The Critical Role of Chemical Pre-Treatment in Ensuring *Cryptosporidium* Removal by Filtration of High Quality Source Water

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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ABSTRACT

Drinking water utilities reliant on surface water utilize chemically-assisted filtration (CAF) as a key barrier against the passage of protozoan pathogens, like *Cryptosporidium* spp. oocysts, to treated water. The goal of this work was to enable system-specific and potentially dynamic assessment of oocyst removal by CAF by using zeta potential as a tool for rapid operational feedback. Specifically, this work focused on systems utilizing high quality, low turbidity (typically <1 NTU) source water, with relatively low *C. parvum* oocyst concentrations and applied full scale coagulant doses (typically <5 mg/L). In these systems, the formation of settleable flocs is not a necessity because source water turbidities are already low and frequently meet treated water criteria. Rather, coagulation is used to enable particle removal through physico-chemical (i.e., chemically -assisted) filtration, as indicated by filter effluent turbidities that may or may not be indicative of optimal particle destabilization and removal by CAF. Accordingly, the identification of "optimal" coagulant doses can be challenging, and becomes even more challenging when process performance is being assessed, such as when *Cryptosporidium* oocysts are added to filter influents to evaluate their removal by CAF processes.

In Phase 1 of this work, the role of oocyst coagulation during CAF performance demonstrations was investigated. It was demonstrated that appropriate coagulation of oocyst seed suspensions is critical to reflecting "well-operated" CAF performance. A protocol for ensuring optimal coagulation of oocyst seed suspensions during such performance demonstrations was developed and demonstrated at pilot-scale. Here, zeta potential was useful in identifying the coagulant doses needed for maximal particle destabilization and removal by CAF. This pilot-scale approach was then validated using lower, environmentally relevant oocyst concentrations (and much longer pilot-scale investigations) during which the entire filtered volume of water was evaluated. Using this protocol, it was demonstrated that a minimum of 3-log oocyst removal could be achieved by CAF (essentially direct filtration) at a variety of operational conditions.

During Phase 2 of this work, the protocol developed in Phase 1 was used to evaluate oocyst passage through CAF processes with different filter designs (bed depths, water temperature) at various operational conditions (suboptimal coagulation, filter ripening, end-of-run operation, and hydraulic surges). Here, because of the high quality, low turbidity source water, adequate coagulation was the dominant control for risk, in contrast to many reported investigations in which more deteriorated source water was investigated and operational period within the filter cycle was a more dominant control over oocyst passage through the CAF process. Here, with the exception of suboptimal coagulation conditions, the pilot-scale filters consistently achieved $\geq$3-log *C. parvum* oocyst removal in an essentially directly filtration mode. Thus, this work demonstrated the critical importance of (1) appropriate particle destabilization by coagulation prior to CAF of low turbidity, low DOC source waters, (2) coagulation of oocysts prior to their addition to filter influent streams during CAF performance demonstrations, and (3) zeta potential as a useful tool for ensuring adequate particle destabilization in situations (i.e. treatment of low turbidity, low DOC source waters) in which extensive particle settling is not likely. In doing so, this work further highlights that *Cryptosporidium* spp. oocyst removal credits of >2.5 log may be warranted for "well-operated" direct filtration processes.

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DEDICATION

To the victims from the December 6th, 1989 École Polytechnique (de Montreal) Massacre

Anne-Marie Edward

Maud Haviernick

Maryse Laganière

Maryse Leclair

Geneviève Bergeron

Hélène Colgan

Nathalie Croteau

Barbara Daigneault

Annie Turcotte

Anne-Marie Lemay

Sonia Pelletier

Michèle Richard

Annie St-Arneault

Barbara Klucznik-Widajewicz

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS

C. parvum – *Cryptosporidium parvum*

CAF – Chemically Assisted Filtration

CDC – Centers for Disease Control Prevention

DOC – Dissolved Organic Carbon

END – End of run experiment

HLR – Hydraulic Loading Rate

HV – High Volume

IESWTR – Interim Enhanced Surface Water Treatment Rule

IFA – Immunofluorescence Assay

LT1ESWTR – USEPA Long Term 1 Enhanced Surface Water Treatment Rule

LT2ESWTR – USEPA Long Term 2 Enhanced Surface Water Treatment Rule

MCLG – Maximum Contaminant Level Goal

MID0 – Middle of run experiment under sub-optimal coagulation (0 mg alum/L in seed suspension)

MID40 – Middle of run experiment using 40 mg alum/L seed suspension

MID5 – Middle of run experiment under sub-optimal coagulation (5 mg alum/L in seed suspension)

NOM – Natural Organic Matter

NTU – Nephelometric Turbidity Units

P1 - 22.5a – Phase 1 Pilot-scale CAF Experiment, 22.5 mg alum/L (May 11th)

P1 - 22.5b – Phase 1 Pilot-scale CAF Experiment, 22.5 mg alum/L (Jun. 8th)

P1 - 40a – Phase 1 Pilot-scale CAF Experiment, 40 mg alum/L (Jun. 9th)

P1 - 40b – Phase 1 Pilot-scale CAF Experiment, 40 mg alum/L (Aug. 3rd)

P1 - 5 – Phase 1 Pilot-scale CAF Experiment, 5 mg alum/L (Aug. 2nd)

PZC –Point of Zero Charge

QMRA – Quantitative Microbial Risk Assessment

RIP – Ripening experiment

SCADA – Supervisory Control and Data Acquisition

SUR – Surge experiment

SUVA – Specific Ultraviolet Absorbance

SW – Settled Water

SW + O – Oocysts added to settled water

SW + O + Alum – Settled water with both oocysts and alum added

TOC – Total Organic Carbon

USEPA – United States Environmental Protection Agency

UV – Ultraviolet

WTP – Water Treatment Plant

Chapter 1 Introduction

Protozoan pathogens, especially *Cryptosporidium* spp., are a key driver of drinking water treatment infrastructure needs in North America. It has been estimated that these pathogens recently cost employers ~$10 million over a three-month period in at least one jurisdiction (Ridderstedt, Widerström, Lindh, & Lilja, 2017). Climate change is expected to exacerbate these risks due to increased frequency and intensity of extreme weather events, which have been linked to increased protozoan pathogen occurrence in source waters (Davies et al., 2004). Most drinking water utilities reliant on surface water in North America utilize chemically-assisted filtration (CAF) as a key barrier against the passage of protozoan pathogens into treated drinking water. Although UV irradiation offers an effective alternative to traditional disinfectants, it is significantly more expensive than widely used chlorination in both capital and operations and maintenance costs (Snicer, Malley, Margolin, & Hogan, 2000); thus, CAF remains a critical and required (MOEE, 2000; USEPA, 2006b) drinking water treatment process for managing protozoan pathogen health risks (Emelko, Huck, & Coffey, 2005; Ramsay, Wagner, Robertson, Smith, & Pollock, 2014).

Current regulations are treatment technique-driven and have necessitated performance demonstrations to quantify oocyst removal by specific treatment configurations or facilities. Performance demonstrations typically require sufficiently high initial concentrations of *Cryptosporidium* oocysts to enable calculation of their removal by treatment processes; thus, oocysts are often added to process influent streams and performance is assessed by evaluating the difference between influent and effluent concentrations, typically on a log basis. The majority reported demonstrations of *Cryptosporidium* oocyst removal by CAF have focused on systems treating relatively more deteriorated source water quality (Dugan, Fox, Owens, & Miltner, 2001; Emelko, 2003; Emelko, Huck, & Douglas, 2003; Huck, Coffey, Emelko, et al., 2002; Nieminski & Ongerth, 1995), rather than those treating high quality (defined herein as low turbidity [on average values are between 0.5 and 3.0 NTU] and low total organic carbon (TOC) [<2 mg/L]) source/raw water.

Systems treating high quality source water from surface-based sources often face unique operational challenges associated with coagulation. Conventional CAF comprised of coagulation, flocculation, and sedimentation if often employed in surface water treatment to receive 3-log credits for the treatment of *Cryptosporidium* spp. and *Giardia* spp. (USEPA, 1999). In these systems, the formation of settleable flocs is not a necessity, however, because source water turbidities are already low and frequently meet treated water criteria. Rather, coagulation is used to enable particle removal through physico-chemical (i.e., chemically -assisted) filtration, as indicated by filter effluent turbidities that may or may not be indicative of optimal particle destabilization and removal by CAF. Accordingly, the identification of "optimal" coagulant doses can be challenging, and becomes even more challenging when process performance is being assessed, such as when *Cryptosporidium* oocysts are added to filter influents to evaluate their removal by CAF processes.

1.1 Research Objectives

Information regarding *Cryptosporidium* oocyst removal by CAF is relatively scant for treatment systems in which low turbidity, low DOC source waters are typically treated by sweep floc coagulation prior to CAF. This information is critical because treatment performance in these high quality systems is likely the most vulnerable to relatively small shifts in source water quality and/or periods of non-ideal operation challenges (e.g., hydraulic surges) because of the associated need to rapidly adjust coagulant dosing. Accordingly, the overall goal of this research was to gain a better understanding of protozoan pathogen removal by CAF in systems treating low turbidity, low dissolved organic carbon (DOC) source water and to provide strategies for better treatment process evaluations and control. With specific application to low turbidity, low DOC source water, the specific objectives of this work were to:

1. Quantitatively evaluate *Cryptosporidium parvum (C. parvum)* oocyst removal by CAF;
2. Develop a protocol for conducting CAF performance demonstrations in which high concentrations of (oo)cysts are introduced to CAF influent streams to (a) enable quantitative evaluation of their removal and (b) adequately reflect optimal chemical pre-treatment (coagulation);

3. Evaluate filter design (depth) and operational (sub-optimal coagulation, ripening, hydraulic surges, etc.) effects on *C. parvum* oocyst passage through CAF to identify key process controls for ensuring optimal performance;
4. Evaluate the adequacy of support infrastructure/tools (i.e., turbidity, zeta potential analysis) for ensuring adequate protozoan pathogen removal by CAF in near-real-time; and,
5. Assess the validity of utilizing high oocyst concentrations in filtration performance demonstrations to quantify the removal of lower/more environmentally relevant oocyst concentrations by CAF.

1.2 Research Approach

While *C. parvum* oocyst removal by CAF has been widely investigated, very few studies have evaluated it in plants treating low turbidity and low DOC source water. To address this important knowledge gap, a two phase research program was devised to (1) develop strategies for conducted performance demonstrations in systems treating this type of source water and (2) evaluate design and operational effects on oocyst passage through CAF processes.

Phase 1 involved the development and validation of a seeding protocol in which *Cryptosporidium* oocysts could be added to the influent stream pilot-scale CAF processes to evaluate their removal in a manner reflecting optimal, "well operated" treatment of low turbidity, low DOC source water. To do this, a jar coagulation investigation was conducted to monitor turbidity and zeta potential during incremental additions of coagulant, aluminum sulfate (alum), to establish optimal particle destabilization and the coagulant dose required to achieve it. This test was followed by a series of pilot-scale CAF tests in which the dominant mechanisms of coagulation were investigated to validate the seeding protocol. To further validate the seeding protocol, a set of more environmentally relevant oocyst concentrations were seeded into the pilot filters over a longer duration using the Environmental Protection Agency (EPA) Method 1623 (USEPA, 2005). The development of this seeding protocol was critical to establishing the conditions under which pilot-scale CAF operation in an essentially direct filtration mode could be considered "well-operated". This protocol—and the capacity to identify appropriate particle (and oocyst) destabilization—was critical given that oocysts were being added to a low turbidity, low DOC source water, thereby

changing water quality substantially in most cases and potentially necessitating shifts in coagulant dosing for adequate particle destabilization.

During Phase 2, the oocyst seeding protocol developed during Phase 1 was used to evaluate oocyst passage through CAF processes with different filter designs (bed depths, water temperature) at various operational conditions (suboptimal coagulation, filter ripening, end-of-run operation, and hydraulic surges). During this phase, zeta potential analysis was further explored as a tool for ensuring optimal particle destabilization and oocyst removal during CAF.

1.3 Thesis Structure

The remainder of the thesis is divided into four chapters, a reference list, and a series of appendices. Chapter 2 provides background information related to the research objectives. It largely focuses on past research related to *Cryptosporidium* spp. oocyst removal by CAF. Following this, Chapter 3, outlines the general research approach including experimental development and rationale. The various methods used throughout the research and their development are described. Chapter 4 contains the results and discussion. Chapter 5, contains conclusions, implications, and recommendations.

Chapter 2 Background

2.1 Cryptosporidium spp.

Although *Cryptosporidium* spp. oocysts were first described in the early 1900's, cryptosporidiosis in humans was reported for the first time in the 1970's (Fayer, 1997). Since then, seventeen species of *Cryptosporidium* have been identified in humans worldwide; the most common species reported in humans are *C. parvum*, and *C. hominis* (Zahedi, Paparini, Jian, Robertson, & Ryan, 2016). While *C. parvum* infects a wide range of host species, *C. hominis* only infects humans (Zahedi et al., 2016). *Cryptosporidium* exists in two forms, the infectious stage inside a host and in an environmentally resistant stage known as an oocyst (i.e. the stage that is of interest to drinking water providers). Regardless of the species, oocysts are generally 4 to 6 μm in diameter and have a relatively low infectious dose that is believed to range from 10 to 30 oocysts (CDC, 2005).

2.1.1 Sources and Outbreaks of Waterborne Diseases

Outbreaks of cryptosporidiosis continue to occur globally (Baldursson & Karanis, 2011; Efstratiou, Ongerth, & Karanis, 2017; Karanis, Kourenti, & Smith, 2007) and have been reported in over 90 countries and on all continents populated by humans (Dillingham, Lima, & Guerrant, 2002; Fayer, Morgan, & Upton, 2000). Notably, the largest cryptosporidiosis outbreak in U.S. history occurred in 1993, in Milwaukee, WI affecting 25% of the population (406,000 individuals). In Canada, the largest recorded outbreak occurred in Kitchener-Waterloo, ON with 23,900 reported cases of cryptosporidiosis; remarkably, this outbreak occurred with no evidence of compromised treatment (Welker et al., 1994).

Prior to 2007, 325 water-associated outbreaks of cryptosporidiosis had been reported; the majority of these occurred in North America and Europe (Karanis et al., 2007). This is likely because more cases go unreported or misdiagnosed in jurisdiction with limited financial resources. Mahmoudi et al. (2017) reported on the prevalence of *Cryptosporidium* spp. occurrence and cryptosporidiosis in Asia, which experienced similar occurrences to other continents. Human infectious *Cryptosporidium* spp. are ubiquitous and increasingly being reported in jurisdictions outside of North America and Europe, underscoring the global need to identify strategies for protecting public health from associated outbreaks of waterborne disease.

2.1.1.1 Outbreaks of Waterborne Disease and Associated Implications for Society

Recently, drinking water outbreaks of disease in Europe, North America, and New Zealand from 2000 to 2014 were reviewed, confirming that the waterborne pathogenic protozoa *Cryptosporidium* spp. and *Giardia* spp. still pose a significant health risk, affecting more consumers than other pathogens; and underscoring that surface water supply contamination remains the leading cause of exposure (Moreira & Bondelind, 2017). Nonetheless, holistic assessments of the societal implications and costs of outbreaks of waterborne diseases such as cryptosporidiosis are relatively scant, though there are a few notable exceptions. A few of these key case studies are discussed below.

The well-known, 1993 Milwaukee cryptosporidiosis outbreak affected a very large number of individuals; specifically, approximately 403,000 of the 1.61 million residents who lived in the city (Davis et al., 1994) were affected by this outbreak. In this case, the source of this outbreak was a deficiency in one of the two drinking water treatment facilities in which CAF treatment performance was inadequate, thereby resulting in *Cryptosporidium* oocyst passage into the treated drinking water supply (Davis et al., 1994). Corso et al. (2003) reported losses due to medical expenses and non-medical expenses (productivity losses) after this outbreak; costs due to litigation, bottled water purchases, and government involvement were included in that assessment. Specifically, they concluded that the total cost of medical care and productivity losses resulting from the outbreak was approximately $96.2 million (USD), based on $31.7 million in medical costs and $64.6 million in productivity losses (i.e., 67% of the total cost of the outbreak). Notably, although only 1% of those infected had severe cases of disease that required hospitalization, these cases accounted for 74% of the total medical costs.

A recent retrospective study in Sweden demonstrated that cryptosporidiosis-related absences from work per sick child shared between parents/guardians over a three-month period in 2010 resulted in an estimated direct cost of €7 million (~$10 million CDN) for employers (Ridderstedt et al., 2017). This assessment accounted for adults who took sick leave due to their own symptoms as well as those who took sick leave to care for children. Notably, adults who took sick days to care for sick children accounted for 25% of the sick days taken by working adults. It was estimated that 45% of the population of 60,000 was affected by an outbreak of *Cryptosporidium hominis*

(Widerström et al., 2014). Here, young age, number of infected family members, amount of water consumed daily, and gluten intolerance were identified as key risk factors associated with illness; importantly, insufficient drinking water treatment was implicated in this outbreak of waterborne disease. The implication of drinking water treatment here further underscores the importance of ensuring that assessments that conclude that "well-operated" drinking water treatment is being practiced, and adequately reflects adequate particle (and therefore protozoan pathogen) destabilization for effect removal during CAF treatment when these widely-implemented processes are implemented.

Unlike other earlier economic analyses of large cryptosporidiosis outbreaks, Chyzheuskaya et al. (2017) included a multitude of costs from both the private and public sectors from the cryptosporidiosis outbreak in Galway, Ireland. This study included costs that are a direct impact from the 242 confirmed cases and includes costs faced by other groups like those in the affected population from the surrounding area that had boiled water advisories over a 158 day period. The total cost of the Galway, Ireland outbreak exceeded €19 million (Chyzheuskaya et al., 2017).

Finally, Adam et al. (2017) reported that in 2014 American insurance covered approximately $1 million (USD) worth of expenses to treat cryptosporidiosis nationwide. The CDC (2010) reported that between 2004 and 2007, the annual cost for hospitalisations in the U.S. caused by cryptosporidiosis totalled $37-145 million. This estimate included the administrative cost to the U.S. government through the delivery of programs like Medicare and Medicaid. These reports underscore that waterborne disease attributable to pathogenic protozoa, and cryptosporidiosis specifically, remains a persistent, 21st century threat to public health that drives the need for a better understanding of treatment options and real- or near-real-time assessments of treatment performance in managing these risks.

2.2 Regulatory Policy Approaches to Managing Risks of Waterborne Diseases Attributable to *Cryptosporidium* spp.

Given the health effects associated with exposure to pathogenic protozoa such as *Cryptosporidium* spp., health-based treatment goals of a minimum 3.0-log removal and/or inactivation of cysts and oocysts are typically implemented in Canada (Health Canada, 2012) and the United States (USEPA, 2006b), as well as other jurisdictions (MOECC, 2016). In general, source water quality

is characterized and then pathogen removal/treatment targets are established to achieve safe finished drinking water quality. Risk assessment approaches that rely upon concepts of maximum acceptable risk levels have been developed to manage these risks (Health Canada, 2012; USEPA, 2006b). Although quite variable across different jurisdictions for ground water supplies, regulatory policy approaches to managing risks of waterborne diseases attributable to pathogenic protozoa such as *Cryptosporidium* spp. in surface water supplies are generally similar across jurisdictions, especially Canada and the United States.

In general, risks to public health attributable to waterborne pathogens in surface water-based drinking water supplies are managed using a combination of treatment strategies; specifically, pathogen removal by physico-chemical treatment processes and inactivation by disinfection processes (Health Canada, 2012; USEPA, 2006b). Further protection of public health from waterborne bacteria and viruses (but not protozoa) is achieved by maintaining an adequate concentration of disinfectant residual throughout the distribution system (Health Canada, 2012; USEPA, 2006b). Although UV irradiation offers effective disinfection of protozoa, it is significantly more expensive in both capital and operations and maintenance costs than widely used chlorination (Snicer et al., 2000); moreover, most surface waters also require removal of turbidity/suspended solids for efficient implementation of disinfection; thus, CAF remains a critical and required (MOEE, 2000; USEPA, 2006a) barrier to protozoan pathogen passage into treated drinking water supplies (Emelko et al., 2005; Ramsay et al., 2014). Accordingly, most drinking water utilities reliant on surface water in North America utilize CAF or equivalent treatment for managing *Cryptosporidium* spp. risks.

Quantitative microbial risk assessment (QMRA) approaches have been developed to better manage these risks, and although they are increasingly used, they are also continuously evolving (Health Canada, 2012; Schmidt & Emelko, 2011). In Canada and the United States, risks from *Cryptosporidium* spp. (and other pathogens) are managed and regulated using a "treatment technique" based approach in which oocyst treatment (i.e., removal or disinfection) credits are allocated for implementation of specific treatment infrastructure and evidence of "well-operated" treatment (e.g., achieving specified treated water quality targets). These frameworks rely on the identification of critical control points that represent a point, step, or procedure at which control can be applied and, as a result, a water safety hazard can be prevented, eliminated, or reduced to

an acceptable level (WHO, 2017). Although an exhaustive review of these continuously evolving frameworks and the associated regulatory policies is beyond the scope of the present investigation, a brief overview of Canadian and U.S. regulatory policies and associated guidelines is provided below.

2.3 Regulatory Framework in North America for Managing Waterborne Diseases

The need for approaches that deliver reliable quantitative data that are representative of full-scale *Cryptosporidium* oocyst removal through CAF treatment processes is rooted in American and Canadian regulatory frameworks focused on protecting public health by ensuring adequately removal of protozoan pathogens during drinking water treatment using a treatment technique-based approach, rather than requiring cost-prohibitive monitoring. These frameworks are discussed in brief below.

2.3.1 Canadian Federal Guidelines

Health Canada publishes the Guidelines for Canadian Drinking Water Quality, which are reviewed on a regular basis. The guidance document entitled "Enteric Protozoa: *Giardia* and *Cryptosporidium*" is a component of those guidelines (Health Canada, 2012). It discusses and details treatment technique driven approaches for managing risks of waterborne diseases attributable to pathogens. As mentioned above, treatment credits awarded for implementation of specific treatment infrastructure and evidence of "well-operated" treatment (e.g., achieving specified treated water quality targets). Different process configurations receive different treatment credits that are prescribed based on general consensus of the research and practitioner communities; thus, an expert-system type of approach is utilized. For example, for the treatment of *Cryptosporidium* spp., "well operated" conventional treatment (i.e., coagulation, flocculation, and clarification followed by CAF) receives 3-log (99.9% removal) treatment credit, whereas direct filtration (i.e., only coagulation and flocculation followed by CAF) receives only 2.5-log (99.7% removal) treatment credit.

Oocyst (and cyst) removal requirements are based on the source water quality; as source water oocyst concentrations increase beyond 10 oocysts/100 L (on a log scale), the required levels of treatment increases proportionally (Health Canada, 2017). Importantly, the treatment credits assigned to CAF filtration processes are largely based on pilot- and full-scale performance

demonstrations reported in the 1990's and early 2000's in which highly variable oocyst concentration, purification, and enumeration techniques were used, and often poor surrogate parameters were relied upon to avoid expensive and laborious enumeration of oocysts (Dugan et al., 2001; Dugan & Williams, 2004; Emelko, 2003; Emelko et al., 2005, 2003; Huck, Coffey, Anderson, et al., 2002; Huck, Coffey, Emelko, et al., 2002; Nieminski & Ongerth, 1995; Ongerth & Pecoraro, 1995; Xagoraraki, Harrington, Assavasilavasukul, & Standridge, 2004a). If required, additional credits can be achieved by reaching specific treated water quality targets (e.g., 0.1 NTU 95^{th} percentile turbidity in combined filter effluents) beyond those that are required (e.g., maximum combined filter effluent turbidity for conventional and direct filtration cannot exceed 0.3 NTU in at least 95% of measurements collected in any given month, with no one measurement exceeding 1 NTU [Health Canada, 2017]) or implementation of additional disinfection processes such as UV irradiation (Health Canada, 2012).

2.3.2 Ontario Provincial Regulations

In Ontario, regulatory requirements for *Cryptosporidium* spp. oocyst removal and disinfection are described in the Procedure for Disinfection of Drinking Water in Ontario (MOECC, 2016). Consistent with the federal guidelines, the province of Ontario uses a removal-based credit system to evaluate drinking water treatment compliance in achieving public health protection goals associated with managing health risks attributable to waterborne *Cryptosporidium* spp. It should be highlighted that Ontario is the only province in Canada that deviates from Health Canada's recommended guideline of achieving a minimum of 3-log treatment of both *Cryptosporidium* spp. and *Giardia* spp. for conventional filtration (Health Canada, 2017). While Ontario uses this same framework for *Giardia* spp., a minimum of only 2-log treatment is required for *Cryptosporidium* spp.; consistent with this, well-operated conventional CAF processes are only awarded 2-log treatment credit (MOECC, 2016). Direct filtration is also awarded 2-log treatment credit by the provincial regulation. The policies specify that treatment (i.e., removal and/or disinfection) requirements may be increased in situations of "higher than typical" source water oocyst or cyst concentrations; for example, in the case of intakes that are exposed to agricultural runoff or wastewater treatment plant discharges (MOECC, 2016).

2.3.3 U.S. Policies

In December 1998, the United States Environmental Agency (USEPA) finalized the Interim Enhanced Surface Water Treatment Rule (IESWTR) that set *Cryptosporidium* treatment standards (USEPA, 1999). To limit public exposure to pathogenic protozoa in treated drinking water, the Rule outlined a maximum contaminant level goal of zero *Cryptosporidium* oocysts and a minimum of 2-log oocyst removal to be achieved by CAF processes. To receive this 2-log treatment credit, the maximum combined filter effluent turbidity could not exceed 0.3 NTU in at least 95% of measurements collected in any given month, with no one measurement exceeding 1 NTU (USEPA, 1999).

In January 2002, the USEPA released the Long-Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR). This was created to supplement the IESWTR by focusing on public water systems serving fewer than 10,000 persons, which were previously not regulated under the SWTR (which addressed only on *Giardia* spp. and preceded the IESWTR). The LT2ESWTR was enacted in 2006 to address risks from *Cryptosporidium* spp. and provide greater clarity in defining higher risk systems and associated treatment requirements (i.e., required treatment credits) and identifying approaches for achieving additional treatment credits. The LT2ESWTR relied upon source water *Cryptosporidium* spp. monitoring that was required as part of the 2001 Information Collection Rule (USEPA, 2001) to identify whether or not additional treatment of *Cryptosporidium* spp. was required. A “bin” approach was developed; it is presented in Table 2-1 and Table 2-2. Similar to the Canadian guidelines and regulations developed by Health Canada (Health Canada, 2017), higher source water concentrations of *Cryptosporidium* spp. require more extensive treatment.

Table 2-1. Bin Classification System used by the USEPA in the LT2ESWTR (USEPA, 2006a)

For systems that are:	Mean *Cryptosporidium* Concentration*	Bin Classification
…required to monitor for *Cryptosporidium*	< 0.075 oocysts/L	Bin 1
	From 0.075 to 1.0 oocysts/L	Bin 2
	From 1.0 to 3.0 oocysts/L	Bin 3
	≥ 3.0 oocysts/L	Bin 4

*Samples must be analyzed by an approved laboratory and use USEPA Method 1622 or 1623

Table 2-2. Additional Treatment Requirements for Filtered Systems used by the USEPA in the LT2ESWTR (USEPA, 2006a)

	If the system uses the following filtration in full compliance with existing requirements, then the additional *Cryptosporidium* treatment requirements are…			
Bin Classification	Conventional filtration treatment (including softening)	Direct Filtration	Slow sand or diatomaceous earth filtration	Alternative filtration technologies
Bin 1	No additional treatment	No additional treatment	No additional treatment	No additional treatment
Bin 2	1-log treatment	1.5-log treatment	1-log treatment	(1)
Bin 3	2-log treatment	2.5-log treatment	2-log treatment	(2)
Bin 4	2.5-log treatment	3-log treatment	2.5-log treatment	(3)

(1) As determined by the state such that the total *Cryptosporidium* removal and inactivation is at least 4.0-log
(2) As determined by the state such that the total *Cryptosporidium* removal and inactivation is at least 5.0-log
(3) As determined by the state such that the total *Cryptosporidium* removal and inactivation is at least 5.5-log

2.3.3.1 Standardized Methods of *Cryptosporidium* spp. Detection

Analytical methods for quantification of *Cryptosporidium* spp. oocysts from water were originally developed from those for *Giardia* spp.; to date, they remain unreliable (i.e. with widely variable recovery), laborious, and expensive. In brief, these methods typically require three steps:

concentration, purification, and enumeration. In 1999, USEPA Method 1623 was published to serve as a standard method for enumerating *Cryptosporidium* spp. and *Giardia* spp. in natural waters (USEPA, 2005). In brief, it involves filtration of relatively large volumes of water, immunomagnetic separation, an immunofluorescence assay (IFA) for identification, and manual enumeration using fluorescence microscopy. Quintero-Betancourt et al. (2002) reviewed laboratory methods for detection and enumeration of *Cryptosporidium* spp. oocysts and noted that the method recovery efficiency ranges from 12-93%, which generally falls within the 21-100% range specified within USEPA's acceptance criteria (USEPA, 2005). However, other techniques that involve filtration of smaller sample volumes directly on membrane filters, IFA, and direct enumeration using epifluorescence microscopy have been widely used in performance demonstrations (Emelko et al., 2003; Hansen & Ongerth, 1991; Huck, Coffey, Emelko, et al., 2002; Lalancette, Di Giovanni, & Prévost, 2010; Rochelle, Johnson, Leon, & Di Giovanni, 2012). The sample volumes processed using these methods can be orders of magnitude smaller than those processed using standard methods such USEPA Method 1623.1 (USEPA, 2005) thereby allowing for more samples to be processed in a shorter amount of time. Importantly, for pilot-scale performance demonstrations in which large numbers of oocysts are added to treatment process influent streams, more expensive methods developed to enable the processing of large sample volumes (e.g. Method 1623.1) are not needed.

2.4 Drinking Water Treatment

Many aspects of drinking water source quality affect treatment design and operations; these include alkalinity, pH, turbidity, natural organic matter (NOM; typically described by total organic carbon (TOC) or dissolved organic carbon (DOC), temperature, colour, and hardness (Crittenden, Trussell, Hand, Howe, & Tchobanoglous, 2012; Edzwald, 2011). It is important to note, however, that turbidity, an indicator of suspended solids, and NOM are the two main aspects of water quality that govern the need to implement certain treatment infrastructure; specifically, CAF or equivalent treatment processes (Crittenden et al., 2012; Edzwald, 1993; Pernitsky & Edzwald, 2006; Van Benschoten & Edzwald, 1990). Critically, aromatic fractions of NOM exert greater coagulant demand than less aromatic or non-aromatic fractions (Crittenden et al., 2012; Edzwald, 2011).

As landscapes change, whether from urban landscape developments, changing industrial practices, or climate change-associated disturbances (e.g., floods, wildfires), source water quality deteriorates, and turbidity and NOM levels are increasingly elevated and more variable (Tufenkji & Emelko, 2011). Thus, source water protection and implementation of resilient water treatment infrastructure and operations are increasingly imperative. These challenges underscore the need to develop tools to better signal periods of higher/lower risk during the provision of potable water, thereby enabling operator responsiveness, more resilient treatment, and enhanced protection of public health. Important aspects of drinking water treatment that affect the removal of particles, and more specifically pathogens are discussed below, especially as they relate to treatment of high quality (low turbidity, low DOC) source water supplies. Finally, key knowledge gaps are highlighted and opportunities to address these gaps are identified—these form the rationale for the research reported in this thesis.

2.4.1 Coagulation Regimes and Physico-chemical Filtration

Coagulation is a vital component of conventional drinking water treatment and has been widely identified as an integral contributor to *Cryptosporidium parvum* oocyst removal by CAF (Barkay-Arbel et al., 2012; Brown & Emelko, 2009; Dai & Hozalski, 2002; James K. Edzwald & Kelley, 1998; Emelko, 2003; Huck et al., 2002; Keegan et al., 2008; Logsdon, 2000; Nieminski & Ongerth, 1995; Ongerth & Pecoraro, 1995; Shaw et al., 2000; Torabian et al., 2008). There are two primary mechanisms of metal salt (e.g., aluminum sulfate [alum], ferric chloride, etc.) coagulation: (1) adsorption and charge neutralization and (2) sweep coagulation (Amirtharajah & Mills, 1982; Lartiges et al., 1997). As described by Pernitsky & Edzwald (2006) and Benjamin & Lawler (2012), colloids and nanoparticles are destabilized by stoichiometric adsorption of charged chemical species (e.g., hydrolyzed species of Al(III) and Fe(III)) that carry a charge opposite to that of the surface of the particle (which is typically negative in natural waters), thereby reducing the surface potential (decreasing repulsion forces) and neutralizing the surface charge. Thus, an overdose of adsorbable species can cause reversal of charge on the particle. In contrast, sweep coagulation involves precipitation of metal hydroxides and enmeshment of particles in those precipitates. In source waters containing low solids concentration, these precipitates also increase contact opportunities for flocculation and/or attachment to surfaces such as filter media. Amirtharajah & Mills (1982) analyzed numerous coagulation investigations and developed a

solubility diagram for aluminum hydroxide illustrates the regions of pH and alum dosage that correspond to the dominant mechanisms of alum coagulation—this figure is reproduced in Figure 2-1 below. Johnson and Amirtharajah (1983) produced a similar diagram for ferric iron.

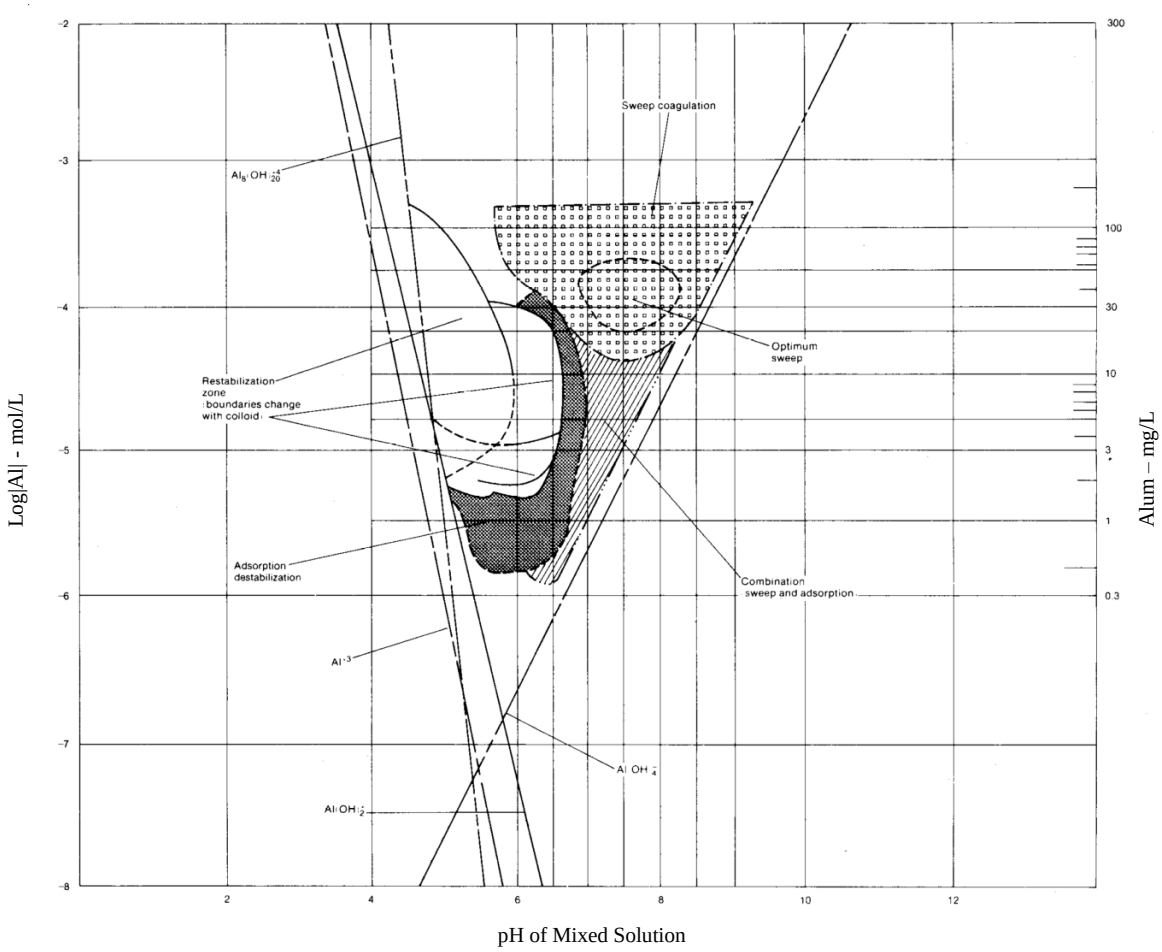


Figure 2-1. Design and operation diagram for alum coagulation (From Amirtharajah & Millls, Journal – American Water Works Association, Copyright © 1982 by John Wiley & Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.)

The relevance of these mechanisms for achieving effective coagulation and physico-chemical filtration (i.e., CAF) has been widely reported (Bustamante, Shanker, Pashley, & Karaman, 2006; Butkus, Bays, & Labare, 2003; Ghernaout, 2015; Xagoraraki & Harrington, 2004). Indeed, these mechanisms enable physico-chemical filtration (which is not a size exclusion process) because they create the conditions that destabilize particles, thereby creating conditions that are

thermodynamically favorable for particle attachment on filter media surfaces. While these approaches offer a useful starting point for determining approximate coagulant dosages that result in different coagulation mechanisms at a given pH, it should be noted that humic and other organic substances (i.e., NOMs) can act as complexing ligands that can lead to higher concentrations of soluble aluminum complexes than those that would be predicted by theoretical solubility (Driscoll & Letterman, 1987).

2.5 Surface Charge

The surface charge of colloidal particles and surfaces is described by zeta potential, which is theoretically defined as "the difference in electric potential at the shear plane and the bulk liquid". Zeta potential is not directly measured; rather, electrophoretic mobility is measured and zeta potential is back calculated using the Smoluchowski equation (McTigue & Symons, 2010):

$$EPM = \left(\frac{\varepsilon_\omega}{\mu}\right)\zeta \tag{1}$$

in which EPM is the electrophoretic mobility, or the velocity of a particle excited by a known electric field (m/s), ε_ω is the dielectric constant of water (F/m), μ is the dynamic viscosity of water (m^2/s), and ζ is the zeta potential (mV). As would be expected, alum concentration, zeta potential, and the removal of *Cryptosporidium parvum* oocyst (or any other particle) are inextricably linked (Bean et al., 1964; Cleasby et al., 1963; Gupta et al., 1973; Neuman, 1981; Riddick, 1961; Xagoraraki & Harrington, 2004). Of course, the proper interpretation of zeta potential data is critical (Neuman, 1981) and must be considered within the broader context of coagulation regime (discussed above) and its relationship to the treatment technology being deployed.

As would be expected, optimal coagulation and CAF occur when negatively charged particles are destabilized such that the zeta potential remains negative and is within a few millivolts of the point of zero charge (PZC) (Bean et al., 1964; Bustamante et al., 2006; Cleasby et al., 1963; Ghernaout, 2015; Gupta et al., 1973; Karaman, Pashley, Bustamante, & Shanker, 1999; Neuman, 1981; Riddick, 1961; Xagoraraki & Harrington, 2004; Xu, Fitzpatrick, & Deng, 2006). It has been suggested that ± 4 mV of the PZC is ideal (Xagoraraki & Harrington, 2004). Properly understanding the use of zeta potential was critical to interpreting and comparing results from this research by providing a range and understanding of zeta potential.

2.6 CAF Performance Demonstrations

Performance demonstrations are conducted to quantitatively and reproducibly demonstrate pathogen removal achieved by treatment processes such as CAF. During these demonstrations, inactivated oocysts are added to unit process influent streams; this typically occurs at either the raw water or filter influent points within the treatment process. In some studies, oocyst seed suspensions were coagulated to destabilize the oocysts prior to their introduction to treatment process influent streams (Amburgey et al., 2005; Emelko, 2001, 2003, Emelko et al., 2005, 2003; Huck, Coffey, Anderson, et al., 2002; Huck, Coffey, Emelko, et al., 2002; Scott, 2008); in others, oocysts introduced to treatment process influent streams were not destabilized by coagulation (Dugan et al., 2001; Dugan & Williams, 2004; Nieminski & Ongerth, 1995; Ongerth & Pecoraro, 1995; Swertfeger, Metz, DeMarco, Braghetta, & Jacangelo, 1999; Tomko & Scheuring, 2002; Xagoraraki, Harrington, Assavasilavasukul, & Standridge, 2004b). Notably, in most of the cases in which oocyst seed suspensions were not pre-coagulated prior to being introduced to treatment process influent streams, they were added prior to the coagulation stage of treatment; thus, they were coagulated during the process evaluations (Dugan et al., 2001; Dugan & Williams, 2004; Nieminski & Ongerth, 1995; Ongerth & Pecoraro, 1995; Swertfeger et al., 1999; Tomko & Scheuring, 2002; Xagoraraki et al., 2004b).

The biggest challenge associated with conducting performance demonstrations in this latter manner is that they are often infeasible because of the high concentrations of oocysts introduced to the raw water in order to demonstrate $\geq$3-log removal of oocysts. This results in a sufficiently high, non-zero number of oocysts in the filter effluent stream (at least 10, as suggested by Emelko et al.,(2008)) so that treatment efficiency can actually be calculated without several orders of magnitude of uncertainty in the estimates. Moreover, this type of analysis would also require the analysis of very large volumes of filter effluent, likely hundreds to thousands of liters, depending on the type and scale of processes being evaluated. The direct addition of oocysts to filter influent streams enables these challenges to be avoided; however, this type of approach also requires appropriate oocyst destabilization. At present, no guidance for achieving oocyst destabilization in seed suspensions during such performance demonstrations is available. As discussed above, all of the performance demonstration reported in the literature to date have utilized full-scale plant coagulant doses in such cases.

2.7 High Quality Source Water

Consistently “high quality” source water can be described as having low turbidity and low TOC/DOC; it is not typically influenced by seasonal fluctuations such as run-off of snowmelt/spring freshet. Plants that treat such source waters typically use low coagulant doses. "Low” turbidity source water is low in suspended solids and has been widely “defined” as 1 NTU or less (Al-Ani, Hendricks, Logsdon, & Hibler, 1986; Brink, Hendricks, & Al-Ani, 1988) though ranges of 0.5 to 5 NTU (Ongerth & Pecoraro, 1995) or 3 NTU or less (Bustamante et al., 2006) also have been suggested. Scott (2008) referred to low turbidity source water when using Lake Ontario water that averaged 0.29 NTU and ranged between 0.06 and 2.97 NTU—this is consistent with Masher & Hendricks (1986) whose source water turbidity ranged from 0.43 to 1.46 NTU in their low turbidity source. Similarly, Zhou (2016) provided a structure for categorizing TOC/DOC. Low TOC water was defined as generally less than 2 mg/L and medium TOC water was described as 2 to 5 mg/L, with >5 mg/L of DOC being described as high TOC. This is contradicted by Edzwald (1993) who describes a system with 30 NTU as low turbidity. All of these ranges are summarized in Table 2-3.

Table 2-3. Publications Referencing Low Turbidity Source Water

Publication	Definition of “low turbidity”
(Edzwald, 1993)	30 NTU
(Brink et al., 1988)	≤ 1 NTU
(Al-Ani et al., 1986)	< 1 NTU
(Ongerth & Pecoraro, 1995)	0.5 to 5 NTU
(Bustamante et al., 2006)	< 3 NTU
(Scott, 2008)	0.06 to 2.97 NTU (0.29 NTU, average)
(Masher & Hendricks, 1986)	0.43 to 1.46 NTU

Regardless of the exact values that are used to define "high quality," "low turbidity," or "low TOC/DOC" source water, it is critical to note that the efficacy of CAF processes is typically solely indicated by achieving filter effluent turbidities below a specified value, typically <0.3 NTU or <0.1 NTU as discussed above in Section 2.3.1. However, when source water turbidity is already near this value and very little NOM is present to exert coagulant demand, it is possible that the achievement of low filter effluent turbidity may not be indicative of or attributable to adequate or optimal particle destabilization during chemical pre-treatment (i.e., coagulation). While the potential for such a scenario is speculative, it would explain why very low *C. parvum* oocyst reductions have been observed during the few available performance demonstrations in which oocysts were added to filter influent streams in which low turbidity, low DOC source water was being treated. Although the addition of oocyst seed suspensions effectively changed filter influent water quality, the seed suspensions were only coagulated at the full-scale plant coagulant dose that was optimized for the matrix prior to oocyst addition or not coagulated at all (Amburgey et al., 2005; Emelko, 2001, 2003, Emelko et al., 2005, 2003; Huck, Coffey, Anderson, et al., 2002; Huck, Coffey, Emelko, et al., 2002; Scott, 2008).

2.8 Key Knowledge Gaps

While well-operated CAF remains a universally recognized, critical treatment process for removing protozoan pathogens from drinking water (Emelko et al., 2005; Ramsay et al., 2014); traditional filtered water monitoring strategies are ineffective at ensuring pathogen removal because:

1. They do not broadly reflect operational capacity and resilience;
2. Methods for the identification and enumeration of *Cryptosporidium* spp. and *Giardia* spp. are not available in real time and fraught with uncertainty, are expensive and extremely time consuming (Health Canada, 2012; Ryan & Hijjawi, 2015); and,
3. No reliable surrogates for (oo)cyst removal by filtration exist (Headd & Bradford, 2015; Payment, Plante, & Cejka, 2001; Tufenkji & Emelko, 2011).

Critically, as discussed above, when CAF performance is sub-optimal, disease outbreaks (continue to) occur (Moreira & Bondelind, 2017), resulting in significant and often undocumented economic burden to society (Ridderstedt et al., 2017). Thus, a key knowledge gap in the water industry is

that tools that better support a CCP-based approach for ensuring protozoan pathogen removal by CAF are still needed.

While monitoring of oocysts and cysts in CAF effluents is neither cost-effective nor possible in real-time, tools for signaling higher/lower risk periods for oocyst and/or cyst passage through CAF are likely available. Online turbidity and newly available online zeta potential analysis offer promise for real-time treatment and CAF performance optimization—the value of online zeta potential analysis was recently demonstrated during the 2013 Calgary flood (Kundert, Emelko, Mielke, Elford, & Ruecker, 2014) and the 2016 Horse River wildfire (Fort McMurray, AB) (Silins & Emelko, 2017). In combination with design and operational factors (e.g. backwash staggering), these tools offer a significant opportunity to address the knowledge gap identified above and develop improved CCP-based approaches for ensuring protozoan removal by CAF. This thesis research endeavors to contribute to addressing that goal.

Chapter 3 Materials and Methods

3.1 General Research Approach

Pilot-scale CAF experiments were conducted at the R.C. Harris Water Treatment Plant in the City of Toronto, Ontario, Canada. Phase 1 of this research involved the development and validation of a seeding protocol in which *Cryptosporidium* oocysts could be added to the influent stream pilot-scale CAF processes to evaluate their removal in a manner reflecting optimal, "well operated" treatment of low turbidity, low DOC source water. To do this, a jar coagulation investigation was conducted to monitor turbidity and zeta potential during incremental additions of coagulant, aluminum sulfate (alum), to establish optimal particle destabilization and the coagulant dose required to achieve it. This test was followed by a series of pilot-scale CAF tests in which the dominant mechanisms of coagulation were investigated to validate the seeding protocol. To further validate the seeding protocol, a set of more environmentally relevant oocyst concentrations were seeded into the pilot filters over a longer duration using the Environmental Protection Agency (EPA) Method 1623.1 (USEPA, 2005).

The development of this seeding protocol was critical to establishing the conditions under which pilot-scale CAF operation in an essentially direct filtration mode could be considered "well-operated". This protocol—and the capacity to identify appropriate particle (and oocyst) destabilization—was further critical given that oocysts were being added to a low turbidity, low DOC source water, thereby changing water quality substantially in most cases and potentially necessitating shifts in coagulant dosing for adequate particle destabilization. Three sets of experiments were conducted during Phase 1. These were:

1. **Jar coagulation experiments** were conducted to evaluate the extent of *C. parvum* oocyst destabilization achieved by coagulation to ensure that the developed experimental approach represented "well-operated" chemical pre-treatment of the low turbidity, low DOC source waters in which oocyst addition could change source water quality substantially;
2. **Pilot-scale CAF experiments** were conducted to demonstrate that (1) sufficient chemical pre-treatment/coagulation of oocysts (or any other particles) is required to effectively neutralize/destabilize their surface charge during performance demonstrations

in order to reflect "well-operated" treatment (even when oocysts are added to clarified water) and (2) zeta potential analysis of coagulated filter influent/seed suspensions during a performance demonstrate can inform "well-operated" chemical pre-treatment prior to filtration; and,

3. **Seeding protocol validation experiments** were conducted using lower/more environmentally relevant oocyst concentrations to confirm the validity of utilizing high oocyst concentrations in CAF performance demonstrations.

The completion of Phase 1 was critical to enabling Phase 2, during which filter design and operational effects on *C. parvum* oocyst removal by CAF of low turbidity, low DOC source water were investigated to address another substantial knowledge gap. A variety of filter bed depths and operational conditions (e.g., hydraulic surges, ripening, end-of-run, increased hydraulic loading rate, and varied source water temperature) were evaluated. These experiments were conducted throughout the year to capture warm and cold water conditions. Based on the literature, the most vulnerable operational conditions, ripening (Logsdon, 2000; Nieminski & Ongerth, 1995), hydraulic surges and sub-optimal oocyst coagulation conditions were evaluated during cold water temperatures. For this system, cold water conditions were defined as settled water at or below 10 °C as practiced by the City of Toronto. In addition to stable operating conditions (with optimal oocyst chemical pre-treatment during jar coagulation), hydraulic surges and sub-optimal oocyst coagulation conditions were evaluated. Zeta potential analysis was conducted during this phase of experimentation to evaluate the extent of chemical pre-treatment (i.e., optimal vs sub-optimal).

3.2 Site Characteristics

3.2.1 Source Water

The experiments described herein were conducted at the City of Toronto's pilot-scale CAF plant located in the Harris WTP. The Harris WTP has two intake pipes that draw Lake Ontario water from 2.3 km offshore at a depth of 15 m. All raw water was pre-chlorinated prior to conventional treatment of which a small fraction was diverted to the pilot plant. The pre-chlorinated water had a typical free chlorine residual of 0.50 mg/L and a total chlorine residual of 0.56 mg/L. A summary of the water characteristics during each experiment from the full- and pilot-scale plants is located in Appendix A and a summary of monthly raw water conditions can be found in Table 3-1.

Table 3-1. Nominal Raw Water Quality at the Harris Water Treatment Plant in Toronto between January 1, 2017 and April 24, 2018 ($n_{pH,\ turbidity}$ = 470, n_{TOC} = 16)

	pH	Turbidity (NTU)	TOC (mg/L)
Value	8.0 ± 0.15	0.24 ± 0.15*	2.0 ± 0.20

*During a single event in April 2018 turbidity rose to 20 NTU

Notably, the raw water turbidity during the experimental period averaged 0.24 NTU which is less than the 0.3 NTU effluent turbidity target set by Health Canada (2017) and the USEPA's LT2ESWTR (2006b), and similar to the typical target of $\leq$0.1 NTU identified by the Partnership for Safe Water (AWWA et al., 2012) that is required by regulations like USEPA's LT2ESWTR for an additional 0.5-log *Cryptosporidium* treatment credit (2006b).

3.2.2 Harris Water Treatment Plant and Pilot Facility

The pilot plant at the Harris WTP mimics the full-scale treatment plant configurations in Toronto's four WTPs. The research described herein involved only the Harris WTP filter configuration which is the largest in Toronto with a treatment capacity of 950 million L/day. The process at this WTP consists of alum coagulation, flocculation, sedimentation, and dual media filtration. There are two treatment buildings: a pump house, and a multi-level building that houses treatment chemicals and 40 anthracite/sand filters (300 mm of anthracite over 300 mm of sand, and 500 mm of support gravel). Typical operational parameters for the full- and pilot-scale Harris WTP are summarized in Table 3-2.

Table 3-2. Typical Range of Full- and Pilot- Scale Filter Operational Conditions in Toronto

Parameter		Full Scale	Pilot Scale
Alum dose (mg/L)		4 to 5	4 to 5
Raw water turbidity (NTU)		0.28	0.28
Raw water TOC (mg/L)		2.0	2.0
Number of filters		40	2
Filter surface area – total (m^2)/filter		190	0.018
Sand	depth (mm)	250 to 300	250 to 300
	effective size (mm)	0.48	0.48
	uniformity co-efficient	1.4	1.4
Anthracite	depth (mm)	250 to 300	250 to 450
	effective size (mm)	0.95	0.95
	uniformity co-efficient	1.3	1.3
Empty bed contact time (min)		13 to 16	13
HLR (m/h)		2.0 to 9.0	2.0 to 5.0

As can be seen in Table 3-2, the pilot plan was able to closely mirror the operational conditions of the full scale plant. The pilot plant is much smaller than the full scale plant; it is a two-story structure within one of the buildings of the Harris WTP. The flexibility of the set-up and smaller size of the pilot plant make it an ideal location to run tests on a variety of operating conditions or treatment configurations. In addition, the pilot allows for up to six filter column configurations on two separate trains (three columns on each train) to be run simultaneously. For the research described in this thesis, two filter columns were used on the same train, one to simulate the existing filters (referred to as shallow for this study) and one to investigate a proposed depth for new filters (referred to as deep for this study). For the purpose of this research, the focus was on the coagulation and filtration aspects of the treatment process, in Figure 3-1.

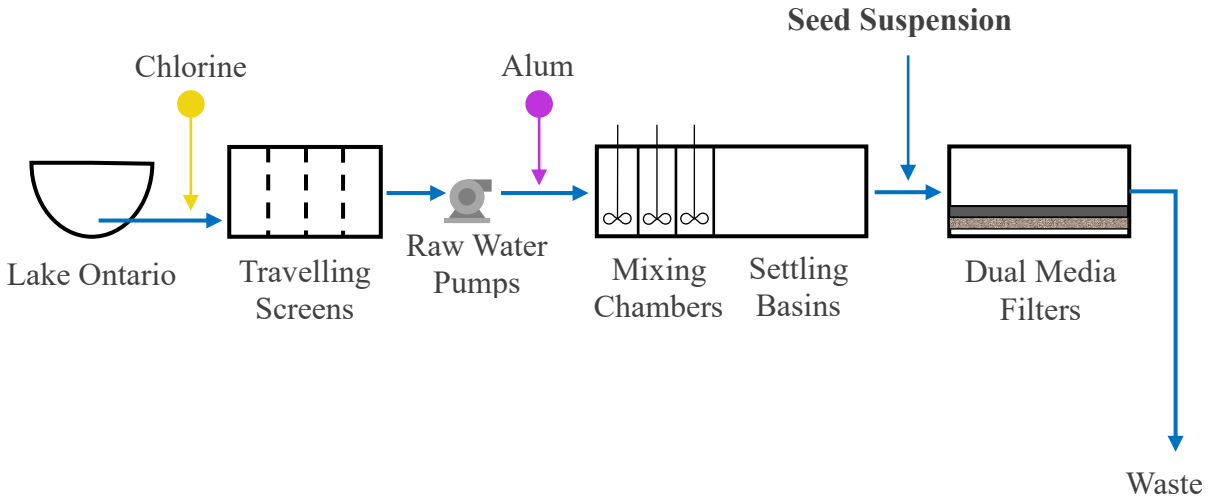


Figure 3-1. Process diagram of treatment stages from source water to pilot plant mimicking the Toronto R.C. Harris Water Treatment Plant process

The CAF process investigated herein is reflected in the treatment from the settling basin to the waste stream in Figure 3-1. Settled water was used to create the seed suspension for each experiment. During the performance demonstrations inactivated *C. parvum* oocysts were added to settled water and coagulated with continuous mixing in beakers. Oocysts were added from a stock suspension of 10^9 oocysts in 1 mL of 5% formalin-inactivated oocysts without Tween (Waterborne, Inc., New Orleans, LA, USA) and were directly pipetted into the seed suspension. The volume of seed suspension created varied based on which experiment was conducted, and thus, the volume of oocyst added from the stock solution also varied by experiment. The aim for each seed suspension was a concentration of 10^7 oocysts/L; exact seed suspension volumes and enumerated oocysts concentrations in the seed suspension can be found in Appendix E. The seed suspension was pumped into the pilot filter influent stream, immediately before it enters the filter columns (Figure 3-2 and Figure 3-3).

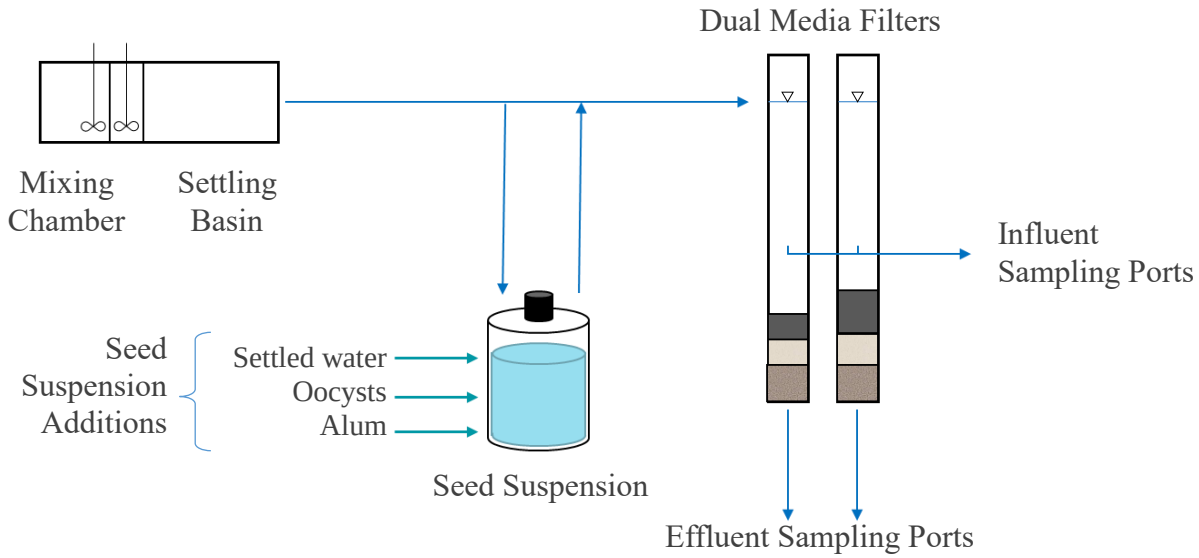


Figure 3-2. Experimental design of the performance demonstrations to evaluate CAF of the Toronto R.C. Harris Water Treatment Plant from Phase 1, Phase 2, and seeding protocol validation experiments.

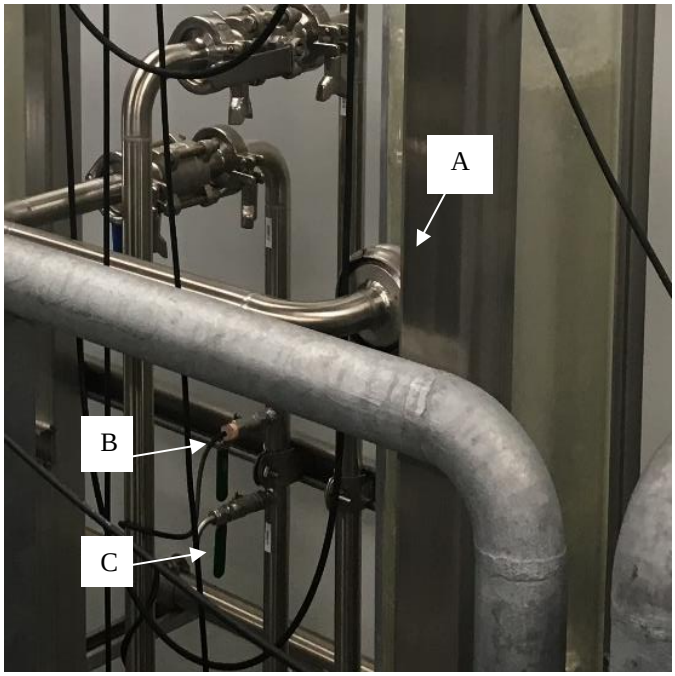


Figure 3-3. Ports used during Phase 1, Phase 2, and Seeding Protocol Validation Experiments performance demonstrations: a) piping where settled water mixed with oocyst seed suspension entered the filter column, b) port where seed suspension was introduced to the settled water stream prior to entering the filter column, and c) port where settled water was collected to create seed suspensions prior to the start of each experiment.

The seed suspension was added at this location (Figure 3-3, b) to allow for the oocysts to mix with the settled water stream in the pilot plant. Based on the small diameter and length of the piping used to add settled water to the filter column it was assumed that this allowed for proper mixing of the seed suspension into the settled water to occur prior to entering the filter column. Constant head was maintained in the filters during the experiments (3.7 m and 3.4 m from the base of each filter, shallow and deep, respectively).

3.3 Phase 1 - Protocol Development for Conducting CAF Performance Demonstrations

Three sets of preliminary experiments were conducted to develop and validate a protocol for creating a seed suspension to conduct performance demonstrations for oocyst removal by CAF of high quality, low turbidity, low DOC source water. These experiments included: (1) jar

coagulation experiments, (2) pilot-scale CAF experiments, and (3) seeding protocol validation experiments. Together, these experiments investigated and validated strategies for achieving appropriate particle destabilization by coagulation prior to CAF of low turbidity, low DOC source waters and addressed the question of whether or not coagulation of oocysts prior to their addition to filter influent streams during CAF performance demonstrations is required. They are detailed below.

3.3.1 Jar Coagulation

These experiments were conducted to evaluate the extent of *C. parvum* oocyst destabilization achieved by jar coagulation prior to their introduction to filter influent streams. This was done to ensure that the experimental approach being utilized represented "well-operated" chemical pre-treatment of low turbidity, low DOC source waters in which oocyst addition could change source water quality (e.g., turbidity) substantially. Zeta potential analysis was utilized to identify the minimum coagulant doses required to achieve adequate oocyst destabilization (i.e., "well-operated" chemical pre-treatment)—this was expected to be a zeta potential of -7 mV, or a value less negative and closer to, but not exceeding the PZC (Pernitsky & Edzwald, 2006).

A jar test apparatus (PB-700, Phipps and Bird, Richmond, VA, USA), turbidimeter (2100AN, Hach, Loveland, CO, USA), ZetaSizer Nano Z, and pH meter were utilized. Turbidity, zeta potential, and pH analysis protocols are also detailed below in Section 3.3.2, 3.3.3, 3.3.4, and 3.4.2. A single 2 L square jar was filled with settled water from the pilot plant. Measurements were taken: (1) prior to the addition of anything to the settled water, (2) following the addition of 10^7 oocysts/L to the settled water, and (3) following alum ($(Al_2(SO_4)_3 \bullet 14(H_2O)$, (ChemTrade Logistics, Toronto, Ontario, Canada) coagulant addition, which was added in 2.5 mg alum/L increments, until a final dose of 20 mg alum/L was achieved. The suspension was mixed at 80 RPM for one min after each alum addition followed by 15 min of settling time prior to taking any measurements.

An additional test was conducted that used the same oocyst concentration and measured the same parameters to see if the results of incremental alum addition were similar to a single higher alum addition. A coagulant dose of 10 mg alum/L was selected. In this jar, measurements were taken prior to alum addition and once again after 10 mg alum/L was added instead of after 2.5, 5, 7.5, and 10 mg alum/L were added.

3.3.2 Pilot-scale CAF Performance Demonstrations

These experiments were conducted to demonstrate the importance of adequate chemical pre-treatment of oocysts (or any other particles) to optimize CAF performance and achieve maximal oocyst removal. Specifically, the ultimate goal of these experiments was to evaluate whether or not jar coagulation of oocysts during CAF performance demonstrations was necessary; and if so, to identify the appropriate coagulant dose that should be utilized during these demonstrations. In conducting these experiments, it was quickly observed that oocyst addition to the low turbidity, low DOC source water investigated frequently resulted in measureable and often substantial changes in turbidity. Therefore, it followed that the coagulant doses applied at the full-scale plant to destabilize particles in the source water might be insufficient for effective destabilization of the charged particles/oocysts in the same source water when oocysts were added at high concentrations (i.e., ~10^7 oocysts/L). In this case, insufficient chemical pre-treatment/coagulation would preclude optimal filtration performance/oocyst removal by CAF during the performance demonstrations. To investigate this possibility, a series of pilot-scale CAF filtration experiments was conducted.

The pilot-scale CAF filtration experiments were conducted using two filter configurations representing those relevant to the full-scale Harris WTP. These included a relatively shallow filter bed consistent with the WTP's full-scale configuration and a deeper bed configuration that could be implemented in the WTP. The pilot-scale filter operational conditions during these experiments are detailed in Table 3-3.

Table 3-3. Parameters for the Pilot Filters during Seed Suspension Protocol Development

Parameter		Value
HLR		4.6 m/h
Flow rate		1.4 L/min
Shallow filter bed	Anthracite	250 mm
	Sand	250 mm
	Gravel	500 mm
Deep filter bed	Anthracite	450 mm
	Sand	300 mm
	Gravel	500 mm

The deep bed filter contained twice as much anthracite as the shallow bed filter (Figure 3-4). Before the beginning of each experiment the effluent sample lines (which were a side-stream off of the main filter effluent lines) were flushed and their flow rates were set to ~250 mL/min to ensure enough sample (~1 L) was collected during each 5 min interval of the 75 min long experiment. The effluent sample ports remained open (flowing) during the experiments. In contrast, the influent sample lines, remained closed between sample times to limit the loss of oocysts reaching the filter bed.

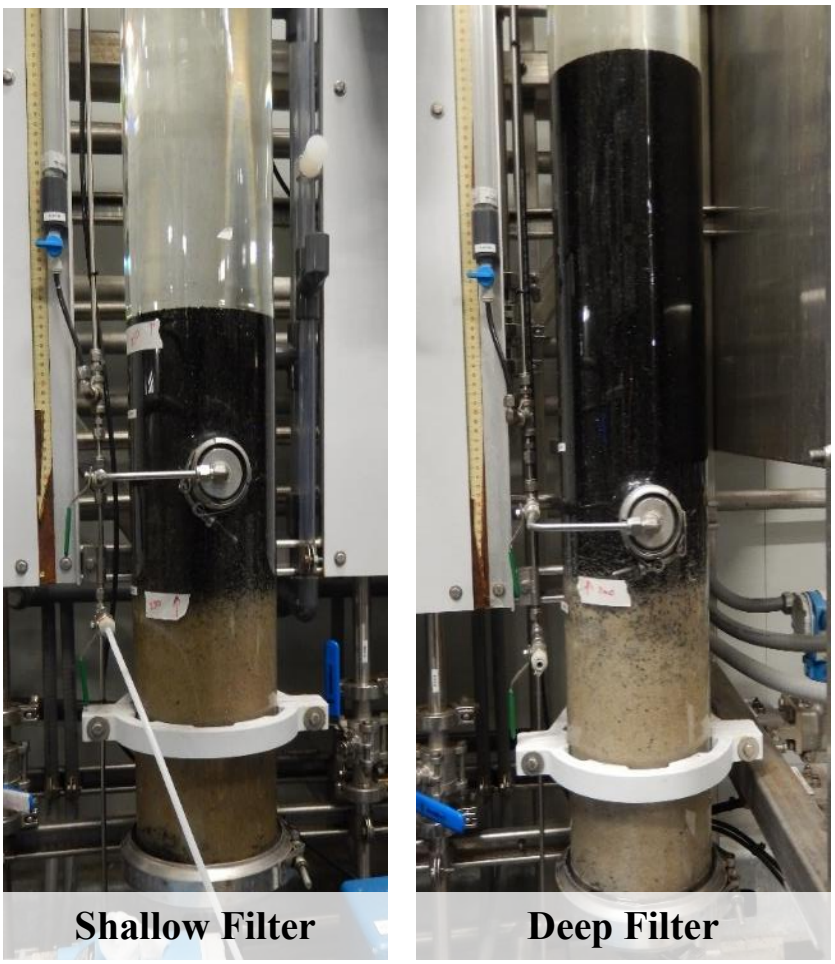


Figure 3-4. Shallow and deep bed filters in the Toronto R.C. Harris Water Treatment pilot plant used during Phase 1, Phase 2, and seeding protocol validation experiments.

A series of five tests were completed during Phase 1, including two sets of back-to-back performance demonstrations. An approach similar to the seeding technique described by Emelko et al. (2003) was used. It included jar coagulation of formalin-inactivated oocysts in settled water prior to introducing the seed suspension into the filter influent stream where it was able to mix inline. Three alum doses (5, 22.5, and 40 mg alum/L) resulting in varying zeta potentials were utilized. Zeta potential was measured throughout the jar coagulation process (i.e., the zeta potentials of settled water, the seed suspension after oocyst addition, and the seed suspension after alum addition were evaluated). The filters were seeded with jar-coagulated oocysts suspended in

settled water for 60 minutes. Filter influent and effluent samples were collected every 15 min starting at time 0, for a total of 75 minutes in sterilized glass (Wheaton) bottles rinsed with eluting solution. Each filter effluent sample (~1 L) was collected over a 5-min period. Influent samples (~250 mL) were collected over a 0.5 min period. The filter influent and effluent sampling schedule during the Phase 1 pilot-scale CAF experiments is summarized in Table 3-4. Each operational condition is defined and discussed below (Sections 3.3.4 and 3.3.4.1).

Table 3-4. Influent and Effluent Sampling Schedule during Phase 1 Pilot-scale CAF Experiments

Experiment Type	Sample Times from Start of Experiment (min)
High HLR	0, 15, 30, 45, 60, 75, 90
Ripening	0, 5, 10, 15, 20, 25, 30
Middle/baseline	0, 15, 30, 45, 60, 75, 90, 105
Low coagulant	0, 15, 30, 45, 60, 75, 90, 105
End of run	0, 15, 30, 45, 60, 75, 90, 105
Hydraulic surge	0, 50, 55, 61, 66, 71, 80, 100

Visual indicators (i.e., pin floc formation) were utilized (to the extent possible) to differentiate between predominant coagulation mechanisms (i.e., sweep coagulation vs. adsorption and charge neutralization) in the seed suspensions (Amirtharajah & Mills, 1982). Notably, due to low concentrations of particles in low turbidity source waters, visual indicators could not be extensively relied upon to differentiate the balance between specific coagulation mechanisms (Brink et al., 1988), although some visualization of pin floc formation was possible with the correct lighting. Irrespective of the specific mechanisms that predominated in achieving optimal CAF performance, zeta potential analysis was used to identify key threshold doses associated with achieving optimal particle and oocyst destabilization in the seed suspensions.

3.3.3 Seeding Protocol Validation Experiments

A third set of experiments was completed using lower, more environmentally relevant oocyst concentrations to validate the use of high oocyst concentrations during pilot-scale CAF performance demonstrations. Two seeding protocol validation experiments were conducted. While

these experiments also involved pilot-scale CAF, they differed from the previous pilot-scale CAF experiments in several ways. Most importantly, the *C. parvum* oocyst concentrations in the filter influent streams in the seeding protocol validation experiments were lower/more environmentally relevant. Specifically, they were ~250 to 320 oocysts/L; approximately two orders of magnitude lower than those (~10^5 oocysts/L) used in the previous experiments. The use of high oocyst concentrations enabled easier, more rapid concentration and enumeration of oocysts by use of direct membrane filtration, IFA staining, and fluorescence microscopy as described in Emelko et al. (2003).

In addition to the use of lower, more environmentally relevant filter influent oocyst concentrations, the entirety of the filter effluent was collected and filtered through EnviroChek® HV cartridges (Pall Gelman Laboratory, Ann Arbor, MI, USA) whereas in the previous experiments multiple, smaller volume samples were collected at prescribed time intervals. Finally, the HLR used during these experiments were consistent with those used in the full-scale WTP and lower than those used during the previous pilot-scale CAF experiments (2.3 vs. 4.6 m/h, respectively). Accordingly, a longer seeding and sample collection period (~11 times longer than in the previous pilot-scale CAF experiments) was required so that at least 3.0 log oocyst removal could be calculated. The main differences in CAF operations between the previous pilot-scale CAF and the seeding protocol validation experiments are summarized in Table 3-5. Similar results from the high and low filter influent oocyst concentrations indicated that the high oocyst concentration protocol developed was a valid method.

Table 3-5. CAF Operational Differences between Phase 1 Pilot-Scale CAF and Seeding Protocol Validation Experiments

Parameter		Pilot-scale CAF	Seeding Protocol Validation
HLR		4.6 m/h	2.3 m/h
Flow rate		1.4 L/min	0.7 L/min
Shallow filter bed	anthracite	250 mm	250 mm
	sand	250 mm	250 mm
	gravel	500 mm	500 mm
Deep filter bed	anthracite	450 mm	
	sand	300 mm	N/A
	gravel	500 mm	

USEPA Method 1623.1 (2005) was used for oocyst identification and enumeration because it allowed processing of the large sample volumes required to complete these experiments. Specifically, four cartridges were used over an 8.5- and 10.5- h period respectively on consecutive days, which allowed for the experiments to be conducted at similar source water conditions. As per the method, ColorSeed™ (BTF, North Ryde BC, NSW, Australia) was used as an internal check standard for quantifying analytical recovery—the associated protocol is detailed in Appendix B. The samples were processed by the City of Calgary's Canadian Association for Laboratory Accreditation (CALA) accredited laboratory where they were processed within the 96 h time frame required by Method 1623.1 (USEPA, 2005). It should be noted that these experiments were only conducted using the shallow bed filter configuration. The two other filters in the filter train were turned off during these experiments so that all of the filter effluent entering the clearwell could be collected and processed (Figure 3-5 and Figure 3-6).

Figure 3-5. Filter effluent collection in Toronto R.C. Harris Water Treatment pilot plant clearwell during Seeding Protocol Validation Experiments.

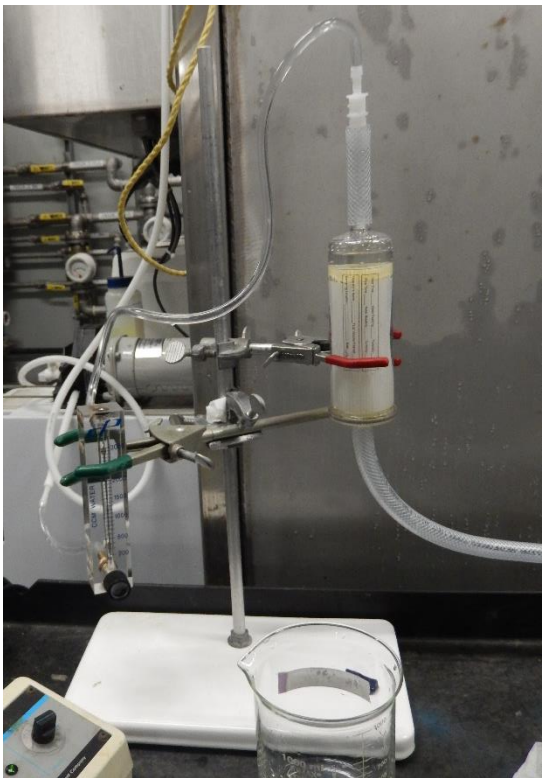


Figure 3-6. EnviroChek® HV cartridge and flow meter in Toronto R.C. Harris Water Treatment pilot plant clearwell during Seeding Protocol Validation Experiments.

3.3.4 Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Phase 2 involved an evaluation of filter design and operational effects on *C. parvum* oocyst removal by CAF of low turbidity, low DOC source water. Summarized in Table 3-6, periods that have been previously identified as especially vulnerable to *Cryptosporidium* spp. oocyst passage through CAF processes including filter ripening (Emelko et al., 2005; Huck, Coffey, Emelko, et al., 2002), end-of run (Emelko, 2001; Emelko et al., 2005, 2003; Huck, Coffey, Emelko, et al., 2002), and sub-optimal coagulation (Dugan et al., 2001; Emelko, 2001; Huck, Coffey, Anderson, et al., 2002; Huck, Coffey, Emelko, et al., 2002; Masher & Hendricks, 1986) were investigated. Hydraulic surges, which are understood to have highly variable effects on *C. parvum* oocyst passage through CAF processes (Emelko, 2001; Emelko et al., 2005, 2003; Huck, Coffey, Emelko, et al., 2002), were also evaluated. All these conditions were investigated during both cold and warm water temperatures entering the filters, with 10 °C marking the cut-off between warm and cold water temperatures. To easily distinguish between the experiments, each experiment was given a unique identifier, as listed in Table 3-7.

Table 3-6. Samples Times and Oocyst Seeding Duration during Phase 2 Pilot-scale CAF Experiments conducted at the R.C. Harris Water Treatment Pilot Plant

Experiment Type	Sample Times from Start of Experiment (min)	Seeding Duration (min)
Ripening	0, 5, 10, 15, 20, 25, 30	0 to 30
Middle/baseline	0, 15, 30, 45, 60, 75, 90, 105	0 to 95
Sub-optimal coagulation	0, 15, 30, 45, 60, 75, 90, 105	0 to 95
End of run	0, 15, 30, 45, 60, 75, 90, 105	0 to 95
Hydraulic surge	0, 50, 55, 61, 66, 71, 80, 100	0 to 90; surge at 60

Table 3-7. Description of Phase 2 Experiments

Identifier	Cold/ Warm	Temp (°C)	Filter Condition	Alum Dose (mg/L)	Zeta Potential Acquired
RIP-1	Warm	12.0	Ripening	40	
RIP-2	Cold	4.1	Ripening	40	
RIP-3	Cold	8.9	Ripening	40	Yes
MID40-1	Cold	7.2	Middle	40	
MID40-2	Cold	9.7	Middle	40	
MID40-3	Cold	4.5	Middle	40	Yes
MID40-4	Cold	4.6	Middle	40	Yes
MID5-5	Cold	4.9	Middle	5	Yes
MID0-6	Cold	5.0	Middle	0	Yes
END	Cold	8.9	End	40	Yes
SUR-1	Warm	13.1	Hydraulic surge	40	
SUR-2	Warm	12.5	Hydraulic surge	40	
SUR-3	Cold	4.3	Hydraulic surge	40	
SUR-4	Cold	4.3	Hydraulic surge	40	
SUR-5	Cold	5.3	Hydraulic surge	40	
SUR-6	Cold	8.8	Hydraulic surge	40	Yes

As illustrated in Table 3-7, settled water in the pilot plant ranged from and 4.1°C to 16.0°C during the Phase 1 and 2 experimental periods (both cold and warm water conditions). Notably and in contrast, the raw water temperature during the Phase 1 and 2 experimental period ranged from 2.2 °C to 15.9 °C. Each experiment detailed in Table 3-7 was conducted concurrently using two filter bed depths in separate filters (Figure 3-4). The two filters were continuously fed from a single oocyst seed suspension. Unlike in Phase 1, the HLR in Phase 2 was set to match the full-scale plant (Table 3-8).

Table 3-8. Differences between Phase 1 and Phase 2 Pilot-scale CAF Experiments

Parameter		Phase 1	Phase 2
HLR		4.6 m/h	2.3 m/h
Flow rate		1.4 L/min	0.7 L/min
Shallow filter bed	anthracite	250 mm	250 mm
	sand	250 mm	250 mm
	gravel	500 mm	500 mm
Deep filter bed	anthracite	450 mm	450 mm
	sand	300 mm	300 mm
	gravel	500 mm	500 mm

Based on the full-scale filter backwashing schedule, the pilot filters at the Harris WTP pilot were backwashed either 96 h after the previous backwash (more typical case) or after the accumulation of 3 m of head. Thus, in consultation with the pilot operator, the end-of-run experiments (END) were conducted as close to after 96 h of filter operation as possible. During the END experiment, the oocyst seed suspension was created as in Phase 1 and sample collection occurred at the same time points as the middle-of-run/stable operation experiments.

During the hydraulic surge experiments, the pumps were shut down and started up as the filters were backwashed; this varied the loading rates in the filters that remained in service. A higher loading rate to the filters was investigated as increased loading may disrupt filter performance, potentially leading to particle detachment from filter beds. In these experiments, the HLR was increased from 2.3 m/h to 4.6 m/h over a 45 second interval at time 60 (min) when the filter was otherwise running optimally. To capture the surge event, two samples were collected prior to the surge (at time 50 and 55 min) to establish the filter's baseline oocyst removal for that day. The surge was followed by 15 min of continuous effluent sampling (samples were collected every 5 min) in an effort to capture any potential disruptions caused by the surge. The remaining samples were collected to describe the tail of the surge event.

MID40-1 to MID40-4 sample ports were sampled using the same method as that used during the pilot-scale CAF experiments in Phase 1; the main difference between these experiments was the temperature because all of them were conducted under optimal coagulation (40 mg alum/L). In

addition to these experiments, two sub-optimal coagulation experiments were conducted at cold water temperatures: MID5-5 (5 mg alum/L) and MID0-6 (0 mg alum/L). These were conducted to evaluate filter performance at sub-optimal and no coagulant conditions to observe changes in zeta potential. They followed the same approach as the other middle of run experiments.

3.3.4.1 Ripening

Ripening experiments (designated RIP-1 through RIP-3) were also conducted during Phase 2. The initial improvement in filter effluent water quality, which typically occurred within the first 30 minutes in the pilot-scale filters, occurs when a filter is put in service following backwash (ripening). The duration of the ripening period was approximately 60 minutes (until filter effluent turbidity reached a pseudo steady state), in both the shallow and deep filters. In order to capture the spike in turbidity, samples were collected in successive 5-minute intervals (Table 3-6). From the top of the filter column, where the oocysts were injected, oocysts travel time typically took 30 minutes to reach a steady concentration at the surface of the filter media which was the entirety of the ripening spike. Therefore, to capture the ripening event and collect as many samples as possible, the tubing used to seed the oocysts into the filter column was lowered, closer to the top of the filter media. This allowed the oocysts to quickly reach the filter media in a timely manner so that samples could be collected to reflect filter performance during ripening.

During ripening a longer set of tubing was needed to reach the top of the filter. Neoprene MasterFlex L/S 13 (ID: 0.8 mm) tubing was used to seed oocysts and neoprene MasterFlex L/S 14 (ID: 1.6 mm) tubing was used for sample collection. Based on the design of the pilot filters, the only access point for additional tubing to reach the filter bed at the same height above both filters (shallow and deep) was from the top of the open filter. An additional challenge faced during the design of this experiment was a metal grate with 4 mm slats half way down each filter column which could not be removed. The tubing had to be moved through the grate in order to reach the top of the filter bed. To compensate for the flexibility of the tubing, a small rod was taped (to cover any exposed metal) to the end of each tube to allow for passage through the metal grate (Figure 3-7).

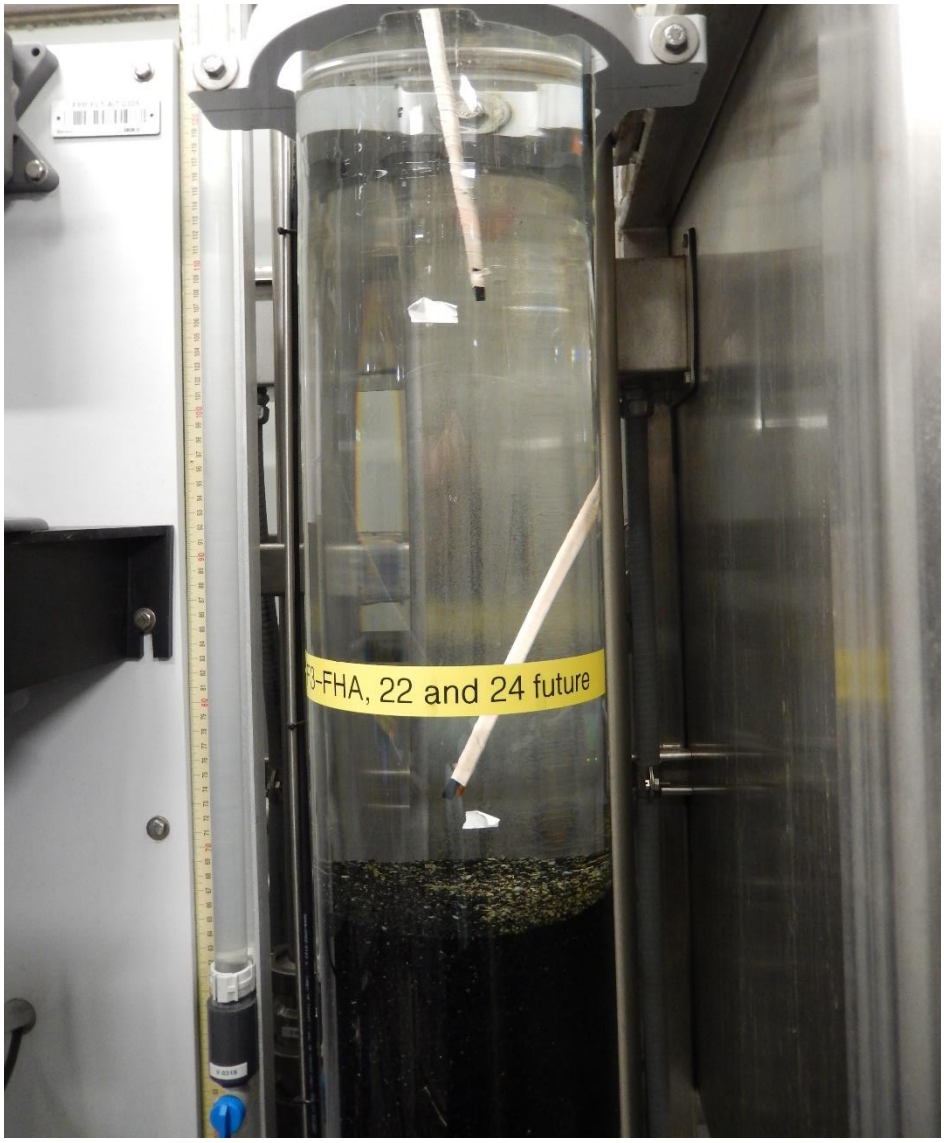


Figure 3-7. The deep bed filter in Toronto R.C. Harris Water Treatment pilot plant during ripening experiment. Tubing with a small rod attached to: fit through metal grate half way down the filter column, and be closer to the filter bed to shorten the oocyst travel time and capture the 30 minute ripening period.

Initially, the influent sampling tubing was positioned to collect samples 76 mm above each filter media surface and the oocyst seeding tubing discharged 305 mm above the filter bed. This was done to have the oocysts reach the filter bed as quickly as possible from the influent tubing. However, after comparing the influent concentrations from the first two ripening experiments with the other experiments that had been conducted, it was determined that there was insufficient time

and/or distance for the oocysts to properly mix with the water above the media. A third experiment was conducted with the influent sampling tube repositioned 127 mm above the filter media surface. The rationale for this was to allow for adequate mixing to occur above the filter.

To ensure that the lower influent oocyst concentration was not due to losses in the longer seeding tubing, recovery tests were conducted and it was found that no significant losses were attributable to the tubing (Appendix C).

3.4 *C. parvum* Oocyst Analyses

3.4.1 Concentration, Purification, and Enumeration Techniques

With the exception of oocyst samples collected during the Seeding Protocol Validation Experiments, all *C. parvum* oocyst concentration, purification, and enumeration analyses in Phases 1 and 2 were conducted following the direct membrane filtration, IFA staining (Crypt-a-Glo, Waterborne Inc., New Orleans, Louisiana, USA), and fluorescence microscopy at 200x magnification (Axioskop 2 plus, Zeiss, Göttingen, Germany) technique described by Emelko et al. (2003). In brief, ~700 mL (on average) of filter effluent and 1 mL of filter influent were measured and filtered through 0.4 μm nominal porosity polycarbonate filter membranes (Whatman Nuclepore®, Whatman Inc., GE Healthcare Life Sciences, Mississauga, ON, CA) placed on top of 8.0 μm nominal porosity cellulose acetate support membranes (Whatman Nuclepore®, Whatman Inc., GE Healthcare Life Sciences, Mississauga, ON, CA) on a manifold or in a syringe filter. Filter influent samples were processed using 13 mm diameter membranes and 25 mm diameter membranes were used for filter effluent samples. Crypt-a-Glo IFA stain was then applied to the filter membrane surfaces and the manifolds were incubated at 35 °C for a minimum of 25 min, as per supplier instructions. The membranes were then mounted on glass slides for oocyst enumeration. Counts of 30 to 100 oocysts per slide were targeted (Emelko et al. 2008). The membrane filtration characteristics and IFA stain volume used to process oocyst samples collected during Phase 1 and 2 pilot-scale CAF Experiments are summarized in Table 3-9.

Table 3-9. Crypt-a-Glo Volume and Membrane Filter Characteristics used to process Oocyst Samples Collected during Pilot Plant Experiments

Parameter		Filter Influent		Filter Effluent	
Technique		Weighted Manifold	Syringe	Weighted Manifold	Glass Manifold
Filter Membrane	Diameter	13 mm	13 mm	25 mm	25 mm
	Pore Size	0.4 μm	0.4 μm	0.4 μm	0.4 μm
	Type	polycarbonate	polycarbonate	polycarbonate	polycarbonate
Support Membrane	Diameter	25 mm	13 mm	25 mm	N/A
	Pore Size	8.0 μm	8.0 μm	8.0 μm	N/A
	Type	cellulose	cellulose	cellulose	N/A
Vol. of Crypt-a-Glo		100 μL	50 μL	200 μL	200 μL

N/A – not applicable, the glass manifold did not require the use of the support membranes

The same process utilized for the influent samples was utilized to enumerate the oocyst concentration of the seed suspensions used during each experiment. Based on the higher concentration of oocysts in the seed suspensions from each experiment, the volume filtered through the syringes was modified to 10 μL.

3.4.1.1 Quantifying Oocyst Removal

To assess removal of *C. parvum* oocysts by filtration, the log reduction between the influent and effluent samples was calculated. In order to accurately describe the removal achieved during each experiment (run), the average oocyst removal was calculated based on the averaged, normalized influent and effluent concentrations.

$$oocyst\ removal = \ log_{10}\left(\frac{influent\ concentration_{avg,normalized}}{effluent\ concentration_{avg,normalized}}\right) \qquad (2)$$

When calculating the oocyst removal for individual time intervals of each run (for box and whisker plots), the following equation was used:

$$oocyst\ removal = \log_{10}\left(\frac{influent\ concentration_{avg,normalized}}{effluent\ concentration_{time\ interval,normalized}}\right) \quad (3)$$

The main difference was that the effluent concentration was specific to the time interval instead of an overall experimental average. Table 3-10 summarizes the samples that were used to calculate the average oocyst removal for each type of experiment. When calculating averages, influent slide concentrations (typically n=5) from the below mentioned periods were averaged before calculating the log removal. This was done because $\log_{10}$ values should not be averaged. Notably, the seed suspension was injected into the settled water influent line that discharges into the top of the filter, about 3 to 4 m above the actual water/media interface. Thus, oocyst travel time in the column needed to be accounted for when calculating the average oocyst removal for each experiment.

Table 3-10. Calculation of Oocyst Removal Averages

Experiment	Sample Times from Start of Oocyst Addition (min)	Period in Average
Middle	0, 15, 30, 45, 60, 75, 90, 105	45 to end
End	0, 15, 30, 45, 60, 75, 90, 105	45 to end
Ripening	0, 5, 10, 15, 20, 25, 30	10 to end
Surge	0, 50, 55, 61, 66, 71, 80, 100	61 to end

In addition to the information in Table 3-10, the influent values used to calculate oocyst removal were also adjusted. Ultimately, each type of experiment used a single influent value to calculate the oocyst removals for both individual time intervals and experimental averages. This was done because each experiment had the same seed suspension concentration and was enumerated using the same technique. This allowed for more consistent comparison between experiments of the same type, between the two filters, and between the various time intervals within sample. By doing this some of the variability between samples within an experiment and between experiments was removed.

When a zero count was encountered, a value of 1 was put in place of it to provide a conservative estimate of the removal achieved. This affected the shape of some box and whisker plots which

resulted in no error/variance bars. Because influent oocyst concentrations were averaged for each run (Equation 3), the removal values were often identical for multiple time intervals. Based on how box and whisker plots are created (i.e. differences between quartile values and maximum/minimum values) in combination with averaging influent concentrations, this sometimes resulted in no boxes on the plots.

3.4.2 Zeta Potential Analysis

Zeta potential was evaluated (whenever possible) using a ZetaSizer Nano Z. Samples were collected prior to oocyst addition to the settled water (SW), after oocyst addition to settled water (SW + O), and after alum addition to the seed suspension (SW + O + alum). Between each reading, the syringe used to collect the samples, and the cartridge used by the ZetaSizer Nano Z were rinsed with settled water from the pilot plant. Each sample value was the average of three individual readings, which were taken consecutively and 10 seconds apart (averaged manually).

3.4.3 Statistical Analysis of Data

Prior to conducting any quantitative comparisons of oocyst removal with different filter configurations or operational conditions, the data were grouped (Table 3-7) and normality was evaluated using a combination of the Shapiro-Wilks test, histograms, and Q-Q plots (Appendix D) using RStudio open source software (RStudio, Boston, MA, USA). The grouped data were further divided into shallow and deep bed configurations. The distributions of oocyst removals achieved by CAF were not always consistent with the normal distribution—this was especially expected during dynamic periods in the filter cycle, including ripening and hydraulic surges. Thus, the non-parametric Mann-Whitney U-test was utilized to evaluate whether or not the sample means from the data sets came from the same or different distributions. The p-value outputs from the Mann-Whitney U-test were compared and assessed at the 5% significance level. Oocysts removal by CAF was quantitatively compared between (1) stable and various CAF operational conditions; (2) cold and warm settled water temperatures; and (3) shallow versus deep filter bed configurations, as summarized in Table 3-11.

Table 3-11. Quantitative Comparisons of *C. parvum* Oocyst Removal Performance by CAF during Phase 2 Pilot-scale CAF Experiments

Condition Evaluated	Base Data Set	Comparison Data Set
Filter Operation	MID40-1 to MID40-4*	RIP-1 to RIP-3
		RIP-3
		MID5-5
		MID0-6
		END-1
		SUR-1 to SUR-5
		SUR-6
		SUR-1 to SUR-6
Settled Water Temperature	(warm)	(cold)
	MID40-1	MID40-2 to MID40-4
	SUR-2 & SUR-3	SUR-1, SUR-4 & SUR-5
Filter Bed Depth	(shallow)	(deep)
	RIP-1 to RIP-3	RIP-1 to RIP-3
	MID40-1 to MID40-4	MID40-1 to MID40-4
	MID5-5 & MID0-6	MID5-5 & MID0-6
	END-1	END-1
	SUR-1 to SUR-6	SUR-1 to SUR-6

*compared with every other condition in *Comparison Data Set* column

The p-value outputs from the Mann-Whitney U-test were compared to test the null hypothesis. If the outputs were less than 0.05 the two sets of data were considered non-identical.

3.5 Lab Equipment Procedures

Between experiments all lab equipment (i.e. not pilot plant equipment) and all sampling bottles were cleaned which were rinsed with acetone, rinsed with deionized water, and autoclaved between uses. Multiple syringe tips and manifold components were rinsed with acetone in a beaker which was filled with deionized water after the initial rinse. After at least 30 minutes had passed, all equipment was rinsed with deionized water and autoclaved for the next sample. Graduated cylinders were cleaned in a similar fashion. Acetone was pipetted into the cylinder, careful to cover the entire interior circumference of the cylinder, and filled with deionized water. Again, after at least 30 minutes they were rinsed 3 times with deionized water and autoclaved for the next sample. Lastly, syringe tips, manifold components, and cylinders were placed in the autoclave.

Prior to removing waste from the sample bottles, the bottles containing the remaining samples were autoclaved on liquid cycle followed by the addition of 3 mL of acetone and were left overnight. The resulting waste was removed from the bottles which were then dish washed and dried. The waste water containing acetone was disposed of using the chemical disposal in the Douglas Wright Engineering building on the University of Waterloo campus. Clean equipment was kept in a common area but labelled for this research. Any items that may have been used by others or mislabelled during cleaning were re-cleaned to ensure the proper steps were taken to avoid cross-contamination.

Prior to each experiment in the Toronto WTP, clean bottles were rinsed with eluting solution, autoclaved, and labelled. The eluting solution was added to all sampling bottles as a means to limit the number of oocyst that could remain attached to the bottle during filtration and IFA processes. The only bottle not rinsed with eluting solution was the bottle used to create the seed suspension during each experiment to eliminate the number of external factors that would interfere with zeta potential measurements.

Chapter 4 Results and Discussion

4.1 Phase 1 Results

Phase 1 involved the design and investigation of a seeding protocol for optimized oocyst removal by CAF of high quality source water. Three sets of experiments were conducted: (1) jar coagulation experiments, (2) pilot-scale CAF experiments, and (3) seeding protocol validation experiments. These results from these experiments are discussed in detail below.

4.1.1 Seed Suspension Protocol Development

These experiments were conducted to evaluate the extent of *C. parvum* oocyst destabilization achieved by coagulation, to ensure that the developed experimental approach represented "well-operated" chemical pre-treatment of the low turbidity, low DOC source waters in which oocyst addition could change source water quality substantially. *C. parvum* oocysts were added to 2 L of settled water to achieve a target concentration of 10^7 oocysts/L. Alum was added in 2.5 mg/L increments and turbidity, pH and zeta potential were analyzed to identify the minimum coagulant dose required to achieve oocyst destabilization (i.e., "well-operated" chemical pre-treatment). As discussed in Sections 2.5 and 3.4.2, this was expected to be a zeta potential of -7 mV or a value less negative, and closer to, but not exceeding the PZC. The turbidity, pH, and zeta potential data collected during the Phase 1 jar coagulation experiment are presented in Table 4-1.

Table 4-1. Oocyst Seed Suspension Characteristics resulting from Increased Coagulant Addition during Jar Coagulation Experiment

Added to settled water	Alum Dosage (mg/L)	Settled Turbidity* (NTU)	pH	Zeta Potential (mV)
--	0.0	0.22	7.7	-18.1
oocysts	0.0	0.70	7.8	-19.7
alum	2.5	0.35	7.5	-15.8
alum	5.0	0.39	7.5	-16.5
alum	7.5	0.52	7.3	-13.7
alum	10.0	0.62	7.2	-14.8
alum	12.5	0.73	7.1	-9.7
alum	15.0	0.79	6.9	-4.5
alum	17.5	0.90	6.9	-2.5
alum	20.0	0.79	6.9	-2.3

* following a 15 min settling time

As would be expected for such a low turbidity source water, oocyst addition substantially increased the seed suspension turbidity from 0.22 to 0.70 NTU. Thereafter, turbidity in the seed suspension increased (up to a point) with increasing alum coagulant addition (Figure 4-1). The initial increase in turbidity was due to the lack of particulate matter initially present in the water matrix. As alum was added to the oocyst seed suspension, the coagulation regime and associated predominant coagulation/particle destabilization mechanisms shifted from a combination of adsorption and charge neutralization, and sweep coagulation (i.e., precipitation of aluminum hydroxide solid [$Al(OH)_3$ (s)] coupled with increased contact opportunities for flocculation resulting from the formation of that solid) to increasingly more sweep coagulation, as described by Amirtharajah & Mills (1982). In the present experiments, as sweep coagulation ultimately became the predominant coagulation mechanism, small, pin flocs formed and were eventually visible. Concurrently, some turbidity reduction during settling was observed. Here, this behavior was observed at alum doses of 15 to 20 mg/L (Figure 4-1).

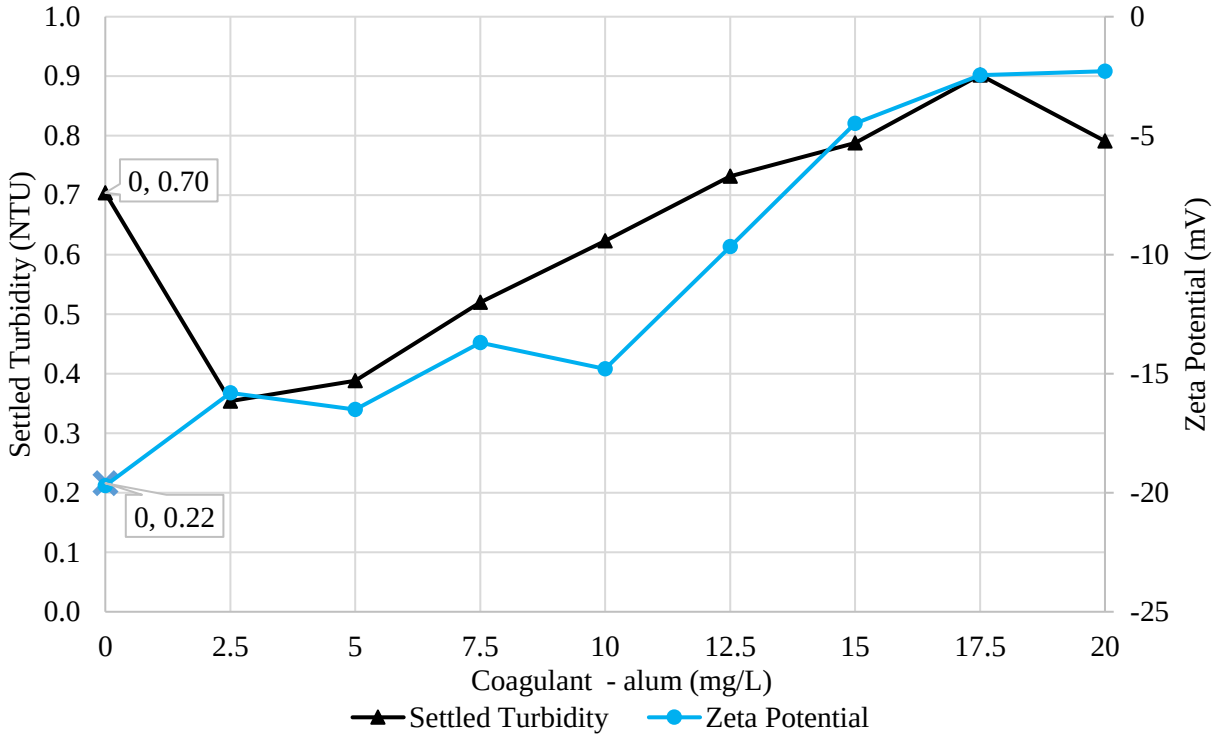


Figure 4-1. Results from Phase 1 jar coagulation investigation

The pH of the seed suspension decreased with increasing alum addition, as would be expected as result of the addition of an acidic coagulant to a water matrix with relatively low alkalinity; notably, the zeta potential became less negative with increased alum addition (Figure 4-1). It was -18.1 mV initially and decreased to -19.7 mV after the addition of the negatively charged oocysts. The seed suspension zeta potential at the full-scale WTP's applied alum dose of 5 mg/L was -16.5 mV. Based on the general suggestion of Pernitsky (2003) and the broader understanding the filter influent zeta potential should be in the vicinity of the PZC, ± 4mV of 0 mV, (but still negative so as not to unnecessarily over-coagulate) to achieve optimal particle and oocyst (Bean et al., 1964; Bustamante et al., 2006; Cleasby et al., 1963; Ghernaout, 2015; Gupta et al., 1973; Karaman et al., 1999; Neuman, 1981; Riddick, 1961; Xagoraraki & Harrington, 2004; Xu, Fitzpatrick, & Deng, 2006) removal by CAF. Using the PZC, these data indicate that full scale plant alum dose of 5 mg/L likely would be insufficient for achieving optimal CAF treatment performance; rather, doses of 15 mg/L or more would be expected to be more likely to result in optimal particle and oocyst removal by CAF when treating this water matrix.

It should be noted that a settling period was used during these experiments to observe the effect of settling during the relatively short (15-minute) settling period; and if so, to identify the associated alum dose. It should be further underscored that the seed suspensions were continuously stirred as they were added to filter influents during subsequent pilot-scale CAF experiments. During those experiments, alum doses (such as the ~15 mg/L dose observed here) resulted in the formation of small pin flocs that were barely visible. Coagulation resulting in pin floc formation is appropriate for achieving optimal particle removal by direct filtration (Crittenden et al., 2012), which is effectively what was conducted in the subsequent phases of this work (Sections 4.1.2 and 4.2) because the oocysts seed suspensions were being added directly to filter influent streams.

An additional experiment was conducted to demonstrate that at the process of incremental alum addition during the jar coagulation experiments described in Section 3.3.1 and presented above (Table 4-1), resulted in comparable turbidity, pH and zeta potential. During this experiment, a 10 mg/L was added directly to the water matrix containing the *C. parvum* oocysts. This resulted in a zeta potential of -14.4 mV and turbidity of 0.963 NTU (-14.8 mV and 0.623 NTU during the incremental coagulant additions). Importantly, these data demonstrate the post-coagulation water quality was not meaningfully impacted by the incremental addition of alum that was required to identify the level of alum addition that was expected to result in optimal particle and oocyst removal by CAF (i.e., "well-operated" treatment) of any given filter influent matrix. This expectation was confirmed during the Phase 1 pilot-scale CAF Experiments described in the next section below (Section 4.1.2).

4.1.2 Pilot-scale CAF Experiments

Pilot-scale CAF experiments were conducted to demonstrate that (1) sufficient chemical pre-treatment/coagulation of oocysts (or any other particles) was required to effectively neutralize/destabilize their surface charge during performance demonstrations in order to reflect "well-operated" treatment (even when oocysts are added to clarified water) and (2) zeta potential analysis of coagulated filter influent/seed suspensions during performance demonstrations can inform "well-operated" chemical pre-treatment prior to filtration. Specifically, five pilot-scale CAF experiments were conducted. In brief, filtration using shallow and deep bed anthracite/sand

filter configurations was investigated. The experimental CAF design and operation details were presented in Section 3.3.2 and Table 3-2.

Following up on the analysis presented in Section 4.1.1, jar coagulation of the oocyst seed suspensions introduced into the filter influent streams was investigated using three alum doses: (1) 5 mg alum/L to mimic the alum dose typically applied at the full-scale Harris WTP, (2) ~22.5 alum mg/L to yield zeta potentials less negative than ~-7 mV and somewhat visible pin floc formation, and (3) 40 mg alum/L to yield negative zeta potentials closer to the PZC than those achieved with the 22.5 mg/L alum dose and clearly visible pin floc formation. After one initial experiment, back-to-back sample dates were chosen for subsequent experiments to ensure that environmental conditions, especially source water quality and temperature, were as consistent as possible so that optimal alum dose concentrations would not vary substantially between the experiments. An initial experiment using 22.5 mg alum/L was conducted on May 11, 2017. The back-to-back experiments were conducted in the following pairings: 22.5 mg alum/L and 40 mg alum /L (on June 8 and 9, 2017), and 5 mg alum /L and 40 mg alum/L (on August 2 and 3, 2017). The repetition of the experiment with the addition of 40 mg/L of alum to the seed suspension served as a common point of comparison between the sets of experiments.

C. parvum oocyst removal by shallow and deep bed filters during the Phase 1 pilot-scale CAF experiments is summarized in a box-and-whisker plot (Figure 4-2) and Table 4-2. In these plots, the line in the center of the box represents the median (50^{th} percentile) oocyst removal by the CAF process. The lower and upper portions of the box respectively indicate the 25^{th} and 75^{th} percentile oocyst removals achieved by the CAF process. The lower and upper portions of the line (whisker), respectively, represent the minimum and maximum oocyst removals achieved by the CAF process In this and all ensuing figures, the 3.0-log oocyst removal that is expected from "well-operated" CAF in most North American drinking water regulatory policies (Health Canada, 2012; USEPA, 2006a) is indicated as a benchmark for what is expected from this treatment process.

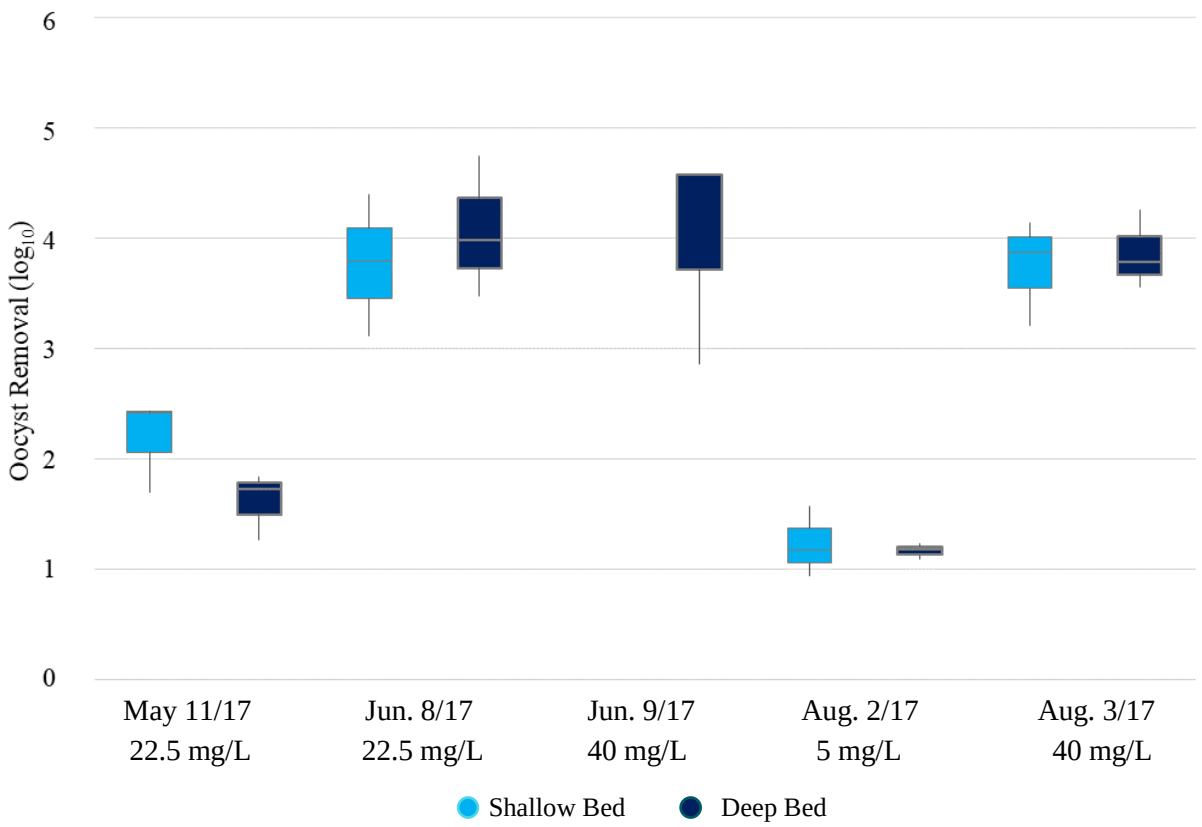


Figure 4-2. Box and whisker plot comparing *C. parvum* oocyst removal in shallow and deep bed filters during Phase 1 Pilot-scale CAF experiments.

Table 4-2. Phase 1 Pilot-scale Experiment Summaries

Exp. ID	Alum Dose (date)	Mean Oocyst Removal (log_{10})		Final Zeta Potential (mV)
		Shallow Bed	Deep Bed	
P1 – 22.5a	22.5 mg alum/L (May 11th)	2.0	1.5	N/A
P1 – 22.5b	22.5 mg alum/L (Jun. 8th)	3.5	3.8	-9.5
P1 – 40a	40 mg alum/L (Jun. 9th)	4.1	3.3	-5.2
P1 – 40b	40 mg alum/L (Aug. 3rd)	3.6	3.8	-7.1
P1 – 5	5 mg alum/L (Aug. 2nd)	1.2	1.2	-15.5

The data in Figure 4-2 generally suggest two operational scenarios: (1) ≥3-log removal of *C. parvum* oocysts when there was sufficient oocyst destabilization by coagulation that enabled optimized oocyst removal by CAF and (2) <3 log removal of *C. parvum* oocysts when there was insufficient oocyst destabilization by coagulation and associated sub-optimal oocyst removal by CAF. Notably, although zeta potential data were not available for the initial experiment conducted on May 11th, 2017, it is likely that oocyst destabilization was insufficient on this occasion—oocyst removal by CAF likely would have been better if a higher alum dose had been utilized. This hypothesis is generally supported by the other data collected later in this study, in which good oocyst removals (i.e., >3-log) by CAF were observed in both the shallow and deep bed filters when seed suspension zeta potentials were generally smaller in magnitude than -10 mV (i.e. between -5.2 and -9.5 mV), while more negative seed suspension zeta potentials (at least -15.6 mV) resulted in lower (<3-log) oocyst removals by CAF (Table 4-2). Notably, although the deep and shallow bed filters generally performed similarly; with the exception of the initial experiment, oocyst removal by the deep bed filter was less variable and frequently better than the achieved by the shallow bed filter (Figure 4-2 and Table 4-2). Mean oocyst removals that were achieved by these different filter configurations were generally similar for a given operational condition, however;

thereby underscoring the critical importance of sufficient chemical pre-treatment/coagulation for effective and optimal particle and potential pathogen removal by CAF processes.

4.1.3 Seeding Protocol Validation Experiments

To confirm the validity of utilizing high oocyst concentrations in CAF performance demonstrations, two experiments were conducted using lower (by two orders of magnitude)/more environmentally relevant oocyst concentrations so that these results could be compared to those obtained from performance demonstrations conducted at the same operational conditions, but with higher oocyst concentrations. Thus, these experiments were designed to validate the use of alum doses that were higher than those used by the full-scale WTP when coagulating oocyst seed suspensions in order to ensure that sufficient particle/oocyst destabilization occurred and well operated CAF was evaluated. The operational conditions and experimental data associated with these experiments are provided in Table 4-3.

Table 4-3. Overview of Operational Conditions and Experimental Data from the Phase 1 Seeding Protocol Validation Experiments

Sample Date	Alum Dose (mg/L)	Zeta Potential (mV)	Capsule #	Total # of *C. parvum* Oocysts Seeded (#)	Volume Filtered (L)	*C. parvum* Oocysts in Effluent* (#)	Oocyst Removal (log_{10})
July 16, 2018	20	- 8.0	1	5.6E+04	231	3	4.7
			2	4.4E+04	180	29	3.6
July 17, 2018	7.5	-11.1	1	3.9E+04	140	41	3.4
			2	6.4E+04	185	2	4.9

* *C. parvum* oocysts recovered from cartridges fed with filter effluent

In contrast to the pilot-scale CAF experiments in which matched filter influent and effluent pairs of data were used to calculate oocyst removals, the entire filter effluent flow was filtered in the present experiments. The total number of oocysts captured in each of two cartridges was compared to the number of oocysts introduced in the filter influent to calculate oocyst removal by CAF which are presented in Table 4-3.

Critically, it must be emphasized that the alum doses used during these experiments were lower than those used in the previous pilot-scale CAF experiments. The alum doses were selected to achieve seed suspension zeta potentials in the approximate vicinity of -7 mV (July 16) and equivalent to those used by the full-scale WTP (July 17). As in the previously reported pilot-scale CAF experiments, alum was added in 2.5 mg/L increments, as shown in Figure 4-3. These experiments demonstrated that similar, excellent (i.e., >3-log) *C. parvum* oocyst removals by CAF were achieved, irrespective of alum dose and filter influent oocyst concentration as long as sufficient oocyst destabilization was achieved in the seed suspension (as evidenced by zeta potential) prior to introducing the seed suspension to the filter influent stream.

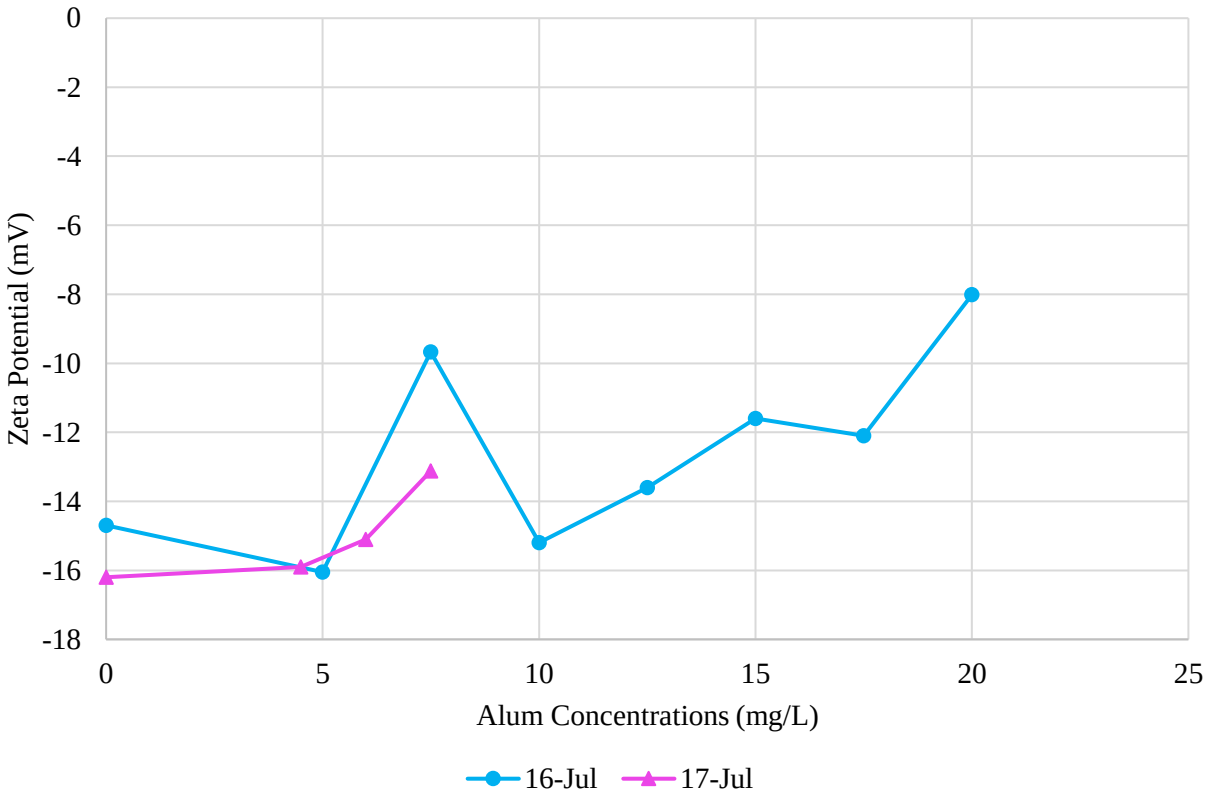


Figure 4-3. July 16 and 17 oocyst seed suspension zeta potential vs alum concentration

Interestingly, it is possible that the change in predominant coagulation regime (i.e., shifting from adsorption and charge neutralization to sweep coagulation) as described by (Edzwald & Lawler, 1983) was observed during the July 16 experiment. The less negative zeta potential observed at an alum dose of 7.5 mg/L may have corresponded to the optimal observed particle/oocyst destabilization by adsorption and charge neutralization and subsequent, more negative zeta potentials that were observed as alum dose further increased could indicate charge reversal. Subsequent improvement in zeta potentials (to less negative values) with additional alum addition would then have been associated with the emergence of sweep coagulation as the predominant particle/oocyst destabilization mechanism. This effect was not investigated during the second experiment (on July 17) because that experiment was exclusively focused on mirroring the full-scale alum dose in the seed suspension; nonetheless, a similar trend in zeta potential did begin to emerge.

4.2 Phase 2 Results

Upon completion of Phase 1, filter design and operational effects on *C. parvum* oocyst removal by CAF of low turbidity, low DOC source water were investigated. All of the experiments conducted during Phase 2 used the same set up as in Phase 1, which included using settled water as the base for the seed suspension. One major difference between the pilot experiments using this protocol in Phase 1 and Phase 2 was the hydraulic loading rate which was reduced to 2.3 m/h (from 4.6 m/h) to mirror that of the Harris full-scale WTP. Based on the range of results observed during Phase 1, a set alum dose of 40 mg/L was used during Phase 2 to ensure optimal particle/oocyst destabilization and pin floc formation (i.e., well-operated CAF with optimal particle/oocyst destabilization) without needing to go through the somewhat labor-intensive process of incremental coagulant addition and manual evaluation of seed suspension zeta potential.

4.2.1 Filter Operational Conditions

A total of 16 experiments were conducted to investigate *C. parvum* oocyst removal by CAF of low turbidity, low DOC water by shallow and deep dual media filters, at warm and cold water temperatures, during hydraulic surges, periods of filter ripening, end-of-run filter operation, and sub-optimal coagulation. These are summarized in (Table 3-7).

4.2.1.1 Stable Filter Operation and Sub-optimal Coagulation during Middle of Run

Four sets of stable filter operation experiments and two sets of sub-optimal coagulation experiments were conducted during the middle portion of the filter cycle. The details of the experiments are summarized in Table 4-4 and Figure 4-4. Detailed results from these experiments can be found in Appendix E. Importantly, quantification of *C. parvum* oocyst removal by CAF during stable filter operation established a baseline for process performance during warm and cold water conditions and also provided a basis for comparison when other operational conditions were investigated.

Table 4-4. Oocyst Removals from Phase 2 Middle of Run Experiments during Stable Filter Operation (MID40-1 to MID40-4) and Sub-optimal Coagulation (MID5-5, MID0-6) (n=5)

Identifier	Temperature (°C)	Alum Dose (mg/L)	Zeta Potential (mV)	Avg. Oocyst Removal (log_{10})	
				Shallow Bed	Deep Bed
MID40-1	7.2	40	N/A	3.8	3.9
MID40-2	9.7	40	N/A	4.3	4.9
MID40-3	4.5	40	0.1	5.4	5.4
MID40-4	4.6	40	2.0	4.7	4.2
MID5-5	4.9	5	-10.0	2.4	1.9
MID0-6	5.0	0	-7.4	1.3	0.9

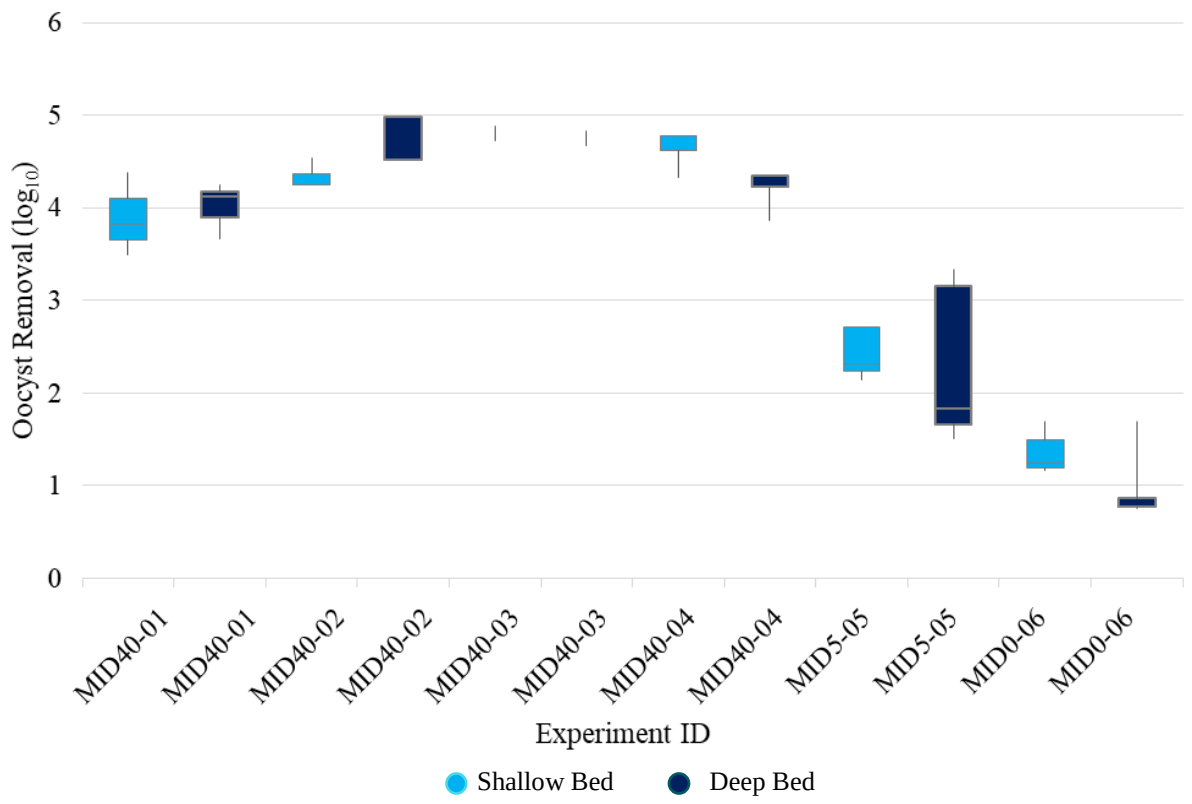


Figure 4-4. *C. parvum* oocyst removal by CAF during Phase 2 middle-of-run, 40 mg alum/L (MID40-1 to MID40-4) and sub-optimal coagulation (MID5-5, MID0-6), 5 and 0 mg alum/L, experiments

During stable filter operation, *C. parvum* oocyst removal by shallow and deep bed CAF ranged from 3.8 to 5.4 log (Table 4-4 and Figure 4-4). As would be expected, *C. parvum* oocyst removals by deep and shallow bed CAF during sub-optimal coagulation conditions were significantly different from those observed during stable filter operation (5% significance level). These results align well with the Phase 1 results (discussed in Section 4.1.2) and highlight the importance of achieving sufficient particle/oocyst destabilization by coagulation to reflect well-operated CAF. Thus, they also underscore the importance of ensuring sufficient jar coagulation of oocyst seed suspensions during CAF performance demonstrations, especially during treatment of low turbidity, low DOC source waters for which filter effluent turbidity alone may not be adequately indicative of sufficiently destabilized particles/oocysts, and therefore, well-operated treatment.

The results from these experiments also are generally consistent with previously reported

C. parvum oocyst removal by CAF data from other systems (Barkay-Arbel et al., 2012; Brown & Emelko, 2009; Dai & Hozalski, 2002; Edzwald & Kelley, 1998; Emelko, 2003; Huck et al., 2002; Keegan et al., 2008; Logsdon, 2000; Nieminski & Ongerth, 1995; Ongerth & Pecoraro, 1995; Shaw et al., 2000; Torabian et al., 2008). Notably, they are consistent with the high levels (overall 5.5 log) of oocyst removal during stable operation by CAF of moderate turbidity, moderate DOC source water, that were previously reported for a system in Ottawa, Canada (Emelko, 2001; Huck et al., 2002). CAF during sub-optimal coagulation conditions (MID5-5 and MID0-6) resulted in median *C. parvum* oocyst removals of less than 3-log. This result also is consistent with previous research (Dugan et al., 2001; Emelko, 2001; Huck, Coffey, Anderson, et al., 2002; Huck, Coffey, Emelko, et al., 2002; Masher & Hendricks, 1986) that demonstrated that oocyst passage through CAF processes can substantially increase during sub-optimal coagulation conditions, especially when coagulant residual (i.e., metal hydroxide precipitate) is not present in treatment units preceding filtration (Emelko, 2001; Huck et al., 2002; Emelko et al., 2003). Table 4-5 provides statistical evidence that, when compared to the experiments conducted under stable filter operation, MID5-5 and MID0-6.

Table 4-5. Comparison of Oocyst Removals during Stable Operation to those during Sub-Optimal Coagulation (MID5-5 and MID0-6) using the Mann-Whitney U-test

Experiment		p-value*	W
MID5-5	Shallow Bed	0.00061	115
	Deep Bed	0.00094	113
MID0-6	Shallow Bed	0.00061	115
	Deep Bed	0.00061	115

*when p-value < 0.05, reject null hypothesis: samples come from the same distribution, no difference in medians

The p-value is lower than 0.05 for both experiments at both filter bed depths which indicates that there is a difference between stable filter operation experiments and the two sub-optimal filter experiments.

4.2.1.2 Ripening

Three sets of ripening experiments were conducted and are summarized in Table 4-6 and Figure 4-5. Full results from ripening and all other Phase 2 experiments, can be found in Appendix E.

Table 4-6. Average Oocyst Removals in Phase 2 Ripening Experiments (n=4)

Identifier	Temperature (°C)	Alum Dose (mg/L)	Zeta Potential (mV)	Avg. Oocyst Removal (log_{10})	
				Shallow Bed	Deep Bed
RIP-1	12.0	40	N/A	2.8	2.7
RIP-2	4.1	40	N/A	2.1	2.6
RIP-3	8.9	40	1.3	3.0	3.3

N/A - not available/not measured

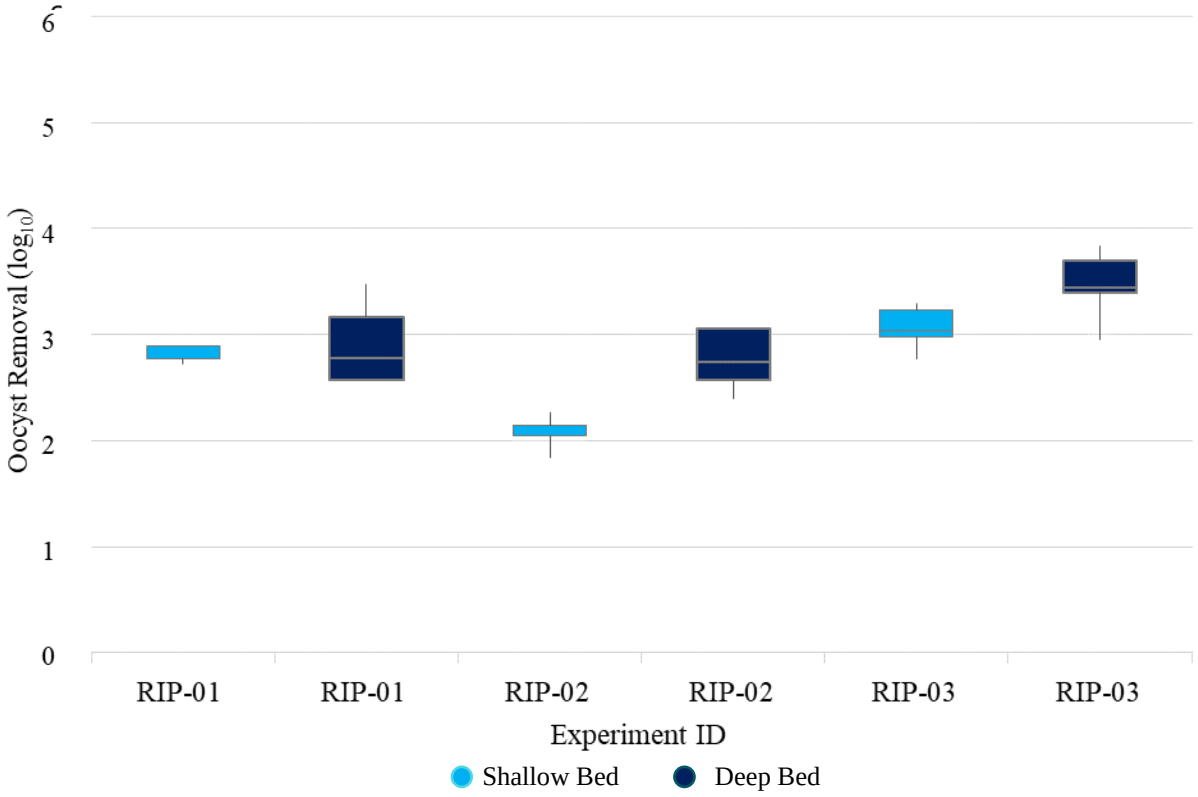


Figure 4-5. *C. parvum* oocyst removal during Phase 2 filter ripening experiments

C. parvum oocyst removal by CAF of low turbidity, low DOC source water did not always exceed the 3-log removal target during filter ripening. It is important to note that the experimental configuration was altered for the RIP-3 experiment, as discussed in Section 3.3.4.1. Briefly, the tube that introduced the oocyst seed suspension to the filter influent was moved to a location 17.8 cm higher above the filter bed, which allowed for additional mixing of the seed suspension in the filter influent stream prior to the collection of filter influent samples. After implementation of this change, median *C. parvum* oocyst removals by CAF during filter ripening were >3.0-log. These results underscore the importance of experimental design during CAF performance demonstrations.

The significance of the ripening test results was evaluated both jointly and with RIP-3 in isolation (Table 4-7). In all cases, *C. parvum* oocyst removal by deep and shallow bed CAF during filter ripening was significantly different than that measured during stable filter operation (5% significance level). The results observed herein are consistent with other reports of *C. parvum* oocyst removal by CAF during filter ripening. For example, Huck et al. (2002) reported a significant increase in oocyst passage during ripening tests conducted at the Britannia WTP in Ottawa, Canada. In that study, average *C. parvum* oocyst removal by CAF during filter ripening was consistently >3-log, but during this period, oocyst removal by CAF decreased by ~0.5 log on average, relative to stable operation. A similar experiment was conducted at the Metropolitan Water District of Southern California pilot plant, but significant differences between oocyst removal during filter ripening and stable filter operation were not observed (Huck, Coffey, Emelko, et al., 2002). The significance of the three ripening test results were first evaluated together. RIP-3 was then evaluated in isolation (Table 4-7). This was done due to the change in method that resulted in removal exceeding 3.0 log.

Table 4-7. Comparison of Oocyst Removals during Stable Operation to those during Ripening using the Mann-Whitney U-test

Experiments		p-value*	W
RIP-1 to RIP-3	Shallow Bed	3.7E-07	343
	Deep Bed	2.3E-06	331
RIP-3	Shallow Bed	9.4E-04	113
	Deep Bed	0.005	105

*when p-value < 0.05, reject null hypothesis: samples come from the same distribution, no difference in medians

The results from the testing in Toronto indicate that at the 5% significance level, filter performance during ripening (RIP-3) and stable filter operation were different for both the shallow and deep bed filters. These results are consistent with what was reported in Ottawa by Huck et al. (2002).

4.2.1.3 End-of-Run

One end-of-run experiment was conducted. The conditions for this experiment are summarized in Table 4-8 and Figure 4-6. Detailed results from these experiments are located in Appendix E.

Table 4-8. Averaged Oocyst Removal from Selected Phase 2, End of Run Experiments (n =5)

Identifier	Temperature (°C)	Alum Dose (mg/L)	Zeta Potential (mV)	Avg. Oocyst Removal (log_{10})	
				Shallow Bed	Deep Bed
END	8.9	40	-2.3	3.2	3.4

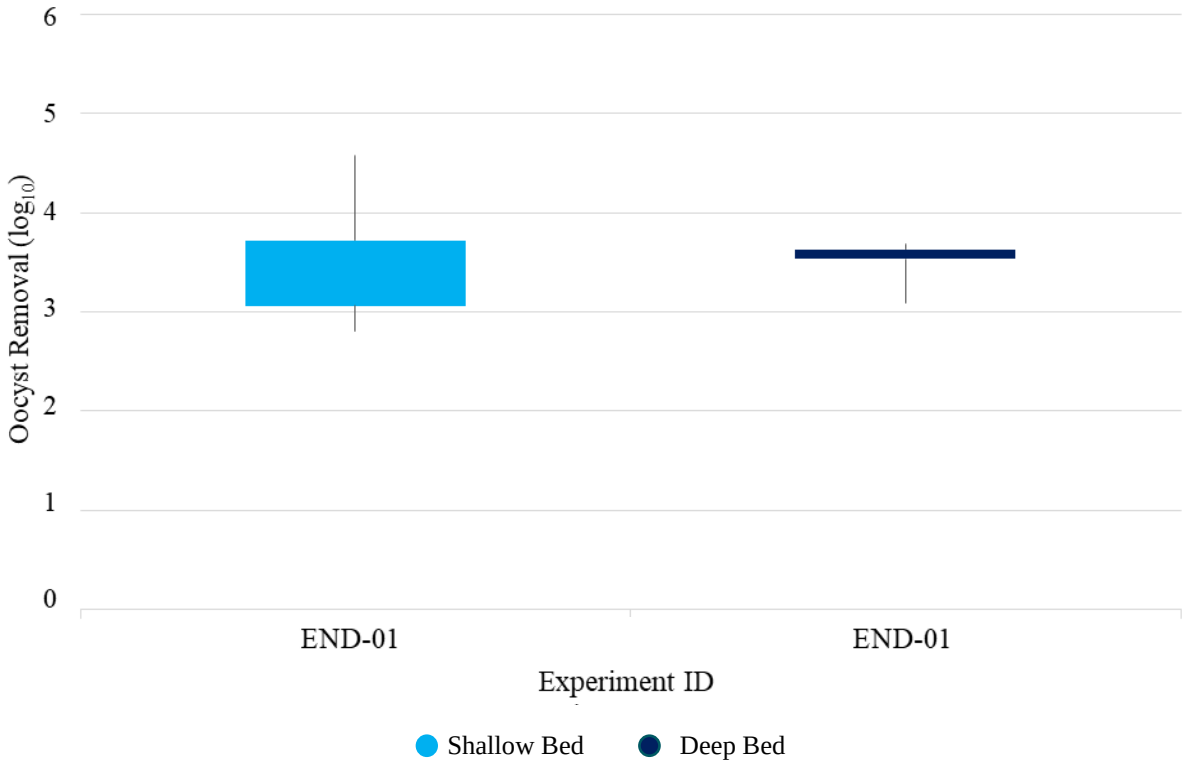


Figure 4-6. *C. parvum* Oocyst removal by CAF during Phase 2 end-of-run experiment (n=5)

C. parvum oocyst removals by shallow and deep bed CAF during end of run operation ranged from 2.9 to 4.1 log (Appendix E). Oocyst removals by deep and shallow bed CAF during end of run conditions were significantly different from those observed during stable filter operation (5% significance level). Although the median oocyst removal was slightly lower, the results from the end of run experiment generally align with those observed during stable filter operation (in Section 4.2.1.1). During the end of run experiment, the median *C. parvum* oocyst removals by shallow and deep bed CAF were >3.0-log, even when the filters had been in service for over 90 h. This is

consistent with a number of other end-of-run studies in the refereed literature (Emelko et al., 2003; Emelko, 2001; Huck et al., 2002).

When compared to stable filter operation (Table 4-9), end-of-run in shallow and deep bed filters were statistically different as the p-values for both the shallow and deep bed filters were less than 0.05.

Table 4-9. Comparison of Oocyst Removals in Stable Operation to those End-of-Run using the Mann-Whitney U-test

Experiment		p-value*	W
END-1	Shallow Bed	0.016	98
	Deep Bed	0.003	107

*when p-value < 0.05, reject null hypothesis: samples come from the same distribution, no difference in medians

4.2.1.4 Hydraulic Surge

A series of 6 sets of deep and shallow bed CAF experiments were conducted to investigate hydraulic surge conditions. The details of the experiments are summarized in Figure 4-7 and Table 4-10.

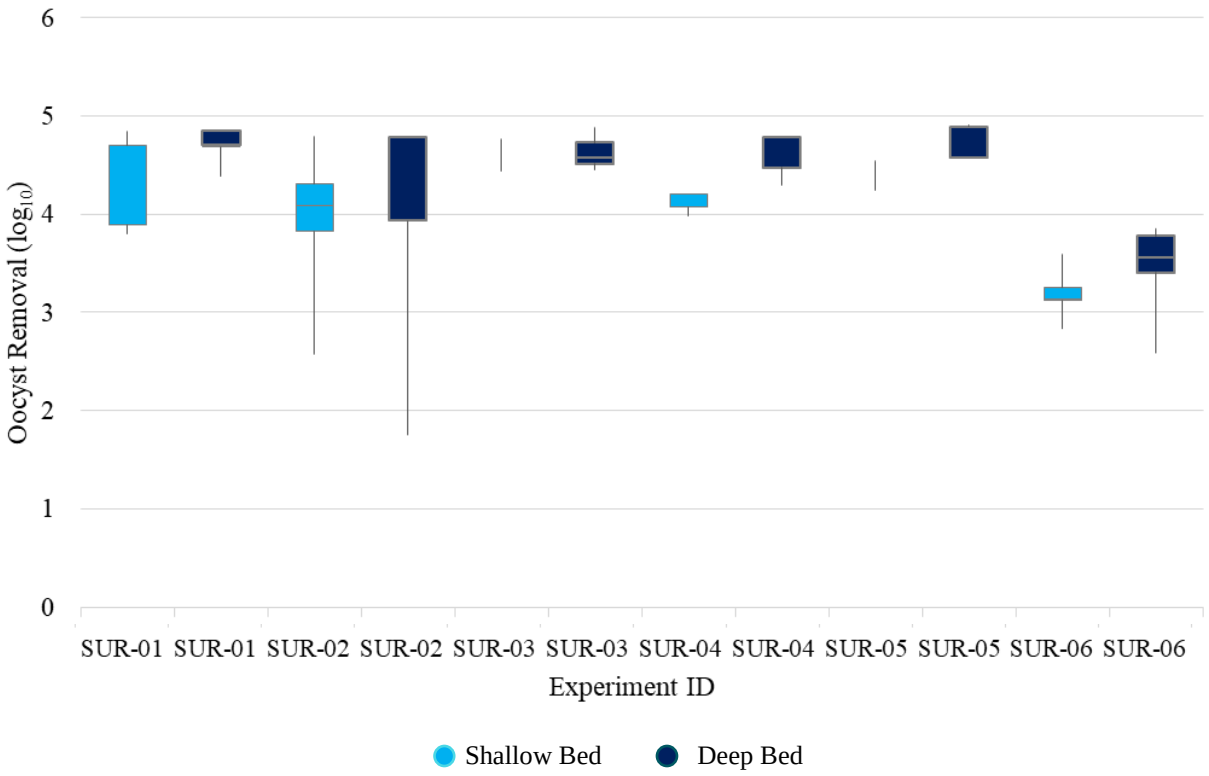


Figure 4-7. *C. parvum* oocyst removal by CAF during Phase 2 hydraulic surges experiments, n=5

Table 4-10. Average Oocyst Removals for Phase 2 Hydraulic Surge Experiments (n=5)

Identifier	Temperature (°C)	Alum Dose (mg/L)	Zeta Potential (mV)	Avg. Oocyst Removal (log_{10})	
				Shallow Bed	Deep Bed
SUR-1	13.1	40	N/A	4.2	4.8
SUR-2	12.5	40	N/A	3.2	4.4
SUR-3	4.3	40	N/A	4.7	4.7
SUR -4	4.3	40	N/A	4.1	4.5
SUR -5	5.3	40	N/A	4.5	4.7
SUR -6	8.8	40	0.5	3.1	3.1

C. parvum oocyst removals by shallow and deep bed CAF during hydraulic surge conditions ranged from 1.8 to 4.9 log (Appendix E). With the exception of SUR-6, oocyst removal by CAF

did not deteriorate during hydraulic surges relative to stable filter operation conditions. A statistical comparison between stable filter operation and the hydraulic surge tests was conducted (Table 4-11). The surge experiments were evaluated as SUR-1 to SUR-5, SUR-6, and as the whole group (SUR-1 to SUR-6) because CAF performance appeared different during SUR-6. The results indicated that oocyst removals during SUR-1 to SUR-5 and SUR-1 to SUR-6 were not significantly different from those observed during stable filter operation (5% significance level). However, when the SUR-6 experiment was independently compared to stable filter operation, the difference in oocyst removal by CAF was statistically significant (p=0.001 and p=0.005 for shallow and deep bed filters respectively). These observations are consistent with one of three experimental observations reported by Emelko (2001) and Huck et al. (2002), which resulted in an average oocyst removals of 4.0-log by CAF, while the remainder of the reported results indicated more deteriorated (2.7- and 0.2-log; Emelko, 2001) performance. Those authors commented specifically about the difficulty in achieving reproducible oocyst removals by CAF during hydraulic surge experiments; in comparison, the results presented herein were substantially more consistent between the replicate experiments.

Table 4-11. Comparison of Oocyst Removals in Stable Operation to those Hydraulic Surges using the Mann-Whitney U-test

Experiment		p-value*	W
SUR-1 to SUR-5	Shallow Bed	0.930	292
	Deep Bed	0.094	206
SUR-6	Shallow Bed	0.001	112
	Deep Bed	0.005	105
SUR-1 to SUR-6	Shallow Bed	0.290	404
	Deep Bed	0.550	311

*when p-value < 0.05, reject null hypothesis: samples come from the same distribution, no difference in medians

4.2.2 Effect of Temperature

For the purpose of this investigation, experiments conducted at settled water temperatures

exceeding 10 °C were considered warm water experiments while those conducted at water temperatures below 10 °C were considered cold water experiments. Cold and warm water experiments were conducted for all operational conditions except during middle of run and sub-optimal coagulation. Those two conditions, during Phase 2, were only evaluated at cold water temperatures. The results from shallow and deep bed filter removals for all experiments are summarized in a box and whisker plot (Figure 4-8).

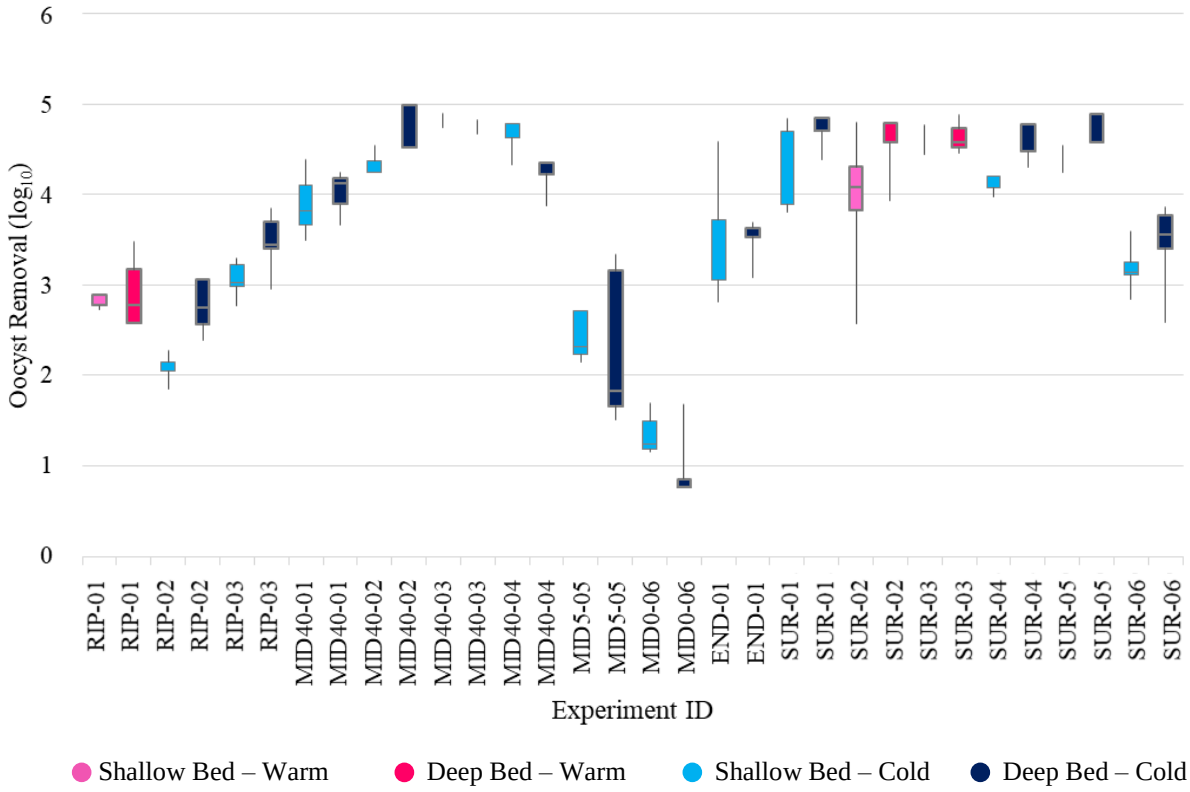


Figure 4-8. *C. parvum* oocyst removal by CAF in warm and cold water during stable operation (MID40), filter ripening (RIP), sub-optimal coagulation (MID0 and MID5), and hydraulic surge (SUR) experiments

The red tones in Figure 4-8 indicate warm water conditions, when settled water was above 10 °C in the pilot plant, and the blue tones indicate conditions where the settled water temperature was below 10 °C. The analysis presented in Table 4-12 reveals that median *C. parvum* oocyst removals by CAF during hydraulic surge conditions were not statistically different from those observed during stable filter operation. As can be seen in Figure 4-8, there were eight instances when filter

performance did not consistently exceed the 3-log oocyst removal target for well-operated treatment. This relatively deteriorated CAF performance can be attributed to specific operational conditions (e.g., sub-optimal coagulation, filter ripening) rather than water temperature.

For example, as previously discussed, the filter ripening experiments that did not allow for adequate mixing of oocysts in filter influent streams prior to filter influent sampling suggested poor oocyst removals by CAF, although they were most likely attributable to the experimental configuration (which was modified for the last of the ripening experiments). The remainder of the instances when oocyst by CAF removal were less than 3-log were performed under sub-optimal coagulation conditions; thus, those results were expected. While the warm water temperatures were not particularly warm (highest was 13.1 °C), water temperature did not affect *C. parvum* oocyst removal by CAF under any circumstances; these results are consistent with similar data reported by Emelko (2001) and Huck et al. (2002), who saw no deterioration during stable filter operation at temperatures as low as 1 °C.

Table 4-12. Comparison of *C. parvum* Oocyst Removal by CAF of Warm and Cold Water using the Mann-Whitney U-test

Experiment Type	Filter Bed Depth	p-value	W
SUR	Shallow Bed	0.843	105
	Deep Bed	0.612	112

*when p-value < 0.05, reject null hypothesis: samples come from the same distribution, no difference in medians

4.2.3 Effect of Filter Bed Depth

After the pilot-scale CAF experiments were completed, and water samples were processed and enumerated in the lab, oocyst removals by CAF were calculated using the time intervals outlined in Table 3-10. The sample collection times used to describe filter performance during the experiments were selected based on periods of consistent filter effluent oocyst counts, which indicated that pseudo steady state operational conditions had been achieved, and accounted for different travel distance in the columns above the media. The normality of these data was assessed using the Shapiro-Wilk test, Q-Q plots, and histograms (Appendix D). In many cases, the data

were not distributed in a manner consistent with the normal distribution, as would be expected during dynamic periods (ripening, hydraulic surges, etcs.) of CAF process operation. Accordingly, a non-parametric signed-rank statistical hypothesis test, the Mann-Whitney U-test, was utilized, and the shallow and deep bed CAF performance data were paired (Table 4-13).

Table 4-13. Summary of Results from Shapiro-Wilk and Wilcoxon Tests to Evaluate Data Set Normality and Compare Filter Performance between Filter Bed Depths, Respectively

Type of Exp.	Filter Pairs	Shapiro Wilk		Mann-Whitney U-test	
		w	p-value	w	p-value
MID40	Shallow	0.93	0.11	241	0.61
	Deep	0.90	0.028		
MID5/MID0	Shallow	0.90	0.14	107	0.26
	Deep	0.81	0.010		
END	Shallow	0.87	0.25	10	0.67
	Deep	0.79	0.073		
RIP	Shallow	0.92	0.18	65	0.051
	Deep	0.95	0.54		
SUR	Shallow	0.90	0.0048	291	0.019
	Deep	0.72	2.7E-06		

In general, these statistical analyses indicated that the shallow and deep bed filters did not perform differently from one another. Notably, there was no consistent trend in which the deviation from the median oocyst removal from the deep bed filter was lower than that in the shallow bed filter. One example in which the MID40-2 and MID40-4 experiments did suggest differences in performance is between the filter bed depths. In MID40-2, the deep bed filter outperformed the shallow bed filter, yet the spread in the data from the deep bed filter was much greater than that of

the shallow bed filter. For MID40-4, data from both the shallow and deep bed filters had similar spread, but the shallow bed filter outperformed the deep bed filter.

The most notable exceptions to comparable performance between the two filter depths were the hydraulic surge (SUR) experiments. Oocyst removal by the shallow and deep filters was statistically different during the hydraulic surge investigations ($p = 0.019$; Figure 4-7, Figure 4-8), with deep bed filters consistently out-performing the shallow bed filters at these conditions.

4.2.4 Zeta Potential

To analyse zeta potential, samples were collected prior to oocyst addition to settled water (SW), after oocysts were added (SW + O), and finally, after the specified alum addition for the experiment (SW + O + alum). Each reading reflects the average of the three individual measurements from a single sample. Zeta potentials and average oocyst removals from Phase 1 and Phase 2 are summarized (Table 4-14).

Table 4-14. Zeta Potentials Measured in Phase 1 and 2 Experiments

Exp. ID.	Zeta Potential (mV)			Oocyst Removal (log_{10})	
	Settled Water (SW)	SW + Oocysts (SW + O)	SW + O + Alum	Shallow Bed	Deep Bed
Phase 1					
P1 - 22.5b**	-22.0	-16.9	-9.5	3.5	3.8
P1 – 40a**	-21.4	-11.8	-5.2	4.1	3.3
P1 - 5	-12.2	-9.7	-15.6	1.2	1.2
P1 – 40b**	-16.0	-10.7	-7.1	3.6	3.8
Phase 2					
MID40-3	-13.2	-5.4	0.1	5.4	5.4
MID40-4	-8.2	-11.0	2.0	4.7	4.2
MID5-5	-11.3	-6.3	-10.0	2.4	1.9
MID0-6	-7.2	-7.4	-7.4*	1.3	0.9
END	-13.6	-10.1	1.3	3.2	3.3
RIP-3	-11.8	-10.5	-2.3	3.0	3.4
SUR-6	-16.3	-12.9	0.5	3.1	3.1

*no alum was added during this experiment, testing effect from no jar coagulation

**a, b distinguish between repeat experiments from Phase 1, experiments were run on two separate occasions with the same alum dose

Generally, settled water exhibited the lowest/most negative zeta potentials (-7.2 to -22.0 mV). These zeta potential measurements became less negative as oocysts (- 5.4 to -16.9 mV) and alum (2.0 to -15.6 mV) were added to the seed suspension. There were two instances in which the zeta potential decreased in the settled water (became less negative) after oocyst addition to the settled water (MID40-4 and MID0-6). These were the only experiments in which zeta potential was less negative than -10 mV in the settled water. The shift in zeta potential here to become more negative is likely due to the more negative nature of the oocysts, which were added to the settled water in

the high quantities necessary for demonstrating oocyst removal by CAF, that resulted in the majority of SW + O readings tending towards -10 to -12 mV.

Another trend in zeta potential was observed in the oocyst only seed suspension (SW + O) and following the alum addition. When 40 mg alum/L was added to the seed suspension, the zeta potential became less negative (closer to 0 mV) than it had been after oocysts were added to settled water (SW + O). The only experiments in which the zeta potential did not increase (i.e. become less negative) between oocyst addition and alum addition were the experiments when only 5 mg alum/L were added (P1 – 5 and MID5-5). These zeta potential data support the conclusions from Phase 1 (Table 4-2) that indicate the importance of adequate particle/oocyst destabilization by coagulation to achieve well operated CAF in which oocyst removal is maximized.

The final seed suspension zeta potential values (SW + O + SW) and oocyst removal were plotted (Figure 4-9 and Figure 4-10). The shallow bed and deep bed filter results were separated to avoid overlapping points and clearly display the results from each filter depth. The zeta potential values for the seed suspensions were the same for the shallow and deep bed filter as both filters used a common seed suspension. Thus, the main difference between Figure 4-9 and Figure 4-10 is the change in oocyst removal achieved by the shallow and deep bed filters. Otherwise, the trends related to zeta potential and oocyst removal are very similar between figures.

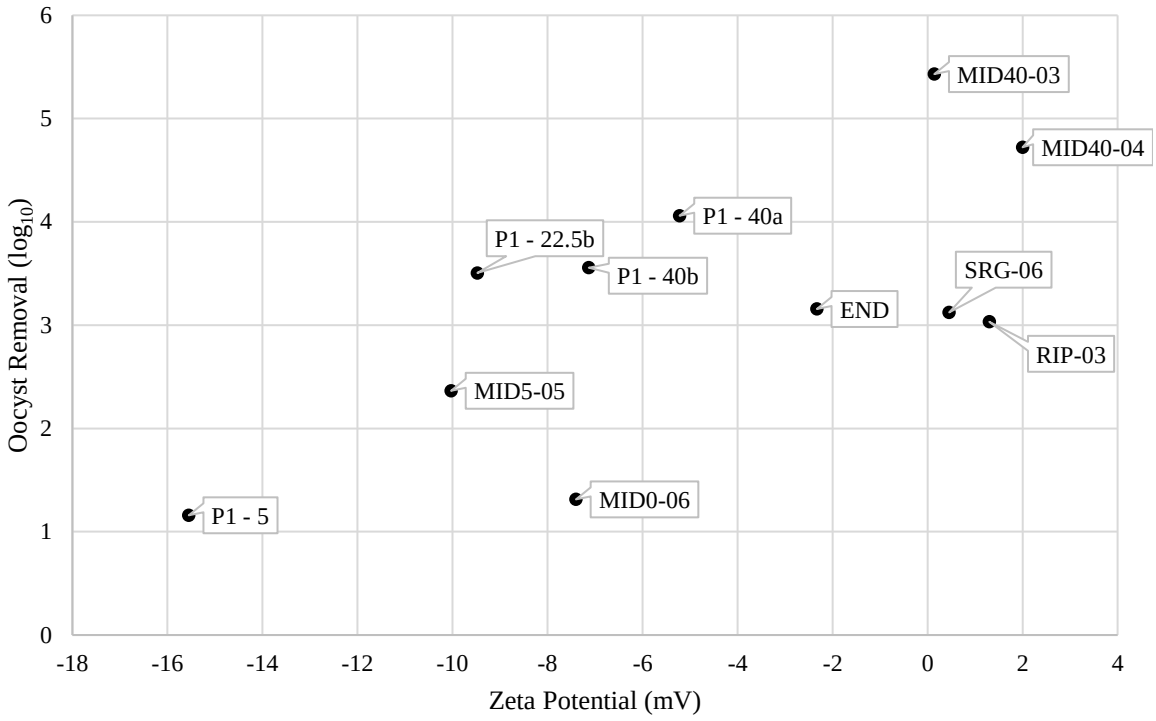


Figure 4-9. Shallow bed filter zeta potential vs oocyst removal for Phase 2 experiments.

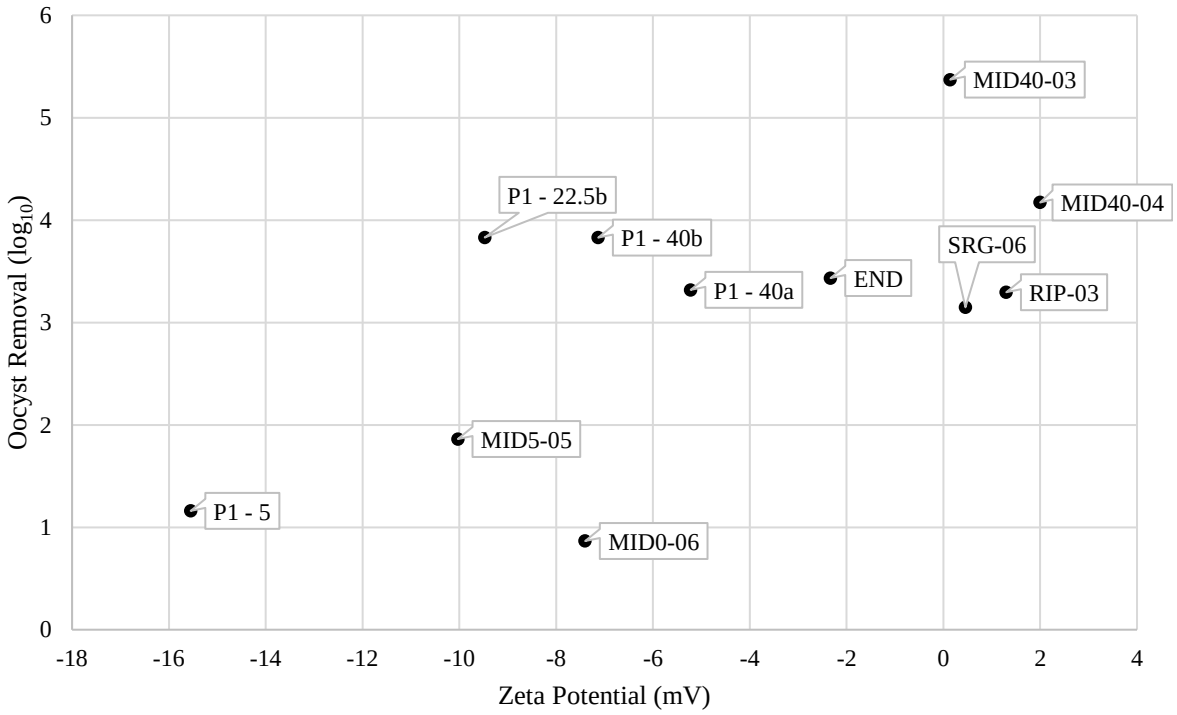


Figure 4-10. Deep bed filter zeta potential vs oocyst removal for Phase 2 experiments

The general trends in Figure 4-9 and Figure 4-10 indicate that all experiments in which coagulation was applied at doses above the typical plant dose (5 mg alum/L) resulted in ≥3 log oocyst removal. In addition, all experiments dosed at 40 mg alum/L produced zeta potentials of -7.1 mV or higher (i.e. less negative). There are three main data groupings in Figure 4-9 and Figure 4-10:

1. P1 – 5, MID5 – 5, MID0 – 6, and P1 – 22.5;
2. P1 – 40b, and MID0 – 6; and
3. RIP – 3, END, and SUR – 6.

The first set of points represent experiments in which sub-optimal coagulation (or no coagulation) resulted in oocyst removal below 3 log. P1 – 22.5 was an exception (Figure 4-9 and Figure 4-10) to this, but it was conducted at a coagulant dose that had previously resulted in poor oocyst removal; the first experiment conducted at 22.5 mg alum/L (P1 – 22.5a) resulted in average oocyst removals of 2.0 and 1.5 log in the shallow and deep bed filters, respectively. Therefore, it was

added to this group on the basis that coagulant doses associated with these experiments had resulted in sub-optimal oocyst removal due to inadequate coagulation.

This data grouping includes sub-optimal conditions which resulted in low oocyst removal and zeta potential values more negative than -4 mV. Zeta potential closer to the ZPC is expected (of course) as a result of more optimal coagulation (Bean et al., 1964; Bustamante et al., 2006; Cleasby et al., 1963; Ghernaout, 2015; Gupta et al., 1973; Karamanet al., 1999; Neuman, 1981; Riddick, 1961; Xagoraraki & Harrington, 2004; Xu et al., 2006). These results compare favorably with Xagoraraki & Harrington (2004) who concluded that charge neutralization was not the dominant mechanism in coagulation, but rather the formation of aluminum hydroxide precipitates. As expected, Figure 4-9 and Figure 4-10 illustrate the trend that relatively higher coagulant doses (and higher oocyst removal) are associated with zeta potentials near the ZPC. Alum speciation in the seed suspension was beyond the scope of this study but this could be done to further understand the relevant chemical phenomena that are occurring.

The second pairing of points mentioned above, P1 – 40b and MID0 – 06, are of interest based on the similarities of their zeta potentials (-7.1 and -7.4 mV, respectively), but associated with different oocyst removals (above and below 3 log, respectively). The main difference between the two experiments here was the coagulant dose: P1 – 40b coagulated at 40 mg alum/L while MID0 – 06 did not involve jar coagulation (i.e. no coagulant was added to the seed suspension). Here, interpretation of the net zeta potential is critical and it must be recognized that the oocysts were not adequately destabilized. This trend could be one reason the oocyst removals in P1 – 40b, and MID0 – 06 were so different; without the addition of at least some alum good oocyst removal will not occur (Dugan et al., 2001; Emelko, 2001; Huck, Coffey, Anderson, et al., 2002; Huck, Coffey, Emelko, et al., 2002; Masher & Hendricks, 1986). These data underscore the importance of recognizing that only net zeta potential is being evaluated and that the absolute value of zeta potential alone is inadequate for assessing particle/oocyst removal by CAF; rather, the zeta potential must be evaluated in parallel with understanding/ensuring that oocysts are being destabilized.

The remaining experiments (RIP-3, END, and SUR-6, pairing 3) were not conducted under stable filter operation conditions. That said, all had near 0 mV zeta potential values in the final seed

suspension and achieved oocyst removal between 3.0 and 3.4 log in both filter bed depths. While these removals were lower than those observed during MID40-3 and MID40-4 (4.2 to 5.4 log, respectively) which had similar zeta potential values, they still exceed 3.0 log removal, the baseline for "well operated" filtration and "good" oocyst removal. It should be noted that more exhaustive analysis is need for definitive conclusions; nonetheless, the RIP and END experiments, (when compared with stable filter operation experiments described in Section 4.2.1), revealed significant deterioration in filter performance during these periods, consistent with previous investigations (Emelko et al., 2005; Huck, Coffey, Emelko, et al., 2002). In contrast to these previous literature, however, these data also indicated that most of these differences could be overcome as long as adequate particle/oocyst destabilization (i.e. coagulation) could be achieved.

Chapter 5 Conclusions, Implications, and Recommendations

The overall goals of this study were to gain a better understanding of *Cryptosporidium* spp. oocyst removal by CAF in systems treating low turbidity, low TOC source water and provide strategies for improving CAF performance demonstrations. A protocol for conducting CAF performance demonstrations was developed and filter design (depth) and operational (sub-optimal coagulation, ripening, hydraulic surges, etc.) effects on *C. parvum* oocyst passage through CAF processes were investigated at pilot-scale. The utility of zeta potential for ensuring adequate protozoan pathogen removal by CAF in near-real-time was evaluated and the validity of utilizing high oocyst concentrations in filtration performance demonstrations to quantify the removal of lower/more environmentally relevant oocyst concentrations by CAF was confirmed.

5.1 Conclusions

Key findings from this research include the following:

1. CAF remains a critical and effective barrier against protozoan pathogen (i.e. *Cryptosporidium* spp. and *Giardia* spp.) passage into treated drinking water. Here, mean *C. parvum* oocyst removals of 4.3- and 4.4-log (ranging from 3.5- to 4.9-log and 4.4- to 5.0-log) were consistently achieved in shallow and deep bed pilot-scale filters respectively, during performance demonstrations conducted at optimal operating conditions (i.e., stable filter operation with appropriate jar coagulation of oocysts prior to filtration). These observations are consistent with what has been reported in other such investigations.

2. Performance demonstrations in which high concentrations of oocysts are introduced to CAF influent streams to quantify their removal, must be conducted carefully to ensure coagulation is not a limiting factor. This is especially important when low turbidity, low TOC (high quality) source waters (typically <0.9 NTU and <2.3 mg TOC/L in the present investigation) are evaluated. Here, turbidity increased from an average of 0.22 NTU to 0.70 NTU after oocysts were added to filter influent water to create the oocyst concentrations needed to demonstrate up to 5.0-log removal by CAF using direct membrane filtration and IFA staining for enumeration. These types of changes in filter influent quality during performance demonstrations have not been previously reported, likely because evaluations of *C. parvum* oocyst removal by CAF of low turbidity, low TOC source waters have

generally been considered to be less likely to contain sufficient quantities of oocysts to warrant expensive challenge testing.

Notably, the consistently observed changes in filter influent water quality (turbidity) associated with oocysts addition to filter influent streams suggest that demonstrations of protozoan pathogen removal by CAF must be conducted with care to ensure that well-operated/optimized filtration conditions are being represented. Thus, this work has two critical implications for conducting CAF performance demonstrations:

(i) coagulation of seed suspensions (i.e., jar coagulation) is likely required to effectively neutralize/destabilize oocyst surface charge (through mechanisms of charge neutralization or enmeshment of oocysts in metal salt precipitates) during performance demonstrations in order to reflect well-operated CAF (even when oocysts are added to clarified water); and

(ii) jar coagulation of oocyst seed suspensions may require higher coagulant doses than those used during regular treatment (i.e., when no oocysts are added to filter influent streams) when the performance of CAF of low turbidity, low TOC source waters is being evaluated.

3. While it is commonly recognized that appropriate chemical pre-treatment is important for achieving well-operated CAF and protozoan pathogen removal, this work demonstrated that it is <u>especially critical</u> to ensuring optimal oocyst removal by CAF in systems treating high quality source water. In these situations, relatively small shifts in source water quality can substantially affect both coagulation regime (i.e., the primary mechanism(s) by which particles are destabilized to enable their removal by CAF: charge neutralization, sweep floc coagulation, enmeshment in metal salt precipitate) and efficacy. Here, excellent oocyst removals (3.7-log on average) were achieved in all cases (i.e., regardless of filter design [depth] and operational conditions [hydraulic surge, ripening, end-of-run, ripening, increased loading rate, water temperature]) as long as appropriate chemical pre-treatment was implemented. When adequate jar coagulation was not implemented, oocyst removals by CAF decreased to 1.3-log on average.

These observations underscore the importance of coagulation as a critical control for ensuring protozoan pathogen removal by CAF in systems treating low turbidity, low TOC source waters. These results markedly differ from reports from systems with higher source water TOC and turbidity, in which chemical pre-treatment had a less significant impact on oocyst passage through CAF than period in the filter cycle (e.g., end-of-run filtration, ripening. This difference underscores the importance of coagulation regime and its relationship to filter performance.

4. To further the previous conclusion, jar coagulation of oocysts was identified as the most important factor pertaining to the deterioration of filter performance as evidenced by MID5-5 and MID0-6. In this research, filter performance during sub-optimal and no jar coagulation conditions resulted in removals of oocysts that were significantly impaired (< 3.0 log) as compared to stable filter operation. These results quantitatively speak to the differences in oocyst log removal when particle/oocyst destabilization was insufficient in the seed suspension.

5. In addition to sub-optimal coagulation, ripening and end of run experiments resulted in a deterioration of oocyst removal (~0.5 log decrease on average) by CAF. These results have been previously observed by others (Huck, Coffey, Emelko, et al., 2002) where a deterioration in filter performance was observed while overall oocyst removals remained above 3.0 log. This further underscores the importance of proper coagulation as a filter operational conditions did not contribute to oocyst passage that resulted in less than 3.0 log removal and were not a limiting factor in CAF performance of oocyst removal.

6. Although filter effluent turbidity is a good indicator of treatment performance, it is not necessarily a good indicator of *C. parvum* oocyst removal by CAF, in systems with low turbidity, low TOC source water, such as the one studied herein. In the present investigation, a very wide range of oocyst removals (ranging from 0.8- to 5.0-log) by pilot-scale CAF was observed when coagulant dose and associated oocyst surface charge (indicated by zeta potential) in the seed suspension was varied, despite filter operation that would be considered “well-operated” with effluent turbidities that were always less than 0.2 NTU and below 0.1 NTU the majority of the time. Such a wide range of oocyst

removals by "well-operated" filters during periods of stable operation has not been previously reported.

Notably, these observations have two important implications:

(i) the criteria for what constitutes "well-operated" treatment (Health Canada, 2017; MOECP, 2018) for ensuring protozoan pathogen removal by CAF must be further clarified and validated; and,

(ii) additional support infrastructure/tools should be developed and validated to better ensure adequate protozoan pathogen removal by CAF in (near) real-time.

7. Zeta potential analysis is useful for ensuring optimal CAF performance in systems treating low turbidity, low TOC source water that requires sweep floc coagulation for adequate particle destabilization. Here, zeta potential values between -5 mV and 0 mV during chemical pre-treatment consistently resulted in mean *C. parvum* oocyst removals of 3.0-log or greater.

   Given that low filter effluent turbidities (< 0.1 NTU) alone were inadequate for ensuring oocyst removal during the pilot-scale investigations reported herein, this work suggests that a combination of zeta potential analysis and turbidity monitoring may offer better control of CAF as a barrier against *C. parvum* oocyst passage into treated drinking water. This combination is likely relevant for systems in which either enmeshment or charge neutralization are the dominant mechanisms of coagulation.

8. Both the deep and shallow filter bed designs (750 mm and 500 mm, respectively) investigated herein can achieve excellent (i.e., >3-log) *C. parvum* oocyst removal at optimal operating conditions. The statistical comparisons between the filters revealed that the deep and shallow bed configurations performed similarly with the exception of during hydraulic surges during which the deep bed filter outperformed the shallower configuration. Thus, in some instances a deeper bed filter design may offer additional operational resilience when influent water quality changes or other operational challenges (e.g., hydraulic surges) occur.

9. High oocyst concentrations can be used in filtration performance demonstrations to quantify the removal of lower/more environmentally relevant oocyst concentrations by

CAF, under the conditions investigated herein. This result is in contrast to another study that reported that *C. parvum* oocyst removal by CAF was affected by influent oocyst concentration (Assavasilavasukul et al., 2008). In the present investigation, two filter influent oocysts concentrations were investigated: ~10^2 oocysts/L (which is a lower, more environmentally relevant oocyst concentration (LeChevallier & Norton, 1995; LeChevallier, Norton, & Lee, 1991) and ~10^5 oocysts/L (which enabled performance demonstrations in which up to 5.0-log oocysts removal by CAF could be quantitatively evaluated using direct membrane filtration and IFA staining). Notably, similar removals of oocysts by CAF were observed, irrespective of influent oocyst concentration.

This result has two critical implications:

(1) it illustrated that filter performance demonstrations in which high concentrations of oocysts are introduced to CAF influent streams to quantify their removal are a valid approach for quantitatively evaluating oocyst removal by CAF; and

(2) it validated the appropriateness of the jar coagulation procedure used during the pilot-scale investigations because comparable results were achieved between the low and high influent oocyst concentration experiments, which also required different coagulant doses due to the associated changes in water quality that resulted from oocyst addition to the filter influent streams.

10. Temperature ranged from 4.1 to 13.1°C over the course of the research, and cold water temperature was determined as water temperature at and below 10°C. The majority of the experiments were conducted during cold water temperatures (thirteen of sixteen) and achieved greater than 3.0 log removal, with the exception of MID5-5 and MID6-0 where sub-optimal and no jar coagulation were present. These results showed that water temperature did not affect *C. parvum* oocyst removal by CAF under any circumstances other than during suboptimal jar coagulation, which is consistent with similar data reported by Emelko (2001) and Huck et al. (2002), who saw no deterioration during stable filter operation.

5.2 Recommendations

The following recommendations for operations and management, and further research are proposed based on the conclusions of this work.

5.2.1 Operations and Management

The following recommendations are proposed for conducting performance demonstrations of oocyst removal by CAF of high quality source water:

1. Monitor clarified water/filter influent zeta potential to ensure adequate charge neutralization prior to filtration;
2. Jar coagulate seed suspensions of *C. parvum* oocysts (or other particles) to ensure that operational conditions during performance demonstrations reflect "well-operated" treatment—this is especially critical when oocyst addition significantly changes filter influent water quality; and,
3. Respond as quickly as possible when sub-optimal coagulation is evident—zeta potential analysis may be more sensitive than turbidity in identifying this situation.

5.2.2 Research

Several suggestions for improvement or further areas to study are listed below to build upon the findings of this thesis research.

1. More data related to non-ideal operational conditions should be collected (specifically during hydraulic surges, ripening, and end-of-run conditions) to build a more comprehensive understanding of zeta potential analysis use at these conditions.
2. Perform additional confirmatory studies using more environmentally relevant/lower concentrations of *Cryptosporidium* oocysts at a wider range of operational conditions.

References

Adam, E. A., Collier, S. A., Fullerton, K. E., Gargano, J. W., & Beach, M. J. (2017). Prevalence and direct costs of emergency department visits and hospitalizations for selected diseases that can be transmitted by water, United States. *Journal of Water and Health*, *15*(5), 673–683. http://doi.org/10.2166/wh.2017.083

Al-Ani, M. Y., Hendricks, D. W., Logsdon, G. S., & Hibler, C. P. (1986). Removing , Giardia Cysts From Low Turbidity Waters by Rapid Rate Filtration. *AWWA Journal*, (5), 66–73.

Amburgey, J. E., Amirtharajah, A., York, M. T., Brouckaert, B. M., Spivey, N. C., & Arrowood, M. J. (2005). Comparison of conventional and biological filter performance for *Cryptosporidium* and microsphere removal. *Journal / American Water Works Association*, *97*(12), 77–91.

Amirtharajah, A., & Mills, K. M. (1982). Rapid-mix design for mechanisms ~ of alum coagulation. *American Water Works Association Journal*, *47*(4), 210–216.

Assavasilavasukul, P., Lau, B. L. T., Harrington, G. W., Hoffman, R. M., & Borchardt, M. A. (2008). Effect of pathogen concentrations on removal of *Cryptosporidium* and *Giardia* by conventional drinking water treatment. *Water Research*, *42*(10–11), 2678–2690. http://doi.org/10.1016/j.watres.2008.01.021

Baldursson, S., & Karanis, P. (2011). Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2004-2010. *Water Research*, *45*(20), 6603–14. http://doi.org/10.1016/j.watres.2011.10.013

Barkay-Arbel, Y., Hatukai, S., Asheri, T., Vaizel-Ohayon, D., & Rebhun, M. (2012). Performance and process mechanisms of a high-rate direct filtration plant targeting 0.1 ntu. *Journal - American Water Works Association*, *104*(12). http://doi.org/10.5942/jawwa.2012.104.0156

Bean, E., Campbeii, S., & Anspach, F. (1964). Zeta Potential Measurements in the Control of Coagulation Chemical Doses. *AWWA Journal*, (2), 214–224.

Brink, D. R., Hendricks, D. W., & Al-Ani, M. Y. (1988). Bench-Scale Evaluation of coagulants for Low Turbidity Water. *AWWA Journal*, (April), 199–204.

Brown, T. J., & Emelko, M. B. (2009). Chitosan and metal salt coagulant impacts on *Cryptosporidium* and microsphere removal by filtration. *Water Research*, *43*(2), 331–338. http://doi.org/10.1016/j.watres.2008.10.035

Bustamante, H. A., Shanker, S. R., Pashley, R. M., & Karaman, M. E. (2006). Interaction Between *Cryptosporidium* Oocysts And Water Treatment Coagulants. *Water Research*, *35*(13), 3179–3189.

Butkus, M. A., Bays, J. T., & Labare, M. P. (2003). Influence of Surface Characteristics on the Stability of. *Applied and Environmental Microbiology*, *69*(7), 3819–3825. http://doi.org/10.1128/AEM.69.7.3819

CDC, C. for D. C. and P. (2010). Waterborne Diseases Could Cost over $500 Million Annually in U.S. Retrieved from https://www.cdc.gov/media/pressrel/2010/r100714.htm

Chyzheuskaya, A., Cormican, M., Srivinas, R., Donovan, D. O., Prendergast, M., Donoghue, C. O., & Morris, D. (2017). Economic Assessment of Waterborne Outbreak of Cryptosporidiosis. *Emerging Infectious Diseases*, *23*(10). http://doi.org/10.3201/eid2310.152037

Cleasby, J. L., Williamson, M. M., & Baumann, E. R. (1963). Effect of Filtration Rate Changes on Quality. *Journal - American Water Works Association*, *55*(7), 869–880.

Crittenden, J. C., Trussell, R. R., Hand, D. W., Howe, K. J., & Tchobanoglous, G. (2012). *MWH's Water Treatment: Principles and Design, 3rd Edition*. Hoboken, New Jersey: John Wiley & Son, Inc.

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). http://doi.org/10.1016/S0043-1354(02)00045-3

Davies, C. M., Ferguson, C. M., Kaucner, C., Krogh, M., Altavilla, N., Deere, D. A., & Ashbolt, N. J. (2004). Dispersion and Transport of *Cryptosporidium* Oocysts from Fecal Pats under Simulated Rainfall Events. *Applied and Environmental Microbiology*, *70*(2), 1151–1159.

Davis, K. L., Thal, L. J., Gamzu, E. R., Davis, C. S., Woolson, R. F., Gracon, S. I., … Doody, R. S. (1994). A Massive Outbreak in Milwaukee of *Cryptosporidium* Infection Transmitted

Through the Public Water Supply. *The New England Journal of Medicine*, *327*, 161–167.

Dillingham, R. A., Lima, A. A., & Guerrant, R. L. (2002). Cryptosporidiosis: Epidemiology and impact. *Microbes and Infection*, *4*(10), 1059–1066. http://doi.org/10.1016/S1286-4579(02)01630-1

Driscoll, C. T., & Letterman, R. D. (1987). *Residual aluminum in filtered water*. *AWWARF and AWWA*. Denver, Colorado.

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*(December 2001), 64–76.

Dugan, N. R., & Williams, D. J. (2004). Removal of *Cryptosporidium* by in-line filtration: Effects of coagulant type, filter loading rate and temperature. *Journal of Water Supply: Research and Technology - AQUA*, *53*(1).

Edzwald, J. K. (1993). Coagulation in drinking water treatment: particules, organics and coagulants. *Water Science & Technology*, *27*(11), 21–35.

Edzwald, J. K. (2011). *Water Quality and Treatment: A Handbook on Drinking Water*, *Sixth Edition*. American Water Works Association, American Society of Civil Engineers, McGraw-Hill.

Edzwald, J. K., & Kelley, M. B. (1998). Control of *Cryptosporidium*: From Reservoirs to Clarifiers to Filters. *Water Science & Technology*, *37*(2), 1–8.

Edzwald, J. K., & Lawler, D. F. (1983). Mechanisms of particle destabilization for polymers in water treatment. In *American Water Works Association Seminar on Use of Organic Polyelectrolytes in Water Treatment*.

Efstratiou, A., Ongerth, J. E., & Karanis, P. (2017). Waterborne transmission of protozoan parasites: Review of worldwide outbreaks - An update 2011–2016. *Water Research*, *114*(January), 14–22. http://doi.org/10.1016/j.watres.2017.01.036

Emelko, M. B. (2001). Removal of *Cryptosporidium parvum* by granular media filtration, 374. Retrieved from http://hdl.handle.net/10012/614

Emelko, M. B. (2003). Removal of viable and inactivated *Cryptosporidium* by dual- and tri-media filtration. *Water Research*, *37*(12), 2998–3008. http://doi.org/10.1016/S0043-1354(03)00113-1

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. http://doi.org/10.1002/j.1551-8833.2003.tb10371.x

Emelko, M. B., Schmidt, P. J., & Roberson, J. A. (2008). Quantification of uncertainty in microbial data - reporting and regulatory implications. *Journal / American Water Works Association*, *100*(4), 167.

Fayer, R. (1997). *Cryptosporidium and Cryptosporidiosis*. Salem: CRC Press LLC.

Fayer, R., Morgan, U., & Upton, S. J. (2000). Epidemiology of *Cryptosporidium*: Transmission, detection and identification. *International Journal for Parasitology*, *30*(12–13), 1305–1322. http://doi.org/10.1016/S0020-7519(00)00135-1

Ghernaout, D. (2015). Controlling Coagulation Process: From Zeta Potential to Streaming Potential. *American Journal of Environmental Protection*, *4*(5), 16. http://doi.org/10.11648/j.ajeps.s.2015040501.12

Gupta, V. S., Bhattacharjya, S. K., & Dutta, B. K. (1973). Zeta-Potential Control for Alum Coagulation. *AWWA Journal*, *12*(5), 21–23.

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.

Headd, B., & Bradford, S. A. (2015). Use of aerobic spores as a surrogate for cryptosporidium oocysts in drinking water supplies. *Water Research*, *90*, 185–202. http://doi.org/10.1016/j.watres.2015.12.024

Health Canada. (2012). *Guidelines for Canadian Drinking Water Quality: Technical Document –*

Enteric Protozoa: Giardia and Cryptosporidium. Retrieved from https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/water-quality/enteric-protozoa-giardia-cryptosporidium.html#a93

Health Canada. (2017). Guidelines for Canadian Drinking Water Quality - Summary Table, (February 2017). Retrieved from 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

Huck, P. M., Coffey, B. M., Anderson, W. B., Emelko, M. B., Maurizio, D. D., Slawson, R. M., … O'Melia, C. R. (2002). Using turbidity and particle counts to monitor *Cryptosporidium* removals by filters. *Water Science and Technology: Water Supply*, *2*(3), 65–71. http://doi.org/10.2166/ws.2002.0086

Huck, P. M., Coffey, B. M., Emelko, M. B., Maurizio, D. D., Slawson, R. M., Anderson, W. B., … O'Melia, C. R. (2002). Effects of filter operation on *Cryptosporidium* removal. *Journal / American Water Works Association*, *94*(6), 97–111.

Karaman, M. E., Pashley, R. M., Bustamante, H. A., & Shanker, S. R. (1999). Microelectrophoresis of *Cryptosporidium parvum* oocysts in aqueous solutions of variousinorganic and surfactant cations. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *146*, 212–221.

Karanis, P., Kourenti, C., & Smith, H. (2007). Fault tree analysis of the causes of waterborne outbreaks. *Journal of Water and Health*, *5*(SUPPL. 1), 1–18. http://doi.org/10.2166/wh.2006.002

Keegan, A., Daminato, D., Saint, C. P., & Monis, P. T. (2008). Effect of water treatment processes on *Cryptosporidium* infectivity. *Water Research*, *42*(6–7), 1805–1811. http://doi.org/10.1016/j.watres.2007.11.008

Kundert, K., Emelko, M. B., Mielke, L., Elford, T., & Ruecker, N. (2014). Alberta Flood 2013 – City of Calgary Water Treatment System Resiliency. In *16th National Conference on Drinking Water* (pp. 26–29). Gatineau, QC, Canada,.

Lalancette, C., Di Giovanni, G. D., & Prévost, M. (2010). Improved Risk Analysis by Dual Direct

Detection of Total and Infectious *Cryptosporidium* Oocysts on Cell Culture in Combination with Immunofluorescence Assay. *Applied and Environmental Microbiology*, *76*(2), 566–577. http://doi.org/10.1128/AEM.01496-09

Lartiges, B. S., Bottero, J. Y., Derrendinger, L. S., Humbert, B., Tekely, P., & Suty, H. (1997). Flocculation of Colloidal Silica with Hydrolyzed Aluminum: An 27 Al Solid State NMR Investigation. *Langmuir*, *13*(15), 147–152. http://doi.org/10.1021/la951029x

LeChevallier, M. W., & Norton, W. D. (1995). Giardia and Cryptosporidium in raw and finished water. *American Water Works Association Journal*, *87*(9), 54–68.

LeChevallier, M. W., Norton, W. D., & Lee, R. G. (1991). Occurrence of Giardia and *Cryptosporidium spp.* in surface water supplies. *Applied and Environmental Microbiology*, *57*(9), 2610–2616.

Logsdon, G. S. (2000). Effective management and operation of coagulation and filtration. *Water, Air, and Soil Pollution*, *123*(1–4), 159–166.

Mahmoudi, M. R., Ongerth, J. E., & Karanis, P. (2017). *Cryptosporidium* and cryptosporidiosis: The Asian perspective. *International Journal of Hygiene and Environmental Health*, *220*(7), 1098–1109. http://doi.org/10.1016/j.ijheh.2017.07.005

Masher, R. R., & Hendricks, D. W. (1986). Rapid Rate Filtration of Low Turbidity Water Using Field-Scale Pilot Filters. *AWWA Journal*, (12), 42–51.

McTigue, N. E., & Symons, J. M. (Eds.). (2010). *The Water Dictionary* (Second). Denver, CO: American Water Works Association.

MOECC. (2016). *Procedure for Disinfection of Drinking Water in Ontario*.

MOEE. (2000). *Ontario Regulation 459/00: Drinking Water Protection – Larger Water Works*.

Moreira, N. A., & Bondelind, M. (2017). Safe Drinking Water and Waterborne Outbreaks. *Journal of Water and Health*, *15*(1), 83–96.

Neuman, W. E. (1981). Optimizing Coagulation with Pilot Filters and Zeta Potential. *AWWA Journal*, (9), 472–475.

Nieminski, E. C., & Ongerth, J. E. (1995). Removing *Giardia* and *Cryptosporidium* by

conventional treatment and direct filtration, (C), 96–106.

Ongerth, J. E., & Pecoraro, J. P. (1995). Removing C*ryptosporidium* using multimedia filters. *Journal / American Water Works Association*, *87*(12), 83–89. http://doi.org/10.1017/CBO9781107415324.004

Payment, P., Plante, R., & Cejka, P. (2001). Removal of Indicator Bacteria, Human Enteric Viruses, Giardia Cysts, and *Cryptosporidium* Oocysts at a Large Wastewater Primary Treatment Facility. *Canadian Journal of Microbiology*, *47*(3), 188–193.

Pernitsky, D. J. (2003). *Coagulation 101*. Retrieved from https://awwoa.ab.ca/home/pdfs/Coagulation 101.pdf%5Cnhttp://medcontent.metapress.com/index/A65RM03P4874243N.pdf%5Cnhttps://www.awwoa.ab.ca/home/pdfs/Coagulation 101.pdf

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), 121–141. http://doi.org/10.2166/aqua.2006.062

Quintero-Betancourt, W., Peele, E. R., & Rose, J. B. (2002). *Cryptosporidium parvum* and Cyclospora cayetanensis: A review of laboratory methods for detection of these waterborne parasites. *Journal of Microbiological Methods*, *49*(3), 209–224. http://doi.org/10.1016/S0167-7012(02)00007-6

Ramsay, C. N., Wagner, A. P., Robertson, C., Smith, H. V., & Pollock, K. G. J. (2014). Effects of Drinking-Water Filtration on *Cryptosporidium* Seroepidemiology, Scotland. *Emerging Infectious Disease Journal*, *20*(1), 70–77.

Ridderstedt, F., Widerström, M., Lindh, J., & Lilja, M. (2017). Sick Leave due to Diarrhea caused by Contamination of Drinking Water Supply with *Cryptosporidium hominis* in Sweden: A Retrospective Study. *Journal of Water and Health*, *In Press–U*, wh2017311. http://doi.org/10.2166/wh.2017.311

Riddick, T. M. (1961). Zeta Potential and Its Application to Difficult Waters. *AWWA Journal*, (8), 1007–1030.

Rochelle, P. A., Johnson, A. M., Leon, R. De, & Di Giovanni, G. D. (2012). Assessing the risk of

infectious *Cryptosporidium* in drinking water. *Journal - American Water Works Association*, *104*(5), 325–336. http://doi.org/10.5942/jawwa.2012.104.0063

Ryan, U., & Hijjawi, N. (2015). New Developments in *Cryptosporidium* Research. *International Journal of Parasitology*, *45*(6), 367–373.

Schmidt, P. J., & Emelko, M. B. (2011). QMRA and decision-making: Are we handling measurement errors associated with pathogen concentration data correctly? *Water Reserach*, *45*(2), 427–438.

Scott, D. J. (2008). *Cryptosporidium and Particle Removal from Low Turbidity Water by Engineered Ceramic Media Filtration*.

Shaw, B. Y. K., Walker, S., & Koopman, B. E. N. (2000). Improving Filtration of Cryptosporidium, (November), 7–9.

Silins, U., & Emelko, M. B. (2017). Wildfire Impacts on Watersheds and Drinking Water Supply and Treatment. In *British Columbia Ministry of Forests, Lands, and Natural Resource Operations*. Kamloops, BC.

Snicer, G. A., Malley, J. P. J., Margolin, A. B., & Hogan, S. P. (2000). UV Inactivation of Viruses in Natural Waters. In *AWWA Research Foundation and American Water Works Association*. Denver, CO.

Swertfeger, J., Metz, D. H., DeMarco, J., Braghetta, A., & Jacangelo, J. G. (1999). Effect of filter media on cyst and oocyst removal. *Journal / American Water Works Association*, *91*(9), 90–100. http://doi.org/10.2166/aqua.2013.104

Tomko, R., & Scheuring, M. (2002). Enhanced coagulation and removal of *Cryptosporidium*. *AWWA Journal*, (November), 67–77.

Torabian, A., Hesam Hasani, A., & Mehrabadi, A. R. (2008). Effect of physical and chemical parameters on water treatment plant effluent quality (particle count and particle size). *WIT Transactions on Ecology and the Environment*, *111*. http://doi.org/10.2495/WP080471

Tufenkji, N., & Emelko, M. B. (2011). Groundwater Pollution: Impacts on Human Health: Fate and Transport of Microbial Contaminants. *Encyclopedia of Environmental Health*, 715–726.

http://doi.org/10.1016/B978-0-444-52272-6.00040-4

USEPA. (1999). *Microbial and Disinfection Byproduct Rules Simultaneous Compliance Guidance Manual*.

USEPA. (2001). National Primary Drinking Water Regulations: Long Term 2 Enhanced Surface Water Treatment Rule. Retrieved from https://www.reginfo.gov/public/do/eAgendaViewRule?pubId=200104&RIN=2040-AD37

USEPA. (2005). *Method 1623: Cryptosporidium and Giardia in Water by Filtration/IMS/FA*.

USEPA. (2006a). *Long Term 2 Enhanced Surface Water Treatment Rule; Final Rule*. Federal Register.

USEPA. (2006b). National Primary Drinking Water Regulations: Long term 2 Enhanced Surface Water Treatment Rule (40 CFR Parts 9, 141, and 142). *Federal Register*, *71*(3), 654–786.

Van Benschoten, J. E., & Edzwald, J. K. (1990). Chemical aspects of coagulation using aluminum salts - II. Coagulation of fulvic acid using alum and polyaluminum chloride. *Water Research*, *24*(12), 1527–1535. http://doi.org/10.1016/0043-1354(90)90087-M

Welker, R., Porter, R., Pett, W. B., Provart, M. R., Schwartz, M., & Kauri, T. (1994). Cryptosporidiosis outbreak in Kitchener-Waterloo: identification, assessment and future prevention. In *In Planning for Tomorrow – Proceedings of the Sixth National Conference on Drinking Water* (pp. 39–60). Ottawa, Ontario, Canada: Canadian Water and Wastewater Association.

WHO. Guidelines for Drinking-Water Quality. Fourth Ed. Including First addendum (2017). Geneva, Switzerland. Retrieved from http://www.who.int/water_sanitation_health/publications/drinking-water-quality-guidelines-4-including-1st-addendum/en/

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 Disease Journal*, *20*(4), 581. http://doi.org/10.3201/eid2004.121415

Xagoraraki, I., & Harrington, G. W. (2004). Zeta potential, dissolved organic carbon, and removal of *Cryptosporidium* oocysts by coagulation and sedimentation. *Journal of Environmental Engineering-Asce*, *130*(12), 1424–1432. http://doi.org/Doi 10.1061/(Asce)0733-9372(2004)130:12(1424)

Xagoraraki, I., Harrington, G. W., Assavasilavasukul, P., & Standridge, J. H. (2004a). Removal of emerging waterborne pathogens and pathogen indicators. *AWWA Journal*, *95*(5), 102–113.

Xagoraraki, I., Harrington, G. W., Assavasilavasukul, P., & Standridge, J. H. (2004b). Removal of emerging waterborne pathogens and pathogen indicators pilot-scale conventional treatment. *Journal / American Water Works Association*, *96*(5), 102–113. http://doi.org/10.1002/j.1551-8833.2004.tb10632.x

Xu, G. R., Fitzpatrick, C. S. B., & Deng, L. Y. (2006). Effects of filtration temperature, humic acid level and alum dose on cryptosporidium sized particle breakthrough. *Water Science and Technology*, *54*(11–12), 353–361. http://doi.org/10.2166/wst.2006.783

Zahedi, A., Paparini, A., Jian, F., Robertson, I., & Ryan, U. (2016). Public health significance of zoonotic *Cryptosporidium* species in wildlife: Critical insights into better drinking water management. *International Journal for Parasitology: Parasites and Wildlife*, *5*(1), 88–109. http://doi.org/10.1016/j.ijppaw.2015.12.001

Appendix A – Summary of Full- and Pilot-Scale Water Characteristics from Each Experiment

Date	Experiment Tag	Temperature - Pilot	Temperature - Full Scale (RW)	pH - Pilot	pH - Full Scale (RW)	Experiment Coagulant Dose - Pilot	Coagulant Dose - Pilot	Coagulant Dose - Full Scale	Exp. Flow Rate Filter 1 - Pilot	Exp. Flow Rate Filter 3 - Pilot	Total Flow Rate - Full Scale
Units		°C	°C			mg/L	mg/L	mg/L	L/min	L/min	L/min
Phase 1 Experiments											
11-May-17	P1 – 22.5a	7.9	6.4	7.5	7.9	22.5	5.0	5.0	1.49	1.48	2.9
08-Jun-17	P1 – 22.5b	12.2	11.1	7.4	7.7	22.5	5.5	5.0	1.48	1.49	3.4
09-Jun-17	P1 – 40a	11.7	9.9	7.4	7.7	40	5.5	5.0	1.47	1.48	3.3
02-Aug-17	P1 – 5	16.2	14.0	7.2	7.7	5	5.5	5.0	1.48	1.48	4.5
03-Aug-17	P1 – 40b	14.8	11.9	7.2	7.6	40	5.5	4.5	1.48	1.48	4.2
Phase 2 Experiments											
11-Dec-17	RIP-1	12.0	4.8	6.8	7.7	40	4.0	4.0	0.68	0.67	3.2
09-Jan-18	RIP-2	4.1	2.5	7.0	7.7	40	4.0	5.0	0.63	0.62	3.5
29-May-18	RIP-3	9.1	6.7	7.2	7.7	40	4.5	4.5	0.68	0.70	3.0
05-Oct-17	MID40-1	16.0	15.9	7.2	7.8	40	5.5	*	0.84	0.67	3.5
23-Oct-17	MID40-2	9.7	6.6	7.0	7.6	40	4.5	*	0.78	0.67	3.5
06-Mar-18	MID40-3	4.5	2.8	6.9	7.7	40	4.5	4.5	0.69	0.69	3.3
19-Mar-18	MID40-4	4.6	2.6	6.9	7.7	40	4.5	*	0.68	0.74	5.8
10-Apr-18	MID5-5	4.9	2.9	7.0	7.7	5	4.3	4.5	0.68	0.79	5.6
23-Apr-18	MID0-6	5.0	3.1	7.1	7.7	0	5.0	5.0	0.68	0.77	4.0
29-May-18	END-1	9.1	6.7	7.2	7.7	40	4.5	4.5	0.68	0.70	3.0
13-Nov-17	SUR-1	13.1	5.1	6.8	7.7	40	4.8	4.0	0.70	0.72	3.3
28-Nov-17	SUR-2	12.5	2.2	6.9	7.9	40	6.0	*	0.70	0.69	3.3
22-Jan-18	SUR-3	4.3	2.1	6.8	7.9	40	4.3	3.9	0.71	0.71	3.0
06-Feb-18	SUR-4	4.3	2.3	6.9	7.7	40	4.5	4.5	0.69	0.69	3.3
21-Feb-18	SUR-5	5.3	2.2	6.9	7.6	40	4.0	4.5	0.68	0.68	3.2
19-Jun-18	SUR-6	8.9	6.7	7.2	7.7	40	4.5	4.5	0.67	0.70	3.0
Method 1623 Experiments											
16-Jul-18	--	11.0	7.7	7.2	7.6	20	4.5	4.5	0.67	--	4.6
17-Jul-18	--	10.7	7.4	7.2	7.7	7.5	4.5	4.5	0.7	--	3.0

Appendix B – Modified USEPA (2005) Method 1623.1

ColorSeed™ Recovery Procedure:

1. Removed Envirochek®HV capsule from packaging and labelled with sample location.
2. Connected the capsule inlet to a flow meter using tubing. Positioned the pump on the upstream side of flow meter connecting it to filtered water.
3. Connected the capsule outlet to the filtered water tubing. This tube drained into a waste drain.
4. Turned the pump on and maintained a flow rate of ~ 2 LPM, and allowed approximately 1 L of filtered water to flow through the capsule prior to adding ColorSeed™.
5. Turned off the pump and placed a clamp on both the inlet and outlet tubing to stop water from draining from the capsule. The filter cartridge was full.
6. Placed the capsule in an upright position using a ring stand with inlet pointing upward.
7. Carefully removed the outlet tubing and allowed for water to drain to the top of pleated white membrane, and reattached the clamp.
8. Added 2 mL of Tween into the ColorSeed™ vial, replaced the cap and vortexed for 20 seconds at maximum speed.
9. Removed the cap and poured the ColorSeed™ into the capsule inlet, ensured it all went onto the filter.
10. Added 3 mL of filtered water to the ColorSeed™ tube. Replaced cap and vortex for 20 seconds at maximum speed.
11. Removed cap and poured the vial content into the capsule inlet.
12. Repeated the above two steps (rinsed vial with 3 mL filtered water) two more times (three total). Ensured the water level remained above the pleated white membrane if not using the cartridge immediately.
13. If the experiment required more than one cartridge, prepared all cartridges with ColorSeed™. Made sure the water level remained above the pleated white membrane and kept the cartridge upright during storage.

Experimental Procedure:

1. Connected the capsule inlet to a flow meter using tubing. The pump went on the upstream side of flow meter connecting it to filtered water source.
2. Connected the capsule outlet to the filtered water tubing. Had this tube drain into a waste drain.
3. Turned the pump on and maintained a flow rate of ~ 2 LPM.
4. Collected effluent in a bucket and used this as the source of filtered water that pumped through the cartridge.
5. Monitored the flow rate and total sample volume filtered.
6. When the required sample volume was filtered, turned off the pump, removed tubing from the capsule outlet, and replaced it with a blue vinyl cap.
7. To ensure the water level remained above the pleated white membrane, turned the pump on and allowed the cartridge to fill before turning off the pump. Placed the other blue vinyl cap on the inlet side after removing the tubing.
8. Recorded the time and sample volume filtered on the cartridge.
9. Placed the capsule in a cooler for transport to the lab.
10. Refrigerated sample at 2-8 °C if not proceeding with Sample Elution.

If multiple cartridges are being used, quickly moved the inlet tubing to the second cartridge once it is removed from the previous cartridge.

Appendix C – Recovery Testing

Ripening Recovery Study

Extra tubing was required during ripening experiments in order to capture the ripening period of the filter. The additional tubing seeded closer to the top of the filter but provided more opportunities for oocysts loss. A recovery study was conducted on the additional seeding and influent sample tubing to quantify losses during this portion experimental work. The tubing used during ripening experiments was more than double the length of the tubing from the seed suspension to the filter bed, and from the filter bed to influent sampling port.

The recovery test was conducted using the same framework as the ripening experiment in order to mimic conditions. A seed suspension was created to test the influent length of tubing while influent samples from previous (non-ripening) experiments were used to evaluate losses through the influent sample length of tubing. A sample volume of 1 mL was initially used during immunofluorescence assay to numerate samples collected from the seeding tubing but slide counts were too numerous to count. The volume used during this process was modified to 0.2 mL for reasonable counts. Results from the recovery study are in Table A-1.

Table A-1. Results from Seed Suspension Tubing during Ripening Recovery Testing

Trial	Sample Volume (mL)	Initial Conc. (oocysts/L)	Normalized Counts (oocysts/L)
1	0.02	25,400,000	19,750,000
2	0.02		11,800,000
3	0.02		3,950,000
4	0.02		14,150,000
5	0.02		13,000,000
6	0.02		16,850,000
7	0.02		11,600,000
8	0.02		15,400,000
9	0.02		16,400,000
Mean			13,000,000

A similar approach was taken to enumerate the samples taken from the influent sampling tubing. A sample volume of 1 mL was processed from 10 different samples. The results are in Table A-2.

Table A-2. Results from Influent Sampling Tubing during Ripening Recovery Testing

Trial	Sample Volume (mL)	Initial Conc. (oocysts/L)	Normalized Counts (oocysts/L)
1	1	60,400	50,000
2	1		40,000
3	1		40,000
4	1		35,000
5	1		43,000
6	1		52,000
7	1		43,000
8	1		31,000
9	1		34,000
10	1		23,000
Mean			39,000

The results in Table A-1 and Table A-2 indicate that the tubing was not the cause for the low oocyst removals calculated during RIP-01 and RIP-02. The losses listed in the tables were not the same order of magnitudes as the difference between RIP-01/-02 and the experiments conducted prior to them. Losses from tubing were ruled as the cause for low oocyst removal during the initial ripening experiments.

Lab Equipment Recovery Study

Recovery testing was also completed to determine losses from each set of lab procedures used to process and enumerate samples collected during Harris WTP experiments. First, a dilution from the stock suspension of *Cryptosporidium* oocysts was enumerated using a haemocytometer. This was completed for a more accurate representation of the dilution. The aim of the dilution was 100 oocysts per 1/1,000 mL (1 μL) on the haemocytometer which ultimately resulted in an average concentration of 84 oocysts/μL in the dilution. Following this, influent and effluent solutions were

made to mirror volumes processed during typical experiments while maintaining concentration of 84 oocysts/μL.

The lab methods that were evaluated for recovery testing were the influent and effluent manifold methods, the syringe influent method, and the glass manifold effluent method. All samples and test equipment were prepared to imitate sample collection and lab procedures. More specifically, water was collected from the respective ports from the pilot plant in Toronto to ensure the same water matrix was used and eluting solution was added to each sterilized bottle prior to oocyst addition. A volume of 3 mL was selected for the influent samples and 300 mL for the effluent samples. Once completed, 10 trials were run using each of the four methods mentioned above. The results from the study are below in Table A-3.

Table A-3. Lab Recovery test results for influent and effluent lab procedures

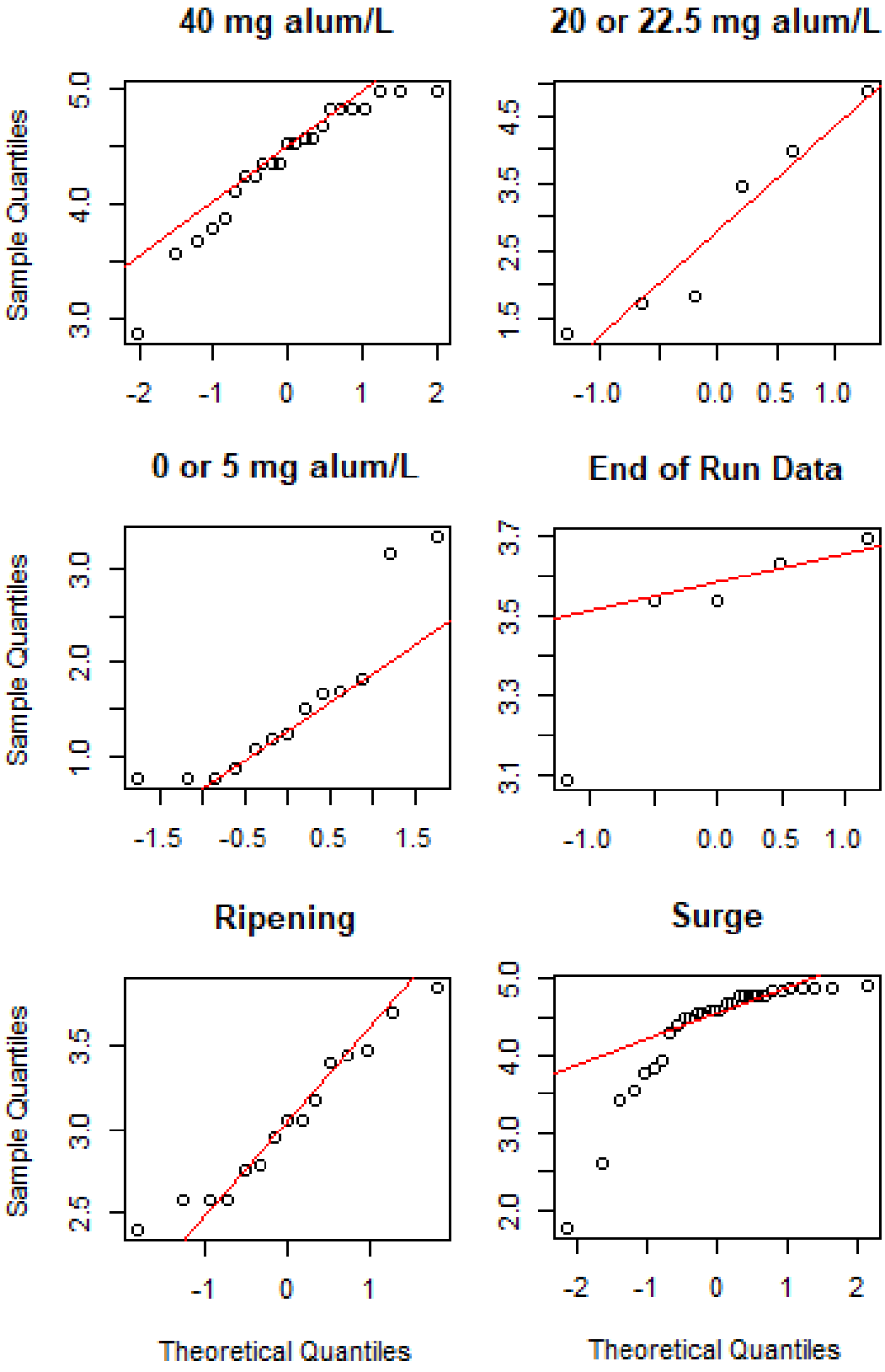
Trial	Influent				Effluent		
	Haemo-cytometer	Initial Conc.	Manifold	Syringe	Initial Conc.	Manifold	Glass
	oocysts/mL	oocysts/L	oocysts/L	oocysts/L	oocysts/L	oocysts/L	oocysts/L
1	96,000	28,056	667	9,333	281	117	230
2	84,000		1,333	10,000		97	190
3	122,000		1,000	4,667		213	213
4	106,000		2,667	13,667		287	203
5	74,000		1,333	15,000		150	187
6	80,000		14,667	23,667		163	143
7	84,000		1,000	11,667		163	137
8	80,000		2,333	10,667		150	247
9	84,000		3,667	2,333		117	163
10	54,000		3,000	1,000		127	160
11	86,000	N/A	N/A	N/A	N/A	N/A	N/A
12	60,000	N/A	N/A	N/A	N/A	N/A	N/A
Mean	84,167	28,056	3,167	10,200	281	158	187
Recovery	N/A	N/A	11%	36%	N/A	56%	67%

The results from Table A-3 show better recovery in the effluent methods than the influent methods. The results from the influent manifold method may not be representative of the actual test results as the majority of the tests conducted using this method, experiments prior to October 5, 2017, used less than 1 mL sample volume which was a volume small enough not to come into contact with the rough walls of the smaller manifold which were introduced during April 2017 experiments. This is supported by the similar influent counts enumerated over the time period the influent manifold was used and the syringe technique used as well as results from August experiments when both influent methods were used to enumerate the influent samples. The losses

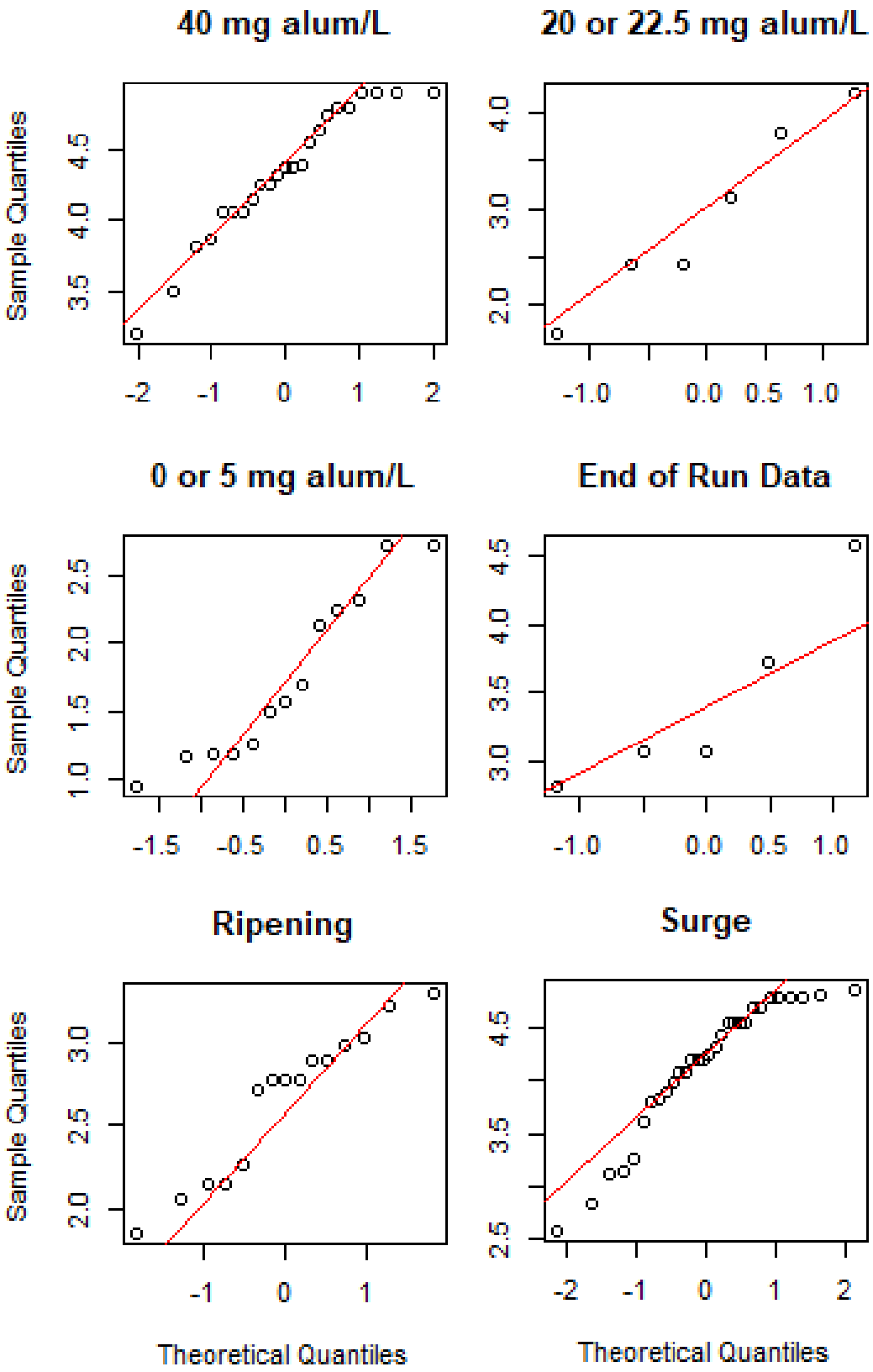
from the influent manifold system were therefore attributed to the surface roughness that resulted from the parts made at the University of Waterloo. These parts were made to accommodate the smaller diameter filter membrane used for influent samples while still using the same manifold and weight system made for a larger diameter filter membrane (22 mm).

Appendix D – Assessment of Normality (Q-Q Plots)

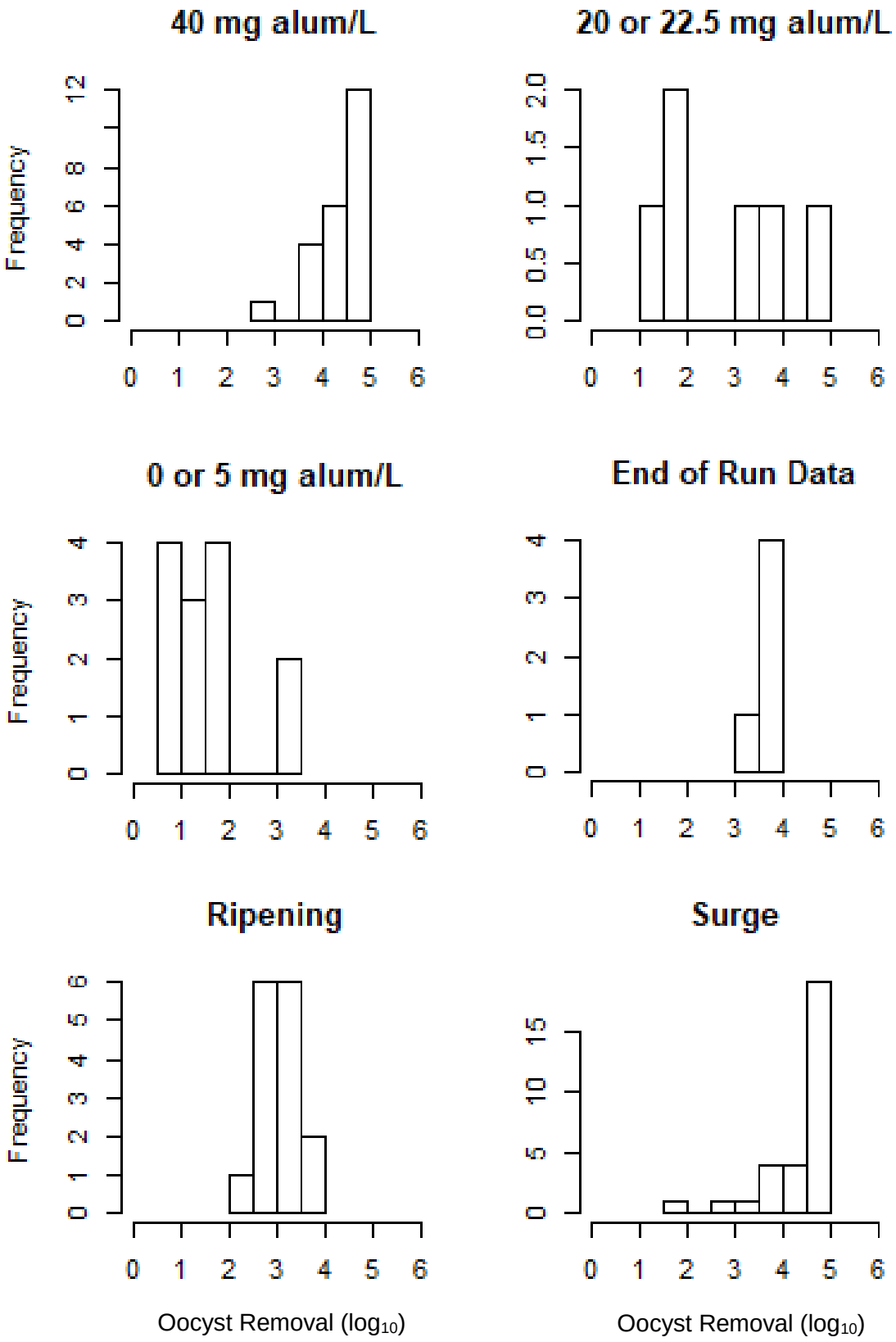
Deep Bed Filter Q-Q Plots



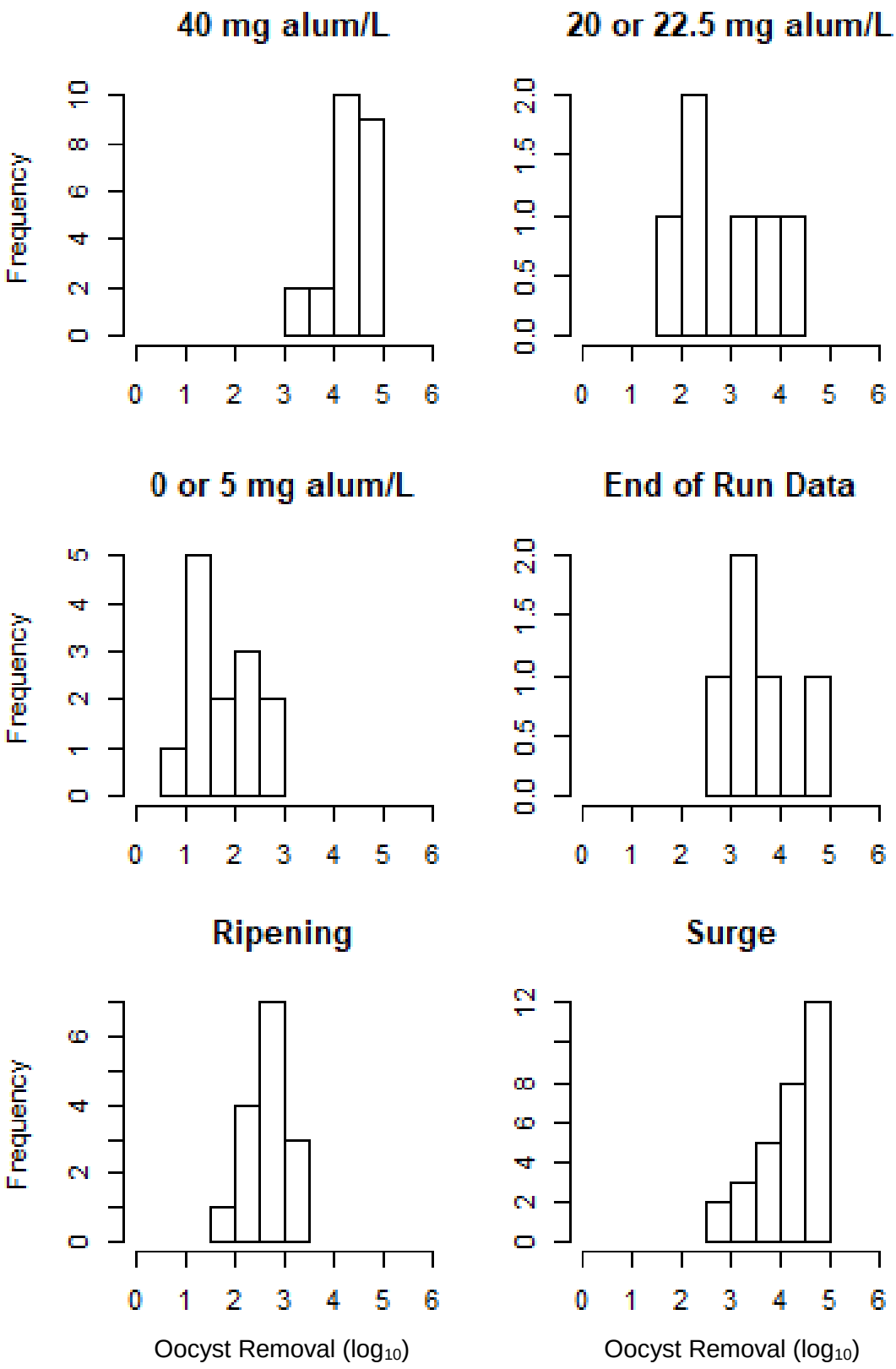
Shallow Bed Filter Q-Q Plots



Deep Bed Filter Histograms



Shallow Bed Filter Histograms



Appendix E – Phase 1 and Phase 2 Results

Date: May 11, 2017

ID: P1 – 22.5a

Type of Experiment: Phase 1 Pilot-scale CAF Performance Demonstration

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentrations (Oocyst/mL)
Shallow	Harris	regular (0.3)	22.5mg/L	5	700mL between both	5.0E+08
Deep	Harris	deep (0.48)	22.5mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Cell Count Influent Oocysts	Cell Count/L Influent Oocysts	Effluent Volume (mL)	Cell Count Effluent Oocysts	Cell Count/L Effluent Oocysts	Log Removal Oocysts
0	01	3	2	667	200	TNTC	519	--
	02	3	0	0	100	0	0	
	03				200	135	675	
15	01	3	4	1,333	200	TNTC	672	0.05
	02	3	3	1,000	200	281	1,405	
30	01	3	2	667	200	TNTC	608	0.2
	02	3	2	667	200	62	310	
45	01	3	32	10,667	200	457	2,285	1.5
	02	3	233	77,667	200	55	275	
60	01	3	278	92,667	200	32	160	2.6
	02	3	268	89,333	200	66	330	
75	01	3	201	67,000	200	43	215	2.4
	02	3	127	42,333	200	52	260	

Deep

Time (min)	Trial	Influent Volume (mL)	Cell Count Influent Oocysts	Cell Count/L Influent Oocysts	Effluent Volume (mL)	Cell Count Effluent Oocysts	Cell Count/L Effluent Oocysts	Log Removal Oocysts
0	01	3	0	0	200	32	160	--
	02	3	7	2,333	200	273	1,365	
	03	3	0	0	100	0	0	
15	01	3	22	7,333	200	28	140	0.7
	02	3	3	1,000	200	336	1,680	
30	01	3	12	4,000	200	8	40	1.3
	02	3	2	667	200	174	870	
	03	3	24	8,000				
	04	3	65	21,667				
45	01	10	125	12,500	100	28	280	1.7
	02	3	97	32,333	200	78	390	
	03	3	7	2,333				
60	01	3	35	11,667	200	79	395	1.0
	02	3	40	13,333	200	307	1,535	
	03	3	4	1,333				
75	01	3	69	23,000	200	20	100	2.1
	02	3	191	63,667	200	83	415	
	03	3	1	333				

Date: June 8, 2017

ID: P1 – 22.5b

Type of Experiment: Phase 1 Pilot-scale CAF Performance Demonstration

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentrations (Oocyst/mL)
Shallow	Harris	regular (0.3)	22.5mg/L	5	750mL between both	5.0E+07
Deep	Harris	deep (0.48)	22.5mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal Oocysts
0	01	6	0	0	700	TNTC	--	--
	02	6	2	333				
	03	6	0	0				
15	01	6	17	2,833	700	1	1	3.4
	02	6	23	3,833				
30	01	1	10	10,000	700	3	4	3.5
	02	1	18	18,000				
45	01	0.6	0	0	700	3	4	3.8
	02	0.6	0	0				
	03	0.6	35	58,333				
	04	0.6	32	53,333				
60	01	0.6	2	3,333	700	13	19	3.2
	02	0.6	0	0				
	03	0.6	39	65,000				
	04	0.6	31	51,667				
75	01	0.6	12	20,000	700	1*	1	>4.0
	02	0.6	7	11,667				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	2	3	--
	02	3	0	0				
15	01	6	3	500	700	1*	1	>3.1
	02	3	9	3,000				
30	01	6	199	33,167	700	3	4	3.9
	02	3	126	42,000				
45	01	1	5	5,000	100	1	10	3.8
	02	6	444	74,000	700	1	1	
60	01	6	293	48,833	100	3	30	3.5
	02	0.6	33	55,000	700	5	7	
75	01	6	432	72,000	700	1*	1	> 4.7
	02	0.6	44	73,333				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Date: June 9, 2017

ID: P1 – 40a

Type of Experiment: Phase 1 Pilot-scale CAF Performance Demonstration

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentrations (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	700mL between both	5.0E+07
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	1	167	700	0	0	--
	02	6	3	500				
15	01	6	19	3,167	700	3	4	2.9
	02	6	23	3,833				
30	01	1	30	30,000	700	2	3	4.0
	02	0.6	20	33,333				
45	01	0.6	1	1,667	700	2	3	3.9
	02	0.6	29	48,333				
60	01	0.6	0	0	700	2	3	4.2
	02	0.6	51	85,000				
75	01	0.6	4	6,667	700	2	3	4.1
	02	0.6	39	65,000				

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	1	1	--
	02	3	0	0				
15	01	6	5	833	700	1	1	3.3
	02	3	13	4,333				
30	01	6	128	21,333	700	3	4	4.0
	02	3	174	58,000				
45	01	6	366	61,000	700	1	1	4.6
	02	0.6	36	60,000				
60	01	6	385	64,167	100	9	90	2.8
	02	0.6	16	26,667	700	39	56	
75	01	6	35	5,833	700	1	1	4.6
	02	0.6	59	98,333				

Date: August 2, 2017

ID: P1 – 5

Type of Experiment: Phase 1 Pilot-scale CAF Performance Demonstration

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentrations (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	700mL between both	5.00E+07
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	1	0	0	100	3	30	--
	02	6	0	0	100	0	0	
15	01	6	1	167	10	1*	1	>3.4
	02	3	10	3,333	10	1*	1	
	03	6	23	3,833				
30	01	6	64	10,667	10	4	400	1.9
	02	3	110	36,667	10	2	200	
45	01	6	160	26,667	10	11	1,100	1.6
	02	3	220	73,333	10	7	700	
	03	3	31	10,333				
60	01	0.1	2	20,000	100	485	4,850	0.7
	02	1	11	11,000	10	28	2,800	
	03	3	114	38,000				
	04	3	33	11,000				
75	01	3	253	84,333	10	27	2,700	1.3
	02	6	268	44,667	10	18	1,800	
	03	3	8	2,667				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01				10	1	100	--
	02	6	0	0	10	0	0	
15	01	6	6	1,000	10	1	100	0.7
	02	6	0	0	10	1	100	
30	01	3	52	17,333	10	3	300	1.6
	02	3	91	30,333	10	8	800	
45	01	3	35	11,667	10	24	2400	1.1
	02	3	185	61,667	10	39	3900	
60	01	3	61	20,333	10	15	1500	0.9
	02	3	59	19,667	10	40	4000	
75	01	3	328	109,333	10	12	1200	1.3
	02	3	179	59,667	10	65	6500	

Date: August 3, 2017

ID: P1 – 40b

Type of Experiment: Phase 1 Pilot-scale CAF Performance Demonstration

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentrations (Oocyst/mL)
Shallow	Harris	regular (0.3)	5mg/L	5	1050mL between both	5.00E+07
Deep	Harris	deep (0.48)	5mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	10	1,667	700	1	1	2.8
	02	6	1	167				
15	01	6	27	4,500	700	1	1	3.2
	02	6	2	333				
30	01				700	5	7	3.6
	02	6	57	9,500				
	03	3	122	40,667				
45	01	3	136	45,333	10	0	0	4.0
	02	6	34	5,667	700	4	6	
60	01	3	130	43,333	100	2	20	3.2
	02	6	9	1,500	700	4	6	
75	01	3	102	34,000	700	1	1	4.1
	02	3	14	4,667				

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
15	01	6	3	500	700	1	1	2.4
	02	6	1	167				
30	01	3	99	33,000	700	2	3	4.0
	02	3	60	20,000				
45	01	3	11	3,667	700	2	3	3.3
	02	3	23	7,667				
60	01	3	130	43,333	400	2	5	3.7
	02	3	12	4,000				
75	01	3	72	24,000	700	1*	1	>4.2
	02							

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Date: December 11, 2017

ID: RIP -1

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentrations (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	1050mL between	5.00E+05
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
5	01	1	298	298,000	700	6	9	4.6
	02	1	315	315,000				
10	01	1	1	1,000	700	8	11	2.2
	02	1	3	3,000				
15	01	1	11	11,000	700	8	11	3.0
	02	1	10	10,000				
20	01	1	5	5,000	700	6	9	2.8
	02	1	5	5,000				
25	01	1	11	11,000	100	2	13	3.0
	02	1	14	14,000	700	4		
30	01	1	3	3,000	700	6	9	2.7
	02	1	5	5,000				

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
5	01	1	1	1,000	700	1	1	3.2
	02	1	4	4,000				
10	01	1	1	1,000	700	1	1	3.6
	02	1	10	10,000				
15	01	1	2	2,000	700	8	11	2.6
	02	1	8	8,000				
20	01	1	8	8,000	700	8	11	2.9
	02	1	7	7,000				
25	01	1	0	0	700	2	3	2.2
	02	1	1	1,000				
30	01	1	4	4,000	700	5	7	2.6
	02	1	2	2,000				

Date: January 9, 2018

ID: RIP -2

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	1050mL between	4.60E+06
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	1	167				
5	01	1	0	0	700	2	3	1.9
	02	2	1	500				
10	01	1	0	0	700	4	6	1.6
	02	2	1	500				
15	01	1	0	0	700	8	11	1.3
	02	2	1	500				
20	01	2	3	1,500	700	3	4	2.5
	02	2	2	1,000				
25	01	2	2	1,000	700	5	7	2.2
	02	2	2	1,000				
30	01	2	2	1,000	700	4	6	2.3
	02	2	3	1,500				

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
5	01	1	4	4,000	700	1	1	3.4
	02	1	3	3,000				
10	01	1	0	0	700	1	1	--
	02	1	0	0				
15	01	1	1	1,000	700	1	1	2.9
	02	1	1	1,000				
20	01	1	3	3,000	700	2	3	2.9
	02	1	2	2,000				
25	01	1	2	2,000	100	1	6	2.5
	02	1	2	2,000				
30	01	1	3	3,000	700	3	4	2.8
	02	1	2	2,000				

Date: May 29, 2018

ID: RIP – 3

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentrations (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	130L between	2.87E+07
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	64	10,667	700	25	36	0.0
	02	10	0	0				
5	01	1	0	0	689	1	1	--
	02	2	0	0				
10	01	1	33	33,000	700	37	53	2.7
	02	2	48	24,000				
15	01	1	66	66,000	700	33	47	3.1
	02	2	92	46,000				
20	01	1	84	84,000	700	21	30	3.4
	02	2	121	60,500				
25	01	1	62	62,000	700	60	86	2.9
	02	2	117	58,500				
30	01	1	19	19,000	700	18	26	3.2
	02	2	110	55,000				

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	9	13	0.0
	02	10	0	0				
5	01	1	25	25,000	700	13	19	3.2
	02	2	77	38,500				
10	01	1	35	35,000	700	11	16	3.4
	02	2	71	35,500				
15	01	1	162	162,000	700	62	89	3.3
	02	2	337	168,500				
20	01	1	80	80,000	700	20	29	3.6
	02	2	220	110,000				
25	01	1	79	79,000	700	22	31	3.4
	02	2	160	80,000				
30	01	1	27	27,000	700	8	11	3.3
	02	2	27	13,500				

Date: October 5, 2017

ID: MID40-1

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	750mL btwn	2.92E+06
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
15	01	6	0	0	700	0	0	0.0
	02	6	0	0				
30	01	3	49	16,333	700	3	4	4.0
	02	3	183	61,000				
45	01	3	270	90,000	700	8	11	3.7
	02	3	48	16,000				
60	01	3	124	41,333	700	2	3	4.6
	02	3	525	175,000				
75	01	3	142	47,333	700	16	23	3.4
	02	3	199	66,333				

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
15	01	6	0	0	700	0	0	0.0
	02	6	0	0				
30	01	3	120	40,000	700	1*	1	>4.3
	02	3	68	22,667				
45	01	3	62	20,667	100	2	11	3.8
	02	3	352	117,333	700	2		
60	01	3	182	60,667	700	2	3	4.2
	02	3	60	20,000				
75	01	3	147	49,000	700	3	4	4.1
	02							

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Date: October 23, 2017

ID: MID40-2

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	1050mL between	2.02E+06
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
15	01	6	3	500	700	1*	1	>2.5
	02	6	3	500				
30	01	6	209	34,833	700	1*	1	>4.3
	02	6	151	25,167				
45	01	3	148	49,333	700	3	4	3.9
	02	6	109	18,167				
60	01	3	547	182,333	700	2	3	4.6
	02	6	424	70,667				
75	01	3	141	47,000	700	3	4	4.3
	02	3	398	132,667				
90	01	3	325	108,333	700	4	6	4.3
	02	3	391	130,333				
105	01	3	240	80,000	700	4	6	4.4
	02	3	544	181,333				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
15	01	6	1	167	700	1	1	2.2
	02	6	2	333				
30	01	6	100	16,667	700	1*	1	>4.0
	02	6	68	11,333				
45	01	6	430	71,667	700	1*	1	>4.6
	02	6	244	40,667				
60	01	6	759	126,500	700	2	3	4.7
	02	3	495	165,000				
75	01	3	102	34,000	700	2	3	4.2
	02	3	173	57,667				
90	01	3	222	74,000	700	1*	1	>4.9
	02	3	446	148,667				
105	01	3	277	92,333	700	1*	1	>4.8
	02	3	279	93,000				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Date: March 6, 2018

ID: MID40-3

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	1050mL between	4.48E+07
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
15	01	1	0	0	700	1*	1	>2.5
	02	1	1	1,000				
30	01	1	14	14,000	700	1	1	4.1
	02	1	18	18,000				
45	01	1	43	43,000	700	1	1	4.5
	02	1	53	53,000				
60	01	1	68	68,000	700	1*	1	>4.6
	02	1	57	57,000				
75	01	1	89	89,000	700	1*	1	>4.8
	02	1	85	85,000				
90	01	1	91	91,000	700	1*	1	>4.8
	02	1	86	86,000				
105	01	1	100	100,000	700	1*	1	>4.9
	02	1	100	100,000				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
15	01	1	2	2,000	700	1	1	3.2
	02	1	2	2,000				
30	01	1	27	27,000	700	1	1	4.3
	02	1	30	30,000				
45	01	1	36	36,000	700	1*	1	>4.4
	02	1	31	31,000				
60	01	1	52	52,000	700	1*	1	>4.5
	02	1	44	44,000				
75	01	1	63	63,000	700	1*	1	>4.6
	02	1	57	57,000				
90	01	1	97	97,000	700	1	1	4.8
	02	1	86	86,000				
105	01	1	101	101,000	700	1*	1	>4.9
	02	1	107	107,000				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Date: March 19, 2018

ID: MID40-4

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	1050mL between	4.35E+07
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
15	01	1	3	3,000	700	1	1	3.3
	02	1	3	3,000				
30	01	1	11	11,000	700	3	4	3.4
	02	1	12	12,000				
45	01	1	30	30,000	700	2	3	4.0
	02	1	31	31,000				
60	01	1	53	53,000	700	1*	1	>4.5
	02	1	44	44,000				
75	01	1	64	64,000	700	1	1	4.6
	02	1	60	60,000				
90	01	1	77	77,000	700	1	1	4.7
	02	1	78	78,000				
105	01	1	82	82,000	700	1*	1	>4.8
	02	1	85	85,000				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6		0				
15	01	1	1	1,000	700	1	1	>2.5
	02	1	0	0				
30	01	1	2	2,000	700	2	3	3.5
	02	1	16	16,000				
45	01	1	9	9,000	700	1	1	3.6
	02	1	3	3,000				
60	01	1	6	6,000	700	3	4	3.7
	02	1	38	38,000				
75	01	1	39	39,000	700			
	02	1	40	40,000				
90	01	1	42	42,000	700	1	1	4.5
	02	1	42	42,000				
105	01	1	51	51,000	700	1	1	4.5
	02	1	49	49,000				

Date: April 10, 2018

ID: MID5-5

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	5mg/L	5	1130L between	4.45E+07
Deep	Harris	deep (0.48)	5mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	30	0	0	0.0
	02	6	0	0				
15	01	1	16	16,000	30	6	200	1.9
	02	1	17	17,000				
30	01	1	29	29,000	30	8	267	2.1
	02	1	32	32,000				
45	01	1	33	33,000	30	4	133	2.5
	02	1	57	57,000				
60	01	1	61	61,000	30	15	500	2.1
	02	1	60	60,000				
75	01	1	56	56,000	30	10	333	2.3
	02	1	69	69,000				
90	01	1	85	85,000	30	4	133	2.8
	02	1	82	82,000				
105	01	1	95	95,000	30	12	400	2.4
	02	1	97	97,000				

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	30	8	267	0.0
	02	6	0	0				
15	01	1	10	10,000	30	1	33	2.5
	02	1	9	9,000				
30	01	1	20	20,000	30	2	67	2.4
	02	1	17	17,000				
45	01	1	38	38,000	30	1.5	50	2.9
	02	1	34	34,000				
60	01	1	42	42,000	30	1	33	3.1
	02	1	44	44,000				
75	01	1	74	74,000	30	32	1,067	1.8
	02	1	75	75,000				
90	01	1	101	101,000	30	47	1,567	1.8
	02	1	90	90,000				
105	01	1	118	118,000	30	66	2,200	1.7
	02	1	100	100,000				

Date: April 23, 2018

ID: MID0-6

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration Aim (Oocyst/mL)
Shallow	Harris	regular (0.3)	0mg/L	5	1100L between	5.00E+07
Deep	Harris	deep (0.48)	0mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	30	4	133	0.0
	02	6	17	2,833				
15	01	1	0	0	30	48	1,600	--
	02	1	0	0				
30	01	1	23	23,000	30	57	1,900	1.0
	02	1	12	12,000				
45	01	1	42	42,000	30	59	1,967	1.3
	02	1	28	28,000				
60	01	1	59	59,000	30	175	5,833	1.0
	02	1	62	62,000				
75	01	1	73	73,000	30	153	5,100	1.2
	02	1	105	105,000				
90	01	1	112	112,000	30	187	6,233	1.3
	02	1	121	121,000				
105	01	1	159	159,000	30	87	2,900	1.7
	02	1	140	140,000				

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	30	118	3,933	0.0
	02	6	2	333				
15	01	1	11	11,000	30	79	2,633	0.3
	02	1	0	0				
30	01	1	25	25,000	30	151	5,033	0.4
	02	1	2	2,000				
45	01	1	42	42,000	30	45	1,500	1.3
	02	1	22	22,000				
60	01	1	91	91,000	30	303	10,100	1.0
	02	1	76	76,000				
75	01	1	49	49,000	30	382	12,733	0.8
	02	1	95	95,000				
90	01	1	99	99,000	30	373	12,433	0.9
	02	1	70	70,000				
105	01	1	133	133,000	30	381	12,700	0.9
	02	1	50	50,000				

Date: May 29, 2018

ID: END-1

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	1100L between	6.90E+06
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	400	38	95	0.0
	02	6	11	1,833				
15	01	1	0	0	700	29	41	1.4
	02	1	2	2,000				
30	01	1	6	6,000	700	6	9	2.9
	02	1	6	6,000				
45	01	1	20	20,000	700	1	1	4.1
	02	1	15	15,000				
60	01	1	46	46,000	700	5	7	3.8
	02	1	50	50,000				
75	01	1	13	13,000	700	23	33	2.9
	02	1	37	37,000				
90	01	1	32	32,000	700	23	33	3.1
	02	1	59	59,000				
105	01	1	41	41,000	700	41	59	3.0
	02	1	64	64,000				

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	5	833	700	0	0	0.0
	02	6	1	167				
15	01	1	0	0	700	23	33	1.2
	02	1	1	1,000				
30	01	1	14	14,000	700	28	40	2.6
	02	1	20	20,000				
45	01	1	12	12,000	700	7	10	3.2
	02	1	18	18,000				
60	01	1	44	44,000	700	28	40	3.0
	02	1	48	48,000				
75	01	1	37	36,500	700	10	14	3.4
	02	1	35	35,000				
90	01	1	74	74,000	700	8	11	3.8
	02	1	60	60,000				
105	01	1	78	78,000	700	10	14	3.8
	02	1	84	84,000				

Date: November 13, 2017

ID: SUR-1

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	1050mL between	4.75E+06
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	1	167	700	0	0	0.0
	02	6	6	1,000				
50	01	1	55	55,000	700	6	9	3.9
	02	1	78	78,000				
55	01	1	53	53,000	700	1	1	4.6
	02	1	57	57,000				
61	01	1	123	123,000	700	1	1	4.7
	02	1	12	12,000				
66	01	1	92	92,000	700	8	11	3.9
	02	1	96	96,000				
71	01	1	91	91,000	700	6	9	4.0
	02	1	80	80,000				
80	01	1	88	88,000	700	1*	1	>4.6
	02	1	34	34,000				
100	01	1	61	61,000	700	1	1	4.6
	02	1	47	47,000				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	1	167	700	0	0	0.0
	02	6	0	0				
50	01	1	65	65,000	700	1*	1	>4.5
	02	1	17	17,000				
55	01	1	71	71,000	700	1*	1	>4.6
	02	1	38	38,000				
61	01	1	43	43,000	700	1	1	4.6
	02	1	83	83,000				
66	01	1	60	60,000	700	2	3	4.4
	02	1	71	71,000				
71	01	1	43	43,000	700	1*	1	>4.6
	02	1	72	72,000				
80	01	1	107	107,000	700	1*	1	>4.9
	02	1	108	108,000				
100	01	1	85	85,000	700	1	1	4.9
	02	1	119	119,000				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Date: November 28, 2017

ID: SUR-2

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	1050mL between	3.03E+06
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	2	333				
50	01	1	90	90,000	700	1	1	4.7
	02	1	58	58,000				
55	01	1	72	72,000	700	3	4	4.3
	02	1	81	81,000				
61	01	1	61	61,000	700	1	1	4.7
	02	1	82	82,000				
68	01	1	87	87,000	700	3	4	4.2
	02	1	59	59,000				
73	01	1	83	83,000	700	5	7	4.1
	02	1	105	105,000				
80	01	1	118	118,000	700	160	229	2.7
	02	1	96	96,000				
100	01	1	90	90,000	700	9	13	3.9
	02	1	123	123,000				

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
50	01	1	36	36,000	700	1	1	>4.6
	02	1	82	82,000				
55	01	1	87	87,000	700	1*	1	>4.7
	02	1	48	48,000				
61	01	1	48	48,000	700	TNTC	TNTC	TNTC
	02	1	74	74,000				
68	01	1	75	75,000	100	2	10	3.9
	02	1	85	85,000	700	0		
73	01	1	134	134,000	700	1	1	4.9
	02	1	97	97,000				
80	01	1	114	114,000	700	1	1	4.9
	02	1	121	121,000				
100	01	1	90	90,000	700	1	1	4.9
	02	1	110	110,000				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

TNTC – too numerous to count

Date: January 22, 2018

ID: SUR-3

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	1050mL between	4.94E+06
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
50	01	1	45	45,000	700	1*	1	>4.5
	02	1	53	53,000				
55	01	1	31	31,000	700	2	3	4.1
	02	1	39	39,000				
61	01	1	51	51,000	700	2	3	4.3
	02	1	60	60,000				
66	01	1	80	80,000	700	1	1	4.7
	02	1	76	76,000				
71	01	1	91	91,000	700	1	1	4.8
	02	1	83	83,000				
80	01	1	101	101,000	700	1	1	4.9
	02	1	140	140,000				
100	01	1	121	121,000	700	1	1	5.0
	02	1	177	177,000				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	2	333				
50	01	1	71	71,000	700	1*	1	>4.6
	02	1	47	47,000				
55	01	1	42	42,000	700	1	1	4.7
	02	1	85	85,000				
61	01	1	78	78,000	700	1	1	4.7
	02	1	79	79,000				
66	01	1	92	92,000	700	2	3	4.5
	02	1	86	86,000				
71	01	1	111	111,000	700	1	1	4.9
	02	1	96	96,000				
80	01	1	171	171,000	700	2	3	4.7
	02	1	133	133,000				
100	01	1	331	331,000	700	2	3	4.9
	02	1	99	99,000				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Date: February 6, 2018

ID: SUR-4

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	1050mL between	1.80E+06
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
50	01	1	42	42,000	700	2	3	4.2
	02	1	41	41,000				
55	01	1	50	50,000	700	2	3	4.2
	02	1	40	40,000				
61	01	1	65	65,000	700	3	4	4.2
	02	1	59	59,000				
66	01	1	80	80,000	700	3	4	4.3
	02	1	81	81,000				
71	01	1	72	72,000	700	3	4	4.2
	02	1	74	74,000				
80	01	1	79	79,000	700	4	6	4.1
	02	1	88	88,000				
100	01	1	90	90,000	700	5	7	4.1
	02	1	88	88,000				

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
50	01	1	25	25,000	700	1	1	4.3
	02	1	27	27,000				
55	01	1	31	31,000	700	1	1	4.4
	02	1	39	39,000				
61	01	1	103	103,000	700	1	1	4.9
	02	1	101	101,000				
66	01	1	55	55,000	700	2	3	4.3
	02	1	59	59,000				
71	01	1	101	101,000	700	1	1	4.9
	02	1	102	102,000				
80	01	1	145	145,000	700	2	3	4.6
	02	1	93	93,000				
100	01	1	187	187,000	700	3	4	4.6
	02	1	131	131,000				

Date: February 21, 2018

ID: SUR-5

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	1050mL between	3.90E+07
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
50	01	1	59	59,000	700	9	13	3.7
	02	1	61	61,000				
55	01	1	38	38,000	700	1	1	4.4
	02	1	43	43,000				
61	01	1	156	156,000	700	2	3	4.7
	02	1	115	115,000				
66	01	1	162	162,000	700	2	3	4.7
	02	1	132	132,000				
71	01	1	108	108,000	700	2	3	4.6
	02	1	113	113,000				
80	01	1	121	121,000	700	4	6	4.3
	02	1	117	117,000				
100	01	1	92	92,000	700	2	3	4.5
	02	1	91	91,000				

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	0	0	0.0
	02	6	0	0				
50	01	1	74	74,000	700	1*	1	>4.7
	02	1	71	71,000				
55	01	1	82	82,000	700	1	1	4.8
	02	1	86	86,000				
61	01	1	144	144,000	700	1	1	4.9
	02	1	104	104,000				
66	01	1	134	134,000	700	2	3	4.6
	02	1	110	110,000				
71	01	1	125	125,000	700	2	3	4.6
	02	1	119	119,000				
80	01	1	113	113,000	700	1	1	4.9
	02	1	138	138,000				
100	01	1	152	152,000	700	1	1	5.0
	02	1	110	110,000				

*actual slide count is 0 but changed to 1 to calculate a conservative oocyst removal

Date: June 19, 2018

ID: SUR-6

Type of Experiment: Phase 2 - Pilot-scale Evaluation of Filter Design and Operational Effects on *C. parvum* Oocyst Removal CAF of High Quality Source Water

Filter	Plant	Filter depth (m of anthracite)	Coagulant Dose	Pump Rate (mL/min)	Seed Suspension Volume (L)	Seed Suspension Concentration (Oocyst/mL)
Shallow	Harris	regular (0.3)	40mg/L	5	1080L between	4.42E+07
Deep	Harris	deep (0.48)	40mg/L	5		

Shallow

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	4	6	0.0
	02	6	6	1,000				
50	01	1	31	31,000	700	27	39	2.9
	02	1	31	31,000				
55	01	1	33	33,000	700	17	24	3.1
	02	1	32	32,000				
67	01	1	40	40,000	700	9	13	3.6
	02	1	72	72,000				
72	01	1	73	73,000	700	27	39	3.1
	02	1	33	33,000				
77	01	1	43	43,000	700	51	73	2.9
	02	1	72	72,000				
86	01	1	52	52,000	700	26	37	3.1
	02	1	48	48,000				
106	01	1	76	76,000	700	20	29	3.4
	02	1	72	72,000				

Deep

Time (min)	Trial	Influent Volume (mL)	Influent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Effluent Volume (mL)	Effluent Cell Count (oocysts)	Normalized Cell Count (oocysts/L)	Log Removal
0	01	6	0	0	700	134	191	0.0
	02	6	0	0				
50	01	1	28	28,000	700	12	17	3.3
	02	1	33	33,000				
55	01	1	35	35,000	700	11	16	3.4
	02	1	44	44,000				
67	01	1	16	16,000	700	6	9	3.6
	02	1	48	48,000				
72	01	1	33	33,000	700	92	131	2.5
	02	1	49	49,000				
77	01	1	33	33,000	700	5	7	3.9
	02	1	73	73,000				
86	01	1	57	57,000	700	14	20	3.6
	02	1	96	96,000				
106	01	1	90	90,000	700	10	14	3.8
	02	1	80	80,000				