surrogates (Huck et al., 2000; Huck et al., 2001; Emelko, 2001; Emeloko et al., 2005). Most turbidity in raw water is caused by suspended inorganic-rich colloidal particles that are less than 0.2 μm in size (Chung, 2012), and it is affected by the numbers, size, shape, and refractive index of the suspended

particles which impacting the light-scattering properties of the suspension (Jakubowski et al., 1996). Therefore, the removal of turbidity may be a poor indicator of oocysts removal. The discrepancy among different studies suggest that turbidity is an inadequate surrogate for predicting *Cryptosporidium* removal by filters, and it is indicative of overall treatment but not of oocyst removal (Huck et al., 2001). The study of Copes et al. (2008) examined the relationships between turbidity in raw water supplies and microbial risk for human health in finished drinking water. The authors stated that as a filtration efficiency parameter, turbidity has poor predictive value for the presence of pathogens.).

2.2.3.2 Particle counting

Particle counting has also been used for treatment optimization because it is more sensitive to larger particles with diameter greater than 2 μm, while turbidity is more sensitive to submicron particles (Hargesheimer, Lewis, & Yentsch, 1992). However, their study also suggested that particle counting is unable to discriminate between particles in the same size range and to detect minor changes in concentration that are associated with *Cryptosporidium*. Particle counts is affected by many factors, such as raw water turbidity, algal counts, and particulate loading rate to a sand filter (Morse et al., 2002). For different source water type, the correlations between removal of oocysts and particle counts could be significant different. Many studies shown that particle counting is indicative of overall treatment but not of oocyst removal (Emelko, 2005). Therefore, site-specific correlations should be obtained and monitored in water treatment plant and the use of particle counting on its own as a surrogate to assess the removal of oocysts by water industry is not appropriate. Although turbidity or 5-20μm particle breakthrough of filters is known to be somewhat related to pathogen breakthrough, pathogen may breakthrough well before measurable turbidity or particle counts are detected by on-line meters (Petterson & Ashbolt, 2016; Verberk et al., 2007). Their studies revealed the poor correlation between pathogens and these physical surrogates in raw waters.

2.2.4. Carboxyl fluorescence polystyrene microspheres

Oocyst-sized polystyrene microspheres have shown promise as non-microbiological surrogates for oocyst removal by filtration (Swertfeger et al., 1999; Emelko, 2001).

Microspheres are synthetic polymeric materials that can be prepared in a particle size similar to that of oocysts. Some functional groups, i.e. carboxylic, sulphonate, amino etc., may attach to the microspheres to provide desired electrical charge or other surface properties (Xu & Logan, 2006a, 2006b). Fluorescence carboxylated polystyrene microspheres have been used as surrogates for oocysts in many studies since 2003 (Dai and Hozalski, 2003) as reflected in the review of Emelko (2005). It gains the popularity for experiments in which a surrogate is needed to assess the efficiency of treatment methodologies. It offers advantages over oocysts, such as decreased cost, reduced processing and analyzing time, enhanced durability, and greater consistency of results (Amburgey et al, 2001). The authors used multiple colors of microspheres during different portions of a filter run, which provided new insights into particle behavior within drinking water filters. They are also available in a wide range of sizes which enhances their applicability in modelling microbes or other colloids (Links, 2015). Table 2-2 in section 2.7 summarizes the recent use of various-sized microspheres as *Cryptosporidium* oocysts surrogates in treatment process(es).

2.2.4.1 Dose concentration

Large variation in selected dose concentration were observed. Gottinger et al., (2013) spiked 10^6 beads/L of 4.5 µm microspheres as surrogates for *Cryptosporidium* oocysts in slow sand biofiltration bed, while Hogan et al., (2013) dosed only 10^3 beads/L for their hydrologic removal experiment. Given the large variation in selected dose concentrations, the dose concentration is irrelevant if the removal is quantifiable (Link, 2015).

2.2.4.2 Composition

Fluoresbrite® carboxylate polystyrene microspheres are composed of polystyrene latex with a carboxylate coating, which were purchased from Polyscience Inc. (Catalog no. 16592-5, USA) with initial concentration 4.99×10^8 particles/ml as a 2.5wt% aqueous suspension. The specific gravity was 1.025 for polystyrene as provided by manufacturer.

2.2.4.3 Fluorescence

Fluorescence microspheres are available in a range of colors, and commonly reported are Yellow-Green (YG), Yellow-Orange (YO), and Bright-Blue (BB), which are believed to have the clearest detection. The Yellow-Green color had excitation wavelength at 441 nm and emission wavelength at 486nm, which are used in many *Cryptosporidium* studies in the literature as summarized in Table 2-2 and used in this study

2.2.4.4 Surface chemistry

These microspheres had the similarity in size, shape and density to oocysts and easy-to-detect property. However, their surface charge is very different than that of oocyst, which often under- or over-predict the oocysts' transport during the filtration studies. To be a better oocyst surrogate and to give more accurate prediction, surface charge of microspheres needs to be modified to approximate that of the oocysts. Biomolecule modified carboxylate polystyrene microspheres as a surrogate for *Cryptosporidium* was first proposed by Pang et al. (2012). By conjugating the glycoprotein to the microspheres, the zeta potential was change from -80 to -25 mv at neutral pH, which is close to the oocyst surface charge in nature environment. Unfortunately, the cost of glycoprotein limited its application in large-scale studies. Other than cost, the hydrophobicity of the oocysts was overlooked by their study. Not only mimicking the surface charge of the oocyst but also modifying the hydrophobicity of the microspheres to be much closer to the oocysts are considered in this study.

2.3 Removal mechanisms of granular media filtration

The removal of suspended particles within a filter is considered to involve at least two distinct and separate processes: (1) the transport of suspended particles to the media grain or to another particle previously retained in the filter bed; (2) the attachment of particles to the grain surface (Yao, Habibian, & O'Melia, 1971). In this review, a critical evaluation of particle deposition theory and an improved understanding of the fundamental mechanisms controlling particle transport and attachment are obtained.

2.3.1 Transport mechanisms

A single particle of filter media is termed a collector, emphasizing that the ultimate purpose of transporting suspended particles from the bulk flow to the external surfaces of media grains in packed beds is the collection of these particles, thereby accomplishing their removal from the water (Yao et al., 1971). When considering a single particle of filter media, it is assumed that the collector is not affected by its neighbors and is fixed in space in the flowing suspension (Figure 2-3). Particles are transported close to the grain surface by numerous processes during filtration. The transport mechanisms were initially described in classical colloidal filtration theory, developed by Yao et al. (1971), and summarized below from Crittenden et al. (2005) and Chung (2012).

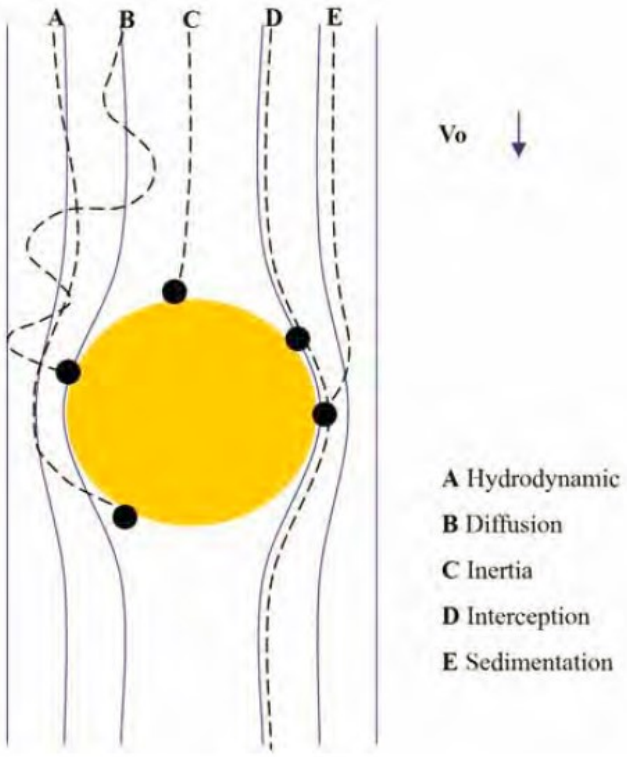

Figure 2-3. Conceptual diagram of particle transport mechanisms (adapted from Chung, 2012).

A. Hydrodynamic transport

The particles are transported with the fluid flow in streamlines to the surfaces of filter media is term as hydrodynamic transport. It is caused by a nonuniform shear distribution

in the filter bed. This streamlining and irregularity of flow patterns results in additional contact between particles and filter media.

B. Diffusion

A particle in suspension is subject to random bombardment by molecules of the suspending medium, resulting in the Brownian movement of the particle (Chung, 2012). This Brownian motion process generates diffusion and is important for submicron (<1μm) sized particles (Chung, 2012). Therefore, it is unlikely an important mechanism for *C. parvum* oocysts or their surrogates' removal in the size range 2-6 μm but plays a leading role for other particles or microorganisms that are very small in size, such as virus (Yao et al., 1971).

C. Inertia

This is the process whereby particles carried by a fluid stream are unable to follow the streamlines over the surfaces of the grains and continue along their previous trajectory to collide with the grains (Stevenson, 1997). It leads particles to deviate from the streamline of fluid around media grains and continue downwards to come into contact with a media grain. This is a significant mechanism in air filtration, where the viscosity is low. With liquids, the much higher viscosity could cause particles to be swept round the grains. Inertia effects are negligible during water filtration (Ives, 1982).

D. Interception

Interception happens when a particle suspended in a fluid stream approaches a collector surface and gets within a distance equivalent to the radius of the particle. Particles will interact with the stationary liquid zone on the surface of the grain and potentially attach to it. The suspended particles larger than about 1 μm are likely transported to the filter media by interception and sedimentation, such as *Cryptosporidium* (Yao et al., 1971).

E. Sedimentation

Sedimentation is in which larger and heavier particles, whose density is greater than that of water, follow a different trajectory other than fluid streamline around the media grain

due to the influence of the gravitational force field, but settle on the grain (Ives, 1970; Yao et al., 1971). Amirtharajah (1988) claimed that the sedimentation mechanism is increasing important for particles in 2-25 μm size range.

Depending on the size of the particles, the major transport mechanisms are interception and sedimentation for *Cryptosporidium*-sized particles (Ives, 1970; Yao et al., 1971; Amitarajah, 1988). The schematic representation of removal efficiency to particle size distribution is described in Figure 2-4 (Yao et al., 1971). The numerical equation below is used to describe the temporal and spatial variation of particle concentration in transport model:

$$\frac{\partial C}{\partial t} + v\nabla C = D_{bm}\nabla^2 C + (1 - \frac{\rho}{\rho_p})\frac{mg}{3\pi\mu d_p}\frac{\partial C}{\partial z} \qquad (2\text{-}1)$$

The first term on the left-hand side of the equation (2-1) represents the temporal variation of C at any point with coordinates x, y, and z; the second term describes the effects of advection on the concentration at that point. On the right-hand side of equation (2-1), the first term describes the effects of diffusion, and the second term describes the effects of gravitational settling on the system. The influence of interception is included in the boundary conditions used in integrating the equation. The form of equation (2-1) has been widely used in engineering field to characterize the fate of pollutants in the atmosphere and in streams and estuaries and has been applied to air and water filtration processes (Yao et al., 1971).

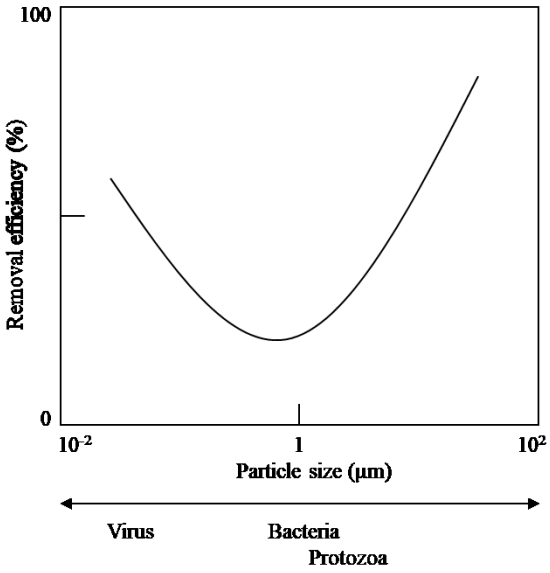

Figure 2-4. Schematic representation of removal efficiency to particle size distribution (adapted from Yao et al, 1971).

2.3.2 Attachment mechanisms

As the particle and collector surfaces come close together (less than 100 nm), several surface interaction forces can be considered for the attachment. Two that have been extensively studied and quantified are the electron double layer (EDL) interaction force and the London-Van der Waals (LVDW) or dispersion force. Some other less understood and less quantified forces, including structural force due to hydration of surface ions, the hydrophobic forces for hydrophobic materials, and the steric interaction from adsorption of polymers (macromolecules) (Tobiason & O'Melia, 1988), are also reviewed in this chapter. In practice, the following interactions are important (Gregory, 2005) and are reviewed in this section.

- LVDW (usually attractive)
- EDL (either attractive or repulsive)
- Hydration effect (repulsive)
- Hydrophobic effect (attractive)
- Steric interaction of adsorbed layers (usually repulsive)

2.3.2.1 DLVO interactions

LVDW force

The universal LVDW force is usually attractive in aqueous system. LVDW force is a result of interactions at short distance between electronic dipoles of the molecules comprising the surfaces and the solution (Tobiason & O'Medlia, 1988). The magnitude of this force depends on interacting particle sizes, the separation distance between particles, and granular collector (Elimelech & O'Melia, 1990).

EDL force

Electrical double layer force is resulting from the interaction of the EDLs of a particle and collector. The magnitude of the force is a function of the separation distance, ionic strength, and charge at each surface (Elimelech & O'Melia, 1990). Depending on the signs of surface charge of particles and filter media, electrical interaction between the target particles and the granular collector can be either attractive or repulsive. Generally, filter media and most environmental particles, including microorganisms, have a net negatively charged surface in water, which is examined by zeta potential. Therefore, the electrostatic force between the surface of the filter media and microorganisms is usually repulsive (Ives, 1970), but can be changed by adding the coagulant and changing in pH to reduce the surface potential and suppress the separation distance to achieve the favorable conditions for microorganism removal by filtration.

2.3.2.2. DLVO theory

The combination of the attractive LVDW force and repulsive EDL force gives rise to the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Pembrey, 2001). Classic DLVO theory attempts to describe the colloid-collector interactions in terms of adhesive and repulsive forces (Molnar et al., 2015). It has been used as both a qualitative and quantitative model to explain microbial behavior and adhesion (Hermansson, 1999). According to the DLVO theory, the net interaction energy (V_T) between a spherical particle and a charged plate is the sum of the attractive van der Waals energy (V_A) and the repulsive electrostatic energy (V_R):

$$V_T = V_A + V_R \qquad (2\text{-}2)$$

$$V_A = -\frac{AR}{6h} \qquad (2\text{-}3)$$

$$V_R = \frac{\varepsilon R(\varphi_P+\varphi_S)}{4}\left[\frac{2\varphi_P\varphi_S}{{\varphi_P}^2+{\varphi_S}^2}\ln\left(\frac{C_1}{C_2}\right) + lnC_3\right] \qquad (2\text{-}4)$$

where $C_1 = 1 + \exp(-kh)$, $C_2 = 1 - \exp(-kh)$, $C_3 = 1 - \exp(-2kh)$, A is the system Hamaker coefficient (ergs), (colloid particles-water-collector), R is the particle radius (meters), h is the distance between the plate and particle (meters), e is the dielectric constant of the suspending medium, and $\varphi_P$and φ_S (volts) are the surface charges of the plate and sphere, respectively. k (meters^{-1}), referred to as the double-layer thickness, is a function of the ionic strength of the suspending medium. The value of V_T determines whether temporary adhesion occurs and whether the opportunity for permanent adhesion exists. It is the function of separation distance as shown in Figure 2-5 (A) (Elimelech, 1998). Depending on the relative contribution of the two interactions, the three patterns of energy profiles with separation distance (h) can be drawn as shown in Figure 2-5 (B) (Elimelech, 1998).

Recently, the traditional DLVO model had been found unable to fully describe colloidal behavior in aqueous media (Grasso et al., 2002) since it simply assumes the surfaces to be monolithic (homogeneous), so the model calculation is based on the bulk surface properties of colloids and collectors. This so-called mean-field DLVO approach does not account for microscale to nanoscale heterogeneity that is expected to exist on real surfaces (Molnar et al., 2015). Several non-DLVO forces were found to contribute to the colloidal interactions by the incorporation of heterogeneity into colloid-surface DLVO interactions (Elimelech, 1998; Hermansson, 1999), including steric repulsion, polymer bridging, hydrophobic interaction, and hydration. Significant work has been focused to extending the precepts of the transitional DLVO model to accommodate these non-DLVO forces (Grasso et al., 2002).

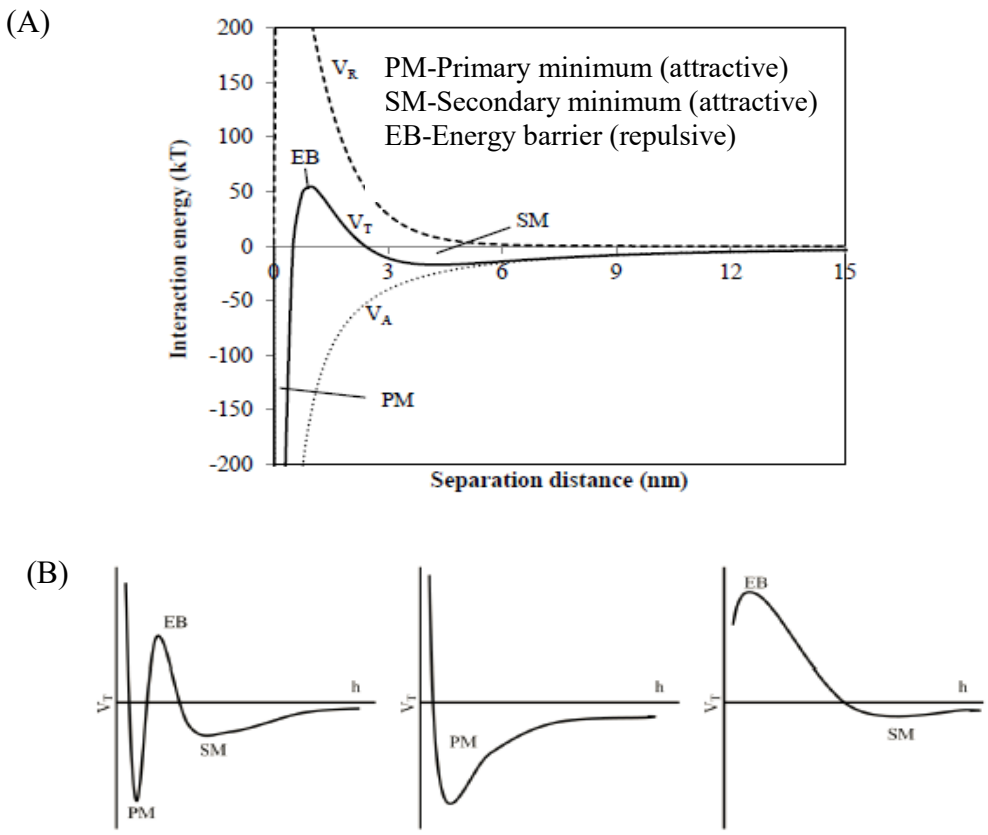

Figure 2-5. Schematic representations of total DLVO interaction energy profiles as a function of separation distance: (A) quantitative example for equal spherical particles of 1µm diameter in 100 mM NaCl, zeta potential=-25mV, A=8.3×10^{-21}J, and (B) the three characteristic types of profiles (adapted from Elimelech, 1998).

2.3.2.3 Non-DLVO Interactions

Structural forces

Hydrophilic (Hydration) effect (repulsion)

The surface of particles of a biological origin is usually hydrophilic due to the presence of hydrophilic molecules, such as protein and polysaccharides, and the hydration of ions at particle surfaces cause extra repulsion (Gregory, 1993). However, the hydrophilic force is short-range force and can only be effective at distance less than 2 nm (Haes et al., 2004).

Hydrophobic effect (attraction)

When the surface of a particle has non-polar or ionic groups or hydrogen-binding sites, its surface has some degree of hydrophobic character when dispersed in water (Gregory, 1993). The hydrophobic effect is long-range force and can be effective at distance more than 2 nm (Lee, 1991).

Steric stabilization (repulsion) or Electrosteric repulsion

Several studies reported the surface macromolecules that are present on the surface of oocysts can give rise to electrosteric repulsion between oocysts and surfaces (Considine et al., 2002; Kuznar & Elimelech, 2006; Kuznar, 2005; Liu et al., 2009). Electrosteric repulsion is relevant to surfaces containing polyelectrolytes where both electrostatic and steric repulsive forces are present. Kuznar and Elimelech (2004) demonstrated that the classic DLVO theory drastically overpredicts the deposition rate of the viable oocysts onto the quartz surface in the presence of a monovalent salt and approves the presence of non-DLVO repulsive force that prevents oocysts attachment. They proposed the presence of a steric repulsive force, originating from the protein layer on the oocysts surfaces, which significantly reduces the oocysts deposition rate. Kuznar and Elimelech (2005) then observed improved attachment efficiency due to the presence of divalent cations and they hypothesized that the charge neutralization of the surface proteins by divalent cations results in the conformational changes and the subsequent collapse of the proteins which can eliminate steric repulsion. Gregory (1993) found that the low negative zeta potential of most natural aquatic colloids is due to the adsorption of natural anionic material from microorganism extracellular polymers. Other study (Considine et al., 2002) also prove that some steric stabilization from the fine hair or brushlike protrusions on the surfaces of fresh oocysts prevent oocyst adhesion to surface.

Polymer bridging (attraction)

In water treatment, anionic polymers are added to improve the flocculation of same negatively charged particles to form aggregation (flocs) by linking carboxylate groups with anionic sites on particle surfaces, even though they repel each other due to the same sign of surface charge (Gregory, 1993).

Born force (repulsive)

Born force is resulting from the overlapping of the electron cloud and therefore is repulsive. Together with structural forces, these repulsive forces play important roles in particle detachment from collector surfaces (Raveendran & Amirtharajah, 1995).

2.3.2.4. Extended DLVO theory

Since the traditional models have been unable to describe environmental colloidal behavior (Grasso* et al., 2002), extension of the DLVO theory is proposed and it is initially suggested by Van Oss (1989), including the hydrophobic/hydrophilic interactions (Lewis acid-base interfacial energy). These interactions are asymmetric; consisting of an electron accepting component (γ^+) and an electro donating component (γ^-). The value of γ^+ and γ^- can be calculated through surface tension measurements (i.e. contact angles) with polar liquids.

$$\Delta G^T = \Delta G^{LW} + \Delta G^{EL} + \Delta G^{AB} \qquad (2\text{-}5)$$

Where ΔG^{LW}, ΔG^{EL} and ΔG^{AB} denote London-van der Waal, electrostatic, and Lewis acid-base interaction energy.

In the review of non-DLVO interactions in environmental colloidal system, Grassi and co-works (2002) concluded that no single approach can be applied to all scenarios in the complex environmental system. The initial assumptions, boundary conditions and parameter estimations techniques used as part of the model must temper the results.

2.3.3 Detachment mechanisms

Detachment refers to the release of particles that have been captured by the grains of the media during the filtration process (Chung, 2012) and it happened when the hydrodynamic forces overcome the adhesion force between the particle and the filter media (Bai & Tien, 1997). Detachment is dependent on both physical and chemical factors. The physical factors include head loss gradient and surface roughness; chemical factors include changes of pH and ionic strength of solution that favoring the occurrence of detachment (Chung, 2012). In order to reduce the potential of detachment and favor the attachment of particles to the filter media, (1) hydrodynamic forces should be lower than the attachment forces;

(2) the thickness of the repulsive double layer should be reduced by increasing the ionic strength or decreasing the pH of solution to compress the electrical double layers. Instabilities caused by incoming particles is also a factor that impact particle detachment. Worth noting, detachment of loosely attached particles may occur during the transport process, but it is not the dominant process since the rate of detachment is much smaller than the deposition rate (Tufenkji, Ryan, & Elimelech, 2002). In summary, the understanding of detachment mechanism provides information on minimizing the detachment and extending filter run times.

2.4 Operational factors affecting particle removal

This section describes the main operational factors that affecting the removal of oocysts and other particles that similar in size in the filtration process, including the chemical pretreatment processes that used to improve the filter influent quality. It is followed by the identification of potential operational factors affecting *Cryptosporidium* removal by granular media filtration and further discussion examined by reviewing the filtration studies. Figure 2-6 listed the potential factors that may affect the filter removal efficiency.

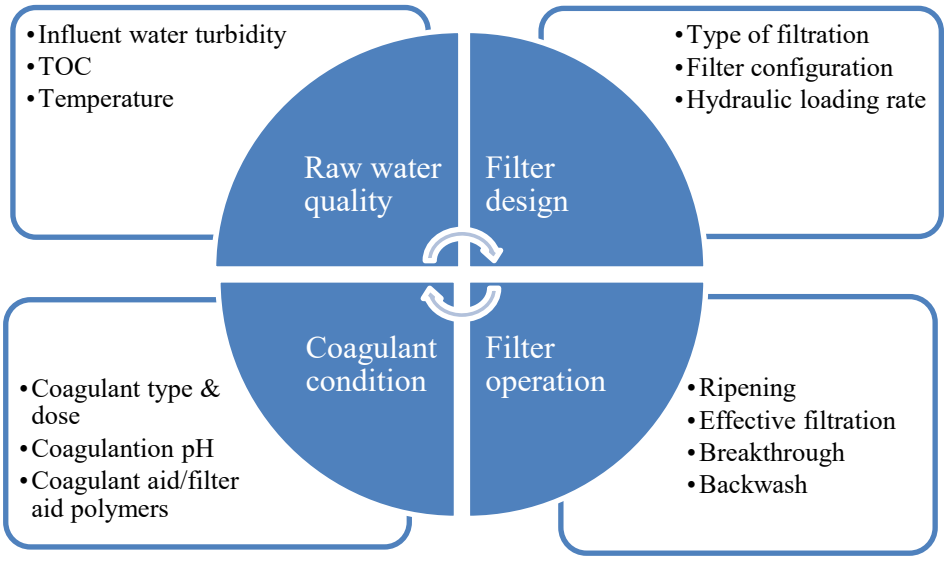

Figure 2-6. Operational factors affecting *Cryptosporidium* removal in filtration.

2.4.1 Raw water quality

2.4.1.1 Influent water turbidity

Influent water turbidity plays a role in filtration because it challenges treatment process effectiveness. Also, turbidity can interfere with *Cryptosporidium* detection and thus potentially influences removals (Zhou, 2016). Higher turbidity of influent water can dramatically shorten the filter run time and thus reduced water treatment capacity.

2.4.1.2 Natural organic matter

The effect of natural organic matter (NOM) on the removal of oocysts has been studied in columns packed with glass beads (Dai & Hozalski, 2002, 2003). They found that the oocyst removal efficiency decreased by 14% in the presence of NOM. Also, they claimed that NOM significantly change the zeta potential oocysts making them more negative. The removal efficiency of oocysts declined significantly. Also, the bench-scale column studies done by Zhang et al., (2017a) indicated that humic acid adsorbed on the grain surfaces greatly enhanced the mobility of microspheres in the packed bed due to increased electrostatic repulsion from the more negative surface charge of microspheres and grain media. Other mechanisms, such as competing with the particles for deposition site, were reported to hinder the aggregation of particles and enhance transport in porous media (Dai & Hozalski, 2002; Abudalo et al., 2010; Yang, Kim, & Tong, 2012).

2.4.1.3 Temperature

Temperature affect the viscosity of the water. The more viscous the water (colder water temperature), the slowly particles move relative to the water to reach a grain surface (Ives and Sholji, 1965), thus the probability of particle removal is reducing. Ives and Sholji (1965) reported considerably decreasing in particle removal at colder water temperatures when other factors remained the same. However, Swertfeger et al. (1999) reported no difference in oocyst removal between summer and winter. Also, Huck et al. (2001) demonstrated that no significant variations in oocyst removals in Ottawa water treatment plants, where experienced a wide range of temperature from 1 to 27˚C. Comparable log removal of oocysts were also observed by States et al. (2002) when conducting various filtration experiments under different temperatures by using ferric chloride as coagulant.

The effect of water temperature on particle transport and removal are not easy to elucidate since many other factors can be affected by temperature change, such as clarification and raw water qualities, which making it almost impossible to ensure certain effects as exclusively temperature-related (Huck et al., 2001).

2.4.2 Filter design

2.4.2.1 Type of filtration (Conventional vs. direct filtration)

Conventional water treatment includes a series of processes, including coagulation, flocculation, clarification through sedimentation, filtration, and disinfection (Geldreich, 1996). Direct filtration is used for the treatment of superior quality of water supplies. It involves the addition of coagulant, rapid mix, flocculation, and filtration with the absence of a sedimentation or flotation between coagulation and filtration. Direct filtration could provide both long filter runs and efficient removal as a way of reducing chemical inputs, residual solids, and operational cost (James et al., 2011). The study of *Cryptosporidium* removal during direct filtration is the focus of this project, with intention to gain more knowledge on the impact of operational factors, such as coagulant type, filter aid polymer type and dose, on the oocyst removal when water has good quality at ice-coved temperature.

2.4.2.2 Filter configuration

There are many types of filter media available in water treatment process. Sand, anthracite, granular activated carbon (GAC), garnet, and ilmenite with various sizes, shapes, roughness, and uniformity coefficient are common in bench-, pilot- and full-scale applications. Some are used alone, and others are used only in combination with other media. Configurations, such as single-, dual-, and multi-media, are usually different from plant to plant. Swertfeger et al. (1999) examine different configurations of filter media to remove parasites and they concluded that “no media type outperformed the other”, means that using parasite removal as a tool to choose the best available medium for the drinking water treatment is not appropriate. Many investigation of media type and design on oocyst removal demonstrated a negligible effect (Dugan et al., 2001; Swertfeger et al, 1999;

Patania, 1995). These data suggest, however, that parasite removal will not be compromised regardless of the medium chosen.

Dual (Anthracite/sand) media filters (or regular bed)

Dual media bed contains two layers. The fine bottom layer is usually silica sand that is used as a polishing layer, while the upper coarse layer usually consists of an anthracite coal which is used as the roughing layer. The two dissimilar materials have different particle sizes and specific gravities, which allow the layers to separate and re-stratify during the high rate water backwash.

Deep dual (anthracite/sand) media filters (or deep bed)

Deep bed is believed to be the most effective and economical way to treat large quantities of liquids containing relatively low solid volume fractions of particles with fine or colloidal size that are less than 30μm in diameter (Tien & Payatakes, 1979). Swertfeger et al. (1999) examined the oocyst removal by 3 types of filters, such as sand only, typical dual anthracite/sand media, deep anthracite/sand media, by spiking heat-inactivated oocysts, and they demonstrated that all of the media types used in this study had very similar removal capabilities, with average oocyst removal of 2.7-3.9 log.

2.4.2.3 Hydraulic loading rate

Hydraulic loading rate can significantly impact the filter performance and it ranges from 2.45 to 15 m/h in published studies (Zhou, 2016). In bench-scale studies, the removal efficiency of oocyst or its surrogates decreased with increasing hydraulic loading rate (Shaw, Walker, & Koopman, 2000; Kim, Walker, & Bradford, 2010; Zhang, 2016), and they concluded that as the water velocity increased the magnitudes of the drag and lift forces that act on the particles increased (Li & Johnson, 2005). Higher hydraulic loading rate resulted in the enhanced transport and subsequently less deposition of particles in the filter column. Contrarily, in many pilot-scale studies, no obvious effect of hydraulic loading rate has on the removal of particles. In the review of Hijnen and Medema (2010), they found at rate less than 20 m/h no apparent relationship between hydraulic loading rate and oocyst removal is exist. Also, Harrington et al. (2003) did not observe the effects of

different hydraulic loading rates (5, 10, 15 and 20 m/h) on oocyst removal and effluent turbidity during stable operation. Undoubtedly, higher hydraulic loading rate has associated with increased headloss and shortened filter run time. The optimal hydraulic loading rate is necessary to determine in the specific plant to assess whether the direct relationship between flow rate and oocyst removal can be obtained.

2.4.3 Coagulation conditions

Studies have demonstrated that *Cryptosporidium* removal throughout all stages of the treatment process is largely influenced by the effectiveness of coagulation pretreatment (Dugan et al., 2001). Without optimal coagulation, the removal efficiency of treatment processes could be significantly dampened. Pretreatment influences the balance between attachment and detachment mechanisms of flocs that accumulate within the filter media (Chung, 2012). Emelko (2001) stated that the pretreatment influences whether the particle/flocs will be retained in filters (attachment), retained and subsequently released from filters (detachment), or pass through filters (non-attachment). Understanding the coagulation conditions, such as coagulant type and dose, pH, the use of coagulant aid and filter aid, to reach the maximum potential of filters to removal particles will improve the overall treatment efficiency.

2.4.3.1 Coagulant type and dose

Aluminium-based salts, iron-based salts (ferric chloride) or organic polymers are the most common water treatment coagulant chemicals (Betancourt & Rose, 2004). Alum (Aluminium sulphate) is the most commonly used coagulant among others. Alum is generally seen to be less effective at low temperatures, which has been attributed to lower density flocs and aggregate size (Hanson & Cleasby, 1990). In direct filtration, the slower rate of flocculation could mean that there has been inadequate time for flocculation prior to filtration during the winter. Hanson and Cleasby (1990) also reported that alum flocs at low temperatures were very vulnerable to break up due to fluid shear. Disadvantages associated with the use of alum are summarized by Gebbie (2001) which includes:

- Limited coagulation pH range: 5.5-6.5

- Supplemental addition of alkalinity to the raw water is often required to achieve the optimum coagulation pH, particularly for soft, colored surface waters;
- Residual alum levels in the treated water can often exceed acceptable limits;
- Alum floc produced is particularly fragile. This is especially important if a coagulant is required to maximize color removal in a microfiltration-based water treatment process;
- Produced sulphuric acid can react with alkalinity in the raw water to produce carbon dioxide, thus depressing the pH.

Many alternative aluminum-based coagulants have been developed for water treatment applications. These compounds have the general formula $(Al_n(OH)_mCl_{(3n-m)})_x$ and have a polymeric structure, totally soluble in water. Polyaluminium chloride (PACl, n=2 and m=3) offers the following advantages, which is summarized by Gebbie (2001) as following:

- PACl consumes less alkalinity than alum; PACl is basic. The higher the basicity, the lower will be the consumption of alkalinity in the treatment process and hence impact on pH.
- They are effective over a broader pH range from 5.0 to 8.0;
- Reduced concentration of sulphate added to the treated water;
- It can work extremely well at low raw water temperatures;
- Flocs formed from alum at low temperatures settle very slowly, whereas flocs formed from Polyaluminium coagulants settle well at both low and normal water temperatures;
- Lower dosed are required to give equivalent results to alum; For example, a dose of 12 mg/L PACl (as 100%) was required for treatment of a colored, low turbidity water (Otway region, Victoria) compared to similar performance obtained when using an alum dose of 55 mg/L;
- Increase in chloride in the treated water is much lower than the sulphate increase from alum, resulting in lower overall increases in the total dissolved solids of the treated water.

One possible disadvantage in using PACl relates to the removal of DOC (dissolved organic carbon) from water (Gebbie, 2001). Another concern with PACl is that it would result in a

higher Cl/SO_4 mass ratio (CSMR) in the treated water. PACl adds chloride to the water thereby increasing CSMR while alum adds sulphate to the water thereby decreasing CSMR (Dudi, 2004; Edwards & Triantafyllidou, 2007; El Henawy, 2009). The higher CSMR from PACl has been associated with galvanic lead corrosion in the distribution system due to increased water conductivity (El Henawy, 2009). It is well reported that effective DOC removal is possible with alum, particularly when coagulating at lower pH values using so-called "enhanced coagulation". Also, alum seems to be a better coagulant relative to polyaluminium coagulants when removal of humic and fulvic color constituents are concerned. It is possible that removal of Trihalomethanes (THM) precursors may not be as complete as with alum because higher coagulation pH is associated with polyaluminium coagulants.

One of the most crucial steps in applying a coagulant is the determination of its optimal dosage. Very small dose many not be sufficient, whereas too large quantity can result in the inversion of the particle charge, re-stabilizing the colloid and consequently poor filtration (Tien & Payatakes, 1979). Typically, a jar test can be applied as a simple practical approach to approximate the optimal dosage of coagulant.

2.4.3.2 Coagulation pH

Krasner and Amy (1995) proposed enhanced coagulation by modifying the conventional treatment through reducing the pH to levels of 5-6 during the coagulation process and/or use higher doses of coagulants to achieve lower pH, which also shown to control the formation of disinfection by-products (DBP) precursors. States et al. (2002) investigated the influence of decreased coagulation pH levels on the removal of oocysts, reduction of total organic carbon (TOC), reduction of turbidity and particle count in the filter effluent. A series of pilot experiments using three different coagulants, including alum, ferric chloride, and polyaluminium chloride, were conducted at various pH levels. The results shown that lowing coagulation pH does not interfere with removal of *Cryptosporidium,* but TOC reduction is significantly enhanced.

2.4.3.3 Coagulant aid or Filter aid

Coagulant aid is used to reduce the dose of coagulant. Due to the lag time of floc formation after coagulant addition, coagulant aid such as cationic polyelectrolytes are added to contribute to the formation of polymer-particle flocs that are larger and stronger than those obtained with conventional chemical pretreatment (coagulant addition alone) (Zhu et al., 1996). Patania (1995) demonstrated that without chemical pretreatment, either coagulant addition alone or coagulant with cationic polyelectrolyte as coagulant aid, a rapid deep bed cannot provide effective barrier to removal microorganisms investigated.

Filter aid polymer (FP) is a polymer added to the flow just before the filter and functions by attaching to filter grains, which facilitate the filter performance by the formed polymer-particle flocs that are larger and stronger than those achieved with chemical pretreatment alone (Zhu, et al., 1996 and Zhou, 2016). The polymer molecules then worked together with filter grains acting as collector for destabilized colloid particles (Hendricks, 2006). FP can improve the quality of filter effluents, resist early breakthrough, and reduce the magnitude and duration of ripening (Zhu et al., 1996). Same as using coagulants, the headloss is associated with the addition of FP. Yao et al. (1971) stated that the filter aid induced particle bridging for particles that originally smaller than 1 μm and thus promoted their removal efficiency but the larger particles were not affected. The investigations on the type and dose of filter aid polymers to improve the removal efficiency of oocyst-sized particles or microorganisms are still needed.

2.4.4 Filter operation

Typically, a filter run follows a characteristic pattern with three distinctive segments, ripening, effective filtration and breakthrough, seen in Figure 2-7.

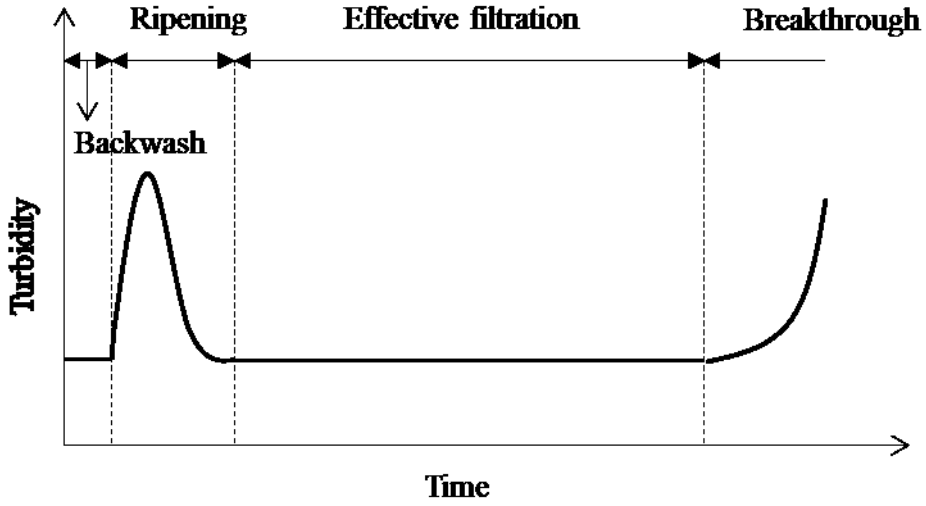

Figure 2-7. Operation of a rapid filter-effluent turbidity versus time (adapted from Crittenden et al., 2005).

2.4.4.1 Ripening

The first segment is the maturation of the filter, called ripening, with the filter effluent turbidity rises to a peak and then falls. In this process, the filter media are considered conditioning with coming particles collected within the clean media. The particles captured during ripening improve the overall efficiency of the filter by providing a better collector surface than uncoated media grains (Crittenden et al., 2005). It has been observed that ripening depends on particle size, with intermediate sized particles (3-7 μm) ripening early and the smaller particles ripened for the longest duration (Moran et al., 1993).

The protozoan passage through granular media filters during ripening were reported in a variety of findings. Logsdon et al. (1981) observed a higher *Giardia* cysts passage during ripening than during stable operation even at low effluent turbidities. Huck et al. (2002) observed minimal or moderate deterioration ($\leqslant$0.5 log10 Unit) of *Cryptosporidium* removal during filter ripening under the conditions of experiments in two pilot-scale plants relative to stable optimized filtration. Their observations are consistent with other findings from literature (LeChevallier & Norton, 1995; Patania, 1995;. Huck, 2001). To ensure the removal of oocysts in drinking water treatment process, the understanding of ripening in filtration is essential; however, it is still not completely understood and the initial particle

breakthrough into the filtered water is not always well managed at water treatment plants (WTPs) (Amburgey et al, 2005).

2.4.2.2 Breakthrough

After ripening, effluent turbidity can be maintained at a steady-state value below 0.1 NTU, which is an indicator of good filter performance (LeChevallier & Norton, 1992; Nieminski & Ongerth, 1995). When the effluent turbidity exceeds 0.1 NTU, it will cause the substantial deterioration in oocyst removal from pilot plant investigation (Zhou, 2017). Logsdon et al. (1981) observed the *Giardia* cyst passage during early breakthrough conditions when the filter effluent turbidity was just above 0.2 NTU. When the filter effluent turbidity changed from 0.1 to 0.2 NTU, Patania et al. (1995) observed *Giardia* passage through filters. Filter effluent turbidity is a readily measured parameter for water utilities, and it is mandatory from regulations and guidelines to ensure health-based pathogen removals. The Guideline for Canadian Drinking Water Quality (2017) requires the single filter effluent turbidity be less than or equal to 0.3 NTU in 95% of measurements and never exceed 1.0 NTU.

However, the use of turbidity as an indicator for protozoa breakthrough during filtration is still a controversy. Low filter effluent turbidity cannot confirm the presence or absence of oocysts nor the magnitude of oocyst removal (Zhou, 2016). No precise relationship between turbidity reduction and that of oocysts has been observed (Health Canada, 2017) even three correlations were reported from the past with correlation coefficient ranging from 0.17 to 0.73 (LeChevallier & Norton, 1992; Nieminski & Ongerth, 1995). Huck et al. (2002a) examined removal from two pilot plants with similar filter effluent turbidity, 2-log removal difference were observed. Also, increase in turbidity under the non-optimized conditions were less directly related to deterioration in oocyst removal. Therefore, traditional filter performance measures (filter effluent turbidity) are not quantitative indicators of oocyst removal capability (Huck et al., 2002), although this parameter is stringently regulated and the attainment of specific values allows a utility to claim *Cryptosporidium* removal credits by maintaining turbidity below the set amount (USEPA, 2006; Health Canada, 2017; Zhou, 2016).

2.4.4.3 Backwash

During breakthrough, the filter contains so many particles that it can no longer retain particles and thus the effluent turbidity increases (Crittenden et al., 2005). Once the filter reached breakthrough, or towards to the end of a filter run, backwash by filtered water flowing upward through the filter bed is required to prevent high-turbidity water from entering the distribution system. The stronger attachment of particle with filter media during the optimization of chemical pretreatment process may make it more difficult to remove particle during subsequent filter backwashing (Emelko et al., 2005). Initial degradation of effluent quality and the potential of pathogen release may be associated with the ineffective media cleaning from backwashing (Amirtharajah & Wetstein, 1980). The flow rate of backwash must be great enough to flush out retained particles but not too high to flush out the media from the filter box (Crittenden et al., 2005). A certain level of bed expansion is required and Kawamura (2000) suggested target expansion rate for anthracite and sand, which is about 25% and 37%, respectively. Ensuring the removal of pathogenic particles thoroughly during the backwashing prevents release of them during ripening or later effective filtration operation (Huck, 2001). The optimal and most common practiced backwashing strategy involves the simultaneous application of air scour with sub fluidization water wash, which proved to reduce the number of oocysts sized particles in filter effluents during ripening (Colton, Hillis, & Fitzpatrick, 1996).

2.5 Pilot- and full-plant studies of *Cryptosporidium* removal from water

To better understand filtration efficiency for *Cryptosporidium* removal, a number of pilot- and full-scale filtration experiments have been conducted. They have been summarized in Table 2-2. Results from published pilot- and full-scale studies illustrate a wide variation in reported oocyst or its surrogate removal, from 0.5 log to 6.0 log by granular media filters only. A review by Emelko et al. (2005) compared many reported log removals and suggested that 3 log *Cryptosporidium* removal can be typically obtained from filtration after optimization of chemical pretreatment. USEPA (2006) assigned a 3-log removal credit of *Cryptosporidium* for conventional filtration processes and a 2.5-log removal credit for direct filtration processes. Health Canada (2017) adopted similar log removal credits for granular media filtration processes after a review of many published studies and its own

review. Hijnen and Medema (2010) proposed microorganism elimination credits (MECs) of 2.6 to 3.0 log for rapid granular filters preceded with coagulation and flocculation. They also speculated that the variability in reported log removal may be attributed from the microorganism type, raw water characteristics, temperature, process set-up and operations. In the study of Huck et al. (2002), analytical reliabilities, sample volume processed, detection limits, and microorganism concentration in filter influent also contribute to the log removal variations in published results.

Table 2-2. Summary of drinking water treatment processes using *Cryptosporidium* surrogates.

References	**Type of treatment**	**Filter bed Media porosity**	**Loading rate**	**Log removal**	**Surrogates used**	**Observations of relevance to present discussion**
Amburgey et al., 2001	Direct filtration with coagulation (Pilot-scale)	Anthracite and GAC (Pilot biofilter); Anthracite (Preozonation and batch coagulation)	568 ML/d (48 mm Diameter)	1.7-2.2	Carboxylated polystyrene beads (4.5 μm); Heat-inactivated oocysts	The removal of carboxylated beads (2.0 log) was consistently greater than that of heat-inactivated oocysts (1.5 log).
Dugan *et al*. 2001	Conventional (full-scale)	Sand or Dual media	5 or 10 m/h	>4.0 log	Turbidity, aerobic endospores, particle counting	Turbidity has no correlation to oocyst removal. Aerobic endospores can be a conservative surrogate for *Cryptosporidium* due to 1-2.5 log less removal observed.
Emelko et al., 2001	Conventional (pilot plant)	Anthracite and sand	9.72 m/h	3.9-5.0	Carboxylated microspheres	Oocysts and microspheres removals were comparable despite varied influent concentrations.
Huck et al., 2001 and 2002	Conventional (pilot plant) High alum dose Low alum dose	Anthracite and sand	9.72 m/h	4.7-5.8 log for high; 2.0-5.0 log for low.	No surrogates. Oocysts dosed.	Oocysts removal were 0.5-1.0 log lower during ripening than during stable operation. Effluent quality deteriorated during end of run and early breakthrough even at turbidity <0.3 NTU.

Harrington et al., 2003)	Conventional (pilot-scale)	Dual media Tri-media		2.0 log	Turbidity, UV_{254}, waterborne pathogens, i.e. aerobic bacteria, virus	
Kim & Zydney, 2004	Coagulation and filtration	Sand			Polymethyl-meta-acryl spheres, polystyrene spheres	Good correlation with similar sized particles.
Emelko, 2003	Conventional (pilot-scale)	Dual media Tri-media		0.5 to 5.0 log	Fluorescence polystyrene microspheres; Formalin-inactivated oocysts	
Hijnen et al., 2004	Slow sand filters (pilot-scale)	Sand		5.0-6.0 log	*C. perfringens*	*C. perfringens* was not suitable surrogates.
Amburgey et al., 2005	Conventional (pilot-scale)	Anthracite	568 ML/d		Microspheres	Microspheres are a good surrogate for oocysts.
Hijnen et al., 2007	Rapid sand filtration-ozonation-GAC filtration (full-scale)	Sand		4.7 log	*C. perfringens*	*C. perfringens* was not suitable surrogates.

Lu & Amburgey, 2016	High rate sand filtration with coagulation (full-scale)	Sand	31-34 m/h	2.0 log	4.5µm polystyrene microspheres	Coagulant addition was effective at increasing the removal of oocysts from full-scale swimming pools in both single dosing and continuously dosing scenarios.
Lu et al., 2017	Rapid sand filtration (full-scale)	Perlite-sand filtration and diatomaceous earth (DE) precoat filtration	3.6 m/h	2.7 log	Fluoresbrite® Carboxylate YG polystyrene microspheres (4.5µm)	Sand filters are relatively ineffective for oocyst and microspheres removal for swimming pools. Either a DE precoat filter or a perlite-sand filter can improve the efficiency of removal of microspheres and oocysts from swimming pools over a standard sand filter.

2.6 Implications from literature findings

The main conclusions from the above literature review are listed as following:

Filtration mechanisms

- There are several filtration mechanisms applicable to filtration using granular media, including transport, attachment, and detachment
- Interception and sedimentation are the predominant mechanisms for the removal of *Cryptosporidium* and other pathogens which similar in size without the addition of coagulant. When the travelling particles are within 100 nm of the filtration media, the surface forces become leading forces to capture the fine particles.
- The capture of the fine particles by filter media can be also driven by a balance between attractive hydrophobic forces or other short-range specific attractive forces and electrostatic repulsion between the similarly charged media grains (negatively charged) and particles (negatively charged).
- For the similarly charged grain media and particles, their interaction can be attractive due to the electrostatic interaction.
- Other attractive forces can be hydrophobic forces, hydrogen bonding and polymer bridging.

Filtration processes

- The criteria to select representative *Cryptosporidium* surrogates in filtration processes, includes their similarities in size, shape, surface properties (surface charge and hydrobiotite), ease to detect, cost-effective, and not-posing hazard to the public.
- The study of modification of microspheres to represent the biomolecule on the surface of oocyst was limited.
- The chemical pretreatment play an important role in the removal of particle in granular media filtration. The operational factor in direct filtration mode was not fully studies for the removal of *Cryptosporidium*.

2.7 Research need

The information gained from this review can provide a basis for water treatment design and operation. Several types of surrogates for the removal of viable oocyst by drinking water treatment processes have been evaluated. These include turbidity, particle counts, spores, yeasts, inactivated oocysts (heat treatment or formalin treatment), microspheres, and glycoprotein modified microspheres. Among these potential surrogates, some (yeast, spores, microspheres) have very different surfaces properties and others (turbidity, particle counts) are indicative of treatment efficiency but not oocyst removal. The modification on the microspheres need to be further evaluated to select the most representative surrogates of *Cryptosporidium*.

The solution chemistry play an important role in the interaction between oocysts and filter media. Ionic strength, pH, and DOC concentration of aqueous solution were main factors that had been evaluated by many researchers. The interactions of modified microspheres have not been assessed previously by using copolymers-modified microspheres. Single-collector contact efficiency and DLVO interaction energy could potentially provide the explanation of transport and retention of surrogates in the porous media.

Pilot-scale filtration experiments were necessary to determine the impact of the chemical pretreatment process and filter configuration on the removal of *Cryptosporidium* surrogates. The operational variables that affect chemical pretreatment processes examined in the direct filtration mode included coagulant type, filter aid polymer type and dose, and filter configuration (i.e. deep bed and regular bed). Direct filtration that had been implemented as a way of reducing chemical inputs, residual solids and operational cost has not yet been fully studied for pathogen removal, specifically *Cryptosporidium*. The relationship between on-line performance parameters (turbidity and particle count) and surrogates removal were evaluated and applied to the development of practical treatment strategies for maximizing *Cryptosporidium* removal by granular media filtration in direct filtration mode.

References

Abudalo, R. A., Ryan, J. N., Harvey, R. W., Metge, D. W., & Landkamer, L. (2010). Influence of organic matter on the transport of Cryptosporidium parvum oocysts in a ferric oxyhydroxide-coated quartz sand saturated porous medium. *Water Research*, *44*(4), 1104–1113. https://doi.org/10.1016/j.watres.2009.09.039

Amburgey, J. E., Amirtharajah, A., Arrowood, M. J., & Spivey, N. C. (2001). Cryptosporidium and Fluorescent Microsphere Surrogate Removals by Conventional and Biological Filters. In Proc. 2001 AWWA WQTC, Denver.

Amburgey, J. E., Amirtharajah, A., York, M. T., Brouckaert, B. M., & et al. (2005). Comparison of Conventional and Biological Filter Performance for Cryptosporidium and microsphere removal. *American Water Works Association. Journal; Denver*, *97*(12), 77–91,10.

Amirtharajah, A. (1988). Some Theoretical and Conceptual Views of Filtration. *Journal (American Water Works Association)*, *80*(12), 36–46.

Amirtharajah, A., & Wetstein, D. P. (1980). Initial degradation of effluent quality during filtration. *Journal (American Water Works Association)*, 518–524.

Atlas, A. B. R. F. (n.d.). Surveillance for Waterborne-Disease Outbreaks—United States, 1995–1996 Cardiovascular Disease Risk Factors and Preventive Practices Among Adults—United States, 1994.

Bai, R., & Tien, C. (1997). Particle Detachment in Deep Bed Filtration. *Journal of Colloid and Interface Science*, *186*(2), 307–317. https://doi.org/10.1006/jcis.1996.4663

Balderrama-Carmona, A. P., Gortáres-Moroyoqui, P., Álvarez-Valencia, L. H., Castro-Espinoza, L., Balderas-Cortés, J. de J., Mondaca-Fernández, I., … Meza-Montenegro, M. M. (2014). Quantitative microbial risk assessment of *Cryptosporidium* and *Giardia* in well water from a native community of Mexico. *International Journal of Environmental Health Research*, 1–13. https://doi.org/10.1080/09603123.2014.989492

Bastos, R. K. X., Viana, D. B., & Bevilacqua, P. D. (2013). Turbidity as a surrogate for *Cryptosporidium* removal by filtration in drinking-water QMRA models. *Water Science and Technology: Water Supply*, *13*(5), 1209–1219. https://doi.org/10.2166/ws.2013.127

Bertolucci, G. C., Gilli, G., Carraro, E., Giacosa, D., & Puppo, M. (1998). Influence of raw water storage on Giardia, Cryptosporidium and Nematodes. *Water Science and Technology*, *37*(2), 261.

Betancourt, W. Q., & Rose, J. B. (2004). Drinking water treatment processes for removal of Cryptosporidium and Giardia. *Veterinary Parasitology*, *126*(1), 219–234. https://doi.org/10.1016/j.vetpar.2004.09.002

Bowen W. Richard, Sabuni Hoze A. M., & Ventham Timothy J. (2004). Studies of the cell‐wall properties of Saccharomyces cerevisiae during fermentation. *Biotechnology and Bioengineering*, *40*(11), 1309–1318. https://doi.org/10.1002/bit.260401104

Brush, C. F., Walter, M. F., Anguish, L. J., & Ghiorse, W. C. (1998). Influence of Pretreatment and Experimental Conditions on Electrophoretic Mobility and

Hydrophobicity of Cryptosporidium parvum Oocysts. *Applied and Environmental Microbiology*, *64*(11), 4439–4445.
Bukhari, Z., Smith, H. V., Sykes, N., Humphreys, S. W., Paton, C. A., Girdwood, R. W. A., & Fricker, C. R. (1997). Occurrence of Cryptosporidium spp oocysts and Giardia spp cysts in sewage influents and effluents from treatment plants in England. *Water Science and Technology*, *35*(11–12), 385.
Bustamante, H. A., Raj Shanker, S., Pashley, R. M., & Karaman, M. E. (2001). Interaction between cryptosporidium oocysts and water treatment coagulants. *Water Research*, *35*(13), 3179–3189. https://doi.org/10.1016/S0043-1354(01)00044-6
Cacciò, S. M., Thompson, R. C. A., McLauchlin, J., & Smith, H. V. (2005). Unravelling Cryptosporidium and Giardia epidemiology. *Trends in Parasitology*, *21*(9), 430–437. https://doi.org/10.1016/j.pt.2005.06.013
Carey, C., Lee, H., & Trevors, J. (2004). Biology, persistence and detection of Cryptosporidium parvum and Cryptosporidium hominis oocyst. *Water Research*, *38*(4), 818–862. https://doi.org/10.1016/j.watres.2003.10.012
Casemore, D. P. (1997). Cryptosporidiosis-human and animal epidemiology. Cryptosporidium and cryptosporidiosis, 65-92.
Chalmers, R. M., & Giles, M. (2010). Zoonotic cryptosporidiosis in the UK - challenges for control: Zoonotic cryptosporidiosis. *Journal of Applied Microbiology*, *109*(5), 1487–1497. https://doi.org/10.1111/j.1365-2672.2010.04764.x
Chatterjee, A., Banerjee, S., Steffen, M., O'Connor, R. M., Ward, H. D., Robbins, P. W., & Samuelson, J. (2010). Evidence for Mucin-Like Glycoproteins That Tether Sporozoites of Cryptosporidium parvum to the Inner Surface of the Oocyst Wall. *Eukaryotic Cell*, *9*(1), 84–96. https://doi.org/10.1128/EC.00288-09
Chauret, C. P., Radziminski, C. Z., Lepuil, M., Creason, R., & Andrews, R. C. (2001). Chlorine Dioxide Inactivation of Cryptosporidium parvum Oocysts and Bacterial Spore Indicators. *Applied and Environmental Microbiology*, *67*(7), 2993–3001. https://doi.org/10.1128/AEM.67.7.2993-3001.2001
Chung, J. (2012). *Development of fluorescently labelled Cryptosporidium oocyst surrogates to test the efficacy of sand filtration processes*. University of New South Wales.
Clancy Jennifer L., Bukhari Zia, Hargy Thomas M., Bolton James R., Dussert Bertrand W., & Marshall Marilyn M. (2000). Using UV to inactivate Cryptosporidium. *Journal - American Water Works Association*, *92*(9), 97–104. https://doi.org/10.1002/j.1551-8833.2000.tb09008.x
Colton, J. F., Hillis, P., & Fitzpatrick, C. S. B. (1996). Filter backwash and start-up strategies for enhanced particulate removal. *Water Research*, *30*(10), 2502–2507. https://doi.org/10.1016/0043-1354(96)00085-1
Considine, R. F., Dixon, D. R., & Drummond, C. J. (2002). Oocysts of Cryptosporidium parvum and model sand surfaces in aqueous solutions: an atomic force microscope (AFM) study. *Water Research*, *36*(14), 3421–3428. https://doi.org/10.1016/S0043-1354(02)00082-9
Considine, R. F., Drummond, C. J., & Dixon, D. R. (2001). Force of Interaction between a Biocolloid and an Inorganic Oxide: Complexity of Surface Deformation,

Roughness, and Brushlike Behavior. *Langmuir*, *17*(20), 6325–6335. https://doi.org/10.1021/la001459+

Crittenden, J. C., & Harza, B. M. W. (2005). Water treatment: principles and design. Wiley

Copes, R., Hrudey, S. E., Payment, P., & Act, D. W. (2008). Turbidity and microbial risk in drinking water. *Prepared for The Minister of Health, Province of British Columbia, pursuant to Section*, *5*. Retrieved from http://www.waterbucket.ca/okw/sites/wbcokw/documents/media/10.pdf

Dai, X, Boll, J., Hayes, M. E., & Aston, D. E. (2004). Adhesion of Cryptosporidium parvum and Giardia lamblia to solid surfaces: the role of surface charge and hydrophobicity. *Colloids and Surfaces B: Biointerfaces*, *34*(4), 259–263. https://doi.org/10.1016/j.colsurfb.2003.12.016

Dai, Xiaojun, & Hozalski, R. M. (2002). Effect of NOM and biofilm on the removal of Cryptosporidium parvum oocysts in rapid filters. *Water Research*, *36*(14), 3523–3532. https://doi.org/10.1016/S0043-1354(02)00045-3

Dai, Xiaojun, & Hozalski, R. M. (2003). Evaluation of Microspheres as Surrogates for Cryptosporidium parvum Oocysts in Filtration Experiments. *Environmental Science & Technology*, *37*(5), 1037–1042. https://doi.org/10.1021/es025521w

D'Antonion RG, Wine RE, Taylor JP, & et al. (1985). A waterborne outbreak of cryptosporidiosis in normal hosts. *Annals of Internal Medicine*, *103*(6_Part_1), 886–888. https://doi.org/10.7326/0003-4819-103-6-886

Dengis, P. B., Nelissen, L. R., & Rouxhet, P. G. (1995). Mechanisms of yeast flocculation: comparison of top-and bottom-fermenting strains. *Applied and Environmental Microbiology*, *61*(2), 718–728.

Dudi, A. (2004). *Reconsidering lead corrosion in drinking water: product testing, direct chloramine attack and galvanic corrosion* (Doctoral dissertation, Virginia Tech)

Dugan, N. R., Fox, K. R., Owens, J. H., & Miltner, R. J. (2001). Controlling "Cryptosporidium" Oocysts Uusing Conventional Treatment. *Journal (American Water Works Association)*, *93*(12), 64–76. https://doi.org/10.2307/41298128

Dumètre, A., Aubert, D., Puech, P.-H., Hohweyer, J., Azas, N., & Villena, I. (2012). Interaction Forces Drive the Environmental Transmission of Pathogenic Protozoa. *Applied and Environmental Microbiology*, *78*(4), 905–912. https://doi.org/10.1128/AEM.06488-11

Edwards, M., & Triantafyllidou, S. (2007). Chloride-to-sulfate mass ratio and lead leaching to water. *Journal (American Water Works Association)*, *99*(7), 96-109.

Efstratiou, A., Ongerth, J., & Karanis, P. (2017). Evolution of monitoring for Giardia and Cryptosporidium in water. *Water Research*, *123*, 96–112. https://doi.org/10.1016/j.watres.2017.06.042

El Henawy, W. (2009). *The Effect of Selected Coagulants on Chloride-to-Sulfate Mass Ratio for Lead Control and on Organics Removal in Two Source Waters* (Master's thesis, University of Waterloo).

Elimelech, M., & O'Melia, C. R. (1990). Kinetics of deposition of colloidal particles in porous media. *Environmental Science & Technology*, *24*(10), 1528–1536.

Elimelech, M., Gregory, J., & Jia, X. (2013). Particle deposition and aggregation: measurement, modelling and simulation. Butterworth-Heinemann.

Emelko, M. B., Huck, P. M., & Coffey, B. M. (2005). A review of Cryptosporidium removal by granular media filtration. *Journal (American Water Works Association)*, *97*(12), 101–115.
Emelko, M. B., Huck, P. M., & Douglas, I. P. (2003). "Cryptosporidium" and microsphere removal during late in-cycle filtration. *Journal (American Water Works Association)*, *95*(5), 173–182.
Emelko, M. B. (2003). Removal of viable and inactivated Cryptosporidium by dual- and tri-media filtration. *Water Research*, *37*(12), 2998–3008.
Emelko, M. B. (2001). Removal of Cryptosporidium parvum by granular media filtration.
Fayer, R, & Ungar, B. L. (1986). Cryptosporidium spp. and cryptosporidiosis. *Microbiological Reviews*, *50*(4), 458–483.
Fayer, Ronald, Farley, C. A., Lewis, E. J., Trout, J. M., & Graczyk, T. K. (1997). Potential Role of the Eastern Oyster, Crassostrea virginica, in the Epidemiology of Cryptosporidium parvum. *Applied and Environmental Microbiology*, *63*(5), 2086–2088.
Fayer, Ronald, Nerad, T., Rall, W., Lindsay, D. S., & Blagburn, B. L. (1991). Studies on Cryopreservation of Cryptosporidium parvum. *The Journal of Parasitology*, *77*(3), 357–361. https://doi.org/10.2307/3283119
Gao, X., & Chorover, J. (2009). In-situ monitoring of Cryptosporidium parvum oocyst surface adhesion using ATR-FTIR spectroscopy. *Colloids and Surfaces B: Biointerfaces*, *71*(2), 169–176. https://doi.org/10.1016/j.colsurfb.2009.02.003
Gebbie, P. (2001). Using polyaluminium coagulants in water treatment. In *Proc. 64th AWIEO Conference*. Citeseer. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.517.5307&rep=rep1&type=pdf
Gottinger, A. M., Bhat, S. V., McMartin, D. W., & Dahms, T. E. S. (2013). Fluorescent microspheres as surrogates to assess oocyst removal efficacy from a modified slow sand biofiltration water treatment system. *Journal of Water Supply: Research and Technology - Aqua*, *62*(3), 129. https://doi.org/10.2166/aqua.2013.104
Grasso*, D., Subramaniam, K., Butkus, M., Strevett, K., & Bergendahl, J. (2002). A review of non-DLVO interactions in environmental colloidal systems. *Reviews in Environmental Science and Biotechnology*, *1*(1), 17–38. https://doi.org/10.1023/A:1015146710500
Gregory, J. (1993). The role of colloid interactions in solid-liquid separation. *Water Science and Technology*, *27*(10), 1–17.
Gregory, J. (2005). *Particles in water: properties and processes*. CRC Press.
Haes, A. J., Zou, S., Schatz, G. C., & Van Duyne, R. P. (2004). Nanoscale Optical Biosensor: Short Range Distance Dependence of the Localized Surface Plasmon Resonance of Noble Metal Nanoparticles. *The Journal of Physical Chemistry B*, *108*(22), 6961–6968. https://doi.org/10.1021/jp036261n
Hancock, C.M., Rose, J.B. and Callahan, M., (1998). Cryptosporidium and Giardia in US groundwater. Journal of American Water Works Association. 90 (3):58-61.
Hansen, J. S., & Ongerth, J. E. (1991). Effects of time and watershed characteristics on the concentration of Cryptosporidium oocysts in river water. *Applied and Environmental Microbiology*, *57*(10), 2790–2795.

Hanson, A. T., & Cleasby, J. L. (1990). The Effects of Temperature on Turbulent Flocculation: Fluid Dynamics and Chemistry. *Journal (American Water Works Association)*, *82*(11), 56–73. https://doi.org/10.2307/41293075

Hargesheimer, E. E., Lewis, C. M., & Yentsch, C. M. (1992). *Evaluation of particle counting as a measure of treatment plant performance*. AWWA. Retrieved from http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=REPIDISCA&lang=p&nextAction=lnk&exprSearch=90333&indexSearch=ID

Harrington Gregory W., Xagoraraki Irene, Assavasilavasukul Prapakorn, & Standridge Jon H. (2003). Effect of Filtration Conditions on Removal of Emerging waterborne pathogens. *Journal - American Water Works Association*, *95*(12), 95–104. https://doi.org/10.1002/j.1551-8833.2003.tb10514.x

Harris, J. R., & Petry, F. (1999). Cryptosporidium parvum: Structural Components of the Oocyst Wall. *The Journal of Parasitology*, *85*(5), 839–849. https://doi.org/10.2307/3285819

Health Canada. (2012). *Guideline for Canadian Drinking Water Quality: Guideline Technical Document-Turbidity.* https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-turbidity.html(accessed on 0.1.15.2018)

Health Canada. (2017). *Guideline for Canadian Drinking Water Quality-Summary Table.* https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/ewh-semt/alt_formats/pdf/pubs/water-eau/sum_guide-res_recom/sum_guide-res_recom-eng.pdf (accessed on 01.15.2018)

Hendricks, D. W. (2006). *Water Treatment Unit Processes: Physical and Chemical*. CRC Press.

Hermansson, M. (1999). The DLVO theory in microbial adhesion. *Colloids and Surfaces B: Biointerfaces*, *14*(1), 105–119. https://doi.org/10.1016/S0927-7765(99)00029-6

Hijnen, W.A.M., Beerendonk, E. F., & Medema, G. J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research*, *40*(1), 3–22. https://doi.org/10.1016/j.watres.2005.10.030

Hijnen, W.A.M., Schijven, J. F., Bonné, P., Visser, A., & Medema, G. J. (2004). Elimination of viruses, bacteria and protozoan oocysts by slow sand filtration. *Water Science and Technology*, *50*(1), 147.

Hijnen, W.A.M, Van Veenendaal, D., van der Speld, W. M, Visser, A., Hoogenboezem, W., & van der Kooij, D. (2000). Enumeration of faecal indicator bacteria in large water volumes using on site membrane filtration to assess water treatment efficiency. *Water Research*, *34*(5), 1659–1665. https://doi.org/10.1016/S0043-1354(99)00311-5

Hijnen, W. A., & Medema, G. J. (2010). Elimination of micro-organisms by water treatment processes. Iwa Publishing.

Hijnen, Wim A. M., Dullemont, Y. J., Schijven, J. F., Hanzens-Brouwer, A. J., Rosielle, M., & Medema, G. (2007). Removal and fate of Cryptosporidium parvum, Clostridium perfringens and small-sized centric diatoms (Stephanodiscus hantzschii) in slow sand filters. *Water Research*, *41*(10), 2151–2162. https://doi.org/10.1016/j.watres.2007.01.056

Hipsey, M.R., & Brookes, J.D. (2017). Pathogen Management in Surface Waters: Practical Considerations for Reducing Public Health Risk.

Hogan, J. N., Daniels, M. E., Watson, F. G., Oates, S. C., Miller, M. A., Conrad, P. A., … Miller, W. A. (2013). Hydrologic and Vegetative Removal of Cryptosporidium parvum, Giardia lamblia, and Toxoplasma gondii Surrogate Microspheres in Coastal Wetlands. *Applied and Environmental Microbiology*, *79*(6), 1859–1865. https://doi.org/10.1128/AEM.03251-12

Huck, P. M., Coffey, B. M., Emelko, M. B., & O'Melia, C. R. (2000). The Importance of Coagulation for the Removal of Cryptosporidium and Surrogates by Filtration. In *Chemical Water and Wastewater Treatment VI* (pp. 191–200). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-59791-6_18

Huck, Peter M. (2001). Filter operation effects on pathogen passage / prepared by Peter M. Huck ... [et al.]; sponsored by Microbial/Disinfection By-Products Research Council. Denver, CO: AWWA Research Foundation and American Water Works Association, c2001.

Huck, Peter M., AWWA Research Foundation, & Microbial/Disinfection By-Products Research Council (U.S.) (Eds.). (2001). *Filter operation effects on pathogen passage*. Denver, CO: AWWA Research Foundation and American Water Works Association.

Huck Peter M., Coffey Bradley M., Emelko Monica B., Maurizio Danielle D., Slawson Robin M., Anderson William B., … O'Melia Charles R. (2002). EFFECTS OF filter operation on Cryptosporidium removal microbial pathogens. *Journal - American Water Works Association*, *94*(6), 97–111. https://doi.org/10.1002/j.1551-8833.2002.tb09493.x

Ives, K. J. (1982, May). Fundamentals of filtration. In *Proceedings of the 21st European Federation of Chemical Engineering Event Symposium on Water Filtration, Antwerp, Belgium*.

Zhang, H., Seaman, J., Wang, Y., Zeng, H., Narain, R., Ulrich, A, & Liu., Y (2017a). Filtration of Glycoprotein-Modified Carboxylated Polystyrene Microspheres as Cryptosporidium Oocysts Surrogates: Effects of Flow Rate, Alum, and Humic Acid. Journal of Environmental Engineering, 143(8). https://doi.org/10.1061/(ASCE)EE.1943-7870.0001201

Ives, K. J., & Sholji, I. (1965). Research on variables affecting filtration. Journal of the Sanitary Engineering Division, 91(4), 1-18.

Ives, K. (1970). Rapid filtration. *Water Research*, *4*(3), 201–223. https://doi.org/10.1016/0043-1354(70)90068-0

Jakubowski Walter, Boutros Susan, Faber Walter, Fayer Ron, Ghiorse William, LeChevallier Mark, … Stewart Mic. (1996). Environmental methods for Cryptosporidium. *Journal - American Water Works Association*, *88*(9), 107–121. https://doi.org/10.1002/j.1551-8833.1996.tb06617.x

James, W., Molla, S., McCrank, M., Craik, S. & Bhargava, A. (2011), *Less water treatment residuals and more options by converting to direct filtration operation during winter.* Presented at Western Canada Water Conference and Exhibit, Saskatoon, SW. September 20-23.

Jenkins, M. B., Eaglesham, B. S., Anthony, L. C., Kachlany, S. C., Bowman, D. D., & Ghiorse, W. C. (2010). Significance of Wall Structure, Macromolecular

Composition, and Surface Polymers to the Survival and Transport of Cryptosporidium parvum Oocysts. *Applied and Environmental Microbiology*, *76*(6), 1926–1934. https://doi.org/10.1128/AEM.02295-09

Karanis, P., Kourenti, C., & Smith, H. (2007). Waterborne transmission of protozoan parasites: A worldwide review of outbreaks and lessons learnt. *Journal of Water and Health*, *5*(1), 1–38. https://doi.org/10.2166/wh.2006.002

Karanis, Panagiotis, Schoenen, D., & Seitz, H. M. (1998). Distribution and removal of Giardia and Cryptosporidium in water supplies in Germany. *Water Science and Technology*, *37*(2), 9.

Kawamura, S. (2000). Integrated design and operation of water treatment facilities. john Wiley & sons.

Kim, H. N., Walker, S. L., & Bradford, S. A. (2010). Coupled factors influencing the transport and retention of Cryptosporidium parvum oocysts in saturated porous media. *Water Research*, *44*(4), 1213–1223. https://doi.org/10.1016/j.watres.2009.09.041

Kim, M., & Zydney, A. L. (2004). Effect of electrostatic, hydrodynamic, and Brownian forces on particle trajectories and sieving in normal flow filtration. *Journal of Colloid and Interface Science*, *269*(2), 425–431. https://doi.org/10.1016/j.jcis.2003.08.004

Korich, D. G., Mead, J. R., Madore, M. S., Sinclair, N. A., & Sterling, C. R. (1990). Effects of ozone, chlorine dioxide, chlorine, and monochloramine on Cryptosporidium parvum oocyst viability. Applied and environmental microbiology, 56(5), 1423-1428.

Krasner Stuart W., & Amy Gary. (1995). Jar ‐ test evaluations of enhanced coagulation. *Journal - American Water Works Association*, *87*(10), 93–107. https://doi.org/10.1002/j.1551-8833.1995.tb06438.x

Kuznar, Z., & Elimelech, M. (2004). Deposition kinetics of cryptosporidium parvum oocysts onto quartz surfaces. *ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY*, *228*, U633–U633.

Kuznar, Zachary A., & Elimelech, M. (2005). Role of Surface Proteins in the Deposition Kinetics of Cryptosporidium parvum Oocysts. *Langmuir*, *21*(2), 710–716. https://doi.org/10.1021/la047963m

Kuznar, Zachary A., & Elimelech, M. (2006). Cryptosporidium oocyst surface macromolecules significantly hinder oocyst attachment. *Environmental Science & Technology*, *40*(6), 1837–1842.

Kuznar, Zachary Andrew. (2005). *Mechanisms controlling the adhesion of Cryptosporidium parvum oocysts to solid surfaces* (Ph.D.). Yale University, United States -- Connecticut. Retrieved from http://search.proquest.com.login.ezproxy.library.ualberta.ca/pqdtglobal/docview/305386558/abstract/1E6E1B26ADD64325PQ/1

LeChevallier, M. W., & Norton, W. D. (1992). Examining Relationships Between Particle Counts and Giardia, Cryptosporidium, and Turbidity. *Journal (American Water Works Association)*, *84*(12), 54–60. https://doi.org/10.2307/41293943

LeChevallier Mark W., & Norton William D. (1995). Giardia and Cryptosporidium in raw and finished water. *Journal - American Water Works Association*, *87*(9), 54–68. https://doi.org/10.1002/j.1551-8833.1995.tb06422.x

Lee, L.-H. (1991). The Chemistry and Physics of Solid Adhesion. In L.-H. Lee (Ed.), *Fundamentals of Adhesion* (pp. 1–86). Boston, MA: Springer US. https://doi.org/10.1007/978-1-4899-2073-7_1

Li, X., & Johnson, W. P. (2005). Nonmonotonic variations in deposition rate coefficients of microspheres in porous media under unfavorable deposition conditions. *Environmental Science & Technology*, *39*(6), 1658–1665.

Links, A. G. (2015). *Utilization of Fluorescent Microspheres as a Surrogate for Cryptosporidium Removal in Conventional Drinking Water Treatment*. Arizona State University.

Lisle, J. T., & Rose, J. B. (1995). Gene exchange in drinking water and biofilms by natural transformation. *Water Science and Technology*, *31*(5–6), 41.

Liu, Y., Janjaroen, D., Kuhlenschmidt, M. S., Kuhlenschmidt, T. B., & Nguyen, T. H. (2009). Deposition of Cryptosporidium parvum Oocysts on Natural Organic Matter Surfaces: Microscopic Evidence for Secondary Minimum Deposition in a Radial Stagnation Point Flow Cell. *Langmuir*, *25*(3), 1594–1605. https://doi.org/10.1021/la803202h

Logsdon, G. S., Symons, J. M., Hoye Jr, R. L., & Arozarena, M. M. (1981). Alternative filtration methods for removal of Giardia cysts and cyst models. Journal (American Water Works Association), 111-118.

Lu, P., & Amburgey, J. E. (2016). A pilot-scale study of Cryptosporidium-sized microsphere removals from swimming pools via sand filtration. *Journal of Water and Health*, *14*(1), 109. https://doi.org/10.2166/wh.2015.047

Lu, P., Amburgey, J. E., Hill, V. R., Murphy, J. L., Schneeberger, C. L., Arrowood, M. J., & Yuan, T. (2017). Removals of cryptosporidium parvum oocysts and cryptosporidium-sized polystyrene microspheres from swimming pool water by diatomaceous earth filtration and perlite-sand filtration. *Journal of Water and Health*, wh2017221. https://doi.org/10.2166/wh.2017.221

Mac Kenzie, W. R., Hoxie, N. J., Proctor, M. E., Gradus, M. S., Blair, K. A., Peterson, D. E., … Davis, J. P. (1994). A Massive Outbreak in Milwaukee of Cryptosporidium Infection Transmitted through the Public Water Supply. *New England Journal of Medicine*, *331*(3), 161–167. https://doi.org/10.1056/NEJM199407213310304

Meisel, J. L., Perera, D. R., Meligro, C., & Rubin, C. E. (1976). Overwhelming watery diarrhea associated with a Cryptosporidium in an immunosuppressed patient. *Gastroenterology*, *70*(6), 1156–1160.

Molnar, I. L., Johnson, W. P., Gerhard, J. I., Willson, C. S., & O'Carroll, D. M. (2015). Predicting colloid transport through saturated porous media: A critical review. *Water Resources Research*, *51*(9), 6804–6845. https://doi.org/10.1002/2015WR017318

Moore, A., Vesey, G., Champion, A., Scandizzo, P., Deere, D., Veal, D., & Williams, K. . (1998). Viable Cryptosporidium parvum oocysts exposed to chlorine or other oxidising conditions may lack identifying epitopes. *International Journal for Parasitology*, *28*(8), 1205–1212. https://doi.org/10.1016/S0020-7519(98)00070-8

Moran, M. C., Moran, D. C., Cushing, R. S., & Lawler, D. F. (1993). Particle Behavior in Deep-Bed Filtration: Part 2—Particle Detachment. *Journal (American Water Works Association)*, *85*(12), 82–93.

Morgan‐Ryan, U. M., Fall, A., Ward, L. A., Hijjawi, N., Sulaiman, I., Payer, R., ... & Xiao, L. (2002). Cryptosporidium hominis n. sp. (Apicomplexa: Cryptosporidiidae) from Homo sapiens. *Journal of Eukaryotic Microbiology*, *49*(6), 433-440.

Morse, A., Wilson, R., Hull, A., & Simms, J. (2002). Monitoring for Cryptosporidium risk: do particle counters help? *Water Science and Technology: Water Supply*, *2*(1), 171.

Mustranta, A., Pere, J., & Poutanen, K. (1987). Comparison of different carriers for adsorption of Saccharomyces cerevisiae and Zymomonas mobilis. *Enzyme and Microbial Technology*, *9*(5), 272–276. https://doi.org/10.1016/0141-0229(87)90002-0

Nanduri, J., Williams, S., Aji, T., & Flanigan, T. P. (1999). Characterization of an immunogenic glycocalyx on the surfaces of Cryptosporidium parvum oocysts and sporozoites. *Infection and Immunity*, *67*(4), 2022–2024.

Neill, M. A., Rice, S. K., Ahmad, N. V., & Flanigan, T. P. (1996). Cryptosporidiosis: An Unrecognized Cause of Diarrhea in Elderly Hospitalized Patients. *Clinical Infectious Diseases*, *22*(1), 168–170. https://doi.org/10.1093/clinids/22.1.168

Nieminski, E. C., & Ongerth, J. E. (1995). Removing Giardia and Cryptosporidium by conventional treatment and direct filtration. *Journal (American Water Works Association)*, *87*(9), 96–106. https://doi.org/10.2307/41295317

O'Donoghue, P. J. (1995). Cryptosporidium and cryptosporidiosis in man and animals. *International Journal for Parasitology*, *25*(2), 139–195. https://doi.org/10.1016/0020-7519(94)E0059-V

Ong, C., Moorehead, W., Ross, A., & Isaac-Renton, J. (1996). Studies of Giardia spp. and Cryptosporidium spp. in two adjacent watersheds. *Applied and Environmental Microbiology*, *62*(8), 2798–2805.

Ongerth Jerry E., & Pecoraro Julie Proctor. (1996). Electrophoretic Mobility of Cryptosporidium Oocysts and Giardia Cysts. *Journal of Environmental Engineering*, *122*(3), 228–231. https://doi.org/10.1061/(ASCE)0733-9372(1996)122:3(228)

Pang, L., Nowostawska, U., Weaver, L., Hoffman, G., Karmacharya, A., Skinner, A., & Karki, N. (2012). Biotin- and Glycoprotein-Coated Microspheres: Potential Surrogates for Studying Filtration of *Cryptosporidium parvum* in Porous Media. *Environmental Science & Technology*, *46*(21), 11779–11787. https://doi.org/10.1021/es302555n

Patania, N. L. (1995). *Optimization of Filtration for Cyst Removal*. American Water Works Association.

Pembrey, R. S. (2001). A critical investigation of microbial cell surface analysis techniques (Order No. 0803885). Available from ProQuest Dissertations & Theses Global. (304739111). Retrieved from http://login.ezproxy.library.ualberta.ca/login?url=https://search-proquest-com.login.ezproxy.library.ualberta.ca/docview/304739111?accountid=14474

Petterson, S. R., & Ashbolt, N. J. (2016). QMRA and water safety management: review of application in drinking water systems. *Journal of Water and Health*, *14*(4), 571–589. https://doi.org/10.2166/wh.2016.262

Pillai, D. R., Melano, R., Rawte, P., Lo, S., Tijet, N., Fuksa, M., … Krajden, S. (2009). *Klebsiella pneumoniae* Carbapenemase, Canada. *Emerging Infectious Diseases*, *15*(5), 827–829. https://doi.org/10.3201/eid1505.081536

Raveendran Palanivel, & Amirtharajah Appiah. (1995). Role of Short-Range Forces in Particle Detachment during Filter Backwashing. *Journal of Environmental Engineering*, *121*(12), 860–868. https://doi.org/10.1061/(ASCE)0733-9372(1995)121:12(860)

Rice, E. W., Fox, K. R., Miltner, R. J., Lytle, D. A., & Johnson, C. H. (1996). Evaluating plant performance with endospores. American Water Works Association.Journal, 88(9), 122.Rose, J. B. (1988). Occurrence and significance of Cryptosporidium in water. *Journal (American Water Works Association)*, 53–58.

Ryan, K. J., Ray, C. G., & Sherris, J. C. (2004). An introduction to infectious diseases. Medical Microbiology, 4, 345-347.

Schaechter, M., Medoff, G., & Eisenstein, B. I. (Eds.). (1993). Mechanisms of microbial disease. Lippincott Williams & Wilkins.

Shaw, K., Walker, S., & Koopman, B. (2000). Improving Filtration of Cryptosporidium. *Journal (American Water Works Association)*, *92*(11), 103–111.

Smith, H. V., Grimason, A. M., Benton, C., & Parker, J. F. W. (1991). The Occurrence of Cryptosporidium Spp. Oocysts in Scottish Waters, and the Development of a Fluorogenic Viability Assay for Individual Cryptosporidium spp. Oocysts. *Water Science and Technology*, *24*(2), 169.

Spano, F., Putignani, L., McLauchlin, J., Casemore, D. P., & Crisanti, A. (1997). PCR-RFLP analysis of the Cryptosporidium oocyst wall protein (COWP) gene discriminates between C. wrairi and C. parvum, and between C. parvum isolates of human and animal origin. *FEMS Microbiology Letters*, *150*(2), 209–217. https://doi.org/10.1111/j.1574-6968.1997.tb10372.x

States, S., Tomko, R., Scheuring, M., & Casson, L. (2002). Enhanced coagulation and removal of Cryptosporidium. *American Water Works Association. Journal; Denver*, *94*(11), 67.

Stevenson, D. G. (1997). Water treatment unit processes. London: Imperial College Press.

Swertfeger, J., Metz, D. H., DeMarco, J., Braghetta, A., & Jacangelo, J. G. (1999). Effect of filter media on cyst and oocyst removal. *American Water Works Association. Journal; Denver*, *91*(9), 90.

Tfaily, R., Papineau, I., Andrews, R. C., & Barbeau, B. (2015). Application of Quantitative Microbial Risk Assessment at 17 Canadian Water Treatment Facilities. *Journal - American Water Works Association*, *107*, E497–E508. https://doi.org/10.5942/jawwa.2015.107.0141

Thonart, P., Custinne, M., & Paquot, M. (1982). Zeta potential of yeast cells: application in cell immobilization. *Enzyme and Microbial Technology*, *4*(3), 191–194. https://doi.org/10.1016/0141-0229(82)90116-8

Tien, C., & Payatakes, A. C. (1979). Advances in deep bed filtration. *AIChE Journal*, *25*(5), 737–759. https://doi.org/10.1002/aic.690250502

Tobiason, J. E., & O'Melia, C. R. (1988). Physicochemical Aspects of Particle Removal in Depth Filtration. *Journal (American Water Works Association)*, *80*(12), 54–64.

Tufenkji, N., Ryan, J. N., & Elimelech, M. (2002). *Peer reviewed: The promise of bank filtration*. ACS Publications.

USEPA. (2001). Cryptosporidium: Human health criteria document. Office of Water, United States Environment Protection Agency, Washington DC. https://www.epa.gov/sites/production/files/2015-10/documents/cryptosporidium-report.pdf (accessed on 01.21.2018)

USEPA. (2006). National Primary Drinking Water Regulations: Long Term 2 Enhanced Surface Water Treatment Rule; Final Rule. Federal Register. 71(3). https://www.epa.gov/dwreginfo/long-term-2-enhanced-surface-water-treatment-rule-documents (accessed on 01.21.2018)

Van Breemen, L. W. C. A., Ketelaars, H. A. M., Hoogenboezem, W., & Medema, G. (1998). Storage reservoirs – a first barrier for pathogenic micro-organisms in the Netherlands. *Water Science and Technology*, *37*(2), 253.

Van Oss, C. J. (1989). Energetics of cell-cell and cell-biopolymer interactions. *Cell Biophysics*, *14*(1), 1–16. https://doi.org/10.1007/BF02797387

Verberk, J. Q. J. C., O'Halloran, K. J., Hamilton, L. A., Vreeburg, J. H. G., & van Dijk, J. C. (2007). Measuring particles in drinking water transportation systems with particle counters. *Journal of Water Supply: Research and Technology - Aqua*, *56*(5), 345. https://doi.org/10.2166/aqua.2007.069

Wallis, P. M., Erlandsen, S. L., Isaac-Renton, J. L., Olson, M. E., Robertson, W. J., & Van Keulen, H. (1996). Prevalence of Giardia cysts and Cryptosporidium oocysts and characterization of Giardia spp. isolated from drinking water in Canada. *Applied and Environmental Microbiology*, *62*(8), 2789–2797.

World Health Organization. (2016). Quantitative Microbial Risk Assessment: Application for Water Safety Management.

Xiao, L., & Ryan, U. M. (2004). Cryptosporidiosis: an update in molecular epidemiology. *Current Opinion in Infectious Diseases*, *17*(5). Retrieved from https://journals.lww.com/co-infectiousdiseases/Fulltext/2004/10000/Cryptosporidiosis__an_update_in_molecular.14.aspx

Xiao, L., Fayer, R., Ryan, U., & Upton, S. J. (2004). Cryptosporidium taxonomy: recent advances and implications for public health. *Clinical microbiology reviews*, *17*(1), 72-97.

Xu, L.-C., & Logan, B. E. (2006a). Adhesion forces between functionalized latex microspheres and protein-coated surfaces evaluated using colloid probe atomic force microscopy. *Colloids and Surfaces B: Biointerfaces*, *48*(1), 84–94. https://doi.org/10.1016/j.colsurfb.2006.01.012

Xu, L.-C., & Logan, B. E. (2006b). Interaction Forces Measured Using AFM between Colloids and Surfaces Coated with Both Dextran and Protein. *Langmuir*, *22*(10), 4720–4727. https://doi.org/10.1021/la053443v

Yang, H., Kim, H., & Tong, M. (2012). Influence of humic acid on the transport behavior of bacteria in quartz sand. *Colloids and Surfaces B: Biointerfaces*, *91*, 122–129. https://doi.org/10.1016/j.colsurfb.2011.10.058

Yao, K.-M., Habibian, M. T., & O'Melia, C. R. (1971). Water and waste water filtration. Concepts and applications. *Environmental Science & Technology*, *5*(11), 1105–1112.

Zhang, H. (2016). *Coupled effects on the transport and deposition of biocolloidal particles in saturated porous media*. University of Alberta. Retrieved from https://era.library.ualberta.ca/files/crx913p93z

Zhou, Y. (2016). Improving the Understanding of Factors Influencing Cryptosporidium Removals Reported for Granular Media Filtration. Retrieved from https://uwspace.uwaterloo.ca/handle/10012/10122

Zhu, H., Smith, D. W., Zhou, H., & Stanley, S. J. (1996). Improving removal of turbidity causing materials by using polymers as a filter aid. *Water Research*, *30*(1), 103–114.

Chapter 3. Development and selection of *Cryptosporidium* surrogates[1]

3.1 Introduction

Due to its widespread occurrence in drinking water supplies and its significant resistance to environmental stresses, *Cryptosporidium* constitutes one of the most important waterborne microbial parasites (Tufenkji et al., 2006). *Cryptosporidium* oocysts possess a relatively high infectivity rate; as few as 10 oocysts can cause infection in a healthy adult (Okhuysen et al., 2002). Notable outbreaks of cryptosporidiosis in Milwaukee, Wisconsin, and more recently in Östersund, Sweden, have been linked to inadequate removal of *Cryptosporidium* spp. from drinking water (Widerström et al., 2014). Removing *Cryptosporidium* oocysts from drinking water treatment processes is challenging because they are resistant to inactivation by many standard chemical disinfectants (Yang et al., 2013). Although *Cryptosporidium* oocysts have shown to be sensitive to ozonation and ultraviolet irradiation treatments, substantial costs are associated with these treatment options. Compared to chemical treatment methods, removal of *Cryptosporidium* by filtration (Emelko, 2003; Emelko et al., 2005) is inexpensive, effective and is the most commonly used method of *Cryptosporidium* removal from drinking water. It is a major barrier against *Cryptosporidium* in drinking water treatment processes.

Evaluating filtration process inevitably involves spiking the water matrix with much higher concentrations (10^2-10^6 higher) of *Cryptosporidium* (or their surrogates) than what is present in the natural environment. Since the risk of infection by using viable *Cryptosporidium* oocysts causing cryptosporidiosis, a reliable, safe and non-expensive surrogate of *Cryptosporidium* is needed to quantify the filtration removal. Previous studies involved using various biological (Emelko, 2003; Emelko et al., 2005) and non-biological surrogates (Dai and Hozalski, 2003; Emelko and Huck, 2004; Pang et al., 2012; Stevenson et al., 2015) for *Cryptosporidium*. Biological surrogates, such as yeast (*Saccharomyces Cerevusuae* Type II), in the size range of 4 and 10 μm, have been considered as an

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indicator of environmental quality (Hagler, 2006) and have shown to be resistant to conventional disinfectants that is comparable to oocysts (Haas et al., 1985). Therefore, it can be used as surrogates for oocysts, offering simple and cost-effective way to cultivation. However, inherent variation of surface properties, complexity of labelling processes and challenges posed by enumeration techniques limit their application as surrogates for *Cryptosporidium*. Studies of oocysts by heat or formalin have shown that the *Cryptosporidium* inactivation process can alter the structure of surface proteins and thus reduce steric repulsion, leading to the low deposition rates and corresponding attachment efficiencies compared with viable oocysts. (Kuznar and Elimelech, 2005). They are not being considered as representative surrogates for oocysts either.

Polystyrene microspheres that are similar to *Cryptosporidium* in size, aspect ratio, and buoyant density have been used as safe surrogates in a variety of bench-scale, pilot-scale, and groundwater field experiments (Amburgey et al., 2005; Emelko & Huck, 2004; Lu et al., 2017). These microspheres are chemically inert, negatively charged, and easy to detect (Behrens et al., 2001). However, previous studies have shown that these polystyrene microspheres exhibit significantly different deposition behaviors from live *Cryptosporidium* in porous media, which may be attributed to the different surface properties, such as surface charge and hydrophobicity (Pang et al., 2012). A change in the original surface charge of oocysts affect their removal in granular media filtration since zeta potential is indicative of the degree of particle destabilization (Emelko et al., 2003). Hydrophobicity change due to conformational changes on the surface bring about the marked difference in the steric repulsion and overall deposition kinetics. A study performed by Pang et al. (2012) evaluated biotin and glycoprotein modified microspheres, and demonstrated that as compared to the unmodified microspheres, glycoprotein-coated microspheres better mimicked the macromolecular structure of *Cryptosporidium* oocysts. More recently, Stevenson et al., (2015) and Zhang et al., (2017a) demonstrated that glycoprotein-coated microspheres had good surface resemblance with viable oocysts. However, significant costs associated with glycoproteins hindered their application at the field scale. More importantly, the hydrophobicity of oocysts, which is known impact on

the oocyst deposition in porous media, cannot be represented by simply using glycoprotein modification

In the present study, three potential biomolecules-coated microspheres are prepared, including glycoprotein-modified, glycopolymer-modified, and copolymers-modified microspheres with diameters similar to *Cryptosporidium* oocysts. Their deposition kinetics and transport behaviors were compared to live *Cryptosporidium* using molecular scale QCM-D studies and laboratory-scale packed-bed columns. Therefore, the main objective of this chapter was to select the representative surrogates for *Cryptosporidium* oocysts in filtration studies.

3.2 Materials and Methods

3.2.1 Materials

3.2.1.1 Particles

Cryptosporidium oocysts used in all experiments were supplied by Waterborne Inc. (USA). According to the supplier, all oocysts (CAS no. 137259-50-8) were shed from the calves that were originally infected with Iowa isolates. The oocysts were purified by sucrose and percoll density gradient centrifugation after initially being extracted with diethyl ether from feces. The oocysts were stored in phosphate buffered saline (PBS) containing antibiotics, including penicillin, streptomycin, and gentamicin, to prevent bacterial growth, and Amphotericin B as a fungicide and 0.01% Tween 20 to prevent cell aggregation. The oocysts suspension (~10^7 oocysts/8mL) was stored at 4°C and used within three months of the shipment date. Prior to each experiment, 1 mL of the stock oocysts sample was centrifuged at 13,000 rpm for one minute using a microcentrifuge (Eppendorf, 5414R, Germany) according to the supplier's specifications. The antibiotic solution was removed, and the pullets were resuspended in pH 8.0 of 1 mM NaCl background solution.

Fluorescent polystyrene microspheres (catalog no. 16592-5) were purchased from Polysciences Inc. The fluorescence yellow-green (YG) color has an excitation wavelength at 441 nm and an emission wavelength at 486 nm. The specific gravity of microspheres

was 1.025 and the mean diameter was 4.3 μm with 7% coefficient of variation as provided by the manufacturer. The surface of the microspheres was functionalized with carboxyl groups.

3.2.1.2 Chemicals

All chemicals were purchased from Sigma-Aldrich Chemicals (Oakville, ON, Canada), and the organic solvents were obtained from Caledon Laboratories Ltd. (Georgetown, ON, Canada). The chain transfer agent, 4-cyanopentanoic acid dithiobenzoate (CTP), 2-lactobionamidoethyl methacrylamide (LAEMA), primary amine containing monomer N-(3-Aminopropyl) methacrylamide hydrochloride (APMA), and zwitterionic monomer sulfobetaine methacrylate (SBMA) were synthesized using the reversible addition-fragmentation chain transfer (RAFT) polymerization described in our previous study (Wang et al. 2014).

3.2.2 Surrogates preparation

3.2.2.1 RAFT Copolymerization of LAEMA with APMA

RAFT polymerization (Deng et al., 2009) was chosen to produce the well-defined glycopolymers. The copolymerization (Wang et al., 2014) was achieved at 70 °C in aqueous media, employing 4,4' azobis (4-cyanovaleric acid) (ACVA) and CTP as the radical initiator and chain transfer agent respectively. Briefly, in a 10-mL Schlenk tube, LAEMA (0.85 g, 1.82 mmol) and APMA (0.15 g, 0.84 mmol) were dissolved in 7 mL of double-distilled deionized water before the addition of CTP (14 mg, 0.05 mmol) and ACVA (2.8 mg, 0.01 mmol) N, N′-dimethylformamide (DMF) stock solution (1 mL). After degassing under a nitrogen atmosphere for 30 min, the flask was placed in a preheated oil bath for 24 h at 70˚C. After precipitation in acetone, the polymer was extensively washed with methanol to remove any residual monomers and then dried under a vacuum to improve the impurity of final product. The conversion and composition of the copolymers were determined by ^{1}H NMR (Nuclear Magnetic Resonance) (Varian 500, USA) in D_2O as a solvent. The polymer molecular weight and molecular weight distributions were evaluated using aqueous gel permeation chromatography (GPC) (Viscotek GPC system, USA) with

a Viscotek model 250 dual detector (refractometer/viscometer) at room temperature, with a flow rate of 1.0 mL/min. Pullulan standards (Mw = 6,200–113,000 g/mol) were used.

3.2.2.2 RAFT Copolymerization of SBMA with APMA

Poly(SBMA-co-APMA) was synthesized using a similar approach to the poly(LAEMA-co-APMA) synthesis. Specifically, 0.78 g of SMBA (2.67 mmol) and 0.22 g of APMA (1.23 mmol) were dissolved in 7 mL of double-distilled deionized water in a Schlenk tube before the addition of a CTP (14 mg, 0.05 mmol) and ACVA (2.8 mg, 0.01 mmol) methanol stock solution (3 mL). The tube was then sealed and degassed under a nitrogen atmosphere for 30 min and placed in a preheated oil bath for polymerization at 70 °C for 24 h. After precipitation in acetone followed by washing with methanol to remove residual monomer, the conversion and composition of the copolymers was determined by ^{1}H NMR using D_2O. The polymer molecular weight and molecular weight distributions were obtained from aqueous GPC as described previously.

3.2.2.3 Conjugation of biomolecules with polystyrene microspheres

The synthetic polymers or glycoproteins were conjugated to carboxyl functionalized polystyrene microspheres through their amine groups by the carbodiimide crosslinker mechanism. A water-soluble carbodiimide cross-linker, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), was used for covalently coupling the primary amine-containing molecules to the carboxyl group on the surface of the microspheres. The coupling procedures were adjusted from the protocols provided by Polysciences Inc. Specifically, 1 mL of 4.3 μm microspheres were centrifuged and washed with 2-(N-morpholino) ethanesulfonic acid (MES) with pH=4 for three times. The microspheres were then resuspended into 5 mL of MES consisting of 25 mg of EDC and 11 mg of N-Hydroxysuccinimide (NHS) for 15 min. Subsequently, the MES buffer was removed by centrifuge and the microspheres were washed three times with a 1×PBS buffer at pH 7.4. The microspheres resuspend in 5 mL 1×PBS buffer. The synthetic polymers, including poly(LAEMA-co-APMA) (18 mg), or poly(LAEMA-co-APMA) (16 mg) with poly(SBMA-co-APMA) (4 mg), or commercial α1-acid glycoprotein (8 mg) (Sigma-Aldrich, Oakville, ON, Canada) were then added to 5 mL microspheres suspension to

conjugate to surface of the microspheres by a reaction in the dark at room temperature for three hours. The final product was obtained by washing the microspheres with double distilled water three times to remove any unreacted coupling agents or polymers. According to the product information, the α1-acid glycoprotein has a molecule weight of 33.0-40.8 kDa (equal to 33000-40800 g/mol) and high solubility in water (1000 mg/100 mL). The microspheres modified by α1-acid glycoprotein is later called glycoprotein-modified microspheres. The microspheres modified by glycopolymer poly(LAEMA-co-APMA) is later called glycopolymer-modified microspheres. The microspheres modified by both poly(LAEMA-co-APMA) and poly(SBMA-co-APMA) in a ratio of 4:1 refer to copolymers-modified microspheres in the later content.

3.2.3 Particle characterization

The oocysts and their potential surrogates were characterized by determining the surface charge and hydrophobicity in a condition similar to drinking water (pH=8.0 of 1 mM NaCl solution). Zeta-potentials (ζ) of particles were approximated from the electrophoretic motility measured by a Zetasizer Nano485 (Malvern, USA). The surface hydrophobicity of microspheres was determined by contact angle measurements using an FTA-200 system (First Ten Angstroms, USA). Then the measured contact angle was converted to percentage of hydrophobicity to compared it with the literature reported viable oocysts hydrophobicity. According to the product information, the α1-acid glycoprotein has a molecule weight of 33.0-40.8 kDa (equal to 33000-40800 g/mol) and high solubility in water (1000 mg/100 mL). The microspheres modified by α1-acid glycoprotein is later called glycoprotein-modified microspheres. The microspheres modified by glycopolymer poly(LAEMA-co-APMA) is later called glycopolymer-modified microspheres. The microspheres modified by both poly(LAEMA-co-APMA) and poly(SBMA-co-APMA) in a ratio of 4:1 refer to copolymers-modified microspheres in the later content.

3.2.4 QCM-D study

The interactions between *Cryptosporidium* oocysts or their surrogates with a silica surface were studied using an E4 QCM-D unit (Q-Sense AB, Gothenburg, Sweden) with silica-coated sensor chips (QSX-303, 5 MHz, AT-cut, Q-Sense AB). All QCM-D experiments

were performed in triplicate under flow-through conditions using a digital peristaltic pump at a fixed flow rate of 200 μL/min. The temperature was maintained at 20±0.2 ˚C during experiments. Prior to the experiment, the system was stabilized by injecting ultrapure water for 10 mins followed by the injection of 1 mM of a salt solution (pH=8.0) for another 10 mins until stable baselines were obtained. The suspension of *Cryptosporidium* or their surrogates (10^5 particles/mL, suspended in 1 mM salt solution) was then injected into the QCM chamber for one hour. Δf and ΔD at 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 9^{th}, and 11^{th} overtones were monitored simultaneously (corresponding to 5, 15, 25, 35, 45, and 55 MHz). Subsequently, the sensor was washed with the same salt solution followed by ultrapure water. Following each experiment, the QCM-D sensor was carefully removed from the flow chamber and visualized by epifluorescence microscopy (Axioskop II microscope, Zeiss, Jena, Germany) equipped with a wide-field fluorescence microscope excitation light source (X-cite 120Q, Lumen Dynamic, ON, Canada), camera (Carl Zeiss Microimaging GmbH, Germany) and image analysis software (ImageJ, USA). To observe the *Cryptosporidium* cell adhering to the silica surface, the sensors were rinsed with a pH 7.4 of 1×PBS buffer and the cells were stained with a diluted cryptosporidium antibody (BEL 0126: FITC NB100-64321, Novus Biologicals, Littleton, CO, USA).

3.2.5 Packed-bed column study

The packed-bed column experiments were performed in Chromaflex® chromatography columns (Kimble Chase, USA), that were 4.8cm (ID) ×15 cm (length) and made of borosilicate glass. Granular sand media, commonly used in a full-scale drinking water treatment filtration process, with an effective size of 0.35-0.45 mm and a uniformity coefficient of less than 1.5, were used as model media in this study. Sands were thoroughly cleaned using acid and base washing, following protocols described by Zhang et al. (2017a). This intensive cleaning procedure yielded very reproducible particle deposition results (Liu et al., 2007). For each experiment, approximately 380 g of freshly cleaned sand was wet packed with mild vibration to minimize any layering or air entrapment to a total depth of 15 cm, resulting in a porosity of 0.43. The column was first equilibrated by pumping 10 pore volumes (PV) of particle-free background electrolyte through the column at 10 mL/min with an upflow mode provided by a peristaltic pump (Cole Parmer, IL). After

that, approximately 5 PV of homogenized oocysts suspension or surrogates containing solutions (~10^6 particle) were injected. The influent concentration was determined in triplicate by a hemocytometer (Hausser Scientific Bright-Line™ counting chamber, USA). A constant influent concentration was maintained by mixing a particle suspension with a magnetic stir bar. Following the particle injection, the column was eluted with an additional 8 PV of the background electrolyte. Every half PV of the column effluent was collected in a 50-mL polystyrene centrifuge tube before enumeration. After the transport experiment, the filter media was evenly dissected into six segments (2.5 cm of each segment) and the retained particles were recovered by 10 minutes sonication in the DI water. Influent samples, effluent samples and retained samples were enumerated by epifluorescence microscopy as described below.

3.2.6 Enumeration of particles

Viable oocysts were enumerated by a modified direct immunofluorescent assay as described by Dai and Hozalski (2002). Specifically, samples were first concentrated onto a polycarbonate membrane (pore size = 0.45 μm, diameter = 25 mm, Fisher Scientific Inc., USA) in the manifold. After filtration, the filter membrane was carefully removed and placed into a six well cell culture plates. Diluted monoclonal antibody solution (100 μL) (Novus Biological. Inc, USA) was applied on the center of the filter and allowed to react in the dark at room temperature for 30 minutes. Oocysts surrogates (prepared from fluorescent microspheres) were counted without further treatment. The fluorescently labeled oocysts or their surrogates were counted in a glass microscope slide at 100× magnification using epifluorescence microscopy described earlier. At least 20 randomly selected fields were counted per filter membrane. In each experiment, the mass balance was determined by summing the total number of eluted particles and the amount of retained particles (i.e., S(X)). The recoveries of oocysts and microspheres using this method ranged from 60% to 98%.

3.2.7 Colloidal filtration theory calculation

To quantitatively compare the overall deposition of the oocysts and their potential surrogates at the study conditions, the deposition rate coefficient K_d was estimated using

the steady state breakthrough concentrations of the particles according to the following equation (Walker and Redelman, 2004):

$$K_d = -\frac{U}{\varepsilon L}\ln\frac{C}{C_0} \qquad (1)$$

Where ε is the bed porosity, U is the approach velocity, L is the column length, and C/C_0 is the normalized breakthrough concentration relevant to "clean bed" conditions, which was obtained from each breakthrough curve by averaging the values measured between four and six pore volumes (Liu et al., 2007b). Colloid filtration theory (CFT) was used to obtain the theoretical particle retention pattern S(X) in the packed columns based on the particle breakthrough curve (Liu and Li, 2008). S is the number of deposited particles per mass of the granular collector, and can be calculated using the following equation (Tufenkji et al., 2003):

$$S(X) = \frac{t_0 \varepsilon K_d C_0}{\rho_b}\exp\left(-\frac{K_d X \varepsilon}{U}\right) \qquad (2)$$

Where t_0 is the duration of continuous particle injection, C_0 is the initial particle concentration, ρ_b is the porous medium bulk density, and X is the column depth.

3.3 Results and Discussions

3.3.1 Biomolecules characterization

The well-defined copolymers were synthesized via the RAFT process, a process earning increasing attention in the synthesis of polymers due to their ideal tolerance to a wide range of reaction conditions and monomers (Deng et al., 2009). The compositions of the copolymers were determined by ^{1}H NMR (Figure 3-1). The structures of synthesized monomers (APMA, LAEMA, and SBMA) and the copolymerization processes are shown in Figure 3-2. The molecular weights of the synthesized copolymers, i.e. poly(LAEMA-co-APMA) and poly(SBMA-co-APMA), are ~13 kDa (Table 3-1), with relatively narrow molecular weight distributions (polydispersity index (PDI) less than 1.20).

Table 3-1. Molecular weight and PDI of polymers synthesized by RAFT

Polymers	**M_n (kDa)**	**PDI (M_w/M_n)**
Poly($LAEMA_{24}$-co-$APMA_{12}$)	13.1	1.16
Poly($SBMA_{36}$-co-$APMA_{18}$)	13.2	1.18

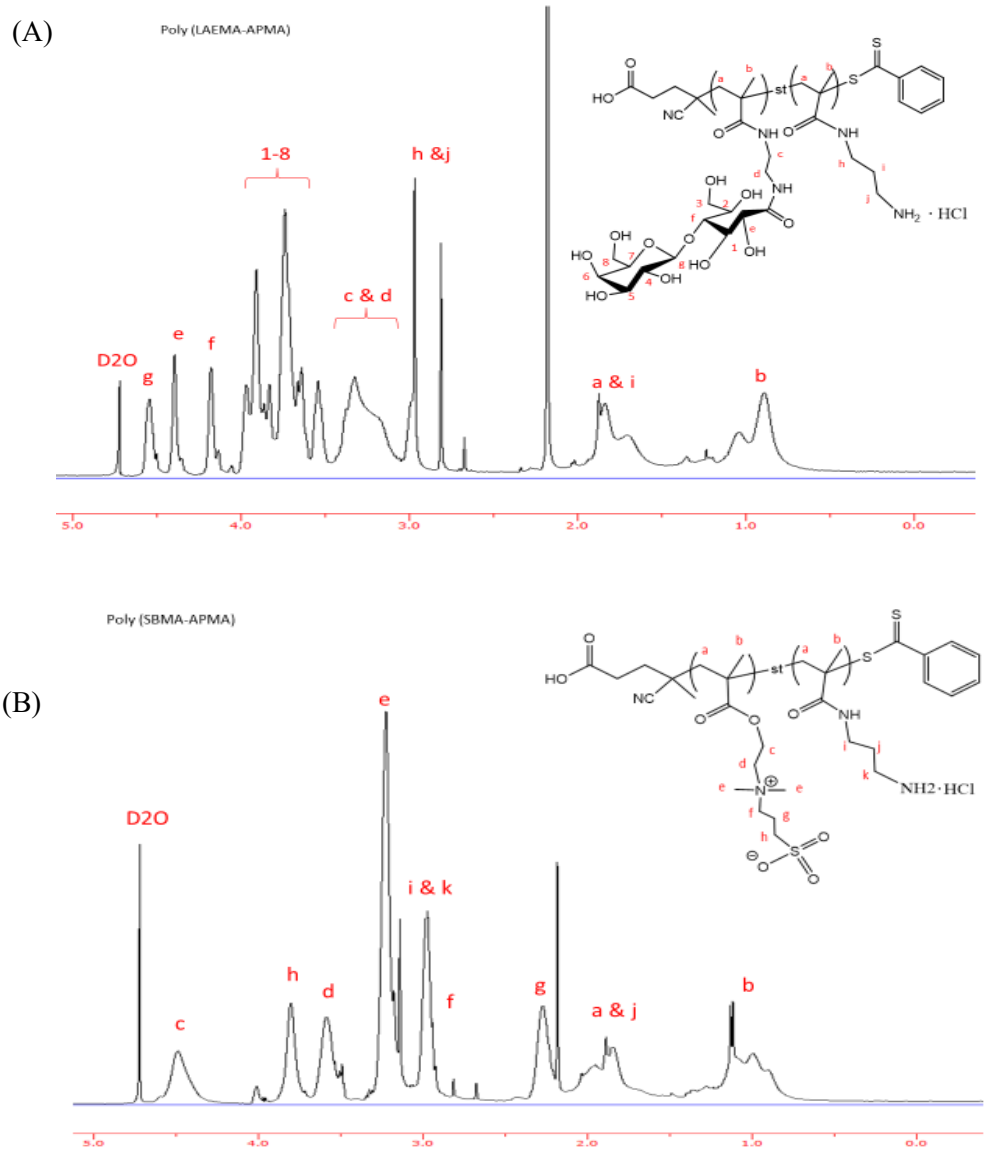


Figure 3-1. ^{1}H NMR spectrum of Poly(LAEMA-co-APMA) (A) and Poly(SBMA-co-APMA) (B).

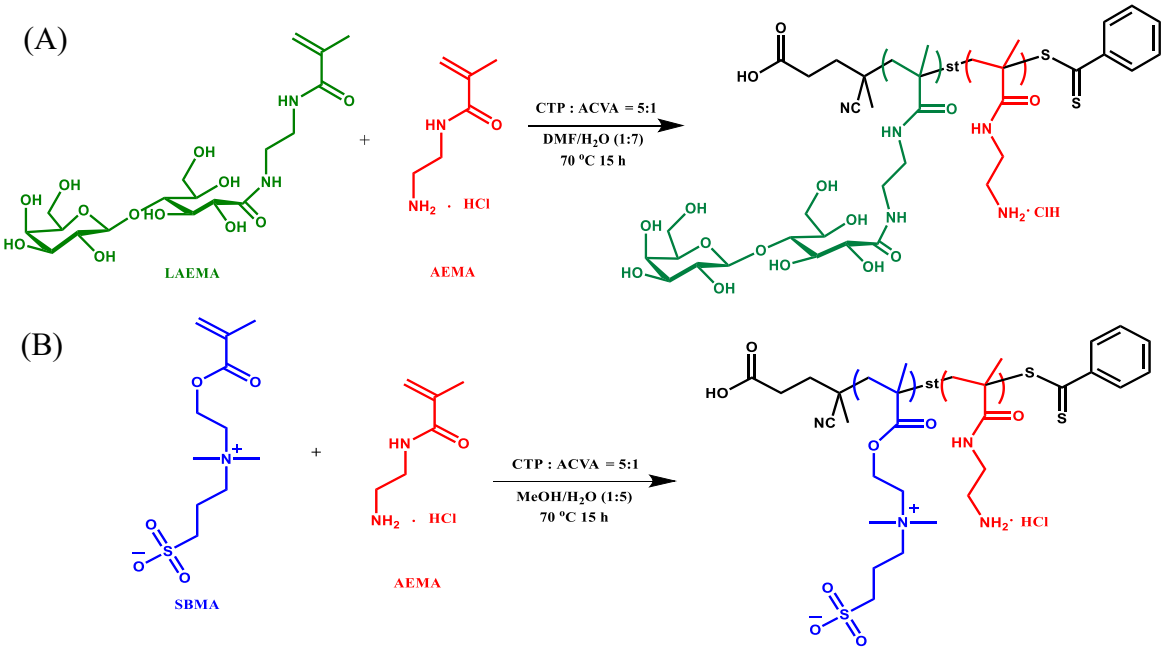


Figure 3-2. (A) RAFT synthesis of poly(LAEMA-co-APMA) and (B) poly(SBMA-co-APMA).

3.3.2 Particle characterization

The schematics of the conjugation processes of biomolecules, including α1-acid glycoprotein, glycopolymer poly(LAEMA-co-APMA), and glycopolymer poly(LAEMA-co-APMA) with zwitterionic polymer poly(SBMA-co-APMA) to the surface of microspheres are illustrated in Figure 3-3. The microscope images of viable *Cryptosporidium* oocysts and modified microspheres are shown in Figure 3-4.

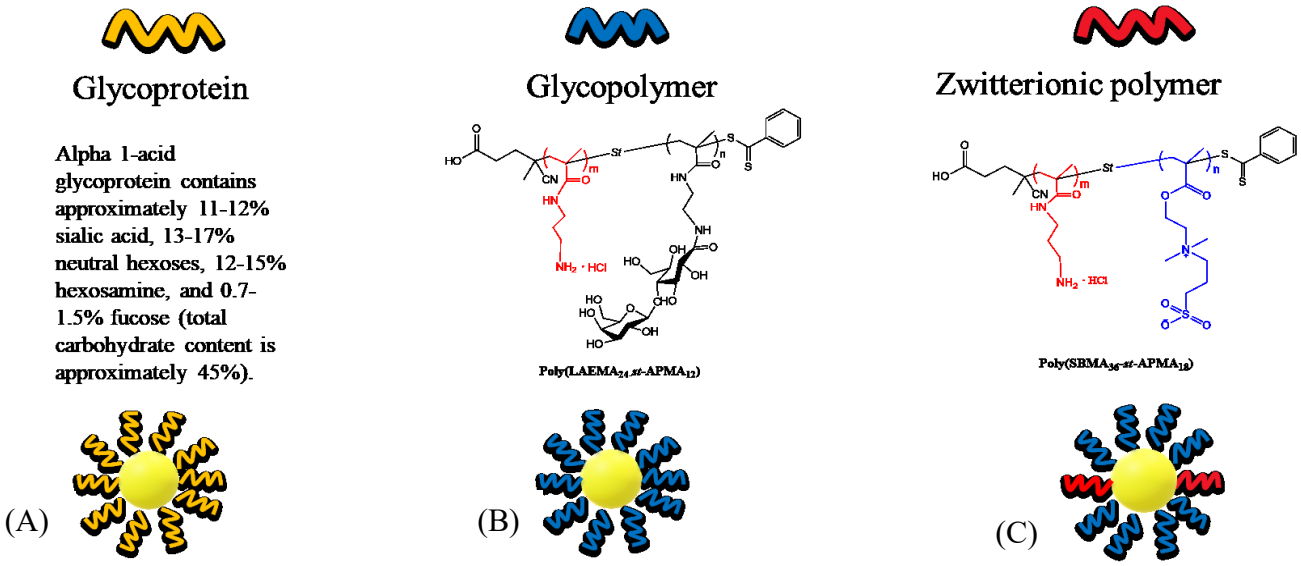
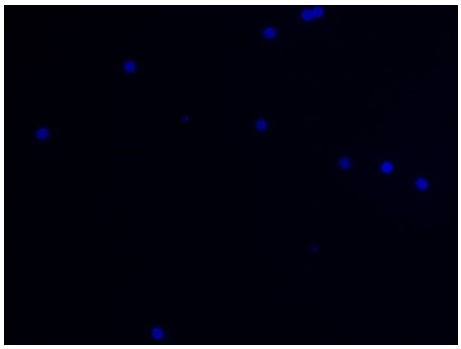
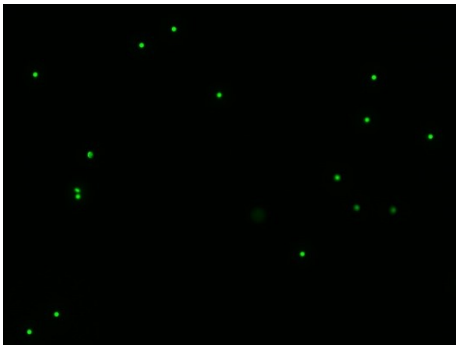


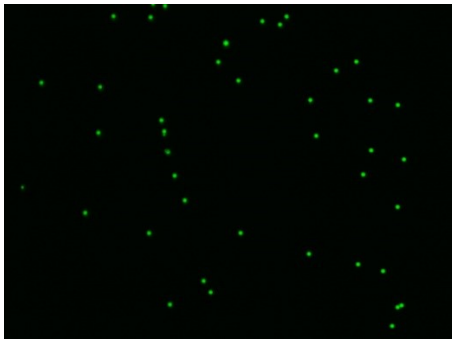
Figure 3-3. Schematic of the conjugation process of biomolecules with microspheres. Glycoprotein-modified microspheres (A), glycopolymer-modified microspheres (B), and copolymers-modified microspheres (C).



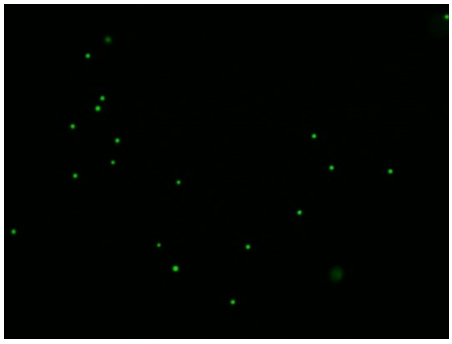
Viable oocyst-400×



Glycoprotein-modified microspheres-200×



Glycopolymer-modified microspheres-200×



Copolymers-modified microspheres-200

Figure 3-4. Microscope images of oocysts and their potential surrogates.

The surface properties of *Cryptosporidium* oocysts were compared with their potential surrogates and unmodified microspheres as shown in Figures 3-5 (A) and (B). Error bars represent the standard deviation of three measurements for the zeta potential. The net negative charge of live oocyst cells was due to the deprotonated carboxyl groups from proteins (Dai and Hozalski, 2003), which was -10.9±1.2 mV in this study under the examined condition. The unmodified microspheres had the most negative zeta potential (-56.5±1.5 mV), which was in agreement with other studies (Dai and Hozalski, 2003; Pang et al., 2012). Modification by glycoprotein increased the zeta potential of microspheres (-36.4±2.1 mV), which was still statistically more negative than that of viable oocysts (P=0.00032). Glycopolymer-modified microspheres and Copolymers-modified microspheres also exhibited a less negative zeta potential than the unmodified ones. It is because the synthesized polymers (containing amine group) conjugated on the surface of the fluorescence microspheres by covalent bonding counteracted a very negative surface charge of the unmodified microspheres. These surface modifications reduced the differences of zeta potentials between modified microspheres and live oocysts (P=0.0026 for glycopolymer-modified microspheres and P=0.0025 for copolymers-modified microspheres).

As shown in Figure 3-5 (B), the surface of live oocysts was very hydrophilic with the lowest percent hydrophobicity (8.4%) (Chung, 2012; Dai and Hozalski, 2003), whereas the glycopolymer-modified microspheres (39.4%) were more hydrophobic among all the surrogates. Compared to the unmodified microspheres (27.2%), the hydrophobicity of glycoprotein-modified microspheres and Copolymers-modified microspheres decreased to 23.7% and 18.0%, respectively. Therefore, after modification by zwitterionic polymer, their surface hydrophobicity was the closest to viable oocysts. The increased hydrophilicity of the copolymers-modified microspheres is due to the presence of synthesized zwitterionic polymers (i.e., poly-SBMA), which possess both cationic and anionic groups making them superhydrophilic (Shao and Jiang, 2015). In summary, the copolymers-modified microspheres and viable oocysts were the most similar of the four potential surrogates evaluated in this study, which make them representative surrogates for viable *Cryptosporidium* oocysts in terms of similar surface characteristics.

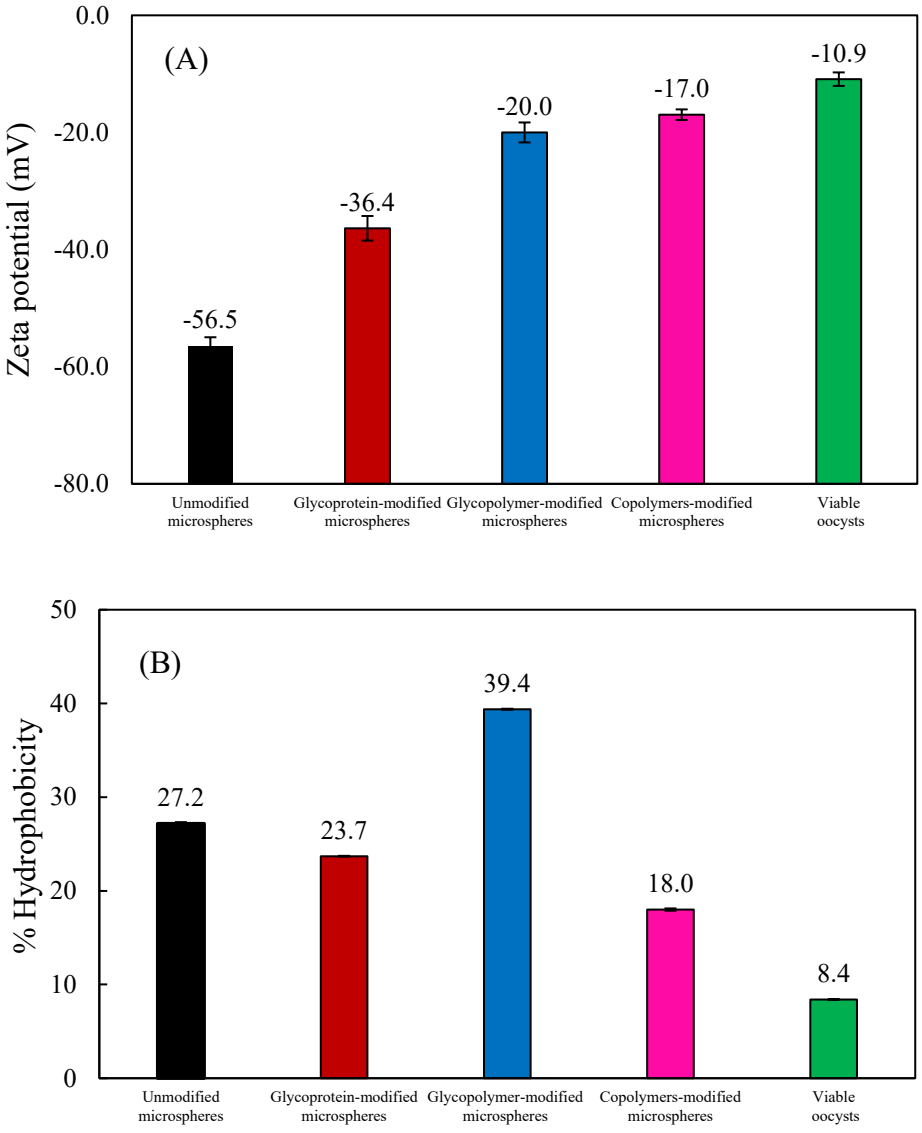


Figure 3-5. (A) Zeta-potentials of viable oocysts and potential surrogates and (B) the % hydrophobicity of viable oocysts and potential surrogates in pH=8.0 of 1 mM NaCl electrolyte solution.

3.3.3 QCM-D signals during particle deposition on a silica surface

Representative QCM-D measurements made when the suspension of oocysts or potential surrogates were being injected into a flow chamber are presented at 3rd overtone shown in Figure 3-6.

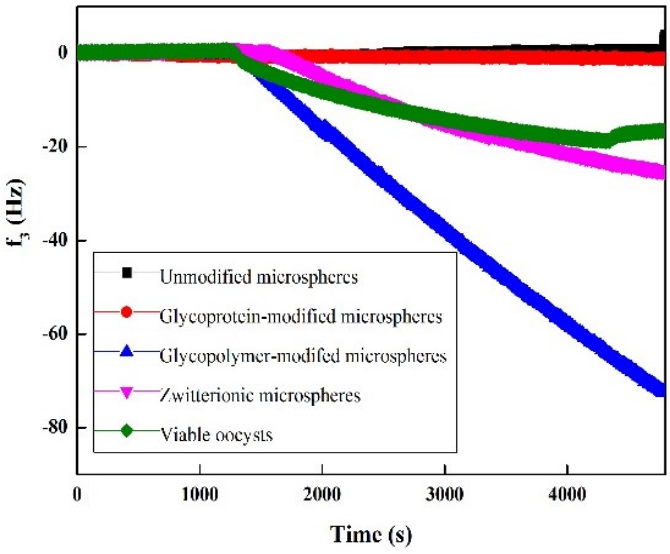


Figure 3-6. Normalized frequency shifts of the third overtone as a function of time on silica surface during the 100 mins experiment.

The shift in frequency for the 3rd overtone (15 MHz) at 80 minutes of experiment is shown in Figure 3-7. The adsorption of the copolymers-modified microspheres was the most similar to that of the viable oocysts. In comparison, low frequencies were observed for the unmodified microspheres (0.2 Hz) and glycoprotein-modified microspheres (0.3 Hz). These low frequency change might be attributed to their significantly higher negative zeta potentials and relatively higher hydrophobicities as compared to those surface characteristics of viable oocysts. In addition, the glycopolymer-modified microspheres (-64.1 Hz) had the highest adsorption observed on the sensor surface (greatest frequency changes), which may be attributed to their highest surface hydrophobicity. The glycopolymer-modified microspheres were suspected of participating in hydrophobic interactions with solid surface (Dai et al., 2004), which underlined a significant role of particle surface hydrophobicity on the adhesion with porous media experimentally. The copolymers-modified microspheres exhibited the most similar frequency change (-20.6 Hz) with viable oocysts (-17.5 Hz). Also, the poly(SBMA-co-APMA) coatings made the surface of the copolymers-modified microspheres more hydrophilic and poly(LAEMA-co-APMA) coating rendered the very negative charge of the polystyrene microspheres.

Therefore, their deposition behaviors at the silica sensor surface were the closest to that of the viable oocysts. It can be concluded from this study that the surface modification using both the zwitterionic polymers and glycopolymer not only simulated the glycan present on the cell surface but generated a zwitterionic polymer layer on the microsphere surface that mimicked the rigid lipid bilayers on an oocyst cell membrane (Bushkin et al., 2013).

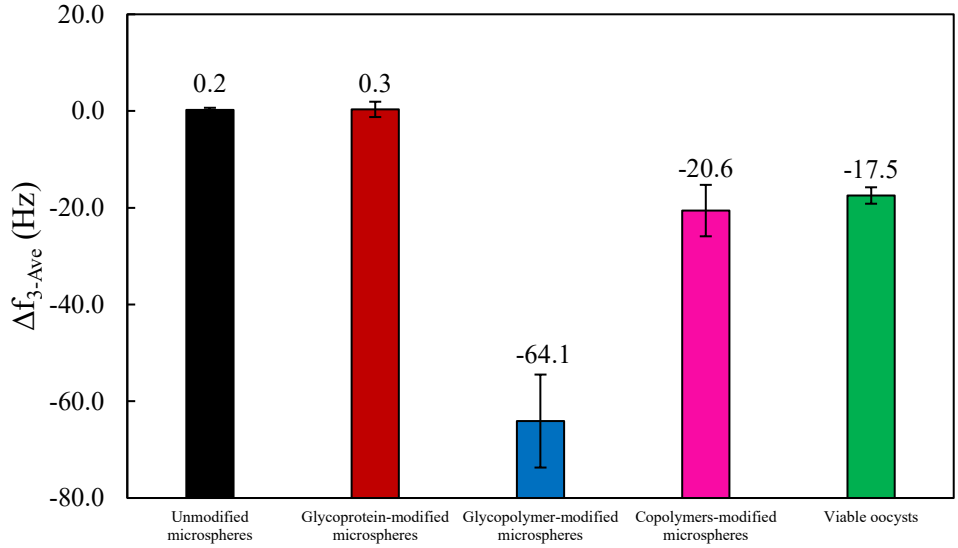


Figure 3-7. Normalized average frequency shifts of the third overtone as a function of time on silica surface at 80-min of experiments. Error bars represent the standard deviation of triplet measurements.

3.3.4 Bench-scale filtration experiments

3.3.4.1 Particles transport in packed-bed column

The breakthrough curves shown in Figure 3-8 (A) compared the transport of viable oocysts, unmodified microspheres, and three types of modified microspheres by plotting normalized particle concentrations (C/C_0) in the column outflow against the number of pore volumes passing through the packed bed. The deposition rate coefficients (K_d) were compared for all the particles under the same conditions, as shown in Figure 3-8 (B). The assumption in this comparison is that the deposition rate coefficient is constant based on the CFT.

The similar transport behavior was observed between copolymers-modified microspheres and viable oocysts in the packed-bed column. The deposition rate coefficient of copolymers-modified microspheres is the closest (11.9 hr^{-1}) to that of viable oocysts (12.2 hr^{-1}), which is in good agreement with our results obtained from QCM-D studies. Our results indicated that the zwitterionic polymer layer (hydrophilic) present on the surface of microspheres plays a key role in mimicking the attachment of live oocysts to the silica surface.

The unmodified microspheres demonstrated a much higher peak breakthrough concentration because the electrostatic repulsion was the highest between the granular sand media (-52.2 mV) and the most negatively-charged unmodified microspheres (-56.5 mV). The very strong electrostatic repulsion in turn promoted the transport of the unmodified microspheres in the packed-bed column. Therefore, the unmodified microspheres had the lowest K_d (8.0 hr^{-1}) based on the breakthrough curve described in Figure 3A, which was in agreement with the very negative surface charge from surface characteristic analysis and the lowest frequency shift from QCM-D studies. Surface charge of unmodified microspheres appears to be more dominant in the sand-packed column than the effect of hydrophobicity in this system. The similar observation was reported in Dai et al. (2004) by examining the adhesion of *Cryptosporidium* to solid surfaces.

The glycopolymer-modified microspheres underpredicted the oocysts' peak concentration as shown in Figure 3A, with highest particle retention achieved in the filter column, which are consistent with the results of QCM-D study. The K_d of glycopolymer-modified microspheres was the largest (15.9 hr^{-1}) among all surrogates tested. The enhanced retention of glycopolymer-modified microspheres could be explained by the increased degree of hydrophobicity due to the presence of outer glycan on their surface. This hydrophobic attraction may play a large role in promoting the adhesion of glycopolymer-modified microspheres with porous sand. There has only been limited research on the role of particle surface hydrophobicity on their adhesion with filter media. This study implies the significance of particle surface hydrophobicity in promoting particle deposition on solid surfaces using both packed-bed column and QCM-D.

The glycoprotein-modified microspheres in the packed-bed column showed comparable transport behavior with viable oocysts, indicating by a similar steady-state breakthrough plateaus with oocysts, as shown in Figure 3A. It might be attributed to the combined effect of high negative zeta potential (-36.4±2.1 mV) and median hydrophobicity (23.7±0.04%), leading to the very comparable deposition rate coefficient (11.0 hr^{-1}) as compared to that of oocysts (12.1 hr^{-1}) in the column study. Adhesion of glycoprotein-modified microspheres with porous media indicated that surface charge and hydrophobicity both played a role in the adhesion to porous media. Interestingly, our observation from column study was not consistent with the previous QCM-D study, which showed very low retention to the silica surface under the study condition. The observed difference in particle deposition on flat solid surfaces and porous media may be attributed to their different system geometry and deposition kinetics. For instance, convective-diffusive transport dominates the particle deposition in QCM-D chamber, while physical straining should be considered in a porous media column. Collector geometry can also attribute to a greater fraction of retained glycoprotein-modified microspheres in the packed-bed column, which is in good agreement with study conducted by Tong et al. (2006). Further surface heterogeneity and the presence of flow stagnation zones in porous media system may also lead to the enhanced microspheres retention in porous media (Tong et al., 2006; Johnson and Hilpert, 2013; Molnar et al., 2015; Hilpert et al., 2017).

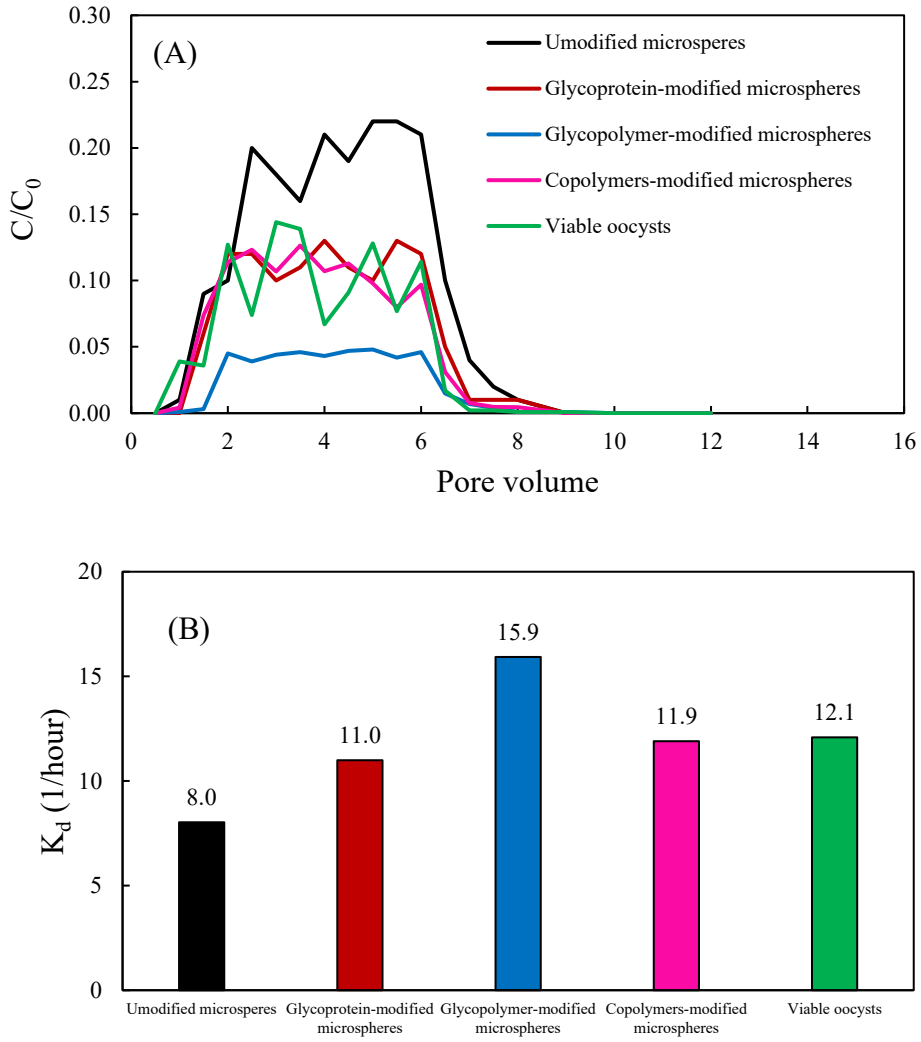


Figure 3-8. (A) Breakthrough curves of oocysts and their potential surrogates under pH=8.0 of 1mM NaCl background solution and (B) comparison of particle deposition rate coefficient (K_d) determined from the breakthrough curves using Eq. 1. Experimental conditions were as follows: approach velocity= 0.0092cm/s and porosity=0.43.

3.3.4.2 Retained particle profiles

Deposition experiments carried out with oocyst and potential surrogates under well-controlled physicochemical conditions in columns packed with sand could provide an improved mechanistic understanding of deposition behavior (Tufenkji et al., 2004; Tufenkji and Elimelech, 2005; Tufenkji et al., 2006b; and Liu et al., 2007a, 2007b). As presented in Figure 3-9, the retained surrogates' profiles were compared to those predicted

by the CFT as implicated in the "clean bed" theory, which assumes a log-linear relationship (a constant deposition rate coefficient K_d) between deposited particles and transport distance.

In the tested unfavorable conditions (presence of energy barrier when the ionic strength of solution is low), the single-rate description model for colloid transport often fail to capture experimentally observed breakthrough and retention behaviors i.e., the non-steady state breakthrough and non-log-linear retention profiles (Molnar et al., 2015). Our observation of retained oocyst profile (green cycles) in Figure 4 deviated from the CFT prediction as indicated in the dotted line. A steeper-than-expected decrease (black squares) in retained concentrations with distance from source was observed for unmodified microspheres, which may be driven by electrostatic repulsion due to the strong negative zeta potentials of unmodified microspheres. In contrast, the modified microspheres in this study showed much less deviations from log-linear behaviors, as indicated in Figure 4. Their profiles were consistent with the filtration theory, indicating that filtration theory captures the essential elements of their deposition (Li et al., 2006a) under the examined condition.

The deviations of deposition patterns from the CFT could be explained by the heterogeneity in particle surface properties and grain surface properties, and distribution in the interaction energies between particles and porous media. Furthermore, when the ratio of the particle diameter to the collector grain diameter was greater than around 0.005, the colloid transport in the heterogeneous systems was primarily controlled by straining (Bradfort et al., 2007). In our study, this ratio is 0.014 and therefore straining likely occurred under the test conditions because the sand media used in the experiments were not uniform (uniformity coefficient of 1.5). Under such conditions, the CFT was no longer valid to adequately describe the deposition profiles (Bradford et al, 2002; Bradford and Bettahar, 2005; Bradford et al., 2006). Overall, an accurate description and modeling of the oocysts' retention process in the packed-bed column will assist in predicting their fate and transport behaviors. It can also help to design new or upgrade current filtration process to effectively remove *Cryptosporidium*, ensuring the safe of treated drinking water.

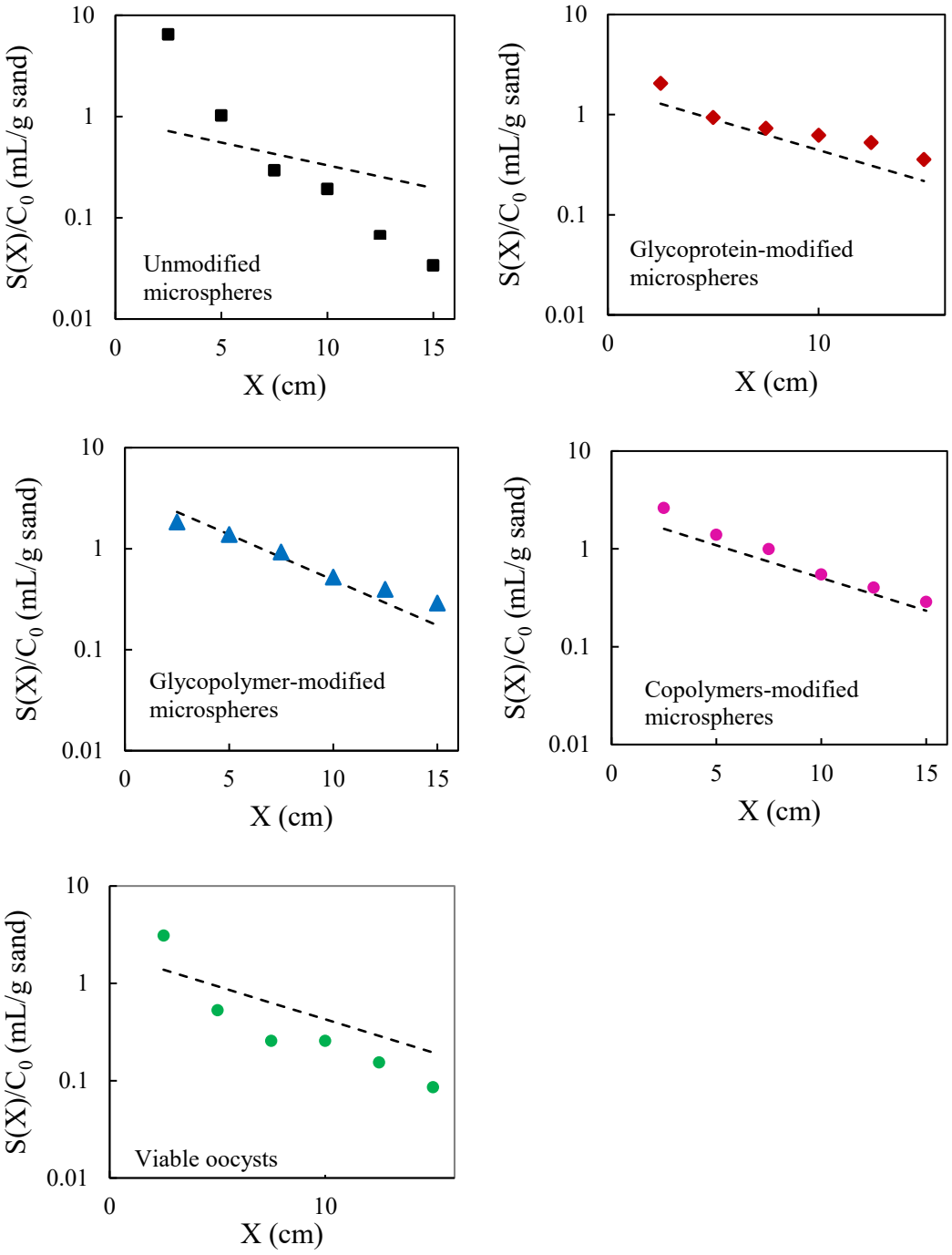


Figure 3-9. The retained particle profiles in the packed-bed column of unmodified microspheres. The dash lines represent predictions based on the CFT using a deposition rate coefficient (K_d) determined using Eq. 2 from the corresponding breakthrough curves. Data are presented in a semilog format. Experimental conditions were as follows: approach velocity= 0.0092cm/s, porosity=0.43, and pH=8.0.

3.4 Conclusion

In this study, we have demonstrated the feasibility of using the QCM-D methodology and packed-bed column experiments for investigating *Cryptosporidium*-silica surface interactions with modified microspheres as surrogates. Our results have demonstrated that modified microspheres are superior to unmodified microspheres as surrogates for studying the filtration removal of *Cryptosporidium* in porous media under the studied conditions. After the modification, the microspheres had similar zeta potential to the viable oocysts, and the surface hydrophilicity increased to a level that was much closer to that of the viable oocysts. Among modified microspheres, zwitterionic microspheres are the most representative surrogates for studying the removal of oocysts in filtration process, which indicated that the glycan and zwitterionic layers on the oocyst's surfaces were both involved in adhesion to the grain media. These microspheres are considered safe, cost-effective, and easy to work with. In addition, they can be detected rapidly with automatic counting techniques (e.g., a spectrofluorimeter, flow cytometer, or particle counter).

These modifications dramatically reduced the material costs by using glycoprotein and made it possible to use large quantities of modified microspheres in a larger scale application. Further validation of these newly developed surrogates in other porous media (such as GAC) and larger scale applications (such as full-scale *Cryptosporidium* surrogate removal efficiency) will be necessary. The potential use of copolymers-modified microspheres to evaluate the efficiency of removing *Cryptosporidium* oocysts in water treatment will improve the understanding of variability on the removal of *Cryptosporidium* surrogates in pilot- and full-scale water treatment processes investigation.

References

Amburgey, J. E., Amirtharajah, A., York, M. T., Brouckaert, B. M., & et al. (2005). Comparison of Conventional and Biological Filter Performance for Cryptosporidium and microsphere removal. *American Water Works Association. Journal; Denver*, *97*(12), 77–91,10.

Behrens, H., Beims, U., Dieter, H., Dietze, G., Eikmann, T., Grummt, T., … Tilkes, F. (2014). Toxicological and ecotoxicological assessment of water tracers. *Hydrogeology Journal*, *9*(3), 321–325. https://doi.org/10.1007/s100400100126

Berglin, M., Olsson, A., & Elwing, H. (2008). The Interaction Between Model Biomaterial Coatings and Nylon Microparticles as Measured with a Quartz Crystal Microbalance with Dissipation Monitoring. *Macromolecular Bioscience*, *8*(5), 410–416. https://doi.org/10.1002/mabi.200700218

Bradford, S A, & Bettahar, M. (2005). Straining, attachment, and detachment of cryptosporidium oocysts in saturated porous media. *Journal of Environmental Quality*, *34*(2), 469–478.

Bradford, S. A., Simunek, J., Bettahar, M., van Genuchten, M. T., & Yates, S. R. (2006). Significance of straining in colloid deposition: Evidence and implications. *Water Resources Research*, *42*(12), W12S15. https://doi.org/10.1029/2005WR004791

Bradford, S., Torkzaban, S., & Walker, S. (2007). Coupling of physical and chemical mechanisms of colloid straining in saturated porous media. *WATER RESEARCH*, *41*(13), 3012–3024.

Bradford, Scott A., Yates, S. R., Bettahar, M., & Simunek, J. (2002). Physical factors affecting the transport and fate of colloids in saturated porous media. *Water Resources Research*, *38*(12), 1327. https://doi.org/10.1029/2002WR001340

Bushkin, G. G., Motari, E., Carpentieri, A., Dubey, J. P., Costello, C. E., Robbins, P. W., & Samuelson, J. (2013). Evidence for a structural role for acid-fast lipids in oocyst walls of Cryptosporidium, Toxoplasma, and Eimeria. *MBio*, *4*(5), e00387-13.

Chung, J. (2012). *Development of fluorescently labelled Cryptosporidium oocyst surrogates to test the efficacy of sand filtration processes*. University of New South Wales.

Dai, X, Boll, J., Hayes, M. E., & Aston, D. E. (2004). Adhesion of Cryptosporidium parvum and Giardia lamblia to solid surfaces: the role of surface charge and hydrophobicity. *Colloids and Surfaces B: Biointerfaces*, *34*(4), 259–263. https://doi.org/10.1016/j.colsurfb.2003.12.016

Dai, Xiaojun, & Hozalski, R. M. (2002). Effect of NOM and biofilm on the removal of Cryptosporidium parvum oocysts in rapid filters. *Water Research*, *36*(14), 3523–3532. https://doi.org/10.1016/S0043-1354(02)00045-3

Dai, Xiaojun, & Hozalski, R. M. (2003). Evaluation of Microspheres as Surrogates for Cryptosporidium parvum Oocysts in Filtration Experiments. *Environmental Science & Technology*, *37*(5), 1037–1042. https://doi.org/10.1021/es025521w

Deng, Z., Ahmed, M., & Narain, R. (2009). Novel well-defined glycopolymers synthesized via the reversible addition fragmentation chain transfer process in aqueous media. *Journal of Polymer Science Part A: Polymer Chemistry*, *47*(2), 614–627. https://doi.org/10.1002/pola.23187

Emelko, M. B. (2003). Removal of viable and inactivated Cryptosporidium by dual- and tri-media filtration. *Water Research*, *37*(12), 2998–3008.
Emelko, M. B., & Huck, P. M. (2004). Microspheres as Surrogates for "Cryptosporidium" Filtration. *Journal (American Water Works Association)*, *96*(3), 94–105.
Emelko, M. B., Huck, P. M., & Coffey, B. M. (2005). A review of Cryptosporidium removal by granular media filtration. *Journal (American Water Works Association)*, *97*(12), 101–115.
Gregory, J. (2005). *Particles in water: properties and processes*. CRC Press.
Haas, C. N., Severin, B. F., Roy, D., Engelbrecht, R. S., Lalchandani, A., & Farooq, S. (1985). Field observations on the occurrence of new indicators of disinfection efficiency. *Water Research*, *19*(3), 323–329.
Hagler, A. N. (2006). Yeasts as indicators of environmental quality. In *Biodiversity and ecophysiology of yeasts* (pp. 515–532). Springer.
Zhang, H., Seaman, J., Wang, Y., Zeng, H., Narain, R., Ulrich, A, & Liu., Y (2017a). Filtration of Glycoprotein-Modified Carboxylated Polystyrene Microspheres as Cryptosporidium Oocysts Surrogates: Effects of Flow Rate, Alum, and Humic Acid. Journal of Environmental Engineering, 143(8). https://doi.org/10.1061/(ASCE)EE.1943-7870.0001201
Jenkins, M. B., Eaglesham, B. S., Anthony, L. C., Kachlany, S. C., Bowman, D. D., & Ghiorse, W. C. (2010). Significance of Wall Structure, Macromolecular Composition, and Surface Polymers to the Survival and Transport of Cryptosporidium parvum Oocysts. *Applied and Environmental Microbiology*, *76*(6), 1926–1934. https://doi.org/10.1128/AEM.02295-09
Kuznar, Z. A., & Elimelech, M. (2005). Role of Surface Proteins in the Deposition Kinetics of Cryptosporidium parvum Oocysts. *Langmuir*, *21*(2), 710–716. https://doi.org/10.1021/la047963m
Kuznar, Z. A., & Elimelech, M. (2006). Cryptosporidium Oocyst Surface Macromolecules Significantly Hinder Oocyst Attachment. *Environmental Science & Technology*, *40*(6), 1837–1842. https://doi.org/10.1021/es051859p
Liu, Y., & Li, J. (2008). Role of Pseudomonas aeruginosa Biofilm in the Initial Adhesion, Growth and Detachment of Escherichia coli in Porous Media. *Environmental Science & Technology*, *42*(2), 443–449. https://doi.org/10.1021/es071861b
Liu, Y., Yang, C.-H., & Li, J. (2007a). Adhesion and retention of a bacterial phytopathogen Erwinia chrysanthemi in biofilm-coated porous media. *Environmental Science & Technology*, *42*(1), 159–165.
Liu, Y., Yang, C.-H., & Li, J. (2007b). Influence of Extracellular Polymeric Substances on Pseudomonas aeruginosa Transport and Deposition Profiles in Porous Media. *Environmental Science & Technology*, *41*(1), 198–205. https://doi.org/10.1021/es061731n
Lu, P., Amburgey, J. E., Hill, V. R., Murphy, J. L., Schneeberger, C. L., Arrowood, M. J., & Yuan, T. (2017). Removals of cryptosporidium parvum oocysts and cryptosporidium-sized polystyrene microspheres from swimming pool water by diatomaceous earth filtration and perlite-sand filtration. *Journal of Water and Health*, wh2017221. https://doi.org/10.2166/wh.2017.221

Okhuysen, P. C., Rich, S. M., Chappell, C. L., Grimes, K. A., Widmer, G., Feng, X., & Tzipori, S. (2002). Infectivity of a Cryptosporidium parvum isolate of cervine origin for healthy adults and interferon-γ knockout mice. *Journal of Infectious Diseases*, *185*(9), 1320–1325.

Pang, L., Nowostawska, U., Weaver, L., Hoffman, G., Karmacharya, A., Skinner, A., & Karki, N. (2012). Biotin- and Glycoprotein-Coated Microspheres: Potential Surrogates for Studying Filtration of *Cryptosporidium parvum* in Porous Media. *Environmental Science & Technology*, *46*(21), 11779–11787. https://doi.org/10.1021/es302555n

Shao, Q., & Jiang, S. (2015). Molecular Understanding and Design of Zwitterionic Materials. *Advanced Materials*, *27*(1), 15–26. https://doi.org/10.1002/adma.201404059

Stevenson, M. E., Blaschke, A. P., Toze, S., Sidhu, J. P. S., Ahmed, W., van Driezum, I. H., … Pang, L. (2015). Biotin- and Glycoprotein-Coated Microspheres as Surrogates for Studying Filtration Removal of Cryptosporidium parvum in a Granular Limestone Aquifer Medium. *Applied and Environmental Microbiology*, *81*(13), 4277–4283. https://doi.org/10.1128/AEM.00885-15

Tufenkji, N., Dixon, D. R., Considine, R., & Drummond, C. J. (2006a). Multi-scale Cryptosporidium/sand interactions in water treatment. *Water Research*, *40*(18), 3315–3331. https://doi.org/10.1016/j.watres.2006.07.036

Tufenkji, N., Dixon, D. R., Considine, R., & Drummond, C. J. (2006b). Multi-scale Cryptosporidium/sand interactions in water treatment. *Water Research*, *40*(18), 3315–3331. https://doi.org/10.1016/j.watres.2006.07.036

Tufenkji, N., & Elimelech, M. (2005). Spatial Distributions of *Cryptosporidium* Oocysts in Porous Media: Evidence for Dual Mode Deposition. *Environmental Science & Technology*, *39*(10), 3620–3629. https://doi.org/10.1021/es048289y

Tufenkji, N., Miller, G. F., Ryan, J. N., Harvey, R. W., & Elimelech, M. (2004). Transport of Cryptosporidium Oocysts in Porous Media: Role of Straining and Physicochemical Filtration. *Environmental Science & Technology*, *38*(22), 5932–5938.

Tufenkji, N., Redman, J. A., & Elimelech, M. (2003). Interpreting Deposition Patterns of Microbial Particles in Laboratory-Scale Column Experiments. *Environmental Science & Technology*, *37*(3), 616–623. https://doi.org/10.1021/es025871i

Walker, M., & Redelman, D. (2004). Detection of Cryptosporidium parvum in soil extracts. *Applied and Environmental Microbiology*, (3), 1827.

Widerström, M., Schönning, C., Lilja, M., Lebbad, M., Ljung, T., Allestam, G., … Lindh, J. (2014). Large Outbreak of Cryptosporidium hominis Infection Transmitted through the Public Water Supply, Sweden. *Emerging Infectious Diseases*, *20*(4), 581–589. https://doi.org/10.3201/eid2004.121415

Yang, R., Murphy, C., Song, Y., Ng-Hublin, J., Estcourt, A., Hijjawi, N., … Ryan, U. (2013). Specific and quantitative detection and identification of Cryptosporidium hominis andC. parvum in clinical and environmental samples. *Experimental Parasitology*, *135*(1), 142–147. https://doi.org/10.1016/j.exppara.2013.06.014

Chapter 4. Factors controlling the *Cryptosporidium* surrogates removal: lab-scale study[2]

4.1 Introduction

The transport and retention of pathogens, specifically *Cryptosporidium*, in an aqueous environment is a complex process, and can be affected by many factors. Classical laboratory-scale packed-bed column experiments have been intensively conducted by many researchers to explore the factors controlling the fate and transport of particles in porous media (Abudalo et al., 2010; Bradford & Bettahar, 2005; Dai & Hozalski, 2002, 2003; Hijnen et al., 2007; Hsu et al., 2001; Mohanram et al., 2012; Tufenkji & Elimelech, 2004a, 2004b, 2005). In this chapter, a surrogate of *Cryptosporidium* oocysts was prepared using copolymers-modified carboxylated polystyrene microspheres, which was demonstrated to be the most representative surrogates for *Cryptosporidium* oocyst as shown in Chapter 3. Solution ionic strength and composition are the most readily controlled conditions for transport experiments. Research has shown that electrolyte with higher ionic strength and/or composed of divalent ions rather than monovalent ions enhanced the aggregation or the deposition of suspended particles due to increased zeta potentials (Kuznar & Elimelech, 2004a; Lerner et al., 2012). Also, the results of oocyst transport experiments demonstrated an increase in oocyst removal with decreasing solution pH (Lytle et al., 2002). The zeta potential of oocysts became less negative with increasing ionic strength or decreasing pH. A decreased zeta potential is usually caused by the compression of the electrical double layers, indicating a less stable colloidal suspension (Chen & Walker, 2007, 2012; Saleh et al., 2008; Zhao et al., 2014). To some degree, oocysts behaved as model colloids in response to changes in solution chemistry (Tufenkji, 2006). Tufenkji et al. (2004a and 2006) claimed that this observed dependence of oocyst attachment with changes in solution salt concentration or pH confirmed the importance of physicochemical filtration playing on oocyst removal in porous media. Gao and Chorover (2009) using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR)

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spectroscopy technique also acknowledged the changes in solution chemistry strongly impact oocyst adhesion behavior in aqueous systems.

Dissolved organic matter (DOM) usually refers to those naturally occurring molecules and macromolecules (i.e. anionic, organic) that are ubiquitous in natural water surface and subsurface waters. Its presence was broadly found to enhance the transport of bacteria (Yang et al., 2012; Zhao et al., 2014), nanoparticles (Wang et al., 2012; Xie et al., 2008), and protozoa such as *Cryptosporidium* (Abudalo et al. 2010). However, increased removal of *Cryptosporidium* oocysts was also reported in rare occasion when *C. parvum* oocysts travelled through organic matter-coated silica surfaces (Liu et al., 2009). The authors claimed that the increased attachment efficiency of oocysts on organic matter-coated silica surfaces were attributed to surface roughness, surface charge heterogeneity and deposition at the secondary minimum energy well. In general, the adsorption of negatively-charged DOM on a sand surface can reversely alter the positively-charged collector surfaces (Abudalo et al. 2010) or significantly increase the magnitude of the negatively-charged oocyst surface or collector surface (Dai and Hozalski 2003). Therefore, repelling force between particles and collectors was increased. Other than solution chemistry, porous media properties, such as size and surface roughness, flow velocity, and oocyst concentrations were previously examined by many researchers and verified to impact the removal of oocyst in the porous media. Well-controlled laboratory experiments are still needed to fully elucidate the influence of the potential factors on the interactions between newly-developed *Cryptosporidium* surrogates and porous media.

In this chapter, factors contributing to the solution chemistry were investigated using *Cryptosporidium* surrogates copolymers-modified microspheres established as shown in Chapter 3; that is ionic strength, pH, and DOC of aqueous solution. Single-collector contact efficiency and DLVO interaction energy were calculated to facilitate the explanation of transport and retention of *Cryptosporidium* oocysts surrogates in the porous media.

4.2 Materials and Methods

4.2.1 *Cryptosporidium* surrogates

Copolymers-modified microspheres were used as surrogates for *Cryptosporidium* oocysts to mimic the size, shape, zeta potential and hydrophobicity of viable oocysts. They were prepared as descript in Chapter 3. The concentration of influent surrogates (2.7-2.8×10^4/mL) remained consistent for each condition examined. The surrogate suspensions were sonicated in a water bath for 10 min prior to the experiments to prevent particle aggregation. A constant surrogate concentration was maintained by agitating the suspension by a magnetic stirring bar during the injection of suspension.

4.2.2 Packed-bed column study

4.2.2.1 Electrolyte conditions

In this chapter, the impacts of solution ionic strength, pH and DOC on the transport and deposition behavior of oocysts surrogates were examined in the laboratory-scale sand packed filter columns. Aqueous sodium chloride background solutions of concentrations of 1.0 and 10.0 mM were prepared using reagent grade NaCl (Fisher Scientific Inc., ON, Canada) and deionized water, thereby creating unfavorable (low ionic strength) and favorable (high ionic strength) conditions for particle deposition, respectively. The pH of the solutions was adjusted to 6.0, 8.0 and 10.0 by the addition of 1.0 mM NaOH or 1.0 mM HCl. The DOC solution was prepared by diluting stock solution of Nordic Reservoir Nature Organic Matter (NOM) (IHSS, MN, USA) to a final DOC concentration equals to 2.0 mg/L, which was a typical concentration in the North Saskatchewan River (NSR) at the cold weather condition during the examination period (winter 2016 and winter 2017). The Nordic Reservoir NOM was selected as a model natural organic matter due to its similarity with the NSR NOM in qualitative Fourier Transform Infrared (FT-IR) analysis. The results were not shown in here. NOM stock solutions were prepared following the method of Yang et al. (2012). The dissolved organic carbon (DOC) content of the stock solution was determined by a Shimadzu TOC-L CPH E100 (Kyoto, Japan) with a detection limit of 4 μg C/L. All the suspensions were prepared at room temperature (20-22˚C). The experimental conditions tested in this study were listed in Table 4-1.

4.2.2.2 Column experiments

The packed-bed column experiments were performed in Chromaflex® chromatography columns (Kimble Chase, USA), that were 4.8 cm (ID) ×15 cm (length) and made of borosilicate glass. Granular sand media, commonly used in a full-scale drinking water treatment filtration process, with an effective size of 0.35-0.45 mm and a uniformity coefficient of less than 1.5, were used as model media in this study. Sands were thoroughly cleaned using acid and base washing, following protocols described by Zhang et al. (2017a). This intensive cleaning procedure yielded very reproducible particle deposition results (Liu et al., 2007a, and 2007b). For each experiment, approximately 380 g of freshly cleaned sand was wet packed with mild vibration to minimize any layering or air entrapment to a total depth of 15 cm, resulting in a porosity of 0.43. The column was first equilibrated by pumping 10 PV of particle-free background electrolyte through the column at 10 mL/min with an upflow mode provide by a peristaltic pump (Cole Parmer, IL). After that, approximately 5 PV of homogenized surrogates containing solutions (2.7-2.8×10^4/mL particle) were injected. The influent concentration was determined in triplicate by a hemocytometer (Hausser Scientific Bright-Line™ counting chamber, USA). A constant influent concentration was maintained by mixing a particle suspension with a magnetic stir bar. Following the particle injection, the column was eluted with an additional 8 PV of the background electrolyte. Every half PV of the column effluent was collected in a 50-mL polystyrene centrifuge tube before enumeration.

After the transport experiment, the filter media was evenly dissected into six segments (2.5 cm of each segment) and the retained particles were recovered for 10 minutes sonication in the DI water. CFT was used to obtain the theoretical particle retention pattern in the packed columns based on microspheres breakthrough curves (Liu et al., 2007). The particle distribution S(X), which is the number of deposited particles per mass of the granular collector, was calculated using the following equation:

$$S(X) = \frac{t_0 \varepsilon K_d C_0}{\rho_b} \exp\left(-\frac{K_d X \varepsilon}{U}\right) \quad (1)$$

Where t_0 is the duration of continuous particle injection, C_0 is the initial particle concentration, ρ_b is the porous medium bulk density, X is the column depth, and K_d is the

deposition rate coefficient, which as estimated based on the steady state breakthrough concentration. The overall recovery (mass balance) of microspheres was determined by summing the percentages of microspheres that exited and that were retained in the column, as shown in Table 4-1.

4.2.3 Detection and analysis

The fluorescence intensity of samples was determined using Varian Fluorescence Spectrophotometer (Cary Eclipse, USA) under emission mode, with the wavelength set from 400 to 500 nm. The excitation wavelength was set at 441 nm according to the manufacture's specification. The scan rate was set at 600 nm/min. The surface modification process does not change the fluorescence intensity of the microspheres, which were verified before and after the modification process. The multi-level standard calibrations of the fluorescence spectrophotometer were performed using the copolymers-modified microspheres. The concentrations of influent, effluent and deposited samples were calculated based on a calibration curve as shown in Appendix A. All the samples were examined in triplicate.

4.2.4 Single-collector contact efficiency and DLVO interaction energy

The theory of colloid filtration was first introduced by Yao et al. (1971). More recently, Tufenkji and Elimelech (2004) developed a new expression (T-E model) for single-collector contact efficiency (η_0), defined as the ratio of the rate at which colloids strike a collector surface to the rate at which particles flow toward the collector (Yao et al., 1971), which was used in this study. Park et al. (2012) found that T-E model is reliable for estimating *Cryptosporidium* oocyst attachment efficiency. Collector removal efficiency (η, Eq. (2)) for a filtration column setup can be found through lab column experiments; an empirical attachment efficiency (α, Eq. (3)) can be established using collector diameter (d_c), porosity (θ), column length (L), and the ratio of influent and effluent particle concentrations ($\frac{C}{C_0}$).

$$\eta = \alpha\eta_0 \qquad (2)$$

$$\alpha = -\frac{2}{3}\frac{d_c}{(1-\theta)L\eta_0} ln\frac{C}{C_0} \quad (3)$$

The DLVO interaction energy was applied to elucidate whether the increased empirical attachment efficiency (α) under acidic condition and higher ionic strength condition was due to the decreased electrostatic and the increased van der Waals forces. Zeta potentials of collector media and surrogates in each condition were measured and used for the calculation of DLVO interaction energy based on the equations 2-2 to 2-4 from Chapter 2

4.3 Results and Discussion

4.3.1 Mass balance

Good recovery rates were obtained from the experiments. The mass recoveries (total from effluent and retained) were from 83.3% to 97.8% (Table 4-1).

Table 4-1. Summary of experimental conditions and recovery of surrogates in packed-bed column studies by conducting microspheres mass balance.

Condition	Ionic strength (mM)	pH	DOC (mg/L)	Recovery (%)
1	1.0	8.0	0	83.3
2	10.0	8.0	0	83.5
3	1.0	6.0	0	86.4
4	1.0	10.0	0	97.8
5	1.0	8.0	2	94.0

4.3.2 Electrokinetic characterization of microspheres and collectors and DLVO interaction energy

The measured zeta potentials of surrogates and clean sands in all tested conditions were represented in Table 4-2. Worth to notice is that the zeta potentials of the sand media were all negative in the tested conditions, which were in agreement with other study (Zhang et al., 2017a). The zeta potentials of sands and surrogates at higher ionic strength were less negative than those at lower ionic strength, which is generally consistent with results reported by others (Bradford et al., 2007; Knappett et al., 2008; Walshe et al., 2010). This could be explained by a reduction in the thickness of the double layer at a greater salt concentration, promoting the surrogate attachment to the collector media (Walshe et al., 2010). The zeta potential of surrogates in acidic conditions (condition 3) was positive,

which implied an attractive electrostatic force with negatively-charged sand media. On the other hand, when the solution pH increased to 10.0, the zeta potential of surrogates and sand decreased dramatically to -44.8 mV and -53.8 mV, respectively. The strong repelling forces between surrogates and sand were suspected to contribute to a decreased interaction energy. The minimum attachment efficiency would be observed at pH=10.0 because the zeta potential of surrogates and sand were the most negative.

Table 4-2. Zeta potential of surrogates and sand media and their DLVO interaction energies.

Condition	Ionic strength (mM)	pH	DOC (mg/L)	Zeta potential (mV)		Depth of secondary energy minimum[a] (kT)	Separation distance to secondary energy well (nm)	Energy barrier (kT)
				Surrogates	Sand			
1	1.0	8.0	0.0	-18.0	-52.2	0.28	109	1470
2	10.0	8.0	0.0	-0.89	-35.5	NA[b]	NA	NB[c]
3	1.0	6.0	0.0	22.4	-36.3	NA	NA	NB
4	1.0	10.0	0.0	-44.8	-53.5	0.24	120.5	6940
5	1.0	8.0	2.0	-49.2	-56.1	0.23	122.5	8150

*[a] value calculated within the separation distance of 150 nm
[b] not applicable
[c] no energy barrier

The DLVO interaction energies between the surrogates and sand media surfaces were calculated based on the equations 2-2 to 2-4 using the measured zeta potential values in five different conditions, as shown in Table 4-2. As expected, no energy barrier was observed for higher ionic strength (10 mM) and acidic condition (pH=6.0), indicating favorable condition for adhesion (Zhang et al., 2017a), which was in good agreement with the measurement of zeta potential. Surrogates were less negative or turned into positive at these two conditions, making the repelling force with the sand media diminished or even turn into attractive. In Table 4-2, the maximum energy barrier (8150 kT) was observed when DOM was present in the background electrolyte solution, with the depth of secondary energy minimum being 0.23 kT and a separation distance of 122.5 nm. Particles at this condition are believed to overcome large energy barrier via “slow” deposition to the

collector (Tufenkji and Elimelech, 2004b). Another notice is that more alkaline solution (pH = 10) had much higher energy barrier (6940 kT) comparing with that at a pH of 8.0 (1470 kT), indicating unfavorable conditions for adhesion under more alkaline condition. Closer inspection demonstrated the presence of secondary energy minimum for both alkaline conditions (pH 8.0 and 10.0) with ionic strength equals to 1.0 mM. There were -0.28 and -0.24 kT at separation distance of 109.0 and 120.5 nm, respectively. Particles at these conditions are believed to get stuck in the secondary energy minimum via "fast" deposition to attach to the collector (Tufenkji and Elimelech, 2004b). Using different scales for the interaction energy, Figure 4-1 and its insert show the repulsive DLVO energy barriers and the secondary minimums of the surrogate-collector interactions in this study, respectively, in the unit of kT.

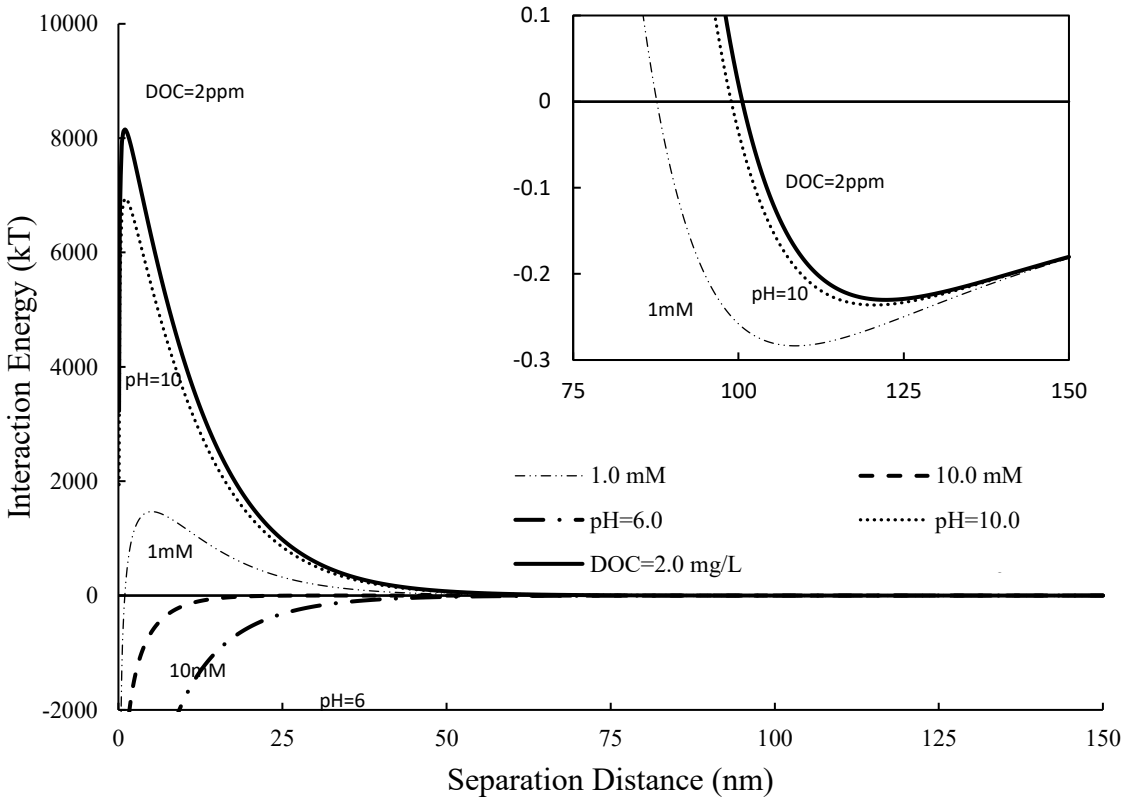

Figure 4-1. The theoretical interaction energies of surrogate transport through the packed-bed sand column.

4.3.3 Observed transport and retention behaviors of surrogates

4.3.3.1 Influence of ionic strength

Ionic strength governs the shape and magnitude of DLVO interaction energy profiles and is critical to particle deposition behavior (Tufenkji and Elimelech, 2004a; Molnar et al., 2015; Torkzaban and Bradford, 2016; Jin et al., 2017). As expected, the higher peak-concentration was observed at lower ionic strength condition (Figure 4-2) by comparing breakthrough curves at two different conditions, consistent with information presented in the DLVO calculation. The increased magnitude of the steady-state breakthrough plateau decreased with increasing ionic strength, indicating a temporal increase in the deposition rate during the course of injection. The single-collector contact efficiency (η) and attachment efficiency (α) were then calculated based on Tufenkji and Elimelech model (2004), which account for van der Waals forces and hydrodynamic forces. It illustrated how the different conditions impact the rate at which surrogates were deposited on the media. Lower ionic strength (1 mM; condition 1) led to more positive DLVO interaction energy and the related forces became repulsive, thereby leading to slightly decreased deposition rate coefficient and attachment efficiency compared for those in higher ionic strength condition (10 mM; condition 2). The K_d and α values were given in Table 4-3. Clearly, the higher K_d (61.3 s^{-1}) was observed at higher ionic strength (10.0 mM), which indicated a favorable condition for the surrogate attachment.

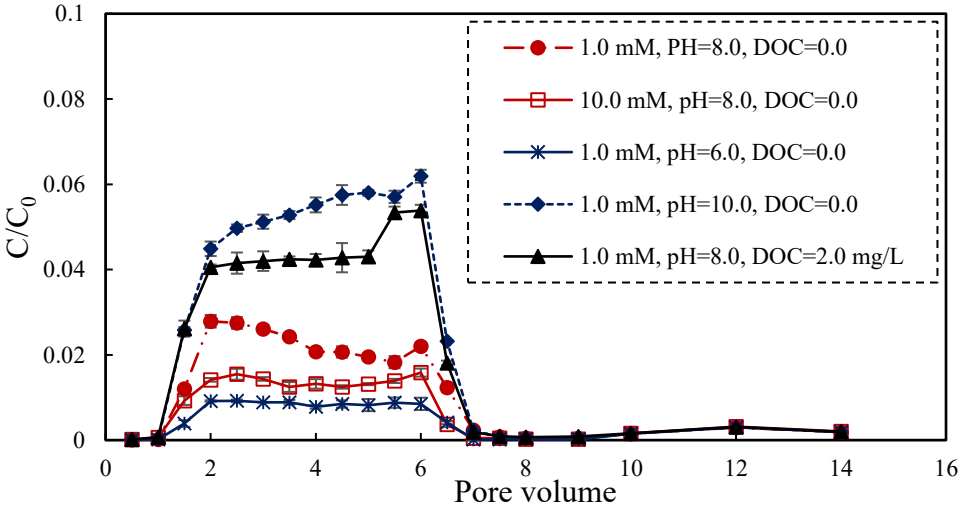

Figure 4-2. Comparison of breakthrough curves in packed-bed column at different conditions.

The retained surrogate profiles compared with those predict by the CFT is shown in Figure 4-3. A log-linear relationship was drawn between the number of retained surrogates and the transport distance in the clean column, indicating a constant K_d as implicated in the "clean-bed" theory. The experimental data deviate apparently from the predicted model. Deviation of the retained profile from expectation was first demonstrated in literature two decades ago (Albinger et al., 1994). Bimodal, power-law, log-normal distribution of deposition rate coefficients had been invoked (Li et al., 2005). This spatial variation of K_d in the column was observed for biological colloids, like bacterial cells (Liu et al., 2007a) and non-biological colloids, like glycoprotein-modified microspheres (Zhang et al., 2017a). The observed deviations could be explained by the heterogeneity in particle surface properties, grain surface properties, distribution in the interaction energies between particles and porous media, straining, and presence of macromolecules on the surface of microspheres. The shape of retained surrogates in low and high ionic strength condition remained monotonic. The different shapes of retained profiles at 1.0 mM and 10.0 mM indicated the sensitivity of deposition rate to ionic strength, which was consistent with the calculation of K_d shown in Table 4-3.

Table 4-3. Attachment efficiency, single-collector contact efficiency and deposition rate coefficient for each condition.

Condition	**Breakthrough concentration** $\frac{C}{C_0}$	**Attachment efficiency** α	**Single-collector contact efficiency** $\eta_0 \times 10^{-2}$	**Deposition rate coefficient** K_d **(1/s)**
1	0.0202	10.9	1.22	55.7
2	0.0137	12.0	1.34	61.3
3	0.0083	13.4	1.49	68.5
4	0.0579	7.9	0.89	40.7
5	0.0470	8.5	0.95	43.7

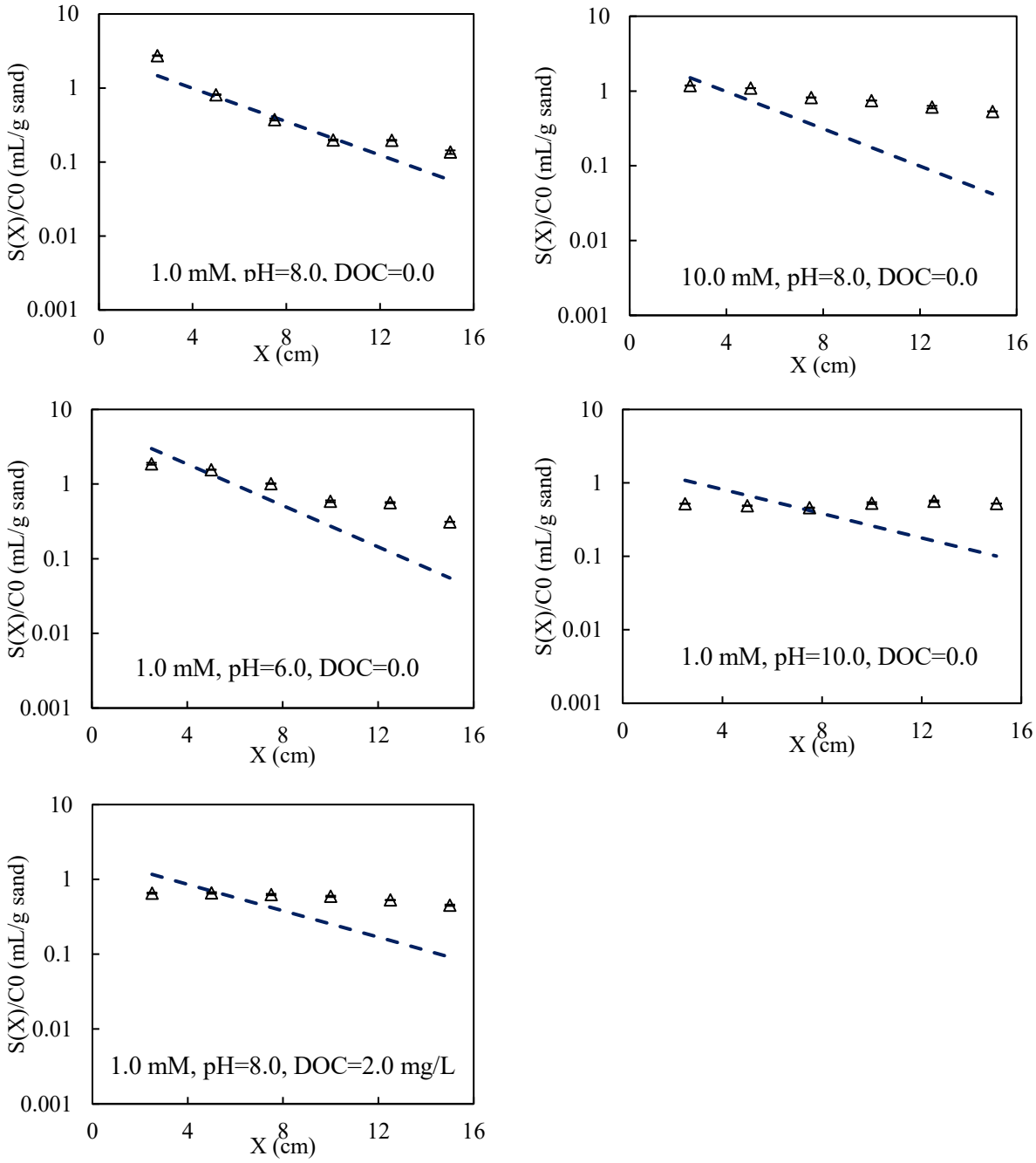

Figure 4-3. Comparison of deposition profiles of surrogates (copolymers-modified microspheres) in packed-bed column at different conditions. The dotted blue line indicated the prediction of deposited surrogates in the filter media as function of travel distance based on CFT.

4.3.3.2 Influence of pH

The effect of pH on the filtration of surrogates was also examined because it also impacts the surface charge of surrogates and collectors by neutralizing the negative zeta potentials of both particles, which are believed to contribute to a crucial factor controlling the deposition of particles in the filter media. It reflected in the DLVO theory that with pH increased the surrogates became less retained in the filter media, indicated by the much higher energy barrier (6940 kT) in alkaline condition, as shown in Table 4-2.

By comparing the breakthrough curves in condition 1, 3 and 4, the peak-concentration was increased significantly at pH 10.0, indicated also by the decreased magnitude of deposition profile, the smallest deposition rate coefficient (K_d=40.7 s^{-1}) and smallest attachment efficiency (α=7.9). It was clearly shown in Table 4-3 that maximum K_d (68.5 s^{-1}) was obtained when the pH deceased to 6.0, whereas the minimum K_d was observed when the background solution pH increased to 10.0. As pH decrease, the surface charges of surrogates and sand media become less negative due to the increasing [H^+] neutralizing the negative charge of their surfaces (Ongerth and Pecoraro, 1996) and their electrostatic repulsion between one another becomes weaker, resulting in a greater attachment of surrogates to sand surface. Unfavorable interaction of deposition was obtained under pH=10.0 condition, as shown in Figure 4-3 (4). This unfavorable condition for adhesion well explained the observed enhanced transported and overall low retention of surrogates as compared to other favorable conditions in the packed sand column (see Figure 4-3 (1) and (3)).

Close inspection of the retained profile for pH=10.0 condition, segments near the column outlet displayed increased retained surrogate concentration relative to preceding segment, which is possible due to surrogate re-deposition. Interestingly, the maximum retained surrogate concentrations were located increasingly down-gradient (close to column exit) when the pH of solution increased to 10.0. The solution pH plays a key role in the change of maximum retained concentration. The sensitivity of retained profiles shape to solution chemistry suggested that surrogate deposition occurs via multiple mechanisms in a given

porous medium, and that the dominant mechanisms yields specific profile shapes (Li et al., 2006b).

4.3.3.3 Influence of DOC

The transport of surrogates in the column enhanced as the concentration of DOC increased, suggested in Figure 4-2 of the breakthrough curve. This also reflected in the decreased retention of surrogates in the deposition profile in Figure 4-3 (5). The filter media in the bottom of the column bed seems lost its ability to retain surrogates, indicated by a decreased magnitude of deposition profile, which could be explained by very shallow secondary energy minimum of the DLVO interaction energy profile (Figure 4-1). Increased DOC concentration also lowers the K_d and α values, as shown in Table 4-3.

The reduced attenuation and enhanced transport of surrogates at DOM present condition (condition 5) can be interpreted as a result of DOM competing with surrogates for available attachment sites (Walshe et al., 2010). The author and coworkers claimed that because of the more negative charge of DOM than surrogates, organic matter would have greater tendency to occupy favorable attachment sites than surrogates, and thus form an electrostatic barrier that inhibits surrogate attachment to the collector media. A site competition by a portion of DOM and the repelling deposition caused by suspended DOM in solution seem to result in the reduced cell retention (Yang et al., 2012). Many other researchers have also reported the enhanced transport of surrogates and *Cryptosporidium* in the presence of organic matter (Dai and Hozalski, 2002 and 2003; Abudalo et al., 2010). The site blocking caused by the pre-equilibrating the porous media with DOM could inhibit the surrogates deposition through steric repulsion and increased electrical repulsion (Foppen et al., 2006 and 2008).

4.4 Conclusions

In this study, the effect of solution chemistry (changes of ionic strength, pH and DOC level in filter influent) on the transport and attenuation of *Cryptosporidium* surrogates in the sand-packed columns were examined. The modification of polystyrene microspheres was

proved to be easy and cost effective, and the macromolecule on the surface of microspheres were representative of *Cryptosporidium*. To date, the filtration study using modified microspheres were still limited (Pang et al., 2012; Stevenson et al., 2015; Zhang et al., 2017a). By changing the composition of the background electrolyte solution, the effect of solution chemistry on the transport and attenuation of surrogates in saturated porous media were examined. In general, the results of surrogate transport experiments demonstrated an increase in surrogate removal with increasing solution ionic strength or decreasing solution pH, which is consistent with other studies (Hsu et al., 2001; Tufenkji et al., 2004a; Tufenkji and Elimelech, 2005). This observed dependence of surrogate attachment with changes in solution salt concentration and pH confirms the significant role of physicochemical filtration (i.e., surface interactions between oocysts and the porous medium) in saturated porous media (Hsu et al., 2001; Tufenkji and Elimelech, 2004b). On the other hand, the DOM had negative effect on the retention of surrogates in the packed-bed column, which emphasized the importance of optimal coagulation to remove DOM for the better filtration performance. This will be further discussed in pilot-scale filtration experiment in Chapter 5. This study validates the copolymers-modified microspheres as representative surrogates for *Cryptosporidium* oocysts in the conditions that relevant to drinking water. Further study can be conducted to test the reliability of surrogates in other relevant environmental conditions.

References

Abudalo, R. A., Ryan, J. N., Harvey, R. W., Metge, D. W., & Landkamer, L. (2010). Influence of organic matter on the transport of Cryptosporidium parvum oocysts in a ferric oxyhydroxide-coated quartz sand saturated porous medium. *Water Research*, *44*(4), 1104–1113. https://doi.org/10.1016/j.watres.2009.09.039

Bradford, S. A., & Bettahar, M. (2005). Straining, attachment, and detachment of cryptosporidium oocysts in saturated porous media. *Journal of Environmental Quality*, *34*(2), 469–478.

Bradford, S., Torkzaban, S., & Walker, S. (2007). Coupling of physical and chemical mechanisms of colloid straining in saturated porous media. *WATER RESEARCH*, *41*(13), 3012–3024.

Chen, G., & Walker, S. L. (2007). Role of Solution Chemistry and Ion Valence on the Adhesion Kinetics of Groundwater and Marine Bacteria. *Langmuir*, *23*(13), 7162–7169. https://doi.org/10.1021/la0632833

Chen, G., & Walker, S. L. (2012). Fecal Indicator Bacteria Transport and Deposition in Saturated and Unsaturated Porous Media. *Environmental Science & Technology*, *46*(16), 8782–8790. https://doi.org/10.1021/es301378q

Chrysikopoulos, C. V., & Syngouna, V. I. (2014). Effect of Gravity on Colloid Transport through Water-Saturated Columns Packed with Glass Beads: Modeling and Experiments. *Environmental Science & Technology*, *48*(12), 6805–6813. https://doi.org/10.1021/es501295n

Dai, X., & Hozalski, R. M. (2002). Effect of NOM and biofilm on the removal of Cryptosporidium parvum oocysts in rapid filters. *Water Research*, *36*(14), 3523–3532. https://doi.org/10.1016/S0043-1354(02)00045-3

Dai, X., & Hozalski, R. M. (2003). Evaluation of Microspheres as Surrogates for Cryptosporidium parvum Oocysts in Filtration Experiments. *Environmental Science & Technology*, *37*(5), 1037–1042. https://doi.org/10.1021/es025521w

Danielson, R. E., & Sutherland, P. L. (1986). Porosity. In *Methods of Soil Analysis: Part 1—Physical and Mineralogical Methods* (pp. 443–461). Madison, WI: Soil Science Society of America, American Society of Agronomy. https://doi.org/10.2136/sssabookser5.1.2ed.c18

Foppen, J. W. A., & Schijven, J. F. (2006). Evaluation of data from the literature on the transport and survival of Escherichia coli and thermotolerant coliforms in aquifers under saturated conditions. *Water Research*, *40*(3), 401–426. https://doi.org/10.1016/j.watres.2005.11.018

Foppen, J. W., Liem, Y., & Schijven, J. (2008). Effect of humic acid on the attachment of Escherichia coli in columns of goethite-coated sand. *Water Research*, *42*(1), 211–219. https://doi.org/10.1016/j.watres.2007.06.064

Gao, X., & Chorover, J. (2009). In-situ monitoring of Cryptosporidium parvum oocyst surface adhesion using ATR-FTIR spectroscopy. *Colloids and Surfaces B: Biointerfaces*, *71*(2), 169–176. https://doi.org/10.1016/j.colsurfb.2009.02.003

Hijnen, W. A. M., Dullemont, Y. J., Schijven, J. F., Hanzens-Brouwer, A. J., Rosielle, M., & Medema, G. (2007). Removal and fate of Cryptosporidium parvum, Clostridium perfringens and small-sized centric diatoms (Stephanodiscus

hantzschii) in slow sand filters. *Water Research*, *41*(10), 2151–2162. https://doi.org/10.1016/j.watres.2007.01.056

Hsu, B.-M., Huang, C., & Pan, J. R. (2001). Filtration behaviors of Giardia and Cryptosporidium—ionic strength and pH effects. *Water Research*, *35*(16), 3777–3782.

Zhang, H., Seaman, J., Wang, Y., Zeng, H., Narain, R., Ulrich, A, & Liu., Y (2017a). Filtration of Glycoprotein-Modified Carboxylated Polystyrene Microspheres as Cryptosporidium Oocysts Surrogates: Effects of Flow Rate, Alum, and Humic Acid. Journal of Environmental Engineering, 143(8). https://doi.org/10.1061/(ASCE)EE.1943-7870.0001201

Jin, C., Zhao, W., Normani, S. D., Zhao, P., & Emelko, M. B. (2017). Synergies of media surface roughness and ionic strength on particle deposition during filtration. *Water Research*, *114*, 286–295. https://doi.org/10.1016/j.watres.2017.02.010

Knappett, P. S. K., Emelko, M. B., Zhuang, J., & McKay, L. D. (2008). Transport and retention of a bacteriophage and microspheres in saturated, angular porous media: Effects of ionic strength and grain size. *Water Research*, *42*(16), 4368–4378. https://doi.org/10.1016/j.watres.2008.07.041

Kuznar, Z. A., & Elimelech, M. (2004). Adhesion Kinetics of Viable Cryptosporidium parvum Oocysts to Quartz Surfaces. *Environmental Science & Technology*, *38*(24), 6839–6845. https://doi.org/10.1021/es0494104

Lerner, R. N., Lu, Q., Zeng, H., & Liu, Y. (2012). The effects of biofilm on the transport of stabilized zerovalent iron nanoparticles in saturated porous media. *Water Research*, *46*(4), 975–985. https://doi.org/10.1016/j.watres.2011.11.070

Liu, Y., Janjaroen, D., Kuhlenschmidt, M. S., Kuhlenschmidt, T. B., & Nguyen, T. H. (2009). Deposition of Cryptosporidium parvum Oocysts on Natural Organic Matter Surfaces: Microscopic Evidence for Secondary Minimum Deposition in a Radial Stagnation Point Flow Cell. *Langmuir*, *25*(3), 1594–1605. https://doi.org/10.1021/la803202h

Lytle, D. A., Johnson, C. H., & Rice, E. W. (2002). A systematic comparison of the electrokinetic properties of environmentally important microorganisms in water. *Colloids and Surfaces B: Biointerfaces*, *24*(2), 91–101. https://doi.org/10.1016/S0927-7765(01)00219-3

Mohanram, A., Ray, C., Metge, D. W., Barber, L. B., Ryan, J. N., & Harvey, R. W. (2012). Effect of Dissolved Organic Carbon on the Transport and Attachment Behaviors of *Cryptosporidium parvum* oocysts and Carboxylate-Modified Microspheres Advected through Temperate Humic and Tropical Volcanic Agricultural soil. *Environmental Science & Technology*, *46*(4), 2088–2094. https://doi.org/10.1021/es2003342

Molnar, I. L., Johnson, W. P., Gerhard, J. I., Willson, C. S., & O'Carroll, D. M. (2015). Predicting colloid transport through saturated porous media: A critical review. *Water Resources Research*, *51*(9), 6804–6845. https://doi.org/10.1002/2015WR017318

Ongerth, J. E., & Pecoraro, J. P. (1996). Electrophoretic mobility of *Cryptosporidium* oocysts and Giardia cysts. *Journal of Environmental Engineering*, *122*(3), 228-231.

Pang, L., Nowostawska, U., Weaver, L., Hoffman, G., Karmacharya, A., Skinner, A., & Karki, N. (2012). Biotin- and Glycoprotein-Coated Microspheres: Potential Surrogates for Studying Filtration of *Cryptosporidium parvum* in Porous Media. *Environmental Science & Technology*, *46*(21), 11779–11787. https://doi.org/10.1021/es302555n

Park, Y., Atwill, E. R., Hou, L., Packman, A. I., & Harter, T. (2012). Deposition of Cryptosporidium parvum Oocysts in Porous Media: A Synthesis of Attachment Efficiencies Measured under Varying Environmental Conditions. *Environmental Science & Technology*, *46*(17), 9491–9500. https://doi.org/10.1021/es300564w

Saleh, N., Kim, H.-J., Phenrat, T., Matyjaszewski, K., Tilton, R. D., & Lowry, G. V. (2008). Ionic Strength and Composition Affect the Mobility of Surface-Modified Fe0 Nanoparticles in Water-Saturated Sand Columns. *Environmental Science & Technology*, *42*(9), 3349–3355. https://doi.org/10.1021/es071936b

Stevenson, M. E., Blaschke, A. P., Toze, S., Sidhu, J. P. S., Ahmed, W., van Driezum, I. H., … Pang, L. (2015). Biotin- and Glycoprotein-Coated Microspheres as Surrogates for Studying Filtration Removal of Cryptosporidium parvum in a Granular Limestone Aquifer Medium. *Applied and Environmental Microbiology*, *81*(13), 4277–4283. https://doi.org/10.1128/AEM.00885-15

Torkzaban, S., & Bradford, S. A. (2016). Critical role of surface roughness on colloid retention and release in porous media. *Water Research*, *88*, 274–284. https://doi.org/10.1016/j.watres.2015.10.022

Tufenkji, N. (2006). Application of a dual deposition mode model to evaluate transport of Escherichia coli D21 in porous media. *Water Resources Research*, *42*(12), W12S11. https://doi.org/10.1029/2005WR004851

Tufenkji, N., & Elimelech, M. (2004a). Correlation Equation for Predicting Single-Collector Efficiency in Physicochemical Filtration in Saturated Porous Media. *Environmental Science & Technology*, *38*(2), 529–536. https://doi.org/10.1021/es034049r

Tufenkji, N., & Elimelech, M. (2004b). Deviation from the Classical Colloid Filtration Theory in the Presence of Repulsive DLVO Interactions. *Langmuir*, *20*(25), 10818–10828. https://doi.org/10.1021/la0486638

Tufenkji, N., & Elimelech, M. (2005). Breakdown of Colloid Filtration Theory: Role of the Secondary Energy Minimum and Surface Charge Heterogeneities. *Langmuir*, *21*(3), 841–852. https://doi.org/10.1021/la048102g

Walshe, G. E., Pang, L., Flury, M., Close, M. E., & Flintoft, M. (2010). Effects of pH, ionic strength, dissolved organic matter, and flow rate on the co-transport of MS2 bacteriophages with kaolinite in gravel aquifer media. *Water Research*, *44*(4), 1255–1269. https://doi.org/10.1016/j.watres.2009.11.034

Wang, C., deKrafft, K. E., & Lin, W. (2012). Pt Nanoparticles@Photoactive Metal–Organic Frameworks: Efficient Hydrogen Evolution via Synergistic Photoexcitation and Electron Injection. *Journal of the American Chemical Society*, *134*(17), 7211–7214. https://doi.org/10.1021/ja300539p

Xie, B., Xu, Z., Guo, W., & Li, Q. (2008). Impact of Natural Organic Matter on the Physicochemical Properties of Aqueous C60 Nanoparticles. *Environmental Science & Technology*, *42*(8), 2853–2859. https://doi.org/10.1021/es702231g

Yang, H., Kim, H., & Tong, M. (2012). Influence of humic acid on the transport behavior of bacteria in quartz sand. *Colloids and Surfaces B: Biointerfaces*, *91*, 122–129. https://doi.org/10.1016/j.colsurfb.2011.10.058

Yao, K.-M., Habibian, M. T., & O'Melia, C. R. (1971). Water and waste water filtration. Concepts and applications. *Environmental Science & Technology*, *5*(11), 1105–1112.

Zhang, H., Seaman, J., Wang, Y., Zeng, H., Narain, R., Ulrich, A, & Liu., Y (2017a). Filtration of Glycoprotein-Modified Carboxylated Polystyrene Microspheres as Cryptosporidium Oocysts Surrogates: Effects of Flow Rate, Alum, and Humic Acid. Journal of Environmental Engineering, 143(8). https://doi.org/10.1061/(ASCE)EE.1943-7870.0001201

Zhao, W., Walker, S. L., Huang, Q., & Cai, P. (2014). Adhesion of bacterial pathogens to soil colloidal particles: Influences of cell type, natural organic matter, and solution chemistry. *Water Research*, *53*, 35–46. https://doi.org/10.1016/j.watres.2014.01.009

Chapter 5. Removal of *Cryptosporidium* surrogates in drinking water direct filtration: pilot-scale study[3]

5.1 Introduction

Cryptosporidium is a waterborne protozoan pathogen often found in surface waters used as drinking water supplies**.** A low dose of the waterborne protozoan *Cryptosporidium* can be infectious and cause diarrheal illness in the host. Notable outbreaks of cryptosporidiosis occurred in Milwaukee, Wisconsin, and more recently in Östersund, Sweden, have been linked to inadequate removal of *Cryptosporidium* spp. from drinking water (Widerström et al., 2014). The challenge with *Cryptosporidium* oocysts removal from drinking water treatment processes is associated with their resistance to inactivation by many standard chemical disinfectants (Yang et al., 2013). Although it has been shown that *Cryptosporidium* oocysts can be inactivated by ozonation and ultraviolet irradiation treatments (Langlais et al., 1991; Morita et al., 2002) , substantial costs of energy-intensive ultraviolet radiation and ozonation are associated with these treatment options. Compared to chemical treatment methods, removal of *Cryptosporidium* by dual media filtration (Emelko, 2003; Emelko et al., 2005) is inexpensive, effective and is the most commonly used method of *Cryptosporidium* removal from drinking water. Properly implemented, filtration is a major barrier against *Cryptosporidium* in drinking water treatment processes.

Direct filtration is often considered in drinking water treatment during conditions when source water turbidity and colour readings are low, i.e. Turbidity < 5 Nephelometric Turbidity Units (NTU) and color < 6 Total Colour Units (TCU). Direct filtration involves coagulation and flocculation directly followed by filtration, without a settling process, unlike conventional treatment where settling occurs before filtration. Direct filtration allows reduced chemical inputs, and residual solids production, and thus reduced operational costs (James et al., 2011). Direct filtration differs from conventional treatment in various ways. For instance, in conventional treatment coagulant addition aims to form

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larger particles that are readily settled. In direct filtration, in contrast, there is no preliminary settling; formation of large particles and aggregates is counter-productive because they cause blockage of filter pores and decrease filter run time. However, if particles are too small, they may not be completely removed during direct filtration (Crittenden et al., 2012; Edzwald et al., 1987; Huck et al., 2000; McCormick and King, 1982; Treweek, 1979). Although full-scale and pilot-scale studies on *Cryptosporidium* oocyst removal from water by conventional filtration systems have been evaluated previously, operational factors governing the removal of *Cryptosporidium* via direct filtration are not well studied. Previous conventional filtration studies at bench-, pilot- and full-scales showed that operational factors, such as influent water quality, coagulant condition, filter aid polymer type and dose, hydraulic loading rate, filter configuration, and temperature play significant roles in controlling *Cryptosporidium* removal (Emelko et al., 2004; Dugan et al., 2001; Bellamy et al.,1993; Shaw et al., 2000; Emelko, 2003; Zhou 2016; States et al., 2002). Such information helps to inform the operational strategies required for direct filtration.

Because in temperate climates direct filtration is often implemented in winter when the supply water has low turbidity, it is critical to evaluate the *Cryptosporidium* removal under low temperature conditions. Previous studies showed that alum is generally less effective at low temperatures, which was an observation that has been attributed to lower density flocs and aggregate size (Hanson and Cleasby, 1990). In comparison, alternative aluminum-based coagulants, such as polyaluminium chloride (PACl), have been shown to be effective under both low and normal water temperatures (Gebbie, 2001). However, the performance of alum and PACl on *Cryptosporidium* removal under low temperature conditions has not been fully investigated. Further, it is important to optimize filter aid polymer (FP) under low temperature conditions for maximizing *Cryptosporidium* removal. FP works together with filter grains to collect destabilized particles from the coagulation process by promoting attachment to filter grains (Hendricks, 2006) and helping to form larger and stronger polymer-particle flocs (Zhu et al., 1996; Zhu et al., 2016), thus playing an important role in improving filter effluent quality, and reducing ripening duration and early breakthrough (Zhu et al., 1996). The types and dosages of FP are crucial to optimize

effective particle removal during filtration. Further, previous studies demonstrated that deep bed filtration was an effective and economical treatment for low turbidity water with fine or colloidal size particles that are less than 30 μm in diameter (Swertfeger et al., 1999; Tien and Payatakes, 1979). However, no studies have been reported on the parallel filter performance comparisons for different filter configurations (regular bed vs. deep bed) under low water temperature conditions.

To address the current research knowledge gaps, this study compared the performance of regular bed and deep bed filters with different coagulants, and FP types and doses for *Cryptosporidium* surrogates removal using pilot-scale filtration systems fed with low temperature water (~0.5˚C). The pilot-scale filtration system was located at the EPCOR E. L. Smith Water Treatment Plant at Edmonton, Alberta, Canada and the source water was the North Saskatchewan River. Zwitterionic polymer and glycopolymer co-modified microspheres (later called copolymers-modified microspheres) were used as surrogates for *Cryptosporidium* oocysts in accordance with our recent study (Liu et al., 2018). The surface properties of the unmodified and *Cryptosporidium* surrogates were compared with viable oocysts under typical drinking water conditions. Deposition profiles of the surrogates were constructed to interpret their deposition behavior in the filter media.

5.2 Materials and Methods

5.2.1 Pilot filtration setup

Four pilot-scale regular bed filters were 152 mm in diameter and 3650 mm in height. These filters were packed with 200 mm anthracite and over 550 mm sand. Further, four deep bed filters were 152 mm in diameter and 5500 mm in height, which were packed with 1400 mm anthracite overlaying 550 mm sand. Figure 5-1 shows schematic diagrams and pictures of the pilot columns. Column dimensions and filter media characterizations are shown in Table 5-1. Column sampling ports had screened inserts to prevent the loss of media and to minimize local flow disruptions during the sampling process. In order to construct the deposition profile in filters, the regular bed columns had seven side ports with 14 cm

intervals and the deep bed filters had five side ports with 40 cm intervals. The filter media sieve analysis report was provided by the supplier (Anthratech Western Inc. filters, Canada)

Table 5-1. Filter column configurations and filter media characteristics

Parameter		**Regular Bed**	**Deep Bed**
Internal Diameter (mm)		152	152
Total Depth (mm)		3650	5500
Total Bed Depth (mm)		750	1950
	Anthracite	200	1400
	Sand	550	550
Effective size (mm)	Anthracite	0.8-0.9	1.2
	Sand	0.35-0.45	0.55
Uniformity coefficient	Anthracite	<1.5	1.46
	Sand	<1.5	1.41
Total Bed Depth Volume (m^3)		0.015	0.035

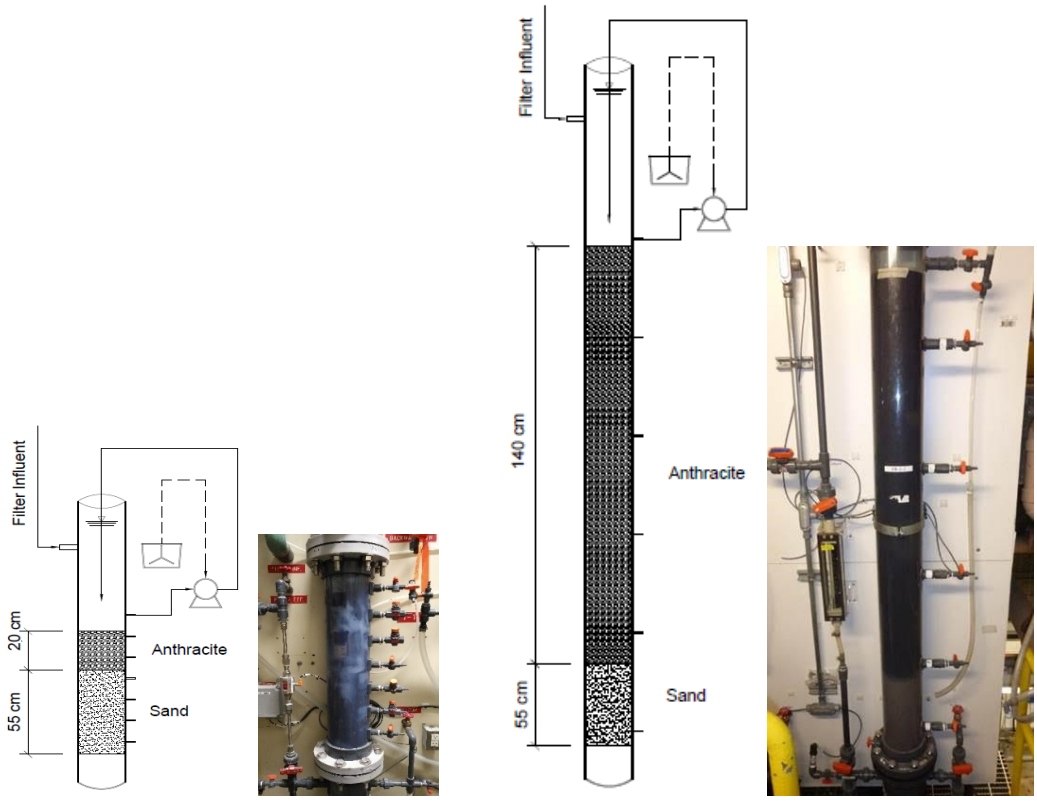


Figure 5-1. Schematic diagrams and photos of pilot-scale filtration columns. The depths of the sand and anthracite media layer were listed (left: regular bed filter; right: deep bed filter).

Pilot-scale filters were operated following declining rate filter strategies. An initial flow rate of 3 L/min corresponded to a hydraulic loading rate of 10 m/h. A turbidimeter (HACH company, USA), a particle counter (Chemtrac Inc., USA), and a pressure sensor (WIKA Instruments Ltd., Canada) were located on the effluent stream of each column. Digital flow meters (IFM Electronic, Germany) mounted on the discharge piping were installed to track the individual column flow. The outputs from these on-line instruments were captured by the plant's Supervisory Control and Data Acquisition (SCADA) system. The filter backwash was performed between trials with an air scouring followed by a 16 L/min of water wash with chlorinated plant service water.

5.2.2 Feed water source and characterization

North Saskatchewan River water was used to supply the pilot plant. Coagulant (alum or PACl) was added upstream of a pilot clarifier that supplied the filtration system. Filter aid polymer (polyamine Magnafloc® LT-7981 or polyDADMAC Magnafloc® LT-7995) was added ahead of two splitter boxes that served as head tanks above each two-column filter train. Raw water samples were analyzed in duplicates to characterize the raw water according to the following parameters: temperature, pH, color, turbidity, alkalinity, and TOC following analytical methods reported previously (Zhang et al., 2017b; Zhang et al., 2017c). Natural organic matter in source water was measured using combined NOM characterization methods. UV absorbance was determined by Varian Cary 50 Bio UV-Visible Spectrophotometer (Palo Alto, California) at wavelength of 254 nm with 1-cm path length of quartz cuvette after filtration through 0.45-µm syringe filters (Fisherbrand, USA). DOC was determined by Shimadzu TOC-L CPH E100 (Kyoto, Japan) with a detection limit of 4 µg C/L after filtration through 0.45-µm syringe filters. Specific ultraviolet light absorbance (SUVA) was then calculated based on measurements of UV_{254} and DOC, which used as an indicator of the molecular weight distribution of the NOM in the water (Edzwald, 2011). It is expressed in units of m^{-1} of absorbance per mg/L of DOC:

$$\text{SUVA} = \frac{\text{UV}_{254}\ (m^{-1})}{\text{DOC}\ (\frac{\text{mg}}{\text{L}})}$$

5.2.3 Comparison of viable oocysts and surrogates

The fabricated glycopolymers (poly(2-lactobionamidoethyl methacrylamide (PLAEMA)), cationic polymers (N-(3-Aminopropyl) methacrylamide hydrochloride (PAPMA)) and zwitterionic polymers (sulfobetaine methacrylate (PSBMA)) were synthesized to coat 4.3 μm fluorescent polystyrene microspheres (Polysciences Inc., Warrington, PA, USA). Reversible addition-fragmentation chain transfer (RAFT) polymerization was chosen to fabricate these well-defined polymers (Deng et al., 2009; Wang et al.,2014). Copolymerization of LAEMA with APMA (poly(LAEMA-co-APMA)) and SBMA with APMA (poly(SBMA-co-APMA)) were performed following the protocols from Wang et al., (2014). Conjugation of copolymers with their amine groups to carboxyl groups on the polystyrene microspheres was achieved by the carbodiimide crosslinker mechanism using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). The fluorescent yellow-green color has an excitation wavelength at 441 nm and an emission wavelength at 486 nm. The specific density was 1.025 as provided by the manufacture. Microspheres were modified following protocols established previously in Chapter 3. It had demonstrated similarities of *Cryptosporidium* surrogates with viable oocysts in terms of their surface characteristics, adsorption kinetics on grain surfaces, and their transport and deposition behaviors in laboratory packed-bed columns.

Live purified *Cryptosporidium parvum* oocysts with an average diameter of 4.5 μm were purchased from Waterborne Inc (USA), and were used to compare the surface properties with their surrogates. According to the supplier, oocysts were purified by sucrose and percoll density gradient centrifugation after initial extraction of feces with diethyl ether. The oocysts were stored in phosphate buffered saline (PBS) solution containing antibiotics with penicillin, streptomycin, and gentamicin to prevent bacterial growth from occurring within the sample, as well as Amphotericin B as fungicide and 0.01% Tween 20 to prevent cell aggregation. The oocysts suspension (~10^7 oocysts/8mL) was refrigerated at 4°C in the dark and used within 3 months after the date of shipment. Prior to each measurement, 1 mL of stock oocysts sample was centrifuged at 13,000 rpm for 1 min using a microcentrifuge (Eppendorf, 5414R, Germany) according to the supplier's specifications.

The antibiotic solution was removed, and the pellets were resuspended in a 1 mM NaCl background solution with pH 8.0.

Surface charge and hydrophobicity of oocysts and their surrogates were measured. The surface charges of viable *Cryptosporidium* oocyst, unmodified microspheres and *Cryptosporidium* surrogates were determined as zeta potential using a Zetasizer (Malvern, USA). Their surface hydrophobicity was determined by contact angle measurements using an FTA-200 system (First Ten Angstroms, USA). The measured contact angle was then converted to % hydrophobicity in order to compare with literature-reported hydrophobicity for viable oocysts. The background electrolyte solution was pH=8.0 of 1 mM NaCl solution to mimic the typical drinking water.

5.2.4 Filtration experiment

The pilot-scale filtration system focused on four variables: coagulant type, FP type, FP dose, and filter configuration. These variables were tested in eight trails (Trials 1-8 in Table 5-2). The concentration of coagulants (alum and PACl) applied were 0.454 mg/L as aluminum content based on typical direct filtration dose setting, which was equivalent to 5.0 mg/L of alum. The dose set points for filter aid polymers (Magnafloc® LT-7981 and FP Magnafloc® LT-7995) were 0.5 and 2.0 mg/L. These setting represent the range of doses applied during previous pilot and full-scale investigations. The characteristics of the two FPs were provided by the manufacturer. Their molecular weight and molecular weight distribution were determined using gel permeation chromatograph (GPC) at room temperature on a Viscotek model 250 dual detector (refractometer/viscometer) in aqueous eluents (0.5 M sodium acetate and 0.5 M acetic acid) with a flow rate of 1.0 mL/min. Filter aid polymer characteristics are compared in Table 5-3.

To quantitatively compare the overall deposition of the *Cryptosporidium* surrogates at each condition, the deposition rate coefficient K_d was estimated using the steady-state breakthrough concentration of the surrogates according to the following equation based on the classic colloid filtration theory (CFT) (Walker and Redelman, 2004).

$$K_d = -\frac{U}{\varepsilon L}\ln\frac{C}{C_0}$$

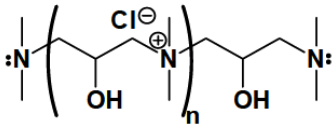
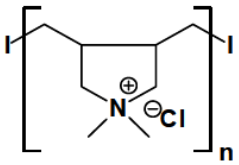
In this equation, ε is the bed porosity, U is the approach velocity, L is the column length, and C/C_0 is the normalized breakthrough concentration relevant to "clean bed" conditions. C/C_0 was obtained by averaging the ratios of influent and effluent concentration after 30 minutes surrogate injection.

Table 5-2. Summary of experimental conditions investigated at pilot plant.

Trial	**FP type Magnafloc®**	**FP Dose (mg/L)**	**Coagulant***	**Filter configuration**
1	LT-7981	0.5	Alum	Regular
	LT-7981	0.5	Alum	Deep
2	LT-7981	2.0	Alum	Regular
	LT-7981	2.0	Alum	Deep
3	LT-7995	0.5	Alum	Regular
	LT-7995	0.5	Alum	Deep
4	LT-7995	2.0	Alum	Regular
	LT-7995	2.0	Alum	Deep
5	LT-7981	0.5	PACl	Regular
	LT-7981	0.5	PACl	Deep
6	LT-7981	2.0	PACl	Regular
	LT-7981	2.0	PACl	Deep
7	LT-7995	0.5	PACl	Regular
	LT-7995	0.5	PACl	Deep
8	LT-7995	2.0	PACl	Regular
	LT-7995	2.0	PACl	Deep

*0.454 mg/L of aluminum content for both coagulants.

Table 5-3. Technical specification of two filter aid polymers (BASF Corporation, 2013).

Filter aid polymers	**Magnafloc® LT-7981**	**Magnafloc® LT-7995**
Chemical name	polyAmine	polyDADMAC
Chemical nature	Aqueous polymer solution of epichlorohydrin amine condensates	Aqueous solution of diallyldimethylammonium chloride homopolymer
Specific gravity	1.14	1.08
Bulk density (lb/gal)	9.51	9.01
pH	6-8	5-8
Supplied viscosity (cp)	100-200	1000-3000
Solid content (%)	50.0	40.0
Concentration (mg/L)	1,080,000	1,140,000
Intrinsic viscosity (dl/g)	0.10	0.50
Number-average molecule weight[a] (Mn)	7,000	30,000
Weight-average molecule weight[a] (Mw)	12,000	147,000
Molecular weight distribution[b] ($\frac{Mw}{Mn}$ =PDI)	1.78	4.92
Repeated units		

[a] determined using gel permeation chromatography (GPC)
[b] with relatively narrow molecular distribution (PDI) less than 1.20

5.2.5 Surrogate injection and sampling protocols

All filtration experiments were performed during the post-ripening period of the filter cycle run (i.e., the effective filtration period), as indicated by the turbidity level that was continuously below 0.08 NTU and the particle count (PC) of less than 45 counts/mL for 2-20 µm particles. The pre-trial samples and surrogate samples were collected from both influent and effluent ports. The influent location was approximately 20 cm above the surface of the filter media, while the effluent was collected from the bottom port. The seed suspension of surrogates of an average initial concentration of 4.0×10^7/L was introduced into the column influent. The injection of seed suspension lasted for 60 minutes. A recirculation loop was used to recirculate feed water above filter media to ensure the feed

water was well mixed. Influent and effluent samples were collected as soon as the surrogate injection started. Influent samples were collected in 1-L polypropylene bottles, and effluent samples were collected in 4-L polypropylene bottles. Within the 60-minute injection period, four influent and effluent sample pairs were collected at 15-minute intervals. In addition, side port samplings were performed to investigate the deposition profiles of surrogates in the filter columns during each trial. The side port samples were collected in 250-mL polypropylene bottles 50 minutes after initial surrogate injection, with minimal flow (~total 50 mL in a plastic centrifuge tube) to minimize flow disruption within the filter media. Backwash samples taken from the backwash overflow were collected in four 10.0-L carboys at 5-minute intervals during the entire 20-minute backwash period. The surrogate injection and sample collection protocol are described and summarized in Table 5-4.

Table 5-4. Surrogate injection and sampling protocols.

Operating condition	**Seeding**	**Sampling**
Immediately after ripening (Turbidity<0.08NTU and PCs<45/mL)	60-min	Samples collected in 15-minute intervals: 0-15, 15-30, 30-45, 45-60 minutes.

5.2.6 Detection methods

The surrogates from the seeding suspension, filter influent port, filter effluent port, and side port as well as backwash sampling ports were assessed by a direct immunofluorescent assay modified from that of Sun et al. (2015). Briefly, water samples were filtered through 25 mm, 0.45 μm nitrocellulose filter membranes (Merck Millipore Ltd., Ireland) with a known volume of water (in the range of 25-1000 mL depending on water quality) on the filtration apparatus (Cole-Parmer®, USA) The filter membranes were then placed on glass microscope slides and observed at 400× magnification using an epifluorescence microscope (Axioskop II microscope, Zeiss, Germany) equipped with a light source (X-cite 120Q, Lumen Dynamic, Canada), a camera (Carl Zeiss Microimaging GmbH, Germany), and image analysis software (ImageJ, USA). A minimum of 20 randomly selected fields were counted per filter membrane. In each experiment, the recovery was calculated by comparing the number of injected surrogates to the sum of deposited

surrogates calculated from the backwash samples and the number of effluent surrogates. The surrogate log removal (log_{10}) was calculated by subtracting the log of the effluent concentration from the log of the influent concentration.

5.2.7 Statistical analysis

To fully understand the impact of examined variables (four variables with two-level experiment design), paired t-test was performed on the log-removal response for filter configuration, coagulant type, and filter aid polymer type and dose (Appendix B). The results of statistical analysis for flow rate reduction in the regular and deep bed columns are described too (Appendix B).

5.2.8 Monitoring parameters: turbidity and particle count

Turbidity was monitored using the on-line turbidimeter, on-line HACH 1720D or 1720E turbidimeter, which was calibrated following the protocol from the manufacturer. Pilot clarifier was ahead of filter bed, where coagulant addition and pH adjustment were achieved. Turbidities were measured for clarifier effluent and filter effluent in the pilot plant. As was the case with turbidity, particle counts were also measured for clarifier effluent and filter effluent. CHEMTRAC LaserTrac™ Particle counter P3400 were used to measure the total particle counts and particle counts in certain size ranges, including 2-4 µm, 4-6 µm, 6-8 µm, 8-10 µm, 10-15 µm, 15-20 µm, 2-20 µm, and >20µm. The detected sizes of the particles ranged from 2 to 750 µm. The concentration limit for particle detection is 20,000 counts/mL for a 2 µm particle.

Particle counter calibrations were verified for two particle sizes: 4.3 µm and 10.0 µm of unmodified microspheres; the calibration curves are shown in Appendix C. The replicated experiment results showed that 4.3 µm microspheres were mainly distributed in 2-4 µm categories and the 10.0 µm microspheres were mainly distributed in 7-10 µm and 10-15 µm size categories. The particle count responses on the viable *Cryptosporidium* cysts and *Giardia* cysts were also examined. The results were consistent with the unmodified

microspheres in both sizes as shown in Appendix D. This study could also provide the reliability of microspheres to be used as surrogates for pathogens.

5.3 Results

5.3.1 Source water characterization

The raw water characteristics were determined, as shown in Table 5-5. Due to the variation in river water quality during the 3 months of study period (January 2017-March 2017), results presented here show the range of water quality tested during these 3 months (sample collected at least twice per month). In general, water quality was stable, with an average temperature of 0.45˚C, pH of 7.99, turbidity of 3.85 NTU, colour of 5.97 TCU, and DOC of 1.93 mg/L. The SUVA value ranges from 2.23 to 3.79 L/cm.mg, with an average of 2.62 L/cm.mg. According to the guidelines for the interpretation of SUVA values (Edzwald & Van Benschoten, 1990), for water supplier with SUVA greater than about 2.5, the NOM from raw water is mixture of humic and non-humic substances. The better understanding of the nature of the NOM in the source water will facilitate the evaluation of effectiveness of coagulation and subsequent filtration for removing organic matter and particles.

5.3.2 Particle characterization

Characterization of *Cryptosporidium* oocysts, unmodified microspheres and *Cryptosporidium* surrogates were compared, including size, shape, zeta potential and hydrophobicity. The size and shape of viable oocysts and their surrogates were observed under a microscope at 400× magnification, as shown in Figure 5-2. The visualization of viable oocysts was obtained by staining the oocysts using diluted monoclonal antibody solution Alexa Fluro® 405 (Novus Biological Inc., USA). The size and shape of *Cryptosporidium* surrogates match well with the viable oocysts.

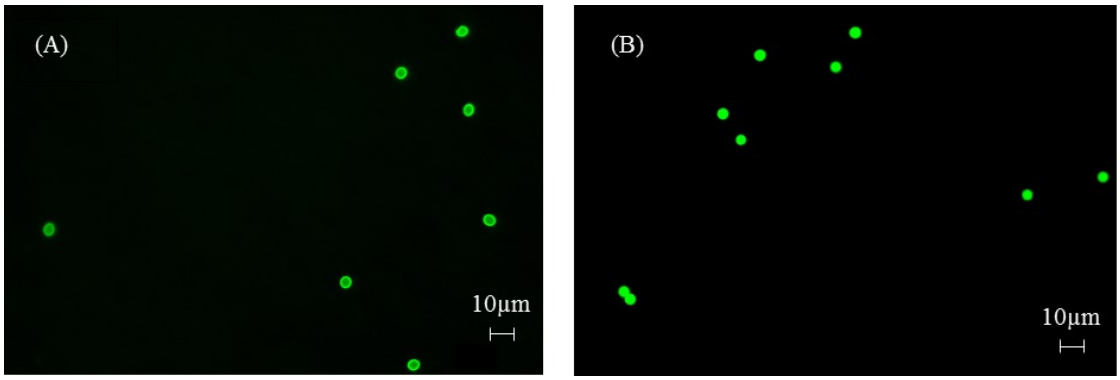


Figure 5-2. (A) The microscope picture of viable *Cryptosporidium* oocysts stained with FITC-conjugated antibody at 400× magnification and (B) copolymers-modified microspheres (*Cryptosporidium* surrogates) at 400× magnification under microscopy.

The zeta potentials oocysts and surrogates were calculated from the measured electrophoretic mobilities using the Smoluchowski equation (Hunter, 1981), as shown in Figure 5-3. The net negative charge of live oocyst cells was -10.9±1.2 mV under the examined condition and was due to the ionized or deprotonated carboxyl groups from protein (Dai and Hozalski, 2003). The unmodified microspheres had the most negative zeta potential (-56.5±1.5 mV), which was in agreement with other studies (Dai and Hozalski, 2003; Pang et al., 2012). The measured zeta potential of the *Cryptosporidium* surrogates (the copolymers-modified microspheres) was -17.0±0.9 mV which was much closer to the zeta potential of viable oocysts than that of unmodified microspheres. Our previous studies (Zhang et al., 2017a and Liu et al., 2018) proved that the synthesized biomolecules conjugated on the surface of fluorescence microspheres counteracted the very negative surface charge contributed by carboxylic groups from their surface, making them less negative.

Table 5-5. Range and average values of raw water characteristics in pilot direct filtration.

Raw Water Characterization	**Results**
Temperature (˚C)	
average	0.45
range	0.39-0.50
pH	
average	7.99
range	7.90-8.11
Turbidity (NTU)	
average	3.85
range	2.36-5.28
Color (TCU)	
average	5.97
range	5.66-6.64
DOC (mg/L)	
average	1.92
range	1.24-2.24
Alkalinity (mg/L as $CaCO_3$)	
average	143
range	124-161
UV_{254} absorbance	
average	0.25
range	0.21-0.27
SUVA (L/cm.mg)	
average	2.62
range	2.23-3.79

Further, as shown in Figure 5-3 (B), the hydrophobicity of *Cryptosporidium* surrogates (18.0%), was much closer to that of viable oocysts (8.4%) (Chung, 2012; Dai and Hozalski, 2003) than that of unmodified microspheres (27.2%). The greater hydrophilicity of the surrogates is due to the presence of synthesized zwitterionic polymer (i.e. poly-SBMA), which possess both cationic and anionic groups making them superhydrophilic (Shao and Jiang, 2015). In summary, the similar surface properties of copolymers-modified microspheres with oocysts make them representative surrogates for viable *Cryptosporidium* oocysts under the conditions tested.

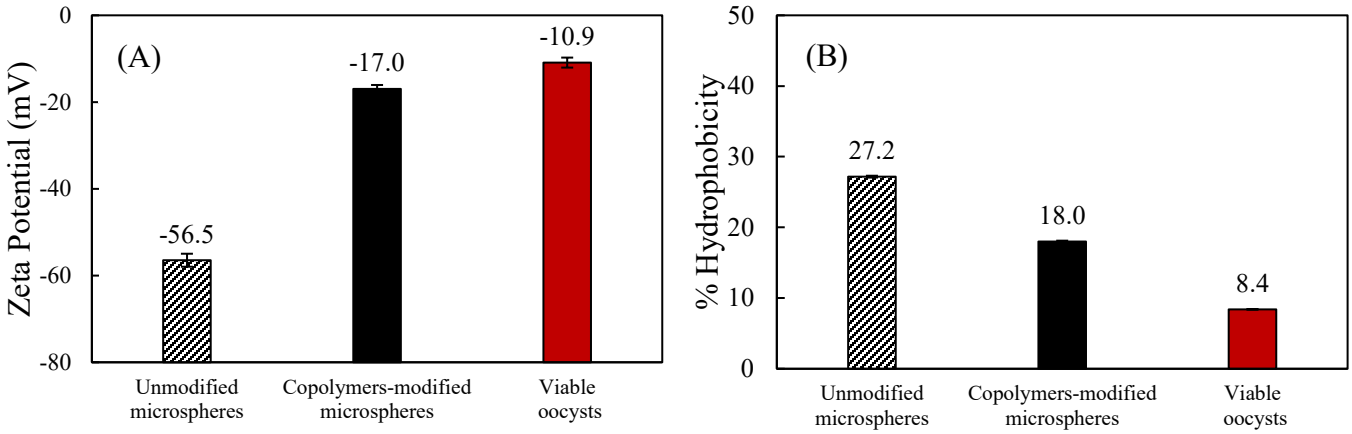


Figure 5-3. (A) Zeta potentials and (B) percentages of hydrophobicity of viable *Cryptosporidium* oocysts, unmodified microspheres and copolymers-modified microspheres (*Cryptosporidium* surrogates) in 1 mM NaCl background solution with pH of 8.0. Error bars represent standard deviations of three measurements for zeta potential and five measurements for contact angle.

5.3.3 Zeta potential responses to two FPs

Bench-scale screening experiments were carried out in which two FPs were added to samples of chlorinated clarifier effluent (representing filter influent) from a full-scale water treatment plant. As shown in Figure 5-4, zeta potential responses to two FPs suggested that, in comparison to Magnafloc® LT-7891, a much lower dose of Magnafloc® LT-7995 could achieve particle charge neutralization. To achieve a near-neutral zeta potential target, a dose of about 0.5 mg/L was required, compared to a dose of 2.0-2.5 mg/L of LT-7981. Based on these bench-scale observations, pilot tests were planned to determine how doses of FP sufficient to neutralize particle surface charges in filter influent would affect surrogate removal.

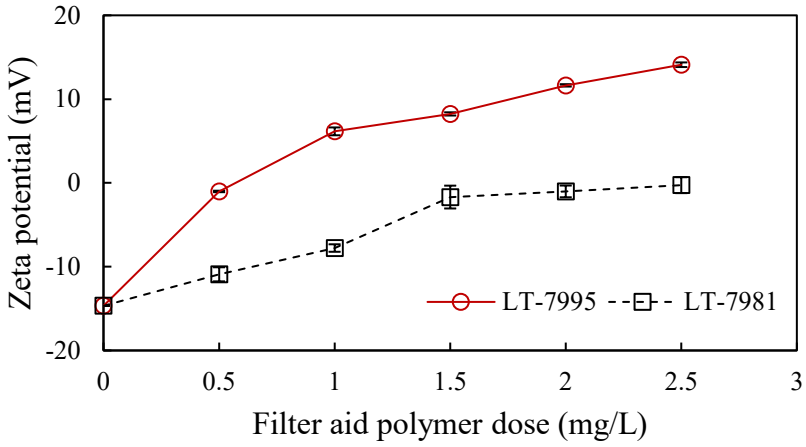


Figure 5-4. Zeta potential responses of two filter aid polymers. Error bar indicated the standard deviations of triplicate measurements.

5.3.4 Impact of operational factors on filter performance

Under all operating conditions tested in pilot plant during Trials 1-8, the average normalized breakthrough concentrations, $C/C_{0\ ave}$, were calculated by averaging the ratios of influent and effluent concentration after 30 mins surrogate injection. The surrogate log removal was then calculated based on the log of $C/C_{0\ ave}$ as shown in Figure 5-5. Overall, surrogate log removal under different conditions ranged from 1.3 to 4.5. The lowest log-removal condition was in Trial 1, in which the chemical pretreatment condition for both regular and deep bed filters was 0.454 mg/L of alum and 0.5 mg/L of polyamine Magnafloc® LT-7981. The maximum log removal of surrogate in the pilot filtration process yielded a result of 4.3 for regular bed filtration (in Trial 4 with 0.454 mg/L of alum and 2.0 mg/L of LT-7995) and 4.5 for deep bed filtration (in Trial 3 with 0.454 mg/L of alum and 0.5 mg/L of LT-7995). The difference in surrogate removal between PACl and alum was not significant at cold temperature conditions tested (P=0.967).

In terms of FP effects, the filtration results demonstrated that compared to Magnafloc® LT-7981 (polyamine), Magnafloc® LT-7995 (polyDADMAC) was associated with greater surrogate removal in the filters (P=0.0212). This finding correlated well with the zeta potential results described above. Also, the higher FP dose was associated with greater log

removal of surrogates. It is hypothesized that higher doses of FP (2.0 mg/L of Magnafloc® LT-7995) neutralized the zeta potential of filter influent particles, allowing filters to achieve higher surrogate deposition due to reduced net repulsive surrogate-collector interactions (Gregory, 1993). Another potential mechanism of the enhanced surrogate removal at higher polymer doses of Magnafloc® LT-7995 is interparticle bridging. Magnafloc® LT-7995 has a much higher average molecular weight and contains various lengths of polymers (long polymer chain and short polymer chain; PDI=4.92) as compare to Magnafloc® LT-7981 (PDI=1.78) (Table 5-3), indicating a higher potential for bridging effects, which could facilitate the removal of surrogates in the succeeding filtration process. Direct comparison of these two types of FPs regarding surrogate or parasite removal efficiency in dual media water treatment filters has never been reported in the literature.

The surrogate log removal in the deep bed filters was associated with higher surrogate log removal than that of in the regular bed filters (P=0.00143), as shown in Figure 5-5 (A). It is noted that the filter flow rate reduction percentages (or head loss percentage) (Fig 5-5 (B)) demonstrated that deep bed filters led to the significantly lower flow rate reductions under all testing conditions (P=0.00120). The flow rate reduction for regular bed filters ranged from 1.8 % to 8.9 % during the surrogate injection period, whereas the maximum flow rate reduction for deep bed filters was only 1.7% (Trials 2, 3, 4 and 6 showed 1.7% flow rate reduction and other trials showed no measurable reductions). The much slower flow rate reductions of deep bed filters would lead to longer filter run times, thus increasing the filtration capacity of the water treatment plants and reducing operational costs associated with frequent backwashes. This is an expected characteristic of deep bed filters compared to regular bed filters.

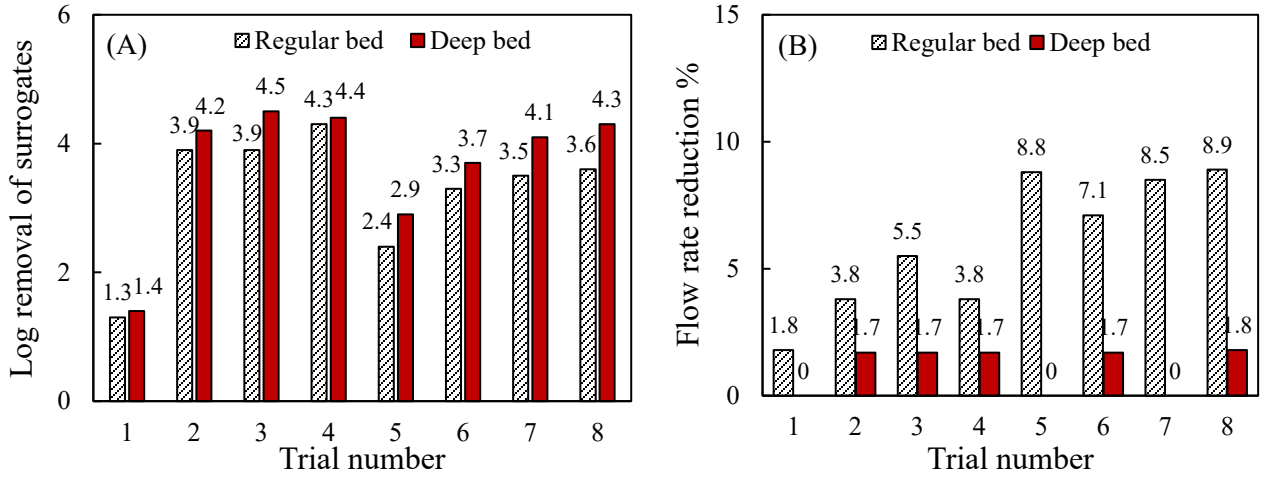


Figure 5-5. (A) Log removals of surrogate in direct filtration using coagulant alum (trials 1-4); Log removals of surrogates in direct filtration using coagulant PACl (trails 5-8). The numbers on the bar chart indicate the log removals of surrogates obtained from single test results. (B) Flow rate reduction percentage after filtration experiments in regular bed and deep bed filters. The numbers on the bar chart indicate the results from single measurement.

5.3.5 Deposition profiles

The percentage of retained surrogates in anthracite and sand layers of regular and deep bed filters was calculated and compared in Figure 5-6. In the regular bed filter, the anthracite retained a greater percentage of surrogates (71.7±12.1%) in the top anthracite layer (20 cm) near the column inlet, while the number of retained surrogates gradually decreased as sand media depth increased. The sand layer functioned as media for further removal of surrogates and accounted for 28.9±12.1 % of the total deposited surrogates. The majority of surrogates deposited in the first few centimeters of bed depth is attributed to ripening (Tong et al., 2008; Jiang et al., 2012), so the pre-deposited particles in the filter media can serve as additional sites for further particle retention. The observation that more surrogates were deposited in anthracite layers is also indicated by a larger K_d in Figure 5-7. As shown in Figure 5-8 (A), the less steep slopes of deposition in the sand layer indicated that K_d decreased with travel distance in the filter, which was in good agreement with previous lab-scale studies (Zhang et al., 2017a and Liu et al., 2018). In the deep bed filter, the anthracite represented the major barrier for surrogate removal with 97.4±2.5% of retention, whereas the sand layer removed only a small proportion of surrogates, representing about

2.6±2.5% of the total retained surrogates. The much higher retention of surrogates in the anthracite layer may be attributed to the greater depth (seven times greater than that in a regular bed filter) which provided more attachment sites for the surrogates to deposit. The variation in surface properties gives rise to a distribution in particle-collector interaction potential, which in turn results in a non-constant or a broad range of deposition rates. Similar observations have been reported previously-where two distinct deposition rates (i.e., fast and slow deposition rates) were observed for the deposition of *Cryptosporidium* oocysts and colloidal particles (3 μm) in laboratory packed-bed columns (Tufenkji and Elimelech, 2004, 2005). As shown in Figure 5-8 (B), the entire depth of the anthracite was utilized, which was demonstrated by the very steep slopes of the deposition profiles in anthracite layer under all tested conditions.

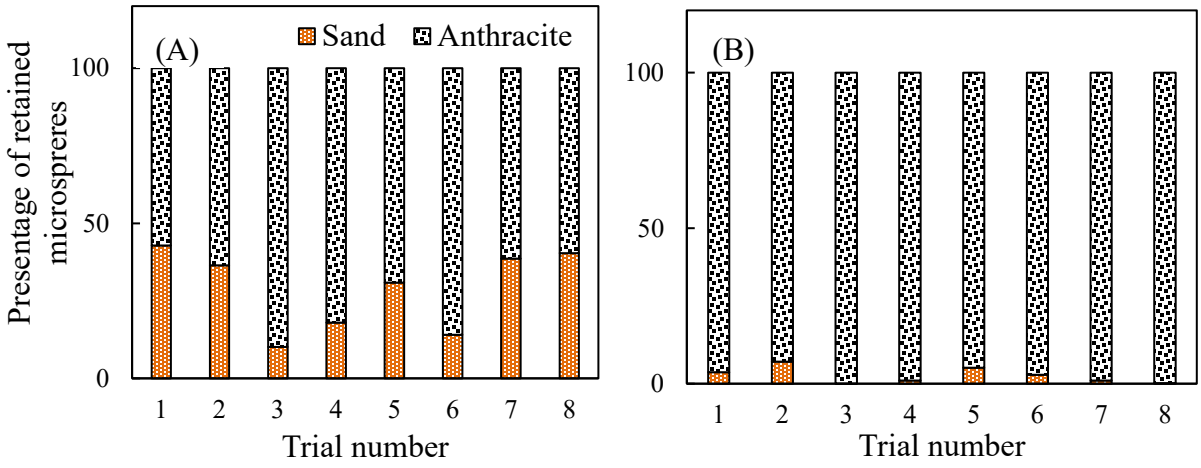


Figure 5-6. (A) Percentage of retained surrogates in anthracite and sand layer of regular bed filters in each trial from 1 to 8. (B) Percent of retained surrogates in anthracite and sand layer of deep bed filters in each trial from 1 to 8.

As shown in Figure 5-7, the deposition rate coefficients (K_d) for anthracite were greater than those for sand. The maximum K_d values for anthracite and sand were observed in the near-optimal chemical pretreatment conditions for the regular bed filters in Trial 4 and for the deep bed filters in Trial 3. These results were consistent with the log reduction of surrogates as reported in Figure 5-5; higher K_d values associated with greater log removal of surrogates in the filter media. Under the same trial conditions, the K_d values for

anthracite and sand were comparable for regular bed and deep bed conditions. This inferred an important role of the chemical pretreatment condition on the retention kinetics of surrogates in filter media.

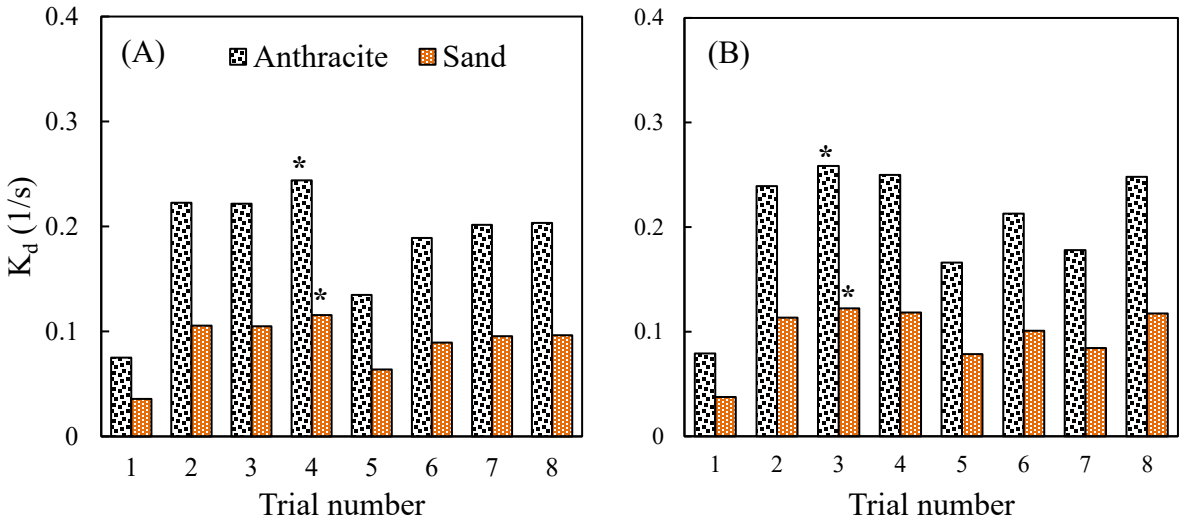


Figure 5-7. (A) Deposition rate coefficient (K_d) of surrogates on anthracite and sand in regular bed filters. (B) Deposition rate coefficient (K_d) of surrogates on anthracite and sand in deep bed filters. * indicated the maximum log removal of surrogates.

Spatial distributions of surrogates in filter bed columns are shown in Figure 5-8. Based on classical CFT (Yao et al., 1997; Tufenkji and Elimelech, 2004), the deposition profile traditionally assumes a constant first-order deposition term in a "clean bed" and predicts an exponential spatial distribution (log-linear) of retained particles with distance from the filter inlet. The deposition of retained surrogates in the saturated porous media demonstrated depth dependency, an observation that agrees with previous studies reported in the literature (Bradford et al., 2002 and 2006; Li et al., 2004; Bradford and Bettahar, 2005, Gargiulo et al., 2008). The spatial deviations of K_d in this pilot study may be explained by the heterogeneity of the collector surface, variation of hydraulic loading rates, distribution of influent particle size other than surrogates, and variation of particle concentrations in theinfluent flow.

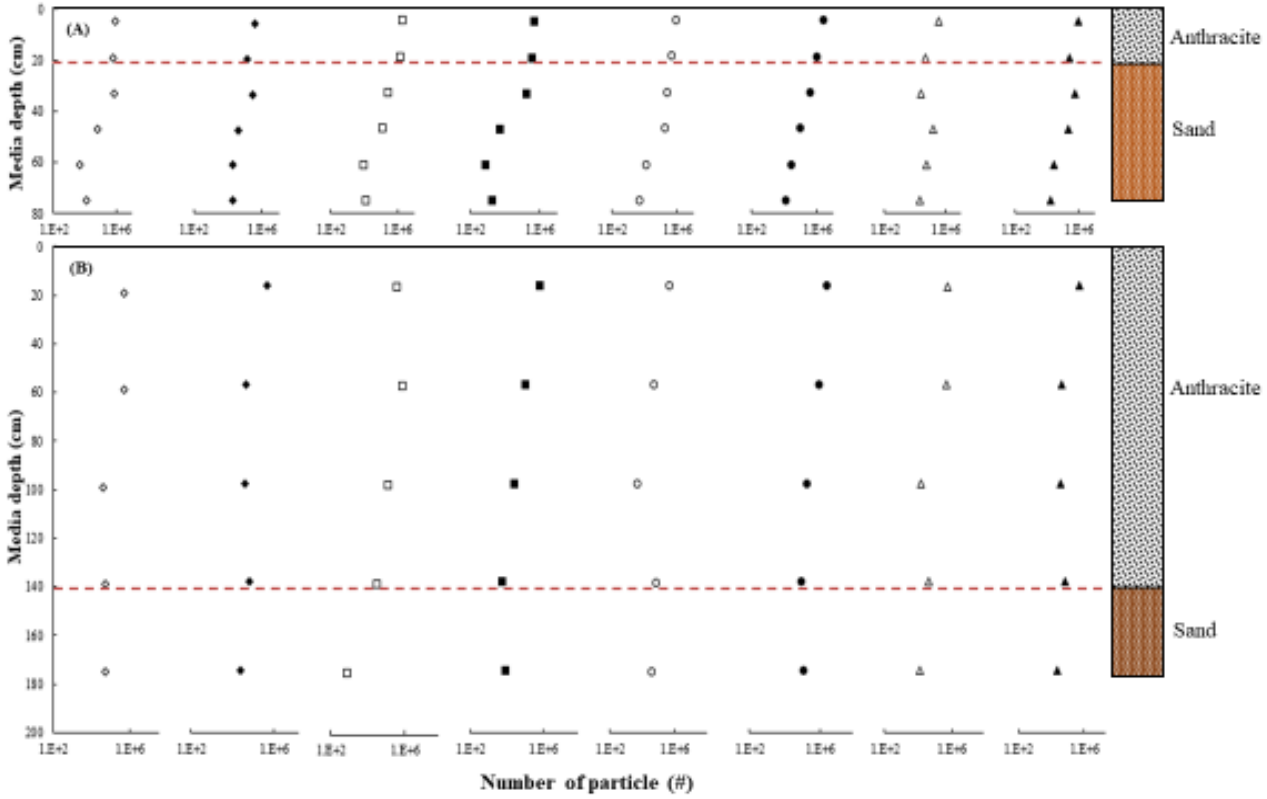


Figure 5-8. (A) Deposition profiles of surrogates in anthracite and sand layer in regular bed filters. (B) Deposition profiles of surrogates in anthracite and sand layer in deep bed filters. The red dish lines indicated the interface of the anthracite and sand in regular and deep bed filters.

5.3.6 Recovery

The recovery of each experiment was calculated by conducting a mass balance, as shown in Table 5-6. The recovery data from regular bed filtration yielded a mean surrogate recovery of 103 % with 19 % RSD, while the recovery data from deep bed filtration yielded a mean surrogate recovery of 104 % with 15 % RSD.

Table 5-6. Surrogate recovery data from the direct filtration at the pilot plant.

Coagulant and FP	Dose (mg/L)	Recovery (%)	
		Regular bed	Deep bed
PACl+LT-7981	Low 0.5	97	88
	High 2.0	129	132
PACl+LT-7995	Low 0.5	103	109
	High 2.0	122	91
Alum+LT-7981	Low 0.5	114	93
	High 2.0	120	108
Alum+LT-7995	Low 0.5	122	110
	High 2.0	86	118
	Mean	112	106
	RSD*	13	14

*relative standard deviation

5.3.7 Correlation with on-line monitoring measurements: turbidity and particle count

In order to examine trends in surrogate removal and determine whether appropriate monitoring parameters could be derived from the aggregation of data in the pilot studies, various relationships were examined based on the removal of the surrogates and other water quality parameters. Specifically, turbidity and particle counts were studied. Although turbidity is universally monitored and accepted as an indicator of filter performance (LeChevallier & Norton, 1992; Nieminski & Ongerth, 1995; Lopes 2008; Bastos et al., 2013;), many studies have shown that turbidity removal lacked a strong correlation with the removal of *Cryptosporidium* (Patania et al., 1995; Huck et al., 2001). This study sought to examine whether turbidity removal is an indicator of *Cryptosporidium* removal. It demonstrated that filter effluent turbidity failed to signal the microsphere breakthrough and respond sensitively to poor water quality treated from the filtration process, as shown in Figure 5-9 (A) and (B). A study by Bastos et al. (2013) also demonstrated that experiments that involve seeding the influent with very high concentrations of *Cryptosporidium* surrogates overestimate oocyst removal (Bastos et al., 2013).

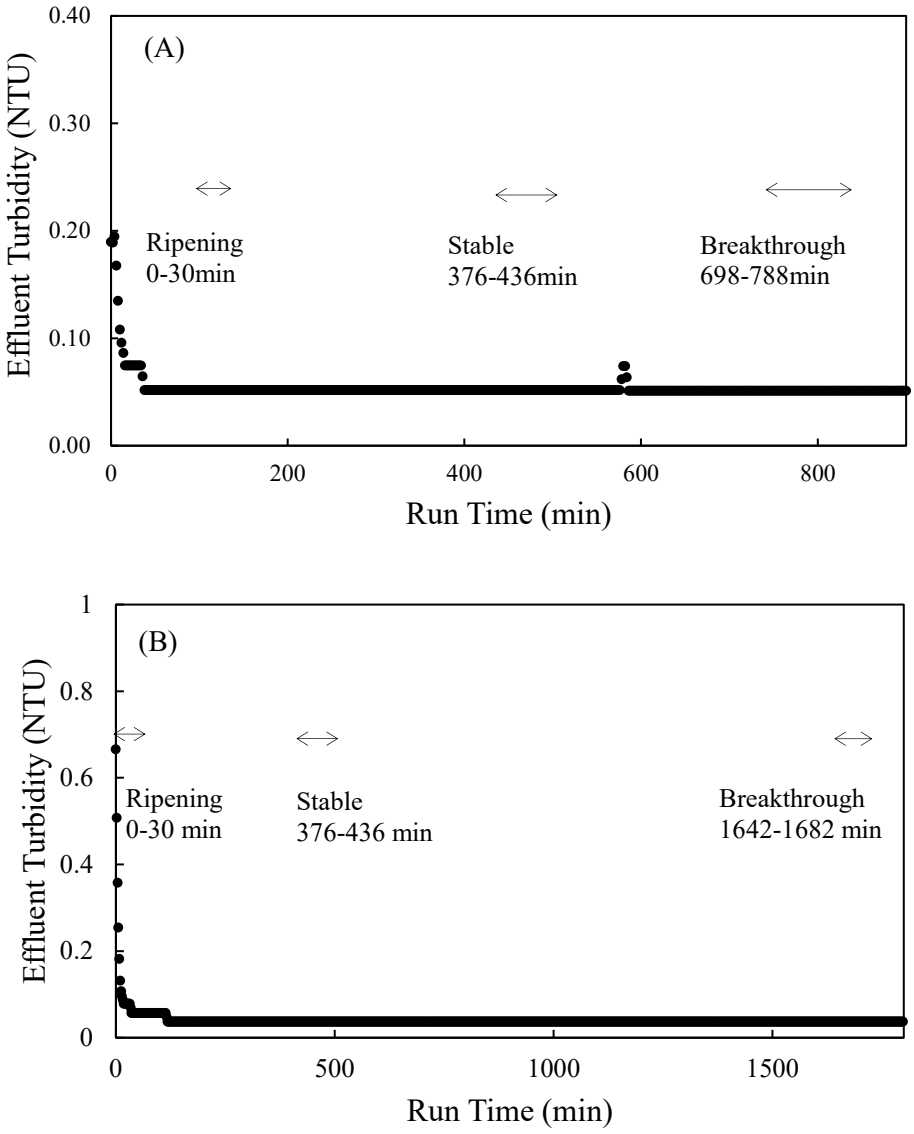
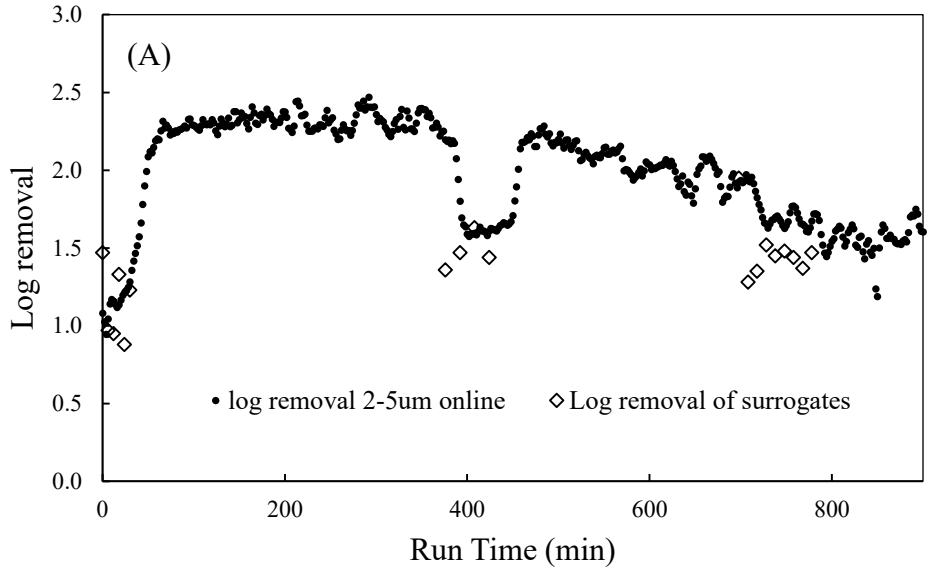


Figure 5-9. (A) Filter effluent turbidity in regular bed filter and in deep bed filter; (B) the arrow indicated the dosing period at different filtration stages, i.e. ripening, stable and breakthrough period.

This study also calculated the total particle reduction through treatment processes based on raw water rather than filter influent values, due mainly to the inability to measure filter influent particle concentration. Similar to turbidity, particle counts can constitute an effective quality control parameter for filtration, thus providing a general index for the removal effectiveness of treatment processes. In Figures 5-10, particle log reduction by on-

line PCs in the 2-5 μm range demonstrated similar trends based on the log removal of microspheres by the direct microscope counting of grab sample method. The strong correspondence between on-line log reductions in particles and lab-measured microspheres reductions were observed for regular bed filtration as shown in Figure 5-10 (A). PCs in the 2-5 μm range underestimated the log removal of microspheres during stable operations but showed similar log removal results during the ripening and end-of-run operation periods for deep bed filtration as shown in Figure 5-10 (B). Therefore, particle counts by size range could comprise a conservative estimation parameter for the log removal of surrogates. Their correlation with pathogen breakthrough will be discussed in the subsequent section.

This study sought to investigate whether total particle reduction served as an indicator of *Cryptosporidium* removal and if the reduction of 2-5μm particles correlates with the *Cryptosporidium* surrogate removal**.** To answer these questions, Table 5-7 summarizes the direct correlation between surrogate removal and particle removal. It clearly demonstrated that the correlations between particle removal and surrogate removal were slightly higher in deep bed filters than that in regular bed filters. The reduction of particle size range may provide a conservative indication of a filter's ability to remove *Cryptosporidium.*



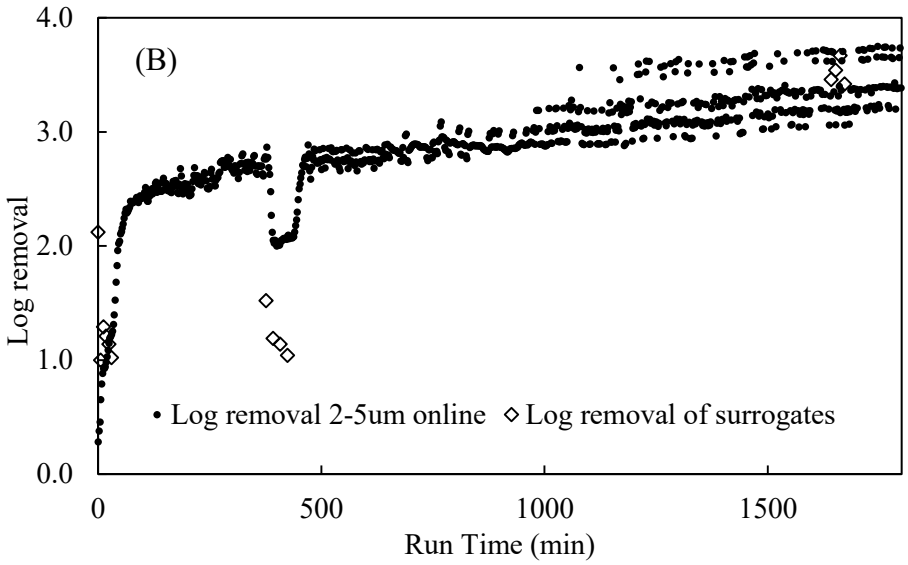


Figure 5-10. (A) Log removal of particles 2-5 µm and log removal of surrogates in regular bed filter. (B) Log removal of particles 2-5 µm and log removal of surrogates in deep bed filter.

Table 5-7. Coefficients for correlations of surrogate removal with particles.

Correlated parameters	**Correlation Coefficient**
Regular bed	
Log removal of microspheres vs. log removal of total particle counts	0.32
Log removal of microspheres vs. log removal of filter effluent 2-5 µm particles	0.30
Deep bed	
Log removal of microspheres vs. log removal of total particle counts	0.52
Log removal of microspheres vs. log removal of filter effluent 2-5 µm particles	0.58

5.3.8 Extend study on surrogate log removal in direct filtration (September 2017-December 2017)

Due to the 3-month short study period, the surrogate log removal study was only performed one time in the pilot filter columns during January to March 2017. The study was then extended in next fall and winter (September to December 2017) at the cold-water

temperature with similar raw water quality compared to previous study to further evaluate the factors impacting the surrogate log removal. The examined variables are limited to FP dose (0.5 versus 2.0 mg/L of PolyDADMAC Magnafloc® LT-7995), pH (8.0 versus 7.5), and filter configuration (regular versus deep bed filter). Four trials were conducted during this period (Trial 1-4 in Table 5-8) and every trial run in replicate.

Table 5-8. Summary of experimental conditions investigated at pilot plant in extend direct filtration.

Trial	FP Dose (mg/L)	pH	Filter configuration
1	0.5	8.0	Regular Deep
2	0.5	7.5	Regular Deep
3	2.0	8.0	Regular Deep
4	2.0	7.5	Regular Deep

Filter influent zeta potential were measured for Trial 1 to 4 as shown in Figure 5-11. When solution pH adjusted to 7.5, the zeta potentials of filter influent samples were less negative than that of in pH 8.0 condition using 0.5 mg/L of FP or even turning positive using 2.0 mg/L of FP when examining deep bed filters. FP may be overdosed and thus result in the charge reverse and the re-stabilization of particles in the suspension, which may induce decreased surrogate reduction in the filtration process. The zeta potential of filter influent shown in Figure 5-11 potentially explained the observed lower log removal in Trial 3 and Trial 4 in the deep bed filters.

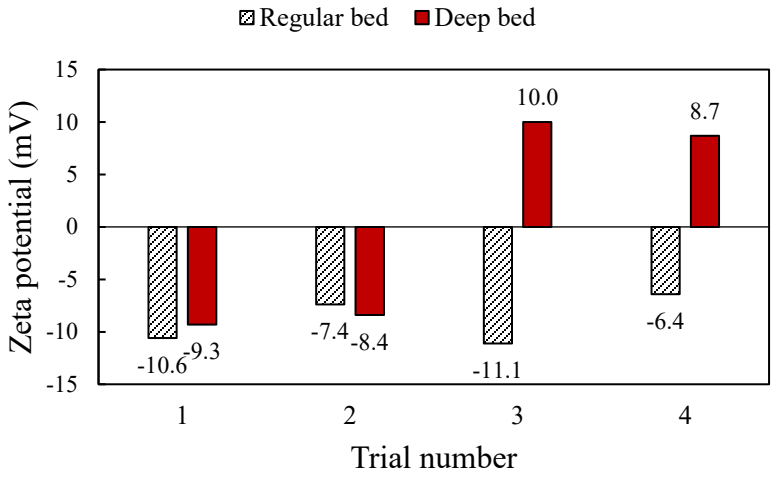


Figure 5-11. Zeta potential of filter influent. Chemical pretreatment condition: 0.636 mg/L of Al using alum with either 0.5 or 2.0 mg/L of Magnafloc® LT-7995.

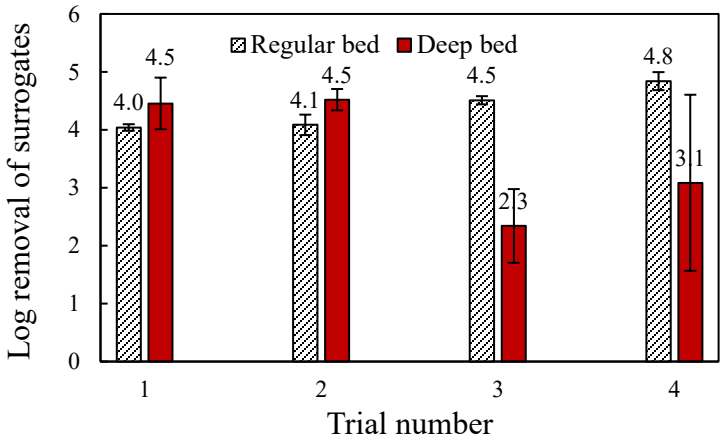


Figure 5-12. Log removal of surrogates in extend direct filtration. Chemical pretreatment condition: 0.636 mg/L of Al using alum with Magnafloc® LT-7995.

In regular bed filters, the results of surrogate log removal were consistent with previous study examined in initial direct filtration; that is, higher dose of FP was associated with higher surrogate log removal. In addition, when pH of filter influent adjusted to 7.5, a higher surrogate log removal was observed, which was due to the less negative of zeta potential of particles in the suspension. Reduced repulsive forces between surrogates and filter media could promote the adhesion and resulted in the greater deposition of surrogates

in the filter media. In deep bed filters, the pH effect was not robust since the large variations of surrogate log removal were observed, which may indicate a different dominant mechanism associated with deep bed filtration. Worth to notice is that overdosed FP deteriorated the filter performance in terms of surrogate reduction in Trial 3 and Trial 4, which manifested an important role of optimized chemical pretreatment condition playing on the particle removal. Except those conditions, the overall log removal of surrogate achieved in the extend direct filtration period was equivalent or slightly higher than in the initial direct filtration period.

5.4 Discussion

5.4.1 Selection of coagulant and filter aid polymer in direct filtration

One of the most crucial steps in optimizing water treatment granular-media filter operation is to determine appropriate types and dosages of coagulants and filter aid polymers. Low coagulant doses may not be sufficient for particle aggregation, whereas high doses may result in the inversion of particle charge, re-stabilizing the particles and consequently poor filtration (Tien and Payatakes, 1979). The selection and use of PACl and alum greatly depended on the concentration of NOM, suggested from a study by Pernitsky and Edzwald (2006). During this study period, the DOC concentration is consistently low (about 2 mg/L) and the nature of NOM in the raw water is mixture of humic and non-humic substances that will impact coagulation. Comparing to alum, PACl was not associated with superior surrogate removal under cold temperature condition as suggested in previous publication (Gebbie, 2001). Thus, from an operational perspective, alum would be a more cost-effective choice of coagulant for direct filtration based on this study. Other considerations of overall cost of coagulation, such as solid residuals, dewatering characteristics and disposal (Pernitsky and Edzwald, 2006) can also be taken into account although they are beyond the scope of this study.

The polymer dose required to destabilize the particles is proportional to the raw water particle concentration or the total negative charge to be neutralized (McEwen, 1998). Studies have shown that the good destabilization should achieve near zero charge

conditions (Pernitsky, 2003). Typically, a jar test can be applied as a simple practical approach to approximate the optimal dosage of coagulant and filter aid polymers. Our results demonstrated that the higher zeta potential of particles in filter influents were associated with higher surrogate log removal. Additionally, Magnafloc® LT-7995 (polyDADMAC) provided a greater dose response than Magnafloc® LT-7981 (polyamine) in terms of increasing zeta potential. Selecting polymers capable of efficiently neutralizing particle charges and promoting interparticle bridging can help optimize filtration while reducing chemical input costs. In addition to surrogate reduction in the water treatment processes, nitrosamine precursor formation from two FPs (polyDADMAC and polyamine) should be considered. Previous research has characterized N-nitrosodimethylamine (NDMA) formation during chloramination from polyamine and polyDADMAC. Studies showed that the yields of NDMA from polyDADMAC were 600 percent less than those from polyamine (Park, 2008). In summary, the results suggest that Magnafloc® LT-7995 (polyDADMAC) is superior as compared to Magnafloc® LT-7981 (polyamine) as a water treatment filtration polymer under the conditions of this study due to the suggested lower NDMA formation potential and observed superior surrogate removal capacity achieved through greater charge neutralization and interparticle bridging.

5.4.2 Selection of filter configuration in direct filtration: deep bed vs. regular bed filters

Under the conditions tested, deep bed columns achieved higher surrogate removal and provided longer filter runs and lower rates of low loss than regular bed columns. In previous study, large anthracite grains in the deep bed columns were associated with longer filter ripening times, lower head loss and slower reductions in filtration rates (James, 2017). When raw water conditions become challenging for direct filtration operation (colour greater than 6 TCU), longer ripening times and earlier breakthrough of particles would be expected. Daily plant wastes, including the volume of filtered water discharged during filter ripening and the volume of treated water used for filter backwashes, would dramatically increase (James, 2017). Deep bed filters could potentially be used to reduce these challenges and extend the direct filtration season by allowing earlier conversion from conventional operation mode in the fall. Benefits would include reducing chemical inputs

and production of coagulant sludge while maintaining treated water quality. This pilot study demonstrated the process advantage of using deep bed filters with optimal chemical pretreatment under cold temperature conditions.

5.4.3 Monitoring parameters: turbidity vs. particle count

The correlation of turbidity with the particle number concentration in suspended matter is difficult to achieve because the size, shape, and refractive index of the particles affect the light-scattering properties of the suspension (Copes et al., 2008). Also, the occurrence of pathogens is usually a rare event that will not always reflect significant changes in turbidity (Copes et al., 2008). In addition, the pathogens may achieve breakthrough before on-line meters can detect measurable turbidity (Petterson & Ashbolt, 2016). In conjunction, these limitations undermine the value of turbidity as an easily-measured parameter of filtration performance for the removal of fine particles. Furthermore, turbidity has a poor predictive value for the presence of pathogens. The observations in this study confirmed the inability of turbidity to predict the removal of copolymers-modified microspheres as *Cryptosporidium* surrogates during the intermittent dosing run in the whole filter operation cycle. Specifically, this study failed to observe a one-to-one correlation between the log removal of turbidity and the log removal of surrogates; however, filter effluent turbidity levels influenced the level of oocyst removal by direct filtration. In addition, the achievement of a filter effluent turbidity goal of 0.1 NTU indicated the treatment performance producing the most effective oocyst removal (Patania et al., 1995). Increases in turbidity during a filter cycle failed to signal deterioration in the removal capacity of surrogates, which occurred during the early breakthrough experiments in the regular bed filter.

Patania et al. (1995) examined four pilot studies whose results suggested a weak relationship between organism removal and particle removal. These various results indicate the limitations of using log removal requirements to ensure adequate microbial quality (Patania et al.,1995). These authors also claimed that the removal level of any factor, such as turbidity, particles, and oocysts, can largely depend on its concentration in

the raw water. This present study only investigated a condition in which high oocyst concentrations were present in waters with relatively low turbidity and particle levels typically in direct filtration mode at cold water temperature condition. This result also concurred with studies previously reported in the literature (LeChevallier et al., 1991; Nieminski and Ongerth. 1995). However, some positive correspondences were observed between on-line particle log reductions based on the particle size measurement (2-5 μm). Such measurements used particle counter and microscopic-based results, which indicated the potential application of the particle counters by size range as the indicator of pathogen removal from filters (James, 2017). Hsu and Yeh (2003) also observed that *Cryptosporidium* oocysts were strongly correlated with 3-5 μm particles in three pilot-scale plants; the results of this study indicated that similar sizes of particles can predict the existence of *Cryptosporidium* oocysts in the water. Many researchers have explored the relationship between oocysts and particles; for example, Nieminski (1994) observed a strong correlation between the removal of *C. parvum* and the removal of 4-7 μm particles (R^2=0.79). Of note, the turbidity ranges of 4 to 23 NTU and 2.5-28 NTU in Nieminski's study exceed the observations of less than 9 NTU found in this study. Nieminski's approximate one-to-one correlation between oocyst removal and particle removal occurred due to the combination of raw waters with higher turbidity, a slightly higher range of particle removal, and a lower overall oocyst removal compared to the results of this pilot study. Specific log removals that are not linked to raw water contaminant concentrations are too simplistic to apply to all conditions.

5.5 Conclusions

The investigations discussed in this pilot study indicated that:

(1) The difference in surrogate log reduction when using alum or PACl in cold water temperature was not significant. The decision to use alum vs. PACl as a coagulant for direct filtration should be based on additional considerations such as the respective environmental impacts and the impact on treated water quality.

(2) Compared to polyamine Magnafloc® LT-7981, polyDADMAC Magnafloc® LT-7995 led to a higher level of surrogate removal in the filters. That was demonstrated

to be the result of greater charge neutralization and interparticle bridging provided by the polyDADMAC polymer.

(3) An increase in the dosage of FP led to a higher log removal of surrogates in direct filtration.

(4) Compared to regular bed filters, deep bed filters were associated with higher surrogate removal, while exhibiting lower rates of flow reduction and longer filter run time.

(5) These studies demonstrated the importance of using zeta potential of filter influent as a polymer dosing indicator. In general, higher doses of FP result in filter influent zeta potential that is closer to neutral or positive and greater surrogate removal.

(6) The deposition rate coefficients (K_d) for anthracite were greater than for sand in all the tested conditions. The maximum K_d values for anthracite and sand were observed in the optimal chemical pretreatment conditions (0.454 mg/L of Al either from PACl or Alum and 2.0 mg/L of polyDADMAC Magnafloc® LT-7995) for both regular and deep bed filters. The spatial deviations of K_d were observed in filtration columns from this pilot study.

(7) The monitoring of filter effluent particle size could represent a potential approach for predicting the presence of particles that are similar in size to oocysts; from this perspective, the reduction of particle size range may provide a conservative indication of a filter's ability to remove *Cryptosporidium*. Future studies need to solidify a reproducible and consistent relationship between oocyst and particle removal with additional experimental datasets.

References

Bellamy, W. D., Cleasby, J. L., Logsdon, G. S., & Allen, M. J. (1993). Assessing treatment plant performance. *American Water Works Association Journal*, *85*(12), 34-38.

Copes, R., Hrudey, S. E., Payment, P., & Act, D. W. (2008). Turbidity and microbial risk in drinking water. *Prepared for The Minister of Health, Province of British Columbia, pursuant to Section*, *5*.

Chung, J. (2012). Development of fluorescently labelled Cryptosporidium oocyst surrogates to test the efficacy of sand filtration processes. University of New South Wales.

Crittenden, J. C., Trussell, R. R., Hand, D. W., Howe, K. J., & Tchobanoglous, G. (2012). MWH's Water Treatment: Principles and Design. John Wiley & Sons.

Dai, X., & Hozalski, R. M. (2003). Evaluation of Microspheres as Surrogates for Cryptosporidium parvum Oocysts in Filtration Experiments. Environmental Science & Technology, 37(5), 1037–1042. https://doi.org/10.1021/es025521w

Dugan, N. R., Fox, K. R., Owens, J. H., & MILTNER, R. J. (2001). Controlling "Cryptosporidium" Oocysts Using Conventional Treatment. *Journal (American Water Works Association)*, *93*(12), 64–76. https://doi.org/10.2307/41298128Edzwald, J. K., & Van Benschoten, J. E. (1990). Aluminum coagulation of natural organic matter. In Chemical water and wastewater treatment (pp. 341–359). Springer.

Edzwald James K., Becker William C., & Tambini Steven J. (1987). Organics, Polymers, and Performance in Direct Filtration. Journal of Environmental Engineering, 113(1), 167–185. https://doi.org/10.1061/(ASCE)0733-9372(1987)113:1(167)

Emelko, M. B. (2003). Removal of viable and inactivated Cryptosporidium by dual- and tri-media filtration. Water Research, 37(12), 2998–3008.

Emelko, M. B., & Huck, P. M. (2004). Microspheres as surrogates for Cryptosporidium filtration. *American Water Works Association. Journal*, *96*(3), 94.

Emelko, M. B., Huck, P. M., & Coffey, B. M. (2005). A review of Cryptosporidium removal by granular media filtration. Journal (American Water Works Association), 97(12), 101–115.

EPCOR Water Services Quality Assurance/PDT. (2014). *Deep Bed Filters for Direct Filtration: Fall 2013-Winter 2014 Pilot Study.* Unpublished internal document.

Or James, W. (2017, Jan 10). Personal communication.

Gebbie, P. (2001). Using polyaluminium coagulants in water treatment. In Proc. 64th AWIEO Conference. Citeseer. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.517.5307&rep=rep1&type=pdf

Gregory, J. (1993). The role of colloid interactions in solid-liquid separation. Water Science and Technology, 27(10), 1–17.

Hanson, A. T., & Cleasby, J. L. (1990). The Effects of Temperature on Turbulent Flocculation: Fluid Dynamics and Chemistry. Journal (American Water Works Association), 82(11), 56–73. https://doi.org/10.2307/41293075

Hendricks, D. W. (2006). Water Treatment Unit Processes: Physical and Chemical. CRC Press.

Huck, P. M., Coffey, B. M., Emelko, M. B., & O'Melia, C. R. (2000). The Importance of Coagulation for the Removal of Cryptosporidium and Surrogates by Filtration. In Chemical Water and Wastewater Treatment VI (pp. 191–200). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-59791-6_18

Hunter, R. J. (1981). Zeta potential in colloid science: principles and applications. London; New York: Academic Press, 1981.

James, W., Molla, S., McCrank, M., Craik, S. & Bhargava, A. (2011), Less water treatment residuals and more options by converting to direct filtration operation during winter. Paper presented at *Western Canada Water Conference and Exhibit,* Saskatoon, Saskatchewan, Canada

James, W., (Jan 10, 2017). Personal communication.

Jiang, X., Tong, M., Lu, R., & Kim, H. (2012). Transport and deposition of ZnO nanoparticles in saturated porous media. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 401, 29–37. https://doi.org/10.1016/j.colsurfa.2012.03.004

Langlais, B., Reckhow, D. A, & Brink, D. R. (1991). Ozone in water treatment: application and engineering: cooperative research report. Chelsea, Mich.: Lewis Publishers

LeChevallier, M. W., Norton, W. D., & Lee, R. G. (1991). Giardia and Cryptosporidium spp. in filtered drinking water supplies. *Applied and Environmental Microbiology*, *57*(9), 2617-2621.

Liu, L., Wang, Y., Narain, R. Liu, Y. (2018). *Functionalized polystyrene microspheres as cryptosporidium surrogates.* Unpublished manuscript. University of Alberta, Canada

McCormick, R. F., & King, P. H. (1982). Factors that affect use of direct filtration in treating surface waters. Journal (American Water Works Association), 74(5), 234–242.

McEwen, J. B. (1998). Treatment Process Selection for Particle Removal. American Water Works Association.

Morita, S., Namikoshi, A., Hirata, T., Oguma, K., Katayama, H., Ohgaki, S., … Fujiwara, M. (2002). Efficacy of UV Irradiation in Inactivating Cryptosporidiumparvum Oocysts. Applied and Environmental Microbiology, 68(11), 5387–5393. https://doi.org/10.1128/AEM.68.11.5387-5393.2002

Nieminski, E. C. (1994). Removing *Giardia* and *Cryptosporidium* cysts through direct filtration and conventional treatment. In *Proc. 1994 AWWA Ann. Conf., New York*.

Nieminski, E. C., Schaefer, F. W., & Ongerth, J. E. (1995). Comparison of two methods for detection of Giardia cysts and Cryptosporidium oocysts in water. *Applied and Environmental Microbiology*, *61*(5), 1714-1719.

Pang, L., Nowostawska, U., Weaver, L., Hoffman, G., Karmacharya, A., Skinner, A., & Karki, N. (2012). Biotin- and Glycoprotein-Coated Microspheres: Potential Surrogates for Studying Filtration of Cryptosporidium parvum in Porous Media. Environmental Science & Technology, 46(21), 11779–11787. https://doi.org/10.1021/es302555n

Park, S. H. (2008). Effect of amine-based water treatment polymers on the formation of N-nitrosodimethylamine (NDMA) disinfection by-product. Georgia Institute of Technology.

Patania, N. L. (1995). *Optimization of filtration for cyst removal*. American Water Works Association.

Pernitsky, D. J., & Edzwald, J. K. (2006). Selection of alum and polyaluminum coagulants: principles and applications. Journal of Water Supply: Research and Technology - Aqua, 55(2), 121. https://doi.org/10.2166/aqua.2006.062

Petterson, S. R., & Ashbolt, N. J. (2016). QMRA and water safety management: review of application in drinking water systems. *Journal of water and health*, *14*(4), 571-589.

Shao, Q., & Jiang, S. (2015). Molecular Understanding and Design of Zwitterionic Materials. Advanced Materials, 27(1), 15–26. https://doi.org/10.1002/adma.201404059

Shaw, K., Walker, S., & Koopman, B. (2000). Improving filtration of Cryptosporidium. *American Water Works Association. Journal*, *92*(11), 103.

States, S., Tomko, R., Scheuring, M., & Casson, L. (2002). Enhanced coagulation and removal of Cryptosporidium. Journal - American Water Works Association, 94(11), 67-77.

Sun, X., Shao, Y., Boluk, Y., & Liu, Y. (2015). The impact of cellulose nanocrystals on the aggregation and initial adhesion to a solid surface of Escherichia coli K12: Role of solution chemistry. Colloids and Surfaces B: Biointerfaces, 136, 570–576. https://doi.org/10.1016/j.colsurfb.2015.09.042

Swertfeger, J., Metz, D. H., DeMarco, J., Braghetta, A., & Jacangelo, J. G. (1999). Effect of filter media on cyst and oocyst removal. American Water Works Association. Journal; Denver, 91(9), 90.

Tien, C., & Payatakes, A. C. (1979). Advances in deep bed filtration. AIChE Journal, 25(5), 737–759. https://doi.org/10.1002/aic.690250502

Tong, M., Ma, H., & Johnson, W. P. (2008). Funneling of Flow into Grain-to-grain Contacts Drives Colloid−Colloid Aggregation in the Presence of an Energy Barrier. Environmental Science & Technology, 42(8), 2826–2832. https://doi.org/10.1021/es071888v

Treweek, G. P. (1979). Optimization of Flocculation Time Prior to Direct Filtration. Journal (American Water Works Association), 71(2), 96–101.

Tufenkji, N., & Elimelech, M. (2004). Deviation from the Classical Colloid Filtration Theory in the Presence of Repulsive DLVO Interactions. Langmuir, 20(25), 10818–10828. https://doi.org/10.1021/la0486638

Tufenkji, N., & Elimelech, M. (2005). Spatial Distributions of Cryptosporidium Oocysts in Porous Media: Evidence for Dual Mode Deposition. Environmental Science & Technology, 39(10), 3620–3629. https://doi.org/10.1021/es048289y

Walker, M., & Redelman, D. (2004). Detection of Cryptosporidium parvum in soil extracts. Applied and Environmental Microbiology, (3), 1827.

Widerström, M., Schönning, C., Lilja, M., Lebbad, M., Ljung, T., Allestam, G., … Lindh, J. (2014). Large Outbreak of Cryptosporidium hominis Infection Transmitted

through the Public Water Supply, Sweden. Emerging Infectious Diseases, 20(4), 581–589. https://doi.org/10.3201/eid2004.121415

Yang, R., Murphy, C., Song, Y., Ng-Hublin, J., Estcourt, A., Hijjawi, N., … Ryan, U. (2013). Specific and quantitative detection and identification of Cryptosporidium hominis andC. parvum in clinical and environmental samples. Experimental Parasitology, 135(1), 142–147. https://doi.org/10.1016/j.exppara.2013.06.014

Zhang, H., Seaman, J., Wang, Y., Zeng, H., Narain, R., Ulrich, A, & Liu., Y (2017a). Filtration of Glycoprotein-Modified Carboxylated Polystyrene Microspheres as Cryptosporidium Oocysts Surrogates: Effects of Flow Rate, Alum, and Humic Acid. Journal of Environmental Engineering, 143(8). https://doi.org/10.1061/(ASCE)EE.1943-7870.0001201

Zhang, Q., Davies, E. G. R., Bolton, J., & Liu, Y. (2017b). Monochloramine Loss Mechanisms in Tap Water. Water Environment Research, 89(11), 1999–2005. https://doi.org/10.2175/106143017X14902968254421

Zhang, Q., Gaafar, M., Yang, R.-C., Ding, C., Davies, E. G. R., Bolton, J. R., & Liu, Y. (2017c). Field data analysis of active chlorine-containing stormwater samples. Journal of Environmental Management, 206, 51–59. https://doi.org/10.1016/j.jenvman.2017.10.009

Zhu, H., Smith, D. W., Zhou, H., & Stanley, S. J. (1996). Improving removal of turbidity causing materials by using polymers as a filter aid. Water Research, 30(1), 103–114.

Zhu, T., Lawler, D. F., Chen, Y., & Lau, B. L. T. (2016). Effects of natural organic matter and sulfidation on the flocculation and filtration of silver nanoparticles. Environ. Sci.: Nano, 3(6), 1436–1446. https://doi.org/10.1039/C6EN00266H

Zhou, Y. (2016). Improving the Understanding of Factors Influencing Cryptosporidium Removals Reported for Granular Media Filtration (master's thesis, University of Waterloo).

Chapter 6. Conclusion, implications and recommendations

6.1 Conclusions

The key conclusions from this project are present below.

- From phase I of this study:
    - Copolymers-modified microspheres have been identified as the most representative surrogates for *Cryptosporidium* oocysts in filtration studies relative to other tested modified microspheres. They have comparable size, shape, surface charge and hydrophobicity, similar adhesion kinetics on solid surface, and similar transport and deposition behaviors in the column as compared to live *Cryptosporidium*. This surrogate offers additional advantages such as easy-to-detect, cost effective and not posing hazards.
- From phase II of the lab-scale sand-packed column experiments:
    - High ionic strength and low pH conditions associated with higher removal of *Cryptosporidium* surrogates; while the presence of NOM can significantly reduce *Cryptosporidium* removal.
    - No energy barrier was observed for higher ionic strength and acidic pH conditions, indicating favorable conditions for the adhesion of surrogates on the media grain.
    - Large energy barrier was observed for DOC present, more alkalic and lower ionic strength conditions, indicating unfavorable conditions for adhesion.
    - DLVO theory captured the main interaction energy between surrogates and media grain in the tested conditions with attention to the secondary minimum that can occur in the unfavorable interactions.
- From phase III of pilot-scale direct filtration experiments:
    - Filter aid polymer polyDADMAC Magnafloc® LT-7995 led to the greater *Cryptosporidium* surrogate removal in both regular and deep bed filters compared to the polyamine Magnafloc® LT-7981.
    - Increasing the dosage of filter aid polymer led to the higher log removal of surrogates.

- Deep bed filters enhanced surrogate removal and extended filter run time as compared to the regular bed filters.
- The observed surrogate deposition behaviors did not follow the classical CFT predictions, but relatively constant deposition coefficients were observed for both regular and deep bed filters.
- The observations from this phase confirmed the inability of turbidity to predict the removal of copolymers-modified microspheres as *Cryptosporidium* surrogates during the intermittent dosing run in the whole filter operation cycle.
- Some positive correspondences were observed between on-line particle log reductions based on the particle size measurement (2-5 μm) and surrogate removal. Particle count is more sensitive monitor parameter as an indicator of *Cryptosporidium* surrogate removal. The potential application of the particle counters as the indictor of pathogen removal from filters can be further investigated.
- This pilot study demonstrated the importance of using zeta potential of filter influent as a filter aid polymer dosing indicator. In general, higher dose of filter aid polymer can contribute to the less negative of filter influent zeta potential and higher surrogate deposition on the filter media.

6.2 Implications

- The findings of this study have implications for the understanding of transport and deposition of *Cryptosporidium* in the filtration process. Both surface charge and hydrophobicity are involved in the adhesion of *Cryptosporidium* on the media grain surface. The effects of solution chemistry (ionic strength, pH and DOC level) from the filter influent, coagulation conditions, and filter configurations control their log removal in the filtration process.
- The pilot-scale study demonstrated the exceed 2.5-log removal of *Cryptosporidium* surrogate (required from Guideline for Canadian Drinking Water Quality) can be achieved in the direct filtration during Edmonton cold-water condition when the

pretreatment processes are optimized using 0.454 mg/L of alum as Al with addition of 0.5 mg/L polyDADMAC.

- The magnitude of surrogate removal was site- and source water-specific. It was also shown that surrogate removal by filtration varies at different pretreatment conditions.
- Turbidity is very loosely related to pathogen levels. Increasing in filter effluent particle counts tent to signal increases in the potential for *Cryptosporidium* surrogate to pass through filters.
- This research has demonstrated the validity of the general approach by the water treatment plant of minimizing the filter effluent turbidity and particle concentration for maximizing the *Cryptosporidium* surrogate removal by filtration.

6.3 Recommendations

6.3.1 Water treatment plant operations and management

- A general recommendation for deep bed filters is made based on their superior performance during direct filtration in terms of surrogate removal and filter run time. The operational cost could be substantially reduced due to increased filtration capacity of deep bed without deteriorating filter effluent water quality, especially the surrogate removal efficiency. Challenges from operation and maintenance of deep bed filters as well as cost benefit analysis should also be considered to determine the suitability of converting regular bed to deep bed filter.
- Much lower dose of polyDADMAC can achieve charge neutralization of filter influent and maintain treated water quality than polyamine. Chemical dose and cost could be decreased by applying polyDADMAC in the examined conditions. Switching polymer from one to another should also consider their respective environmental impacts, which need further investigation.
- Passage of *Cryptosporidium* through filters could not be captured by the obvious increased of these parameters. Do not rely on turbidity or particle removal as surrogate for *Cryptosporidium* removal by filtration. Collecting samples routinely to develop a database of pathogen occurrence in the watershed and to monitoring

microbial levels are recommended for utilities. Viability and infectivity of *Cryptosporidium* or *Giardia* should be conducted in addition to the microbial monitoring program.

6.3.2 Water treatment research

- All the experiment conducted in this project from both bench- and pilot-scale studies involves spiking dramatically higher concentration of *C. parvum* oocyst or its surrogates compared to their concentrations in the natural environment. Naturally occurring oocysts in the raw water have extremely low concentration or even below the current detection limit, which poses difficulties to accurately evaluate the treatment removal efficiency. The removal achieved in this study whether are representative of those achieved with natural lower concentrations is questionable. The removals from spiked seeding experiments could be relied on for optimizing treatment process to maximize the oocysts removal.
- Additional studies are necessary and possible to further investigate the correlation between surrogate removal by independent microscope enumeration and particle removal by on-line particle counter with relevant size ranges. A new on-line particle counter to monitoring the filter influent particle counts after spiking could be considered in the pilot plant. The removal of particle in the relevant size range can be captured by knowing the concentrations of particles from filter influent and effluent. The correlation between particle removal and surrogate removal could be established in this further investigation.

References

Abudalo, R. A., Ryan, J. N., Harvey, R. W., Metge, D. W., & Landkamer, L. (2010). Influence of organic matter on the transport of Cryptosporidium parvum oocysts in a ferric oxyhydroxide-coated quartz sand saturated porous medium. *Water Research*, *44*(4), 1104–1113. https://doi.org/10.1016/j.watres.2009.09.039

Amburgey, J. E., Amirtharajah, A., York, M. T., Brouckaert, B. M., & et al. (2005). Comparison of Conventional and Biological Filter Performance for Cryptosporidium and microsphere removal. *American Water Works Association. Journal; Denver*, *97*(12), 77–91,10.

Amirtharajah, A. (1988). Some Theoretical and Conceptual Views of Filtration. *Journal (American Water Works Association)*, *80*(12), 36–46.

Amirtharajah, A., & Wetstein, D. P. (1980). Initial degradation of effluent quality during filtration. *Journal (American Water Works Association)*, 518–524.

Atlas, A. B. R. F. (n.d.). Surveillance for Waterborne-Disease Outbreaks—United States, 1995–1996 Cardiovascular Disease Risk Factors and Preventive Practices Among Adults—United States, 1994.

Bai, R., & Tien, C. (1997). Particle Detachment in Deep Bed Filtration. *Journal of Colloid and Interface Science*, *186*(2), 307–317. https://doi.org/10.1006/jcis.1996.4663

Balderrama-Carmona, A. P., Gortáres-Moroyoqui, P., Álvarez-Valencia, L. H., Castro-Espinoza, L., Balderas-Cortés, J. de J., Mondaca-Fernández, I., … Meza-Montenegro, M. M. (2014). Quantitative microbial risk assessment of *Cryptosporidium* and *Giardia* in well water from a native community of Mexico. *International Journal of Environmental Health Research*, 1–13. https://doi.org/10.1080/09603123.2014.989492

Bastos, R. K. X., Viana, D. B., & Bevilacqua, P. D. (2013). Turbidity as a surrogate for *Cryptosporidium* removal by filtration in drinking-water QMRA models. *Water Science and Technology: Water Supply*, *13*(5), 1209–1219. https://doi.org/10.2166/ws.2013.127

Behrens, H., Beims, U., Dieter, H., Dietze, G., Eikmann, T., Grummt, T., … Tilkes, F. (2014). Toxicological and ecotoxicological assessment of water tracers. *Hydrogeology Journal*, *9*(3), 321–325. https://doi.org/10.1007/s100400100126

Bellamy, W. D., Cleasby, J. L., Logsdon, G. S., & Allen, M. J. (1993). Assessing treatment plant performance. *American Water Works Association Journal*, *85*(12), 34-38.

Bertolucci, G. C., Gilli, G., Carraro, E., Giacosa, D., & Puppo, M. (1998). Influence of raw water storage on Giardia, Cryptosporidium and Nematodes. *Water Science and Technology*, *37*(2), 261.

Betancourt, W. Q., & Rose, J. B. (2004). Drinking water treatment processes for removal of Cryptosporidium and Giardia. *Veterinary Parasitology*, *126*(1), 219–234. https://doi.org/10.1016/j.vetpar.2004.09.002

Bowen W. Richard, Sabuni Hoze A. M., & Ventham Timothy J. (2004). Studies of the cell‐wall properties of Saccharomyces cerevisiae during fermentation.

Biotechnology and Bioengineering, *40*(11), 1309–1318. https://doi.org/10.1002/bit.260401104

Bradford, S A, & Bettahar, M. (2005). Straining, attachment, and detachment of cryptosporidium oocysts in saturated porous media. *Journal Of Environmental Quality*, *34*(2), 469–478.

Bradford, S. A., Simunek, J., Bettahar, M., van Genuchten, M. T., & Yates, S. R. (2006). Significance of straining in colloid deposition: Evidence and implications. *Water Resources Research*, *42*(12), W12S15. https://doi.org/10.1029/2005WR004791

Bradford, S., Torkzaban, S., & Walker, S. (2007). Coupling of physical and chemical mechanisms of colloid straining in saturated porous media. *WATER RESEARCH*, *41*(13), 3012–3024.

Bradford, Scott A., Yates, S. R., Bettahar, M., & Simunek, J. (2002). Physical factors affecting the transport and fate of colloids in saturated porous media. *Water Resources Research*, *38*(12), 1327. https://doi.org/10.1029/2002WR001340

Brush, C. F., Walter, M. F., Anguish, L. J., & Ghiorse, W. C. (1998). Influence of Pretreatment and Experimental Conditions on Electrophoretic Mobility and Hydrophobicity of Cryptosporidium parvum Oocysts. *Applied and Environmental Microbiology*, *64*(11), 4439–4445.

Bukhari, Z., Smith, H. V., Sykes, N., Humphreys, S. W., Paton, C. A., Girdwood, R. W. A., & Fricker, C. R. (1997). Occurrence of Cryptosporidium spp oocysts and Giardia spp cysts in sewage influents and effluents from treatment plants in England. *Water Science and Technology*, *35*(11–12), 385.

Bushkin, G. G., Motari, E., Carpentieri, A., Dubey, J. P., Costello, C. E., Robbins, P. W., & Samuelson, J. (2013). Evidence for a structural role for acid-fast lipids in oocyst walls of Cryptosporidium, Toxoplasma, and Eimeria. *MBio*, *4*(5), e00387-13.

Bustamante, H. A., Raj Shanker, S., Pashley, R. M., & Karaman, M. E. (2001). Interaction between cryptosporidium oocysts and water treatment coagulants. *Water Research*, *35*(13), 3179–3189. https://doi.org/10.1016/S0043-1354(01)00044-6

Cacciò, S. M., Thompson, R. C. A., McLauchlin, J., & Smith, H. V. (2005). Unravelling Cryptosporidium and Giardia epidemiology. *Trends in Parasitology*, *21*(9), 430–437. https://doi.org/10.1016/j.pt.2005.06.013

Carey, C. ., Lee, H., & Trevors, J. . (2004). Biology, persistence and detection of Cryptosporidium parvum and Cryptosporidium hominis oocyst. *Water Research*, *38*(4), 818–862. https://doi.org/10.1016/j.watres.2003.10.012

Chalmers, R. M., & Giles, M. (2010). Zoonotic cryptosporidiosis in the UK - challenges for control: Zoonotic cryptosporidiosis. *Journal of Applied Microbiology*, *109*(5), 1487–1497. https://doi.org/10.1111/j.1365-2672.2010.04764.x

Chatterjee, A., Banerjee, S., Steffen, M., O'Connor, R. M., Ward, H. D., Robbins, P. W., & Samuelson, J. (2010). Evidence for Mucin-Like Glycoproteins That Tether Sporozoites of Cryptosporidium parvum to the Inner Surface of the Oocyst Wall. *Eukaryotic Cell*, *9*(1), 84–96. https://doi.org/10.1128/EC.00288-09

Chauret, C. P., Radziminski, C. Z., Lepuil, M., Creason, R., & Andrews, R. C. (2001). Chlorine Dioxide Inactivation of Cryptosporidium parvum Oocysts and Bacterial

Spore Indicators. *Applied and Environmental Microbiology*, *67*(7), 2993–3001. https://doi.org/10.1128/AEM.67.7.2993-3001.2001

Chen, G., & Walker, S. L. (2007). Role of Solution Chemistry and Ion Valence on the Adhesion Kinetics of Groundwater and Marine Bacteria. *Langmuir*, *23*(13), 7162–7169. https://doi.org/10.1021/la0632833

Chen, G., & Walker, S. L. (2012). Fecal Indicator Bacteria Transport and Deposition in Saturated and Unsaturated Porous Media. *Environmental Science & Technology*, *46*(16), 8782–8790. https://doi.org/10.1021/es301378q

Chung, J. (2012). *Development of fluorescently labelled Cryptosporidium oocyst surrogates to test the efficacy of sand filtration processes*. University of New South Wales.

Clancy Jennifer L., Bukhari Zia, Hargy Thomas M., Bolton James R., Dussert Bertrand W., & Marshall Marilyn M. (2000). Using UV to inactivate Cryptosporidium. *Journal - American Water Works Association*, *92*(9), 97–104. https://doi.org/10.1002/j.1551-8833.2000.tb09008.x

Colton, J. F., Hillis, P., & Fitzpatrick, C. S. B. (1996). Filter backwash and start-up strategies for enhanced particulate removal. *Water Research*, *30*(10), 2502–2507. https://doi.org/10.1016/0043-1354(96)00085-1

Considine, R. F., Dixon, D. R., & Drummond, C. J. (2002). Oocysts of Cryptosporidium parvum and model sand surfaces in aqueous solutions: an atomic force microscope (AFM) study. *Water Research*, *36*(14), 3421–3428. https://doi.org/10.1016/S0043-1354(02)00082-9

Considine, R. F., Drummond, C. J., & Dixon, D. R. (2001). Force of Interaction between a Biocolloid and an Inorganic Oxide: Complexity of Surface Deformation, Roughness, and Brushlike Behavior. *Langmuir*, *17*(20), 6325–6335. https://doi.org/10.1021/la001459+

Copes, R., Hrudey, S. E., Payment, P., & Act, D. W. (2008). Turbidity and microbial risk in drinking water. *Prepared for The Minister of Health, Province of British Columbia, pursuant to Section*, *5*. Retrieved from http://www.waterbucket.ca/okw/sites/wbcokw/documents/media/10.pdf

Crittenden, J. C., Trussell, R. R., Hand, D. W., Howe, K. J., & Tchobanoglous, G. (2012). *MWH's Water Treatment: Principles and Design*. John Wiley & Sons.

Dai, X, Boll, J., Hayes, M. E., & Aston, D. E. (2004). Adhesion of Cryptosporidium parvum and Giardia lamblia to solid surfaces: the role of surface charge and hydrophobicity. *Colloids and Surfaces B: Biointerfaces*, *34*(4), 259–263. https://doi.org/10.1016/j.colsurfb.2003.12.016

Dai, Xiaojun, & Hozalski, R. M. (2002). Effect of NOM and biofilm on the removal of Cryptosporidium parvum oocysts in rapid filters. *Water Research*, *36*(14), 3523–3532. https://doi.org/10.1016/S0043-1354(02)00045-3

Dai, Xiaojun, & Hozalski, R. M. (2003). Evaluation of Microspheres as Surrogates for Cryptosporidium parvum Oocysts in Filtration Experiments. *Environmental Science & Technology*, *37*(5), 1037–1042. https://doi.org/10.1021/es025521w

D'Antonio RG, Winn RE, Taylor JP, & et al. (1985). A waterborne outbreak of cryptosporidiosis in normal hosts. *Annals of Internal Medicine*, *103*(6_Part_1), 886–888. https://doi.org/10.7326/0003-4819-103-6-886

Deng, Z., Ahmed, M., & Narain, R. (2009). Novel well-defined glycopolymers synthesized via the reversible addition fragmentation chain transfer process in aqueous media. *Journal of Polymer Science Part A: Polymer Chemistry*, *47*(2), 614–627. https://doi.org/10.1002/pola.23187

Dengis, P. B., Nelissen, L. R., & Rouxhet, P. G. (1995). Mechanisms of yeast flocculation: comparison of top-and bottom-fermenting strains. *Applied and Environmental Microbiology*, *61*(2), 718–728.

Dudi, A. (2004). *Reconsidering lead corrosion in drinking water: product testing, direct chloramine attack and galvanic corrosion* (Doctoral dissertation, Virginia Tech)

Dugan, N. R., Fox, K. R., Owens, J. H., & MILTNER, R. J. (2001). Controlling "Cryptosporidium" Oocysts Using Conventional Treatment. *Journal (American Water Works Association)*, *93*(12), 64–76. https://doi.org/10.2307/41298128

Dumètre, A., Aubert, D., Puech, P.-H., Hohweyer, J., Azas, N., & Villena, I. (2012). Interaction Forces Drive the Environmental Transmission of Pathogenic Protozoa. *Applied and Environmental Microbiology*, *78*(4), 905–912. https://doi.org/10.1128/AEM.06488-11

Edwards, M., & Triantafyllidou, S. (2007). Chloride-to-sulfate mass ratio and lead leaching to water. *Journal (American Water Works Association)*, *99*(7), 96-109.Edzwald, J. K., & Van Benschoten, J. E. (1990). Aluminum coagulation of natural organic matter. In *Chemical water and wastewater treatment* (pp. 341–359). Springer.

Edzwald James K., Becker William C., & Tambini Steven J. (1987). Organics, Polymers, and Performance in Direct Filtration. *Journal of Environmental Engineering*, *113*(1), 167–185. https://doi.org/10.1061/(ASCE)0733-9372(1987)113:1(167)

Efstratiou, A., Ongerth, J., & Karanis, P. (2017). Evolution of monitoring for Giardia and Cryptosporidium in water. *Water Research*, *123*, 96–112. https://doi.org/10.1016/j.watres.2017.06.042

El Henawy, W. (2009). *The Effect of Selected Coagulants on Chloride-to-Sulfate Mass Ratio for Lead Control and on Organics Removal in Two Source Waters* (Master's thesis, University of Waterloo)

Elimelech, M., & O'Melia, C. R. (1990). Kinetics of deposition of colloidal particles in porous media. *Environmental Science & Technology*, *24*(10), 1528–1536.

Emelko, M. B., & Huck, P. M. (2004). Microspheres as Surrogates for "Cryptosporidium" Filtration. *Journal (American Water Works Association)*, *96*(3), 94–105.

Emelko, M. B., Huck, P. M., & Coffey, B. M. (2005). A review of Cryptosporidium removal by granular media filtration. *Journal (American Water Works Association)*, *97*(12), 101–115.

Emelko, M. B., Huck, P. M., & Douglas, I. P. (2003). "Cryptosporidium" and microsphere removal during late in-cycle filtration. *Journal (American Water Works Association)*, *95*(5), 173–182.

Emelko, Monica B. (2003). Removal of viable and inactivated Cryptosporidium by dual- and tri-media filtration. *Water Research*, *37*(12), 2998–3008.

Emelko, Monica Beata. (2001). Removal of Cryptosporidium parvum by granular media filtration.

Fayer, R, & Ungar, B. L. (1986). Cryptosporidium spp. and cryptosporidiosis. *Microbiological Reviews*, *50*(4), 458–483.

Fayer, Ronald, Farley, C. A., Lewis, E. J., Trout, J. M., & Graczyk, T. K. (1997). Potential Role of the Eastern Oyster, Crassostrea virginica, in the Epidemiology of Cryptosporidium parvum. *Applied and Environmental Microbiology*, *63*(5), 2086–2088.

Fayer, Ronald, Nerad, T., Rall, W., Lindsay, D. S., & Blagburn, B. L. (1991). Studies on Cryopreservation of Cryptosporidium parvum. *The Journal of Parasitology*, *77*(3), 357–361. https://doi.org/10.2307/3283119

Foppen, J. W. A., & Schijven, J. F. (2006). Evaluation of data from the literature on the transport and survival of Escherichia coli and thermotolerant coliforms in aquifers under saturated conditions. *Water Research*, *40*(3), 401–426. https://doi.org/10.1016/j.watres.2005.11.018

Foppen, J. W., Liem, Y., & Schijven, J. (2008). Effect of humic acid on the attachment of Escherichia coli in columns of goethite-coated sand. *Water Research*, *42*(1), 211–219. https://doi.org/10.1016/j.watres.2007.06.064

Gao, X., & Chorover, J. (2009). In-situ monitoring of Cryptosporidium parvum oocyst surface adhesion using ATR-FTIR spectroscopy. *Colloids and Surfaces B: Biointerfaces*, *71*(2), 169–176. https://doi.org/10.1016/j.colsurfb.2009.02.003

Gebbie, P. (2001). Using polyaluminium coagulants in water treatment. In *Proc. 64th AWIEO Conference*. Citeseer. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.517.5307&rep=rep1&type=pdf

Gottinger, A. M., Bhat, S. V., McMartin, D. W., & Dahms, T. E. S. (2013). Fluorescent microspheres as surrogates to assess oocyst removal efficacy from a modified slow sand biofiltration water treatment system. *Journal of Water Supply: Research and Technology - Aqua*, *62*(3), 129. https://doi.org/10.2166/aqua.2013.104

Grasso*, D., Subramaniam, K., Butkus, M., Strevett, K., & Bergendahl, J. (2002). A review of non-DLVO interactions in environmental colloidal systems. *Reviews in Environmental Science and Biotechnology*, *1*(1), 17–38. https://doi.org/10.1023/A:1015146710500

Gregory, J. (1993). The role of colloid interactions in solid-liquid separation. *Water Science and Technology*, *27*(10), 1–17.

Gregory, J. (2005). *Particles in water: properties and processes*. CRC Press.

Haas, C. N., Severin, B. F., Roy, D., Engelbrecht, R. S., Lalchandani, A., & Farooq, S. (1985). Field observations on the occurrence of new indicators of disinfection efficiency. *Water Research*, *19*(3), 323–329.

Haes, A. J., Zou, S., Schatz, G. C., & Van Duyne, R. P. (2004). Nanoscale Optical Biosensor: Short Range Distance Dependence of the Localized Surface Plasmon Resonance of Noble Metal Nanoparticles. *The Journal of Physical Chemistry B*, *108*(22), 6961–6968. https://doi.org/10.1021/jp036261n

Hagler, A. N. (2006). Yeasts as indicators of environmental quality. In *Biodiversity and ecophysiology of yeasts* (pp. 515–532). Springer.

Hansen, J. S., & Ongerth, J. E. (1991). Effects of time and watershed characteristics on the concentration of Cryptosporidium oocysts in river water. *Applied and Environmental Microbiology*, *57*(10), 2790–2795.

Hanson, A. T., & Cleasby, J. L. (1990). The Effects of Temperature on Turbulent Flocculation: Fluid Dynamics and Chemistry. *Journal (American Water Works Association)*, *82*(11), 56–73. https://doi.org/10.2307/41293075

Hargesheimer, E. E., Lewis, C. M., & Yentsch, C. M. (1992). *Evaluation of particle counting as a measure of treatment plant performance*. AWWA. Retrieved from http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=REPIDISCA&lang=p&nextAction=lnk&exprSearch=90333&indexSearch=ID

Harrington Gregory W., Xagoraraki Irene, Assavasilavasukul Prapakorn, & Standridge Jon H. (2003). Effect of Filtration Conditions On Removal of Emerging waterborne pathogens. *Journal - American Water Works Association*, *95*(12), 95–104. https://doi.org/10.1002/j.1551-8833.2003.tb10514.x

Harris, J. R., & Petry, F. (1999). Cryptosporidium parvum: Structural Components of the Oocyst Wall. *The Journal of Parasitology*, *85*(5), 839–849. https://doi.org/10.2307/3285819

Health Canada. (2012). *Guideline for Canadian Drinking Water Quality: Guideline Technical Document-Turbidity.* https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-turbidity.html(accessed on 0.1.15.2018)

Health Canada. (2017). *Guideline for Canadian Drinking Water Quality-Summary Table.* https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/ewh-semt/alt_formats/pdf/pubs/water-eau/sum_guide-res_recom/sum_guide-res_recom-eng.pdf (accessed on 01.15.2018)Hendricks, D. W. (2006). *Water Treatment Unit Processes: Physical and Chemical*. CRC Press.

Hermansson, M. (1999). The DLVO theory in microbial adhesion. *Colloids and Surfaces B: Biointerfaces*, *14*(1), 105–119. https://doi.org/10.1016/S0927-7765(99)00029-6

Hijnen, W.A.M., Beerendonk, E. F., & Medema, G. J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research*, *40*(1), 3–22. https://doi.org/10.1016/j.watres.2005.10.030

Hijnen, W.A.M., Schijven, J. F., Bonné, P., Visser, A., & Medema, G. J. (2004). Elimination of viruses, bacteria and protozoan oocysts by slow sand filtration. *Water Science and Technology*, *50*(1), 147.

Hijnen, W.A.M, Van Veenendaal, D. ., van der Speld, W. M. ., Visser, A., Hoogenboezem, W., & van der Kooij, D. (2000). Enumeration of faecal indicator bacteria in large water volumes using on site membrane filtration to assess water treatment efficiency. *Water Research*, *34*(5), 1659–1665. https://doi.org/10.1016/S0043-1354(99)00311-5

Hijnen, Wim A. M., Dullemont, Y. J., Schijven, J. F., Hanzens-Brouwer, A. J., Rosielle, M., & Medema, G. (2007). Removal and fate of Cryptosporidium parvum, Clostridium perfringens and small-sized centric diatoms (Stephanodiscus hantzschii) in slow sand filters. *Water Research*, *41*(10), 2151–2162. https://doi.org/10.1016/j.watres.2007.01.056

Hipsey, M.R., & Brookes, J.D. (2017). Pathogen Management in Surface Waters: Practical Considerations for Reducing Public Health Risk.

Hogan, J. N., Daniels, M. E., Watson, F. G., Oates, S. C., Miller, M. A., Conrad, P. A., … Miller, W. A. (2013). Hydrologic and Vegetative Removal of Cryptosporidium parvum, Giardia lamblia, and Toxoplasma gondii Surrogate Microspheres in Coastal Wetlands. *Applied and Environmental Microbiology*, *79*(6), 1859–1865. https://doi.org/10.1128/AEM.03251-12

Hsu, B.-M., Huang, C., & Pan, J. R. (2001). Filtration behaviors of Giardia and Cryptosporidium—ionic strength and pH effects. *Water Research*, *35*(16), 3777–3782.

Hsu, B.-M., & Yeh, H.-H. (2003). Removal of Giardia and Cryptosporidium in drinking water treatment: a pilot-scale study. *Water Research*, *37*(5), 1111–1117. https://doi.org/10.1016/S0043-1354(02)00466-9

Huck, P. M., Coffey, B. M., Emelko, M. B., & O'Melia, C. R. (2000). The Importance of Coagulation for the Removal of Cryptosporidium and Surrogates by Filtration. In *Chemical Water and Wastewater Treatment VI* (pp. 191–200). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-59791-6_18

Huck, Peter M. (2001). Filter operation effects on pathogen passage / prepared by Peter M. Huck ... [et al.] ; sponsored by Microbial/Disinfection By-Products Research Council. Denver, CO : AWWA Research Foundation and American Water Works Association, c2001.

Huck, Peter M., AWWA Research Foundation, & Microbial/Disinfection By-Products Research Council (U.S.) (Eds.). (2001). *Filter operation effects on pathogen passage*. Denver, CO: AWWA Research Foundation and American Water Works Association.

Huck Peter M., Coffey Bradley M., Emelko Monica B., Maurizio Danielle D., Slawson Robin M., Anderson William B., … O'Melia Charles R. (2002). EFFECTS OF filter operation on Cryptosporidium removal microbial pathogens. *Journal - American Water Works Association*, *94*(6), 97–111. https://doi.org/10.1002/j.1551-8833.2002.tb09493.x

Ives, K. (1970). Rapid filtration. *Water Research*, *4*(3), 201–223. https://doi.org/10.1016/0043-1354(70)90068-0

Ives, K. J. (1982, May). Fundamentals of filtration. In *Proceedings of the 21st European Federation of Chemical Engineering Event Symposium on Water Filtration, Antwerp, Belgium*.Jakubowski Walter, Boutros Susan, Faber Walter, Fayer Ron, Ghiorse William, LeChevallier Mark, … Stewart Mic. (1996). Environmental methods for Cryptosporidium. *Journal - American Water Works Association*, *88*(9), 107–121. https://doi.org/10.1002/j.1551-8833.1996.tb06617.x

Jenkins, M. B., Eaglesham, B. S., Anthony, L. C., Kachlany, S. C., Bowman, D. D., & Ghiorse, W. C. (2010). Significance of Wall Structure, Macromolecular Composition, and Surface Polymers to the Survival and Transport of Cryptosporidium parvum Oocysts. *Applied and Environmental Microbiology*, *76*(6), 1926–1934. https://doi.org/10.1128/AEM.02295-09

Jiang, X., Tong, M., Lu, R., & Kim, H. (2012). Transport and deposition of ZnO nanoparticles in saturated porous media. *Colloids and Surfaces A:*

Physicochemical and Engineering Aspects, *401*, 29–37. https://doi.org/10.1016/j.colsurfa.2012.03.004

Jin, C., Zhao, W., Normani, S. D., Zhao, P., & Emelko, M. B. (2017). Synergies of media surface roughness and ionic strength on particle deposition during filtration. *Water Research*, *114*, 286–295. https://doi.org/10.1016/j.watres.2017.02.010

Karanis, P., Kourenti, C., & Smith, H. (2007). Waterborne transmission of protozoan parasites: A worldwide review of outbreaks and lessons learnt. *Journal of Water and Health*, *5*(1), 1–38. https://doi.org/10.2166/wh.2006.002

Karanis, Panagiotis, Schoenen, D., & Seitz, H. M. (1998). Distribution and removal of Giardia and Cryptosporidium in water supplies in Germany. *Water Science and Technology*, *37*(2), 9.

Kim, H. N., Walker, S. L., & Bradford, S. A. (2010). Coupled factors influencing the transport and retention of Cryptosporidium parvum oocysts in saturated porous media. *Water Research*, *44*(4), 1213–1223. https://doi.org/10.1016/j.watres.2009.09.041

Kim, M., & Zydney, A. L. (2004). Effect of electrostatic, hydrodynamic, and Brownian forces on particle trajectories and sieving in normal flow filtration. *Journal of Colloid and Interface Science*, *269*(2), 425–431. https://doi.org/10.1016/j.jcis.2003.08.004

Knappett, P. S. K., Emelko, M. B., Zhuang, J., & McKay, L. D. (2008). Transport and retention of a bacteriophage and microspheres in saturated, angular porous media: Effects of ionic strength and grain size. *Water Research*, *42*(16), 4368–4378. https://doi.org/10.1016/j.watres.2008.07.041

Krasner Stuart W., & Amy Gary. (1995). Jar‐test evaluations of enhanced coagulation. *Journal - American Water Works Association*, *87*(10), 93–107. https://doi.org/10.1002/j.1551-8833.1995.tb06438.x

Kuznar, Z., & Elimelech, M. (2004). Deposition kinetics of cryptosporidium parvum oocysts onto quartz surfaces. *ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY*, *228*, U633–U633.

Kuznar, Zachary A., & Elimelech, M. (2004). Adhesion Kinetics of Viable Cryptosporidium parvum Oocysts to Quartz Surfaces. *Environmental Science & Technology*, *38*(24), 6839–6845. https://doi.org/10.1021/es0494104

Kuznar, Zachary A., & Elimelech, M. (2005). Role of Surface Proteins in the Deposition Kinetics of Cryptosporidium parvum Oocysts. *Langmuir*, *21*(2), 710–716. https://doi.org/10.1021/la047963m

Kuznar, Zachary A., & Elimelech, M. (2006a). Cryptosporidium Oocyst Surface Macromolecules Significantly Hinder Oocyst Attachment. *Environmental Science & Technology*, *40*(6), 1837–1842. https://doi.org/10.1021/es051859p

Kuznar, Zachary A., & Elimelech, M. (2006b). Cryptosporidium oocyst surface macromolecules significantly hinder oocyst attachment. *Environmental Science & Technology*, *40*(6), 1837–1842.

Kuznar, Zachary Andrew. (2005). *Mechanisms controlling the adhesion of Cryptosporidium parvum oocysts to solid surfaces* (Ph.D.). Yale University, United States -- Connecticut. Retrieved from

http://search.proquest.com.login.ezproxy.library.ualberta.ca/pqdtglobal/docview/305386558/abstract/1E6E1B26ADD64325PQ/1
LeChevallier, M. W., & Norton, W. D. (1992). Examining Relationships Between Particle Counts and Giardia, Cryptosporidium, and Turbidity. *Journal (American Water Works Association)*, *84*(12), 54–60. https://doi.org/10.2307/41293943
LeChevallier Mark W., & Norton William D. (1995). Giardia and Cryptosporidium in raw and finished water. *Journal - American Water Works Association*, *87*(9), 54–68. https://doi.org/10.1002/j.1551-8833.1995.tb06422.x
Lee, L.-H. (1991). The Chemistry and Physics of Solid Adhesion. In L.-H. Lee (Ed.), *Fundamentals of Adhesion* (pp. 1–86). Boston, MA: Springer US. https://doi.org/10.1007/978-1-4899-2073-7_1
Lerner, R. N., Lu, Q., Zeng, H., & Liu, Y. (2012). The effects of biofilm on the transport of stabilized zerovalent iron nanoparticles in saturated porous media. *Water Research*, *46*(4), 975–985. https://doi.org/10.1016/j.watres.2011.11.070
Li, X., & Johnson, W. P. (2005). Nonmonotonic variations in deposition rate coefficients of microspheres in porous media under unfavorable deposition conditions. *Environmental Science & Technology*, *39*(6), 1658–1665.
Links, A. G. (2015). *Utilization of Fluorescent Microspheres as a Surrogate for Cryptosporidium Removal in Conventional Drinking Water Treatment*. Arizona State University.
Lisle, J. T., & Rose, J. B. (1995). Gene exchange in drinking water and biofilms by natural transformation. *Water Science and Technology*, *31*(5–6), 41.
Liu, Yang, & Li, J. (2008). Role of Pseudomonas aeruginosa Biofilm in the Initial Adhesion, Growth and Detachment of Escherichia coli in Porous Media. *Environmental Science & Technology*, *42*(2), 443–449. https://doi.org/10.1021/es071861b
Liu, Yang, Yang, C.-H., & Li, J. (2007a). Adhesion and retention of a bacterial phytopathogen Erwinia chrysanthemi in biofilm-coated porous media. *Environmental Science & Technology*, *42*(1), 159–165.
Liu, Yang, Yang, C.-H., & Li, J. (2007b). Influence of Extracellular Polymeric Substances on Pseudomonas aeruginosa Transport and Deposition Profiles in Porous Media. *Environmental Science & Technology*, *41*(1), 198–205. https://doi.org/10.1021/es061731n
Liu, Yuanyuan, Janjaroen, D., Kuhlenschmidt, M. S., Kuhlenschmidt, T. B., & Nguyen, T. H. (2009). Deposition of Cryptosporidium parvum Oocysts on Natural Organic Matter Surfaces: Microscopic Evidence for Secondary Minimum Deposition in a Radial Stagnation Point Flow Cell. *Langmuir*, *25*(3), 1594–1605. https://doi.org/10.1021/la803202h
Lu, P., & Amburgey, J. E. (2016). A pilot-scale study of Cryptosporidium-sized microsphere removals from swimming pools via sand filtration. *Journal of Water and Health*, *14*(1), 109. https://doi.org/10.2166/wh.2015.047
Lu, P., Amburgey, J. E., Hill, V. R., Murphy, J. L., Schneeberger, C. L., Arrowood, M. J., & Yuan, T. (2017). Removals of cryptosporidium parvum oocysts and cryptosporidium-sized polystyrene microspheres from swimming pool water by diatomaceous earth filtration and perlite-sand filtration. *Journal of Water and Health*, wh2017221. https://doi.org/10.2166/wh.2017.221

Lytle, D. A., Johnson, C. H., & Rice, E. W. (2002). A systematic comparison of the electrokinetic properties of environmentally important microorganisms in water. *Colloids and Surfaces B: Biointerfaces*, *24*(2), 91–101. https://doi.org/10.1016/S0927-7765(01)00219-3

Mac Kenzie, W. R., Hoxie, N. J., Proctor, M. E., Gradus, M. S., Blair, K. A., Peterson, D. E., … Davis, J. P. (1994). A Massive Outbreak in Milwaukee of Cryptosporidium Infection Transmitted through the Public Water Supply. *New England Journal of Medicine*, *331*(3), 161–167. https://doi.org/10.1056/NEJM199407213310304

McCormick, R. F., & King, P. H. (1982). Factors that affect use of direct filtration in treating surface waters. *Journal (American Water Works Association)*, *74*(5), 234–242.

McEwen, J. B. (1998). *Treatment Process Selection for Particle Removal*. American Water Works Association.

Meisel, J. L., Perera, D. R., Meligro, C., & Rubin, C. E. (1976). Overwhelming watery diarrhea associated with a Cryptosporidium in an immunosuppressed patient. *Gastroenterology*, *70*(6), 1156–1160.

Mohanram, A., Ray, C., Metge, D. W., Barber, L. B., Ryan, J. N., & Harvey, R. W. (2012). Effect of Dissolved Organic Carbon on the Transport and Attachment Behaviors of *Cryptosporidium parvum* oocysts and Carboxylate-Modified Microspheres Advected through Temperate Humic and Tropical Volcanic Agricultural soil. *Environmental Science & Technology*, *46*(4), 2088–2094. https://doi.org/10.1021/es2003342

Molnar, I. L., Johnson, W. P., Gerhard, J. I., Willson, C. S., & O'Carroll, D. M. (2015). Predicting colloid transport through saturated porous media: A critical review. *Water Resources Research*, *51*(9), 6804–6845. https://doi.org/10.1002/2015WR017318

Moore, A. ., Vesey, G., Champion, A., Scandizzo, P., Deere, D., Veal, D., & Williams, K. . (1998). Viable Cryptosporidium parvum oocysts exposed to chlorine or other oxidising conditions may lack identifying epitopes. *International Journal for Parasitology*, *28*(8), 1205–1212. https://doi.org/10.1016/S0020-7519(98)00070-8

Moran, M. C., Moran, D. C., Cushing, R. S., & Lawler, D. F. (1993). Particle Behavior in Deep-Bed Filtration: Part 2—Particle Detachment. *Journal (American Water Works Association)*, *85*(12), 82–93.

Morgan‐Ryan, U. M., Fall, A., Ward, L. A., Hijjawi, N., Sulaiman, I., Payer, R., ... & Xiao, L. (2002). Cryptosporidium hominis n. sp. (Apicomplexa: Cryptosporidiidae) from Homo sapiens. *Journal of Eukaryotic Microbiology*, *49*(6), 433-440.

Morita, S., Namikoshi, A., Hirata, T., Oguma, K., Katayama, H., Ohgaki, S., … Fujiwara, M. (2002). Efficacy of UV Irradiation in Inactivating Cryptosporidiumparvum Oocysts. *Applied and Environmental Microbiology*, *68*(11), 5387–5393. https://doi.org/10.1128/AEM.68.11.5387-5393.2002

Morse, A., Wilson, R., Hull, A., & Simms, J. (2002). Monitoring for Cryptosporidium risk: do particle counters help? *Water Science and Technology: Water Supply*, *2*(1), 171.

Mustranta, A., Pere, J., & Poutanen, K. (1987). Comparison of different carriers for adsorption of Saccharomyces cerevisiae and Zymomonas mobilis. *Enzyme and Microbial Technology*, *9*(5), 272–276. https://doi.org/10.1016/0141-0229(87)90002-0

Nanduri, J., Williams, S., Aji, T., & Flanigan, T. P. (1999). Characterization of an immunogenic glycocalyx on the surfaces of Cryptosporidium parvum oocysts and sporozoites. *Infection and Immunity*, *67*(4), 2022–2024.

Neill, M. A., Rice, S. K., Ahmad, N. V., & Flanigan, T. P. (1996). Cryptosporidiosis: An Unrecognized Cause of Diarrhea in Elderly Hospitalized Patients. *Clinical Infectious Diseases*, *22*(1), 168–170. https://doi.org/10.1093/clinids/22.1.168

Nieminski, E. C., & Ongerth, J. E. (1995). Removing Giardia and Cryptosporidium by conventional treatment and direct filtration. *Journal (American Water Works Association)*, *87*(9), 96–106. https://doi.org/10.2307/41295317

O'Donoghue, P. J. (1995). Cryptosporidium and cryptosporidiosis in man and animals. *International Journal for Parasitology*, *25*(2), 139–195. https://doi.org/10.1016/0020-7519(94)E0059-V

Okhuysen, P. C., Rich, S. M., Chappell, C. L., Grimes, K. A., Widmer, G., Feng, X., & Tzipori, S. (2002). Infectivity of a Cryptosporidium parvum isolate of cervine origin for healthy adults and interferon-γ knockout mice. *Journal of Infectious Diseases*, *185*(9), 1320–1325.

Ong, C., Moorehead, W., Ross, A., & Isaac-Renton, J. (1996). Studies of Giardia spp. and Cryptosporidium spp. in two adjacent watersheds. *Applied and Environmental Microbiology*, *62*(8), 2798–2805.

Ongerth Jerry E., & Pecoraro Julie Proctor. (1996). Electrophoretic Mobility of *Cryptosporidium* Oocysts and *Giardia* Cysts. *Journal of Environmental Engineering*, *122*(3), 228–231. https://doi.org/10.1061/(ASCE)0733-9372(1996)122:3(228)

Pang, L., Nowostawska, U., Weaver, L., Hoffman, G., Karmacharya, A., Skinner, A., & Karki, N. (2012). Biotin- and Glycoprotein-Coated Microspheres: Potential Surrogates for Studying Filtration of *Cryptosporidium parvum* in Porous Media. *Environmental Science & Technology*, *46*(21), 11779–11787. https://doi.org/10.1021/es302555n

Park, S. H. (2008). *Effect of amine-based water treatment polymers on the formation of N-nitrosodimethylamine (NDMA) disinfection by-product*. Georgia Institute of Technology.

Park, Y., Atwill, E. R., Hou, L., Packman, A. I., & Harter, T. (2012). Deposition of Cryptosporidium parvum Oocysts in Porous Media: A Synthesis of Attachment Efficiencies Measured under Varying Environmental Conditions. *Environmental Science & Technology*, *46*(17), 9491–9500. https://doi.org/10.1021/es300564w

Patania, N. L. (1995). *Optimization of Filtration for Cyst Removal*. American Water Works Association.

Pernitsky, D. J., & Edzwald, J. K. (2006). Selection of alum and polyaluminum coagulants: principles and applications. *Journal of Water Supply: Research and Technology - Aqua*, *55*(2), 121. https://doi.org/10.2166/aqua.2006.062

Petterson, S. R., & Ashbolt, N. J. (2016). QMRA and water safety management: review of application in drinking water systems. *Journal of Water and Health*, *14*(4), 571–589. https://doi.org/10.2166/wh.2016.262

Pillai, D. R., Melano, R., Rawte, P., Lo, S., Tijet, N., Fuksa, M., … Krajden, S. (2009). *Klebsiella pneumoniae* Carbapenemase, Canada. *Emerging Infectious Diseases*, *15*(5), 827–829. https://doi.org/10.3201/eid1505.081536

Raveendran Palanivel, & Amirtharajah Appiah. (1995). Role of Short-Range Forces in Particle Detachment during Filter Backwashing. *Journal of Environmental Engineering*, *121*(12), 860–868. https://doi.org/10.1061/(ASCE)0733-9372(1995)121:12(860)

Rose, J. B. (1988). Occurrence and significance of Cryptosporidium in water. *Journal (American Water Works Association)*, 53–58.

Saleh, N., Kim, H.-J., Phenrat, T., Matyjaszewski, K., Tilton, R. D., & Lowry, G. V. (2008). Ionic Strength and Composition Affect the Mobility of Surface-Modified Fe0 Nanoparticles in Water-Saturated Sand Columns. *Environmental Science & Technology*, *42*(9), 3349–3355. https://doi.org/10.1021/es071936b

Shao, Q., & Jiang, S. (2015). Molecular Understanding and Design of Zwitterionic Materials. *Advanced Materials*, *27*(1), 15–26. https://doi.org/10.1002/adma.201404059

Shaw, K., Walker, S., & Koopman, B. (2000). Improving Filtration of Cryptosporidium. *Journal (American Water Works Association)*, *92*(11), 103–111.

Smith, H. V., Grimason, A. M., Benton, C., & Parker, J. F. W. (1991). The Occurrence of Cryptosporidium Spp. Oocysts in Scottish Waters, and the Development of a Fluorogenic Viability Assay for Individual Cryptosporidium spp. Oocysts. *Water Science and Technology*, *24*(2), 169.

Spano, F., Putignani, L., McLauchlin, J., Casemore, D. P., & Crisanti, A. (1997). PCR-RFLP analysis of the Cryptosporidium oocyst wall protein (COWP) gene discriminates between C. wrairi and C. parvum, and between C. parvum isolates of human and animal origin. *FEMS Microbiology Letters*, *150*(2), 209–217. https://doi.org/10.1111/j.1574-6968.1997.tb10372.x

States, S., Tomko, R., Scheuring, M., & Casson, L. (2002). Enhanced coagulation and removal of Cryptosporidium. *American Water Works Association. Journal; Denver*, *94*(11), 67.

Stevenson, M. E., Blaschke, A. P., Toze, S., Sidhu, J. P. S., Ahmed, W., van Driezum, I. H., … Pang, L. (2015). Biotin- and Glycoprotein-Coated Microspheres as Surrogates for Studying Filtration Removal of Cryptosporidium parvum in a Granular Limestone Aquifer Medium. *Applied and Environmental Microbiology*, *81*(13), 4277–4283. https://doi.org/10.1128/AEM.00885-15

Sun, X., Shao, Y., Boluk, Y., & Liu, Y. (2015). The impact of cellulose nanocrystals on the aggregation and initial adhesion to a solid surface of Escherichia coli K12: Role of solution chemistry. *Colloids and Surfaces B: Biointerfaces*, *136*, 570–576. https://doi.org/10.1016/j.colsurfb.2015.09.042

Swertfeger, J., Metz, D. H., DeMarco, J., Braghetta, A., & Jacangelo, J. G. (1999). Effect of filter media on cyst and oocyst removal. *American Water Works Association. Journal; Denver*, *91*(9), 90.

Tfaily, R., Papineau, I., Andrews, R. C., & Barbeau, B. (2015). Application of Quantitative Microbial Risk Assessment at 17 Canadian Water Treatment Facilities. *Journal - American Water Works Association*, *107*, E497–E508. https://doi.org/10.5942/jawwa.2015.107.0141

Thonart, P., Custinne, M., & Paquot, M. (1982). Zeta potential of yeast cells: application in cell immobilization. *Enzyme and Microbial Technology*, *4*(3), 191–194. https://doi.org/10.1016/0141-0229(82)90116-8

Tien, C., & Payatakes, A. C. (1979). Advances in deep bed filtration. *AIChE Journal*, *25*(5), 737–759. https://doi.org/10.1002/aic.690250502

Tobiason, J. E., & O'Melia, C. R. (1988). Physicochemical Aspects of Particle Removal in Depth Filtration. *Journal (American Water Works Association)*, *80*(12), 54–64.

Tong, M., Ma, H., & Johnson, W. P. (2008). Funneling of Flow into Grain-to-grain Contacts Drives Colloid−Colloid Aggregation in the Presence of an Energy Barrier. *Environmental Science & Technology*, *42*(8), 2826–2832. https://doi.org/10.1021/es071888v

Torkzaban, S., & Bradford, S. A. (2016). Critical role of surface roughness on colloid retention and release in porous media. *Water Research*, *88*, 274–284. https://doi.org/10.1016/j.watres.2015.10.022

Treweek, G. P. (1979). Optimization of Flocculation Time Prior to Direct Filtration. *Journal (American Water Works Association)*, *71*(2), 96–101.

Tufenkji, N. (2006). Application of a dual deposition mode model to evaluate transport of Escherichia coli D21 in porous media. *Water Resources Research*, *42*(12), W12S11. https://doi.org/10.1029/2005WR004851

Tufenkji, N., Dixon, D. R., Considine, R., & Drummond, C. J. (2006a). Multi-scale Cryptosporidium/sand interactions in water treatment. *Water Research*, *40*(18), 3315–3331. https://doi.org/10.1016/j.watres.2006.07.036

Tufenkji, N., Dixon, D. R., Considine, R., & Drummond, C. J. (2006b). Multi-scale Cryptosporidium/sand interactions in water treatment. *Water Research*, *40*(18), 3315–3331. https://doi.org/10.1016/j.watres.2006.07.036

Tufenkji, N., & Elimelech, M. (2004a). Correlation Equation for Predicting Single-Collector Efficiency in Physicochemical Filtration in Saturated Porous Media. *Environmental Science & Technology*, *38*(2), 529–536. https://doi.org/10.1021/es034049r

Tufenkji, N., & Elimelech, M. (2004b). Deviation from the Classical Colloid Filtration Theory in the Presence of Repulsive DLVO Interactions. *Langmuir*, *20*(25), 10818–10828. https://doi.org/10.1021/la0486638

Tufenkji, N., & Elimelech, M. (2005a). Breakdown of Colloid Filtration Theory:? Role of the Secondary Energy Minimum and Surface Charge Heterogeneities. *Langmuir*, *21*(3), 841–852. https://doi.org/10.1021/la048102g

Tufenkji, N., & Elimelech, M. (2005b). Spatial Distributions of *Cryptosporidium* Oocysts in Porous Media: Evidence for Dual Mode Deposition. *Environmental Science & Technology*, *39*(10), 3620–3629. https://doi.org/10.1021/es048289y

Tufenkji, N., Miller, G. F., Ryan, J. N., Harvey, R. W., & Elimelech, M. (2004). Transport of Cryptosporidium Oocysts in Porous Media: Role of Straining and Physicochemical Filtration. *Environmental Science & Technology*, *38*(22), 5932–5938.

Tufenkji, N., Redman, J. A., & Elimelech, M. (2003). Interpreting Deposition Patterns of Microbial Particles in Laboratory-Scale Column Experiments. *Environmental Science & Technology*, *37*(3), 616–623. https://doi.org/10.1021/es025871i

Tufenkji, N., Ryan, J. N., & Elimelech, M. (2002). *Peer reviewed: The promise of bank filtration*. ACS Publications.

van Breemen, L. W. C. A., Ketelaars, H. A. M., Hoogenboezem, W., & Medema, G. (1998). Storage reservoirs – a first barrier for pathogenic micro-organisms in the Netherlands. *Water Science and Technology*, *37*(2), 253.

Van Oss, C. J. (1989). Energetics of cell-cell and cell-biopolymer interactions. *Cell Biophysics*, *14*(1), 1–16. https://doi.org/10.1007/BF02797387

Verberk, J. Q. J. C., O'Halloran, K. J., Hamilton, L. A., Vreeburg, J. H. G., & van Dijk, J. C. (2007). Measuring particles in drinking water transportation systems with particle counters. *Journal of Water Supply: Research and Technology - Aqua*, *56*(5), 345. https://doi.org/10.2166/aqua.2007.069

Walker, M., & Redelman, D. (2004). Detection of Cryptosporidium parvum in soil extracts. *Applied and Environmental Microbiology*, (3), 1827.

Wallis, P. M., Erlandsen, S. L., Isaac-Renton, J. L., Olson, M. E., Robertson, W. J., & Van Keulen, H. (1996). Prevalence of Giardia cysts and Cryptosporidium oocysts and characterization of Giardia spp. isolated from drinking water in Canada. *Applied and Environmental Microbiology*, *62*(8), 2789–2797.

Walshe, G. E., Pang, L., Flury, M., Close, M. E., & Flintoft, M. (2010). Effects of pH, ionic strength, dissolved organic matter, and flow rate on the co-transport of MS2 bacteriophages with kaolinite in gravel aquifer media. *Water Research*, *44*(4), 1255–1269. https://doi.org/10.1016/j.watres.2009.11.034

Wang, C., deKrafft, K. E., & Lin, W. (2012). Pt Nanoparticles@Photoactive Metal–Organic Frameworks: Efficient Hydrogen Evolution via Synergistic Photoexcitation and Electron Injection. *Journal of the American Chemical Society*, *134*(17), 7211–7214. https://doi.org/10.1021/ja300539p

Widerström, M., Schönning, C., Lilja, M., Lebbad, M., Ljung, T., Allestam, G., … Lindh, J. (2014). Large Outbreak of Cryptosporidium hominis Infection Transmitted through the Public Water Supply, Sweden. *Emerging Infectious Diseases*, *20*(4), 581–589. https://doi.org/10.3201/eid2004.121415

Xiao, L., & Ryan, U. M. (2004). Cryptosporidiosis: an update in molecular epidemiology. *Current Opinion in Infectious Diseases*, *17*(5). Retrieved from https://journals.lww.com/co-infectiousdiseases/Fulltext/2004/10000/Cryptosporidiosis__an_update_in_molecular.14.aspx

Xiao, L., Fayer, R., Ryan, U., & Upton, S. J. (2004). Cryptosporidium taxonomy: recent advances and implications for public health. *Clinical microbiology reviews*, *17*(1), 72-97.

Xie, B., Xu, Z., Guo, W., & Li, Q. (2008). Impact of Natural Organic Matter on the Physicochemical Properties of Aqueous C60 Nanoparticles. *Environmental Science & Technology*, *42*(8), 2853–2859. https://doi.org/10.1021/es702231g

Xu, L.-C., & Logan, B. E. (2006a). Adhesion forces between functionalized latex microspheres and protein-coated surfaces evaluated using colloid probe atomic

force microscopy. *Colloids and Surfaces B: Biointerfaces*, *48*(1), 84–94. https://doi.org/10.1016/j.colsurfb.2006.01.012

Xu, L.-C., & Logan, B. E. (2006b). Interaction Forces Measured Using AFM between Colloids and Surfaces Coated with Both Dextran and Protein. *Langmuir*, *22*(10), 4720–4727. https://doi.org/10.1021/la053443v

Yang, H., Kim, H., & Tong, M. (2012). Influence of humic acid on the transport behavior of bacteria in quartz sand. *Colloids and Surfaces B: Biointerfaces*, *91*, 122–129. https://doi.org/10.1016/j.colsurfb.2011.10.058

Yang, R., Murphy, C., Song, Y., Ng-Hublin, J., Estcourt, A., Hijjawi, N., … Ryan, U. (2013). Specific and quantitative detection and identification of Cryptosporidium hominis andC. parvum in clinical and environmental samples. *Experimental Parasitology*, *135*(1), 142–147. https://doi.org/10.1016/j.exppara.2013.06.014

Yao, K.-M., Habibian, M. T., & O'Melia, C. R. (1971). Water and waste water filtration. Concepts and applications. *Environmental Science & Technology*, *5*(11), 1105–1112.

Zhang, H. (2016). *Coupled effects on the transport and deposition of biocolloidal particles in saturated porous media*. University of Alberta. Retrieved from https://era.library.ualberta.ca/files/crx913p93z

Zhang, H., Seaman, J., Wang, Y., Zeng, H., Narain, R., Ulrich, A, & Liu., Y (2017a). Filtration of Glycoprotein-Modified Carboxylated Polystyrene Microspheres as Cryptosporidium Oocysts Surrogates: Effects of Flow Rate, Alum, and Humic Acid. Journal of Environmental Engineering, 143(8). https://doi.org/10.1061/(ASCE)EE.1943-7870.0001201

Zhang, Q., Davies, E. G. R., Bolton, J., & Liu, Y. (2017b). Monochloramine Loss Mechanisms in Tap Water. *Water Environment Research*, *89*(11), 1999–2005. https://doi.org/10.2175/106143017X14902968254421

Zhang, Q., Gaafar, M., Yang, R.-C., Ding, C., Davies, E. G. R., Bolton, J. R., & Liu, Y. (2017c). Field data analysis of active chlorine-containing stormwater samples. *Journal of Environmental Management*, *206*, 51–59. https://doi.org/10.1016/j.jenvman.2017.10.009

Zhao, W., Walker, S. L., Huang, Q., & Cai, P. (2014). Adhesion of bacterial pathogens to soil colloidal particles: Influences of cell type, natural organic matter, and solution chemistry. *Water Research*, *53*, 35–46. https://doi.org/10.1016/j.watres.2014.01.009

Zhou, Y. (2016). Improving the Understanding of Factors Influencing Cryptosporidium Removals Reported for Granular Media Filtration. Retrieved from https://uwspace.uwaterloo.ca/handle/10012/10122

Zhu, H., Smith, D. W., Zhou, H., & Stanley, S. J. (1996). Improving removal of turbidity causing materials by using polymers as a filter aid. *Water Research*, *30*(1), 103–114.

Zhu, T., Lawler, D. F., Chen, Y., & Lau, B. L. T. (2016). Effects of natural organic matter and sulfidation on the flocculation and filtration of silver nanoparticles. *Environ. Sci.: Nano*, *3*(6), 1436–1446. https://doi.org/10.1039/C6EN00266H

Appendix A. Calibration Curves of fluorescence spectrophotometer

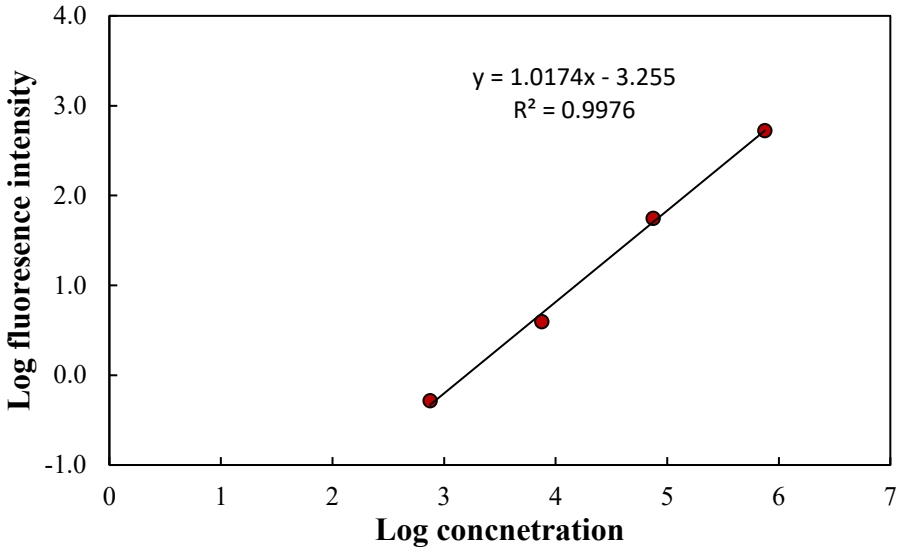


Figure A. Calibration curves of fluorescence spectrophotometer using multilevel concentration of copolymers-modified microspheres.

Appendix B. Statistical analysis for pilot direct filtration study

Table B-1. Statistical analysis of surrogate log removal.

Dependent Variable	**Filter configuration**		**Coagulant**		**Filter aid polymer**		**Filter aid polymer dose**	
Category	Regular bed	Deep bed	PACl	Alum	Polyamine LT-7981	PolyDADMAC LT-7995	0.5 mg/L	2.0 mg/L
Mean	3.275	3.688	3.475	3.488	2.888	4.075		
Variance	0.951	1.118	0.379	1.787	1.221	0.139		
Observations	8	8	8	8	8	8	8	8
Pearson Correlation	0.978		0.891		0.0857		0.382	
Hypothesized Mean Difference	0		0		0		0	
df	7		7		7		7	
T Stat	-5.083		-0.0423		-2.957		-2.420	
P(T<=t) one-tail	0.000713		0.484		0.0106		0.0230	
t critical one-tail	1.895		1.895		1.895		1.895	
P(T<=t) two-tail	**0.00143**		**0.967**		**0.0212**		**0.0461**	
t critical two-tail	2.365		2.365		2.365		2.365	

Table B-2. Statistical analysis of flow rate reduction in regular and deep bed columns.

Dependent variable	**Flow rate reduction**	
Category	In regular bed	In deep bed
Mean	6.025	1.063
Variance	7.325	0.774
Observations	8	8
Pearson Correlation		
Hypothesized Mean Difference	0	
df	7	
T Stat	4.787	
P(T<=t) one-tail	0.000998	
t critical one-tail	1.895	
P(T<=t) two-tail	**0.00120**	
t critical two-tail	2.365	

Appendix C. Calibration curves of on-line particle counters

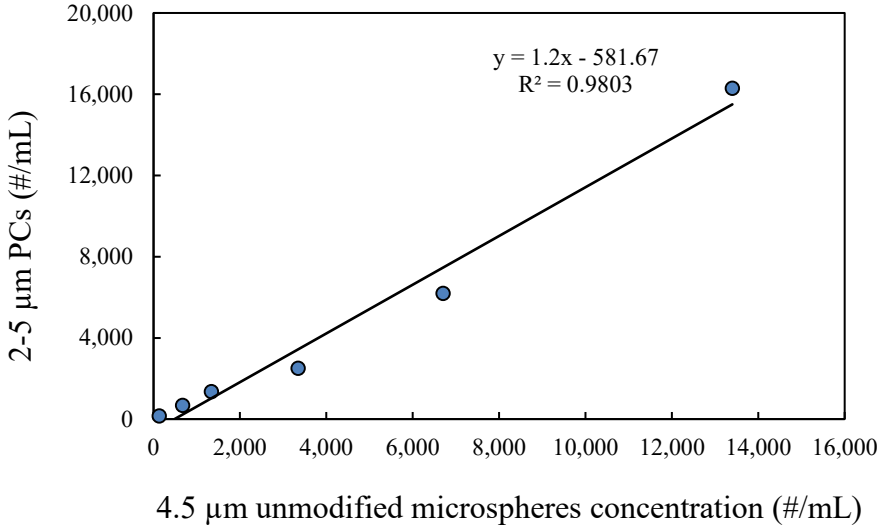


Figure C-1. Calibration curves of on-line particle counters using 4.3 µm unmodified polystyrene microspheres.

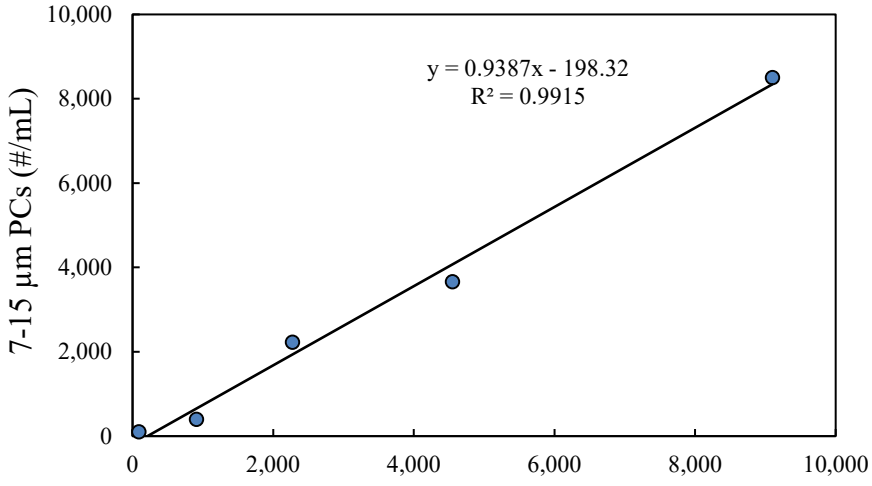


Figure C-2. Calibration curves of on-line particle counters using 10.0 µm unmodified polystyrene microspheres.

Appendix D. Particle counter responses to viable *Cryptosporidium* oocysts and *Giardia* cysts

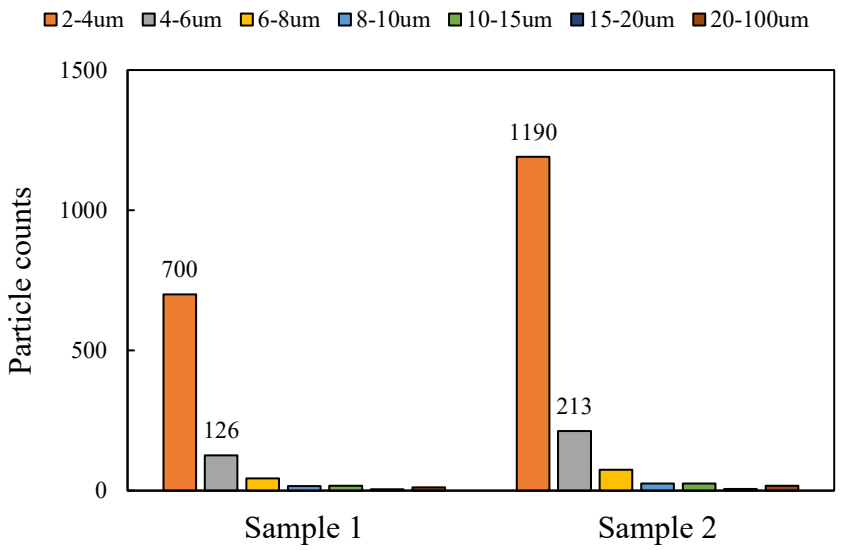


Figure D-1. Particle counts responses to viable *Cryptosporidium parvum* oocysts.

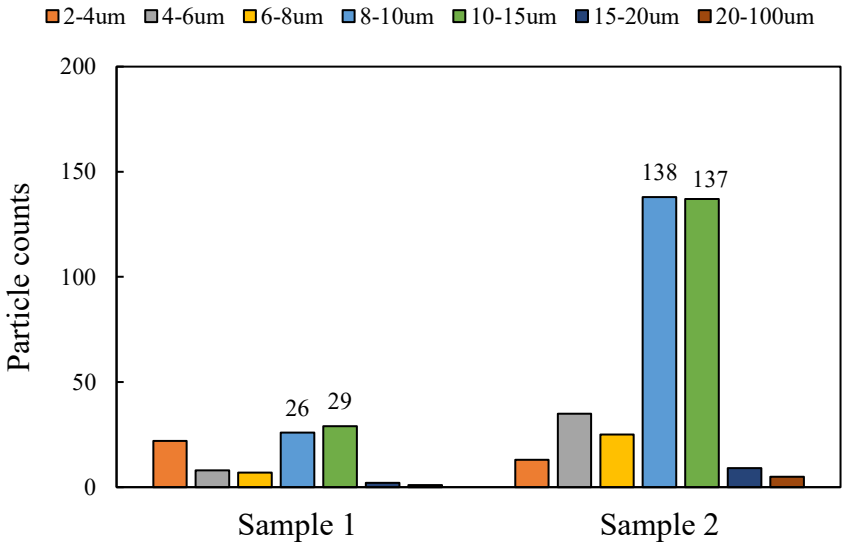


Figure D-2. Particle counter responses to viable *Giardia lamblia* cysts.