REMOVAL OF *CRYPTOSPORIDIUM PARVUM* BY GRANULAR MEDIA FILTRATION

by

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ABSTRACT

Increasingly stringent regulations for drinking water quality have placed increased emphasis on a multi-barrier approach for providing protection from waterborne pathogens. Widely found in surface waters, the pathogen *Cryptosporidium parvum* is particularly resistant to chemical disinfectants commonly used in drinking water treatment, underscoring the need for multiple treatment strategies for inactivation or removal of *C. parvum* from drinking water. When operation is optimized, granular media filtration systems are particularly effective barriers against *C. parvum* passage into potable water; however, less is known about the pathogen removal capacity of these systems at the beginning and end of the filter cycle (*i.e.*, filter ripening and breakthrough) or when particle removal processes are challenged (*e.g.*, coagulation upsets, hydraulic changes, *etc.*).

The research presented in this thesis examined the passage of *C. parvum* and potential surrogates for *C. parvum* through granular media filters during periods of optimal and non-optimal filter operation. A thorough review of the relevant filtration and *C. parvum* literature emphasized the difficulty in accurately enumerating *C. parvum* from water samples. A relatively simple analytical method for concentrating and enumerating *C. parvum* during filtration studies was implemented and optimized. Then, to address the uncertainty or reliability of *C. parvum* concentration and removal data, a new quantitative tool that incorporated several sources of error (representative sampling, random analytical error, and non-constant analytical recovery) was developed. The statistical model assumed a Poisson distribution for the true sample counts, a binomial distribution for modeling the recovered fraction of oocysts, and a Beta distribution for describing the uncertainty of oocyst recovery. A numerical technique (Gibbs sampler) was then applied to the statistical model to determine confidence intervals for *C. parvum* concentration and removal data. This method of describing the uncertainty associated with *C. parvum* data (confidence intervals calculated via the Gibbs sampler) was used throughout this thesis research because it allowed for comparison between different data sets with, in some cases, different analytical recoveries.

Bench-scale experiments were performed to determine if viable and chemically-inactivated *C. parvum* oocysts were similarly removed by granular media filters at a variety of operating conditions. These experiments were critical because of the potential health risks associated with the experimental use and release of viable oocysts. Since uncoagulated, chemically-inactivated oocysts have demonstrated slightly different surface charge properties (described by zeta potential) than viable oocysts, it had been speculated that the different oocysts might also be removed differently by granular media filters. Dual- (anthracite/sand) and tri-media (anthracite/sand/garnet) investigations demonstrated similar removals of viable and chemically-inactivated *C. parvum* oocysts during optimized operation, filter ripening, and coagulation failure. While *C. parvum* removals were moderately lower (by ~0.5 to 1-log) during ripening than during stable operation, the *C. parvum* removal capacity of both dual- and tri-media filters was severely and significantly compromised during coagulation failure when it decreased by >3-log relative to stable operation. *C. parvum* removals were not statistically different in

dual- and tri-media filters, though increased replication may yield statistically significant differences in the marginally higher removals achieved by tri-media filtration.

Pilot-scale experiments represented the majority of the experimental efforts and focused on investigating design and operational strategies for maximizing *C. parvum* removal by filtration. Multiple research platforms permitted investigation of different types of raw waters, water temperatures, coagulation regimes, and filter designs. Formalin-inactivated *C. parvum* oocysts were seeded at all of the experimental locations. In addition to turbidity and particle concentration evaluations, polystyrene microspheres were evaluated as potential surrogates for *C. parvum* because they were similar to oocysts in size and easy to identify and enumerate.

The pilot-scale experiments demonstrated that excellent removals (>5-log) of *C. parvum* could be achieved during optimal operating conditions, even at temperatures as low as 1°C and during spring runoff conditions. These removals deteriorated substantially (by 3- to 4-log) during end-of-run and early breakthrough filtration, even at filter effluent turbidities below 0.1 NTU. This result suggested that filter operation during breakthrough, as measured by turbidity or perhaps even particles, should be avoided. Coagulation failure and sub-optimal coagulation conditions (reductions in coagulant dose) also resulted in deteriorated *C. parvum* removals. Relatively rapid changes in hydraulic loading demonstrated varied effects on *C. parvum* removal by filtration, though in most cases only little to no deterioration in filter effluent *C. parvum* concentrations occurred. Turbidity monitoring proved more useful than particle counting in gauging the effects of hydraulic steps on *C. parvum* passage through filters. These events should be investigated further to better define how and when they impact pathogen passage. *C. parvum* removals by filtration were moderately (~0.5-1 log) lower during ripening than during stable operation, these less substantial differences occurred over a relatively short duration of the ripening period. During most of these operating conditions, oocyst-sized polystyrene microspheres appeared to be reasonable surrogates for *C. parvum* removal by filtration; however, they should continue to be evaluated relative to oocysts to better define the limits of their applicability as surrogates. The pilot-scale investigations resulted in several operational and design implications and strategies for maximizing *C. parvum* removal by granular media filtration, the most notable of which were the importance of optimized chemical pretreatment (coagulation prior to filtration) and the potential for increased pathogen passage during end-of-run operation.

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CHAPTER 1
INTRODUCTION

1.1 *CRYPTOSPORIDIUM* AND DRINKING WATER TREATMENT

The primary goal of drinking water supply systems is to protect public health by supplying water with acceptably low concentrations of microbial and chemical contaminants. Increasingly stringent regulations for drinking water quality necessitate a multi-barrier approach for providing protection from waterborne pathogens. Widely found in surface waters, the pathogen *Cryptosporidium parvum* is particularly resistant to chemical disinfectants commonly used in drinking water treatment. It has been suggested that *C. parvum* may require up to ten times the ozone dose required for effective inactivation of *Giardia lamblia* cysts (Owens *et al.*, 1994), a level which can push the limits of economic feasibility and disinfection by-product compliance for many utilities.

The considerable costs and practical limitations of adequate inactivation of *C. parvum* by traditional disinfection processes have underscored the importance of multiple treatment strategies for inactivation or removal of *C. parvum* from drinking water. Although alternative treatment processes such as pressure-driven membranes offer excellent removal capabilities of parasitic pathogens, they are not currently economical for treatment of flows greater than 1 to 5 MGD (Wiesner *et al.*, 1994). A common component of conventional water treatment operations, filtration has demonstrated efficacy as a barrier against *C. parvum*. Full-scale *C. parvum* removals from 2 to >4 log have been reported in the literature (Baudin and Laîné, 1998; Nieminski and Ongerth, 1995). This range of reported removals may be in part explained by differences in operating conditions which can greatly affect oocyst removal by filters (Patania *et al.*, 1995). To date, most of the reported investigations of *C. parvum* removal by filtration have focused on optimal operating conditions. Pathogen passage during vulnerable

periods in the filter cycle when particle removal processes are challenged (*e.g.*, ripening, breakthrough, *etc.*) has been less thoroughly investigated.

Originally described by Huck *et al.* (2001), a conceptual representation of the relationship between pathogen passage and traditional filter performance parameters such as turbidity and particle counts is presented in Figure 1.1. The left vertical axis shows turbidity or particle counts while the right vertical axis shows pathogen concentration in filter effluents. The horizontal axis represents the time of approximately one filter cycle.

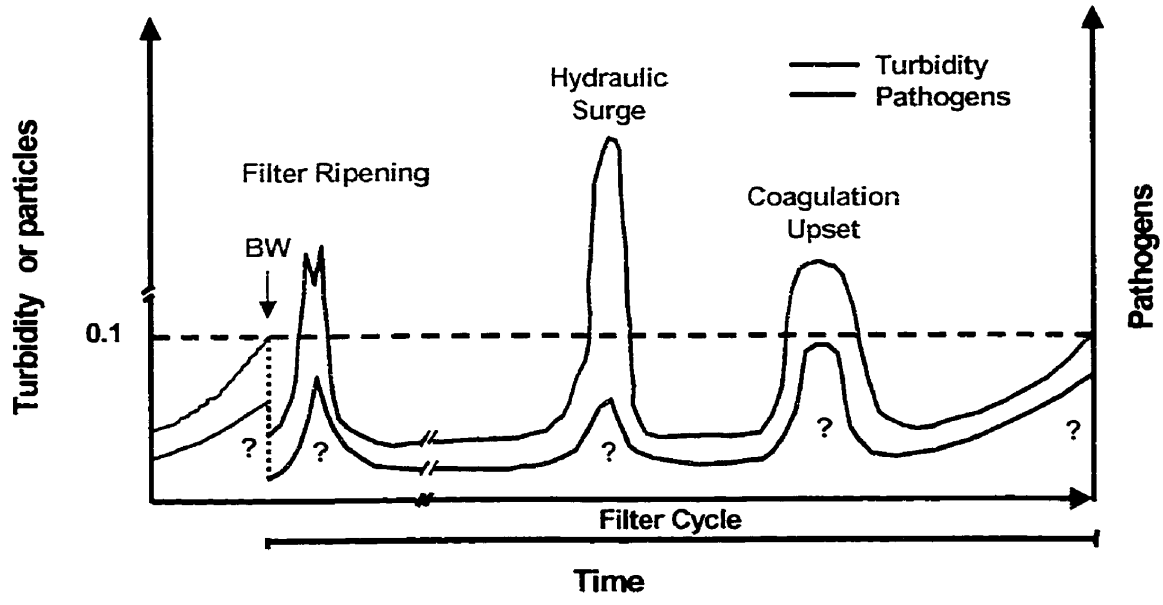


Figure 1.1 Conceptual relationship between pathogen passage and traditional operational parameters such as turbidity and particle counts (modified from Huck *et al.*, 2001).

As shown in Figure 1.1, regulatory guidelines and treatment objectives often necessitate that filter effluent turbidity must always remain below a specified goal or standard. After backwash, both turbidity and particle counts increase during filter ripening. Pathogen passage through the filters may increase during this time for the same reasons. Events such as coagulation upsets result in sub-optimal pretreatment and may consequently cause filter effluent concentrations of turbidity, particles, and pathogens to increase as a result of non-attachment. Similarly, events such as hydraulic surges may result in the release or detachment of particles including pathogens. As the filter becomes loaded, particles and perhaps pathogens may also break through the filter, resulting in the initiation of a backwash cycle. The primary focus of this thesis research is to address the passage of *C. parvum* through filters relative to measurements of turbidity, particle counts, and potential surrogates (polystyrene microspheres and *B. subtilis* spores) during various phases and events occurring throughout typical filter cycles.

A full understanding of the ability of filters to remove *C. parvum* (or any other pathogen) can only be attained when the reliability of experimentally obtained data is understood and quantified. Collecting *C. parvum* data and expressing the reliability of those data has been one of the greatest challenges associated with studying and optimizing water treatment processes for the removal of this pathogen. Reliable *C. parvum* removal data are necessary so that they can be related to on-line performance parameters that can be measured, such as turbidity and particle counts, that can be responded to in real time. To that end, this thesis research also endeavored to provide a reliable analytical method for evaluating *C. parvum* concentration and removal during treatment process challenge studies. The reliability of these data was demonstrated with a quantitative tool developed for assessing the reliability of *C. parvum* data. The relationships between on-line performance parameters and oocyst removal were evaluated and contributed to the development of practical treatment strategies for maximizing *C. parvum* removal by filters. Since surrogate parameters could also be very useful in developing such operational strategies, polystyrene microspheres and *B. subtilis* spores were also assessed as potential surrogates for *C. parvum* removal by filtration.

1.2 RESEARCH OBJECTIVES

The overall goal of this research was to evaluate the impact of filter design and operational parameters on pathogen removal during drinking water treatment so that conceptual mechanistic models for maximizing pathogen removal by filtration could be developed and incorporated into practical treatment strategies.

Specific objectives in pursuit of this goal were:

1. To implement a reliable, state-of-the-art method for concentration and enumeration of *C. parvum* oocysts from water during treatment process challenge studies during which oocysts are spiked into the treatment process.

2. To develop a quantitative statistical tool that describes the reliability of, or uncertainty associated with, *C. parvum* (and other discrete particle) concentration and removal data.

3. To determine if the removal of formalin-inactivated *C. parvum* oocysts by filtration is a reliable surrogate for the removal of viable oocysts.

4. To evaluate the impact of several design and operational factors on *C. parvum* oocyst removal by granular media filters.

5. To evaluate the removal of polystyrene microspheres as surrogates for the removal of *C. parvum* by granular media filtration.

6. To provide practical design and operational strategies for maximizing the removal of *C. parvum* by granular media filters.

1.3 RESEARCH APPROACH

Physico-chemical water treatment processes for the removal of parasitic pathogens such as *C. parvum* have been receiving increased attention because of the difficulty of chemically inactivating such microorganisms. Although numerous investigations have examined the removal of these and other pathogens through filtration processes, those studies have generally been conducted under optimized operating conditions. Several studies of *C. parvum* and surrogate removal by filtration have included some examination of vulnerable operating periods; however, they have been limited in scope and have not focused on thoroughly assessing vulnerable periods of operation and providing design and operational strategies for maximizing *C. parvum* removal by filtration.

The approach used for the completion of this thesis research was based on defining *C. parvum* and potential surrogate removals during vulnerable periods of filter operation, relating them quantitatively to removals during stable operation, and identifying general strategies for maximizing *C. parvum* removal by filtration. This research approach, as it addresses the experimental objectives outlined in Section 1.2, is shown in Figure 1.2 and summarized below.

After a thorough review of the relevant filtration and particle removal literature, several key studies were identified which focused on analytical methods for concentration and enumeration of *C. parvum*, approaches for expressing *C. parvum* concentration data, and removals of *C. parvum* and potential surrogates by granular media filtration. A wide range of methods and recoveries were identified, demonstrating the need for a statistical framework for incorporating analytical uncertainty into assessments of *C. parvum* removal by water treatment processes. Limited information regarding *C. parvum* removal at various points in the filter cycle or during sub-optimal operating conditions was available, emphasizing the need for investigation of design and operational effects on *C. parvum* removal by filtration.

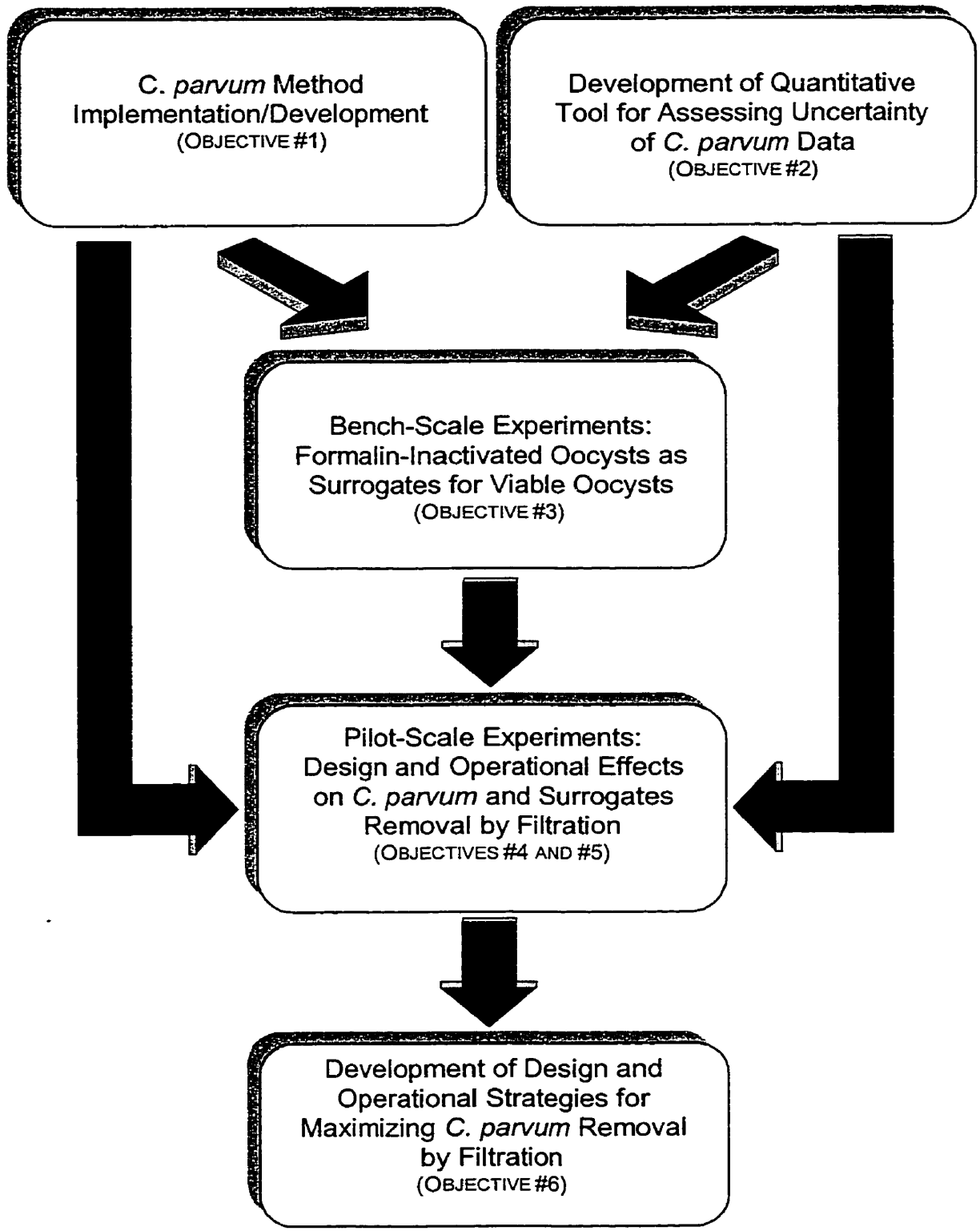


Figure 1.2 Research approach.

Prior to actually investigating *C. parvum* removals by filters, it was necessary to develop and implement a reliable oocyst concentration and enumeration method. Given the ultimate goal of assessing oocyst removal efficiencies of filters at several operating conditions, it was considered critical that oocysts be present in both the filter influent and effluent in reliably countable concentrations. High influent oocyst concentrations were particularly critical for evaluating the oocyst removal capacity of filters under optimal operating conditions when, as indicated by the literature, filter effluent oocyst concentrations were expected to be low. Therefore, it was essential to have high concentrations of oocysts in the filter influent during the thesis investigations.

Analytical methods for the concentration and identification of *C. parvum* are generally laborious and fraught with uncertainty. In the present research, a method reported in the literature was optimized so that it could be used in conjunction with a seeding protocol that provided high enough filter influent oocyst concentrations so as to ensure reliably countable numbers of oocysts in the filter effluent. The burden of implementing a reliable analytical method for enumerating *C. parvum* during such studies was considerably less than it would have been for evaluation of indigenous concentrations. Seeding *C. parvum* oocysts into the filter at a high concentration (several orders of magnitude higher than indigenous concentrations) allowed for the collection and processing of small water sample volumes. Although this approach necessitated the assumption that filters would remove comparable levels of oocysts regardless of influent concentration, it was the only approach feasible given the available state-of-the-art analytical techniques for *C. parvum* enumeration.

Large-scale filtration investigations with viable *C. parvum* were impractical because of the health risks associated with the handling and discharge of such a pathogen. It has typically been assumed that inactivated oocysts are suitable surrogates for viable oocysts during filtration investigations, however, it has also been demonstrated that chemical inactivation changes the surface charge properties of oocysts, thereby possibly affecting removal by filtration. The first experimental phase of this thesis research was designed to demonstrate whether or not comparable removals of viable and formalin-inactivated oocysts could be expected by filtration at various operating conditions.

The second experimental phase of this research represented a large majority of the experimental work and was designed to investigate design and operational strategies for maximizing *C. parvum* removal by filtration. This research component was crucial to establishing the extent to which elevated turbidity or particle counts in filter effluents might be accompanied by increased *C. parvum* passage through filters. Experiments were conducted at the Ottawa, Windsor, and University of Waterloo pilot plants. The multiple research platforms permitted investigation of different types of raw waters, water temperatures, coagulation regimes, and filter designs. Formalin-inactivated *C. parvum* oocysts were seeded at all of the experimental locations. Polystyrene microspheres were evaluated as potential surrogates for *C. parvum* because they were similar to oocysts in size and easy to identify and enumerate. Microspheres were seeded at Ottawa and the University of Waterloo. Microsphere removals by filtration were compared to *B. subtilis* spore removals by filtration and total particle reductions through the treatment process, both of which are indicators of treatment performance, but not qualitative surrogates for *C. parvum* removal by filtration.

The experimental data were evaluated using a statistical approach that was developed during the course of this thesis research. The statistical approach incorporated analytical recovery and uncertainty of recovery and yielded confidence intervals on both measured *C. parvum* concentrations and removals by filtration. These data were then evaluated relative to traditional performance data (*e.g.* turbidity and particle counts) to provide clear design and operational strategies for maximizing *C. parvum* removal by filtration.

The overall research approach employed in this thesis was designed to provide clear outcomes of practical value to the water industry while elucidating some of the fundamental mechanistic processes governing the removal of microbiological particles such as *C. parvum* by filtration processes. A more detailed discussion of the experimental design and statistical framework can be found in Chapters 3 and 4, respectively. Chapter 8 provides a summary of the practical outcomes and recommendations for the water treatment industry.

1.4 THESIS ORGANIZATION

Chapter 2 presents background information regarding the significance of the proposed research and the theoretical concepts employed therein. An extensive review of previous research regarding *C. parvum* occurrence, detection, and removal during drinking water treatment has been included in that chapter. Chapter 2 concludes with a summary of the limitations of current knowledge regarding the removal of *C. parvum* by filtration. Chapter 3 outlines the experimental design, research platforms, and analytical methods employed during the course of this research. A statistical framework for describing the reliability of *C. parvum* concentration and removal data is developed in Chapter 4. Bench-scale data focused on establishing that formalin-inactivated oocysts are adequate surrogates for viable oocysts are discussed in Chapter 5; a preliminary evaluation of design strategies for maximizing *C. parvum* removal by filtration (*e.g.*, by comparing tri-media to dual-media filtration) is also included in that chapter. The pilot-scale experiments address the main objective of this research by investigating *C. parvum* and potential surrogate (*B. subtilis* and polystyrene microsphere) removals by various filter designs during several operating conditions; these experiments are discussed in Chapter 6. In Chapter 7, the bench- and pilot-scale *C. parvum* and microsphere data are discussed relative to traditional performance measures (turbidity and particle counts) and surrogates in the broader context of water treatment optimization and practice. In Chapter 8, the experimental data are then integrated to provide design and operational strategies for maximizing *C. parvum* removal by filtration. This chapter also summarizes the conclusions, implications, and contributions of this thesis research to the water industry.

Chapter 2
Background

2.1 *Cryptosporidium* in Drinking Water

2.1.1 Importance and Epidemiology

Pathogenic microorganisms interact with hosts to cause disease. Among the most common pathogen-associated illnesses are acute gastrointestinal infections that are typically manifested as diarrhea (Ryan *et al.,* 1994). Only exceeded in frequency by respiratory tract infections such as the common cold, most gastrointestinal infections do not receive medical attention because they are usually self-limiting, often within hours or days (Ryan *et al.,* 1994). However, diarrheal illness is of profound worldwide significance as it is a major cause of morbidity and mortality among infants and children. As many as five billion episodes of diarrhea and five to ten million diarrhea-associated deaths have been reported annually in Asia, Africa, and Latin America (Current, 1988). Depending on socioeconomic and nutritional factors, diarrhea-associated death rates of children under the age of 7 can be as high as 50% in these areas (Current, 1988). Mortality associated with diarrhea in developed nations is considerably lower, but still significant (Ryan *et al.,* 1994).

Recognized as a major worldwide health problem in 1976, *Cryptosporidium* infections are the third or fourth most common cause of diarrhea worldwide (ASM News, 1996). Diarrhea from *Cryptosporidium* infections can last from three to twelve days in most well-nourished persons (Current, 1988), however in some cases symptoms can last for more than one year after the acute phase (ASM News, 1996). Persistent diarrhea can lead to poor absorption of foods and severe malnutrition in children. It has been estimated that in developing nations such as Brazil, *Cryptosporidium* infection rates in children

may exceed 90% (ASM News, 1996). *Cryptosporidium* oocysts have been detected in 1-4% of gastroenteritis cases in young children in western countries whereas these rates have ranged from 4-11% in developing nations; adults suffer from gastroenteritis at rates approximately one third of those reported in children (Ryan *et al.*, 1994).

Infection rates increase in immunocompromised populations. *Cryptosporidium* has been identified in 15% of American AIDS patients suffering from diarrhea and as many as 50% of such individuals in Haiti and Africa (Ryan *et al.*, 1994). Other enteric pathogens such as *Giardia lamblia* have been recovered only from a minority of these patients (Ryan *et al.*, 1994). Cryptosporidiosis is an important and dangerous disease for both immunocompromised and immunocompetent individuals since it has no effective treatment (ASM News, 1996).

2.1.2 Life Cycle

Cryptosporidium is an obligate parasite that replicates in the intestinal tract of a wide range of host mammals including humans. Like other sporozoans, these 3-6 μm (in diameter) spherical parasites exhibit alternating cycles of sexual and asexual reproduction that are completed within the gastrointestinal tract of a single host (Ryan *et al.*, 1994). Parasitized animals excrete *Cryptosporidium* oocysts, which are environmentally resilient. Infection is acquired by ingesting oocysts which can release four infective sporozoites that attach to epithelial cells of the gastrointestinal tract and transform into trophozoites that subsequently go through merogony (asexual reproduction). The reproductive process involves multiple fission (schizogony) of the sporozoites to form schizonts containing eight daughter cells known as Type 1 merozoites. A second generation of Type 1 merozoites is produced when the daughter cells are released from the schizont, attach to further epithelial cells, and repeat schizogony. Figure 2.1 depicts the life cycle of *Cryptosporidium*.

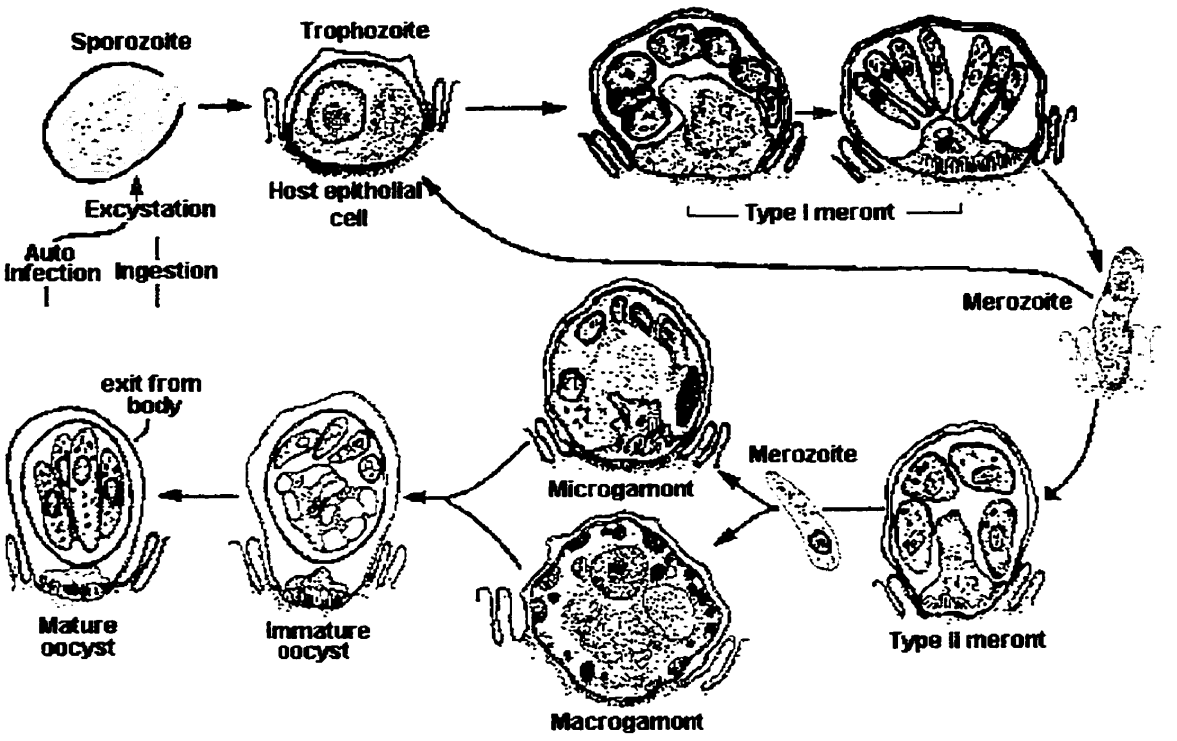


Figure 2.1 Life cycle of *Cryptosporidium* (*after* Fayer and Ungar, 1986)

Oocysts typically undergo one cycle of asexual reproduction and produce a self-limited diarrhea (Schaechter *et al.*, 1993). After asexual reproduction, schizonts containing four Type 2 merozoites incapable of asexual reproduction are formed. During gametogony, the Type 2 merozoites undergo sexual reproduction. Following fertilization, the resulting zygotes develop into oocysts that are subsequently excreted (Ryan *et al.*, 1994).

Approximately 80% of the oocysts formed from zygotes are of the environmentally resilient nature that, upon finding a new host, undergo the same life cycle described above. The remaining 20% of oocysts form only a thin wall and initiate an autoinfective cycle within the original host. The immunity of immunocompetent hosts dampens the production of merozoites and thin-walled oocysts, stopping autoinfection and terminating the acute infection (Ryan *et al.*, 1994). In the immunocompromised, oocysts undergo numerous cycles of sexual and asexual reproduction that may result in severe infections that last indefinitely (Schaechter *et al.*, 1993).

2.1.3 Sources and Occurrence of *C. parvum* in Water

Cryptosporidium has worldwide distribution and occurs in several host species including mammals, birds, and fish. *Cryptosporidium parvum* is associated with most human infections and is also common in livestock (Rose, 1988). *C. parvum* oocysts have been detected in surface waters in concentrations as high as 10^4 /100 L and as low as 0.3/100 L (LeChevallier and Norton, 1995; Lisle and Rose, 1995; Smith *et al.*, 1991), regardless of whether the waters are pristine or impacted by human and animal activity. Several studies have indicated that watershed character and protection influence parasite contamination (Ong *et al.*, 1996; Hansen and Ongerth, 1991). Human cryptosporidiosis is believed to involve both anthroponotic and zoonotic cycles of transmission (Casemore *et al.*, 1997). Transmission in humans is either direct through the fecal-oral route or indirect through water contamination. Treated wastewater effluents and agricultural runoff are often significant sources of oocysts in surface waters (Rose, 1988).

At least two distinct genotypes of *C. parvum* are currently linked to human cryptosporidiosis (Peng *et al.*, 1997; Patel *et al.*, 1998; Widmer *et al.*, 1998). Peng *et al.* (1997) demonstrated that genotypes 1 and 2 were infective to humans, but only genotype 2 isolates were infective to mice and cattle under routine laboratory conditions; this demonstrated the occurrence of two distinct *C. parvum* transmission cycles in humans. Waterborne outbreaks of cryptosporidiosis have been historically attributed in significant part to agricultural runoff and were considered to be associated with *C. parvum* of genotype 2. Several recent investigations have challenged this assumption by demonstrating that a majority of human infections of cryptosporidiosis from waterborne outbreaks were linked to genotype 1 (Patel *et al.*, 1998; Sulaiman *et al.*, 1998; Widmer *et al.*, 1998). In one study from the United Kingdom, however, similar frequencies of genotype 1 and 2 isolates from sporadic human infections were reported (McLauchlin *et al.*, 1999).

The compelling evidence suggesting that *C. parvum* oocysts of genotype 1 are the predominant cause of waterborne outbreaks of human cryptosporidiosis in North America may result in increased focus on domestic wastewaters and may lead to some re-evaluation of watershed protection strategies aimed at minimizing oocyst contamination

of drinking water supplies. Methodologies used to evaluate treatment efficacy for removing or inactivating oocysts may also have to be re-examined. Despite the apparent epidemiological significance of genotype 1, (the predominant cause of human cryptosporidiosis), type 1 isolates are not typically used in removal, viability, or infectivity investigations because of the inability of maintaining them in the laboratory. Although the genotype 1 oocysts may be the predominant cause of waterborne outbreaks of cryptosporidiosis, the results of investigations utilizing genotype 2 are not necessarily invalidated. Since isolates of the same genotype can vary significantly in their infectivity in cultured cells and in animal models, it is possible that results obtained with genotype 2 may be similar to those that might be obtained with genotype 1 (Chappell *et al.*, 1999). In the future, investigations with genotype 1 may be possible. Recent research has demonstrated the first successful serial propagation of genotype 1 *C. parvum*; type 1 isolates were successfully adapted to propagate in gnotobiotic piglets (Widmer *et al.*, 2000).

2.1.4 Waterborne Outbreaks of Cryptosporidiosis

Outbreaks of waterborne cryptosporidiosis have been well documented in North America and abroad (Fox and Lytle, 1996; Roefer *et al.*, 1996; Welker *et al.*, 1994); drinking water has been implicated in several worldwide outbreaks. The most famous outbreak was the 1993 Milwaukee outbreak, during which more than 400,000 people were affected, making it the largest outbreak of waterborne disease ever recorded in the United States (Solo-Gabriele and Neumeister, 1996). The Las Vegas outbreak was also significant since 20 immunocompromised individuals died as a result of infection (Roefer *et al.*, 1995; 1996). Outbreaks of waterborne cryptosporidiosis are of interest to the water treatment industry because of their regulatory and engineering implications as well as their public health consequences. During the documented outbreaks in the United States, each of the implicated water treatment plants was in compliance with federal and local regulations (Solo-Gabriele and Neumeister, 1996). Table 2.1 summarizes some of the documented waterborne outbreaks of cryptosporidiosis.

Table 2.1

Waterborne Outbreaks of Cryptosporidiosis

Location	Est. # of People Affected (Confirmed)	Source Water	Water Treatment Strategy	Suspected Source of Oocysts	Reference
Braun Station, TX 1984	5900 (2006)	ground	chlorination	sewage contaminated well	D'Antonio *et al.* (1989)
Cobham, Surrey, UK 1983 1985	NA (16) NA (50)	spring	chlorination, softening	NA	Barer and Wright (1990) Lisle and Rose (1995)
Sheffield, UK 1986	NA (84)	surface	NA	cattle feces in runoff	Lisle and Rose (1995)
Carrollton, GA 1987	32400 (12960)	river	conventional	raw sewage, cattle runoff	Hayes *et al.* (1989)
Ayrshire, UK 1988	24000 (27+)	NA	chlorination, filtration minimally	pipe cross connection, cattle feces	Smith *et al.* (1989)
Swindon/ Oxford-shire,UK 1989	741092 (516)	surface	conventional	filter BW recycle, cattle feces	Richardson *et al.* (1991)
Loch Lommond, UK 1990	NA (147)	surface	NA	NA	Barer and Wright (1990) Lisle and Rose (1995)
Isle of Thanet, UK 1990	177300 (47)	surface	conventional	treatment deficiencies	Joseph *et al.* (1991)
Berks County, PA 1991	NA (551)	ground	chlorination	septic tank effluent, creek	Moore *et al.* (1994)
South London, UK 1991	44	—	—	—	Maguire *et al.* (1995)
Jackson County, OR 1992	15000 (NA)	spring/ river	chlorination	river, runoff, wastewater	Leland *et al.* (1993)
Milwaukee, WN 1993	1600000 (403000)	lake	conventional	cattle /slaughter waste, sewage	MacKenzie *et al.* (1994)
Kitchener-Waterloo, ON 1993	NA (225)	river, ground	conventional + biofiltration	—	Welker *et al.* (1994)
Yakima County, WA 1993	7 (3)	well	—	runoff - elk, cattle, sheep	Solo-Gabriele and Neumeister (1996)

Table 2.1

Waterborne Outbreaks of Cryptosporidiosis (Continued)

Location	Est. # of People Affected (Confirmed)	Source Water	Water Treatment Strategy	Suspected Source of Oocysts	Reference
Cook County, MN 1993	NA (108)	lake	—	sewage or septic tank effluent	Solo-Gabriele and Neumeister (1996)
Las Vegas, NV 1994	NA (120+)	lake	conventional, pre- and post-chlorination	treated wastewater, boat sewage	Roefer *et al.* (1995, 1996)
Walla Walla County, WA 1994	86 (15)	well	—	treated wastewater	Solo-Gabriele and Neumeister (1996)
South and West Devon, UK 1995	(575)	river and gravel wells		treated wastewater	*Cryptosporidium* Capsule (1998a) Patel *et al.* (1998)
Northern Italy 1995	294	—	—	—	*Cryptosporidium* Capsule (1997a) Pozio *et al.* (1997)
Kelowna, BC 1996	1136	—	—	—	*Cryptosporidium* Capsule (1996a,b)
Ogose, Japan 1996	9000	—	—	—	*Cryptosporidium* Capsule (1996c)
Collingwood, ON 1996	182	spring	—	—	*Cryptosporidium* Capsule (1996e)
London and Hertfordshire, UK 1997	(345)	ground	—	treated wastewater	Patel *et al.* (1998)
North Thames, England 1997	244	ground	filtration and ozonation	unknown	*Cryptosporidium* Capsule (1996d, 1997b)
Shoal Lake, ON 1997	100 (11)	lake	chlorination	unknown	*Cryptosporidium* Capsule (1997c)
Brushy Creek, TX 1998	1300 (32)	ground	—	Sewage contamination of creek/wells	*Cryptosporidium* Capsule (1998b)
NW England, UK 1999	360	surface	unfiltered	—	*Cryptosporidium* Capsule (1999a,b)

2.2 RECOVERY AND DETECTION OF *CRYPTOSPORIDIUM* FROM WATER

Analytical methods for quantification of *C. parvum* oocysts from water were originally developed from those for *Giardia*. Most of these methods require three steps: concentration, purification, and enumeration. Oocyst concentrations are typically low in natural waters, necessitating the concentration of oocysts from large volumes of water to achieve consistently countable numbers. Typically at least 100 L of source waters and ≥1000 L of finished waters are recommended for *C. parvum* analyses (Jakubowski *et al.*, 1996). Oocyst concentration is typically achieved via some form of cartridge or membrane filtration under mild vacuum pressure (ASTM 1993; USEPA, 1999a).

It can be difficult to distinguish between oocysts and other particles. Since oocysts represent a small fraction of the particles and microorganisms found in natural waters, purification steps are often incorporated to separate oocysts from the remaining debris in water concentrates. One of the challenges associated with detection of *C. parvum* has been poor and highly variable oocyst recovery (Clancy *et al.*, 1994). Oocyst purification becomes additionally important because the analytical recovery of several common detection methods is greatly impacted by the amount and type of debris present (LeChevallier *et al.*, 1990; 1991a,b,c). Flotation by density gradients (Hansen and Ongerth, 1991; LeChevallier *et al.*, 1995) and flocculation (Vesey *et al.*, 1993a) have been used for oocyst separation from concentrated water samples. More recently, immunomagnetic separation has been demonstrated as a more reliable method of oocyst purification (Rossomondo *et al.*, 1994; Bukhari *et al.*, 1998).

Once oocysts are concentrated and purified, they must be enumerated. Immunofluorescence assays (IFAs) commonly used to detect and quantify *Cryptosporidium* oocysts allow good discernment between oocysts and other particles. Both monoclonal and polyclonal antibodies (LeChevallier *et al.*, 1995; Ongerth and Stibbs, 1987) have been used successfully in direct and indirect IFAs for *C. parvum* (Musial *et al.*, 1987; ASTM, 1993). In these IFAs, the FITC (fluorescein isothiocyanate) label appears bright apple green during epifluorescence microscopy (Nieminski *et al.*,

1995). Some of the difficulties associated with IFAs can include background fluorescence and false positives (Rodgers *et al.*, 1995).

FITC-IFAs only identify oocysts; they do not indicate whether or not the oocysts are viable and infectious. Vital dye (Roberston *et al.*, 1992) and *in vitro* excystation (Robertson *et al.*, 1993) assays have been developed to indicate oocyst viability, but they do not measure infectivity. Finch *et al.* (1993) demonstrated that the analytical methods with which oocyst inactivation and infectivity are determined could potentially underestimate assessments of inactivation. Their work contributed to the general acceptance of *in vivo* animal infectivity assays as the standard method for assessing *C. parvum* inactivation. Several comparisons of *in vitro* methods such as excystation and DAPI/PI (4',6-diamidino-2-phenylindole/propidium iodide) to *in vivo* animal infectivity have revealed that *in vitro* assays often over-estimate oocyst viability compared to *in vivo* infectivity data (Bukhari *et al.*, 1999).

One of the first *C. parvum* concentration and identification methods was proposed by the American Society for Testing and Materials (ASTM) and involved the passage of large volumes of water through a polypropylene yarn-wound cartridge filter, flotation on a Percoll-sucrose gradient, and IFA enumeration (ASTM, 1993). The literature strongly and frequently indicates that the ASTM method is inadequate because it typically results in highly variable and low oocyst recoveries (Hargy *et al.*, 1996). LeChevallier *et al.* (1995) examined the ASTM method and revealed that the centrifugation and clarification steps could each result in losses as high as 30%. Further difficulties with the ASTM method can result from the presence of algae because numerous species can fluoresce and result in false positive counts (Rodgers *et al.*, 1995). Despite the lack of a more accurate and consistent method, the ASTM method was incorporated into the United States Environmental Protection Agency's (USEPA's) Information Collection Rule (ICR) as the standard protocol for *C. parvum* detection and enumeration from water (USEPA, 1996).

Several alternatives to the ASTM protocol have been examined (Nieminski *et al.* 1995; Whitmore and Carrington, 1993; Vesey *et al.*, 1993a). Most notably, the USEPA introduced Method 1622 which requires filtration, immunomagnetic separation of

oocysts, and an IFA for determination of oocyst concentrations, with confirmation through DAPI staining and differential interference contrast (DIC) microscopy (USEPA, 1999a). Almost identical to Method 1622, Method 1623 was introduced for simultaneous detection of *Cryptosporidium* and *Giardia* (USEPA, 1999b). Method 1622 is a significant improvement over the ICR method and demonstrated mean oocyst recoveries of >70% in early trials (Clancy *et al.*, 1997; Bukhari *et al.*, 1999). Other studies of Method 1622, such as the USEPA validation experiments, have yielded recoveries of approximately 35% with 13% relative standard deviation (Clancy *et al.*, 1999). More detailed discussions of analytical methods for the detection and enumeration of *C. parvum* are available in the literature (Jakubowski *et al.*, 1996; Vesey *et al.*, 1991).

Due to continued low and non-constant analytical recoveries, evaluations of *C. parvum* removal by water treatment processes usually involve seeding the water to be treated with oocyst concentrations higher than those typically present in raw water. This approach is analytically advantageous because it allows for smaller sample volumes that do not necessarily require purification. Seeding high concentrations of oocysts can also be critical to accurately assessing the oocyst removal capacity of treatment processes, which requires oocysts present in reliably countable concentrations in the effluent. Log removal estimates may be limited by influent concentration when effluent concentrations are low or based on non-detects because treatment processes cannot remove more oocysts than those with which they have been spiked. Comparisons between studies should be evaluated with caution because oocyst recovery is sensitive to seeded concentration (Straub *et al.*, 1996; Musial *et al.*, 1987), oocyst quality (Dawson *et al.*, 1993), sample volume processed (Nieminski *et al.*, 1995), flow rate (Musial *et al.*, 1987), and water quality (Shepherd and Wyn-Jones, 1996).

Ensuring that spiked oocyst concentrations are accurately measured contributes to the consistency of analytical recoveries (Straub *et al.*, 1996). Several methods for determining spike doses of *Cryptosporidium* and *Giardia* have been evaluated. Among the methods evaluated by Straub *et al.* (1996) were well-slide IFA and hemocytometer counts. Theoretically, hemocytometer counts should provide the most accurate and consistent results because sample processing is not necessary and detection does not rely

on staining. Despite initial parity between the two methods, the authors concluded that well-slide IFA provided the most accurate results; however, they speculated that the unexpected relative performance of the hemocytometer was likely due to an additional sample transfer and holding step (Straub *et al.*, 1996). A second study with consistent sample handling indicated that hemocytometer counts generally yielded higher recoveries and better precision than well-slide IFA counts; for any given trial, no significant differences among analysts were observed, regardless of the counting method (Jackson *et al.*, 1997).

2.3 QUANTIFYING THE RELIABILITY OF *CRYPTOSPORIDIUM* DATA

A drinking water limit of approximately one *Cryptosporidium* oocyst per 34,000 L (one per 34 m^3) has been suggested (Lisle and Rose, 1995). Such a low limit, or even one several orders of magnitude higher, would be difficult to implement because current methods for measuring *Cryptosporidium* concentrations are unreliable, laborious, and expensive. Methodological difficulties are likely more pronounced when attempting to quantify indigenous concentrations, since high concentrations of oocysts (typically 10^3-10^6 oocysts/L) are commonly used in experimental evaluations of treatment processes.

To date, few studies of *Cryptosporidium* concentration and removal efficiency have addressed the issue of data reliability beyond stating average analytical recovery. The lack of information regarding the reliability of *Cryptosporidium* data is likely associated with the expense and difficulty of processing samples. It is also commonly understood that means and standard deviations calculated from replicate samples do not account for all of the uncertainty in the data. For example, they do not account for variability resulting from sampling strategy or analytical methodology. The complexity of incorporating these additional sources of variability into a statement of data reliability such as a confidence interval is increased by the fact that the normal distribution is often inappropriate for describing distributions of microorganisms such as *Cryptosporidium* in water samples.

Haas and Rose (1996) showed that naturally occurring oocyst densities could be described adequately by the Poisson distribution, as would be expected for a sample from a uniform suspension of such microorganisms in water that was enumerated by an ideal method. Atherholt and Korn (1999) presented Poisson methods for sample counts in the context of the ICR protocol and suggested that a more complex distribution than the Poisson distribution may be required to account for the various errors in the analytical process. Nahrstedt and Gimbel (1996) developed such a statistical framework for calculating confidence intervals by assuming a Poisson distribution for the true sample counts, a binomial distribution for modeling the recovered fraction of oocysts, and a Beta distribution for describing the uncertainty of recovery. They also noted that the time and location of sampling influence error, but suggested that this contribution cannot be statistically determined. As defined primarily by Nahrstedt and Gimbel (1996), the statistically describable errors that influence the concentration and enumeration of *Cryptosporidium* (and other discrete particles) are summarized below.

2.3.1 Representative Sampling

The number of oocysts in a sample from a uniform suspension of oocysts in water should have a Poisson distribution because Poisson events are those occurring in time or space where the probability of success (or occurrence) is independent and constant in each unit of time or space (Blom, 1989). According to the Poisson model, the probability of N oocysts occurring in a sample is,

$$P(N) = \frac{\lambda^N}{N!} e^{-\lambda} \tag{2.1}$$

where λ is the expected value or mean of the distribution. The parameter λ represents the true mean count for the population and can be thought of as the product of the true concentration of oocysts (c) in the water body and the volume of water sample evaluated (V) given by,

$$\lambda = c\ V \tag{2.2}$$

The Poisson distribution (Equation 2.1) describes random sampling error resulting from the fact that the entire water body is not evaluated. In other words, λ represents the oocyst count that would be expected if an infinite number of random samples were collected. This was described by Nahrstedt and Gimbel (1996) and is illustrated in Figure 2.2, where a true concentration of 1 oocyst/500 L of water is assumed. If three random 500 L samples were collected, the actual number of oocysts (N) in each sample might be different; in this case, $N_1 = 1$ oocyst, $N_2 = 2$ oocysts, and $N_3 = 0$ oocysts. As the number of samples approaches infinity, the expected value (calculated by Equation 2.2) approaches 1.

The Poisson approach may be limited in applicability because the true oocyst concentration (c) is not known and methodological losses cannot be accurately incorporated because loss is sample specific. Deviations from the Poisson distribution can occur due to factors such as non-representative sampling or imperfect analytical methods. A relevant example of deviation from a Poisson distribution is non-independence of observations due to oocyst aggregation.

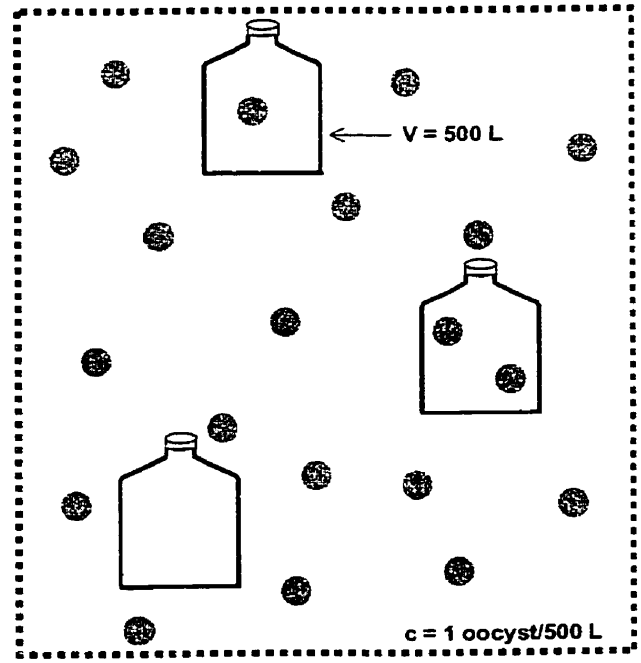


Figure 2.2 Sampling from a water body (*after* Nahrstedt and Gimbel, 1996)

Observed *Cryptosporidium* counts have been adequately represented by a Poisson distribution in at least two cases (Haas and Rose, 1996; Parkhurst and Stern, 1998). However, it has also been suggested that the Poisson model may not be directly applicable for real data sets where methodological error may play a substantial role (Nahrstedt and Gimbel, 1996). Since analytical methods for the detection of *Cryptosporidium* are often highly uncertain over a range of recoveries, several researchers have suggested that a distribution more complex than a Poisson distribution may be necessary to account for methodological error (Nahrstedt and Gimbel, 1996; Atherholt and Korn, 1999).

2.3.2 Random Analytical Error

Given an imperfect analytical method, the oocyst counts observed after *Cryptosporidium* processing represent a portion of a sample's true counts. Only the recovered fraction of oocysts (p) is observed under the microscope (Figure 2.3). The number of observed oocysts is a sub-sample from the original Poisson distribution of oocysts in the water sample. The probability of observing oocysts in the sub-sample follows a binomial distribution with probability p that each of the original oocysts will be observed (Fisher and van Belle, 1993; Nahrstedt and Gimbel, 1996).

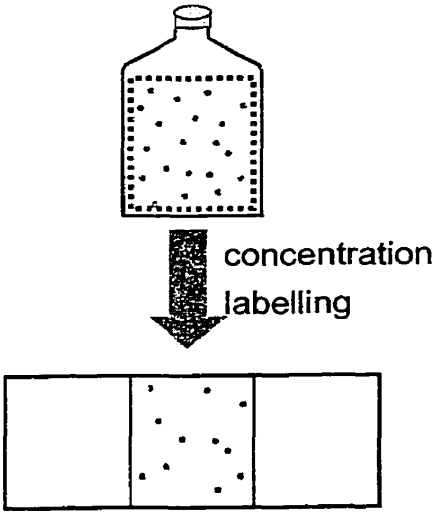


Figure 2.3 Effect of sample preparation on number of observed oocysts (*after* Nahrstedt and Gimbel, 1996)

The probability of detecting X oocysts from a water sample containing N oocysts by using an analytical method with recovery p is described by the binomial distribution

$$P(X;N:p)=\binom{N}{X}p^{X}(1-p)^{N-X} \tag{2.3}$$

Parkhurst and Stern (1998) demonstrated that a binomially distributed sub-sample drawn from a Poisson-distributed sample also has a Poisson distribution. This observed Poisson distribution has a mean (λ_{obs}) described by,

$$\lambda_{obs} = p\,cV \tag{2.4}$$

which is similar to Equation 2.2. Based on this analysis, the mean count of observed oocysts can be scaled to account for a constant recovery of p.

Numerous investigators have reiterated the finding that oocyst recovery can be affected by numerous factors such as water quality for several analytical methods (Shepherd and Wyn-Jones, 1996). Therefore, recovery studies to determine p should always be performed under conditions comparable to experimental conditions. This includes accounting for matrix effects (*e.g.*, turbidity, presence of coagulant, *etc.*), using the same sample volumes, and quantifying the recovery of anticipated counts.

When performing recovery studies, the initial mode of oocyst quantification must also be considered reliable and reproducible. Precautions should be taken to minimize oocyst losses during dilution steps prior to the processing of the samples used in the recovery study. The most common methods for quantifying oocysts (when they are present in high concentrations) are hemocytometer counts and well-slide counts employing an IFA (Straub *et al.*, 1996; Jackson *et al.*, 1997). Due to the numerous and complex individual steps associated with oocyst concentration and enumeration, it is often difficult to maintain highly reproducible oocyst recovery. The non-constant analytical recovery of oocysts may therefore require description beyond that of a single value.

2.3.3 Non-Constant Analytical Recovery

Nahrstedt and Gimbel (1996) addressed the issue of non-constant analytical recovery by using a Beta distribution for the uncertainty (or scattering) of recovery. They introduced two additional parameters (v and w) to account for the scattering of recovery. Atherholt and Korn (1999) pointed out that the experimental and mathematical determination of these parameters was not developed in Nahrstedt and Gimbel's (1996) research.

In the present discussion, the Beta distribution parameters are referred to as a and b. These parameters are constants that affect the spread and height of the Beta distribution. The probability of recovery (p) depends on these parameters and is described by,

$$P(p) = p^{a-1}(1-p)^{b-1}\frac{\Gamma(a+b)}{\Gamma(a)\Gamma(b)} \tag{2.5}$$

where Γ represents the gamma function.

The Beta distribution is useful for describing oocyst recovery because the recovery is bound between 0 and 1 (*i.e.*, $0 \leq p \leq 1$) and the distribution is very flexible, allowing for the description of a variety of recovery profiles. Figure 2.4 describes the effects of two combinations of a and b on the recovery probability density function; the Beta parameters are those that Nahrstedt and Gimbel (1996) used to describe the recovery profiles of LeChevallier *et al.* (1991c) and Vesey *et al.* (1993a). As can be seen in Figure 2.4, larger values of a and b result in more narrow distributions. When $a>b$, the distribution is skewed to the right; when $a<b$, the distribution is skewed to the left. Therefore, analytical methods that consistently demonstrate high recoveries will be described by Beta distributions with large values of a and b where $a>b$.

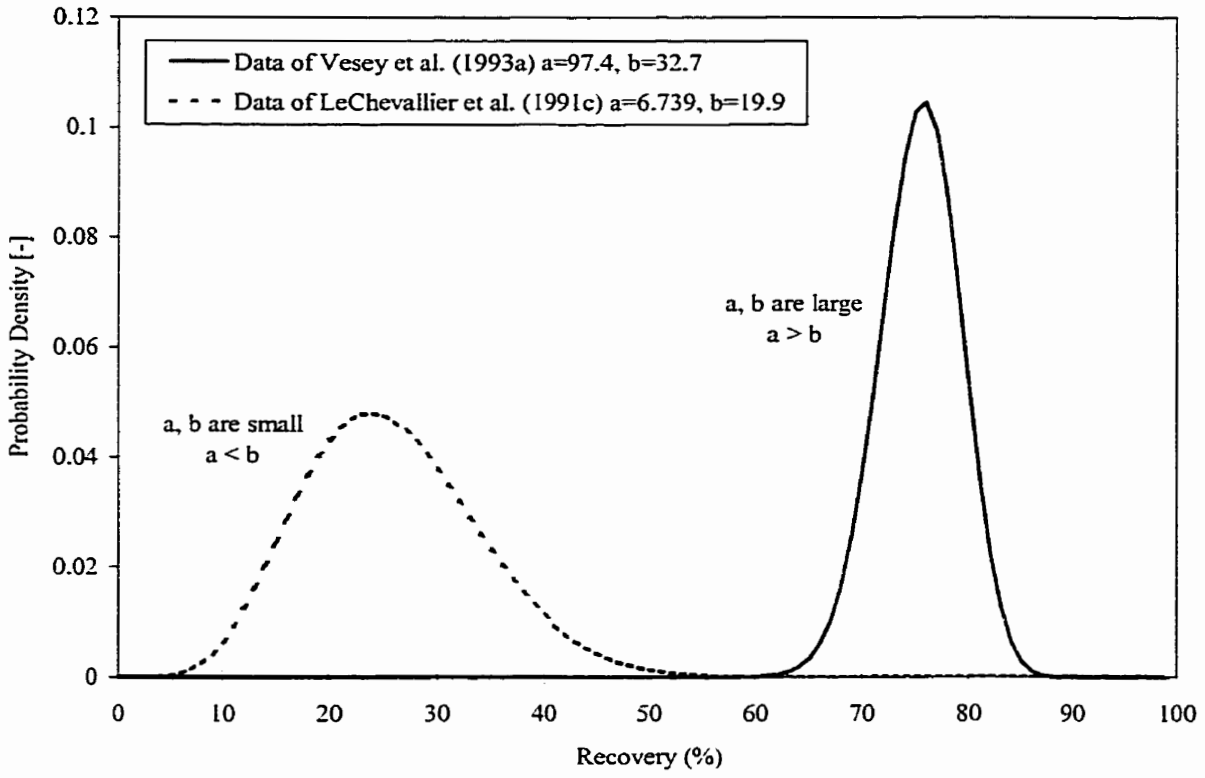


Figure 2.4 Effect of Beta parameters *a* and *b* on recovery probability density function

2.4 TREATMENT OPTIONS FOR *CRYPTOSPORIDIUM*

Studies of *C. parvum* inactivation have indicated that, compared to *Giardia* cysts exposed to the same disinfection conditions, oocysts were 30 times more resistant to ozone and fourteen times more resistant to chlorine dioxide disinfection, while chlorine and monochloramine were found to be considerably less effective (Korich *et al.* 1990). Other studies have concluded that *Cryptosporidium* oocysts were approximately ten times more resistant to ozone inactivation than were *Giardia* cysts (Owens *et al.* 1994). Regardless of the magnitude of the increased level of disinfection necessary for oocyst inactivation relative to cyst inactivation, it is generally accepted that ozone is the most effective oxidant used against *C. parvum* in water treatment disinfection. As a sole barrier, however, ozone does not provide adequate inactivation of *C. parvum* oocysts at practical operating conditions.

Finch *et al.* (1994) and Gyürék *et al.* (1996) indicated disinfectant synergy when the sequential application of ozone and chlorine species was employed. These studies concluded that previous research based on the disinfection capabilities of chemicals used singly grossly underestimated the disinfection efficacy of chemicals used sequentially, which is often done in water treatment practice. The data indicated that the current practice of post-ozonation chloramination might provide a partial disinfection barrier against *C. parvum*.

UV disinfection technologies have demonstrated over 3-log (99.9%) inactivation of *Cryptosporidium* (Campbell *et al.*, 1995; Clancy *et al.* 1996; Clancy *et al.*, 2000), but their operation is most effective in very clean waters such as filter effluents (Bukhari *et al.*, 1999). The use of UV for disinfection of unfiltered waters, however, may not be consistently effective (Bukhari *et al.*, 1999). Despite progress in the development of disinfection technologies that can achieve reasonable *C. parvum* inactivation, such as UV, the traditional physico-chemical barriers used in drinking water treatment remain critical to achieving desirable levels of *Cryptosporidium* removal.

Raw water storage reservoirs are often the first physico-chemical barrier against *Cryptosporidium* and other microorganisms during drinking water treatment. Bertolucci *et al.* (1998) demonstrated that approximately 0.7-log removal of oocysts could be achieved in a reservoir with a theoretical detention time of 18 days. Studies of reservoir-stored river water indicated 1.3- and 1.7-log removal of oocysts stored for 10 and 24 weeks respectively (van Breemen *et al.*, 1998). Such studies indicate that reservoirs can act as a barrier against *Cryptosporidium* passage into water treatment plants.

Coagulation and clarification processes provide another physico-chemical barrier against *Cryptosporidium*. Several bench-, pilot- and full-scale studies have demonstrated that conventional coagulation and sedimentation processes can achieve approximately 0.5- to 2-log removal of oocysts, with average removals in the 0.5- to 1-log range (Kelley *et al.*, 1995; Baudin and Laîné, 1998; Fox *et al.*, 1998; Dugan *et al.*, 1999). Coagulation coupled with dissolved air flotation clarification has demonstrated 0.6- to >3.1-log removal of oocysts (Edzwald *et al.*, 1996; Edzwald and Kelley, 1998; Edzwald *et al.*,

1999). Coagulation combined with other clarification systems such as solids blanket and ballasted clarifiers has respectively yielded 1.8- to 3.7- and 2.5- to 4.2-log removal of oocysts (Alvarez *et al.*, 1999).

While optimized coagulation and clarification contribute a substantial barrier against *Cryptosporidium*, filtration is one of the most critical and successful physico-chemical barriers against *Cryptosporidium* passage through water treatment. Although pressure-driven membranes offer excellent removal capabilities of parasitic pathogens, they are not necessarily economical for treatment of more than 1 to 5 MGD (*e.g.*, Wiesner *et al.*, 1994). A component of conventional water treatment operations, rapid granular media filtration has demonstrated efficacy as a barrier against *Cryptosporidium*. Full-scale *Cryptosporidium* removals from 2- to >4-log have been reported in the literature (Baudin and Laîné, 1998; Nieminski and Ongerth, 1995).

2.5 Removal Mechanisms of Granular Media Filtration

Consideration of the particle removal mechanisms of filtration is a reasonable starting point for evaluating pathogen removal by filtration because the particles found in natural water supplies occur in a wide variety of shapes and sizes; they typically include microorganisms, algae, and clay (Montgomery, 1985). Particle removal during filtration occurs when particles deviate from the fluid streamlines due to gravitational forces, diffusion gradients, and inertial effects of momentum (O'Melia and Stumm, 1967). The relative impact of these effects depends on water quality and physical characteristics of the particles and filter media.

Particle removal can occur either by straining or attachment (Montgomery, 1985). Particles larger than the pore space of media can be removed by straining. Particles smaller than the typical pore distances between media grains can also be removed by straining as particles collect and close the pores. Upon consideration of the relative dimensions of representative particles, collectors, pore sizes, and particle-collector separation distances, Amirtharajah (1988) concluded that particle removal during filtration is predominately a function of non-straining, rather than straining, mechanisms.

The non-straining mechanisms of granular media filtration involve three distinct steps: transport, attachment, and detachment. These mechanisms highlight the physico-chemical nature of the filtration process. The physical nature of filtration requires the transport of suspended particles to the vicinity of the filter grain (collector). The chemical nature of filtration is evidenced in the ability of particles to attach to the surface of collectors. Particles detach when adhesive forces are exceeded by shear forces (that increase as a filter clogs). Attachment of a given particle can occur more than once, however, and particles may reattach at further depths within the filter.

Particle capture during granular media filtration can be estimated from knowledge of particle mechanics under the influence of hydrodynamic and physicochemical forces in porous media. The mechanistic basis for removal by particle deposition requires particle transport to distances close to collectors (*i.e.*, media grains, with or without previously deposited particles) where attachment can occur (O'Melia and Stumm, 1967; Elimelech, 1991). Particles deposited in filters can also detach and be transported to the bulk fluid. Detached particles may re-attach at further depths or pass through the filter and appear in the filter effluent (Amirtharajah, 1988). Figure 2.5 schematically describes the mechanisms of filtration.

2.5.1 Transport

O'Melia (1985) and Amirtharajah (1988) provided thorough summaries of filtration theory. The mechanistic basis for particle removal from suspensions requires that particles transported past collectors must deviate from the fluid streamlines in the bulk of the fluid to distances close to the collector surfaces where attachment is possible. Described schematically in Figure 2.6, particle transport mechanisms include hydrodynamic action, diffusion, sedimentation, and inertia. Though sometimes listed as a transport mechanism and included in this figure, interception is more appropriately considered a boundary condition that results in deposition.

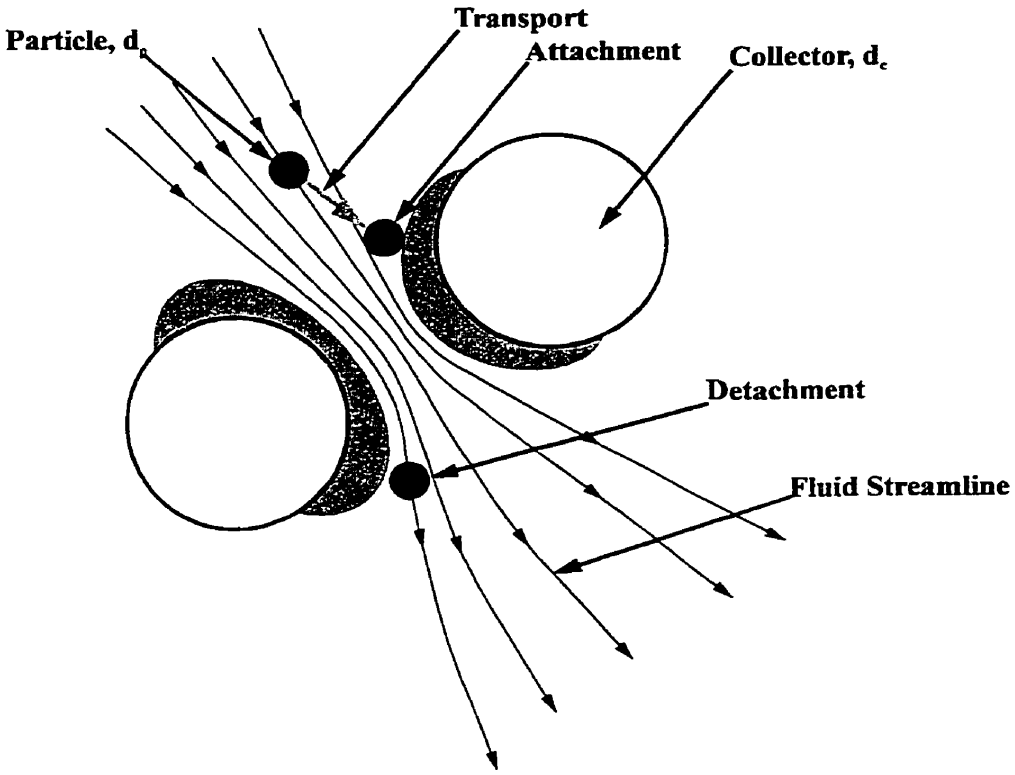


Figure 2.5 Filtration mechanisms (*after* Amirtharajah, 1988)

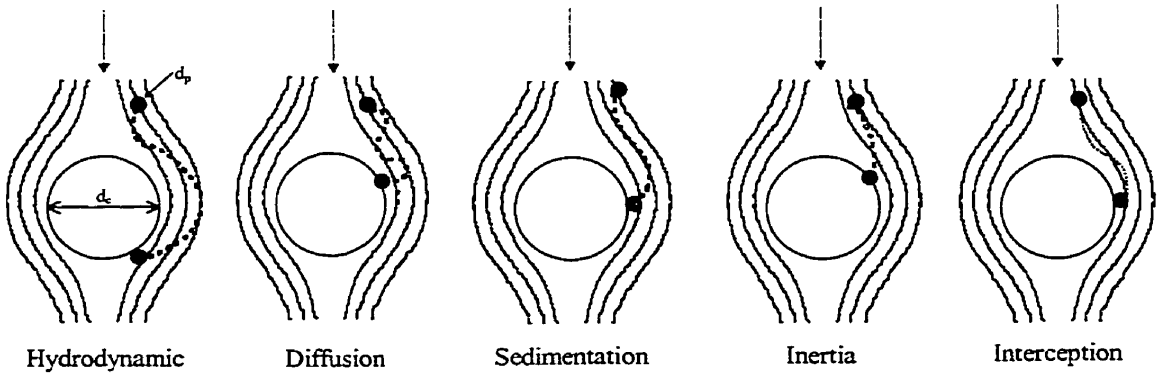


Figure 2.6 Particle transport mechanisms (*after* Ives, 1982; Amirtharajah, 1988)

Diffusion and sedimentation are the dominant transport mechanisms during water filtration (Ives, 1982). Diffusion resulting from Brownian motion is relevant to particles less than 1 μm in size, whereas sedimentation from gravity and the associated particle settling velocity is more significant for particles greater than 1 μm in size (Amirtharajah, 1988). The combination of these two transport mechanisms yields a minimum net transport efficiency for particles that are approximately 1 μm in size (Amirtharajah, 1988). Given that *Cryptosporidium* oocysts are typically 3-6 μm in size, they are near the minimum net transport efficiency.

Amirtharajah (1988) incorporated interception as a boundary condition for attachment resulting from diffusion and sedimentation. Interception occurs when a particle along a streamline is close enough to a collector for attachment to occur. Although hydrodynamic action has not yet been quantitatively described, it involves particle movement across streamlines and is a function of particle shape and interaction in the fluid field. Inertial effects are negligible during water filtration (Ives, 1982).

O'Melia and Stumm (1967) first presented the concept of trajectory analysis for water filtration. The fundamental principle of trajectory analysis views granular beds as an assembly of collectors onto which deposition occurs as a suspension flows past or through them. Tien and Payatakes (1979) specified that the analysis requires knowledge of the geometry and size of the collectors, the flow field around and through the collectors, the nature and magnitude of the forces acting on the particles in the suspension, and the criteria for particle attachment. The concept of particle collection efficiency (η) (for a single collector) is defined as the total rate of particle contact with a single collector divided by the rate of particle flow toward the projected area of the collector. The transport mechanisms of diffusion, sedimentation, and interception can be described in terms of their single-collector removal efficiency that is the sum of the individual collector efficiencies.

Physical parameters impacting particle transport (*e.g.*, particle size and density, media size, fluid temperature, and filtration rate) were incorporated into several theoretical trajectory theory models (Levich, 1962; Yao, 1968; Yao *et al.*, 1971; Rajagopolan and

Tien, 1976; *etc.*) which have been supported with experimental data (Rajagopolan and Tien, 1976; Tien and Payatakes, 1979). These transport models (and others) are not alone able to precisely predict filter performance, however, because they assume particle destabilization such that there is no repulsive potential between the particles and collectors. Vaidyanathan and Tien (1988) indicated that collection efficiency under a repulsive double layer potential shows a gradual decline rather than the sudden decline predicted by trajectory theory (Payatakes *et al.*, 1974), emphasizing that trajectory analysis is limited in its ability to predict the results of filtration with unfavorable surface interactions.

2.5.2 Attachment

Particle attachment to either a media grain or previously retained particles depends on the surface properties of these materials. O'Melia and Stumm (1967), Tobiason and O'Melia (1988), and Raveendran and Amirtharajah (1995) specified several mechanisms that affect attachment during filtration. Summarized in Figure 2.7, they include: London-van der Waals (LVDW) forces, electrical double-layer (EDL) forces, hydrodynamic forces, steric forces, Born forces, structural forces, and chemical or bridging forces.

The LVDW and EDL forces are considered long-range forces (Raveendran and Amirtharajah, 1995). Due to interactions between electronic dipoles of the surfaces and the solution, the LVDW force is typically attractive in aqueous systems (Tobiason and O'Melia, 1988). Spielman (1978) showed the increased importance of London-van der Waals forces at small separation distances between particles and collectors. As particles approach collectors, hydrodynamic retardation slows them down and would preclude attachment without these attractive forces.

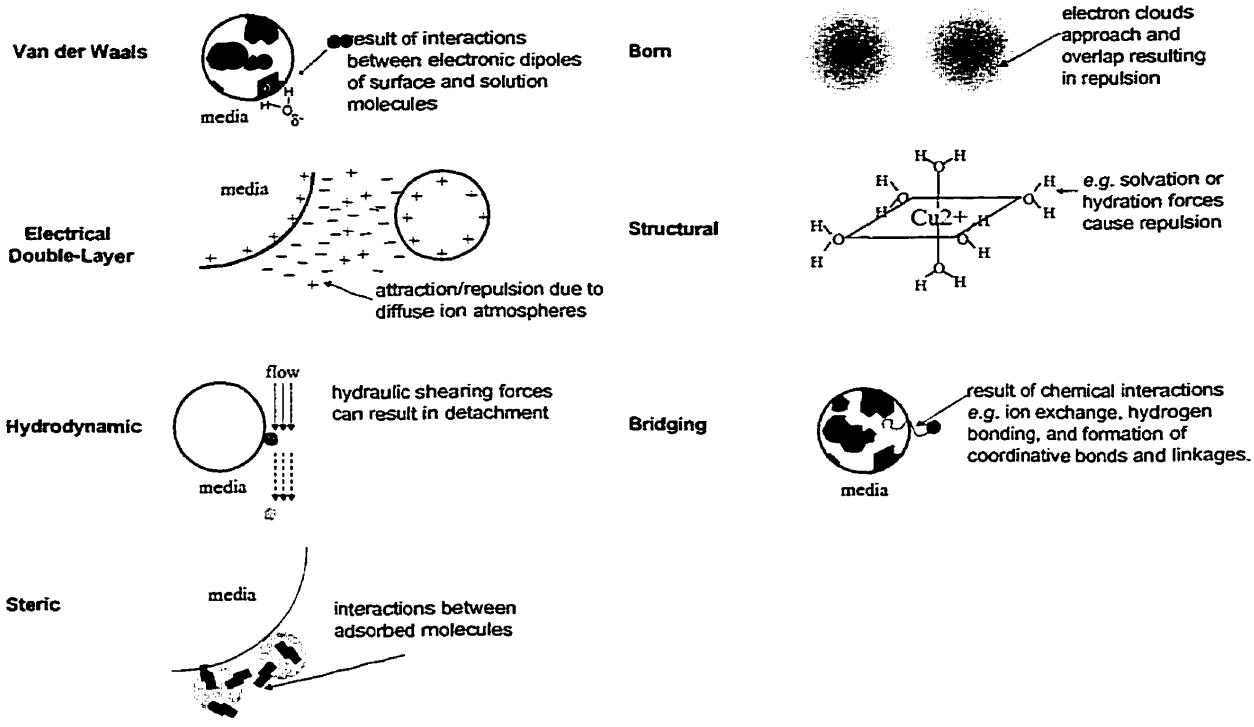


Figure 2.7 Mechanisms affecting attachment during filtration

The EDL force results from the two diffuse ion atmospheres surrounding suspended particles and collectors; these atmospheres interact as particles approach collectors. This interaction is attractive when the double layers are of opposite charge, otherwise the interaction is repulsive (O'Melia and Stumm, 1967). The magnitude of the EDL force depends on the separation distance between the particle and collector, the ionic strength, and the potential or charge at each surface (Tobiason and O'Melia, 1988). The steric interactions of adsorbed macromolecules can also result in similar repulsive forces (Tobiason and O'Melia, 1988).

Water passing through filter media exerts hydrodynamic forces, which can result in the detachment of particles. Hydrodynamic forces consist of lift and drag forces (Raveendran and Amirtharajah, 1995). Detachment occurs when these forces exceed adhesive forces. Specific modes of detachment are discussed below (Section 2.5.3).

Born and structural forces are considered short-range forces that are necessary to explain the initial events of detachment (Raveendran and Amirtharajah, 1995). Born forces are

repulsive and result from the overlap of electron clouds; they determine how close two atoms or molecules can ultimately approach one another. Between particles, this repulsive interaction can be determined by summing the individual interactions between molecules or atoms (Raveendran and Amirtharajah, 1995).

At short ranges, structural forces are often much stronger than LVDW and EDL forces; they arise from disruption of the ordering of liquid molecules during the approach to a second surface (Raveendran and Amirtharajah, 1995). When water is the medium, they are termed hydration forces and are repulsive whenever water molecules strongly bind to surfaces containing hydrophilic groups (Raveendran and Amirtharajah, 1995). Raveendran and Amirtharajah (1995) demonstrated that the inclusion of such short-range forces in theoretical force calculations helped to qualitatively explain experimental results describing attachment and detachment behavior during filter backwashing.

O'Melia and Stumm (1967) pointed out that chemical bridging forces often outweigh electrostatic forces. The forces might include interactions such as ion exchange, hydrogen bonding, and the formation of coordinative bonds and linkages (O'Melia and Stumm, 1967). An example of chemical bridging in water treatment is the adsorption of anionic polymers on negatively charged surfaces (Black *et al.*, 1965).

2.5.3 Detachment

The detachment process is analogous to attachment in that it can be considered as a detachment step followed by a transport step into the flowing liquid. As particles are removed from the bulk flow and attach to collectors, they accumulate and can act as additional collectors (O'Melia and Stumm, 1967). When the magnitude of the hydrodynamic forces (consisting of lift and drag) on particles exceeds that of adhesive forces, the particles are detached and reach lower depths within the filter; an avalanche effect of arriving particles may be an alternative mechanism of detachment (Figure 2.8). Attachment and detachment occur simultaneously in filter layers that have reached a saturated but metastable configuration of deposited particles (Amirtharajah, 1988). Many studies have demonstrated that optimized particle destabilization minimizes "premature" detachment (Roebeck *et al.*, 1964; O'Melia, 1985).

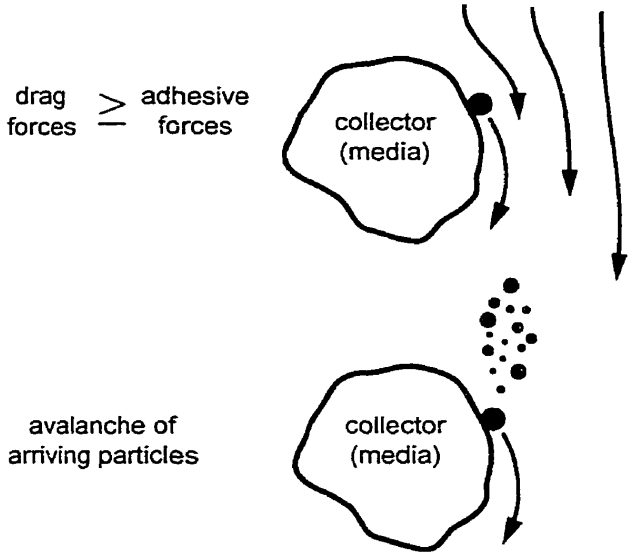


Figure 2.8 Modes of detachment during filtration

Ionic strength and solution pH have been identified as significant factors affecting particle detachment when a constant hydrodynamic force is applied for detachment (McDowell-Boyer, 1982; Ryan and Gschwend, 1994). By varying the hydrodynamic force, Hubbe (1984) and Sharma *et al.* (1992) theoretically and experimentally addressed physical aspects of particle detachment. These investigations demonstrated that particle detachment increased with flow rate and particle size.

Interstitial velocities increase as deposits build up in filters, resulting in less effective removal. As deposits build up, detachment increases. Though particles may re-attach at lower depths, ultimately, the filter bed depth is inadequate for providing desirable filter effluent quality and particle breakthrough occurs. During breakthrough there are several possible sources of particles in filter effluents. Particles may pass through the filter directly from the influent, attach and detach from the filter, or enter the filter at a smaller size, form flocs on the surface of the media, and then detach (Lawler *et al.*, 1995).

Graham (1988) defined pore flocculation as inter-particle aggregation an attempted to quantify it with a computer model, and so concluded that filter pore flocculation, while appreciable, was less important than particle-grain attachment and particle-particle

attachment by previously retained particles. This investigation only addressed pore flocculation resulting primarily from polymer addition, however. Ginn *et al.* (1992) speculated that filter pore flocculation can likely be ignored in systems exclusively using an inorganic coagulant such as alum because particle-grain and particle-particle bonds are weaker in such systems.

The particle size distribution data of Ginn *et al.* (1992) demonstrated a decrease in removal efficiency of larger particles as particle deposits in the bed increased, however, this trend was not evidenced in turbidity measurements. This finding was consistent with that of Moran *et al.*, (1993a) who concluded that ripening and breakthrough were strongly dependent on particle size. That investigation demonstrated that while removal of smaller particles increased (or ripened) for the longest duration, removal of intermediate sizes (such as those of *Cryptosporidium*) ripened early but decreased substantially as deposits in the bed increased (Moran *et al.*, 1993a). Moran *et al.*, (1993b) also concluded that particle detachment was predominant in intermediate and large particle size ranges.

Two mechanisms could have possibly caused the observed particle size increases in the filter effluents: the detachment of deposits from collectors and/or pore flocculation of suspended particles (Ginn *et al.*, 1992; Moran *et al.*, 1993a,b). Based on a substantial investigation of particle size distribution data, Ginn *et al.* (1992) concluded that detachment was the major source of particles in the studied filter effluents and that the size distribution shifted to larger particle sizes. This conclusion was generally consistent with those of Moran *et al.*, (1993b), however, the later investigations suggested some detachment of smaller particles as parts of larger flocs.

The mechanistic studies conducted by Ginn *et al.* (1992) and Moran *et al.* (1993b) concluded that detachment of previously retained particles or flocs contributed substantially to breakthrough. Moran *et al.* (1993b) additionally speculated that the input of new particles may have been necessary for breakthrough to occur (*i.e.*, avalanche effect described in Figure 2.8). Ginn *et al.* (1992) proposed a conceptual model of filtration consisting of four phases (Figure 2.9). From a mechanistic standpoint, this

model was generally consistent with the findings of Moran *et al.* (1993a) that indicated that ripening and breakthrough were not distinct stages of filter operation, but occurred simultaneously for different sized particles.

The first phase of the Ginn *et al.* (1992) model is filter ripening during which attachment increases as particles are deposited and subsequently act as collectors. Assuming a clean filter with no deposits, there is essentially no detachment at the start of a filter cycle; detachment slowly increases as deposits begin to accumulate in the filter. Effective filtration is the second phase of the model during which increasing interstitial flow velocities result in increased detachment and decreased attachment; however, attachment is sufficient to remove many of the influent particles.

Effluent turbidity begins to breakthrough during the third phase of the filtration model, as a result of increasing interstitial flow velocities caused by clogging of pores. Attachment continues to decrease while detachment increases. Preferential tubular passages (wormholes) of flow begin to form; because of their high pore velocities, these passages result in the significant decrease of particle attachment. Detachment peaks and begins to decrease because of the continued concentration of flow in the wormhole passages in which little new deposition occurs. The fourth phase of the model occurs after breakthrough, when wormhole flow begins to dominate and the filtering capacity of the bed is almost exhausted. At this time, attachment and detachment do not occur and filter effluent turbidity and particles rapidly approach their influent values.

Several important ramifications for water treatment may be drawn from the reported experimental and theoretical work regarding particle detachment during filtration. The experimental findings of Ginn *et al.* (1992) and Moran *et al.*, (1993a,b) underscored the inadequacy of particle count data as a sole tool for assessing filter removals of pathogens such as *Cryptosporidium*. The demonstrated deterioration in removals (or early breakthrough) of oocyst-sized particles in the later portions of filter cycles suggested that end-of-run operating conditions may be particularly vulnerable in terms of *Cryptosporidium* removal, or more specifically, *Cryptosporidium* detachment (Ginn *et al.*, 1992; Moran *et al.*, 1993a,b).

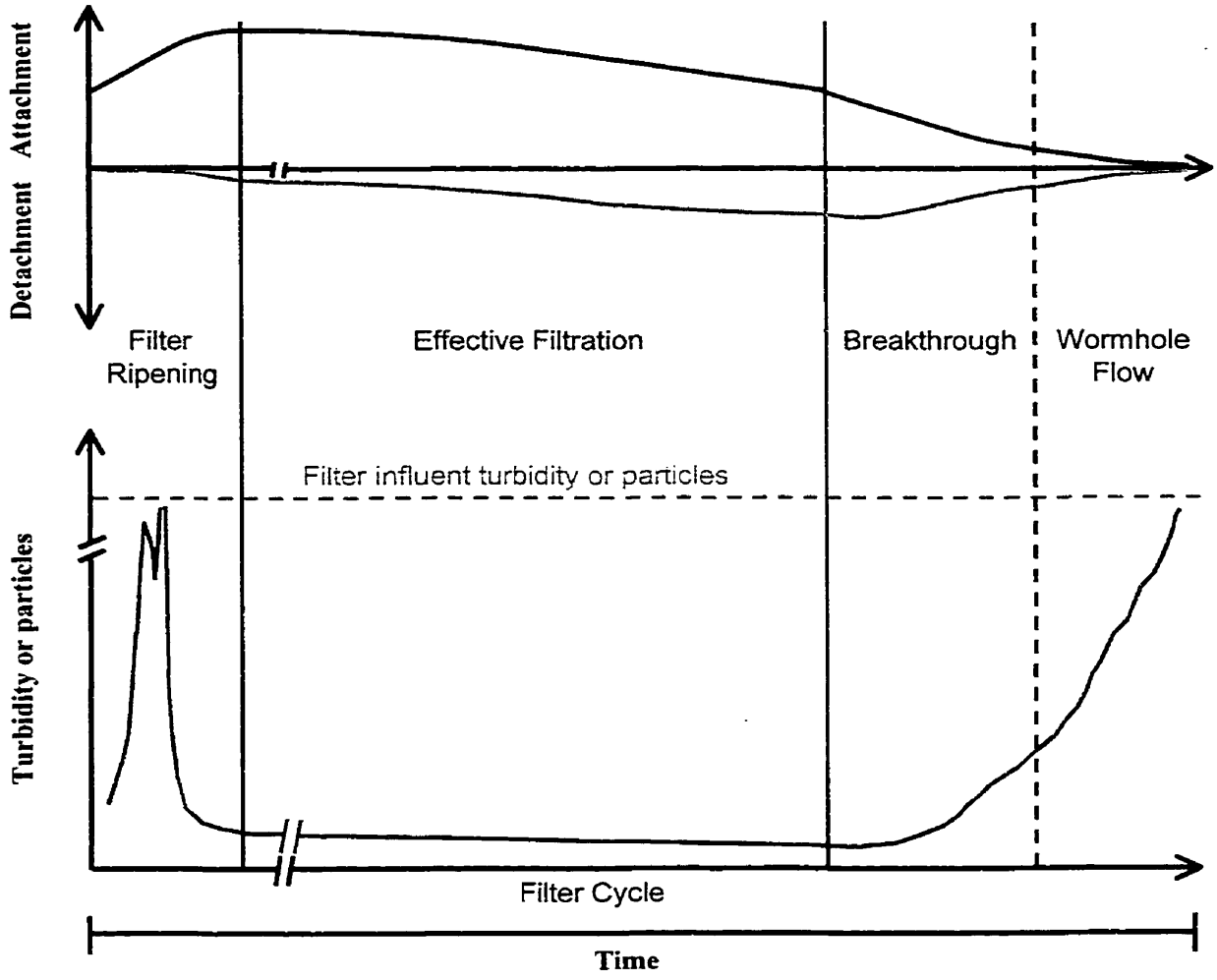


Figure 2.9 Conceptual model of attachment and detachment during filtration (*modified from* Ginn *et al.*, 1992)

Limited experimental data suggested the potential for pore flocculation of smaller particles to form intermediate sized particles (Moran *et al.*, 1993a,b), suggesting that *Cryptosporidium* oocysts in filter effluents would not necessarily be found exclusively in the oocyst-sized range of particles (*i.e.*, they could also flocculate to form larger particles). As pointed out by Ginn *et al.* (1992), these data also indicated the possibility of passage of destabilized particulate matter through well-operated deep bed filters. Microorganisms bound in such aggregates could be more difficult to inactivate in

subsequent disinfection processes that relied on direct contact with the individual microorganisms.

It is very difficult to determine the source and history of particles exiting filters. Regardless of the degree to which pore flocculation may occur for certain types of particles, several studies have demonstrated that particle detachment occurs continuously and influences particle removal during filtration (Ginn *et al.*, 1992; Moran *et al.*, 1993a,b). Since detachment and attachment are critical to understanding particle and microorganism removal, their respective roles during various operating conditions must be considered when optimizing removal of specific particles such as *Cryptosporidium* oocysts. The filtration model proposed by Ginn *et al.* (1992) provides a conceptual framework for attachment and detachment mechanisms during filtration that can be generally applied to understanding and predicting operational effects on particle, and more specifically, *Cryptosporidium* removal by filters.

2.6 Operational Factors Affecting Particle Removal

The operating mode and conditions of water treatment processes can affect particle removal. Both pretreatment conditions (*e.g.*, coagulation) and filtration (*e.g.*, filter aid, hydraulic changes, backwash strategy, media type, ripening filtration, breakthrough filtration) conditions directly impact the particle removal efficiency of filters. Table 2.2 summarizes operational conditions and their impacts on particle removal and passage mechanisms.

Table 2.2

Operational Factors and Related Particle Removal and Passage Mechanisms

Operational Factors	Particle Removal/Passage Mechanism			Key References
	Transport	Attachment	Detachment	
Coagulation	✔	✔	✔	Roebeck *et al.*, 1964 O'Melia and Stumm, 1967 O'Melia, 1985
Filter Aid		✔	✔	Black *et al.*, 1965 Zhu *et al.*, 1996
Hydraulic Changes	✔	✔	✔	Cleasby *et al.*, 1963 Tuepker and Bauescher, 1968 Logsdon *et al.*, 1981a Sharma *et al.*, 1992
Backwash Strategy	✔		✔	Amirtharajah, 1988 Raveendran and Amirtharajah, 1995 Colton *et al.*, 1996
Temperature	✔			Ives and Sholji, 1965 Ives, 1982
Ripening		✔		O'Melia and Ali, 1978 Amirtharajah, 1985 Moran *et al.*, 1993a
Breakthrough		✔	✔	Ginn *et al.*, 1992 Moran *et al.*, 1993a,b Lawler *et al.*, 1995
Media		✔	✔	Ives and Sholji, 1965 Trussell *et al.*, 1980

2.6.1 Coagulation

Although considerable reductions in both particles and turbidity can occur during filtration without chemical pretreatment, it has been repeatedly demonstrated that proper coagulation is critical in maintaining good particle removal during filtration (O'Melia and Crapps, 1964; Roebeck *et al.*, 1964; Trussell *et al.*, 1980). As discussed previously, several particle attachment mechanisms occur during filtration (O'Melia and Stumm, 1967; O'Melia, 1985), including particle destabilization forces, which are directly affected by coagulation. The relative strength of attachment forces affects the degree of non-attachment that occurs in filters; however, the relative ratio of attachment to detachment (hydrodynamic shear) forces dictates detachment. Chemical parameters such as pH and ionic strength, which are impacted by chemical pretreatment, have been experimentally identified as significant factors affecting particle detachment in packed bed filters when a constant hydrodynamic force is applied (McDowell-Boyer, 1992; Ryan and Gschwend, 1994; Nocito-Gobel and Tobiason, 1995).

The impact of coagulation on filtration is considerable because coagulation affects the balance between fundamental attachment and detachment mechanisms that dictate whether or not particles will be retained in filters (attachment), retained and subsequently released from filters (detachment), or pass through filters (non-attachment). The difficulty in understanding and optimizing coagulant effects during filtration is complicated by the different roles of attachment and detachment mechanisms occurring during different periods of the filter cycle such as ripening and breakthrough, which occur simultaneously but at different rates for different sized particles (Moran *et al.*, 1993a,b). In some cases, chemical pretreatment can affect competing objectives such as extent of ripening period, extent of backwash period, etc.

It stands to reason that coagulation would have a similar impact on the removal of pathogenic colloidal particles such as *Cryptosporidium* oocysts. The importance of coagulation processes for improving filter removal efficiencies of *Giardia* cysts has been well documented. Several investigations have demonstrated little (<1-log) to no removal of *Giardia* cysts by GAC filters (Patania *et al.*, 1995), sand and dual-media filters (Al-

Ani *et al.*, 1986), and tri-media filters (Horn *et al.*, 1988) during no coagulation conditions. Even sub-optimal coagulation conditions have been shown to affect the pathogen removal efficiency of filters. In a pilot-scale direct filtration plant, Logsdon *et al.* (1981a) found that mean *Giardia muris* removals decreased by approximately 1- to 2.5-log during periods of sub-optimal and minimal coagulation compared to optimal operating conditions. Similar decreases in cyst removal as a result of sub-optimal coagulation conditions have been demonstrated at other direct (Ongerth and Pecoraro, 1995) and conventional pilot plants (Patania *et al.*, 1995).

2.6.2 Filter Aid

Filter aids can considerably improve the quality of filter effluents. When a polymer is used as a filter aid, both transport and attachment mechanisms can be facilitated by the formation of polymer-particle flocs which are generally larger and stronger than those achieved with conventional chemical pretreatment alone (Zhu *et al.*, 1996). Studies have demonstrated that polymer addition during filtration is similar to coagulation in that particle destabilization must be optimized (Zhu *et al.*, 1996). The use of filter aids has provided improvements in filtrate quality (Conley and Hsiung, 1969), resistance to early breakthrough (Conley and Pitman, 1960), and reduction in the magnitude and duration of ripening (Tuepker and Bauescher, 1968), however, it can also result in significant increases in head loss. Appropriate addition of filter aids should result in higher attachment forces and therefore should be concurrent with optimized backwashing (Amirtharajah, 1988).

Patania *et al.* (1995) investigated of the impact of filter aid addition on *Giardia* removal by GAC/sand filters and concluded that the use of filter aid did not improve cyst removal under the conditions studied; this conclusion was consistent with the findings of Ongerth *et al.* (1989). This apparent discrepancy between improved filtrate turbidity but unchanged cyst/oocyst removal may suggest that filter aid improved filtrate quality by enhancing the removal of non-oocyst/cyst sized particles. It is possible that filter aid induced particle bridging considerably increases the size of particles originally in the 1 μm range, which are known to have a minimum transport efficiency (Yao *et al.*, 1971),

thereby increasing their transport and removal efficiencies while the removals of larger particles remained largely unaffected.

2.6.3 Hydraulic Changes

Several studies have concluded that different filtration rates do not necessarily result in different protozoan removals by filters. Al-Ani *et al.* (1986) performed pilot-scale sand and dual-media filtration experiments of *G. lamblia* removal at rates ranging from 2 to 8 gpm/ft^2 (5 to 19 m/h) and concluded that the range of filtration rates had little impact on cyst removal by the filters. Similar findings were reported by Horn *et al.* (1988) who investigated *G. lamblia* removals by tri-media filters operated at rates from 5 to 10 gpm/ft^2 (12 to 24 m/h); removals ranging from 0.7 to 3-logs were reported for two different water types.

Hydraulic conditions can significantly impact the quality of filter effluents. It is generally recognized that filter performance is adversely affected by non-steady flow (Trussell *et al.*, 1980). Hydraulic changes can occur suddenly or gradually and at the extreme they encompass sudden starts and stops in operation. In general, hydraulic changes disrupt the equilibrium between particle attachment forces and hydraulic shear (detachment) forces (Logsdon *et al.*, 1999). Therefore, it is reasonable to surmise that the relative impacts of hydraulic changes are inextricably linked to other operational factors such as coagulation conditions; however, some general conclusions regarding the impact of hydraulic changes on filtrate quality can be drawn.

Cleasby *et al.* (1963) and Tuepker and Bauescher (1968) showed that large flow rate changes cause deterioration of filtered water quality by the detachment of previously retained particles. The degree of deterioration was related to the magnitude and rapidity of the rate change and independent of the duration of the disturbance. These relationships support observations that declining rate filters may provide better performance than constant rate filters (Hudson, 1959; DiBernardo and Cleasby, 1980). Subsequent experiments by Hilmoe and Cleasby (1986) found no significant differences between declining rate and constant rate filters, however. The authors speculated that the previously reported poorer effluent quality achieved by constant rate filtration might have

been caused by the constant rate control system used by DiBernardo and Cleasby (1980), which might have inadvertently resulted in continuous flow rate fluctuations or surges.

Cleasby *et al.* (1963) also revealed additional complexity in speculating on the risk of pathogen passage through filters when flow rate changes or hydraulic steps are applied because they demonstrated that particle passage through filters following a disturbance was dependent on the composition of the filter influent. This result underscores that the balance between attachment and detachment forces is affected by multiple factors such as coagulation, the ongoing ripening and breakthrough of different sized particles, floc strength, and hydraulics. Fitzpatrick *et al.* (1999) demonstrated that large and sudden changes in flow dramatically deteriorated particle removal by filters while smaller changes that were implemented gradually did not necessarily increase particle counts. Filtration rate increases of 50-150%, when flocs were not strengthened with filter aid, resulted in increased *Giardia* passage that was considerably higher than the increases in turbidity (Logsdon *et al.*, 1981a). These results suggested that increases in *Cryptosporidium* passage through filters could likely be expected under similar operating conditions.

2.6.4 Backwashing Strategy

During backwashing, hydrodynamic shear is primarily responsible for detachment of particles. Raveendran and Amirtharajah (1995) empirically and theoretically described the interactions between suspended particles and media grains coated with previously deposited particles under fluidization conditions. A calculation of the interaction forces that control particle detachment indicated that solution chemistry affects particle detachment during backwashing; however, optimization of operating conditions for stronger attachment during normal filter operation may possibly make it more difficult to remove particles during subsequent filter backwashing. If media cleaning is ineffective it will lead to poorer quality effluent during the initial stages of filtration, mudball formation, and other long-term problems in the filter bed (Amirtharajah and Wetstein, 1980; Amirtharajah, 1993). Ensuring thorough removal of pathogenic particles during backwashing may also prevent them from release during subsequent ripening or normal

filter operation. The simultaneous application of air scour with subfluidization water wash (collapse pulsing) has been identified as an optimal backwashing strategy (Amirtharajah and Wetstein, 1980; Amirtharajah *et al.*, 1991; Amirtharajah, 1993). The use of collapse-pulsing backwash strategies has been shown to reduce the number of oocyst-sized particles in filter effluents during ripening (Colton *et al.*, 1996).

2.6.5 Temperature

Since particles move relative to water to reach a grain surface, attach, and be removed, they experience a viscous drag proportional to water viscosity (Ives and Sholji, 1965). The more viscous the water (*e.g.*, colder temperatures), the more slowly particles move relative to the water to reach the grain surface, reducing the probability of removal (Ives and Sholji, 1965). Experiments conducted by Ives and Sholji (1965) demonstrated that colder water temperatures resulted in considerable decreases in particle removal when all other factors (*e.g.*, raw water quality, chemical pretreatment, *etc.*) remained constant.

In normal water treatment practice the effects of water temperature on particle transport and subsequent removal by filters are not easy to delineate because other operations such as clarification are also affected by temperature. Seasonal changes also often result in considerably different raw water qualities making it almost impossible to identify certain effects as exclusively temperature-related. The importance of evaluating the role of temperature as it affects pathogen passage through filters is twofold. First, for given water treatment plants it is important to determine stable operation removals across the range of seasonal conditions experienced. Second, it is important to determine if other operational conditions or events such as coagulation upsets, ripening, and breakthrough have similar implications for pathogen passage during warm and cold water conditions.

2.6.6 Ripening

It has been suggested that 90% of the particles that pass through a well-operated filter do so during ripening (O'Melia and Ali, 1978; Amirtharajah, 1985). The two peaks of particle passage that occur during ripening result from backwash water remnants (Amirtharajah, 1988) and the period that particles are being retained and subsequently act

as collectors (O'Melia and Ali, 1978); during this second peak particle passage is primarily due to non-attachment. It is likely that these mechanisms also affect pathogen passage through filters during this period.

A variety of findings regarding protozoan passage through granular media filters during ripening have been reported. Logsdon *et al.* (1981a) reported that *Giardia* cyst passage through filters was significantly higher during ripening than during stable operation, even at low effluent turbidities. Similar findings were obtained at two pilot plants studied by Patania *et al.* (1995); the differences between cyst removals during stable filter operation and ripening were less dramatic, however. At a third pilot plant studied by Patania *et al.* (1995), *Giardia* removals during ripening were comparable to those achieved during stable filter operation. These data suggest that multiple factors may affect pathogen removal during ripening.

2.6.7 Breakthrough

Possible sources of breakthrough during filtration include particles that pass through directly from the influent (non-attachment) or particles that become detached (Lawler *et al.*, 1995). According to the model presented in Figure 2.9 and other studies (Moran *et al.*, 1993b), non-attachment and detachment occur during breakthrough conditions. As particle detachment and non-attachment increase, increased pathogen passage through filters would also be expected.

Logsdon *et al.* (1981a) demonstrated that turbidity breakthrough at the end of a filter cycle (when filter effluent turbidity was above 0.4 NTU) could be accompanied by a tremendous passage of *Giardia* cysts, even if they were not present in the filter influent. A considerable increase in cyst passage was also observed during early breakthrough conditions when filter effluent turbidity was just above 0.2 NTU. Patania *et al.* (1995) investigated *Giardia* passage through filters during breakthrough when effluent turbidities increased from 0.1 NTU to 0.2 NTU or higher. They found that *Giardia* removal was approximately 0.5-log lower during breakthrough than during stable operation. These data suggested that increased *Cryptosporidium* passage could also be expected during breakthrough, especially at filter effluent turbidities above 0.2 NTU.

2.6.8 Media

Important aspects of media specifications are size, shape, depth, uniformity, and the choice of single-, dual-, or multi-media (Trussell *et al.*, 1980). Media size affects the length of time to turbidity breakthrough and limiting headloss (Trussell *et al.*, 1980). Media shape affects head loss (rate of head loss build up) and is associated with the ability of filters to remove particles (Trussell *et al.*, 1980). Angular media have demonstrated better turbidity and particle removal compared to smoother media (Trussell *et al.*, 1980). Lower uniformity coefficients of anthracite media have also demonstrated improved removal of oocyst-sized particles (Yohe *et al.*, 1999).

Filtration theory and practice has indicated that reverse-graded multi-media typically provide better particulate (Rimer, 1968; Conley, 1972) and protozoan removal (Logsdon *et al.*, 1985) at the cost of faster headloss buildup. Other studies, however, have indicated comparable particulate removal by dual- and tri-media filters (Tate and Trussell, 1978, Billica *et al.*, 1999), suggesting that tri-media may not necessarily offer a *C. parvum* removal advantage over dual-media. It is further possible that media selection may offer *C. parvum* removal advantages only under specific operating conditions.

2.7 SURROGATES FOR *CRYPTOSPORIDIUM* REMOVAL DURING WATER TREATMENT

Several different types of surrogates for viable *C. parvum* oocysts have been evaluated and include surrogates for occurrence (indigenous concentrations), disinfection, and removal. An ideal surrogate for cyst or oocyst removal should provide at least a semi-quantitative indication of cyst/oocyst removal by the process during a range of operating conditions that can be encountered during typical operation. Potential surrogates for cyst and oocyst removal by drinking water treatment processes that have reported in the literature include: turbidity, particle counts, heterotrophic bacterial counts (HPC), aerobic spores (typically *Bacillus subtilis*), UV_{254}, dissolved organic carbon (DOC), polystyrene microspheres, and chemically inactivated *C. parvum* oocysts. Table 2.3 lists and summarizes the main observations from several studies that evaluated potential surrogates for the removal of *C. parvum* oocysts during water treatment.

Table 2.3

Surrogate Parameters for Removal of *Cryptosporidium*

Study	Surrogate Parameters										Major Observations
	Cryptosporidium	Turbidity	Particle range	Total particles	*Giardia*	HPC	*Bacillus* spores	UV_{254}	DOC	Microspheres	
LeChevallier *et al.*, 1991c	✔	✔		✔[m]	✔						Turbidity/particles not indicative of oocyst removal. *Giardia* and *Cryptosporidium* removed similarly.
LeChevallier and Norton, 1992	✔	✔		✔[i,m]							Turbidity/particles not indicative of oocyst removal.
Nieminski, 1994	✔	✔	✔[g]		✔	✔					Turbidity/particles indicative of cyst/oocyst removal. *Cryptosporidium* more difficult to remove than *Giardia*. HPCs not good surrogate for cyst/oocyst removal.
West *et al.*, 1994	✔	✔	✔[c]								Particle removal underestimated oocyst removal.
Charles *et al.*, 1995	✔				✔						*Cryptosporidium* removed at similar, but lower levels than *Giardia*.
Kelley *et al.*, 1995	✔	✔	✔[d]								Turbidity/particles not indicative of oocyst removal.
Nieminski and Ongerth, 1995	✔	✔	✔[g]		✔	✔					Turbidity/particles not indicative of oocyst removal. Particles more indicative of oocyst removal than turbidity. HPCs not reliable surrogates for oocyst removal. *Cryptosporidium* removed at similar, but lower levels than *Giardia*.
Ongerth and Pecoraro, 1995	✔	✔			✔						Similar *Cryptosporidium* and *Giardia* removals.
Patania *et al.*, 1995	✔	✔	✔[a,b,h]		✔						Turbidity/particles not indicative of oocyst removal. Similar *Cryptosporidium* and *Giardia* removals, not directly related.

Table 2.3

Surrogate Parameters for Removal of Cryptosporidium (Continued)

Study	Surrogate Parameters										Major Observations
	Cryptosporidium	Turbidity	Particle range	Total particles	*Giardia*	HPC	*Bacillus* spores	UV_{254}	DOC	Microspheres	
Plummer *et al.*, 1995	✔	✔						✔	✔		Turbidity, UV_{254}, and DOC not indicative of oocyst removal.
Coallier *et al.*, 1996		✔					✔				Spore removals more sensitive than turbidity and similar to particles.
Lytle *et al.*, 1996	✔	✔	✔[a,f]	✔[l]			✔			✔	Spore removals closely parallel particle removals. Spores removals decline more rapidly than particles and turbidity. Oocyst-sized particles easiest to remove, not good surrogate.
Rice *et al.*, 1996		✔	✔[e]	✔[l]			✔				Spore removals useful for determining treatment efficiency. Spore removal closely and conservatively match particle removal.
Swaim *et al.*, 1996	✔	✔	✔[e,h]		✔						Cyst and oocyst removals not well correlated with particle removals. Removal of cyst sized particles lower than cyst removal. Comparable *Cryptosporidium* and *Giardia* removals.
Scott *et al.*, 1997	✔	✔	✔[b]	✔[k]			✔				Turbidity/particles not indicative of oocyst removal. Spores conservative indicator of oocyst removal.
Yates *et al.*, 1997	✔	✔		✔[NS]			✔				Spore removal not directly related to particle and turbidity removal.
Baudin and Laîné, 1998	✔	✔		✔[j]	✔						Turbidity/particles not indicative of oocyst removal.
Edzwald and Kelley, 1998	✔	✔	✔[d]								Turbidity/particles not indicative of oocyst removal.

Table 2.3

Surrogate Parameters for Removal of Cryptosporidium (Continued)

	Cryptosporidium	Turbidity	Particle range	Total particles	*Giardia*	HPC	*Bacillus* spores	UV_{254}	DOC	Microspheres	
Fox *et al.*, 1998	✔	✔	✔[f]	✔[j]			✔				Trends in turbidity and particle removal through clarifier generally indicative of oocyst removal.
Dugan *et al.*, 1999	✔	✔		✔[j]			✔				Positive correlation between oocyst removals and particle and turbidity reductions through coagulation and settling. Turbidity removal most conservative indicator of oocyst removal through filtration.
Edzwald *et al.*, 1999	✔	✔	✔[d]	✔[j]							Particle and cyst removal trends generally consistent across clarification and filtration. Particle removal provides a conservative estimate of oocyst removal.
Nieminski and Bellamy, 1998	✔	✔		✔[k]	✔	✔	✔				Turbidity, particles and spores indicative of water quality, not oocyst removal. Mostly non-detects of cysts/oocysts in finished water.
Swertfeger *et al.*, 1999	✔	✔		✔[k]	✔		✔			✔	Microsphere and spore removals were more similar to cyst and oocyst removals than were turbidity and particle removals. Turbidity and particle removals lower than cyst/oocyst removals.

[a] 1-2 µm size range.
[b] 2-5 µm size range.
[c] 2-6 µm size range.
[d] 2-15 µm size range.
[e] 3-5 µm size range.
[f] 3-6 µm size range.
[g] 4-7 µm size range.
[h] 5-15 µm size range.
[i] Other particle size measurements were also made.
[j] Cumulative particles ≥ 1 µm.
[k] Cumulative particles ≥ 2 µm.
[l] Cumulative particles ≥ 3 µm.
[m] Cumulative particles ≥ 5 µm.
[NS] Not stated.

Particle counting allows for real-time monitoring and is more sensitive than turbidity for treatment optimization (Hargesheimer *et al.*, 1992; Arora *et al.*, 1992). However, particle counting lacks the ability to discern between particles in the same size range and to detect very small changes in concentration that would likely be associated with *Cryptosporidium* concentrations (Hargesheimer *et al.*, 1992). Several studies have indicated that while turbidity and particle removals are good indicators of general water quality, they are not quantitatively indicative of *Cryptosporidium* removal by treatment processes (LeChevallier *et al.*, 1991c; Nieminski and Ongerth, 1995; Swaim *et al.*, 1996).

Several pilot- and full-scale studies have demonstrated that organism-sized particles and turbidity are approximate indicators of pathogen removal by drinking water treatment processes, but not reliable surrogates (LeChevallier and Norton, 1992; Nieminski and Ongerth, 1995). Plummer *et al.* (1995) reached similar conclusions about turbidity, as well as UV_{254} and DOC. Patania *et al.* (1995) indicated that achieving a goal of 0.1 NTU was indicative of effective cyst/oocyst removal. Although the risk of *Cryptosporidium* passage appeared to increase with increasing filtrate turbidity in several studies (Hall *et al.*, 1995; Nieminski and Ongerth, 1995), Fuller *et al.* (1995) did not observe significant oocyst passage during the first hour of operation after backwash when filter effluent turbidity was high (filter ripening); the same result was reported at one of three pilot plant plants studied by Patania *et al.* (1995). Similar discrepancies between increases in turbidity during breakthrough and increases in *Giardia* cyst passage at some plants (Logsdon *et al.*, 1981a) and not others (Patania *et al.*, 1995) have also been reported. These data suggest that turbidity is an inadequate surrogate for predicting cyst and oocyst passage through filters.

HPCs yield an estimate of the total number of viable bacteria that can be successfully grown on plates and have been used to determine treatment efficiencies and distribution system integrity in several studies (Ferguson *et al.*, 1990). Evaluating treatment processes for pathogen removal based on HPCs may be difficult because of the varying composition of bacterial populations that constitute HPCs. Several pilot- and full-scale studies have demonstrated that removal of heterotrophic bacteria were not an effective

surrogate for predicting cyst/oocyst removal (Nieminski; 1994; Nieminski and Ongerth, 1995; Nieminski and Bellamy, 1998).

Aerobic spores, primarily *Bacillus*, have been used for evaluating treatment efficiency (Coallier *et al.*, 1996; Rice *et al.*, 1996). They are present in most surface waters, pose no public health threats, and are not indicative of fecal contamination (Jakubowski *et al.*, 1996). The spores are approximately 1 μm in size, close to the size range of *Cryptosporidium* oocysts (Rice *et al.*, 1996). They are also highly resistant to disinfection and their removals closely parallel particle removal (Coallier *et al.*, 1996; Rice *et al.*, 1996).

Studies performed by Scott *et al.* (1997) evaluated *Bacillus* spores, turbidity, and particle counts as surrogates for pathogen removal. Turbidity and particle counts yielded conclusions similar to those discussed above (Nieminski and Ongerth, 1995; Patania *et al.*, 1995) whereas spores demonstrated a significant correlation with *Cryptosporidium* removal at both pilot- and full-scale. Lytle *et al.* (1996), Nieminski and Bellamy (1998), and Swertfeger *et al.* (1999) also concluded that aerobic spores were indicative of treatment efficiency, however, they did not conclude that the spores were adequate surrogates for oocyst removal. These studies suggested that although spores could be considered a more conservative indicator of treatment efficiency than particles or turbidity, they were not necessarily indicative of *Cryptosporidium* removal during water treatment.

Fluorescent polystyrene microspheres in the size range of *Cryptosporidium* oocysts were tested by Li *et al.* (1997) in field scale bag filtration systems. A nearly linear correlation between log removals of microspheres and oocysts was established. During these studies the relationships between *Cryptosporidium* removal and other parameters such as particle counts and turbidity were also very linear. Fox *et al.* (1996) included poiystyrene microspheres in evaluations of *Cryptosporidium* removals by granular media filtration processes; however, neither microspheres nor oocysts were found in the filter effluents. Swertfeger *et al.* (1999) had more success with microspheres in one experiment that

indicated polystyrene microspheres might show promise as a surrogate for *Cryptosporidium* removal.

Due to the lack of adequate surrogates for the removal of viable *C. parvum* oocysts, chemically inactivated oocysts have been commonly used for treatment evaluations. The use of chemically inactivated oocysts is preferable to that of viable oocysts because of the potential health risks associated with the use and release of viable *C. parvum*. It has been suggested that chemically inactivated oocysts may not be ideal surrogates for viable oocysts due to differences in surface charge, described by zeta potential (Lytle and Fox, 1994). Change in colloidal zeta potential can affect removal during granular media filtration because zeta potential is indicative of particle destabilization (Amirtharajah and Mills, 1982). Particles are most readily removed when zeta potential is near zero, corresponding to the particles' isoelectric point (Amirtharajah, 1988). Chemical inactivation can change oocyst zeta potential in a manner that might affect coagulation (Lytle and Fox, 1994; Ongerth and Pecoraro, 1996) and therefore filtration. During water treatment, however, oocyst zeta potential is affected by multiple factors such as water quality, coagulant type and dosage, and pH in addition to chemical inactivation prior to treatment.

2.8 *CRYPTOSPORIDIUM* REMOVAL DURING DRINKING WATER TREATMENT

Filtration is one of the most critical and successful physico-chemical barriers against *Cryptosporidium* passage through water treatment. Several studies have assessed *C. parvum* oocyst removal by granular media filters operated at or near to optimized stable operating conditions. Summarized in Table 2.4, these studies include investigations of both *C. parvum* removal by granular media filtration alone (with removals based on filter influent and effluent oocyst concentrations) and as part of the water treatment process (with removals based on upstream, but not filter influent, and filter effluent concentrations). Unless specified in the table footnotes, the *C. parvum* removals in Table 2.4 are removals by granular media filtration alone.

Table 2.4

Removal of *Cryptosporidium* During Stable Operation

Study	Type of Treatment	Filter Type	Filter Loading (gpm/ft^2)	*C. parvum* Log Removal	Seeded Conc. (#/L)*	Observations of Relevance to Present Study
LeChevallier *et al.*, 1991c	Varied - 66 full scale plants	varied	varied	>2.4‡	–	Rapid sand and GAC effluents had higher oocyst concentrations than dual- and tri-media effluents - possibly related to different raw water quality.
	2 pilot-scale conventional plants	GAC/sand	0.75 - 1.2	>5.3	NS	Rapid sand more effective than GAC for oocyst removal. Many non-detects in filter effluent samples.
LeChevallier & Norton, 1992	3 full scale, conventional plants	anth./sand	NS	>2.4	–	Oocyst/cyst occurrence related to raw water levels.
		GAC/sand	NS	>2.5	–	Many non-detects in filter effluent samples.
		anth./sand	NS	>2.3	–	
Nieminski, 1994	0.5-gpm conventional pilot-scale	anth./sand	2.5	avg. 2.8	~10^4**	No difference between conventional and direct filtration for cyst and oocyst removal.
	0.5-gpm direct filtration pilot-scale	anth./sand	2.5	avg. 2.9	~10^4**	
	600-gpm conventional full-scale	anth./sand	NS	avg. 2.1	~10^7††	Direct filtration yielded better cyst and oocyst removal, but influent quality was very different.
	600-gpm direct filtration full-scale	anth./sand	NS	avg. 2.7	~10^7††	
West *et al.*, 1994	Pilot-scale direct filtration	anth.	6	2.1 - 3.3	~10^2-10^3†	Many non-detects in filter effluent samples.
		anth.	14	1.9 - 3.2	~10^2-10^3†	
Charles *et al.*, 1995	Bench-scale direct filtration	mixed	4	2.3 - 3.3‡	~10^4†	Lack of coagulant decreased removal by >2 -logs.
	2-gpm conventional pilot plant	mixed	4	2.2 - 4.5‡	~100†	Rapid mix conditions impacted removal by <1-log. Stable/optimal removals not specified.
Hall *et al.*, 1995	Pilot-scale dissolved air flotation (DAF)	sand	2.5	2.9 - 3.4‡	~2×10^4†	
	followed by rapid gravity	anth./sand	2.5	3.9‡	2×10^4†	
	filtration (RGF)	GAC	2.5	4.4‡	2×10^4†	
		sand	2.5	>3.6‡	300-800†	No differences between single, dual, and GAC filters.
		anth./sand	2.5	3.1 - >4.4‡	300-800†	Lower removal during ripening (~0.4-log)
		GAC	2.5	3.2 - 4.1‡	300-800†	~ Half the effluent samples were non-detects.
		sand	2	>3.4‡	400†	
		sand	4.1	3.5‡‡	400†	
	Pilot floc blanket clarification and RGF	sand	2.5	3.9‡	700†	

Table 2.4

Removal of *Cryptosporidium* During Stable Operation (Continued)

Study	Type of Treatment	Filter Type	Filter Loading (gpm/ft^2)	*C. parvum* Log Removal	Seeded Conc. (#/L)*	Observations of Relevance to Present Study
Kelley *et al.*, 1995	1 MGD conventional plant	sand	NS	>1.0	–	Many non-detects in filter effluent samples.
	3 MGD conventional plant	sand	NS	>0.9	–	
Nieminski & Ongerth, 1995	0.5-gpm conventional pilot-scale	anth./sand	5.8	1.9 - 4.0	~10^4**	Fluctuating turbidity resulted in variable cyst and oocyst concentrations.
	0.5-gpm direct filtration pilot-scale	anth./sand	5.8	1.3 – 4.0	~10^4**	
	600-gpm conventional full-scale	anth./sand	4.8	1.9 - 2.8	~10^7††	Direct filtration yielded better cyst and oocyst removal, but influent quality was very different.
	600-gpm direct filtration full-scale	anth./sand	4.8	2.6 - 2.9	~10^7††	
Ongerth & Pecoraro, 1995	1-gpm direct filtration pilot plant	tri-media	5	2.5 - 3.2	~400-3000	Inadequate coagulation decreases removal to ~1.5-log
Patania *et al.*, 1995	Conventional pilot-plant	GAC/sand	3-6	–0.2 - 3.1‡‡	~10^3-10^4†	Chemical pretreatment critical for oocyst removal.
		anth./sand	3-6	0.4 - 1.1‡‡	~10^3-10^4†	Media design, filter aid, filtration rate: less important.
	Pilot-scale in-line filtration	anth./sand	8	2.3 - 3.7††	~10^3†	During ripening, cyst /oocyst removals ~0.5- to 1-log lower than during stable operation.
	Conv. pilot w/ low-rate surface filtration	sand	~2	2.2 - 4.7‡‡	~10^3-10^4†	Ripening effect observed at 2 pilot plants. At a third pilot pilot plant, no difference between ripening/stable.
						Stable and breakthrough removals ~ comparable.
						Many influent and effluent non-detects.
						Large range of filter influent concentrations.
Timms *et al.*, 1995	Pilot-scale slow sand filtration	slow sand	0.12 - 0.16	>4.5	~4000	No oocysts found in filter effluent.
Edzwald *et al.*, 1996	Pilot-scale contact filtration	anth./sand	3	avg. 4.7	7210	Filter influent concentrations changed due to chemical pretreatment conditions and affected log removals.
	Pilot-scale dissolved air flotation (DAF)	anth./sand	3	~2 - 4.1	2 - 631	
	followed by RGF	anth./sand	6	~2 - 2.2	2 - 631	
Swaim *et al.*, 1996	Pilot-scale direct filtration	deep anth.	12	3.7 - >4.3‡	~10^3†	During ripening, cyst /oocyst removals ~0.5- to 1-log lower than during stable operation.
		deep anth.	16	3.6 - >4.3‡	~10^3†	
		deep dual	12	3.5 - 4.1‡	~10^3†	Cyst and oocyst removals >3-log during ripening.
		deep dual	16	3.6 - >4.3‡	~10^3†	
Scott *et al.*, 1997	6-gpm conventional pilot plant	dual/tri	1.3, 6	~1.7 - 4.3	~10^8††	Did not distinguish between dual- and tri-media removals.

Table 2.4

Removal of *Cryptosporidium* During Stable Operation (Continued)

Study	Type of Treatment	Filter Type	Filter Loading (gpm/ft^2)	*C. parvum* Log Removal	Seeded Conc. (#/L)*	Observations of Relevance to Present Study
Yates *et al.*, 1997	Pilot-scale direct filtration (alum)	anth./sand	6.0	avg. 3.7	$\sim10^{8}$††	$FeCl_3$ coagulation provided better removals than alum.
	Pilot-scale direct filtration ($FeCl_3$)	anth./sand	6.0	avg. 4.5	$\sim10^{8}$††	
Baudin and Lainé, 1998	12.7 MGD conventional full-scale	anth./sand	4.1	>4	–	During ripening, oocyst removals ~1-log lower than during stable operation.
	31.7 MGD conventional full-scale	GAC	2.9	2.0 – 3.0	–	Stable and breakthrough removals ~comparable.
						Ripening and breakthrough turbidities not specified.
Edzwald and Kelley, 1998	Pilot DAF ($FeCl_3$) and RGF	anth./sand	3.0	2	2510†	Coagulant selection affected DAF removal of oocysts and subsequent filter influent concentration.
			6.0	2	2510†	
	Pilot DAF (alum) and RGF	anth./sand	3.0	3.9	2510†	
			6.0	2.2	2510†	
Fox *et al.*, 1998	Conventional pilot plant	anth./sand	2.0	2 – 3	$\sim10^{5}$	Study focused on clarifier removals, filtration only generally mentioned. Stable/optimal removals not specified.
Dugan *et al.*, 1999	Conventional pilot plant	sand	2.0	~3.0 – 3.7	~84-480	Under coagulation resulted in lower removals.
		anth./sand	2.0	~3.0 – 3.7	~84-480	Comparable removals in sand and dual-media filters.
		anth./sand	4.1	~1.6 – 3.1	~84-480	Removal decreased by > 1-log over time at higher rates.
						Increased media depth did not consistently result in increased oocyst removal.
						Stable/optimal removals not specified.
Edzwald *et al.*, 1999	DAF pilot (alum/polymer) T=2-3°C	anth./sand	6	>3.7	$\sim10^{7}$§§	No oocysts/cysts were found in filter effluent.
	Lamella pilot (alum/polymer) T=2-3°C	anth./sand	6	>4.7	$\sim10^{6}$§§	Filter influent concentrations and subsequent log removals were different due to different removals by clarifiers.
	Lamella pilot plant (alum) T=2-3°C	anth./sand	6	>4.8	$\sim10^{6}$§§	
	DAF pilot (alum/ polymer) T=13-14°C	anth./sand	6	>3.0	$\sim10^{7}$§§	
	Lamella pilot (alum/polym.) T=13-14°C	anth./sand	6	>4.3	$\sim10^{6}$§§	
	Lamella pilot (alum) T=13-14°C	anth./sand	6	>3.9	$\sim10^{6}$§§	

Table 2.4

Removal of *Cryptosporidium* During Stable Operation (Continued)

Study	Type of Treatment	Filter Type	Filter Loading (gpm/ft^2)	*C. parvum* Log Removal	Seeded Conc. (#/L)*	Observations of Relevance to Present Study
Swertfeger *et al.*, 1999	Conventional pilot plant (summer)	sand	2.5	1.8 - 3.3	~10^5-$10^{6\dagger\dagger}$	All media configurations statistically comparable.
		anth./sand	5.0	1.6 - 3.4	~10^5-$10^{6\dagger\dagger}$	Removals adjusted (empty column removals subtracted).
		deep dual	5.0	3.4 - 4.2	~10^5-$10^{6\dagger\dagger}$	Almost entire effluent processed.
	Conventional pilot plant (winter)	sand	2.5	2.5 - 3.0	~10^5-$10^{6\dagger\dagger}$	Polystyrene bead removal somewhat consistent with
		anth./sand	5.0	3.1 - 3.2	~10^5-$10^{6\dagger\dagger}$	oocyst removal in one experiment.
		deep dual	5.0	2.9 - 4.0	~10^5-$10^{6\dagger\dagger}$	Oocyst were not coagulated, only pretreated with a small amount (0.1-0.5 mg/L) of ferric sulphate as filter aid.

- Not applicable.
- NS Not stated.
- * Measured concentration in filter influent unless otherwise stated.
- † Seeded concentration in raw water.
- § Unclear as to whether measured or calculated filter influent concentrations were used.
- ** Theoretical influent concentration based on hemocytometer counts and dilution calculations. Filter influent concentration was not stated.
- †† Total number of cysts/oocysts seeded at filter influent.
- §§ Total number of cysts/oocysts seeded at clarifier influent.
- ‡ Removals based on raw water concentration (not filter influent concentration).
- ‡‡ Removals based on influent clarifier concentration (not filter influent concentration).

Full-scale *C. parvum* removals have been reported anywhere from 2- to 3-log (*e.g.*, Kelley *et al.*, 1995; Nieminski and Ongerth, 1995) to >4-log (*e.g.*, Baudin and Laîné, 1998). Pilot-scale *Cryptosporidium* removal data have suggested that filters can achieve anywhere from 2- to 3-log (*e.g.*, West *et al.*, 1994; Kelley *et al.*, 1995; Fox *et al.*, 1998), 3- to 4-log (Yates *et al.*, 1997), 4- to 5-log (*e.g.*, Patania *et al.*, 1995), and >5-log (*e.g.*, LeChevallier *et al.*, 1991c) removal of oocysts. Several studies that have evaluated *C. parvum* removal by filtration are summarized in Table 2.4. Although this table includes a wide range of filtration scenarios (*e.g.*, different raw waters, different modes of filtration, different scales, etc.), the listed *C. parvum* removals predominantly correspond to optimized filtration; results from non-optimal operating conditions are noted and summarized when applicable.

When considering the removal of *C. parvum* (as in Table 2.4) or any other pathogen by filtration, experimental conditions and methods must be considered. Differences in analytical reliability, processed sample volume, method detection limits, and influent microorganism concentrations can all contribute to inter-study differences in *C. parvum* removal by filters. The range of oocyst removals described in Table 2.4 underscores the need for accurate and thorough description of experimental conditions and methodologies so that *C. parvum* removals by filters and other processes can be thoroughly assessed. Several of the methodological factors that can critically affect the interpretation of *C. parvum* (and other microorganism) removal data are listed in Table 2.5 along with relevant questions that should be considered when evaluating pathogen removal data.

The *C. parvum* removal studies included in Table 2.4 were evaluated in the context of the methodological factors and questions listed in Table 2.5; several of the methodological factors relevant to those studies are listed in Table 2.6. Many of the points highlighted in Table 2.6 are referred to in the critical discussion of currently available *C. parvum* removal data below. Although some of the points seem obvious or redundant, they underscore the need for clear interpretation and understanding of limitations of the currently available *C. parvum* removal data in the literature.

Table 2.5

Methodological Factors and Relevant Questions to Consider When Evaluating Pathogen Removal Data

Methodological Factor	Relevant Questions
Method Recovery	What was the analytical method recovery? How was recovery described? How was recovery determined? Was recovery the same for all of the waters studied?
Data Handling	Were the data adjusted to account for recovery? If yes, how? How were non-detects handled? How did non-detects affect maximum possible removals? How was pathogen removal calculated? What are the implications of reporting geometric vs. arithmetic means?
Operating Conditions	Were several pretreatment conditions evaluated? Did pretreatment affect influent concentrations of pathogens? Did pretreatment change in response to varying raw water conditions? Were operating conditions optimal? Were various process configurations/types compared? Were all relevant operating conditions specified?
Pathogen Concentrations	Were many non-detects found? Did processed sample volume affect the results? Were influent and effluent concentrations high enough to evaluate maximum removal (*i.e.*, no non-detects)? Were pathogens seeded into the treatment process? How were seeded concentrations determined? Were filter influent and effluent concentrations determined by the same analytical method? Were results obtained from experiments with atypically high seeded concentrations or concentrations comparable to indigenous levels?

Table 2.6

Summary of Critical Information from Studies Evaluating Process Removals of *C. parvum*

Study	Recovery	Removal Adjusted for Recovery	Seeded Conc. (#/L)*	FI Sample Volume	FE Sample Volume	Non-Detects in Filter Effluent	Observations of Relevance to Present Study
LeChevallier *et al.*, 1991c	13-59%	no	–	NS	3785 L FE	many	Geometric means reported for oocyst densities.
		no	NS	NS	NS	NS	Stable/optimal removals not specified.
LeChevallier & Norton, 1992	RP	no	–	NS	NS	many	Geometric means reported for oocyst densities. Stable/optimal removals not specified.
Nieminski, 1994	81%	no	~10^{4}**	1 L	60 L	NS	Only used data for samples in which oocysts were found.
			~10^{7}††	20 L	20 L	NS	Different influent quality between experiments.
West *et al.*, 1994	10-40%	not clear	~10^{2}-10^{3}†	50 mL	500 mL	many	
Charles *et al.*, 1995	NS	–	~10^{4}†	10 mL	NS	no	Stable/optimal removals not specified.
	NS	–	~100†	NS	NS	no	
Hall *et al.*, 1995	10-30%	no	~2×10^{4}§	≥ 100L	≥ 100L	no	Unclear whether nor not recovery corresponds to specific waters studied.
			300-800§	≥ 100L	~1000 L	some	
			400§	≥ 100L	~1000 L	some	
			700§	≥ 100L	1470 L	no	
Kelley *et al.*, 1995	6-14%	yes	–	570 L	760 L	many	Many non-detects in filter effluent samples.
Nieminski & Ongerth, 1995	avg. 9% raw avg. 12% FE	no	~10^{4}**	1 L	60 L	NS	Different influent quality between experiments. Only used data for samples in which oocysts were found.
	5-35% FE	no	~10^{7}††	NS	NS	NS	Lower recovery during higher turbidity conditions.
Ongerth and Pecoraro, 1995	avg. 17% FI avg. 29% FE	yes	~400-3000	50 mL	10 L	NS	Variable filter influent and effluent recovery profiles.
Patania *et al.*, 1995	avg. 15-35%	no	~10^{3}-10^{4}†	5-30 L	20-80 L	many	Recovery in all waters assumed equivalent Recovery only measured from raw waters. Many influent and effluent non-detects, large influent range.
Timms *et al.*, 1995	NS	–	~4000	50 mL	750 mL	all	No oocysts were found in the filter effluents.

Table 2.6

Summary of Critical Information from Studies Evaluating Process Removals of *C. parvum* (Continued)

Study	Recovery	Removal Adjusted for Recovery	Seeded Conc. (#/L)*	FI Sample Volume	FE Sample Volume	Non-Detects In Filter Effluent	Observations of Relevance to Present Study
Edzwald *et al.*, 1996	NS	–	7210	NS	NS	NS	Filter influent concentrations changed due to chemical pretreatment and affected log removals.
			2 - 631	NS	NS	NS	
Lytle *et al.*, 1996	NS	–	6900[†]	1 L	1 L	all	No calculations of removal were made due to non-detects.
Swaim *et al.*, 1996	NS	no	~10[3†]	NS	NS	some	Recovery in raw and filtered waters assumed equivalent.
Scott *et al.*, 1997	NS	–	~10[6††]	0.1-10 mL	5 - 500 mL	NS	
Yates *et al.*, 1997	NS	–	~10[6††]	1, 10 mL	300 mL	NS	
Baudin and Laîné, 1998	NS	–	–	NS	NS	many	Ripening and breakthrough turbidities not specified.
Edzwald and Kelley, 1998	NS	–	2510[†]	NS	NS	NS	Pretreatment affect filter influent concentrations.
Fox *et al.*, 1998	NS	–	~10[5]	250 mL	5 L	no	Focused on clarifier removals, filtration generally mentioned. Stable/optimal removals not specified.
Dugan *et al.*, 1999	NS	–	~84-480	250 mL	5 L	no	Stable/optimal removals not specified.
Edzwald *et al.*, 1999	NS	yes	~10[7§§]	NS	NS	all	Pretreatment affect filter influent concentrations.
Swertfeger *et al.*, 1999	NM	–	~10[5]-10[6††]	53-106L	24 L	no	Removals adjusted (empty column removals subtracted).

[-] Not applicable.
[NS] Not stated.
[RP] Reference provided.
[NM] Not measured.
[*] Concentration units unless otherwise stated.
[†] Seeded concentration in raw water.
[§] Unclear as to whether measured or calculated filter influent concentrations were used.
[**] Theoretical influent concentration based on hemocytometer counts and dilution calculations. Filter influent concentration was not stated.
[††] Total number of cysts/oocysts seeded at filter influent.
[§§] Total number of cysts/oocysts seeded at clarifier influent.
[‡] Removals based on raw water concentration (not filter influent concentration).
[‡‡] Removals based on clarifier influent concentration (not filter influent concentration).

2.8.1 Effect of Analytical Recovery on Interpretation of *C. parvum* Removal Data

Perhaps the most commonly cited source of uncertainty associated with *C. parvum* removal data is the lack of consistency in analytical recovery of oocysts from water. Although standard deviations and coefficients of variation can be used to describe the uncertainty associated with oocyst recovery, tools that incorporate this information into *C. parvum* removal data are currently lacking. Therefore, most reported oocyst removal data are analyzed with little if any consideration of analytical recovery and the resulting conclusions are essentially based on the assumption of consistently high recovery (*i.e.*, not much uncertainty associated with the data).

Improved detection methods for *C. parvum* will undoubtedly increase the reliability of oocyst removal data collected during future investigations. Many of the currently available methods are adequate for drawing general conclusions about process removals of oocysts, however. Various analytical methods for *C. parvum* exist; the less consistent the analytical recovery, the more difficult it is to attribute differences in removal data to differences in treatment processes because it is unknown whether differences in treatment or uncertainty associated with recovery cause the observed differences in removal. Presentation of all of the recovery data (not just means and standard deviations) relevant to a particular study is therefore the optimal form of recovery description because it will allow for subsequent reassessment of data reliability once tools are in place to integrate that information with measured removal data.

It is worth noting that a lack of recovery information does not completely invalidate findings from challenge studies. For example, when investigations comparing two filtration scenarios treating the same water are conducted, it is reasonable to assume that the filter influent waters to each filter have similar oocyst recovery profiles (since the filters treat exactly the same water). If the filter effluents produce waters of similar quality (*e.g.*, turbidity and particle counts), it is also likely that the filter effluents will have similar oocyst recovery profiles, although these profiles will not necessarily be the same as those obtained from filter influents. If statistical differences are not observed when recovery is ignored (*i.e*, when recovery is assumed ideal), the incorporation of increased uncertainty of recovery will

likely make it more difficult to demonstrate statistical differences. Therefore, inferences drawn from such data are essentially the same; regardless of recovery which only affects the magnitude of the reported removals. When investigated water qualities are similar, it is only when differences in removal are observed that the investigators must question whether or not they originate from inherent differences between the oocyst removal capacities of the different processes or from the uncertainty and inconsistencies associated with the analytical methodologies. Regardless of whether or not differences between removals are observed, real differences in oocyst removals between compared processes may exist. In such cases, the only way that the differences can be determined is by repeating the experiments and processing samples with more accurate analytical methods.

If oocyst recoveries vary between influents and/or effluents from treatment processes (perhaps due to the presence of algae, turbidity, etc), recovery can affect both the magnitude of reported oocyst removals and the conclusions of comparisons drawn between different processes or operating conditions. The reporting of all relevant recovery data under these conditions can be critical to identifying the limitations of inferences that can be drawn from the removal investigations. Furthermore, the recovery data can be subsequently integrated with the removal data when tools are developed to incorporate methodological uncertainty into removal data.

2.8.2 Mode of Filter Operation

Pilot- and full-scale studies performed by Nieminski (1994) and Nieminski and Ongerth (1995) indicated comparable pilot-scale removals of *Cryptosporidium* by conventional and direct filtration processes. Differences were observed at full-scale where direct filtration removals surpassed those of conventional filtration. The authors speculated that the differences between the full-scale oocyst removals were attributable to differences in filter influent quality and the associated difference in recovery from higher turbidity waters (such as those encountered during conventional treatment experiments). The authors also clearly stated that non-detects were not included in the calculation of log removals; however, it is possible that if larger samples had been processed that oocysts would have been found eventually, thereby increasing mean removals. The inclusion of recovery and sample

handling information was critical to appropriately evaluating the *C. parvum* removal capacity of the two modes of filtration.

C. parvum removal data by various modes of filtration were reported by Patania *et al.* (1995). The authors studied two conventional pilot plants, including a pilot plant with in-line filtration and a conventional pilot plant with low rate surface filtration. Although very high (>4- and often >5-log) removals of oocysts were observed at both pilot plants, the filters alone achieved anywhere from essentially no removal to 4.7-log removal of oocysts (Table 2.4). Considered without methodological information, these data might have suggested that the conventional pilot plant with low rate surface filtration and the pilot plant with in-line filtration offered superior oocyst removal when compared to the conventional pilot plants (Table 2.4). The authors provided critical methodological data that precluded the conclusion that one form of filtration was superior over another, however. As is pointed out in Table 2.6, the *C. parvum* recoveries in all of the waters were assumed equivalent, which was not *necessarily* accurate, as demonstrated by Nieminski and Ongerth (1995). In addition, the experiments described by Patania *et al.* (1995) were conducted over a wide range of influent *C. parvum* concentrations associated with seeding oocysts into the raw water, prior to pretreatment. While the filter influent concentrations were as high as 10^3 oocysts/L during the in-line and low rate surface filtration experiments, they were as low as <0.22 oocysts/L during the conventional filtration experiments, with several influent and effluent non-detects. Although the inappropriateness of concluding that in-line filtration was superior to conventional filtration for oocyst removal is perhaps obvious, the methodological information clearly provided justification for the limitations of the conclusions that could be drawn from the *C. parvum* removal data.

2.8.3 Media Type and Design

Investigations of media type and design have demonstrated that these parameters have little impact on oocyst removals by filters. Hall *et al.* (1995) did not find performance differences between sand and dual media filters when the filters had similar filtrate quality (measured by turbidity). This finding was not incontrovertible due to the presence of non-detects in some

of the filter effluents, however; in addition, no analytical recovery information was specified by the authors, allowing for the possibility of different recoveries from different waters.

Pilot-scale studies conducted by Patania *et al.* (1995) indicated that GAC, GAC/sand, and anthracite/sand (with both 1.0 mm and 1.5 mm effective size of anthracite) filters also achieved essentially comparable oocyst removals by the various types of filters. The authors emphasized that the filter influent oocyst concentrations were below detection limits in one of the media comparisons (GAC/sand versus anthracite sand), thereby precluding a true comparison of oocyst removals by the two filters. In addition, Patania *et al.* (1995) also assumed that oocyst recoveries from the different waters were equivalent. Such a difference could have possibly, though not necessarily, impacted the interpretation of their oocyst removal data.

Comparisons of *C. parvum* removals by sand and dual-media filters by Dugan *et al.* (1999) also resulted in similar oocyst removals; the presence of oocysts in all of the filter effluents further substantiated the conclusion that media type did not have a large effect on oocyst removal by filtration. Swertfeger *et al.* (1999) drew the same conclusion by demonstrating that oocyst removals by filters of sand and dual-media could not be statistically distinguished from one another. This conclusion was also based on experiments that were conducted to yield oocysts in all of the filter effluents. Although the Dugan *et al.* (1999) and Swertfeger *et al.* (1999) investigations produced reliably countable numbers of oocysts in both the filter influents and effluents, the lack of analytical recovery information allowed for some speculation regarding the reliability of the data. However, the comparative conclusions (between media types) that could be drawn from these investigations were essentially unaffected by the recovery specifics because, as was discussed in Section 2.8.1, the filters in each study treated the same influent and produced effluents of similar water quality (based on turbidity and particle counts).

Dugan *et al.* (1999) and Swertfeger *et al.* (1999) also investigated the effects of relative media depth on *C. parvum* removal by filters. Both studies investigated dual-media filters and concluded that media depth did not substantially impact oocyst removal. Dugan *et al.* (1999) specified that their data failed to consistently demonstrate increased oocyst removal

with increased media depth, but noted that their filter cycles were stopped at 36 hours. The authors speculated that differences in oocyst removal between filters with different media depths might have been observed more consistently if the filters had been operated beyond 36 hours.

2.8.4 Filtration Rate

Pilot-scale investigations of sand filtration conducted by Hall *et al.* (1995) did not demonstrate any differences in oocyst removal when the filter was operated at 2 and 4.1 gpm/ft^2 (5 and 10 m/h). The authors noted, however, that there were several non-detects at the lower rate and no non-detects at the higher rate, suggesting that differences might have been observed between the two rates if larger samples had been processed. Patania *et al.* (1995) also investigated the effects of filtration rate on oocyst removal in several types of filters. The authors studied GAC/sand filters operated at rates of 3 and 6 gpm/ft^2 (7 and 15 m/h) and anthracite/sand filters at 5 and 8 gpm/ft^2 (12 and 20 m/h). They concluded that *C. parvum* removals were not greatly affected by the range of filtration rates studied; these data were strengthened by the presence of oocysts in almost all of the filter effluent samples. The authors speculated that as long as pretreatment conditions were optimized, the different filtration rates would have a minimal impact on oocyst removal by the filters. Increases in filtration rate also showed no adverse effects on oocyst removals by slow sand filters (Timms *et al.*, 1995); however, these data were inconclusive because no oocysts were found in any of the filter effluent samples.

Dugan *et al.* (1999) investigated the effects of filtration rate over time in dual-media filters. The authors demonstrated that oocyst removals by filters operated at 2 and 4.1 gpm/ft^2 (5 and 10 m/h) were comparable for the first 30 to 40 hours of operation. Afterward, oocyst removal decreased by >1-log during filter operation at the higher rate. Although recovery information was not specified, oocysts were found in all of the filter effluent samples. These findings did not contradict those of Hall *et al.* (1995) and Patania *et al.* (1995), but rather suggested that additional operational factors must be considered when evaluating the impact of filtration rates on *C. parvum* removals by filtration. They also might have considerable implications for filter operation practices, particularly if the analytical method employed by

Dugan *et al.* (1999) was considered relatively reliable; however, speculation on this issue was not possible because no recovery information was provided. Minimally, these data suggested that the effects of different filtration rates on oocyst removal over time should be further investigated.

2.8.5 Filter Aid

Patania *et al.* (1995) investigated of the impact of filter aid addition on *Cryptosporidium* and *Giardia* removal by GAC/sand filters and concluded that the use of anionic polymer filter aid did not improve the removal of either microorganism under the conditions studied. The authors noted that the filter influent *Cryptosporidium* concentrations were inconclusive since they were below their method detection limit. Swertfeger *et al.* (1999) also discussed the impacts of filter aid on oocyst removal. During these investigations, pilot-scale filters received settled water from a full-scale treatment plant. Non-pretreated (non-coagulated) oocysts were seeded into the filter influent and ferric sulphate was added as a filter aid at doses of 0.5 and 1.0 mg/L. Oocyst removals of > 2.5-log were achieved by the pilot-scale filters.

Although Swertfeger *et al.* (1999) did not investigate oocyst removals by filtration without filter aid, they speculated that filtration with filter aid might be capable of substantial pathogen removal in the event of a limited breakdown in the coagulation process or if cysts/oocysts were introduced into the treatment process after coagulant addition. The latter speculation was supported by the authors' data, although the impact of filter aid on the achieved oocyst removals was unclear. The combined impacts of sub-optimal coagulation and the use of filter aid on oocyst removal by filtration were not discussed during this study; therefore, the speculation on mitigating oocyst passage through filters with the use of filter aid during periods of sub-optimal coagulation represents a potential area of further research that was not substantiated by the data presented by the authors.

2.8.6 Pretreatment

A variety of pretreatment options prior to filtration are possible. Hall *et al.* (1995) conducted pilot-scale investigations that demonstrated that good *C. parvum* removals could

be achieved by rapid gravity filtration following either dissolved air flotation or floc blanket clarification. Recovery information was not clearly specified and non-detects occurred in some of the filter effluents, however, thereby allowing for some speculation regarding the amount of uncertainty associated with these data.

Patania *et al.* (1995) similarly demonstrated that various pretreatment strategies in the form of different coagulant combinations (ferric chloride or alum, with and with cationic polymer) applied to and optimized for the same water could produce equally effective removal of oocysts by GAC/sand and anthracite/sand filtration. It should be noted, however, that the resulting oocyst concentrations in the filter effluents included several non-detects. The authors indicated that their conclusion was supported by other investigations that demonstrated that different coagulation schemes applied to the same water could produce equally effective removal of turbidity and cysts (Logsdon *et al.*, 1985; Al-Ani *et al.*, 1986; LeChevallier *et al.*, 1991c). The authors also speculated that different coagulation conditions did not greatly impact oocyst removal through the entire treatment process because each coagulation condition had been optimized with jar tests prior to application. These findings and speculations were reasonable given that effective filtration is inextricably linked to effective pretreatment during typical water treatment conditions.

Several studies have indicated the importance of coagulation processes for improving filter removal efficiencies of *C. parvum*. The difficulty in understanding and optimizing coagulant effects during filtration is complicated by the different roles of attachment and detachment mechanisms occurring during different periods of the filter cycle such as ripening and breakthrough. It has been demonstrated that a complete lack of chemical pretreatment can result in very poor to no *Cryptosporidium* removal by conventional filters (Charles *et al.*, 1995; Patania *et al.*, 1995); this result underscored the link between effective filtration and pretreatment during water treatment.

Although non-ideal analytical methods can make it difficult to measure low concentrations of oocysts, Patania *et al.* (1995) found relatively high oocyst concentrations in filter effluents during *Cryptosporidium* seeding studies conducted when no chemical pretreatment preceded filtration. This result was demonstrated regardless of filter pre-conditioning with

effectively coagulated water prior to the no coagulation conditions. Although all of the recovery information was not explicitly provided, the oocyst removal data were convincing for even a moderately consistent analytical method because the filter effluent concentrations were so high relative to those obtained during typical stable operation (it difficult to imagine somewhat consistent recovery differences of several log). The critical importance of adequate coagulation for effective filtration of oocysts was further supported by the relatively consistent findings in replicate experiments conducted by the authors.

Charles *et al.* (1995) concluded that rapid mix conditions were less critical than coagulation and impacted oocyst removals by <1-log. The authors also demonstrated considerable deterioration in *C. parvum* removals when no coagulant was used prior to filtration; however, they did not specify if the filters had been pre-conditioned with effectively coagulated water prior to oocyst seeding during the no coagulation investigations. Although Patania *et al.* (1995) did not observe any differences between oocyst removals obtained during no coagulation filtration by pre-conditioned and non-pre-conditioned filters, it is likely that pre-conditioning effects can be somewhat site-specific, much like coagulation conditions.

Sub-optimal coagulation (non-optimal chemical pretreatment) occurs due to factors such as mis-dosing or sudden water quality changes. The frequency of sub-optimal coagulation events varies from plant to plant. Although not as severe as complete coagulation failure, sub-optimal coagulation conditions have consistently demonstrated >1-log decreases in *C. parvum* removal by filtration. Ongerth and Pecoraro (1995) conducted pilot-scale direct filtration investigations during optimized and sub-optimal coagulation conditions. Oocyst removals averaged only 1.5-log during the sub-optimal coagulation experiment, a >1-log decrease relative to stable (or optimized) operating conditions. This experiment was only performed once; given that somewhat different mean oocyst recoveries were obtained from the filter influent and effluent waters, replication would strengthen this result. The data of Ongerth and Pecoraro (1995) strongly suggested that oocyst removals deteriorated during sub-optimal coagulation since the three oocyst removals measured during the sub-optimal coagulation experiment were the lowest of all eleven measured removals.

Dugan *et al.* (1999) reported similar findings when they investigated pilot-scale *C. parvum* removal during periods of sub-optimal coagulation. The authors demonstrated that under-coagulation of moderately and highly turbid waters and moderate coagulation of low turbidity waters resulted in at least 1-log deterioration in oocyst removals relative to those obtained during optimized operating conditions. Although no recovery information was provided, oocysts were detected in all of the filter effluents; oocyst removals by filtration were also consistently lower during non-optimal coagulation experiments than during experiments performed at optimized operating conditions. The various coagulation scenarios that these authors examined underscored that the general term "sub-optimal coagulation" can be interpreted to mean a variety of operating conditions that result in a large range of filter influent and effluent water qualities. The effects of sub-optimal coagulation may also be site specific and result in a range of impacts on *C. parvum* removal by filtration. In general, all of the reported investigations of *C. parvum* removal by filtration during sub-optimal coagulation and no coagulant conditions have demonstrated that optimal chemical pretreatment is critical to maximizing oocyst removal during filtration.

2.8.7 Ripening

Several pilot-scale studies have demonstrated oocyst removals approximately 0.5- to 1-log lower during filter ripening than during stable filter operation. Lower removals during filter ripening or maturation could be expected because ripening is a period during which the initial improvement of filtered water quality occurs in typical filter cycles. Hall *et al.* (1995) demonstrated slightly lower oocyst removal during filter ripening (~0.4-log lower) than during stable operation by sand filtration preceded by dissolved air flotation. The authors only presented data for one experiment, however. The generalized recovery data and a lack of replicate data made it difficult to speculate whether or not this small difference between oocyst removals during ripening and stable operation was due to methodological variability.

Patania *et al.* (1995) also investigated *C. parvum* removals by filtration at three different pilot plants. Two of the pilot plants yielded moderately lower (~0.4- to 0.9-log lower) oocyst removals during ripening than during stable filter operation, a result similar to that reported by Hall *et al.* (1995). No discernable difference between oocyst removals during

ripening and stable filter operation was observed at the third pilot plant. The authors speculated that the deep filter media at the third pilot plant resulted in virtually no maturation with respect to microorganisms. It was also possible that this result was due to methodological variability, rather than media effects, however. Swaim *et al.* (1996) also reported slightly lower mean oocyst removals during ripening compared to stable filter operation; their data were additionally complicated by several non-detects in the filter effluents during both ripening and stable operation experiments. Data such as those of Hall *et al.* (1995), Patania *et al.* (1995), and Swaim *et al.* (1996) emphasize the need for a tool to quantify the uncertainty associated with *C. parvum* analytical methods. These findings also emphasize that multiple operational and design factors must always be considered when evaluating *C. parvum* removal data from filtration processes.

Findings similar to the pilot-scale results discussed above were presented by Baudin and Laîné (1998) who demonstrated oocyst removals ~1-log lower during filter ripening than during stable operation at two full-scale plants. Only one stable operation and one ripening experiment were conducted at each plant; no methodological recovery data were presented. The authors specified that oocyst removals during filter ripening depended on raw water concentrations at one of the plants. Fluctuating filter influent oocyst concentrations at one plant and lacking recovery information made it difficult to speculate about the cause of the observed ~1-log difference in oocyst removals during ripening and stable operation. LeChevallier *et al.* (1991c) also reported slightly lower oocyst removals during filter ripening than during stable operation at two full-scale plants. Numerous non-detects in the filter effluents and somewhat variable analytical recoveries also made it difficult to conclude whether or not the observed differences were due to experimental and methodological variability.

2.8.8 Breakthrough

Pilot-scale investigations of *C. parvum* removal by filtration during turbidity breakthrough were performed by Patania *et al.* (1995). The authors studied turbidity breakthrough when filter effluent turbidities increased from 0.1 NTU to 0.2 NTU or higher. Substantial differences between oocyst removals during stable operation and breakthrough were not

observed, although the authors speculated that oocyst removal might have deteriorated if sampling had continued beyond the point at which filter effluent turbidities were ~0.2 NTU. Oocysts were detected in almost all of the samples collected during the stable operation and breakthrough experiments at the particular pilot plant where turbidity breakthrough was investigated; replicate experiments strengthened the findings.

Similar results were obtained during two full-scale investigations reported by Baudin and Laîné (1998). Little deterioration in oocyst removals was observed during filter breakthrough at either plant. The authors indicated that oocyst removal during breakthrough at both plants depended on the filtration rate. Fluctuating filter influent oocyst concentrations at one plant during the stable operation experiment, unspecified filter effluent turbidities during the breakthrough experiments, and a general lack of recovery information, made it difficult to draw inferences from the oocyst removal data collected during the breakthrough and stable operation experiments.

2.9 RESEARCH NEEDS

Despite progress in the development of disinfection technologies that can achieve reasonable *C. parvum* inactivation, the traditional physico-chemical processes used in drinking water treatment remain critical to achieving desirable levels of *Cryptosporidium* removal. One of these important barriers is filtration. Currently, the amount of oocyst-removal protection provided by filtration processes is not fully known (Solo-Gabriele and Neumeister, 1996). Full- and pilot-scale *C. parvum* removals for a variety of filtration regimes have been reported anywhere in the range from 2- to 3-log (Nieminski and Ongerth, 1995; Fox *et al.*, 1998) to >5-log (Baudin and Laîné, 1998; LeChevallier *et al.*, 1991c). Although several challenge studies evaluating the ability of specific filtration regimes to remove *C. parvum* during optimized treatment conditions exist in the literature, limited data are available regarding the *C. parvum* removal capability of granular media filters during vulnerable periods of operation. Moreover, a strategy or framework for assessing and describing the overall ability of granular media filters to remove *C. parvum* (and other pathogens) during periods of both optimized operation and process upset is lacking. Such a framework would be instrumental in providing guidance to water treatment professionals and regulators.

Many of the limitations of available *C. parvum* removal data are due to the limitations of analytical methods for the concentration and identification of oocysts from natural waters. Differences in analytical reliability, processed sample volume, method detection limits, and influent microorganism concentrations can all contribute to inter-study differences in *C. parvum* removal by filters. Many of the *C. parvum* removal studies summarized in Table 2.4 employed analytical methods that were similar to the proposed ASTM (1993) method. The limitations of this method were underscored by a blind survey of the accuracy and reproducibility of the method which revealed that 6 of 12 laboratories failed to detect any oocysts in spiked samples and that the recovery among laboratories ranged from only 1.3 to 5.5% (Clancy *et al.*, 1994).

Several alternatives to the ASTM protocol have been examined, the most notable of which is USEPA Method 1622. Method 1622 offered significant improvements over the proposed ASTM and ICR methods; it demonstrated mean oocyst recoveries of >70% in early trials (Clancy *et al.*, 1997; Bukhari *et al.*, 1999). Subsequent studies of Method 1622, such as the USEPA validation experiments, yielded recoveries of approximately 35% with 13% relative standard deviation (Clancy *et al.*, 1999). Although this method is a clear improvement over previously used methods, these data indicate that considerable analytical limitations continue to challenge the evaluation and interpretation of *C. parvum* concentration and removal data.

Until a reliable method for *C. parvum* analysis is available, the challenges associated with the concentration and enumeration of oocysts from natural waters will undoubtedly result in the continued application of numerous methods for *C. parvum* analysis. The development of new methods is supported within the framework of Method 1622. While method development is critical, the use of various methods with variable and low to moderate recoveries makes it difficult to interpret and synthesize the results from multiple *C. parvum* removal studies. The lack of reliable analytical methods particularly necessitates the need for accurate and thorough description of experimental conditions and methodologies so that *C. parvum* concentrations and removals by filters and other processes can be thoroughly assessed.

Describing the methodological limitations of *C. parvum* removal data is critical to both interpreting the data and to quantitatively describing the uncertainty associated with the data. Although it is commonly accepted that mean concentrations or removals used to calculate confidence intervals do not account for all of the uncertainty in the *C. parvum* data (*i.e.* they do not account for sampling strategy, method recovery, variability associated with the analytical method), few studies of *Cryptosporidium* concentration and removal efficiency have addressed the issue of data reliability beyond stating average analytical recovery. Incorporation of this uncertainty into a statement of data reliability like a confidence interval can be quite complicated because the normal distribution is often inappropriate for describing distributions of microorganisms such as *Cryptosporidium* in water samples.

Haas and Rose (1996) showed that naturally occurring oocyst densities could be described adequately by the Poisson distribution. Parkhurst and Stern (1998) also suggested Poisson confidence intervals for describing oocyst data. Atherholt and Korn (1999) presented Poisson methods for sample counts in the context of the ICR protocol and suggested that a distribution more complex than the Poisson distribution may be required to account for the various errors in the analytical process; Nahrstedt and Gimbel (1996) developed such a statistical framework. The disadvantage of the Nahrstedt and Gimbel (1996) model is its increased level of complexity, which resulted in a computer program rather than a simple statistical table; the scope of this approach was also limited to calculating confidence intervals on *Cryptosporidium* concentrations, not removals. Developments such as the Nahrstedt and Gimbel (1996) model represent tremendous progress toward handling *C. parvum* concentration data in a statistically rigorous manner. Accessibility to and application of such tools by water treatment professionals is still lacking, however, perhaps due to a need for tools to evaluate oocyst removals by treatment processes, rather than concentrations.

The *C. parvum* removal efficiencies of treatment processes can be evaluated by monitoring the removal of indigenous oocyst concentrations through the treatment processes or by seeding the processes with *C. parvum* oocysts or surrogates. Although most representative of real operating conditions, monitoring the removal of indigenous oocysts is not currently feasible due to methodological limitations that preclude the processing of the very large

water volumes (often >>1000 L) necessary to achieve reliably countable concentrations of oocysts in waters such as filter effluents. The other alternative for assessing treatment process removals of *C. parvum* is to seed influent waters with concentrations of oocysts high enough to yield reliably countable numbers of oocysts in the treated waters; such evaluations can also be performed with surrogates for oocyst removal.

Several different types of surrogates for the removal of viable *C. parvum* oocysts by drinking water treatment processes have been evaluated. These include turbidity, particle counts, HPCs, aerobic spores (typically *Bacillus subtilis*), UV_{254}, DOC, polystyrene microspheres. Of these potential surrogates, some are inadequate (HPCs, UV_{254}, DOC) and others are indicative of treatment efficiency but not oocyst removal (turbidity, particle counts, aerobic spores), the remainder need to be further evaluated (polystyrene microspheres).

Due to the lack of adequate surrogates for the removal of viable *C. parvum* oocysts, chemically inactivated oocysts have been commonly used for treatment evaluations. Differences in surface charge, described by zeta potential, exist between chemically inactivated and viable oocysts of *C. parvum* (Lytle and Fox, 1994); it is not presently known whether or not these differences are substantial enough to result in significantly different removals by granular media filtration processes. Information related to the relationship between viable and inactivated oocyst removals during filtration is necessary for proper interpretation of research evaluating process removals of *C. parvum*.

Unfortunately it is usually impossible to evaluate the *C. parvum* removal efficiency of granular media filtration processes with indigenous oocyst concentrations because existing analytical methods lack the ability to process the volumes of water typically necessary to achieve reliably countable numbers of oocysts in filter effluents. Process evaluations that involve seeding (or spiking) atypically high concentrations of oocysts must therefore assume that the removals achieved with high influent concentrations are the same as those that would be achieved with indigenous influent concentrations. The conclusions that can be drawn from *C. parvum* removal studies are therefore limited in that they are not necessarily

representative of those that would be obtained under similar operating conditions with indigenous oocyst concentrations.

The work described in this thesis endeavored to address some of the research needs discussed above. The primary focus of this thesis research was to assess the removal of *C. parvum* oocysts by granular media filtration processes relative to measurements of turbidity, particle counts, and potential surrogates (polystyrene microspheres) during various phases and events occurring throughout typical filter cycles. A full understanding of the ability of filters to remove *C. parvum* (or any other pathogen) could only be attained when the reliability of experimentally obtained data was understood and quantified. To that end, a statistical tool was developed to describe the reliability of *C. parvum* concentration and removal data collected during treatment process challenge studies by integrating methodological uncertainty with oocyst removal data. The reliability of the data collected during this thesis research was demonstrated with that quantitative tool. The relationships between on-line performance parameters and oocyst removal were evaluated and applied to the development of practical treatment strategies for maximizing *C. parvum* removal by granular media filtration. Polystyrene microspheres were also assessed as potential surrogates for further studies of *C. parvum* removal by filtration.

Chapter 3
Materials and Methods

3.1 Experimental Design

The experimental approach employed during this research included defining the removals of pathogens and surrogates during vulnerable periods of filter operation, relating them quantitatively to removals during stable operation, and investigating design and operational strategies for maintaining removals as high as possible during potable water production. The research approach included three experimental tasks designed to provide concrete outcomes of practical value to the water industry while elucidating some of the fundamental mechanistic processes governing the removal of microbiological particles such as *C. parvum* by filtration processes. These tasks included:

1) bench-scale experiments investigating the applicability of formalin-inactivated *C. parvum* as surrogates for viable *C. parvum* during studies of oocyst removal by filtration,

2) pilot-scale experiments investigating the influence of design and operational effects on *C. parvum* and potential surrogate (polystyrene microsphere) passage in benchmark systems, and

3) pilot-scale experiments investigating mechanistic issues associated with *C. parvum* removal by filtration.

The three experimental components of this research are summarized in Table 3.1. This table is organized according to the research platform at which the experiments were performed.

Table 3.1

Summary of bench- and pilot-scale experiments

Research platform	Experimental objectives	Operational conditions
UW bench-scale	compare removal of viable and inactivated oocysts by filtration	stable operation
		ripening
	compare dual- and tri-media	
		coagulation failure
Ottawa pilot-scale	BASE CASE	stable operation (cold and warm water conditions)
	compare several operating conditions to stable filter operation	stable operation during spring runoff
	evaluate microsphere removal as surrogate for *Cryptosporidium* removal by filters	filter ripening
		sub-optimal coagulation
		coagulation failure (no alum/silicate) after a period of stable operation
		coagulation failure (no alum/silicate) since backwash
		partial coagulation failure (no silicate)
		hydraulic step
		end-of-run (filter effluent turbidity <0.1 NTU)
		early breakthrough (filter effluent turbidity 0.1-0.3 NTU)
		late breakthrough (filter effluent turbidity >0.3 NTU)
Windsor pilot-scale	compare effects of different raw water quality	stable operation
	compare constant and declining rate filtration	
UW pilot-scale	compare dual- and tri-media	stable operation
	evaluate microsphere removal as surrogate for *C. parvum* removal by filters	hydraulic step

The first experimental phase of this thesis research was designed to demonstrate whether or not comparable removals of viable and formalin-inactivated oocysts could be expected by filtration at various operating conditions encountered during typical water treatment. Given the requirements associated with the handling and disposal of viable oocysts, these experiments were planned for bench-scale at the University of Waterloo. Specifically, the experiments were designed to examine the relative removals of oocysts by dual- and tri-media filters during stable operation, ripening, and coagulation failure.

Both dual- (anthracite/sand) and tri-media (anthracite/sand/garnet) filters were studied to examine media effects on removals of viable and inactivated *C. parvum* oocysts. Stable operation was evaluated first because it represented baseline removals at optimal operating conditions (*e.g.*, optimized chemical pretreatment, particle removal, etc.), to which other operating conditions with possibly compromised *C. parvum* removal would be compared. Coagulation failure was selected as one such compromised operating period. It was considered a critical vulnerable period for study because oocyst surface charge is least affected by the presence of coagulant during this period. Any differences between filter removals of viable and inactivated oocysts would most likely be demonstrated during this period. Ripening was evaluated because it typically represents a brief period of high turbidity and particle passage through most filters and it occurs during most filter cycles. It is also a period during which differences in the attachment ability of oocysts may affect their passage through filters; however, this effect was considered less likely to result in a difference between removals of viable and inactivated oocysts since coagulant is typically present during ripening. Particle and turbidity breakthrough also would have been interesting to study; like ripening, this period is associated with a relatively rapid deterioration in filter effluent quality. Unfortunately, available head limitations precluded the study of this operating condition during this phase of the experiments.

The second experimental task evaluated the removals of *C. parvum*, potential surrogates (polystyrene microspheres), and performance indicators (turbidity, particle counts, and *B. subtilis* spores) in benchmark systems (*i.e.*, typical design and operating conditions). As is outlined in Table 3.2, they were conducted at the Ottawa, Windsor, and University of Waterloo (UW) pilot plants in Canada. Inactivated *Cryptosporidium parvum* oocysts and pure-cultured *Bacillus subtilis* spores were seeded at these locations; in addition, fluorescent polystyrene microspheres were seeded during some of the experiments at Ottawa and UW. The experiments were designed to assess the *C. parvum* removal that could be reasonably expected from typical rapid filtration systems and to evaluate the sensitivity of *C. parvum* removal during typical filter cycle events and conditions of process stress. Experiments were developed to capture most of the common (and some less common), dynamic events that occur during typical water treatment operation. Seven basic conditions were investigated during these experiments. They were:

1. stable filter operation,
2. no coagulation,
3. sub-optimal coagulation,
4. ripening,
5. hydraulic step,
6. end of run, and
7. breakthrough.

Each condition was studied at least in triplicate; several sub-categories of the seven general conditions were also examined. Most of the experiments were conducted at Ottawa, allowing for the relative comparison of operating conditions; they are summarized in Table 3.2 along with their rationale and specific experimental conditions. As was noted in Table 3.1, additional experiments were performed at UW and Windsor that permitted the evaluation of different filtration regimes (constant vs. declining rate at Windsor) and different media (UW) and raw water (Windsor) types.

Table 3.2

Operating conditions examined during pilot-scale experiments at Ottawa

Operating Condition	Specific Experiments	Experimental Rationale
Stable operation	warm water	defines highest attainable removal and acts as baseline to which other operating conditions are compared
	cold water (T < 3°C)	defines highest attainable removal under cold water conditions
	spring runoff	gauges effect of sudden water quality change
	seeding at rapid mix	establishes if pre-coagulation in a jar followed by subsequent seeding to filter influent is representative of pilot-plant coagulation and subsequent filtration
No coagulation	no coagulants since backwash	determines extent of coagulant impact on filtration (i.e., worst case scenario)
	temporary coagulation failure	addresses effect of temporary coagulation failure (e.g., pump failure)
	no pre-coagulation of pathogen seed suspension	determines if coagulant in seed suspension (fed into filter influent) impacts pathogen removal
	pre-coagulation of seed suspension only (no plant coagulation)	determines if coagulant in seed suspension impacts pathogen removal without plant-level coagulation
Sub-optimal coagulation	coagulant underfeed	demonstrates the role of coagulant
	loss of coagulant aid	addresses the relative importance of coagulant aid in pathogen removal (i.e., *settling* aid)
Ripening	peak filter effluent turbidity during ripening	determines pathogen passage associated with the operational period that potentially represents one of the most significant types of deterioration in filtrate quality
Hydraulic step	sudden increase in flow	determines any deterioration in pathogen removal associated with rapid changes in filtration rate (e.g., filter out of service) that are known to cause deterioration of filtrate quality
End of run	first sign of change in filter effluent turbidity (< 0.1 NTU)	determines when pathogen passage increases relative to baseline at the end of a filter cycle and relative to indicators like turbidity
Breakthrough	early breakthrough (0.1 - 0.3 NTU) late breakthrough (> 0.3 NTU)	determines degree of pathogen passage at the end of a filter cycle relative to small changes in indicators like turbidity

The filters at the Ottawa and Windsor pilot plants contained media depths and sizes typical of the utilities' full-scale plants (and typical of many existing treatment plants). The operational mode chosen was essentially conventional treatment with inclined plate sedimentation and dual-media filtration. The filter media specifics at the UW pilot plant were also typical of many existing treatment plants. These filters were operated in an in-line flocculation (contact filtration) mode and included both dual- and tri-media filtration. Further operating details regarding the research platforms are specified in Section 3.2.

It should be noted that this thesis research was funded in part by the American Water Works Association Research Foundation (AWWARF) – without this funding, such an extensive investigation as described in this thesis would not be possible. A substantial portion of the *C. parvum* and *B. subtilis* experiments at Ottawa concurrently contributed to the AWWARF report "Filter Operation Effects on Pathogen Passage," by Huck *et al.* (2001). The collaborative effort associated with that project contributed directly to the experimental design at Ottawa, as well as the seeding and sampling protocols described in Section 3.3. Collaboration with the Metropolitan Water District of Southern California provided a starting point for the *C. parvum* analytical method refinement described in Appendix A (Yates, 1997; Yates *et al.*, 1997; Huck *et al.*, 2001).

3.2 EXPERIMENTAL SET-UP

The majority of the pilot-scale experiments were performed at Ottawa where the raw water required a relatively high coagulant dose designed for combined TOC and particle removal. Virtually identical to the pilot plant in Ottawa in design and construction, the Windsor pilot plant also employed a relatively high coagulant dose; however, the raw water quality was substantially different. In comparison to Ottawa, the raw water at Windsor had a considerably lower TOC and higher alkalinity and turbidity (which could spike as high as 350 NTU during the spring). The raw water treated at UW was synthetic, comprised of dechlorinated tap water with kaolinite-induced turbidity. The coagulation regime at UW required a low coagulant dose designed for particle removal. The pilot-plant process configurations and nominal raw water qualities are listed in Table 3.3 and Table 3.4 respectively.

Table 3.3

Process configurations at the various research platforms

Location	Ottawa	Windsor	Waterloo	
Experimental Scale	pilot	pilot	pilot	bench
Design capacity (gpm)	16 (8/train)	16 (8/train)	0.5	n/a
Preoxidation				
Chlorine	yes	yes	n/a	n/a
Chemicals				
Alum	yes	yes	5 mg/L	5 mg/L
Sodium Silicate	yes	no	n/a	n/a
Perchol LT-24	no	yes	n/a	n/a
Rapid Mix				
G, sec^{-1}	in-line	variable	in-line	in-line
Hydraulic detention time (min)	0.02	1.8		
Flocculation				
G for stages 1, 2, and 3 (sec^{-1})	60, 40, 20	60, 40, 20	n/a	n/a
Hydraulic detention time (min)	30	30	n/a	n/a
Sedimentation				
Hydraulic detention time (min)	100	100	n/a	n/a
Filtration				
Loading (m/h)	6.6		10.4	7.5
(gpm/ft^2)	2.7		4.2	3.0
Surface area (ft^2)	0.20	0.20	0.022	0.022
Filter 1 -				
operating mode	constant rate	constant rate	declining rate*	declining rate*
media	anthracite/sand	anthracite/sand	anthracite/sand	anthracite/sand
depth (mm)	450/275	430/300	508/203	650/350
ES (mm)	anthracite 1.07		anthracite 0.98	anthracite 0.98
	sand 0.515		sand 0.5	sand 0.5
UC	anthracite 1.35		anthracite 1.5	anthracite 1.5
	sand 1.32		sand 1.5	sand 1.5
Filter 2 -				
operating mode	n/a	declining rate	declining rate*	declining rate*
media	n/a	anthracite/sand	anthracite/sand/garnet	anthracite/sand/garnet
depth (mm)	n/a	450/300	470/165/76	600/300/100
ES (mm)	n/a	anthracite	anthracite 0.98	anthracite 0.98
		sand	sand 0.5	sand 0.5
			garnet 0.32-0.38	garnet 0.32-0.38
UC	n/a	anthracite	anthracite 1.5	anthracite 1.5
		sand	sand 1.5	sand 1.5
			garnet 1.4	garnet 1.4

* Filter operation was essentially constant rate during the relatively brief experimental periods.

Table 3.4

Nominal raw water quality at the various research platforms

Location	Ottawa	Windsor	Waterloo	
Experimental Scale	pilot	pilot	pilot	bench
Source water	Ottawa River	Detroit River	tap water	tap water
Qualitative description of source water	Few upstream inputs, some logging in past	Great Lakes supply	Dechlorinated tap water with kaolinite induced turbidity	Dechlorinated tap water with kaolinite induced turbidity
Temperature (°C)				
nominal value	11	12	-	-
range	1-27	1-29	17-21	15-17
TOC/DOC (mg/L)				
nominal value	6.0	1.8	<1.0	1.8
range	5-7	1.6-2.3		1.6-2.3
Turbidity (NTU)				
nominal value	3	14	1.5	3.5
range	1-30	<1-40 spikes to 350		
pH				
nominal value	7.2	8.2	-	-
range	6.7-7.6	8.1-8.3	7.3-7.5	7.3-7.5
Alkalinity (mg/L as $CaCO_3$)				
nominal value	22	90	-	-
range	15-40	85-120	300-330	300-330

3.2.1 Bench-Scale Filtration Apparatus

A schematic of the bench-scale filtration apparatus is presented in Figure 3.1. It included a glass filter column (50 mm in diameter) containing one meter of media. The filter was operated in a constant head, declining rate mode during bench-scale experiments. The dual-media filter consisted of 700 mm of anthracite over 300 mm of sand. The tri-media filter consisted of 650 mm of anthracite over 250 mm of sand over 100 mm of garnet. Three layers of gravel supported the media; each layer was 5 cm thick. Media specifics such as effective size (ES) and uniformity coefficient (UC) are available in Table 3.3. All of the media were all riffled to ensure uniformity between experiments.

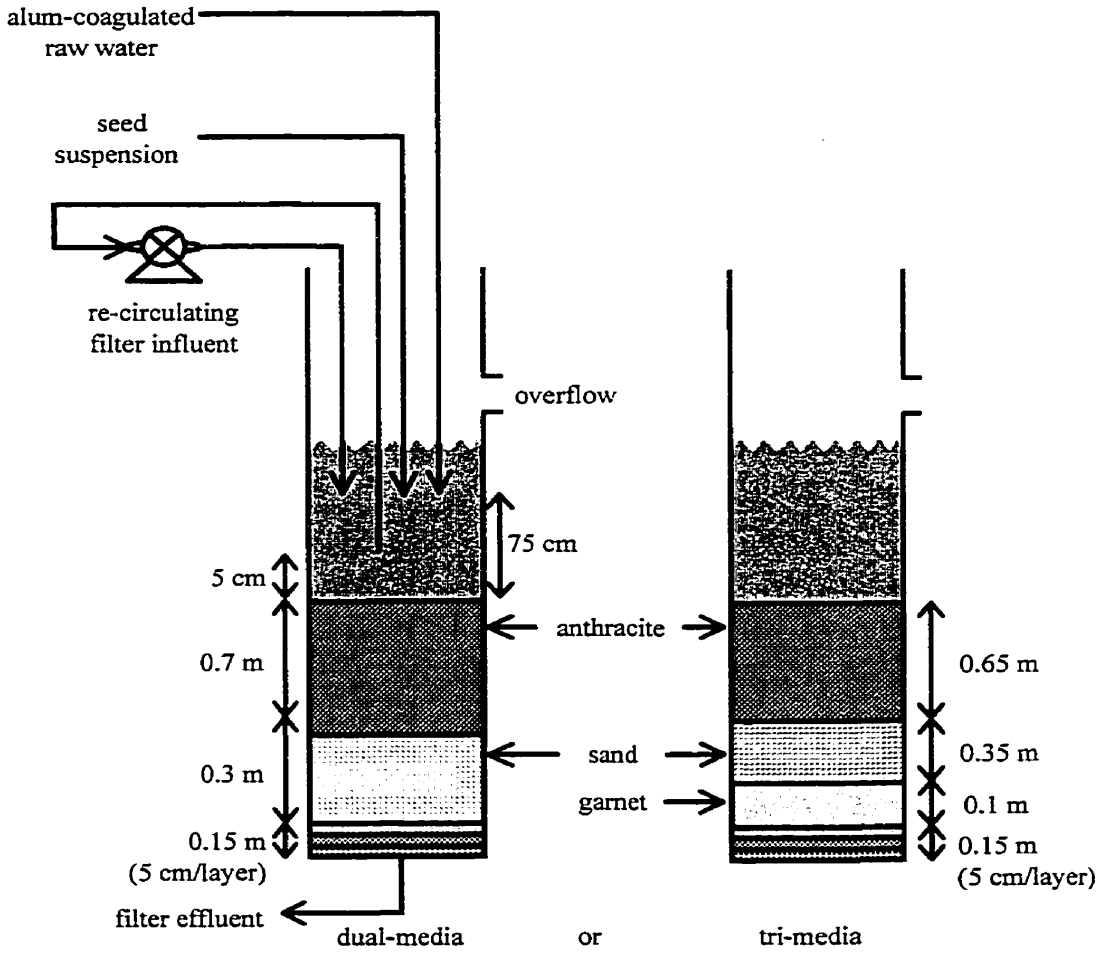


Figure 3.1 Bench-scale filtration apparatus.

Peristaltic pumps (Cole Parmer, distributed by Labcor Inc., Ste. Anjou, Québec) were used to introduce the coagulated raw water and microorganism seed suspension to the filter. To ensure reasonable mixing of the seed suspension with the influent water prior to filter influent sampling, the seed suspension introduction point was situated approximately 75 cm (2.5 ft) above the filter media. A peristaltic pump was also used to re-circulate water from approximately 5 cm (2 in) above the media; this acted as the filter influent sampling location. The filter effluent sampling location was located at the outlet of the column, directly after the support gravel. The process configuration and raw water quality details were listed in Table 3.3 and Table 3.4 respectively.

3.2.2 Ottawa and Windsor Pilot Plants

The Ottawa and Windsor pilot plants are virtually identical. The pilot plants are constructed only of stainless steel, glass and inert fluorocarbons. Raw water was typically pumped at 50-60 L/min to a constant head tank. From the constant head tank, the flow was split between two identical process trains. During this research, Side 1 was operated with pre-chlorination.

Metering pumps injected treatment chemicals into the feed line; the chemicals were mixed in-line. The water then entered a 3-cell under/over flocculation tank. Flocculated water passed into an inclined plate sedimentation tank. The pretreatment units are shown in Figure 3.2. Settled water was collected in a settled water storage tank, before being sampled and fed to a dual-media filter (anthracite/sand). The filter could operate in either the constant rate or declining rate mode. The filter columns are shown in Figure 3.3. Filtrate was collected in a backwash water storage tank with dedicated storage cells. Water for backwashing was then pumped from the dedicated cell back to the filter on the side of the plant from which it was collected. An air compressor and injection port allowed for air-scour during backwashing. On-line data (turbidity, particle counts, flow rates, and headloss) were recorded at 1-minute (Ottawa) or 10-minute (Windsor) intervals by a SCADA data-logging program. The process configuration and raw water quality details were listed in Table 3.3 and Table 3.4 respectively.

Figure 3.2 Pretreatment at the Ottawa Pilot Plant.

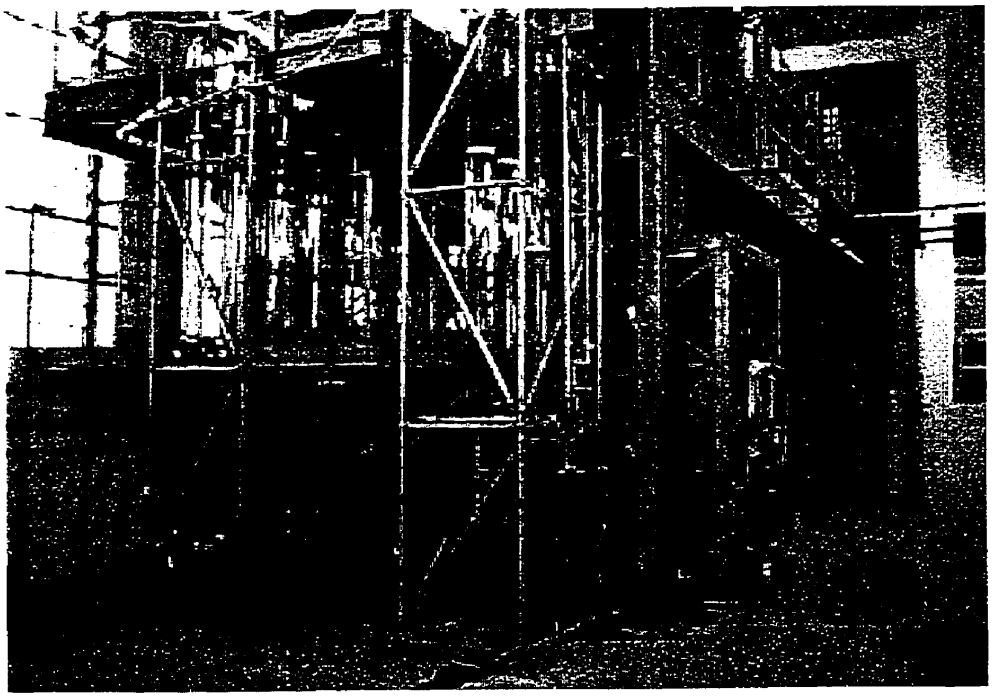


Figure 3.3 Filter columns at the Ottawa Pilot Plant.

3.2.3 University of Waterloo Pilot Plant

The University of Waterloo pilot plant is constructed only of stainless steel, lucite, glass, and inert fluorocarbons. During this research, the University of Waterloo pilot plant was operated in direct filtration mode with in-line flocculation (contact filtration). It treated synthetic raw water comprised of dechlorinated tap water with kaolinite-induced turbidity. The raw water was coagulated in-line with alum and then filtered by both dual- and tri-media filters. The filters were backwashed by simultaneous air scour with subfluidization water wash (collapse pulsing) with dechlorinated tap water. The pilot plant is equipped with continuous on-line turbidity and particle count measurements that are recorded at 1-minute intervals. The process configuration and raw water quality details were listed in Table 3.3 and Table 3.4 respectively.

3.3 SEEDING AND SAMPLING

All of the bench- and pilot-scale seeding experiments employed continuous seeding of microorganisms during specific points of the filter cycle. Almost all of the seeding experiments conducted during this study involved microorganism seeding at the filter influent; a very limited number of experiments examined microorganism seeding at the rapid mix.

Prior to seeding, *C. parvum* and *B. subtilis* concentrations in the stock suspensions were determined by triplicate counts on a hemocytometer (Petroff-Hausser Bacterial Counting Chamber, Hausser Scientific Corporation, Horsham, PA); this method is described in Sections 3.4.1 and 3.4.2. Polystyrene microspheres were determined by the method provided by the manufacturer (Polysciences Inc., Warrington, PA); this method is discussed in Section 3.5. At Ottawa, the feedstock microorganism/microsphere suspension was prepared by adding the microorganism stock suspensions (typically ~8 mL volume) and microsphere aliquots (typically ~150 µL volume) to a 1.5 L seed suspension of chlorinated and quenched raw water. The suspension was added to raw water at Windsor and UW. Each vial containing the concentrated microorganism stock was rinsed into the feedstock ten times with chlorinated/quenched raw water (Ottawa) or

raw water (Windsor and UW). Additional samples were collected from the feedstock to confirm microorganism concentration. At Ottawa and Windsor, the targeted filter influent concentrations of the seeded microorganisms were typically 10^5-10^6 oocysts per liter (*C. parvum*) and 10^4-10^6 CFUs per liter (*B. subtilis*). At UW, the targeted filter influent concentrations of the seeded microorganisms were typically 10^5 oocysts per liter (*C. parvum*) and 10^5-10^6 CFUs per liter (*B. subtilis*). When microspheres were seeded at Ottawa and UW, they were seeded at concentrations comparable to those of the *C. parvum* oocysts.

Microorganism pre-treatment was achieved via a jar-coagulation method described below (Section 3.3.1). In most cases, the jar coagulation conditions were identical to those in the pilot plant during the specific experiments. Some experiments were performed to specifically examine the effects of the coagulant added to the seed suspension (*e.g.*, potential filter aid effects). During these experiments, the jar coagulation conditions were different than those in the pilot plant. The feedstock microorganism suspensions were added at the filter influent (except in a few experiments where they were added at the rapid mix) immediately after jar coagulation.

3.3.1 Pilot Plant Coagulation and Jar Coagulation Protocol

During pilot-scale testing of stable operation, liquid aluminum sulfate (alum, $Al_2(SO_4)_3 \cdot 18H_2O$) was used either alone (UW) or in combination with cationic polymer (Windsor) or activated silica (Ottawa) to achieve the desired conditions for turbidity and particle removal. The cationic polymer or activated silica was dosed during rapid mix simultaneously with alum to improve charge neutralization. Chlorine was added at rapid mix at a dosage of ~2 mg/L (Ottawa). The chlorination dosage was to achieve the benefits of pre-oxidation, but was not necessarily sufficient to meet disinfection requirements such as those required by the Surface Water Treatment Rule (USEPA, 1989). The microorganism jar coagulation protocol mimicked the pilot-scale coagulation conditions; it is provided in Table 3.5. It included the use of sodium thiosulphate ($Na_2S_2O_3$) at a final 2:1 molar ratio of $Na_2S_2O_3$:Cl to quench the chlorine residual in the microorganism/microsphere stock suspension (to prevent any potential disinfection).

Table 3.5

Jar coagulation protocol

1. While stirring pilot plant raw water (~6.0 L) on a stir plate, add NaOCl to a final chlorine concentration of 2 mg/L (Ottawa).

↓

2. Stir chlorinated sample on stir plate for 15 minutes (Ottawa).

↓

3. Quench chlorine by adding sodium thiosulphate ($Na_2S_2O_3$) to a final 2:1 molar ratio of $Na_2S_2O_3$:Cl, adding 2× more $Na_2S_2O_3$ as a safety factor (Ottawa).

↓

4. Stir chlorinated/thiosulphate-quenched sample on stir plate for 5 minutes (Ottawa).

↓

5. Pour 1.0 L of chlorinated/quenched raw water (Ottawa) or raw water into a 2-L jar.

↓

6. Vigorously shake and add the microorganism stock (~8 mL) to the 1.0 L of chlorinated/quenched raw water (Ottawa) or raw water.

↓

7. Using an additional 0.5 L of chlorinated/quenched raw water (Ottawa) or raw water, rinse the microorganism spike container 10 times, adding all of the rinse water to the 2-L jar (for a final volume of ~1.5 L).

↓

8. Jar-coagulate the samples with the jar test apparatus. This step is site specific.

Ottawa:	Windsor:	UW:
- add alum	- add alum	- add alum
- 5 sec @ 100 rpm	- 2 min @ 100 rpm	- 60 sec @ 100 rpm
- add silicate	- add polymer	- 15 min @ 50 rpm
- 15 min @ 30 rpm	- 10 sec @ 100 rpm	- 15 min @ 15 rpm
- 15 min @ 15 rpm	- 10 min @ 20 rpm	- 60 min settling
- 60 min settling	- 10 min settling	

↓

9. After settling, gently mix jar coagulated suspension to break up any flocs.

3.3.2 Calculation of Microorganism and Microsphere Concentration and Removal

Filter influent and effluent microorganism concentrations were determined by the same analytical method for a given microorganism. In the case of *C. parvum*, if a reasonably countable number of oocysts was present (< ~3000 per slide), the *C. parvum* concentration was simply calculated as the number counted per volume processed. During several of the initial experiments at Ottawa, filter influent *C. parvum* concentrations were estimated based on the examination of fifty random fields of view because the number of oocysts on the slides was typically high (~10^4 to 10^5 oocysts/slide). In these cases, the *C. parvum* concentration was calculated by the following general equation:

$$\frac{\text{microorganisms}}{\text{volume processed}} = \frac{\text{microorganisms}}{\text{field of view}} \times \frac{\text{fields of view}}{\text{filter membrane}} \times \frac{\text{filter membrane}}{\text{volume processed}} \quad (3.1)$$

in which the number microorganisms per field of view is the average of fifty random fields of view. The number of fields of view per membrane is a function of the microscope, optics, and magnification utilized during the specific experiment. The last component of this equation is the sample volume passed through the filter membrane. This method of enumerating filter influent *C. parvum* concentrations was only used for a few experiments because it only provided estimates of filter influent oocyst concentrations and required the assumption that the oocysts were uniformly distributed on the filter membrane. For most of the experiments, smaller sample volumes of filter influent were processed, allowing for enumeration of oocysts on the entire filter membrane. The approaches used to enumerate the microspheres were the same as those used for *C. parvum*.

Microorganism and microsphere removals ($\log_{10}$) were calculated by subtracting the log of the filter effluent concentration from the log of the influent concentration. In the subsequent discussion, microorganisms are used as an example. When no

microorganisms were detected, the concentration was reported as 0; however, removal was calculated by using a concentration of 1 microorganism per sample volume processed. For example, a value of 1 oocyst per L would be used in the log removal calculation if no oocysts were found in a 1-L sample. A value of 2 oocysts per L would be used in the calculation if no oocysts were found in a 500-mL sample (1 oocyst per 0.5 L equals 2 oocysts per L).

3.3.3 Bench- and Pilot-Scale Seeding Protocol

A peristaltic pump was used to add the feedstock to the pilot plant filter influent water (and in some cases the raw water). The seed suspensions were introduced into the filter influent water approximately 2.5 feet above the filter media so that some mixing with the filter influent water would occur prior to filtration. Microorganisms were typically seeded into the filter influent for one hour at a rate of 25 mL/min. A limited number of experiments required microorganism seeding for longer periods of time (5 hours). This was achieved by reducing the seed flow to 5 mL/min. The microorganism seed suspension was continuously fed to the bench- or pilot-scale plant filter influent until the last set of filter influent and effluent samples was collected. In some experiments (*e.g.*, those examining detachment-related phenomena), additional samples were collected after microorganism seeding had ceased. A second peristaltic pump was continuously re-circulated filter influent water from a few inches above the surface of the filter media. To prevent carryover of microorganisms between experiments, both this line and the microorganism feed line were flushed for at least ten minutes after the completion of an experiment. These lines were also flushed for several minutes while the flow rates were confirmed prior to the start of each experiment.

3.3.4 Bench- and Pilot-Scale Sampling Protocol

Microorganism samples were collected from the filter influent and effluent. The filter influent location was approximately 5 cm (2 in) above the surface of the filter media (Figure 3.4); the effluent was collected at the column exit immediately after passage through the support gravel (upstream of the turbidimeter and particle counter). Prior to

the seeding experiments, 1-L negative controls were collected from the filter influent and effluent. Additional QA/QC samples were collected from the microorganism feedstock suspension. The samples were collected in 250-mL and 1-L glass Wheaton bottles respectively. Aliquots from the microorganism feedstock were collected in 5-mL glass chromatography vials. All sampling containers were washed, autoclaved, and rinsed with a few milliliters of a buffered detergent solution (1× phosphate buffered saline [PBS] with final concentrations of: 0.1% sodium dodecyl sulphate, 0.1% Tween 80, and 0.01% Sigma Antifoam A and final pH of 7.4) prior to use. The excess surfactant solution was discarded and sodium thiosulphate ($Na_2S_2O_3$) was added to each sample bottle for a final concentration of 0.01%.

The bench-scale experiments were performed during the first four hours of filter operation. Either viable or formalin-inactivated *C. parvum* oocysts were seeded for one hour, with samples collected 15, 30, 45, and 55 minutes after the start of seeding. Additional seeding and sampling details for the bench-scale experiments are provided in Chapter 5.

Most of the pilot-scale experiments were performed during the early to mid portion of the filter cycle after at least four hours of filter operation; ripening and breakthrough conditions were an exception to this criterion. With the exception of ripening, all experiments were conducted after a period of stable operation during which filter effluent turbidities were continuously below 0.1 NTU. Microorganisms (and sometimes microspheres) were typically seeded for one hour, with samples collected 15, 30, 45, and 55 minutes after the start of seeding. Seeding and sampling information for the pilot-scale experiments at Ottawa, Windsor, and UW is summarized in Table 3.6. Additional seeding and sampling specifics for the pilot-scale experiments are presented in Chapter 6.

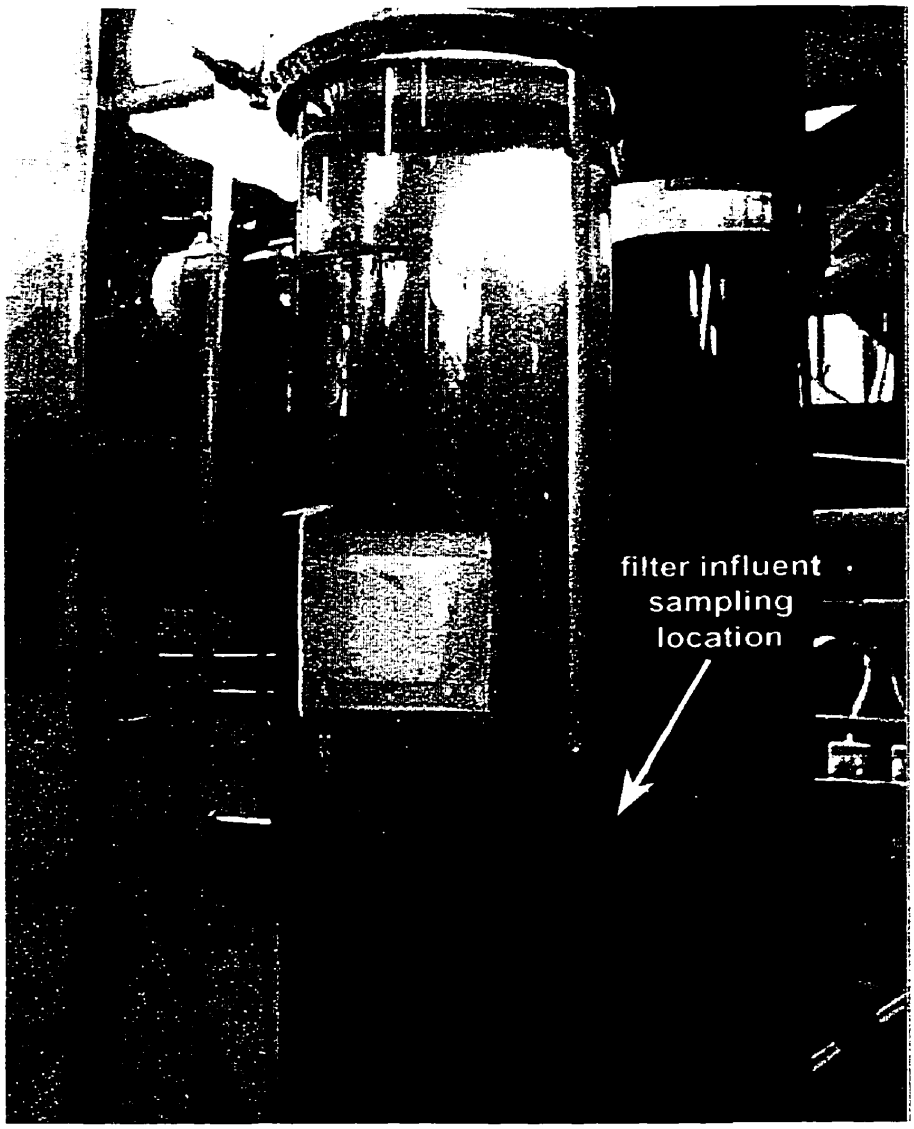


Figure 3.4 Filter influent sampling location at Ottawa.

Table 3.6

Pilot-scale seeding and sampling specifics

General Operating Condition	Seeding	Sampling
Stable operation	1-hour seeding period (0-60 minutes) seeding during first half of filter cycle after at least 4 hours of operation filter effluent turbidity < 0.1 NTU	samples collected at 15, 30, 45, and 55 minutes (Ottawa and Windsor) samples collected at 20, 40, and 55 minutes (UW)
Sub-optimal coagulation	1-hour seeding period (0-60 minutes) seeding during first half of filter cycle after at least 4 hours of operation	samples collected at 15, 30, 45, and 55 minutes (Ottawa)
No coagulation	1-hour seeding period (0-60 minutes) seeding during first half of filter cycle after at least 4 hours of operation	samples collected at 15, 30, 45, and 55 minutes
Ripening	30-minute seeding period capturing both before and after peak filter effluent turbidity during ripening	samples collected at 5, 10, 15, 20, and 25 minutes
Hydraulic step	seeding during first half of filter cycle after at least 4 hours of operation	
Ottawa	5-hour (300 minute) seeding period hydraulic step initiated at 300 minutes	samples collected at 280, 295, 300, 305, 310, 320, and 360 minutes
UW	1-hour (0-60 minutes) *C. parvum* seeding 1-hour (60-120 minutes) *B. subtilis* seeding hydraulic step initiated at 65 minutes	*C. parvum* collected at 45, 60, 70, and 80 minutes *B. subtilis* collected at 60, 70, and 80 minutes
End of run	1-hour seeding period (0-60 minutes)	samples collected at 15, 30, 45, and 55 minutes
Breakthrough	1-hour seeding period (0-60 minutes)	samples collected at 15, 30, 45, and 55 minutes
December 1999 experiments	1-hour (-60-0 minutes) *B. subtilis* seeding 1-hour (0-60 minutes) *C. parvum* seeding	samples collected at 15, 30, 45, and 55 minutes

3.3.5 Microorganism Losses to Seeding Apparatus

Control experiments were conducted to determine microorganism losses to the seeding apparatus and pilot plant equipment. Performed at Ottawa and UW, these experiments consisted of removing the media and seeding the columns with microorganisms (*C. parvum* and *B. subtilis*) to determine system losses. All of the media were removed at UW; at Ottawa 2 to 3 cm of gravel could not be removed. No coagulants were used in the pilot plants or the jar coagulation step during these experiments. Given that the Windsor and Ottawa pilot plants are identical in construction, it was assumed that the Ottawa control results were applicable to Windsor. Similarly, the UW pilot- and bench-scale columns were also almost identical and the pilot-scale control data were considered applicable to the bench-scale system.

The control experiments at UW demonstrated 0.03 ± 0.06 and 0.04 ± 0.05 log loss (mean ± standard deviation) of *C. parvum* and *B. subtilis* respectively. Despite the few remaining centimeters of gravel at Ottawa, the *C. parvum* and *B. subtilis* losses were only 0.09 ±0.12 and 0.15 ± 0.06 log respectively. Overall, these results suggested that the oocyst and spore removals observed during the subsequent pilot scale experiments were due to filtration, with only minimal system losses. Combined with the analytical method recovery data discussed below, these data demonstrated considerable reliability of the microorganism removal data presented in this thesis.

The probability density functions for *C. parvum* losses at Ottawa are presented in Figure 3.5. The $\log_{10}$ oocyst loss was based on four replicate samples; however, since one of the samples resulted in negative removals, it was treated as 0-log removal. The 95% confidence intervals (with highest posterior density - HPD) for each of the data points depicted in Figure 3.5 were calculated. The range between the lowest and highest of these values represents the range of removals, or in this case losses, that could be expected during this operating period given the uncertainty resulting from the analytical method. Based on this analysis, it can be concluded that system losses were minimal, approximately 0- to 0.25-log.

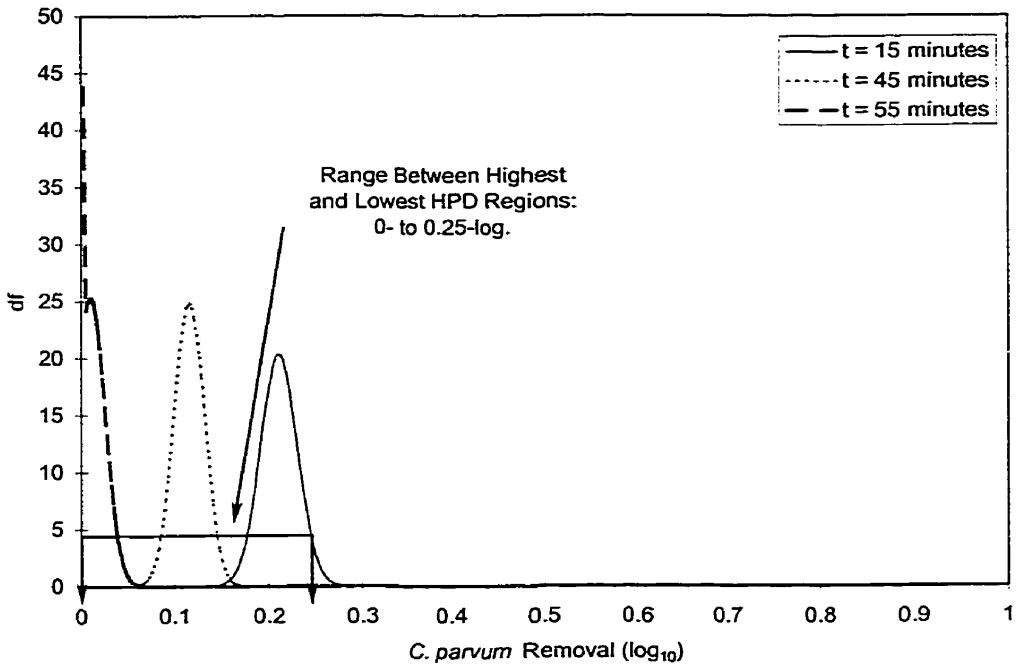


Figure 3.5 *C. parvum* losses to seeding apparatus and equipment during no coagulant and no media control experiments.

3.4 MICROBIOLOGICAL PARAMETERS

3.4.1 *C. parvum*

C. parvum inactivation and preservation

Samples of *C. parvum* were preserved in a penicillin/streptomycin solution because recent research has indicated that oocysts stored in this preservative may more closely represent oocyst behavior in the natural environment (Li *et al.* 1997). Obtained from a commercial laboratory (Waterborne, Inc., New Orleans, LA. or University of Arizona, Department of Veterinary Science, Tucson, AZ.), the oocysts were bovine in origin and were provided in a clean, purified form. For each experiment, ~10^8 (Ottawa and Windsor) or 10^7 (UW) oocysts were obtained; they were inactivated with 5% formalin (final concentration) for *C. parvum* in approximately 8 mL of 1× PBS with 0.01% Tween 20 to prevent oocyst clumping. All microorganism stocks were refrigerated at 4°C in the dark until use.

Enumeration of spike suspension

Prior to *C. parvum* seeding, the stock suspension was briefly vortexed and a small portion of the suspension (< 100 µL in total) was removed to enumerate the oocyst concentration. The stock concentration was determined by averaging triplicate counts with a hemocytometer (Petroff-Hausser Bacterial Counting Chamber, Hausser Scientific Corporation, Horsham, PA). The entire grid (1 mm^2) was used for oocyst enumeration at 400× magnification (Nikon Labophot 2A, Nikon Canada Inc., Toronto, ON or Zeiss Axioskop 2, Empix Imaging, Mississauga, ON). The oocyst concentration in the spike suspensions was determined by the equation below.

$$\text{number of oocysts/L} = \frac{\text{number oocysts counted}}{1\ \text{mm}^2\ \text{grid}} \times \frac{1}{50\ \text{mm depth}} \times \frac{1\ \text{mm}^3}{1\ \text{mL}} \qquad (3.2)$$

Analytical Protocol

Filter influent and effluent concentrations of *C. parvum* were measured. Filter influents were analyzed in 100, 10, 5, and 2.5 mL volumes. Filter effluents were analyzed in volumes ranging from 5 mL to 1 L, depending on the operating condition studied. Sample volumes were chosen to yield between 10 and 2000 oocysts per membrane. All pipettes and glassware were pre-rinsed with the buffered detergent solution (described in Section 3.3.4) to prevent oocyst losses. Samples were filtered through 25 mm, 0.40 µm polycarbonate membranes (Corning, Acton, MA). The filter membranes were placed on top of 25 mm diameter, 8.0µm nitrocellulose support membranes (Millipore Canada Ltd., Nepean, ON) placed on a manifold (Hoefer Scientific, San Francisco, CA) and maintained at a vacuum of 5 in Hg. Weights held the membranes in place. Two milliliters of 1% bovine serum albumen (BSA) were passed through the filter membranes, then the samples were filtered. The glassware that had contained the samples was then rinsed with the buffered detergent solution. This was followed by filtration of 2 mL of BSA and then the immunofluorescence assay (IFA) described below. The membranes were kept wet with 1× PBS and covered until mounting on slides. The concentration and enumeration protocol is presented in Appendix A. A schematic of the direct vacuum filtration apparatus is provided in Figure 3.6.

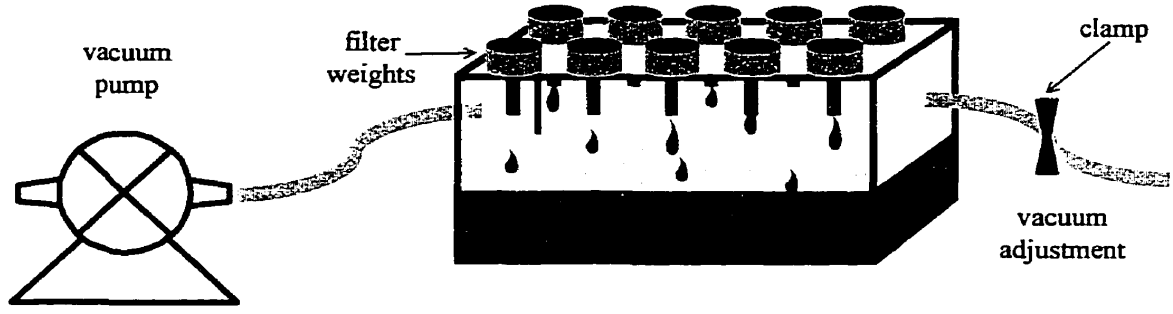


Figure 3.6 Direct vacuum filtration apparatus for processing *C. parvum*.

All *C. parvum* identification was performed using the Hydrofluor™ Combo *Cryptosporidium* and *Giardia* Kit (Strategic Diagnostics, Newark, DE). Presumptive microscopic analysis for *C. parvum* enumeration was performed at 400× magnification at the University of Waterloo (Nikon Labophot 2A, Nikon Canada Inc., Toronto, ON or Zeiss Axioskop 2, Empix Imaging, Mississauga, ON); the fluorescein isothiocyanate (FITC)-stained oocysts did not fluoresce with sufficient intensity for enumeration at 100× magnification (Nikon Labophot 2A, Nikon Canada Inc., Toronto, ON or Zeiss Axioskop 2, Empix Imaging, Mississauga, ON). A limited number of slides were shipped to a commercial laboratory (CH Diagnostic and Consulting Services, Inc., Loveland, CO), for presumptive microscopic analysis as a component of the quality assurance and quality control (QA/QC) program.

Recovery

The *C. parvum* data reported throughout this thesis are presented in both raw and adjusted (for method recovery) forms. The statistical method for adjusting concentration and log removal data for analytical recovery and calculating the endpoints of the associated confidence intervals is developed in Chapter 4. Recovery experiments were performed to determine the parameters (a and b from the Beta distribution) necessary for describing the methodological recovery profile and integrating this information into a confidence interval that describes the reliability of the *C. parvum* removal and concentration data.

C. parvum recovery experiments were performed on water from the Ottawa and UW pilot plants. Recoveries were based on at least five replicate samples of oocysts spiked into filter influent and effluent from each pilot plant; spike concentrations were determined with a hemocytometer (as described above). The processed sample volumes were those typically used during the experiments (1-10 mL of influent and 0.5-1 L of effluent). *C. parvum* recoveries for Ottawa and UW waters respectively ranged from 56 to 86%, and 51 to 93% with overall mean recoveries of 74 and 73%. The detailed *C. parvum* recovery data are presented and discussed in Appendix A.

The high oocyst recoveries were likely attributable to the direct filtration method that did not require elution steps. The use of this method was possible due to the high seeded oocyst concentrations and associated small sample volumes. There were no substantial deviations in recovery between filter influent and effluent samples, so the resulting log removals were essentially unaffected by methodological recovery.

QA/QC

Sample handling, identification, preservation, transportation and storage were completed according to established procedures as described in Section 3.4.1 and following the ICR Methods for Protozoa Analysis (USEPA, 1996). Samples were transported on ice and shipped via overnight courier (if necessary) for processing. The samples were processed immediately or refrigerated at 4°C as specified in the ICR methodology.

The quality assurance/quality control (QA/QC) program ensured that accurate protozoan data were produced. The program included recovery studies and the analysis of negative controls from the waters studied, method blanks, and positive controls. Analyses of method blanks and positive controls were performed each time samples were processed for *C. parvum*. Filter influent and effluent negative controls were collected during each experiment. No *C. parvum* oocysts were found in any of the method blanks or filter influent and effluent negative controls. The control data suggested appropriate sample handling during the *C. parvum* analyses. The lack of oocysts in the filter influent and effluent negative control samples suggested no substantial outside (non-seeded) sources of oocysts. Detailed *C. parvum* QA/QC data are available in Appendix B.

To ensure comparable slide reading of processed *C. parvum* samples, several samples were read at both the University of Waterloo and a commercial laboratory (CH Diagnostic and Consulting Services Inc., Loveland, CO). Limited comparisons included sending slides twice to each laboratory. Table 3.7 summarizes the results of the blind inter-laboratory slide reading comparison; these data suggest general agreement between both laboratories over the entire range of counts observed during this study. Although some differences were observed between samples counted at the two laboratories, the replicated counts at a given laboratory were generally internally consistent. As is demonstrated in Table 3.7, the relative impact of the inter-laboratory differences in counts typically had a negligible impact on $\log_{10}$ counts which were used to calculate the $\log_{10}$ removals that formed the basis of the conclusions in this thesis.

Table 3.7

C. parvum QA/QC data comparing slides read at the University of Waterloo and CH Diagnostic and Consulting Services Inc. (Loveland, CO).

Sample	UW Count		CH Diagnostics Count		Difference to UW	Relative Log Difference
	1st count (#/slide)	2nd count (#/slide)	1st count (#/slide)	2nd count (#/slide)	(±%)	(log UW - log CH)*
Filter influent	6542	—	6780	—	-4	-0.02
	6219	—	6445	—	-4	-0.02
	6859	—	6208	—	9	0.04
	6632	—	6003	—	9	0.04
	21	—	13	—	38	0.21
	9	13	2	12	36	0.20
	297	266	185	220	28	0.14
	175	—	150	—	14	0.07
	79	—	72	—	9	0.04
	71	—	101	—	-42	-0.15
	9	—	7	—	22	0.11
	1176	—	883	—	25	0.12
Filter effluent	761	—	790	—	-4	-0.02
	14	—	9	—	36	0.19
	24	23	14	23	21	0.10
	20	—	23	—	-15	-0.06
	19	19	3	0	92	1.10
	43	45	53	45	-11	-0.05
	0	—	0	—	0	0
	0	—	0	—	0	0

*Average log difference is calculated for samples read twice by each laboratory.

3.4.2 *B. subtilis*

B. subtilis Handling

Samples of *B. subtilis* spores were stored in superclean (Milli Q ™) water. The spores were derived from vegetative cells of *B. subtilis* grown in a nutrient broth consisting of 25% soil extract (77 g african violet soil and 0.2 g Na_2CO_3 to200 mL water). The *B. subtilis* stocks were refrigerated at 4°C in the dark until use.

Enumeration of Spike Suspension

Prior to pilot-scale *B. subtilis* seeding, the stock suspension was briefly vortexed and a small portion of the suspension (< 100 µL in total) was removed to enumerate the oocyst concentration. The stock concentration was determined by averaging triplicate counts with a hemocytometer (Petroff-Hausser Bacterial Counting Chamber, Hausser Scientific Corporation, Horsham, PA). The entire grid was (1 mm^2) used for spore enumeration at 400× magnification (Nikon Labophot 2A, Nikon Canada Inc., Toronto, ON or Zeiss Axioskop 2, Empix Imaging, Mississauga, ON). The hemocytometer formula used to calculate the *C. parvum* concentrations (Equation 3.1) was also used to calculate the *B. subtilis* concentrations.

Analytical Protocol

The analysis for *B. subtilis* (ATCC 6051) was performed according to a method described by Rice *et al.* (1996). This method consists of filtration of samples onto 47 mm diameter, 0.45 µm gridded cellulose acetate membranes (Pall Gelman Corporation #66278, Ann Arbor, MI) and growth at 37°C for 24 hours on plates of nutrient agar with trypan blue (0.015g/L). Spores were identified by their blue color. Typically, duplicate sample volumes of 90 mL and 1.0 L were used to enumerate filter influent and effluents respectively. Serial dilutions were performed as necessary.

3.5 MICROSPHERES

Fluoresbrite™ carboxylated YG fluorescent-dyed polystyrene microspheres (Polysciences Inc., Warrington, PA.) were used as non-biological surrogate indicators for *C. parvum* removal. In a limited number of experiments evaluating detachment during breakthrough, Fluoresbrite™ caboxylated BB fluorescent-dyed polystyrene microspheres (Polysciences Inc., Warrington, PA.) were also used. The microspheres had an average diameter of 4.675 ± 0.208 μm and a density of 1.045 g/mL. The YG dye is a proprietary chemical that is hydrophobic (to prevent dye leaching from the particles into the aqueous phase) and matches the fluorescence filter settings of fluorescein (*i.e.*, maximum excitation at 458 nm and maximum emission at 540 nm; same as FITC for *C. parvum*). The BB dye is also a proprietary chemical that is hydrophobic and matches the fluorescence filter settings of coumarin (*i.e.*, maximum excitation at 365 nm and maximum emission at 468 nm). The Material Safety Data Sheets (MSDS) provided by the manufacturer indicated that neither of the microsphere products contained any hazardous components.

The manufacturer provided the polystyrene microspheres in suspensions of 2.5% aqueous solids in de-ionized water; neither biocides nor stabilizers were added to the suspensions. The microspheres were stored at 4°C until their use. The weight to volume packaging allowed for the calculation of the particle concentration per milliliter by the following equation (Polysciences Inc., 1995):

$$\text{Number of particles per mL} = \frac{6W \times 10^{12}}{p \times \pi \times \varnothing^3} \qquad (3.3)$$

W = grams of polymer per mL in latex (0.025 g / mL for 2.5% solids)
$\varnothing$ = diameter in microns of latex particles
p = density of polymer in grams per mL (1.045 g / mL for polystyrene).

According to Equation 3.3, the concentration of a stock suspension of 4.675 μm microspheres was 4.45×10^8 spheres/mL. This concentration was also confirmed with hemocytometer enumeration (Petroff-Hausser Bacterial Counting Chamber, Hausser Scientific Corporation, Horsham, PA) and Equation 3.2. Due to the high microsphere concentration, 1/10 of the grid (0.1 mm^2) was used in the enumeration process. The average microsphere concentration was 4.55×10^8 spheres/mL after fifteen replicate analyses with the hemocytometer; these data are presented in Table 3.8. A two-tailed *t*-test ($\alpha = 0.05$) failed to demonstrate a statistical difference between hemocytometer-enumerated and weight to volume calculated microsphere concentrations; therefore, the manufacturer's weight to volume method was used to determine the volume of stock microsphere suspensions that was added to the feedstock suspension during seeding experiments.

Table 3.8

Hemocytometer Enumeration of Microspheres

Trial	Number of Microspheres in 1/10 of grid (0.1 mm^2)	Microsphere Concentration (spheres/mL)
1	936	4.68×10^8
2	1056	5.28×10^8
3	1080	5.40×10^8
4	672	3.36×10^8
5	1080	5.40×10^8
6	744	3.72×10^8
7	672	3.36×10^8
8	960	4.80×10^8
9	648	3.24×10^8
10	1080	5.40×10^8
11	864	4.32×10^8
12	864	4.32×10^8
13	888	4.44×10^8
14	1248	6.24×10^8
15	864	4.32×10^8
	Average Concentration	4.55×10^8
	Standard Deviation	8.88×10^7

Microspheres were concentrated and enumerated by the same method used for *C. parvum* (described in Section 3.4.1), with the exception that antibody staining was not necessary for samples that did not contain oocysts. FITC-stained *C. parvum* oocysts and YG microspheres shown at 400× magnification in Figure 3.7, were clearly distinguishable. Although approximately the same size and shape, the microsphere appears larger than the oocyst in Figure 3.7, as a result of the halo-effect associated with the strong intensity of the YG dye. This intensity permitted microsphere enumeration at 100× magnification, as demonstrated in Figure 3.8, which depicts the BB microspheres. Microspheres were enumerated at 100× magnification in samples that were collected prior to *C. parvum* seeding, otherwise they were enumerated concurrently with *C. parvum* oocysts at 400× magnification (FITC-stained oocysts did not fluoresce with enough intensity for enumeration at 100× magnification).

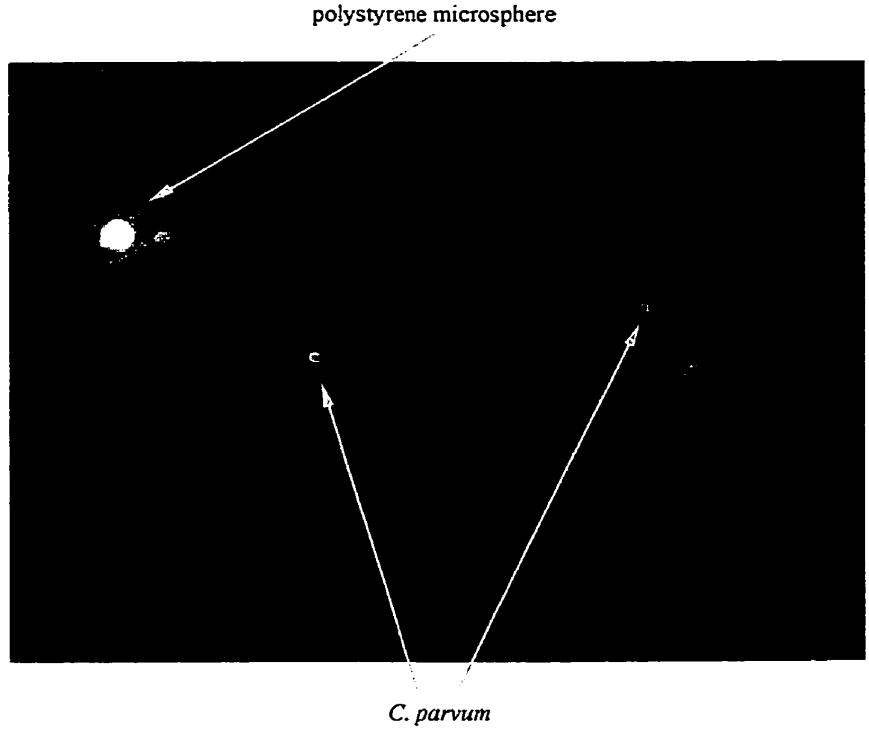


Figure 3.7 *C. parvum* oocysts and YG polystyrene microsphere (400× magnification, Nikon Labophot 2A, Nikon Canada Inc., Toronto, ON).

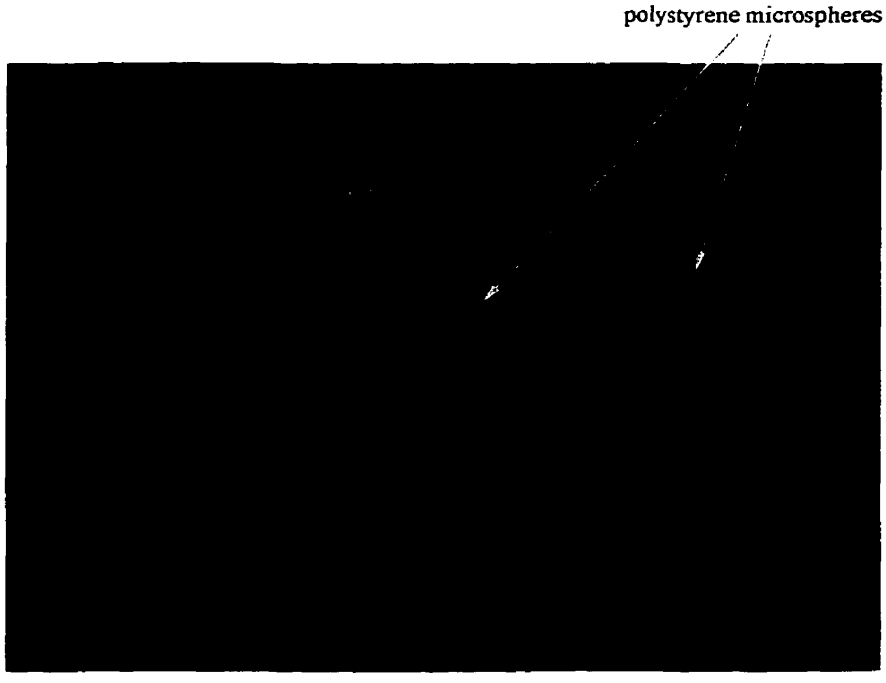


Figure 3.8 BB polystyrene microspheres (100× magnification, Nikon Labophot 2A, Nikon Canada Inc., Toronto, ON).

3.6 PHYSICAL AND CHEMICAL PARAMETERS

3.6.1 Headloss

Differential pressure transducers continuously measured headloss.

3.6.2 Particle Counts

A standard protocol was used at all sites to verify the calibration of the particle counters using commercially available, calibrated, mono-disperse polymer microspheres (Duke Scientific Corp.; Palo Alto, CA.). Each particle counting instrument was calibrated (by the manufacturer) according to ASTM F 658-87 and met the resolution requirements of USP 788. The particle counters measured total particles from 2-150 μm, with the data reported as total particles ≥2 μm. IBR particle counters (IBR, Grass Lake MI) were used at Ottawa and UW; Windsor used a Chemtrac model PC2400D particle counter (distributed by SUMMA Engineering, Mississauga, ON).

3.6.3 Turbidity

At pilot-scale, turbidity was monitored using on-line turbidimeters that were calibrated using dilute formazin solutions as specified by the manufacturer. Calibration was routinely checked by comparison with a bench-top turbidimeter (Ottawa, Windsor, and UW) with an accuracy of $\pm 2\%$, using standards of 0.80 and 6.6 NTU. Hach model 1720C turbidimeters (Hach Co., Loveland, CO.) were used at plant influent, filter influent, and filter effluent locations at Ottawa and Windsor; they were also used at the filter effluent at UW. An additional turbidity meter was used at the filter effluent sampling location in Ottawa (ABB Model 7997/201, Calgary, AB). Filter influent turbidity was measured by grab samples analyzed with a Hach Model 2100P hand held turbidimeter at UW (Hach Co., Loveland, CO.). This turbidimeter was routinely calibrated with formazin standards of 0.5, 1, and 5 NTU.

3.6.4 pH

Sample pH was measured with grab samples analyzed by a pH meter that was calibrated daily, using pH 7.00 and 9.18 buffer solutions.

Chapter 4
Quantifying the Reliability of Pathogen Data

4.1 *Cryptosporidium* Distribution in Natural Waters

In recent years, the protozoan pathogen *Cryptosporidium parvum* has been the causative agent of several outbreaks of waterborne illness, the largest of which resulted in over 400,000 cases of cryptosporidiosis in Milwaukee in 1993 (MacKenzie *et al.*, 1994). Currently there is no effective treatment for cryptosporidiosis (ASM News, 1996). Consequently, these organisms are of significant concern to public health authorities and the drinking water industry. The environmental resilience and ubiquitous nature of *Cryptosporidium* oocysts have resulted in intensive international research on its occurrence in different water sources and its removal and/or inactivation by various water treatment processes.

In the United States, the information collection rule (ICR) required large utilities to monitor their surface water for pathogens such as *Cryptosporidium* and *Giardia*, as well as viruses (USEPA, 1996). The information collected contributes to setting potential drinking water standards for these microorganisms. The surface water treatment rule (SWTR) addresses *Giardia* and viruses (USEPA, 1989). The interim enhanced surface water treatment rule (IESWTR) is a first step toward addressing *Cryptosporidium* (USEPA, 1998). Methods for quantifying the reliability of pathogen data are critical to the long-term success of such regulatory programs.

This chapter proposes guidelines for reporting pathogen, and in particular *Cryptosporidium*, data. Some researchers have suggested strategies for reporting *Cryptosporidium* concentration data; however, these techniques are not directly applicable to the reporting of oocyst removals by treatment processes (as will likely be useful for determining regulatory requirements). Statistical approaches for the calculation of confidence intervals, more appropriately called probability intervals, for both *Cryptosporidium* concentrations and removals by treatment processes are developed below. For the purposes of discussion, the term "confidence intervals" will be used. The strategies described are relevant for any microorganisms or discrete particles; however, they are discussed in the context of *Cryptosporidium* because the difficulty in sampling and measurement of this pathogen necessitate a rigorous approach to quantifying the reliability of such data.

4.2 NECESSITY OF RELIABLE STATISTICAL METHODS

Cryptosporidium oocysts can be frequently isolated from surface waters as well as occasionally from finished waters. It is difficult to precisely measure the occurrence and removal of oocysts from natural waters because they are present in varied and often low concentrations. Oocysts have been found in surface waters in concentrations as high as 10^4 /100 L and as low as 0.3/100 L (LeChevallier and Norton, 1995; Lisle and Rose, 1995; Smith *et al.*, 1991), when detected at all. Traditional water treatment processes can decrease oocyst concentrations by several orders of magnitude, necessitating relatively large sample volumes of treated water so that statistically meaningful data can be obtained.

A drinking water limit of approximately one *Cryptosporidium* oocyst per 34,000 L has been suggested (Lisle and Rose, 1995). Such a low limit, or even one several orders of magnitude higher, would be difficult to implement because current methods for measuring *Cryptosporidium* concentrations are unreliable, laborious, and expensive. Recovery efficiencies for these analytical methods are often low and highly variable (Nieminski *et al.*, 1995). Increasing the volume of processed water to obtain higher counts of oocysts is often difficult, particularly in raw waters, due to the presence of other

particles. The presence of other microorganisms such as algae can further interfere with oocyst identification (Rodgers *et al.*, 1995). Although these methodological difficulties are relevant to sampling indigenous and seeded populations of oocysts, they are likely more pronounced when quantifying indigenous concentrations, since high concentrations of oocysts (typically 10^3-10^6 oocysts/L) are commonly used in experimental evaluations of treatment processes (Patania *et al.*, 1995; Nieminski and Ongerth, 1995).

Although it is also commonly accepted that mean concentrations or removals used to calculate confidence intervals do not account for all of the uncertainty in the *C. parvum* data (*e.g.*, they do not account for sampling strategy, method recovery, variability associated with the analytical method, *etc.*), few studies of *Cryptosporidium* concentration and removal efficiency have addressed the issue of data reliability beyond stating average analytical recovery. The lack of information regarding the reliability of *C. parvum* data is likely associated with the expense and difficulty of processing samples. The complexity of incorporating these additional sources of variability into a statement of data reliability such as a confidence interval is increased by the fact that the normal distribution is often inappropriate for describing distributions of microorganisms such as *Cryptosporidium* in water samples.

4.3 EXAMPLE DATA

To illustrate the statistical methods developed here, several of the recovery data described in Chapter 3 and part of a data set collected during the course of experimental evaluations of *Cryptosporidium* passage through drinking water filters were used. Table 4.1 includes three sets of recovery data originating from two different raw water sources. During the recovery studies, a hemocytometer was used to enumerate stock oocyst concentrations so that they could be diluted to concentrations similar to those that would be expected during subsequent investigations (*i.e.*, evaluations of oocyst removal by filters). The recovery study samples were processed in the same volumes as were processed during the challenge studies described in Chapters 5 and 6. The samples were processed according to the *C. parvum* protocol described in Chapter 3.

Table 4.1

Example *C. parvum* Recovery Data

Water Type	Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number (oocysts)	Observed Number (oocysts)	Recovery (%)
Ottawa Filter Influent	1.0E+06	0.1	0.5	500	351	70
				500	360	72
				500	427	85
				500	409	82
				500	355	71
Ottawa Filter Effluent	1000	0.5	100	500	357	71
				500	366	73
				500	431	86
				500	378	76
				500	345	69
Ottawa Filter Effluent	100	0.5	100	50	36	72
				50	42	84
				50	35	70
				50	38	76
				50	37	74

Illustrative experimental data are summarized in Table 4.2. These data are part of a data set collected after pre-coagulated, formalin-inactivated *Cryptosporidium* oocysts were seeded into an anthracite/sand filter. In this example, stable filter was evaluated at four time intervals. During each time interval, a filter influent and effluent sample were collected. The influent sample volume (V_i) was 2.5 mL and the effluent sample volume (V_e) was 1000 or 700 mL. The oocysts were concentrated and enumerated by the same direct vacuum filtration/immunofluorescence assay described previously.

Table 4.2

Example *C. parvum* Experimental Data

Water Type	Sample Volume (mL)	Sample Time (minutes)	Observed Number in Sample Volume (oocysts)	Observed Concentration (oocysts/L)	Operating Condition
Ottawa	2.5	15	1062	4.2E+5	Stable
Filter	2.5	30	1104	4.4E+5	
Influent	2.5	45	1249	5.0E+5	
	2.5	55	1215	4.9E+5	
Ottawa	1000	15	2	2	Stable
Filter	1000	30	2	2	
Effluent	1000	45	1	1	
	700	55	5	7	

4.4 ADEQUACY OF POISSON ESTIMATION OF CONFIDENCE INTERVALS

4.4.1 Calculating Poisson Confidence Intervals

Haas and Rose (1996) showed that the number of *Cryptosporidium* oocysts in a sample could be described adequately by the Poisson distribution (Equation 2.1), as would be expected for a sample from a uniform suspension of such microorganisms in water that was enumerated by an ideal method (Blom, 1989). Parkhurst and Stern (1998) suggested Poisson confidence intervals for describing oocyst data. Atherholt and Korn (1999) presented Poisson methods for sample counts in the context of the ICR protocol. This approach consists of determining the mean observed oocyst counts for a given sample and then simply determining the endpoints of the confidence interval from the following equations,

$$\Pr\left(X \le CI_U\right) = \alpha/2 = \Pr(X = 0) + \Pr(X = 1) + \cdots + \Pr\left(X = CI_U\right) \tag{4.1}$$

$$\Pr\left(X \ge CI_L\right) = \alpha/2 = 1 - \left[\Pr(X = 0) + \Pr(X = 1) + \cdots + \Pr\left(X = \left[CI_L - 1\right]\right)\right] \tag{4.2}$$

where CI_U is the upper endpoint of the confidence interval, CI_L is the lower endpoint of the confidence interval, and α is the desired significance level. The endpoints represent values of λ that just produce significance at the $\alpha/2\%$ level for the null hypotheses $\lambda=CI_U$ and $\lambda=CI_L$ testing against the respective alternative hypotheses $\lambda>CI_L$ and $\lambda<CI_U$; these values can also be readily looked up in statistical tables. In repeated sampling, $(100-\alpha)\%$ of the intervals calculated in this way include the true value of λ. Therefore, at the 5% significance level, 95% of the intervals calculated with Equations 4.1 and 4.2 include the true value of λ.

Some of the advantages of calculating Poisson confidence intervals on *Cryptosporidium* concentrations are that the necessary statistical tables are readily available and that observations of zero microorganisms (non-detects) can be handled. Naturally, non-detects are not desirable because they lead to large confidence intervals. Caution should be taken when analyzing data that includes non-detects because they are specific to the sample volume examined. A better approach may be to take advantage of the additive property of the Poisson distribution by combining all appropriate replicates and normalizing to the total sample volume examined. This can be done because when individual samples from a Poisson distribution are combined, the resulting distribution is also Poisson with a mean equal to the sum of the means of individual samples (Box *et al.*, 1978). This is essentially what Parkhurst and Stern (1998) recommended when they proposed summing oocyst counts for several samples and dividing this sum by the sum of the effective volumes (the definition of which is developed in their paper).

4.4.2 Limitations of Poisson Confidence Intervals

The limitation of approaches such as that of Parkhurst and Stern (1998) is that they do not incorporate uncertain analytical recovery into the confidence interval calculation. Nahrstedt and Gimbel (1996) used several reported analytical recovery data and demonstrated that a distribution of recoveries may be considerably more appropriate than a single estimate. The disadvantage of the Nahrstedt and Gimbel (1996) model is its increased level of complexity that resulted in a computer program rather than a simple statistical table. During this study, Monte Carlo simulations were performed with the

Nahrstedt and Gimbel (1996) model to generate data sets and examine them for agreement with the Poisson distribution. This was done to determine whether or not such additional complexity was necessary.

The Monte Carlo simulations generated a set of simulated counts of oocysts that one would expect to count on a microscope slide given method recovery information described by a and b, the Beta distribution parameters that describe the uncertainty associated with recovery. To complete these simulations, a common algorithm for random number generation was used. The algorithm is based on three congruential generators and is fully described in Press *et al.* (1989). Three levels of expected values or true oocyst counts (λ=1000, 100, and 10) were simulated. These true counts correspond to the number of oocyst one would expect to count on a microscope slide given an ideal method that always achieves 100% recovery. Only a fraction of these true counts would be enumerated when using an imperfect method, the profile of which is described by the Beta distribution. The first pair of Beta parameters (a = 6.739, b = 19.9) were those calculated for the LeChevallier *et al.* (1991c) data by Nahrstedt and Gimbel (1996). The second set of Beta parameters (a = 92.43, b = 33.79) corresponded to a recovery profile similar to that described for Vesey *et al.* (1993a) by Nahrstedt and Gimbel (1996). Triplicate data sets of 100 samples (n) were simulated for each set of parameters (λ, a, and b). Fisher's chi-squared (χ^2) test (Fisher, 1938) was used to assess whether or not the simulated data sets (i) were consistent with the Poisson distribution. This test was applied by calculating the index of dispersion described by,

$$\chi_i^2 = \frac{\Sigma(x-\bar{x})^2}{\bar{x}} \tag{4.3}$$

where x represented the observed counts and $\bar{x}$ represented the mean observed counts. The simulated data and calculated values of χ_i^2 are presented in Table 4.3.

Table 4.3
Simulated Data Using Nahrstedt and Gimbel (1996) Model

Beta Parameters				a = 6.739		b = 19.9			
λ	1000	1000	1000	100	100	100	10	10	10
	261	308	268	19	30	34	0	2	5
	276	276	229	32	29	29	4	3	10
	236	290	297	27	25	23	3	1	2
	309	288	270	31	21	24	3	3	3
	318	265	320	26	29	28	3	5	2
	267	297	289	36	32	31	3	3	2
	234	301	249	31	24	22	4	1	0
	297	295	244	22	21	31	1	4	2
	216	287	313	25	22	19	2	1	6
	249	327	267	33	29	36	3	2	3
	256	288	286	33	31	27	0	2	4
	285	327	264	25	33	30	4	5	3
	272	271	255	41	15	39	3	6	0
	318	248	313	30	41	32	6	1	10
	317	301	258	32	39	33	2	2	2
	282	248	267	37	34	21	4	4	3
	316	297	241	31	24	26	6	6	3
	311	302	262	21	26	22	2	1	1
	313	250	285	31	18	21	1	5	3
	331	309	313	37	18	33	7	3	5
	307	286	295	26	22	20	4	1	3
	204	259	238	30	23	28	2	4	3
	281	331	285	28	37	28	2	3	2
	297	293	302	28	27	23	4	3	4
	266	247	281	23	37	33	2	0	0
	287	245	265	33	27	27	2	2	5
	240	254	246	23	15	28	6	1	1
	263	254	319	24	21	30	1	4	2
	276	297	292	19	20	32	1	2	1
	328	235	277	32	38	31	5	2	0
	252	259	260	21	28	26	8	2	0
	261	262	313	33	39	36	3	2	6
	272	341	336	32	29	21	1	2	2
	263	269	287	25	28	17	2	3	7
	279	314	271	32	35	29	4	3	2
	253	288	243	29	38	37	2	3	1
	293	255	326	27	17	22	2	3	2
	286	288	264	24	21	36	3	6	4
	294	263	234	26	30	32	5	4	2
	250	306	280	30	16	35	7	1	7
	300	281	247	36	21	47	1	4	4
	188	321	304	23	21	23	2	2	2
	329	319	287	29	23	27	1	5	1
	250	309	267	24	36	41	2	5	3
	232	277	324	31	36	31	4	1	1
	301	275	313	32	33	35	0	5	4
	310	289	318	24	19	20	2	4	1
	264	232	334	27	22	28	4	3	1
	265	306	268	30	28	30	1	2	3
	323	285	278	32	32	31	7	4	1
	356	324	236	33	32	35	4	2	2
	362	225	331	26	29	30	1	2	2
	255	273	276	28	35	21	4	7	0
	292	273	270	34	25	32	0	3	1

Table 4.3

Simulated Data Using Nahrstedt and Gimbel (1996) Model (Continued)

Beta Parameters				a = 6.739			b = 19.9		
λ	1000	1000	1000	100	100	100	10	10	10
	278	236	320	27	29	14	5	3	4
	308	316	244	28	31	37	1	3	1
	342	318	338	25	29	26	3	1	4
	233	204	261	26	22	25	6	1	0
	241	301	256	28	34	28	2	2	2
	294	295	355	25	34	32	3	1	2
	295	275	332	27	27	34	6	4	0
	280	242	302	21	22	33	7	3	2
	277	276	304	36	34	42	2	3	2
	316	314	241	28	26	30	5	4	2
	299	328	341	34	25	27	0	4	4
	206	306	278	30	39	29	5	3	2
	282	283	263	42	25	37	6	2	5
	329	261	260	28	26	36	5	4	1
	270	251	242	32	21	36	8	4	1
	252	317	342	22	22	31	4	2	3
	293	223	272	26	36	16	1	0	3
	341	255	325	38	27	34	3	2	0
	258	324	338	26	24	27	5	7	1
	278	262	256	22	26	26	4	1	3
	225	330	249	31	25	27	4	7	2
	244	269	276	19	25	29	4	2	2
	257	298	283	28	22	28	2	1	2
	316	323	318	30	29	23	2	4	3
	294	273	255	27	28	26	0	2	0
	247	295	337	25	25	38	5	1	4
	311	241	335	29	32	26	2	6	5
	249	327	322	26	29	26	4	2	3
	244	323	301	36	23	35	5	3	3
	259	290	275	21	27	23	3	4	0
	227	262	235	22	36	17	6	2	1
	240	310	298	25	32	28	1	3	1
	271	372	237	36	30	30	4	4	3
	276	336	248	28	39	22	3	5	4
	240	252	277	27	24	25	2	5	3
	236	275	279	23	31	27	3	6	3
	257	232	295	20	30	27	3	4	1
	241	285	270	21	23	28	2	1	3
	310	273	320	37	21	25	2	2	2
	270	247	302	23	31	29	2	2	3
	284	310	308	22	23	29	2	5	9
	256	238	251	34	25	30	2	0	4
	304	312	270	37	15	26	6	1	1
	243	245	273	23	20	18	2	4	3
	261	285	293	24	20	32	2	5	1
	223	221	254	18	28	37	1	1	2
mean, n = 100	276.00	283.21	283.88	28.17	27.33	28.74	3.15	2.96	2.59
χ2, df = 99	428.138	357.963	346.395	96.277	140.143	123.982	117.063	92.514	153.741
Pr{χ^2 <= S}	**100.000**	**100.000**	**100.000**	**44.123**	**99.587**	**95.461**	**89.612**	**33.586**	**99.965**
Reject Poisson at α = 0.05	**X**	**X**	**X**		**X**				**X**
mean, n = 30	280.43	282.53	276.33	28.87	26.93	28.03	3.00	2.73	3.00
χ2, df = 29	126.059	79.364	61.018	26.651	38.561	20.860	31.333	37.933	29.333
Pr{χ^2 <= S}	**100.000**	**100.000**	**99.954**	**40.944**	**88.963**	**13.581**	**65.012**	**87.617**	**55.219**
Reject Poisson at α = 0.05	**X**	**X**	**X**						

Table 4.3

Simulated Data Using Nahrstedt and Gimbel (1996) Model (Continued)

Beta Parameters				a = 92.43		b = 33.79			
λ	1000	1000	1000	100	100	100	10	10	10
	702	711	790	74	73	49	9	4	9
	809	735	726	66	77	70	10	16	8
	737	764	728	71	72	88	8	8	6
	791	755	818	73	65	62	11	5	9
	779	793	782	88	69	99	7	8	4
	727	668	755	82	78	82	11	8	6
	712	781	778	72	91	76	6	4	2
	803	798	793	77	78	76	7	5	6
	727	817	757	89	68	78	8	7	8
	787	797	800	78	70	80	6	13	7
	746	732	765	73	69	79	10	4	4
	768	763	728	85	68	67	11	10	10
	711	708	751	100	93	78	8	8	3
	709	761	733	73	87	85	6	10	8
	737	796	784	65	91	50	7	4	9
	798	800	832	71	89	71	6	9	6
	795	753	777	80	67	84	10	11	9
	745	784	770	73	72	89	6	7	7
	724	738	700	68	85	67	14	10	10
	742	784	780	69	78	74	12	10	12
	778	766	727	80	75	79	13	11	7
	738	739	728	78	86	85	2	9	9
	711	727	721	82	82	63	8	11	8
	726	791	709	87	78	78	9	5	7
	770	786	748	70	69	69	6	8	7
	742	758	700	77	58	79	7	8	9
	771	818	750	64	70	73	7	9	9
	788	747	777	67	80	75	8	6	6
	731	808	692	78	91	87	7	9	5
	777	793	846	68	87	83	9	7	8
	814	753	723	57	86	83	7	4	7
	740	757	724	57	65	72	4	6	9
	779	765	762	82	89	83	2	5	8
	794	761	742	66	73	75	7	9	5
	702	742	726	78	80	70	4	10	8
	748	719	712	80	76	81	9	6	3
	765	712	828	80	59	74	8	12	8
	710	778	765	82	76	77	7	10	9
	762	811	726	65	71	82	7	4	12
	784	728	758	66	91	75	8	7	6
	761	820	795	63	83	68	9	5	11
	765	762	794	90	66	85	11	5	4
	745	779	778	69	66	59	11	10	6
	779	718	786	81	81	84	10	4	7
	736	737	759	76	80	62	9	4	8
	723	798	745	69	90	85	10	5	9
	735	737	717	84	79	81	11	9	2
	736	704	782	81	67	98	8	9	6
	707	794	747	61	60	86	5	6	7
	792	772	805	77	77	84	8	5	3
	736	777	737	82	77	73	7	3	5
	820	756	778	84	78	73	4	6	9
	755	738	764	70	78	77	7	6	7
	757	830	776	66	66	79	9	3	5

Table 4.3

Simulated Data Using Nahrstedt and Gimbel (1996) Model (Continued)

Beta Parameters				**a = 92.43**		**b = 33.79**			
λ	**1000**	**1000**	**1000**	**100**	**100**	**100**	**10**	**10**	**10**
	778	743	784	77	72	59	13	9	6
	786	757	742	87	60	67	8	9	6
	773	757	775	70	81	75	12	5	6
	835	714	761	85	87	84	12	5	10
	759	735	756	74	78	65	13	13	11
	763	780	742	89	74	67	9	5	12
	731	697	811	76	73	79	5	13	5
	798	739	749	79	60	79	9	7	12
	747	822	747	83	78	73	8	6	11
	755	746	785	91	76	65	9	7	8
	743	749	754	78	90	69	7	7	8
	800	794	731	84	85	75	6	11	6
	738	735	768	67	81	73	9	10	7
	728	773	761	76	82	66	9	12	6
	762	767	703	70	62	74	6	4	9
	747	748	741	73	79	79	10	10	5
	741	755	749	70	83	77	10	8	10
	750	779	717	83	80	75	6	11	7
	782	740	738	70	62	88	11	5	14
	733	728	739	74	77	65	12	3	9
	719	783	741	71	98	96	7	7	3
	771	758	765	80	81	84	9	6	6
	757	755	729	67	79	72	5	9	15
	748	741	740	75	82	83	12	5	9
	811	786	801	77	86	76	11	11	7
	785	759	783	69	85	90	10	7	5
	714	812	774	69	87	81	9	8	9
	754	786	711	71	83	77	6	7	12
	811	740	791	62	64	68	2	8	11
	737	765	778	85	77	60	9	2	5
	770	812	722	92	70	71	7	5	7
	790	749	745	71	57	73	2	13	11
	721	747	742	73	89	73	8	6	6
	733	741	699	76	72	71	9	8	8
	747	768	717	68	71	78	7	11	8
	760	775	723	82	80	74	4	9	12
	722	791	807	84	77	74	10	10	14
	754	757	715	74	94	66	6	12	9
	778	777	723	88	84	60	5	4	5
	730	769	740	68	73	66	6	2	6
	811	734	761	70	56	65	15	5	6
	722	743	741	83	77	88	5	15	4
	765	787	755	73	88	81	9	10	5
	732	756	772	79	75	57	4	4	5
	793	752	780	69	80	65	7	6	10
	738	723	710	78	82	80	8	8	11
mean, n = 100	756.48	761.43	754.92	75.54	76.97	75.24	8.07	7.55	7.59
χ2, df = 99	120.761	122.570	135.041	86.085	110.574	114.836	86.556	114.801	93.042
Pr{χ^2 <= S}	**93.220**	**94.570**	**99.060**	**18.058**	**79.940**	**86.805**	**19.039**	**86.758**	**35.028**
Reject Poisson at α = 0.05			**X**						
mean, n = 30	752.70	765.70	758.17	75.93	77.20	75.83	8.30	8.13	7.27
χ2, df = 29	40.819	48.032	61.147	34.629	38.412	47.002	45.575	38.911	15.763
Pr{χ^2 <= S}	**92.859**	**98.541**	**99.956**	**78.303**	**88.656**	**98.139**	**97.415**	**89.662**	**2.189**
Reject Poisson at α = 0.05		**X**	**X**			**X**			**X**

Using the values of χ_i^2, it can be determined whether or not the expected Poisson distribution has been obtained. For a data set containing a number (n) of samples, the probability that χ^2 for a given number of degrees of freedom (n-1) is less than or equal to χ_i^2 can be calculated by the χ^2 integral (Blom, 1989),

$$Pr\left(\chi^2 \le \chi_i^2\right) = \int_0^{\chi_i^2} \frac{1}{2^{(n-1)/2}\Gamma\left((n-1)/2\right)} \chi^{2\left((n-1)/2-1\right)} e^{-\chi^2/2} d\chi^2 \qquad (4.4)$$

in which Γ is the gamma function. The calculation of this function is readily available in most statistical software packages and spreadsheets. Table 4.3 includes probability calculations (using Equation 4.4) for each of the simulated sample sets.

For a selected significance level (α), the null hypothesis that a distribution is consistent with the Poisson distribution cannot be rejected when,

$$\tfrac{1}{2}\alpha < Pr\left(\chi^2 \le \chi_i^2\right) < \left(1 - \tfrac{1}{2}\alpha\right) \qquad (4.5)$$

If a distribution is Poisson at a significance level of 5% (α = 0.05), the probability calculated using Equation 4.4 should be between 2.5% and 97.5% based on Equation 4.5. This general approach has been previously described (Haas and Rose, 1996; Parkhurst and Stern, 1998). Using this method, one would expect that approximately 1 in 20 distributions (or sets of oocyst counts) would have a probability (calculated with Equation 4.4) outside of the 2.5 – 97.5% range.

A 5% significance level was used to determine whether the simulated data sets were consistent with the Poisson distribution. Data sets that were not consistent with the Poisson distribution were marked with an "X" in Table 4.3; several of these scenarios were inconsistent with the Poisson distribution. If the number of simulated samples were substantially increased to 1000 or 10000, then likely even more of the resulting distributions would be inconsistent with the Poisson distribution.

When enumerating *C. parvum*, it is uncommon to collect 100 or more samples with the same expected value and recovery profile. Therefore, a second analysis was performed

using only the first thirty data points from each sample set. The results of the Fisher's chi-squared test indicated that several of the 30-sample data sets were also inconsistent with the Poisson distribution at the 5% significance level (Table 4.3). Overall, the data suggest that Poisson assumption might be more appropriate for some recovery profiles than others; however, in the general case, it does not adequately describe data obtained using an analytical method with non-constant recovery. Therefore, the above exercise suggests that a distribution more complex than the Poisson distribution may be required to account for the various analytical errors associated with *C. parvum* enumeration.

4.5 Sources of Error During *Cryptosporidium* Concentration and Enumeration

Although Atherholt and Korn (1999) presented Poisson methods for describing sample counts, they also suggested that a distribution more complex than the Poisson distribution may be required to account for the various analytical errors. Nahrstedt and Gimbel (1996) developed such a statistical model by assuming a Poisson distribution for the true sample counts, a binomial distribution for modeling the recovered fraction of oocysts, and a Beta distribution for describing the uncertainty of recovery. They also noted that the time and location of sampling influence error, but suggested that this contribution cannot be statistically determined. These sources of error were described in Chapter 2.

4.6 Approximation of Beta Parameters

The Beta distribution is useful for describing oocyst recovery because the recovery is bound between 0 and 1 (*i.e.*, $0 \le p \le 1$) and the distribution is very flexible, allowing for the description of a variety of recovery profiles. The Beta probability density function (pdf) was presented in Equation 2.5. With more detail, the Beta pdf is described by a and b, the parameters of the Beta distribution, such that:

$$P(p) = p^{a-1}(1-p)^{b-1}\frac{\Gamma(a+b)}{\Gamma(a)\Gamma(b)} \qquad (2.5)$$

where,

$$\text{mean or expected value of } p \quad = \quad \mathrm{E}(p) = \frac{a}{a+b} \tag{4.6}$$

and,

$$\text{variance of } p \quad = \quad \mathrm{Var}(p) = \frac{ab}{(a+b)^2(a+b+1)} \tag{4.7}$$

Recovery studies must be performed to determine the Beta parameters a and b that describe the overall recovery profile. Each replicate sample represents a trial from which each counted oocyst represents a success. Button and Reilly (2000) presented a method for determining overall Beta distributions for multiple trials. The authors explained that overall Beta pdfs were necessary because each set of trials provides a different estimate of the Beta distribution for the variable of interest (in this case p, the analytical recovery). The authors emphasized that simply summing all of the data (in the case of oocysts, using the total number of oocysts counted from all of the slides and the total volume of water processed to make all of the slides) does not reflect the uncertainty between data sources and times periods. For example, different analysts increase uncertainty, potentially resulting in different recovery profiles (Beta pdfs). Button and Reilly (2000) presented an overall Beta distribution that had the same mean and variance as the mixture of Beta distributions from the different sets of trials; it was described by

$$\text{overall Beta distribution} \quad = \quad \mathrm{pdf}\left(p \,;\, p_1, p_2, p_3, \cdots, p_k\right) = \sum_{i=1}^{k} \lambda_i \,\mathrm{pdf}\left(p_i; x_i, y_i\right) \tag{4.8}$$

where

p	=	overall probability of success
p_i	=	probability of success on each trial (i)
k	=	number of trials
x_i	=	number of successes in trial i
y_i	=	number of failures in trial i
pdf(p_i, x_i, y_i)	=	Beta pdf for the ith trial
λ_i	=	weighting factor for the ith Beta distribution, where $\sum_{i=1}^{k} \lambda_i = 1$ and the value of each λ_i is proportional to its variance^{-1}.

This method weighted the Beta distribution from each trial in proportion to its precision. The mean and variance of the overall Beta pdf were calculated using Equations 4.9-4.12 as follows:

$$\mathrm{E}(p) = \frac{\sum_{i=1}^{k} \frac{\mu_i}{\sigma_i^2}}{\sum_{i=1}^{k} \frac{1}{\sigma_i^2}} \tag{4.9}$$

$$\mathrm{Var}(p) = \frac{k + \sum_{i=1}^{k} \frac{\mu_i^2}{\sigma_i^2}}{\sum_{i=1}^{k} \frac{1}{\sigma_i^2}} - \left[\mathrm{E}(p)\right]^2 \tag{4.10}$$

where

$$\mu_i = \frac{x_i}{x_i + y_i} \tag{4.11}$$

$$\sigma_i^2 = \frac{x_i y_i}{(x_i + y_i)^2 (x_i + y_i + 1)} \tag{4.12}$$

Once the mean and variance of the overall Beta pdf were calculated, the values of the parameters a and b for the overall Beta distribution were calculated from the standard expressions for the mean and variance of the Beta distribution; these expressions were provided in Equations 4.6 and 4.7 respectively. The overall Beta distribution was fit to the example recovery data from Table 4.1. The overall mean and variance were calculated using Equations 4.9-4.12; some of the key data are summarized in Table 4.4. Using the overall mean and variance, Equations 4.6 and 4.7 were used to calculate the Beta parameters a and b, 28.12 and 8.43 respectively. The corresponding pdf and cumulative density function (cdf) for the recovery data from Table 4.1 are presented in Figure 4.1 and Figure 4.2. There are fifteen trials in this example (k = 15). This approach was used to calculate the Beta parameters used in this thesis (Appendix C).

Table 4.4

Calculated Data Used for Describing the Overall Recovery Profile (Beta pdf)

for Recovery Data in Table 4.1

Seeded Number of Oocysts	Observed Number of Oocysts	x_i	y_i	E(p)	Var(p)
500	351	351	149	0.70	4.176 E-4
500	360	360	140	0.72	4.024 E-4
500	427	427	73	0.85	2.489 E-4
500	409	409	91	0.82	2.972 E-4
500	355	355	145	0.71	4.110 E-4
500	357	357	143	0.71	4.076 E-4
500	366	366	134	0.73	3.916 E-4
500	431	431	69	0.86	2.374 E-4
500	378	378	122	0.76	3.682 E-4
500	345	345	155	0.69	4.269 E-4
50	36	36	14	0.72	3.953 E-3
50	42	42	8	0.84	2.635 E-3
50	35	35	15	0.70	4.118 E-3
50	38	38	12	0.76	3.576 E-3
50	37	37	13	0.74	3.773 E-3

overall mean = 0.7693
overall variance = 0.0047

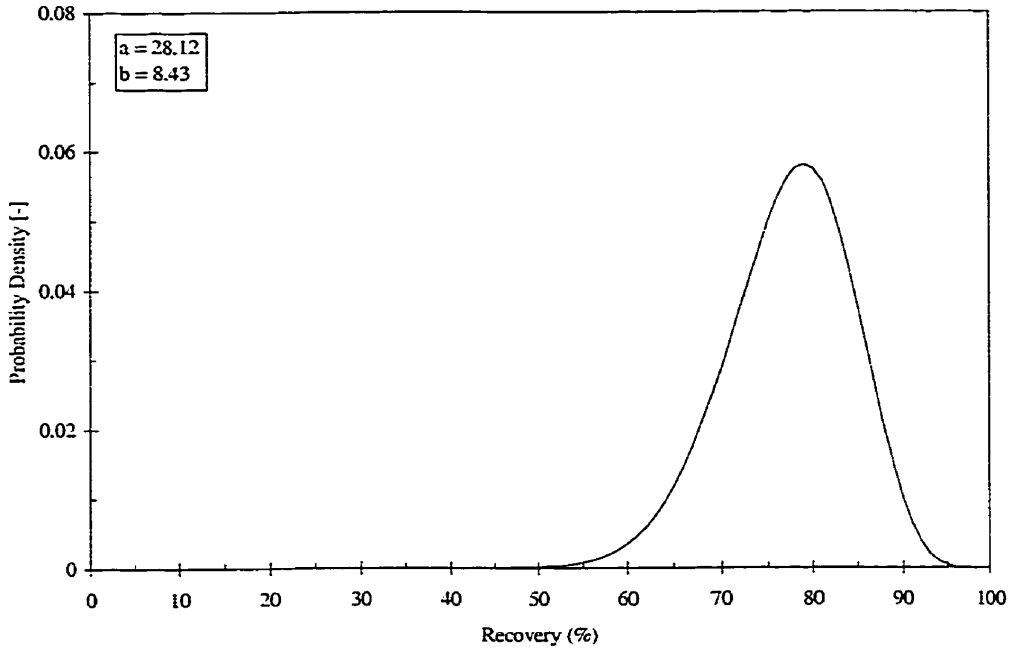


Figure 4.1 Overall Recovery Profile (Beta pdf) for Recovery Data in Table 4.1.

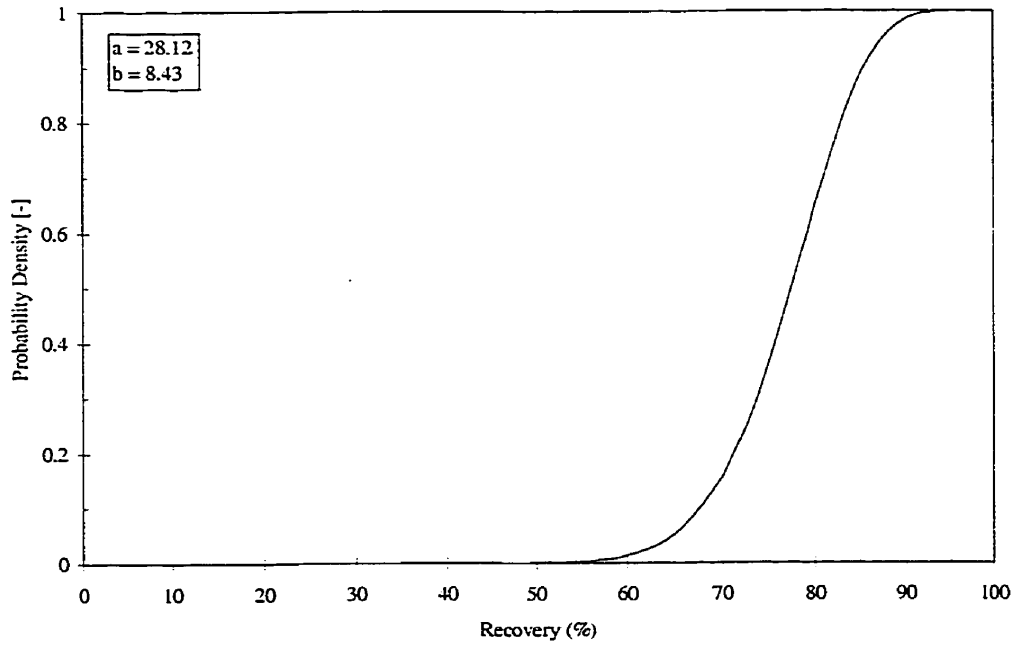


Figure 4.2 Overall Recovery Profile (Beta cdf) for Recovery Data in Table 4.1.

4.7 CALCULATING CONFIDENCE INTERVALS

The approach described in this chapter is similar to that previously described since a Poisson model is used to describe the original distribution of microorganisms in the water body. The issue of analytical recovery is also addressed by incorporating the binomial and Beta distributions. This work is unique in that strategies are presented for quantifying the reliability of both concentrations and removals of microorganisms.

In most cases where oocysts (or any other discrete particles) are being enumerated, a number of oocysts (X) is observed from a processed volume of water (V). The value of the parameter N is an estimate of the true number of oocysts in the water (λ). If the distribution of true counts of oocysts in the water can be described by a Poisson distribution and if a constant fraction (p) of the oocysts are recovered from the water, then the probability of observing X oocysts is described by,

$$P(X|\lambda, N, p) = \frac{\lambda^N e^{-\lambda}}{N!} \frac{N!}{X!(N-X)!} p^X (1-p)^{N-X} \tag{4.13}$$

In other words, Equation 4.13 is the product of the Poisson distribution (Equation 2.1) and the binomial distribution (Equation 2.3) and describes the probability of observing X oocysts under given experimental conditions described by λ, N, and p. N is an unknown parameter representing the true count of oocysts in the sample drawn from the water body. Information regarding p helps to estimate N. This information is necessary to determine the true count of oocysts in the water (λ) and the parameter of interest.

Recovery studies can be performed to describe the uncertainty of p by distributions such as the Beta (Equation 2.5), however, they must somehow be incorporated when drawing inferences about the true count of oocysts (λ) based on the observed count (X). Bayesian inference allows for explicit incorporation of assumptions describing the complexity of recovery (p) that is free from impediment from purely technical limitation (Box and Tiao, 1973). While relatively simple solutions can be obtained from other inferential theories when assumptions such as normality and independence of errors are possible, the solutions are often intractable (Box and Tiao, 1973) in cases such as describing

Cryptosporidium concentrations and removals, where such assumptions are not necessarily valid.

Bayes' theorem can be applied to make inferences about c (the true concentration of oocysts in the water body) and therefore λ (the true count of oocysts in the sample), given information about V_i, p_i (in the form of a and b from the Beta distribution described by Equation 2.5) and X_i. According to Bayes' theorem,

$$Df(c, N_i, p_i|X_i) \propto Df(c, N_i, p_i) \times Df(X_i|c, N_i, p_i) \tag{4.14}$$

where $i = 1,2,\cdots,n$. According to Equation 4.14, in order to describe the density function (Df) of the true oocyst concentration c given the observed oocyst count X, prior information regarding the density function of c, N, and p is required ($Df(c, N_i, p_i)$) for all values of i.

If n replicate samples of volume V are take from a water body of oocyst concentration c, substituting Equation 2.2 into Equation 4.13 yields,

$$P(X_i|c, N_i, p, i = 1,2,\cdots,n) = \frac{(cV_i)^{N_i} e^{-cV_i}}{N_i!} \frac{N_i!}{X_i!(N_i - X_i)!} p^{X_i}(1-p)^{N-X_i} \tag{4.15}$$

where V_i is known. Equation 4.15 does not require that all of the water samples have the same volume (*i.e.*, V_1 does not necessarily equal V_2, etc). It should be noted that Equation 2.4 could be used which would eliminate N ($\lambda_{obs} = N$), but subsequent sampling would be awkward. The likelihood function describes information about c, N, and p that has been derived from the experimental data and is proportional to Equation 4.13.

The prior density function describes what is known about c, N, and p prior to looking at the data. It can be described by,

$$Df(c, N_i, p_i, i = 1,2,\cdots n) \propto \frac{1}{c} \prod_{i=1}^{n} {p_i}^{a-1}(1-p_i)^{b-1} \tag{4.16}$$

According to Equation 4.16, no information regarding c is known in advance. Since $c \geq 0$ (negative oocyst counts and concentrations are not possible) a Jeffreys prior ($1/c$) is used to indicate that all outcomes are equally likely (Jeffreys, 1961). A uniform prior of 1 is used for N, indicating no prior information about N. Finally, the Beta distribution from Equation 2.5 is used as an informative prior that describes the recovery profile of p_i.

Using Bayes' theorem from Equations 4.14, Equations 4.15 and 4.16 are combined to yield the posterior density function,

$$Df\left(c, N_i, p_i | X_i, i = 1,2,\cdots,n\right) \propto \frac{1}{c}\prod_{i=1}^{n} {p_i}^{a-1}\left(1-p_i\right)^{b-1}\frac{\left(cV_i\right)^{N_i}e^{-cV_i}}{N_i!}\frac{N_i!}{X_i!\left(N_i-X_i\right)!}{p_i}^{X_i}\left(1-p_i\right)^{N-X_i} \tag{4.17}$$

which describes c, N, and p given knowledge of the data X where $c>0$, $N \leq X$, $0 \leq p \leq 1$, and $i = 1,2,\cdots,n$.

Monte Carlo methods often estimate features of the posterior or predictive distributions such as the one presented in Equation 4.17 by using samples drawn from that distribution. As described by Smith and Roberts (1993), however, "generating samples from an arbitrary, often highly dimensional joint distribution is not often possible, thus seemingly making sample-based approaches of limited use." The Gibbs sampler is based on Markov Chains and represents an indirect approach to the required sampling that can overcome that problem (Smith and Roberts, 1993).

Introduced by Geman and Geman (1984), the Gibbs sampler, as described by Casella and George (1992) is "a technique for generating random variables from a marginal distribution indirectly, without having to calculate the density." The authors explain that supposing a given a joint density $f(x, y_1, \ldots, y_p)$ and an interest in obtaining the characteristics of the marginal density $[f(x) = \int\cdots\int f\left(x, y_1,\cdots, y_p\right)dy_1\cdots dy_p]$ such as the mean or variance, the typical approach would be to calculate $f(x)$ and use it to obtain the desired characteristic. In the present context, Equation 4.17 describes the joint density for the true concentration of oocysts in the water body (c), the oocyst number in the

sample (N, an estimate of the true number of oocysts in the water body, λ), and the probability of recovery (p) given a count on the slide (X).

The integrations in $f(x)$ are extremely difficult to perform either analytically or numerically in many cases, such as Equation 4.17; in such cases, the Gibbs Sampler provides and alternative method for obtaining $f(x)$ (Casella and George, 1992). The Gibbs sampler does not require the direct computation or approximation of $f(x)$, rather, it effectively allows for the generation of a sample $X_1, \ldots, X_m \sim f(x)$ without requiring $f(x)$, the marginal density (Casella and George, 1992). This is achieved by generating a sample by from $f(x)$ by sampling from the conditional distributions; in the case of a pair of random variables (X, Y), the conditional distributions are $f(x|y)$ and $f(y|x)$ (Casella and George, 1992). The mean, variance or any other characteristic of $f(x)$ can be calculated to the desired degree of accuracy by simulating a large enough sample (Casella, and George, 1992). Several detailed discussions of the Gibbs sampler are available in the literature (Casella, and George, 1992; Smith and Roberts, 1993).

The Gibbs sampler can be used to estimate values of c, N, and p given knowledge of the data X. This is accomplished by sampling from the distributions described by the conditional probability density functions (*Dfc*) of each of the parameters. The conditional probability density functions of c, N_i, and p_i are respectively described by,

$$Dfc(c) \propto c^{-1+\sum N_i} e^{-c\sum V_i} \rightarrow c\sum V_i\text{:Gamma}(\sum N_i) \tag{4.18}$$

$$Dfc(p_i) \propto p_i^{X_i+a-1}(1-p_i)^{N_i-X_i+b-1} \rightarrow p_i\text{:Beta}(X_i+a, N_i-X_i+b) \tag{4.19}$$

$$Dfc(N_i) \propto \frac{cV_i(1-p_i)^{N_i-X_i}\, exp\left[-cV_i(1-p_i)\right]}{(N_i-X_i)!} \rightarrow N_i-X_i\text{:Poisson}\left[cV_i(1-p_i)\right] \tag{4.20}$$

Although each of the conditional probability density functions of c, N, and p can be described, it is the oocyst concentration (c) that is of interest. Confidence intervals on oocyst concentration can be calculated by binning the generated data to yield the pdf.

The two unique points with equal height (density) that are a distance (β) apart on the pdf are the endpoints of the (100β)% confidence interval for oocyst concentration. This confidence interval represents the region of highest posterior density in which no point outside of the interval has a higher posterior density than the points inside the interval.

Confidence intervals for log removals can be calculated using the same method used for oocyst concentrations. This is achieved by concurrently simulating the conditional density functions on influent (c_i) and effluent (c_e) concentrations. The $\log_{10}$ of c_e/c_i is calculated and stored for each iteration, resulting in the density function of log removal through the treatment process being evaluated.

The 95% confidence intervals for the *C. parvum* removal data listed in Table 4.2 were calculated. These intervals were calculated for each of the individual data points as well as the overall (combined or pooled) data set. The intervals for the t = 15, t = 30, t = 45, and t= 55 samples and the overall data set ranged from 4.79- to 6.12-log, 4.81- to 6.13-log, 4.97- to 7.03-log, 4.39- to 5.17-log, and 4.89- to 5.42-log respectively. Of the four samples, the lowest of the highest posterior density (HPD) region endpoint was 4.39-log, while the highest endpoint was 7.03-log. This range of removals is practically relevant because it represents the range of removals that could be reasonably expected during the conditions investigated, based on the measured oocyst counts and the uncertainty associated with the analytical method. This range of removals is also indicated in Figure 4.3, which depicts the pdfs for the evaluated data points and combined data set.

The pdfs in Figure 4.3 demonstrate that the combination of data points to yield an overall pdf results in a less diffuse result with less uncertainty. The pooled data resulted in a 95% confidence (probability) interval with a range of 4.89- to 5.42-log removal of *C. parvum*, a considerably smaller range than the overall range of 4.39- to 7.03-log described above. The pooling of replicate data results in a smaller confidence interval because of the increased number of observations (oocyst counts) associated with the replicate analyses. Since Poisson distributions are additive it is the number of actual observations (counts) rather than the number of replicate experiments that decreases the uncertainty associated with the enumeration of discrete particles such as oocysts.

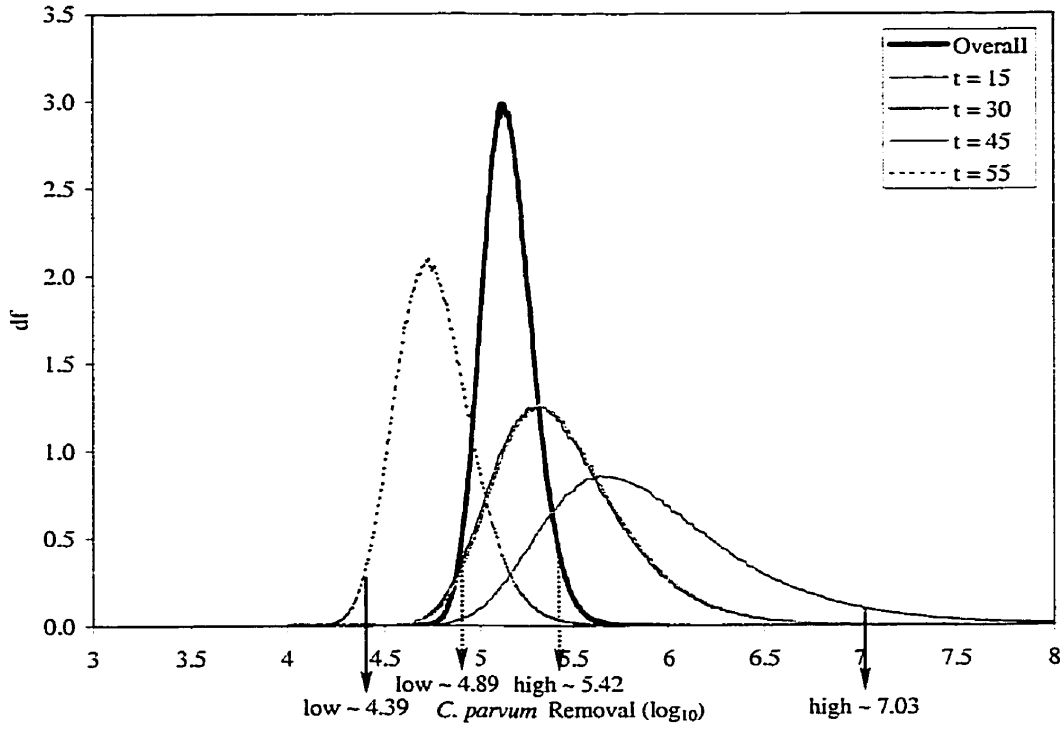


Figure 4.3 Probability density functions (pdfs) for *C. parvum* Removal Example Data in Table 4.2 (Stable Operation at Ottawa).

The relative effect of observations (counts) and replicate samples is demonstrated in Figure 4.4. Two types of data are presented in this figure. The data represented by the black bars correspond to three fictional pairs of filter influent and effluent counts that all resulted in an unadjusted (for recovery) removal of 4-log. The three pairs of data vary in that they each correspond to different counts of oocysts in the filter influent and effluent. The effluent counts indicated in Figure 4.4 correspond to counts of 1, 10, and 100 oocysts/L. The respective influent concentrations were 10^4, 10^5, and 10^6 oocysts/L to yield 4-log removal from each influent-effluent pair. From Figure 4.4, it is clear that the range of the confidence interval is much smaller in the sample with higher counts (100 oocysts/L in the filter effluent). While counting 1 oocyst/L in the filter effluent resulted in a confidence interval that spanned approximately 2.1-log, counts of 10 and 100 oocysts/L resulted in confidence intervals that spanned approximately 0.6- and 0.2-log respectively. This result clearly demonstrates that a considerable amount of uncertainty can be eliminated by enumerating >10 oocysts in filter effluent samples.

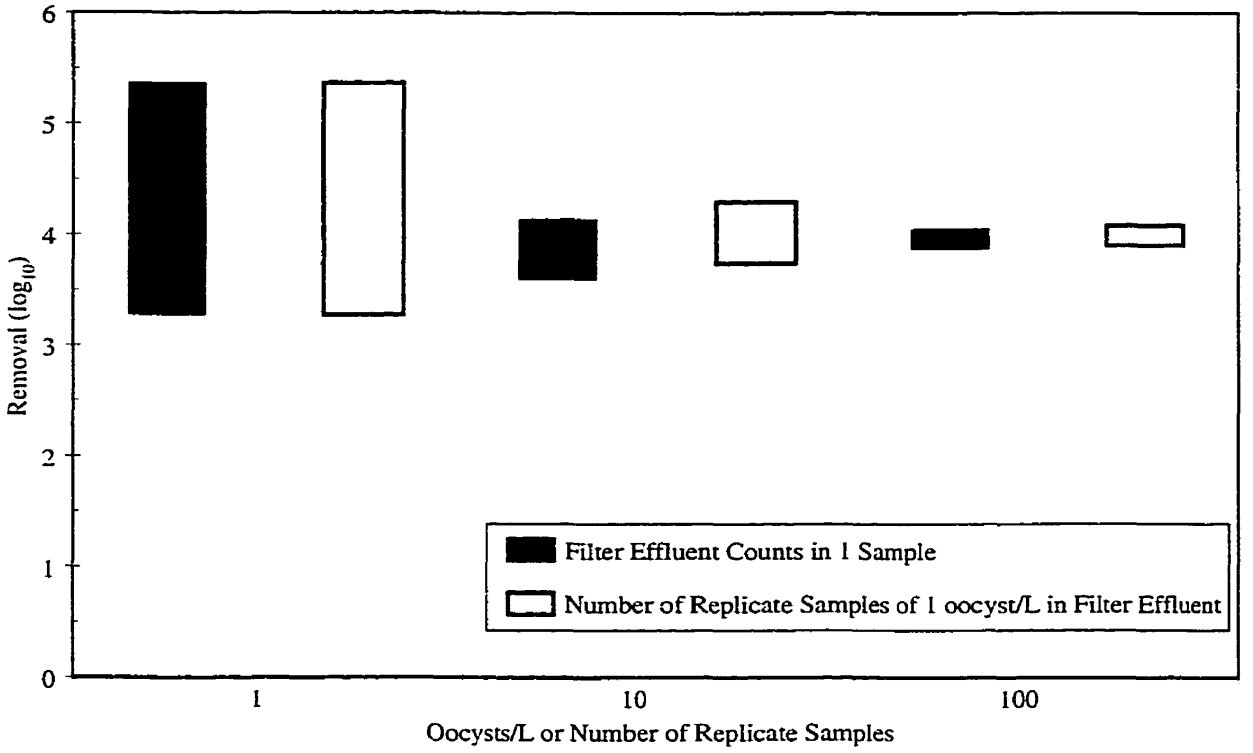


Figure 4.4 Effect of observations (counts) and replicate samples on confidence interval range.

The data represented by the white bars in Figure 4.4 correspond to replicate samples containing 1 oocyst/L in the filter effluent and 10^4 oocysts/L in the filter influent, again to yield an unadjusted (for recovery) removal of 4-log. The three bars correspond to pooled sets of influent-effluent pairs. The first bar from the left (1 replicate sample) represents the confidence interval calculated for one sample containing 1 oocyst/L in the filter effluent and 10^4 oocysts/L. The second bar corresponds (10 replicate samples) corresponds to ten replicate pairs of data in which the influent and effluent *C. parvum* concentrations are each 10^4 oocysts/L and 1 oocyst/L respectively. The third bar similarly represents the confidence interval for one hundred replicate pairs. A comparison of the black and white bars in bars in Figure 4.4 clearly demonstrates that the number of observations (counts) affects the uncertainty. Simply stated, counting at least ten oocysts in filter effluent samples (assuming a higher concentration in the filter influent) will substantially decrease the uncertainty associated with calculations of log removals, regardless of whether the ten oocysts are from one sample containing ten

oocysts or from ten samples each containing one oocyst. This result is a practical demonstration of the additivity of Poisson data. Given the time and cost associated with processing water samples containing *C. parvum*, the desirability of analytical methods that have the ability to process large enough volumes to yield at least ten oocysts is obvious from Figure 4.4.

Another important question also arises from the discussion of pooled data. Although statistically valid, the pooling of data averages out differences in *C. parvum* removal that occur during a given sampling period, with samples with higher counts skewing the pdf toward lower removals. So when is it appropriate to pool data or consider data points as replicates? In this work, the removals calculated from influent-effluent data pairs were considered replicates when the independent variables (settled water turbidity, filter loading rate, pretreatment conditions, etc.) were constant during the sampling period. In these cases, the removal data were pooled. When the independent variables were not constant (e.g., sub-optimal coagulation conditions when settled water turbidity was changing, end-of-run operation when the balance between attachment and detachment of particles within the filter was changing and obviously affecting water quality, etc.), the removal data were not pooled. In these cases, the range of removals was based on the lowest and highest HPD endpoints calculated from each of the individual pdfs. This approach for defining replicates and pooling data will be used in subsequent chapters for evaluating subsequent *C. parvum* and polystyrene microsphere removal data.

Chapter 5
Inactivated Oocysts as Surrogates for Viable Oocysts

5.1 Introduction

Traditional filtration performance parameters such as turbidity (USEPA, 1989) and particle counts are commonly considered adequate surrogate measures for evaluating the removal of microbial pathogens such as *Giardia* by filtration (USEPA, 1991). Although turbidity and particle counts are reliable indicators of treatment efficiency, they are not reliable quantitative surrogates for *Cryptosporidium* removal (Nieminski and Ongerth, 1995; Patania *et al.*, 1995; Fox *et al.*, 1998). While removals of other microbial parameters, such as aerobic spores of *Bacillus subtilis*, have been correlated with the filter removal efficiency of *C. parvum* in some studies, no adequate surrogate for removal of the pathogen has yet been identified. Accordingly, water treatment process evaluations of C. *parvum* removal are often performed using inactivated (non-viable) *C. parvum* oocysts. High concentrations (Nieminski and Ongerth, 1995; Fox *et al.*, 1998) of chemically inactivated oocysts are typically used during these studies because of the potential health risks associated with the use and release of viable oocysts.

Several researchers have noted that chemically inactivated *C. parvum* oocysts may not necessarily be ideal surrogates for viable oocysts due to differences in surface charge (Lytle and Fox, 1994). A change in the original surface charge (described by the zeta potential) of colloidal particles like *C. parvum* might affect their removal during granular

media filtration because zeta potential is indicative of the degree of particle destabilization (Amirtharajah and Mills, 1982). Particles are most readily removed when their zeta potential is near zero, corresponding to their isoelectric point (Amirtharajah, 1988). It has been demonstrated that chemical inactivation can change oocyst zeta potential (Ongerth and Pecoraro, 1996). As a result, some researchers have speculated on how this change might affect coagulation and subsequent filtration (Lytle and Fox, 1994). During water treatment, the zeta potential of *C. parvum* oocysts is affected by multiple factors such as water quality, coagulant type and dosage, and pH, in addition to chemical inactivation prior to treatment.

Amirtharajah (1988) described charge neutralization by demonstrating that colloidal zeta potential is different in coagulated and non-coagulated waters (Figure 5.1). Following chemical pretreatment (coagulation), the surface charge of oocysts and other colloids becomes a function of the pH, coagulant type, and coagulant concentration rather than the specific colloidal zeta potential prior to coagulation, as the positively charged hydrolysis species attach to the particles and neutralize or reduce the net particle surface charge. Similarly, during high coagulant dosing and sweep floc coagulation, oocysts become enmeshed in the precipitating hydroxide solids and the surface charge relevant for oocyst removal becomes a function of the entire floc rather than a single oocyst. Differences in oocyst zeta potential prior to coagulation do not likely impact overall coagulation chemistry because oocyst surface area is essentially insignificant relative to that of other particles present in water. In many cases, this is true for the artificially high oocyst concentrations used in treatment optimization investigations (including the present study).

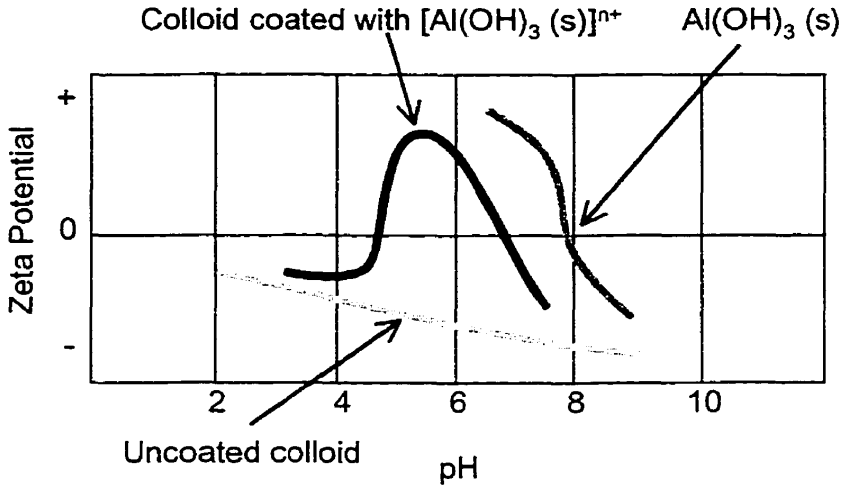


Figure 5.1 Impact of coagulant on colloidal zeta potential (Amirtharajah, 1988).

As filtration is a dynamic process, it is critical to identify key operational and design strategies for maximizing *C. parvum* removal during filtration, especially during periods when the process is challenged. As described in Chapter 2, numerous investigations have been conducted to evaluate the removal of *C. parvum* through filtration under normal operating conditions where chemical pretreatment and filtration processes are performing at optimal or near-optimal conditions. Full-scale *Cryptosporidium* removals from 2 to >4-log have been reported in the literature (*e.g.*, Kelley *et al.*, 1995; Nieminski and Ongerth, 1995; Baudin and Laîné, 1998). Pilot-scale oocyst removal data have suggested that filters can achieve anywhere from 2 to >5-log removal of oocysts (*e.g.*, Patania *et al.*, 1995; Fox *et al.*, 1998). Pathogen passage through filters during vulnerable points in the filter cycle (*e.g.*, ripening, breakthrough, etc.) or when particle removal processes are challenged (*e.g.*, hydraulic surges, coagulation upsets, *etc.*), however, has been less thoroughly investigated.

It has been suggested that maintaining optimal chemical pretreatment is critical to maximizing *C. parvum* removals during filtration. Patania *et al.* (1995) demonstrated that filtration was ineffective for oocyst removal without chemical pretreatment. Other pilot-scale studies also indicated that sub-optimal coagulation decreased oocyst removal by filters by at least 1-log (Charles *et al.*, 1995; Ongerth and Pecoraro, 1995; Dugan *et*

al., 1999). These findings concur with theoretical arguments that suggest proper coagulation conditions are necessary to achieve adequate particle destabilization for attachment by charge neutralization (Figure 5.1) or enmeshment in precipitates that can be subsequently removed by sedimentation or attachment during filtration. When coagulation is not optimized, filter performance deteriorates resulting in higher filter effluent turbidities and particle concentrations compared to stable or optimized operation. Differences between removals of viable and inactivated oocysts by filtration would be most likely to occur under operating conditions such as coagulation failure, when the differences in oocyst zeta potential are least influenced by factors such as coagulant type and concentration.

Filter ripening is another potentially vulnerable period of filter operation because filter effluent turbidities and particle concentrations are also high during ripening compared to stable operation. Amirtharajah (1998) suggested that >90% of the particles that pass through a well-operated filter do so during ripening. Several pilot-scale studies have demonstrated that although filter effluent turbidities can be quite high during ripening, log removals of *C. parvum* only deteriorated by ~0.5 to 1-log compared to those obtained during stable filter operation (Patania *et al.*, 1995; Charles *et al.*, 1995). These findings were confirmed at full-scale by Baudin and Laîné (1998) who demonstrated an ~1-log deterioration in oocyst removals during filter ripening. Despite considerable filter effluent turbidities and particle concentrations during this period, these reported findings suggested that oocyst removals are not particularly vulnerable during filter ripening.

Non-attachment of particles partially contributes to the higher filter effluent turbidities and particle concentrations that occur during ripening (Amirtharajah, 1988). Filter ripening represents a relatively brief operating period during which an increase in non-attachment of particles is expected relative to stable operation. If they exist, subtle differences between attachment efficiencies of viable and inactivated oocysts could potentially be revealed during this period. Although end-of-run breakthrough represents another period of increased non-attachment relative to stable operation, this operating condition could not be achieved in the bench-scale filter due to available head limitations.

Investigations of media type and design have demonstrated that these parameters have little impact on oocyst removals by filters. Hall *et al.* (1995) did not find performance differences between sand and dual-media filters when the filters had similar filtrate quality (measured by turbidity). Other pilot studies have also failed to demonstrate statistically significant differences between *C. parvum* removals by sand, anthracite/sand, and GAC/sand filters (Dugan *et al.*, 1999; Swertfeger *et al.*, 1999). In this study, evaluations of various media types were performed at bench-scale during operating periods when an increase in non-attachment of particles was expected relative to stable operation. These experiments were performed to elucidate any differences between attachment efficiencies of viable and inactivated oocysts that resulted from the media specifically, rather than from the operating conditions.

5.2 Experimental Objectives

The first objective of the bench-scale experiments described in this chapter was to determine if formalin-inactivated *C. parvum* oocysts were adequate surrogates for viable oocysts during both optimal and vulnerable periods of filter operation. Coagulation failure was selected as a critical vulnerable period for study because oocyst zeta potential was least likely affected by the presence of coagulant during this period; any differences between filter removals of viable and inactivated oocysts would most likely be demonstrated during this period. Despite limited data suggesting only marginal deterioration of oocyst removal by filtration during ripening, this period was studied because high filter effluent turbidities, particle passage, and non-attachment of particles relative to stable operation were expected. Subtle differences between attachment efficiencies of viable and inactivated oocysts could potentially be revealed during this period.

The second objective of this study was to identify operational and design strategies for minimizing oocyst passage through filters. In addition to the operational impacts of coagulant and operation during ripening, dual- and tri-media filter oocyst removals were compared to elucidate any potential media type (design) advantages during either stable or vulnerable operating conditions.

5.3 EXPERIMENTAL METHODS

Bench-scale experiments investigated dual- and tri-media filter removals of viable and formalin-inactivated *Cryptosporidium parvum* oocysts during stable operation, ripening, and coagulation failure. Specific information regarding the seeding conditions and filtration apparatus is provided in Chapter 3 (Tables 3.3, Section 3.3, and Figure 3.5). The filters were operated at a loading rate of ~7.5 m/h (3.1 US gpm/ft^2) in a constant head, declining rate mode during these evaluations. To ensure that no oocysts were carried over between experiments, the media were replaced between all experiments. Figure 5.2 generally describes the experimental configuration and conditions.

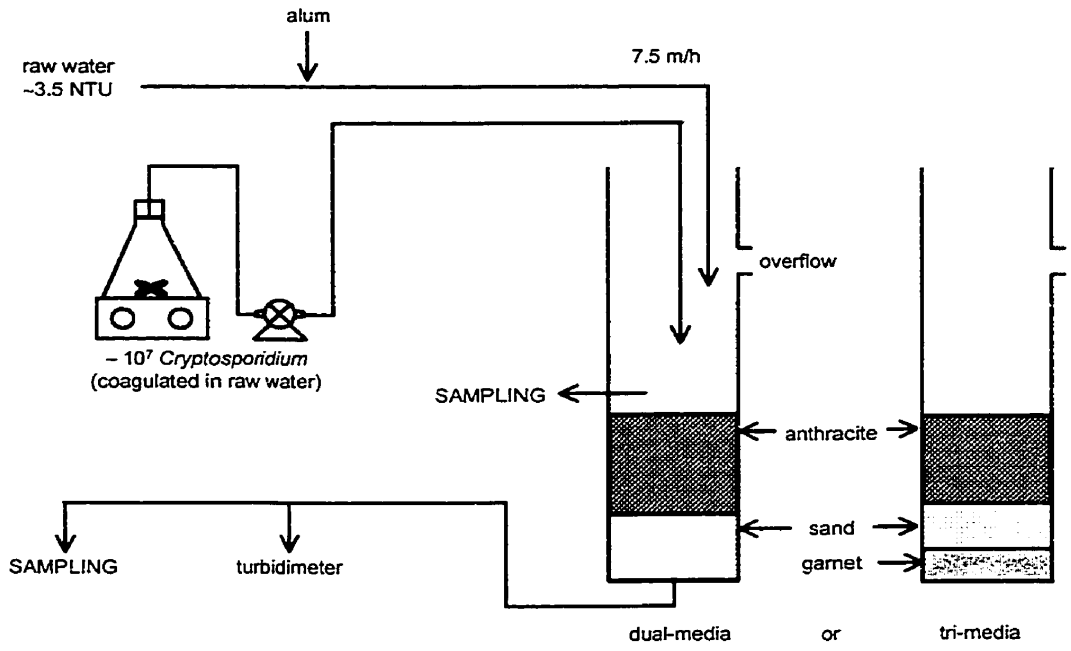


Figure 5.2 Bench-scale experimental configuration

Only one operating condition (stable operation, ripening, or coagulation failure) was evaluated per experiment. During the ripening experiments, the filters were seeded with oocysts during the first 30 minutes of operation; ripening was defined as the period from the start of filter operation to the point when filter effluent turbidity decreased to ~0.2 NTU after peaking. The seeded oocysts were jar-coagulated at the same conditions as the

raw water (5 mg/L alum ($Al_2(SO_4)_3 \cdot 18H_2O$) at pH 6.9). The coagulation conditions were determined using the lowest coagulant dose that achieved a filtered water quality of <0.1 NTU (~0.05 NTU).

Raw water coagulated at the same conditions as described for the ripening experiments pre-conditioned the filters prior to oocyst dosing during the stable operation and coagulation failure experiments. The filter pre-conditioning period was defined as the 2-hour period after filter effluent turbidity peaked during ripening. The seeded oocysts were jar-coagulated at the same conditions as the raw water during the stable operation experiments. During the coagulation failure experiments, raw water coagulation ceased after two hours of filter pre-conditioning and non-coagulated oocysts were seeded into the non-coagulated raw water. The filters were dosed with the oocyst suspension for one hour, during the third hour of operation. Filter performance was evaluated by monitoring filter influent and effluent turbidity every three minutes during ripening and every five minutes subsequent. Coagulation conditions and sampling periods during the bench-scale experiments are summarized in Figure 5.3.

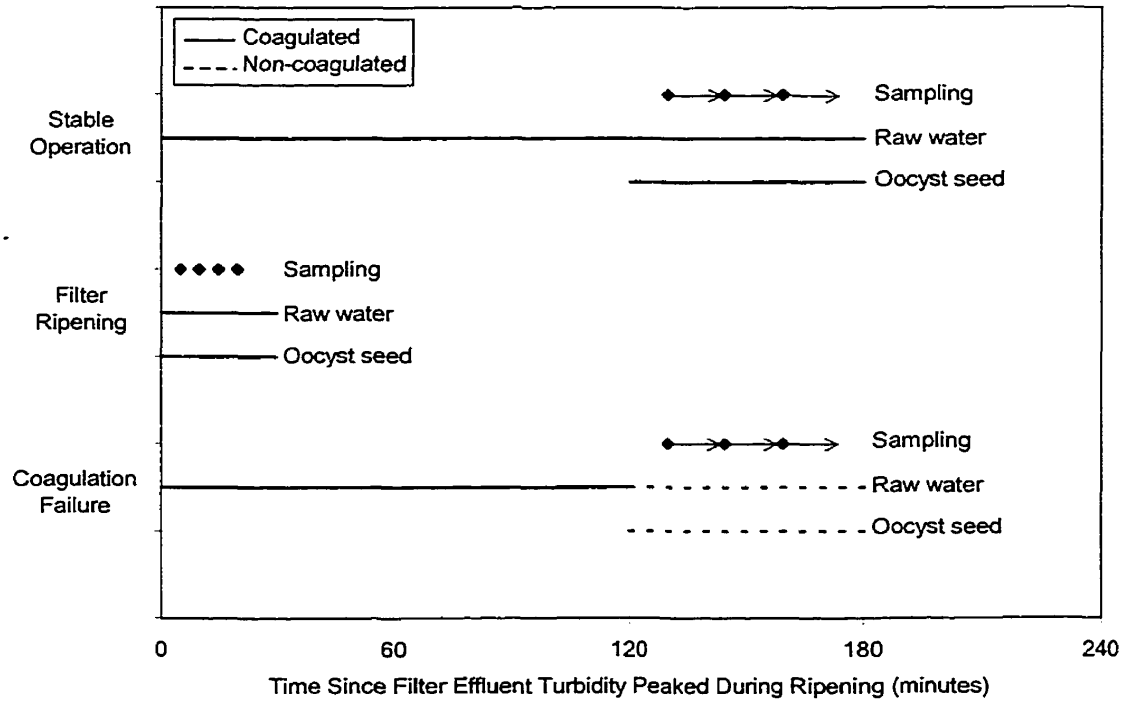


Figure 5.3 Summary of sampling times and coagulation conditions during bench-scale experiments.

The filters were seeded with a total of ~10^7 viable or formalin-inactivated oocysts. The oocyst concentration of the original spike suspension was determined directly with a hemocytometer and phase contrast microscopy as described in Chapter 3. Oocysts in samples of the seed suspension, filter influent, and filter effluent were concentrated by direct vacuum filtration and enumerated on the membranes using the immunofluorescence assay described in Chapter 3.

During the stable operation and coagulation failure experiments, filter influent and effluent samples were collected for *C. parvum* analysis during three consecutive 15-minute periods beginning at 10, 25, and 40 minutes after the start of the 1-hour seeding period. During ripening, filter influent and effluent samples were collected during four 5-minute periods beginning at 5, 10, 15, and 20 minutes after the start of the 30-minute seeding period. All of the sampling times are indicated on Figure 5.3. 250-mL samples of filter influent were collected during each sampling period; of these samples, 10 mL were analyzed for *C. parvum*. Almost all of the filter effluent during the oocyst dosing period was collected (~3 × 3.75 L during stable operation and coagulation failure and ~4 × 1.25 L during ripening) and analyzed for *C. parvum*.

5.4 RESULTS

Turbidity data were collected to confirm comparable filter performance between experiments. Turbidity data from the dual-media experiments using formalin-inactivated oocysts are presented in Figure 5.4. These data indicated that several filter runs (each containing new media) produced similar turbidity removals during the first three hours of operation when seeding occurred. The filter effluent turbidity during the dual-media filter experiments conducted during stable filter operation ranged from 0.03-0.07 NTU, with a mean of 0.05 NTU. Filter effluent turbidity trends during the ripening experiments were relatively reproducible and ranged from 0.04-0.87 NTU. The turbidity trends were also generally consistent between replicate coagulation failure experiments; filter effluent turbidities ranged from 0.03-0.69 NTU during these experiments.

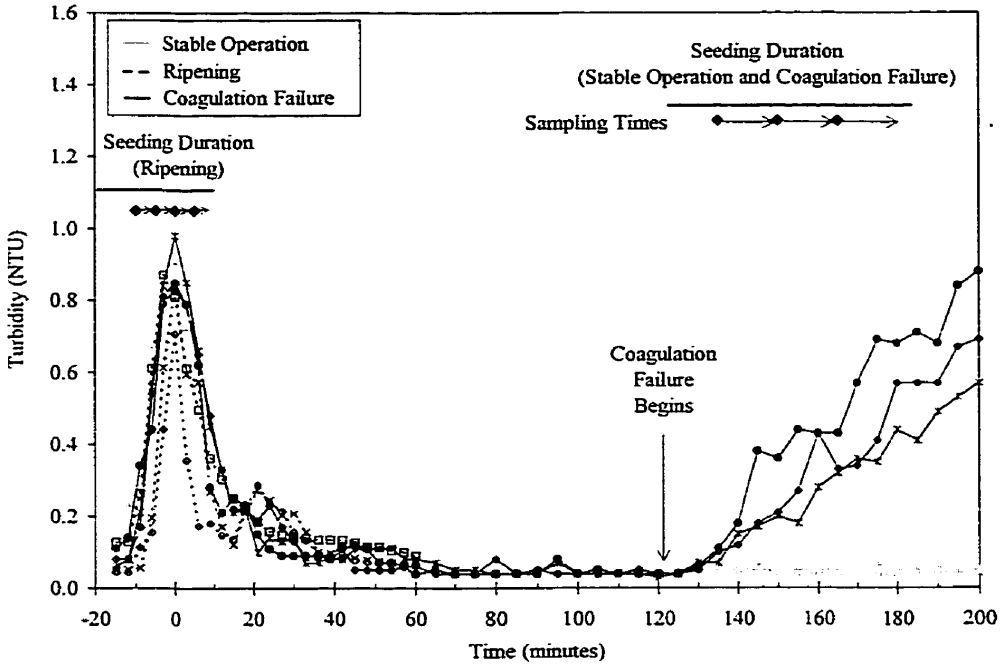


Figure 5.4 Filter effluent turbidity, seeding period, and sampling times during dual-media filter experiments with formalin-inactivated oocysts.

The filter effluent turbidity data in Figure 5.4 indicated that turbidity removal was relatively consistent during replicate filter runs of the three studied conditions, suggesting that media replacement between runs did not result in substantial differences in particle removal trends. Dual-media filter effluent turbidity data collected during the stable operation, ripening, and coagulation failure experiments are summarized in Table 5.1 to Table 5.3 respectively.

Similar filter performance and reproducibility of turbidity removal was observed during the dual-media experiments using viable oocysts (Figure 5.5). During the experiments conducted with viable oocysts, the dual-media filter turbidity during stable filter operation ranged from 0.04-0.06 NTU, with a mean of 0.05 NTU. Filter effluent turbidity trends during the ripening experiments ranged from 0.05-0.89 NTU. The turbidity trends were also generally consistent between replicate coagulation failure experiments; filter effluent turbidities ranged from 0.04-0.60 NTU during these experiments.

Table 5.1
Dual- and Tri-Media Filter Influent and Effluent *C. parvum* Concentrations and Effluent Turbidity During Stable Operation.

Experiment and Oocyst Viability	Sample Time (min)	Dual-Media *Cryptosporidium* (oocysts/L)		Turbidity (NTU)	Tri-Media *Cryptosporidium* (oocysts/L)		Turbidity (NTU)
		FI	FE	FE	FI	FE	FE
Inactivated 1	10	5.5×10^5	6	0.05	5.9×10^5	5	0.03
	25	5.7×10^5	3	0.04	6.2×10^5	1	0.03
	40	4.8×10^5	5	0.04	6.0×10^5	2	0.03
Inactivated 2	10	4.8×10^5	1	0.05	4.5×10^5	2	0.05
	25	5.5×10^5	10	0.05	5.3×10^5	6	0.05
	40	5.6×10^5	2	0.05	5.2×10^5	5	0.04
Inactivated 3	10	5.0×10^5	7	0.03	5.5×10^5	2	0.07
	25	4.7×10^5	2	0.04	5.1×10^5	2	0.05
	40	4.9×10^5	2	0.04	5.9×10^5	1	0.05
Viable 1	10	5.4×10^5	3	0.06	5.7×10^5	1	0.05
	25	4.8×10^5	7	0.05	4.5×10^5	1	0.06
	40	5.8×10^5	5	0.05	4.8×10^5	1	0.05
Viable 2	10	4.9×10^5	2	0.05	3.7×10^5	8	0.09
	25	4.6×10^5	1	0.04	3.6×10^5	2	0.11
	40	4.6×10^5	10	0.05	4.0×10^5	9	0.12
Viable 3	10	3.8×10^5	7	0.05	3.3×10^5	2	0.08
	25	4.0×10^5	1	0.04	3.7×10^5	2	0.07
	40	4.1×10^5	3	0.04	3.3×10^5	4	0.06

[FI] Filter influent.
[FE] Filter effluent.

Table 5.2
Dual- and Tri-Media Filter Influent and Effluent *C. parvum* Concentrations and Effluent Turbidity During Ripening.

Experiment and Oocyst Viability	Sample Time (min.)	Dual-Media *Cryptosporidium* (oocysts/L)		Turbidity (NTU)	Tri-Media *Cryptosporidium* (oocysts/L)		Turbidity (NTU)
		FI	FE	FE	FI	FE	FE
Inactivated 1	5	3.7×10^5	5	0.11	4.3×10^5	3	0.15
	10	3.9×10^5	11	0.16	4.5×10^5	5	0.24
	15	3.5×10^5	32	0.71	4.6×10^5	14	0.65
	20	3.8×10^5	10	0.17	4.6×10^5	8	0.44
Inactivated 2	5	4.1×10^5	3	0.06	3.8×10^5	2	0.33
	10	3.9×10^5	9	0.20	3.9×10^5	2	0.47
	15	4.0×10^5	43	0.84	3.6×10^5	22	0.52
	20	3.7×10^5	12	0.57	3.7×10^5	11	0.31
Inactivated 3	5	4.2×10^5	4	0.26	5.5×10^5	3	0.49
	10	4.2×10^5	16	0.61	5.7×10^5	5	0.69
	15	4.5×10^5	37	0.81	5.3×10^5	30	0.79
	20	4.3×10^5	10	0.50	5.3×10^5	11	0.75
Viable 1	5	5.5×10^5	2	0.25	4.0×10^5	2	0.31
	10	4.9×10^5	7	0.31	4.8×10^5	7	0.38
	15	5.3×10^5	56	0.89	4.3×10^5	16	0.89
	20	5.6×10^5	8	0.51	4.6×10^5	1	0.61
Viable 2	5	4.3×10^5	4	0.15	---	---	---
	10	4.7×10^5	9	0.44	---	---	---
	15	4.6×10^5	25	0.72	---	---	---
	20	4.4×10^5	7	0.57	---	---	---
Viable 3	5	6.0×10^5	5	0.46	---	---	---
	10	5.8×10^5	8	0.59	---	---	---
	15	6.0×10^5	44	0.85	---	---	---
	20	5.5×10^5	38	0.69	---	---	---

[FI] Filter influent.
[FE] Filter effluent.

Table 5.3
Dual- and Tri-Media Filter Influent and Effluent *C. parvum* Concentrations and Effluent Turbidity During Coagulation Failure.

Experiment and Oocyst Viability	Sample Time (min.)	Dual-Media *Cryptosporidium* (oocysts/L)		Turbidity (NTU)	Tri-Media *Cryptosporidium* (oocysts/L)		Turbidity (NTU)
		FI	FE	FE	FI	FE	FE
Inactivated 1	10	5.1×10^5	4.8×10^4	0.07	5.2×10^5	2.9×10^4	0.07
	25	5.3×10^5	7.8×10^4	0.20	5.9×10^5	6.6×10^4	0.13
	40	5.9×10^5	1.1×10^5	0.32	5.8×10^5	1.0×10^5	0.32
Inactivated 2	10	4.2×10^5	6.7×10^4	0.11	4.8×10^5	4.2×10^4	0.10
	25	4.6×10^5	1.0×10^5	0.36	5.0×10^5	5.6×10^4	0.16
	40	4.6×10^5	1.3×10^5	0.43	4.4×10^5	1.1×10^5	0.36
Inactivated 3	10	6.0×10^5	3.0×10^4	0.10	5.4×10^5	4.2×10^4	0.15
	25	6.2×10^5	8.5×10^4	0.21	5.5×10^5	9.3×10^4	0.30
	40	5.6×10^5	1.2×10^5	0.33	6.2×10^5	1.3×10^5	0.42
Viable 1	10	5.4×10^5	4.3×10^4	0.07	3.8×10^5	5.8×10^4	0.15
	25	5.5×10^5	9.2×10^4	0.23	3.1×10^5	9.8×10^4	0.24
	40	4.5×10^5	1.3×10^5	0.32	3.9×10^5	1.3×10^5	0.36
Viable 2	10	5.8×10^5	6.2×10^4	0.10	3.7×10^5	1.2×10^3	0.07
	25	5.9×10^5	1.5×10^5	0.25	4.0×10^5	7.9×10^3	0.14
	40	5.1×10^5	2.1×10^5	0.41	4.2×10^5	6.3×10^3	0.17
Viable 3	10	4.6×10^5	2.7×10^4	0.06	3.8×10^5	3.9×10^4	0.17
	25	4.6×10^5	1.5×10^5	0.15	4.0×10^5	6.5×10^4	0.27
	40	5.0×10^5	2.0×10^5	0.28	4.0×10^5	8.8×10^4	0.29

[FI] Filter influent.
[FE] Filter effluent.

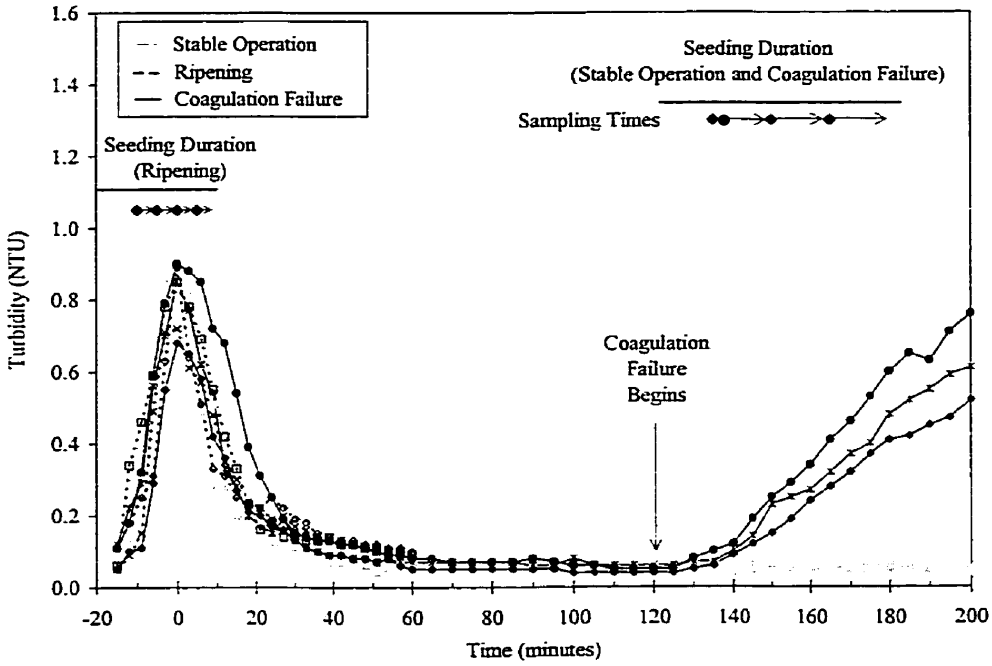


Figure 5.5 Filter effluent turbidity, seeding period, and sampling times during dual-media filter experiments with viable oocysts.

Despite considerably less consistent turbidity profiles during the first 60 minutes of operation (ripening), the tri-media stable operation and coagulation failure experiments achieved filter effluent turbidities comparable to those from the dual-media experiments (Figure 5.6 and Figure 5.7). During the experiments conducted with inactivated oocysts, the tri-media filter effluent turbidities ranged from 0.03-0.08 NTU, with a mean of 0.05 NTU during stable filter operation. The turbidity profiles during ripening were not highly reproducible in the tri-media filter. During ripening, the filter effluent turbidities ranged from 0.08-0.79 NTU. Filter effluent turbidities were also less reproducible during coagulation failure in the tri-media filter, ranging from 0.04-0.52 NTU during those experiments. Although the filter effluent turbidity data were less reproducible between triplicate experiments in the tri-media filter than in the dual-media filter, the filter effluent turbidity trends were generally consistent between replicates and media types during the oocyst seeding periods. The tri-media filter effluent turbidity data for stable operation, ripening, and coagulation failure experiments are summarized in Table 5.1 to Table 5.3 respectively.

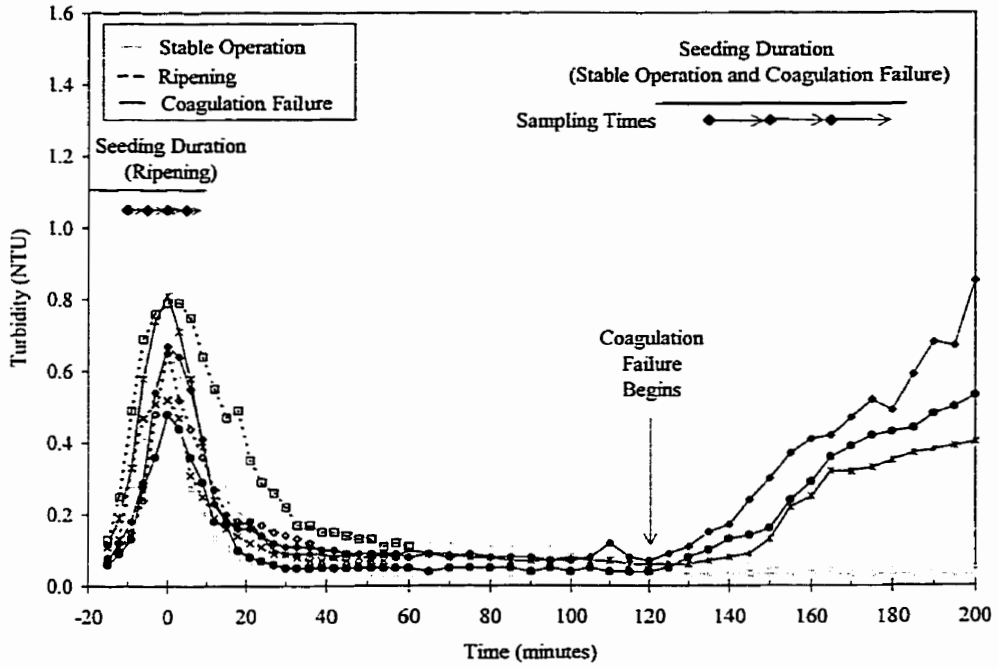


Figure 5.6 Filter effluent turbidity, seeding period, and sampling times during tri-media filter experiments with formalin-inactivated oocysts.

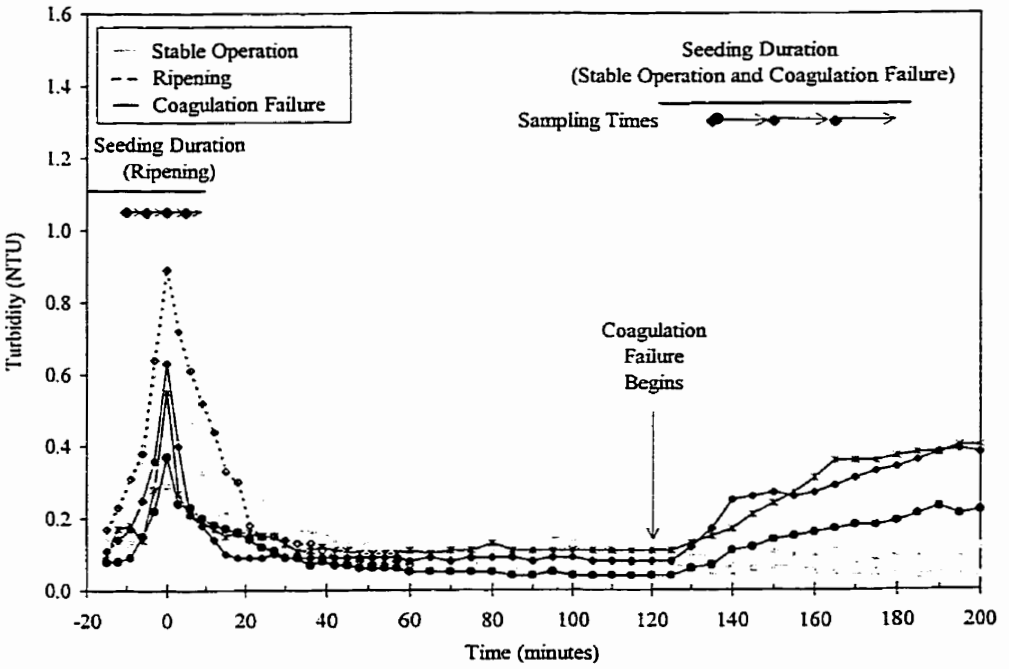


Figure 5.7 Filter effluent turbidity, seeding period, and sampling times during tri-media filter experiments with viable oocysts.

The filter effluent turbidity data collected during the tri-media filter experiments conducted with viable oocysts are presented in Figure 5.7. During these experiments the filter effluent turbidities during stable operation ranged from 0.03-0.12 NTU, with a mean of 0.07 NTU. As was experienced during the experiments with inactivated oocysts, the ripening periods were difficult to reproduce in the tri-media filter. During the one ripening experiment performed with viable oocysts, the filter effluent turbidities ranged from 0.17-0.89 NTU. Filter effluent turbidities were also less reproducible during the tri-media coagulation failure experiments with viable oocysts, ranging from 0.04-0.37 NTU. The tri-media filter effluent turbidity data for stable operation, ripening, and coagulation failure experiments are summarized in Table 5.1 to Table 5.3 respectively.

Each of the stable operation and coagulation failure experiments was performed in triplicate and consisted of three pairs of filter influent and effluent data per experiment. The ripening experiments were also performed in triplicate (with the exception of the tri-media ripening experiment with viable oocysts which was performed only once) and consisted of four pairs of filter influent and effluent data per experiment. Measured filter influent oocyst concentrations were consistently maintained at ~10^5 oocysts/L, regardless of experimental conditions and media type. Regardless of the media type, filter effluent *C. parvum* concentrations were typically ~1-10 oocysts/L during stable filter operation. Marginally worse oocyst removals were observed during ripening in both dual- and tri-media filters, when filter effluent oocyst concentrations were ~1-50 oocysts/L. Filter effluent oocyst concentrations of ~10^4-10^5 oocysts/L indicated substantial deterioration of *C. parvum* removal by both filter types during coagulation failure. Filter influent and effluent *C. parvum* concentrations, measured during stable operation, ripening, and coagulation failure, are presented in Table 5.1 to Table 5.3 respectively.

Log_{10} removals of *C. parvum* were calculated from the individual pairs of measured filter influent and effluent oocyst concentrations that were presented in Table 5.1 to Table 5.3. Calculated log removals for the experiments discussed in this chapter are available in Appendix D (Table D.1). The dual-media filter removals of viable and inactivated oocysts were summarized in a box-and-whisker plot (Figure 5.8). In these plots, the square in the center represents the median removal (50^{th} percentile). The lower and upper

portions of the box respectively indicate the 25th and 75th percentile removals. The lower and upper portions of the line (whisker) respectively indicate the minimum and maximum removals observed.

Coagulated, formalin-inactivated oocyst removal by the dual-media filter ranged from 4.7 to 5.7-log during stable operation, with a median removal of 5.3-log; coagulated, viable oocyst removal also ranged from 4.7 to 5.7-log with a median removal of 5.1-log. Dual-media removals of coagulated, formalin-inactivated oocysts decreased somewhat during ripening when removals ranged from 4.0 to 5.1-log, with median removal of 4.6-log; viable oocyst removals similarly ranged from 4.0 to 5.4-log with a median removal of 4.8-log. The most substantial decrease in oocyst removal by the dual-media filter occurred during coagulation failure when removal of non-coagulated formalin-inactivated oocysts ranged from 0.6 to 1.3-log, with a median removal of 0.8-log. Dual-media removals of non-coagulated viable oocysts ranged from 0.4 to 1.2-log during coagulation failure, with a median removal of 0.6-log.

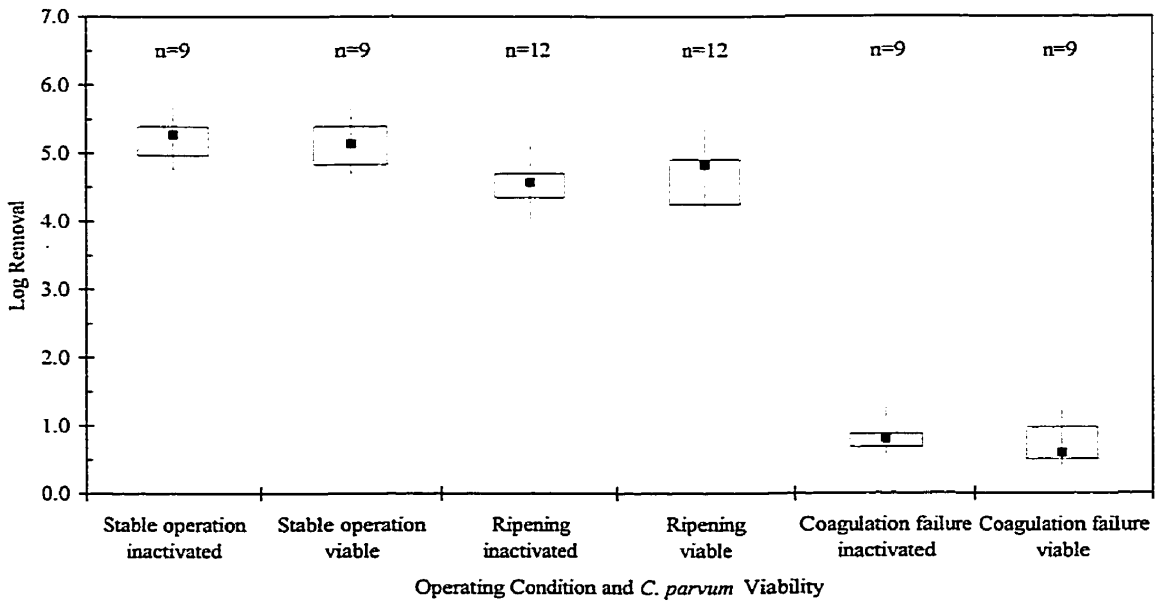


Figure 5.8 Dual-media filter removals of viable and inactivated *Cryptosporidium* during stable operation, ripening, and coagulation failure.

Tri-media filter removals of viable and inactivated oocysts were also summarized in a box-and-whisker plot (Figure 5.9). Coagulated, formalin-inactivated oocyst removal by the tri-media filter ranged from 4.9 to 5.8-log during stable operation, with a median removal of 5.4-log; coagulated, viable oocyst removal ranged from 4.6 to 5.8-log with a median removal of 5.3-log. Similar to dual-media filtration, the removal of coagulated, formalin-inactivated oocysts by tri-media filtration was lower during ripening when removals ranged from 4.2 to 5.3-log, with a median removal of 5.0-log; viable oocyst removals similarly ranged from 4.4 to 5.7-log with a median removal of 5.1-log. During coagulation failure, 0.6 to 2.2-log removal of non-coagulated formalin-inactivated oocysts was achieved by the tri-media filter, with a median removal of 1.1-log; removals of non-coagulated viable oocysts ranged from 0.5 to 2.5-log in the tri-media filter, with a median removal of 0.8-log. Of the operating conditions studied, coagulation failure resulted in the greatest deterioration of *Cryptosporidium* removal by tri-media filters, a result that was consistent with the findings of the dual-media experiments.

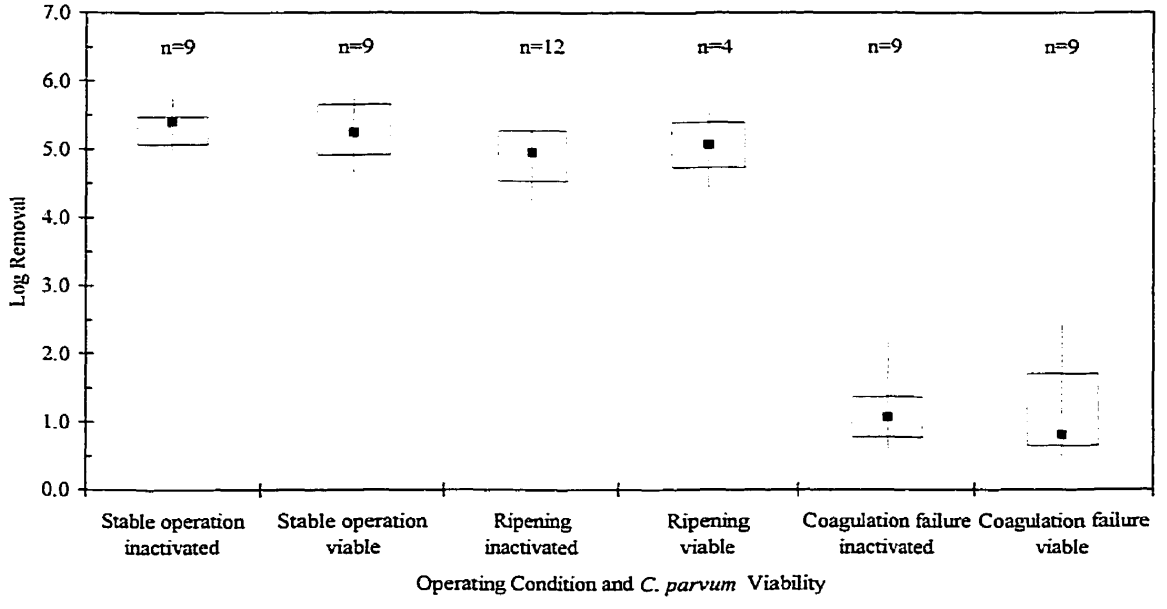


Figure 5.9 Tri-media filter removals of viable and inactivated *Cryptosporidium* during stable operation, ripening, and coagulation failure.

Since both the dual- and tri-media filters did not demonstrate substantial differences between removals of viable and formalin-inactivated oocysts, *Cryptosporidium* removals during dual- and tri-media stable operation, ripening, and coagulation were also compared by pooling the viable and inactivated oocyst removal data. The pooled dual- and tri-media data in the box-and-whisker plot in Figure 5.10 illustrated that tri-media filter removal of oocysts was only marginally better than dual-media removal of oocysts during stable filter operation. The same result was found during filter ripening. During coagulation failure, tri-media removal of oocysts was considerably more variable than in the dual-media filter. The pooled data did not demonstrate substantial differences between the dual- and tri-media removals of *C. parvum* during coagulation failure (α=0.05); however, the tri-media removals were slightly higher.

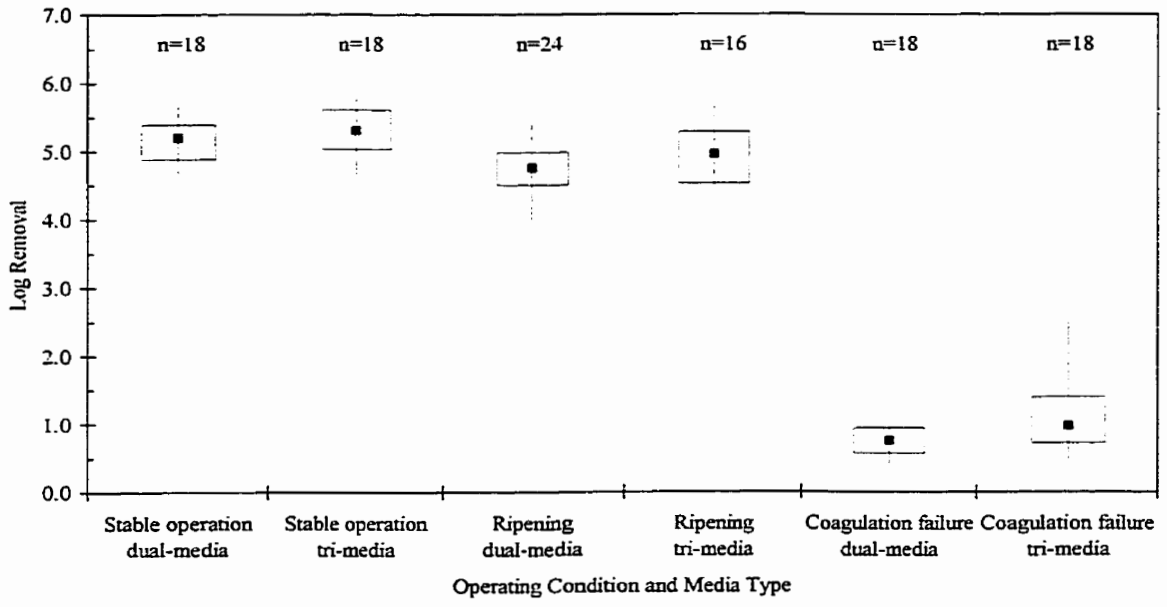


Figure 5.10 Pooled dual- and tri-media filter removals of *Cryptosporidium* during stable operation, ripening, and coagulation failure.

The theoretical filter influent oocyst concentration ($C_{theoretical}$) was calculated for each experiment by the equation

$$C_{theoretical} = \frac{\frac{C_{spike} V_{spike}}{V_{seed}} Q_{seed}}{Q_{filter} + Q_{seed}} \quad (5.1)$$

in which C_{spike} is the original oocyst concentration (determined with a hemocytometer) present in a volume of V_{spike}. The oocyst spike was added to a water volume of V_{seed}; this constituted the seed suspension that was added to the filter influent at a volumetric flow rate of Q_{seed}. Specific seeding conditions and detailed information necessary for the calculation of $C_{theoretical}$ are available in Appendix D, Table D.2.

Equation 5.1 describes the expected filter influent oocyst concentration by accounting for dilution of the seed suspension as it is introduced into the filter influent flow stream. Measured filter influent oocyst concentrations (C_{FI}) during the experiments were consistently ~75% of the theoretical filter influent oocyst concentration ($C_{theoretical}$). These concentrations ranged from 50% to 91% of the theoretical filter influent oocyst concentration. Consistent with the analytical recovery studies described in Appendix A, an assumption of 75% analytical recovery for the direct vacuum filtration/IFA method used to enumerate the measured filter influent concentrations (C_{FI}) and inspection of the $C_{theoretical}/C_{FI}$ ratio suggests essentially no loss of oocysts to the seeding apparatus. From these data it can be concluded that the observed *C. parvum* removals were due to filtration, rather than system losses. These data also demonstrated that the seeding practices consistently resulted in comparable ratios of $C_{FI}/C_{theoretical}$ and coefficients of variation, suggesting that oocyst seeding was comparable in the dual- and tri-media filters during all of the operational conditions investigated. The theoretical and measured filter influent data for all of the experiments discussed in this chapter are reported in Table 5.4.

Table 5.4
Theoretical and Measured Filter Influent *C. parvum* Concentration Data

Experiment	Dual-Media			Tri-media		
	$C_{theoretical}$ (oocysts/L)	C_{FI} (oocysts/L)	$C_{FI}/C_{theoretical}$ (-)	$C_{theoretical}$ (oocysts/L)	C_{FI} (oocysts/L)	$C_{FI}/C_{theoretical}$ (-)
Stable Inactivated 1	714333	5.52E+05	0.77	714333	5.86E+05	0.82
	714333	5.66E+05	0.79	714333	6.22E+05	0.87
	714333	4.84E+05	0.68	714333	5.98E+05	0.84
Stable Inactivated 2	714333	4.79E+05	0.67	714333	4.49E+05	0.63
	714333	5.52E+05	0.77	714333	5.34E+05	0.75
	714333	5.58E+05	0.78	714333	5.20E+05	0.73
Stable Inactivated 3	714333	5.03E+05	0.70	714333	5.52E+05	0.77
	714333	4.66E+05	0.65	714333	5.08E+05	0.71
	714333	4.87E+05	0.68	714333	5.85E+05	0.82
Stable Viable 1	690333	5.36E+05	0.78	627340	5.69E+05	0.91
	690333	4.77E+05	0.69	627340	4.51E+05	0.72
	690333	5.78E+05	0.84	627340	4.80E+05	0.76
Stable Viable 2	690333	4.91E+05	0.71	627340	3.69E+05	0.59
	690333	4.64E+05	0.67	627340	3.56E+05	0.57
	690333	4.64E+05	0.67	627340	3.98E+05	0.64
Stable Viable 3	690333	3.82E+05	0.55	627340	3.27E+05	0.52
	690333	3.97E+05	0.57	627340	3.68E+05	0.59
	690333	4.10E+05	0.59	627340	3.28E+05	0.52
Ripening Inactivated 1	561000	3.68E+05	0.66	561000	4.26E+05	0.76
	561000	3.94E+05	0.70	561000	4.47E+05	0.80
	561000	3.51E+05	0.63	561000	4.61E+05	0.82
	561000	3.77E+05	0.67	561000	4.57E+05	0.81
Ripening Inactivated 2	561000	4.11E+05	0.73	561000	3.79E+05	0.68
	561000	3.88E+05	0.69	561000	3.89E+05	0.69
	561000	4.04E+05	0.72	561000	3.60E+05	0.64
	561000	3.66E+05	0.65	561000	3.70E+05	0.66
Ripening Inactivated 3	561000	4.24E+05	0.76	698000	5.53E+05	0.79
	561000	4.24E+05	0.76	698000	5.69E+05	0.82
	561000	4.53E+05	0.81	698000	5.26E+05	0.75
	561000	4.25E+05	0.76	698000	5.34E+05	0.77

Table 5.4
Theoretical and Measured Filter Influent *C. parvum* Concentration Data
(Continued)

Experiment	Dual-Media			Tri-media		
	$C_{theoretical}$ (oocysts/L)	C_{FI} (oocysts/L)	$C_{FI}/C_{theoretical}$ (-)	$C_{theoretical}$ (oocysts/L)	C_{FI} (oocysts/L)	$C_{FI}/C_{theoretical}$ (-)
Ripening	690333	5.53E+05	0.80	627340	4.01E+05	0.64
Viable 1	690333	4.91E+05	0.71	627340	4.78E+05	0.76
	690333	5.32E+05	0.77	627340	4.25E+05	0.68
	690333	5.58E+05	0.81	627340	4.57E+05	0.73
Ripening	690333	4.26E+05	0.62	---	---	---
Viable 2	690333	4.69E+05	0.68	---	---	---
	690333	4.64E+05	0.67	---	---	---
	690333	4.44E+05	0.64	---	---	---
Ripening	690333	5.98E+05	0.87	---	---	---
Viable 3	690333	5.75E+05	0.83	---	---	---
	690333	6.00E+05	0.87	---	---	---
	690333	5.50E+05	0.80	---	---	---
Coagulation Failure	714333	5.05E+05	0.71	698000	5.24E+05	0.75
Inactivated 1	714333	5.31E+05	0.74	698000	5.87E+05	0.84
	714333	5.85E+05	0.82	698000	5.80E+05	0.83
Coagulation Failure	714333	4.21E+05	0.59	698000	4.84E+05	0.69
Inactivated 2	714333	4.64E+05	0.65	698000	5.04E+05	0.72
	714333	4.60E+05	0.64	698000	4.38E+05	0.63
Coagulation Failure	714333	5.96E+05	0.83	698000	5.44E+05	0.78
Inactivated 3	714333	6.21E+05	0.87	698000	5.52E+05	0.79
	714333	5.62E+05	0.79	698000	6.21E+05	0.89
Coagulation Failure	690333	5.38E+05	0.78	627340	3.75E+05	0.60
Viable 1	690333	5.50E+05	0.80	627340	3.12E+05	0.50
	690333	4.48E+05	0.65	627340	3.87E+05	0.62
Coagulation Failure	690333	5.78E+05	0.84	627340	3.71E+05	0.59
Viable 2	690333	5.91E+05	0.86	627340	4.00E+05	0.64
	690333	5.09E+05	0.74	627340	4.20E+05	0.67
Coagulation Failure	690333	4.62E+05	0.67	627340	3.75E+05	0.60
Viable 3	690333	4.55E+05	0.66	627340	4.00E+05	0.64
	690333	4.94E+05	0.72	627340	3.96E+05	0.63
AVERAGE			**0.73**			**0.71**
COEFF. VARIATION			**9%**			**14%**

5.5 DISCUSSION

The >5-log pilot-scale removals achieved during the stable filter operation experiments discussed in this chapter are on the higher end of *C. parvum* removals that have been reported in the literature. Full-scale oocyst removals have typically been reported in the range of 2 to 3-log (*e.g.*, Kelley *et al.*, 1995; Nieminski and Ongerth, 1995). Pilot-scale *C. parvum* removal data have suggested that filters can achieve anywhere from 2-3 log (*e.g.*, Ongerth and Pecoraro, 1995; Kelley *et al.*, 1995; Patania *et al.*, 1995), to 3-4 log (Yates *et al.*, 1997), or >5-log (*e.g.*, Patania *et al.*, 1995; LeChevallier *et al.*, 1991c) removal of oocysts. Several explanations are possible for this range of differences in *C. parvum* removal by filtration.

When discussing oocyst removal data, it is important to note that log removal estimates are often limited by influent concentration. Assuming no background levels of oocysts, a filter cannot remove more oocysts than are naturally present in the raw water or have been spiked into the water. Furthermore, the removal efficiency of a filter can only be fully evaluated when oocysts are present in the effluent in reliably countable concentrations (a count of at least 10 oocysts per sample was suggested in Chapter 4). When no oocysts are found in filter effluent samples, detection limits are commonly used to calculate log removals; several different practices for reporting detection limits have been used in the literature. Reported log removals may be highly inaccurate in such cases and larger sample volumes or higher influent concentrations may have been necessary for achieving adequate numbers of oocysts in filter effluent samples. Ensuring high seeded oocyst concentrations can be particularly critical when evaluating the oocyst removal capacity of filters under optimal operating conditions where filter effluent oocyst concentrations are often low (*e.g.*, Table 5.1 to Table 5.3).

Analytical differences may also contribute to reported variability in the *C. parvum* removal capacities of filters between studies. Several analytical methods with varied reliabilities have been reported in the literature (Vesey *et al.*, 1993a; Nieminski *et al.*, 1995; Clancy *et al.*, 1999). When comparing *C. parvum* data from different studies, it is critical to consider such factors as analytical recovery (and whether or not it is

incorporated into the final assessment of oocyst removal) and uncertainty associated with recovery. To date, very few studies have incorporated analytical recovery into calculations of oocyst removal. Although a few authors have suggested methods for incorporating analytical recovery and uncertainty associated with recovery (Nahrstedt and Gimbel, 1996; Parkhurst and Stern, 1998), the suggested approaches have been rarely, if ever, applied in the literature. The considerable differences in the reported range of *C. parvum* removals in the literature are not surprising given the lack of reliable analytical methods and statistical tools for describing data uncertainty associated with analytical inconsistencies.

In addition to analytical issues, *C. parvum* removal data could also be substantially affected by the operating conditions during which removal assessments were performed; the limited reporting of such operational data has in part prompted the research described in this thesis. The present study concurs with others (Patania *et al.*, 1995; Charles *et al.*, 1995) that suggested that some periods during the filter cycle (*e.g.*, sub-optimal coagulation) have a higher likelihood of pathogen passage than others (*e.g.*, optimized operation). Deviations from optimal operating conditions might have contributed to the range of *Cryptosporidium* removals reported in the filtration literature. Filter media depth and size distribution might also play a role. The differences in oocyst removals during the operating conditions discussed in the present study underscored the need for accurate and thorough description of experimental operational and design conditions and further investigation of *C. parvum* removals by filters; this type of research was performed and is discussed in Chapter 6.

The present bench-scale study indicated that formalin-inactivated oocysts were reliable surrogates for viable oocysts during removal studies. The stable operation and ripening experiments did not demonstrate substantial differences between removals of jar-coagulated, viable and formalin-inactivated *C. parvum* when the filters treated optimally coagulated water. Without coagulation of either the raw water or the oocysts, inactivated oocysts were removed somewhat more readily than viable oocysts, possibly due to differences in surface charge; however, a larger range of removals was observed for the viable oocysts. This result was particularly important because differences in oocyst zeta

potential were likely most pronounced during coagulation failure. The relatively small difference between zeta potentials of viable and inactivated oocysts that has been reported in the literature (Ongerth and Pecoraro, 1996) may contribute to differences in oocyst removal by filtration with increased replication. Although such a finding might be useful in understanding laboratory results obtained from filters challenged with non-coagulated oocysts, it is unlikely to be of practical significance at full-scale where oocyst surface charge is impacted by multiple factors including coagulation.

It is important to note that during the present investigation the filters were conditioned with optimally coagulated water for two hours prior to the stable operation and coagulation failure experiments. In accordance with filtration theory, it was expected that the conditioning period prior to oocyst seeding resulted in the capture of particles that subsequently acted as collectors within the filter. The captured particles were expected to contribute to continued particle (and oocyst) removal during seeding. Thus, the stable operation experiments realistically represented the optimal filtration conditions. During the coagulation failure experiments, the two-hour conditioning period likely favored more oocyst removal than would have occurred if the filter media had not been exposed to coagulated water prior to oocyst seeding. The relative impact of the conditioning period on oocyst removal in a specific water type and coagulation regime not currently understood, however. The relative importance of filter conditioning at stable operating conditions prior to coagulation upsets is discussed in greater detail in Chapter 6.

If the filtered water had not been coagulated at all, differences in oocyst zeta potential might have resulted in different removals of viable and inactivated oocysts by filtration. Although coagulant-free conditions do not occur during normal water treatment, investigations of coagulant-free conditions may be warranted. The coagulation failure experiments conducted during the present research were representative of a coagulant feed failure. These experiments successfully demonstrated a worst-case assessment of the adequacy of using formalin-inactivated oocysts as surrogates for viable during challenge studies.

Coagulation failure represented the period of greatest oocyst passage of the conditions investigated, regardless of media type (Figure 5.10). *C. parvum* removal by filtration decreased by >3-log as a result of coagulation failure; oocyst removals during this period were dramatically lower than during either ripening or stable operation. The tremendous deterioration in oocyst removal during coagulation failure concurred with other studies that also demonstrated that optimized coagulation was critical for achieving good *C. parvum* removal by filters (Patania *et al.*, 1995; Charles *et al.*, 1995; Ongerth and Pecoraro, 1995; Dugan *et al.*, 1999). Perhaps most importantly, the coagulation failure data presented in this chapter revealed that substantial *C. parvum* passage through filters could occur at turbidities below 0.3 NTU (Figure 5.11 to Figure 5.14), which is the current filter effluent turbidity standard mandated by the U.S. EPA's Interim Enhanced Surface Water Treatment Rule (USEPA, 1998). These data emphasized that prompt response to coagulation upsets can be an important strategy for minimizing *Cryptosporidium* and likely other pathogen passage through filtration. In addition, these data also underscored the generally accepted idea that while traditional performance measures such as turbidity are indicative of treatment performance, they are not necessarily quantitative indicators of *C. parvum* removal by filtration. This point is emphasized when coagulation failure is contrasted with ripening in dual-media filters (Figure 5.11 to Figure 5.14); indicating that the same filter effluent turbidities could correspond to very different oocyst removals by filtration.

It should be noted that the data in Figure 5.11 to Figure 5.14 were collected when filter effluent turbidities were rapidly changing. Although the oocyst concentrations and log removals originated from samples collected over 15 minutes during coagulation failure, the filter effluent turbidity values were those measured at the start of the 15-minute sampling period. This is less of an issue when examining the ripening data because *C. parvum* samples were collected every five minutes while turbidity was measured every three minutes. A more detailed assessment of the relationships between traditional performance measures (turbidity and particle counts) and oocyst removal by filters is presented in Chapters 6 and 7.

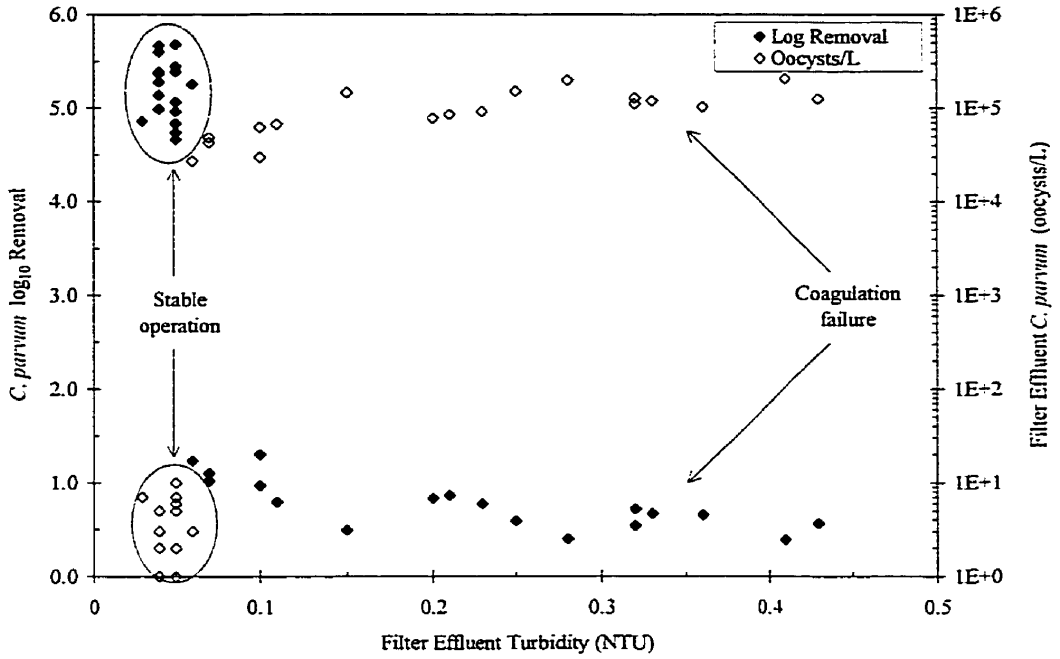


Figure 5.11 Dual-media filter effluent oocyst concentrations and oocyst log removals as a function of filter effluent turbidity during stable operation and coagulation failure.

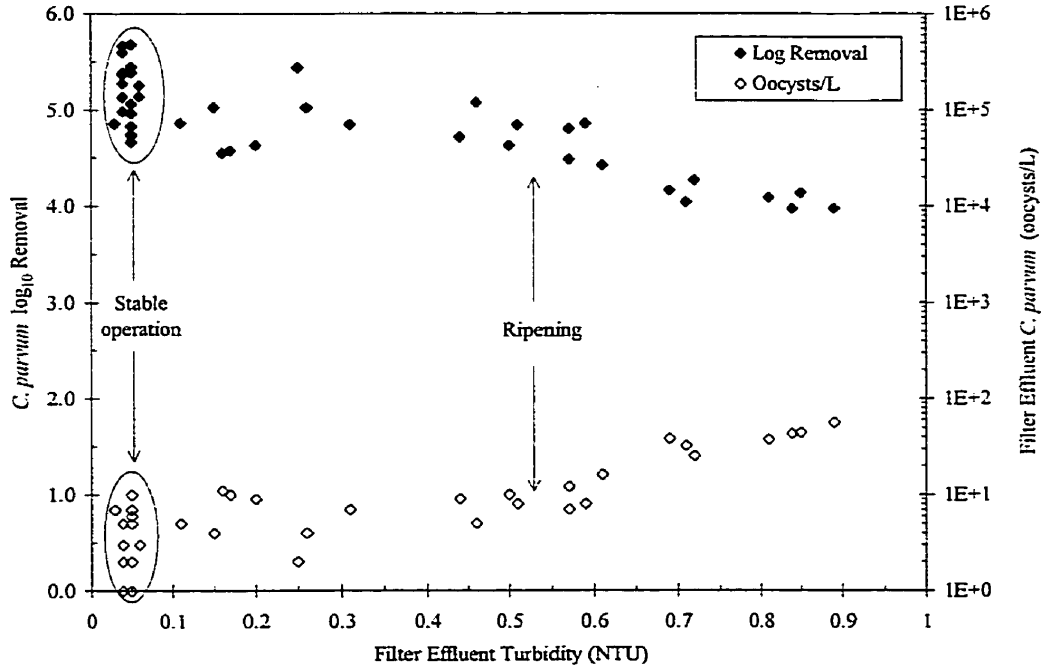


Figure 5.12 Dual-media filter effluent oocyst concentrations and oocyst log removals as a function of filter effluent turbidity during stable operation and ripening.

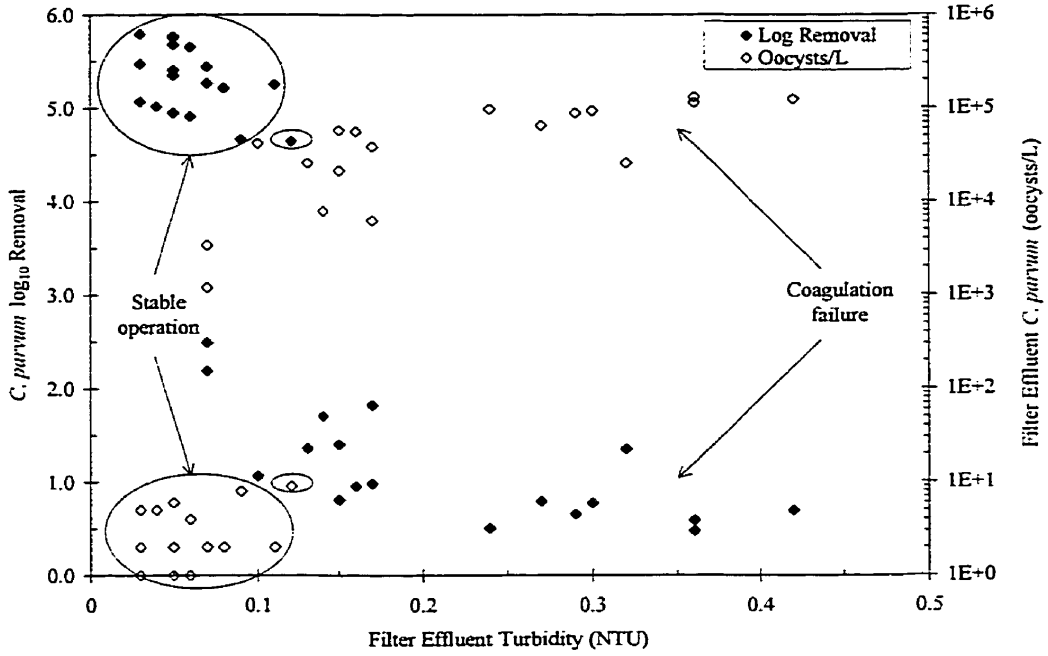


Figure 5.13 Tri-media filter effluent oocyst concentrations and oocyst log removals as a function of filter effluent turbidity during stable operation and coagulation failure.

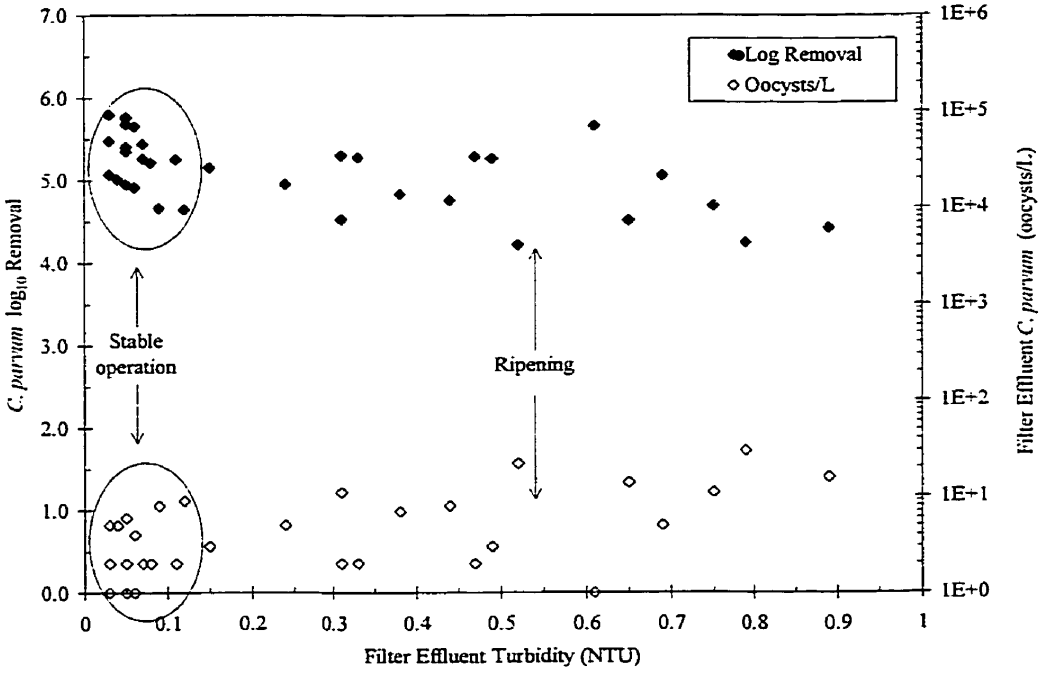


Figure 5.14 Tri-media filter effluent oocyst concentrations and oocyst log removals as a function of filter effluent turbidity during stable operation and ripening.

Summarized in Table 5.4, measured filter influent oocyst concentrations (C_{FI}) during each of the replicate experiments were consistently ~75% of the theoretical filter influent oocyst concentration ($C_{theoretical}$). The recovery data presented in Chapter 3 indicated that the analytical method for *C. parvum* consistently achieved approximately 75% recovery of oocysts from both filter influent and effluent samples from the water matrix studied during the investigations described in this chapter. Assuming that an analytical recovery of 75% contributed to the difference between the theoretical ($C_{theoretical}$) and measured (C_{FI}) filter influent *C. parvum* concentrations presented in Table 5.4, it could be concluded that almost all of the seeded oocysts reached the filter (*i.e.*, negligible loss of oocysts to the seeding equipment). This was considered critical for achieving consistent results and reliably countable oocyst concentrations in the filter effluent, especially during stable operation.

Unaffected by losses to the seeding apparatus, log removal calculations were based on measured influent oocyst concentration rather than theoretical seeded concentration. Log_{10} removals of *C. parvum* were calculated from the individual pairs of measured filter influent and effluent oocyst concentrations. As discussed in Chapter 3, the analytical method for *C. parvum* yielded comparable recovery profiles from the filter influent and effluent water matrices studied during the present investigation. Therefore, the recovery profiles did not greatly impact confidence intervals for oocyst removal because the inherent methodological uncertainty of recovery was consistent.

5.6 CONCLUSIONS

This investigation has yielded several important insights into *Cryptosporidium parvum* removal by drinking water filters. The following conclusions can be drawn from the research presented in this chapter:

1. Formalin-inactivated *C. parvum* oocysts were found to be reliable surrogates for viable oocysts during filtration studies conducted during stable operation, ripening, and coagulation failure in both dual- and tri-media filters, suggesting that formalin-inactivated oocysts are good surrogates for viable oocysts. This is a very important finding because virtually all filtration studies investigating *C. parvum* removal are conducted with inactivated oocysts for practical reasons.

2. *C. parvum* removals by filtration were moderately lower (~0.5 to 1-log) during ripening than during stable operation.

3. During the coagulation failure conditions investigated, the *C. parvum* removal capacity of both dual- and tri-media filters was severely and significantly compromised relative to both filter ripening and stable (optimized) operation. Coagulation failure decreased *C. parvum* removal by >3-log relative to stable operation.

4. *C. parvum* removals were not substantially different in dual- and tri-media filters during stable filter operation, ripening, and coagulation failure. Increased replication may reveal any significance of the marginally higher median removals achieved by tri-media filtration.

Chapter 6
C. parvum and Potential Surrogate Removal by Filtration

6.1 Introduction

Traditional filtration performance parameters such as turbidity (USEPA, 1989) and particle counts are good indicators of filtered water quality, however, they are not reliable surrogates for *C. parvum* removal during drinking water treatment (Nieminski and Ongerth, 1995; Patania *et al.*, 1995; Fox *et al.*, 1998). While removals of other microbial parameters such as aerobic spores of *Bacillus subtilis* have correlated somewhat with the removal of *C. parvum* by physico-chemical processes such as granular media filtration, no adequate quantitative surrogate for removal of the pathogen has yet been identified. The research described in this chapter investigates design and operational strategies for maximizing *C. parvum* removal by filtration. A secondary objective of this work was to evaluate the adequacy of oocyst-sized polystyrene microspheres as surrogates for the removal of *C. parvum* by filtration and to demonstrate how their removal compares to that of *B. subtilis* spores.

The experimental approach employed during this research consisted of:

1. identifying benchmark removals of pathogens and surrogates during stable (optimized) operation;

2. investigating pathogen removals during vulnerable periods of filter operation and relating them quantitatively to removals during stable operation; and,

3. investigating design and operational strategies for maximizing pathogen removals during potable water production.

Details regarding the experimental approach, experimental facilities, raw water qualities, and analytical methods are provided in Chapter 3. Key points are summarized below.

Pilot-scale experiments were conducted at three research platforms: the Ottawa pilot plant, the Windsor pilot plant, and the University of Waterloo (UW) pilot plant. The multiple research platforms allowed for the investigation of different raw water types, water temperatures, coagulation regimes, and filter designs. Formalin-inactivated *C. parvum* oocysts were seeded to a final filter influent concentration of ~10^5 oocysts/L at each experimental location. Microspheres were evaluated as potential surrogates for *C. parvum* because they were oocyst-sized, easy to identify, and relatively easy to enumerate; they were seeded at Ottawa and the UW. Spores of *B. subtilis* were also examined as potential surrogates for *C. parvum* removal by filtration because they had previously demonstrated some success as surrogates for *C. parvum* removal (Scott *et al.*, 1997) and could act as benchmark surrogates to which the microsphere removals could be compared. Turbidity, particle counts, flow rates, and head loss were monitored during each experiment. The optimized treatment conditions were selected to meet the 0.1 NTU turbidity goal of the Partnership for Safe Water, a voluntary treatment optimization program sponsored by the U.S. Environmental Protection Agency and the American Water Works Association.

Except for one experiment specified later, microorganisms seeding occurred at the filter influent. In many experiments this transpired over a one-hour period, although in a few experiments the seeding period was extended. During seeding, several (typically three or four) pairs of filter influent and effluent samples were taken for the enumeration of the seeded microorganisms and microspheres; removals were calculated from these pairs. Each experimental condition was replicated several times.

The non–optimal operating conditions investigated were sub-optimal coagulation and a sudden increase in flow to the filter (hydraulic step). Both of these operational conditions may occur to varying degrees in real treatment plants. The ripening and breakthrough portions of the filter cycle were also investigated because of the potential for decreased microorganism removals during these periods. Ripening is an essentially unavoidable part of every filter cycle; in some plants the water produced during this period is filtered to waste rather than sent to the distribution system. In principle, potable water production during end-of-run turbidity or particle breakthrough can be avoided by terminating filter operation sufficiently early. Of course when this is done, shorter filter cycles result and increase costs due to an increased number of backwashes. The breakthrough experiments focused on identifying how long filters could be left in service without compromising *C. parvum* removal while filter effluent quality, though deteriorating, might still be considered acceptable by traditional turbidity or particle criteria.

As discussed in Chapter 3, samples (three, four, or five) were collected at the filter influent and filter effluent locations during each of the experiments. Sampling typically occurred during the period when the seed suspension was added at the filter influent. The statistical approach described in Chapter 4 was used to calculate the 95% confidence intervals and removal ranges for each experiment and operating condition. Statistical comparisons were made by calculating the upper and lower limits of the 95% confidence interval for each sample; differences between data sets were statistically different at the 5% significance level when the confidence intervals did not overlap. Overall removal ranges (described in Chapter 4), rather than overall confidence intervals were calculated for the experiments for which data could not be pooled due to dynamic operating conditions. For both the confidence interval and removal range calculations, the beta distribution parameters used to describe the *C. parvum* recovery profile were the overall Ottawa ($a = 23.91$, $b = 8.26$) and overall UW-1.5 NTU ($a = 11.05$, $b = 4.42$) parameters presented in Appendix C. The Ottawa recovery parameters were also used in assessing the Windsor data. All of the figures and tables containing data that incorporate the uncertainty associated with analytical recovery are clearly identified. Although mode and mean removals are presented in the tables, mode removals are used and presented in the figures because they represent the most likely removals (*i.e.*, the highest probability of

occurrence). The detailed experimental schedules, raw water quality data, performance data, microorganism and microsphere data, 95% confidence interval endpoints, and adjusted ranges (for non-pooled data) are provided in Appendix D.

6.2 OTTAWA PILOT PLANT INVESTIGATIONS

The majority of the pilot-scale *C. parvum*, *B. subtilis*, and polystyrene microsphere removal investigations were conducted at the Britannia Pilot Plant in Ottawa, Ontario, Canada. Described in Chapter 3, all of the seven general operating conditions investigated during this thesis research (stable filter operation, no coagulation, sub-optimal coagulation, ripening, hydraulic step, end-of-run, and breakthrough) were investigated at Ottawa, allowing for the relative comparison of *C. parvum* removal during the various operating conditions. Each condition was studied at least in triplicate; several sub-categories of the seven general conditions were also examined. The rationale and specific seeding conditions for each of the experiments were summarized in Chapter 3 and are elaborated upon below.

6.2.1 Stable (Optimized) Operation

Pilot-scale *C. parvum* removal data reported in the literature have suggested that filters can achieve anywhere from 2 to >5-log removal of oocysts (Patania *et al.*, 1995; Fox *et al.*, 1998). To determine the *C. parvum* removal capacity of the pilot filter in Ottawa, eight stable operation experiments were performed throughout the approximate two-year period of *C. parvum* removal investigations at the pilot plant. The stable operation experiments represented optimized pretreatment (coagulation, flocculation, and sedimentation) and filtration conditions (*i.e.*, no perturbations or upsets).

6.2.1.1 Experimental Design

The purpose of these experiments was to document the highest *C. parvum* (and *B. subtilis* and polystyrene microsphere) removals that could be obtained by the pilot-scale filter during optimal operating conditions. As mentioned previously, seeding and sampling were conducted after at least four hours of filter operation, during the early to middle

portion of the filter cycle. These experiments were repeated several times because they provided a baseline against which the other operating conditions were compared.

Eight stable operation experiments were conducted at Ottawa. Jar-coagulated *C. parvum* oocysts were seeded into the filter influent for one hour during these experiments. *B. subtilis* spores and yellow polystyrene microspheres were also seeded during some of these experiments. Samples were collected at 15, 30, 45, and 55 minutes after the start of seeding. Stable operation at Ottawa was considered as the period during which filter effluent turbidities and total particle concentrations ≥2μm were consistently <0.1 NTU and <5 particles/mL respectively during the entire filter cycle (after ripening).

One additional stable operation investigation was conducted in which seeding occurred at the rapid mix location. The purpose of this experiment was to document filter performance when the seeded microorganisms had been exposed to the sequence of coagulation, flocculation and sedimentation in the pilot plant. As discussed in Chapter 3, this approach could not be used on a regular basis because it would likely result in filter effluent *C. parvum* concentrations below the detection limit of the analytical method used during this research. A pulse-input of 10^8 *C. parvum* oocysts and 10^9 *B. subtilis* spores was used during this experiment. Though no microorganisms were expected in the filter effluent, this experiment was conducted to determine if there were gross differences in *C. parvum* removal by the pilot-filter as a result of jar-coagulation and direct seeding into the filter influent.

Two additional stable filter operation experiments were conducted during spring runoff conditions when changes in raw water quality impacted the chemical pretreatment process prior to filtration. Since the filters were able to maintain excellent filtered water quality (consistently <0.1 NTU filter effluent turbidity) during spring runoff, it was speculated that the stable operation microorganism removals during runoff would be similar to those achieved during non-runoff periods. The runoff experiments were conducted in a manner identical to the other stable operation experiments, with the exception that *B. subtilis* spores were not seeded. All of the stable operation experiments and seeding conditions are summarized in Table 6.1.

Table 6.1

Summary of Stable Operation Experiments at the Ottawa Pilot Plant

Experiment	Date	*C. parvum*	*B. subtilis*	Blue Spheres	Yellow Spheres
Stable Operation	08/06/98	✓			
	09/09/98	✓			
	09/22/98	✓			
	10/06/98	✓			
	03/09/99	✓	✓		
	05/31/99	✓	✓		
	07/27/99	✓	✓		
	01/19/00	✓	✓		✓
Stable Operation During Runoff	04/05/99	✓			
	04/12/99	✓			
Stable Operation Seeded at Rapid Mix	07/20/99	✓	✓		

6.2.1.2 Results

Filter effluent turbidity and particle counts were consistently low (~0.04 NTU and <5 particles/mL respectively) during the stable operation experiments. Filter effluent turbidity and particle counts, *C. parvum*, *B. subtilis*, and polystyrene microsphere removals, and total particle reductions through the treatment process during stable operation at Ottawa are summarized in Table 6.2. The general filter performance data and detailed instantaneous turbidity and particle data are available in Appendix D (Tables D.4 and D.5 respectively). The detailed instantaneous *C. parvum* and *B. subtilis* data are available in Table D.6 while detailed microsphere data are available in Table D.7.

Table 6.2

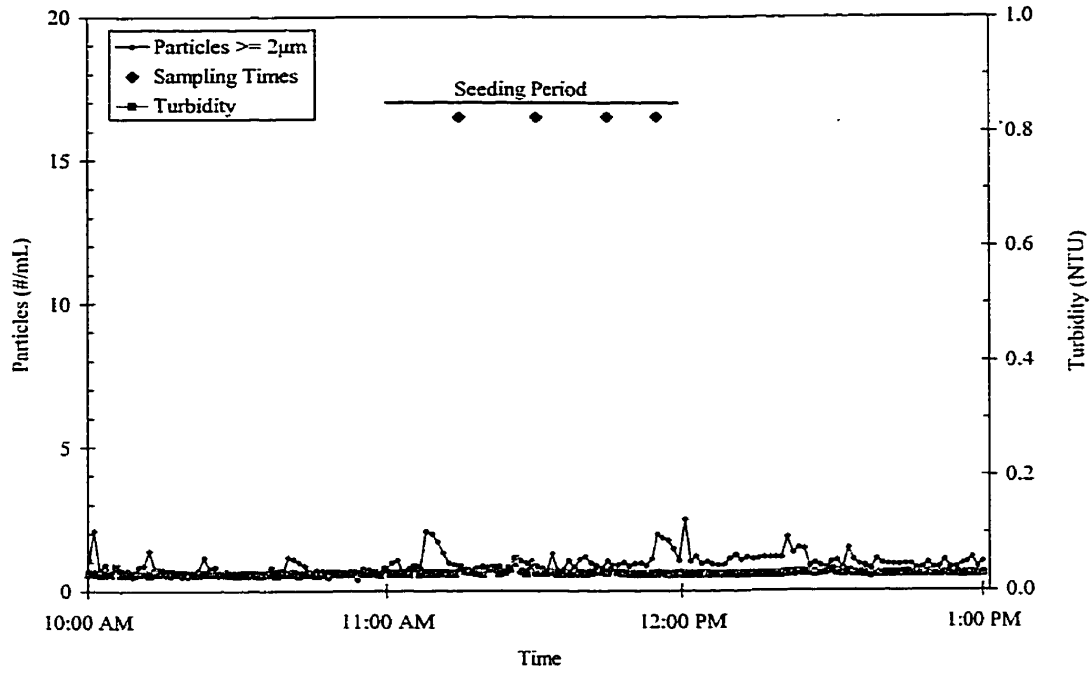
Filter Performance During Stable Operation at Ottawa

Date	Log_{10} Removal (mean ± standard deviation)				Log_{10} Red.*	Effluent Concentration	
	C. parvum	*B. subtilis*	Blue Spheres	Yellow Spheres	Particles ≥ 2μm	Particles (#/mL)	Turbidity (NTU)
08/06/98	4.9 ± 0.21	---	---	---	3.2 ± 0.29	3.7 ± 2.9	0.02 ± 0.00
09/09/98	5.7 ± 0.06	---	---	---	3.8 ± 0.10	0.9 ± 0.2	0.02 ± 0.00
09/22/98	5.8 ± 0.03	4.3 ± 1.51	---	---	2.8 ± 0.24	8.7 ± 5.6	0.03 ± 0.00
10/06/98	5.8 ± 0.15	---	---	---	4.6 ± 0.18	0.2 ± 0.1	0.02 ± 0.00
03/09/99	5.2 ± 0.38	2.1 ± 0.14	---	---	4.1 ± 0.10	0.4 ± 0.1	0.03 ± 0.00
05/31/99	5.6 ± 0.20	4.6 ± 0.05	---	---	3.7 ± 0.18	1.2 ± 0.6	0.03 ± 0.00
07/27/99**	>5.6 ± 0.02	4.5 ± 0.24	---	---	3.0 ± 0.22	5.1 ± 1.5	0.04 ± 0.00
01/19/00	5.3 ± 0.36	4.2 ± 0.01	---	4.9 ± 0.22	---	4.8 ± 0.6	0.03 ± 0.00
04/05/99	5.3 ± 0.29	---	---	---	4.0 ± 0.09	1.3 ± 0.2	0.04 ± 0.00
04/12/99	5.4 ± 0.18	---	---	---	3.9 ± 0.07	2.1 ± 0.3	0.03 ± 0.00
07/20/99**	>1.3 ± 0.93	1.1 ± 0.92	---	---	3.0 ± 0.10	5.7 ± 1.5	0.06 ± 0.01
Overall* **	**5.5 ± 0.37**	**3.9 ± 1.13**	**—**	**4.9 ± 0.22**	**3.6 ± 0.63**	**3.1 ± 3.5**	**0.03 ± 0.01**

*Log reduction of particles through treatment process (plant influent to filter effluent).
**All of filter effluent samples were non-detects – this is indicated by ">" symbol here.
***All experiments except for 04/05/99 (runoff), 04/12/99 (runoff), and 07/20/99 (seeded at rapid mix).

Traditional performance measures (turbidity and total particle counts) and filter effluent microorganism and microsphere concentrations were relatively constant during the stable operation investigations and consistent between replicate experiments. Filter effluent turbidity and total particle counts rarely exceeded 0.06 NTU and 5 particles/mL during stable operation at Ottawa. The turbidity and particle responses of the Ottawa filter during the May 31, 1999 stable operation experiment are shown in

6.1, which demonstrates the excellent and consistent filter effluent turbidity and particle concentrations achieved by the pilot filter during stable operation. This figure also indicates the seeding period and sampling times. The filter effluent turbidity and particle counts were generally consistent between the eight replicate stable operation experiments at Ottawa. The instantaneous turbidity and particle data and microorganism data are available in Appendix D (Tables D.5 and D.6, respectively).



6.1 Filter effluent turbidity and particle concentration during May 31, 1999 stable filter operation experiment at Ottawa.

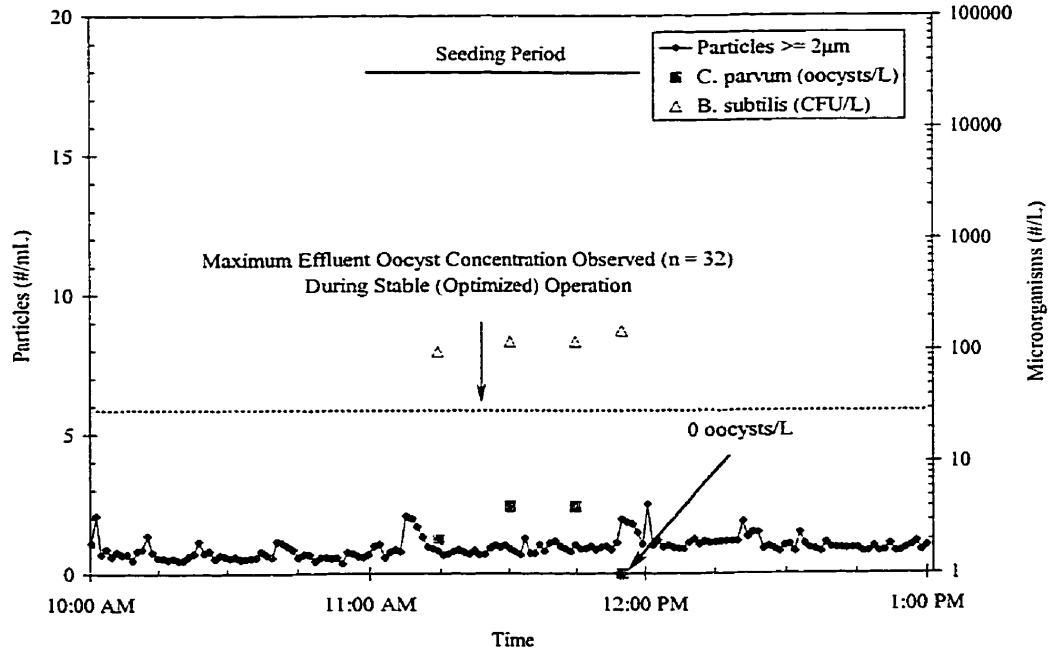


Figure 6.2 Filter effluent particle, *C. parvum*, and *B. subtilis* concentrations during May 31, 1999 stable filter operation experiment at Ottawa.

C. parvum passage through the Ottawa filter during stable operation was consistently low, with filter effluent concentrations often near the method detection limit. Filter effluent particle, *C. parvum*, and *B. subtilis* concentrations from the Ottawa filter during the May 31, 1999 stable experiment are shown in Figure 6.2. While filter influent *C. parvum* concentrations were typically 10^5 to 10^6 oocysts/L, the filter effluent concentrations were consistently less than 10 oocysts/L and often <1 oocyst/L. Though they were more variable between experiments, the filter influent *B. subtilis* concentrations were roughly comparable to those of *C. parvum*, particularly in the later experiments following the implementation of improvements to the *B. subtilis* analytical method. *B. subtilis* spores were invariably detected in the filter effluent samples at higher concentrations than the *C. parvum* oocysts, however, as shown in Figure 6.2, *B. subtilis* spore passage through the Ottawa filter was generally consistent with the *C. parvum* data.

Similar *C. parvum* oocyst and polystyrene microsphere removals were observed during the January 19, 2000 stable operation experiment at Ottawa. The filter effluent turbidity and total particle concentrations during the stable operation experiment investigating oocyst and microsphere removals are presented in Figure 6.3. The filter influent oocyst and microsphere concentrations during the stable operation experiment were similar (means of ~4.6×10^5 oocysts/L and ~6.5×10^5 microspheres/L respectively). Both *C. parvum* and microspheres were found in all of the effluent samples collected during this experiment (Figure 6.4). The filter effluent *C. parvum* concentrations during the January 19, 2000 experiment were less than 10 oocysts/L, which was consistent with the other stable operation experiments conducted at Ottawa. Though the filter effluent microsphere concentrations were more variable than those of *C. parvum*, they were generally of the same order of magnitude, with effluent concentrations of less than 13 microspheres/L. These data indicated that polystyrene microsphere passage through the Ottawa filter was also generally consistent with the *C. parvum* data. However, since only one experiment examined microsphere removals during stable operation, further investigations would be necessary to address the relationship between *C. parvum* and microsphere removals during this operating condition. The instantaneous *C. parvum*, *B. subtilis*, and microsphere data are available in Appendix D (Tables D.6 and D.7).

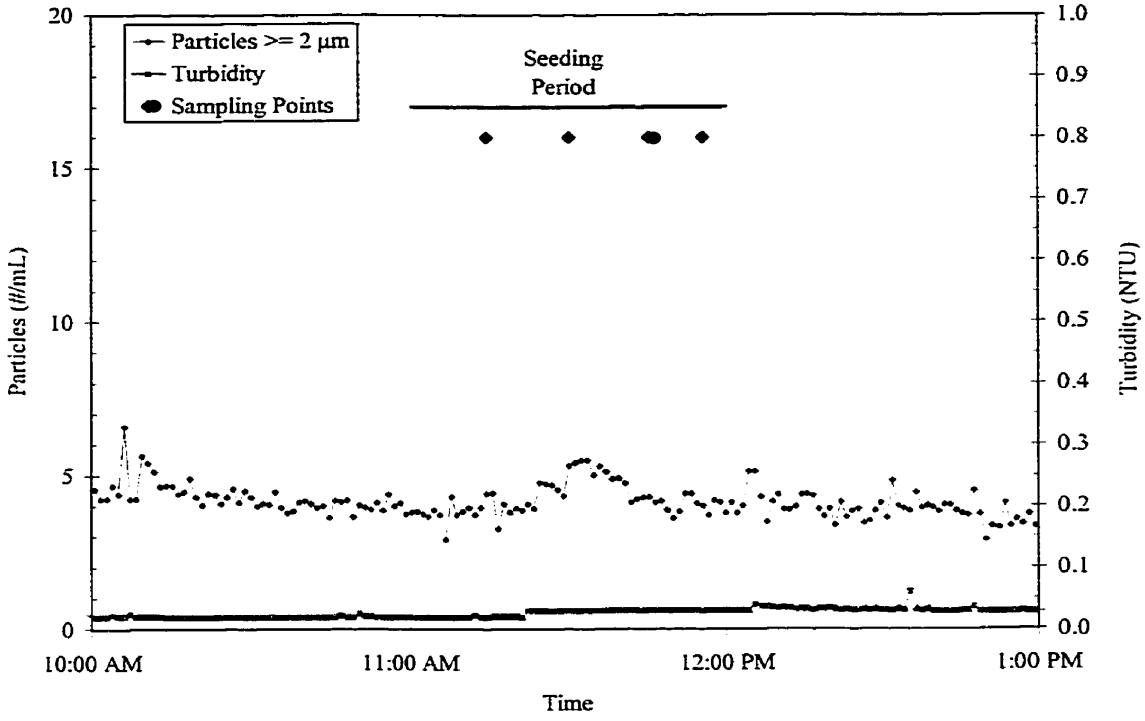


Figure 6.3 Filter effluent turbidity and particle concentration during January 19, 2000 stable filter operation experiment at Ottawa.

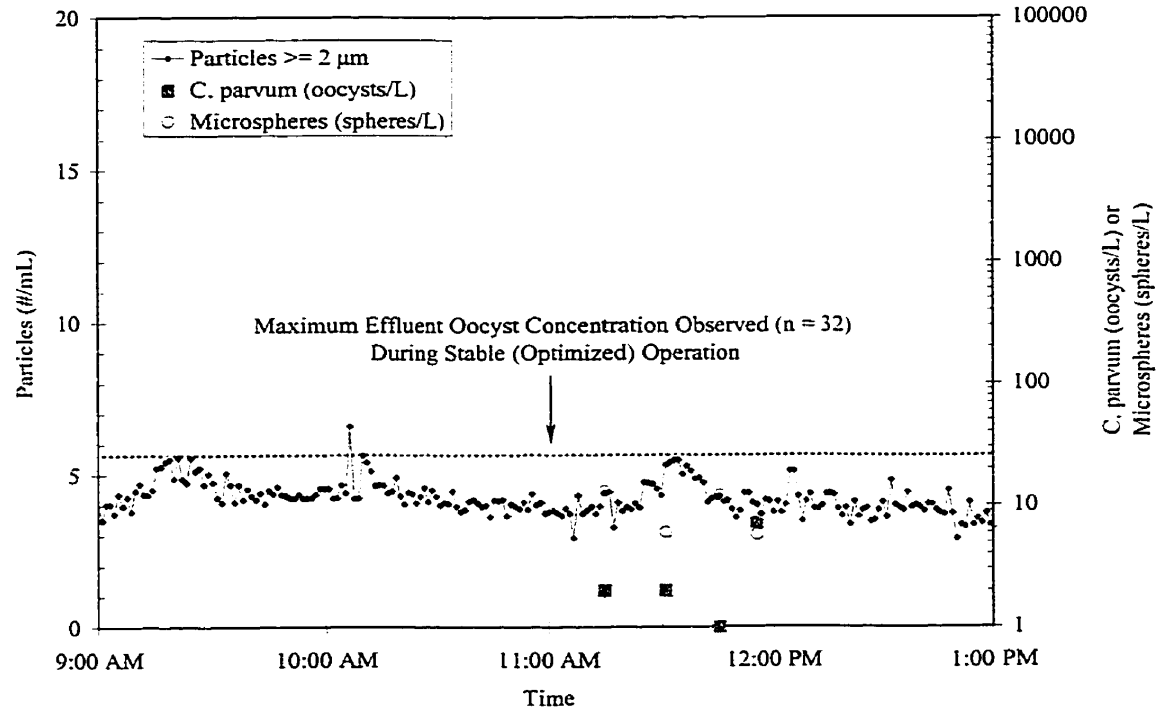


Figure 6.4. Filter effluent particle, *C. parvum*, and microsphere concentrations during January 19, 2000 stable filter operation experiment at Ottawa.

C. parvum removals during stable (optimized) operation ranged from 4.7 to 5.8-log, with a mean oocyst removal of 5.5-log (32 pairs in total). The experiments were conducted at several raw water temperatures (ranging from 1 to 24°C; Table C.4); no deterioration in *C. parvum* removals was observed at temperatures as low as 1 °C. *B. subtilis* removals ranged from 2.0 to 5.1-log, with a mean removal of 3.9-log (20 pairs in total); microsphere removals ranged from 4.7 to 5.1-log, with a mean removal of 4.9-log (4 pairs in total). The removal data are summarized in Table 6.2. Although there was some variation in removals calculated on the basis of individual influent-effluent sample pairs of microorganisms or spheres, the removals during a given experiment were fairly reproducible (Table 6.2). The single exception to the consistency of microorganism removals was an experiment (9/22/98) in which *B. subtilis* removals demonstrated a particularly high standard deviation; however, this variability was attributed to analytical difficulties. The subsequent standard deviations of stable operation experiments involving *B. subtilis* spores were considerably lower (Table 6.2).

B. subtilis removals by filtration were generally lower than *C. parvum* removals. This result is not surprising given that the *B. subtilis* spores are ~1 μm in size which is at the minimum transport efficiency for particle removal by filtration. Performing linear regression and comparing the *C. parvum* and *B. subtilis* removals during stable operation demonstrated that they were only weakly correlated, with a coefficient of determination (R^2) of only 0.16 or 0.19, depending on whether or not the 9/22/98 data set was included. (Figure 6.5). While spore removals were generally lower than oocysts removals, the lack of a relationship between oocyst and spore removals suggested that *B. subtilis* removal data were not quantitatively indicative of the filter's ability to remove *C. parvum*.

Polystyrene microsphere removals were generally lower than *C. parvum* removals during the stable operation experiment performed with microspheres. Though the negative slope of the best fit line achieved by linear regression suggests that microsphere removals were not a good surrogate for *C. parvum* removal by filtration, however, only one such experiment was performed during stable operation. More data would be necessary to better elucidate the relationship between *C. parvum* and microsphere removals during this operating period.

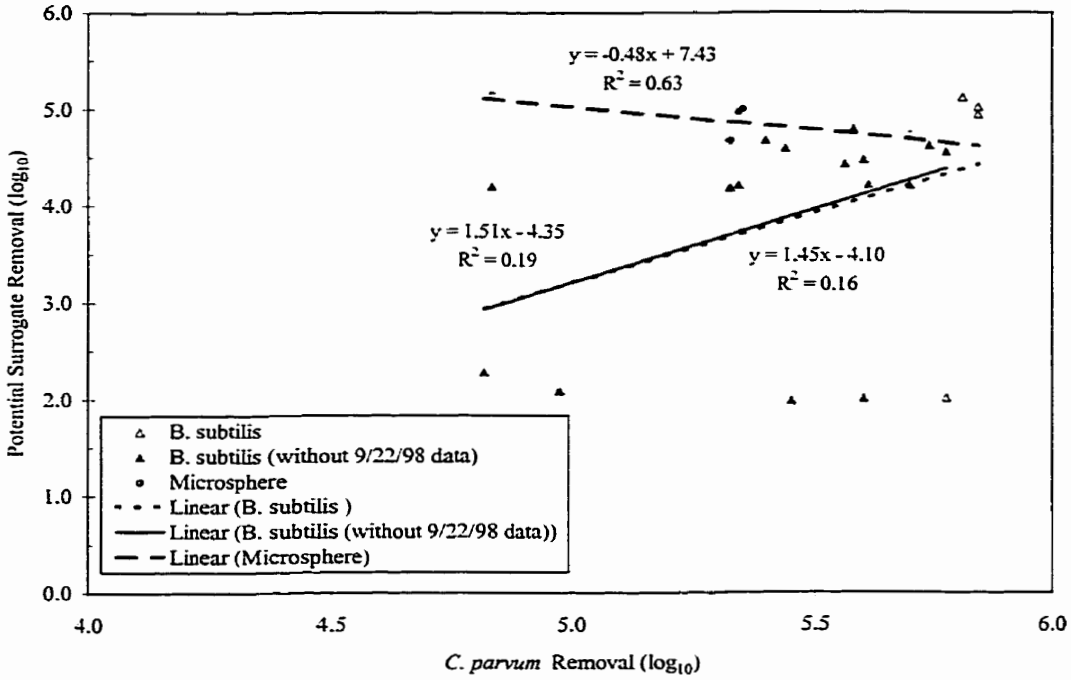


Figure 6.5 Relationship between *C. parvum*, *B. subtilis*, and microsphere removals by filtration during stable operation at Ottawa.

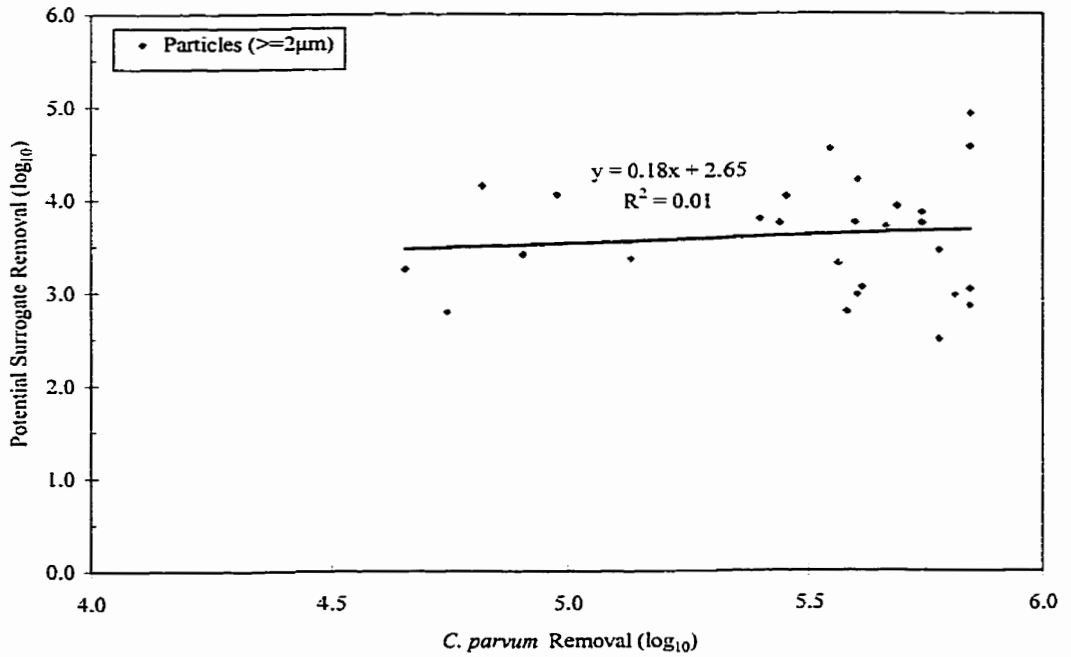


Figure 6.6 Relationship between *C. parvum* removal by filtration and total particle (≥2 µm) reductions through the plant during stable operation at Ottawa.

Although total particle reductions (≥ 2μm) through the treatment process were calculated (Table 6.2), it should be recalled that these removals were based on raw water rather than filter influent values. Filter influent particle concentrations could not be measured due to floc breakage and accumulation on and in the influent particle counter sensor chamber. As with *B. subtilis*, examination of the data in Table 6.2, might suggest that particle reduction through the treatment process was a conservative indicator of a filter's ability to remove *C. parvum* during stable operating conditions. As would be expected, a comparison of *C. parvum* removals and particle reductions during stable operation indicated that these parameters were not correlated (Figure 6.6). This result is not surprising given that particles can change in size in, be removed by, and attach and detach from media in the various treatment processes between the raw water and filter effluent sampling locations.

The two stable operation experiments conducted during spring runoff conditions were critical from an operational perspective because they were conducted during a period when chemical pretreatment was challenged due to a significant variation in the raw water quality. This ultimately resulted in considerably higher settled water turbidities (3-4 NTU during stable operation during spring runoff as compared to 0.5-2.5 NTU during non-runoff periods). Despite elevated raw and settled water turbidities (Table C.3), filter effluent turbidities and particle concentrations remained low during runoff operations and were comparable to other periods of stable filter operation (Table 6.2). *C. parvum* removals by filtration during spring runoff also appeared comparable to those achieved during the other stable operation periods (Table 6.2).

C. parvum and *B. subtilis* removal data for the experiment during which seeding occurred at the rapid mix are also presented in Table 6.2. The *C. parvum* and *B. subtilis* removals during this experiment were clearly lower than during the other stable operation experiments that involved microorganism seeding into the filter influent. The lower microorganism removals were associated with the considerably lower filter influent concentrations during this experiment (Table C.6) that resulted from microorganism removal during coagulation, flocculation, and sedimentation. In the case of *C. parvum*,

no oocysts were detected in the filter effluent samples during this experiment. The handling of non-detects is described in Chapter 3.

6.2.1.3 Statistical Analysis

Confidence intervals for the individual *C. parvum* log removals during stable operation were calculated using the method described in Chapter 4, and incorporated the uncertainty of analytical recovery. The endpoints of the 95% confidence intervals for oocyst removal are summarized in Table 6.3. Almost all of these confidence intervals overlap, thereby failing to demonstrate statistically significant differences between most of the data collected during stable operation (Table 6.3, α=0.05). Similarly the stable operation during spring runoff experiments also failed to demonstrate significant differences in *C. parvum* removals between the replicate experiments (Table 6.3, α=0.05). The paired removal data for each of these conditions were pooled because the data from each were expected to be replicates (since operational conditions and water quality were essentially consistent throughout the experiments) and failed to demonstrated significant differences in microorganism/microsphere removals (when the individual confidence intervals were compared). The overall 95% confidence interval for *C. parvum* removal during stable operation was 5.4- to 5.6-log (32 influent-effluent pairs of data), during spring runoff it was 5.1- to 5.4-log. The endpoints of the range indicate the lowest and highest endpoints of the individual 95% confidence intervals for the *C. parvum* removals observed during the operating period. The overall 95% confidence interval for the pooled microsphere removals was 4.6- to 4.9-log.

The *C. parvum* removal range was 4.0- to 7.2-log during stable operation and 4.4- to 6.6-log during stable operation during runoff. The range for the individual experiments is summarized in Table 6.3. Comparison of the removal ranges and the overall confidence intervals (calculated with pooled data) demonstrates how increased counts (achieved by either larger processed volumes of water or replicate samples) substantially decrease uncertainty. Although pooling was possible during stable operation, it would not be possible during more dynamic operating conditions.

A comparison of the confidence intervals for the stable operation and stable operation during runoff data also failed to demonstrate statistically significant differences between *C. parvum* removals during these periods (Table 6.3, α=0.05), suggesting that the filter could compensate for the challenged pretreatment conditions during this period. A comparison of the overall confidence intervals for *C. parvum* and microsphere removals did demonstrate significant differences at the 5% significance level, suggesting that the microspheres were removed to a lesser degree than oocysts.

Table 6.3

95% Confidence Intervals and *C. parvum* Removal Ranges

During Stable Operation at Ottawa

Experiment	Date	Sample Time	CI_{lower} (log_{10})	CI_{upper} (log_{10})	R_{lower} (log_{10})	R_{upper} (log_{10})
Stable Operation	08/06/98	t = 15	4.18	6.23	**4.02**	**6.45**
		t = 30	**4.02**	6.07		
		t = 45	4.12	5.44		
		t = 55	4.41	**6.45**		
	09/09/98	t = 15	5.01	**7.07**	**4.87**	**7.07**
		t = 30	**4.87**	6.93		
		t = 45	4.95	7.01		
		t = 55	4.93	6.99		
	09/22/98	t = 15	5.05	7.11	**5.11**	**7.17**
		t = 30	5.11	7.17		
		t = 45	5.09	7.15		
		t = 55	**5.11**	**7.17**		
	10/06/98	t = 15	5.11	7.17	**5.01**	**7.17**
		t = 30	5.11	7.17		
		t = 45	5.11	**7.17**		
		t = 55	**5.01**	6.33		
	03/09/99	t = 15	5.06	**6.38**	**4.43**	**6.38**
		t = 30	4.54	5.10		
		t = 45	**4.43**	4.90		
		t = 55	4.93	5.97		
	05/31/99	t = 15	5.01	7.07	**4.86**	**7.11**
		t = 30	**4.86**	6.18		
		t = 45	4.89	6.22		
		t = 55	5.05	**7.11**		
	07/27/99	t = 15	**4.84**	6.91	**4.84**	**6.96**
		t = 30	4.89	6.94		
		t = 45	4.84	6.91		
		t = 55	4.89	**6.96**		

Table 6.3

95% Confidence Intervals and C. parvum *Removal Ranges*

During Stable Operation at Ottawa (Continued)

Experiment	Date	Sample Time	CI_{lower} (log_{10})	CI_{upper} (log_{10})	R_{lower} (log_{10})	R_{upper} (log_{10})
	01/19/00	t = 15	4.79	6.12	**4.39**	**7.03**
		t = 30	4.81	6.13		
		t = 45	4.97	**7.03**		
		t = 55	**4.39**	5.17		
Stable Operation During Runoff	04/05/99	t = 15	**4.42**	5.01	**4.42**	**6.33**
		t = 30	4.82	5.70		
		t = 45	4.83	6.16		
		t = 55	5.01	**6.33**		
	04/12/99	t = 15	4.97	**6.29**	**4.66**	**6.29**
		t = 30	4.93	6.26		
		t = 45	**4.66**	5.44		
		t = 55	4.87	5.91		

6.2.1.4 Discussion

The stable filter operation experiments demonstrated that *C. parvum* removals of >5-log could be consistently achieved by the Ottawa filter at water temperatures ranging from 1 to 24°C. The filter was also able to compensate for higher settled water turbidities that occurred during stable operation during spring runoff conditions. These data provided the baseline removals to which other operating conditions at Ottawa were compared.

Although the microsphere removals during stable operation at Ottawa were significantly lower than the oocyst removals, this result does not necessarily suggest that microspheres are poor surrogates for *C. parvum* removal by filtration. It should be recalled that only one stable operation experiment was performed with microspheres and that a 1:1 relationship is not necessarily required for a good surrogate. The issue of microspheres as overall surrogates for *C. parvum* removal by filtration is discussed in Chapter 7.

6.2.2 Ripening

It has been suggested that 90% of the particles that pass through a well-operated filter do so during ripening (O'Melia and Ali, 1978; Amirtharajah, 1985; Cranston and

Amirtharajah, 1987). To assess the passage of *C. parvum* and *B. subtilis* through filters during ripening, several experiments were conducted during filter ripening at the Ottawa pilot plant after periods of stable operation. Relative to stable operation, lower microorganism removals were expected during the period of particle passage that is typically associated with non-attachment during ripening (O'Melia and Ali, 1978).

6.2.2.1 Experimental Design

During ripening at Ottawa, the filter effluent turbidity and particle concentration typically increased during the first 10 minutes of the experiment and then decreased toward the end of the seeding period. The seeding period during the ripening experiments was only 30 minutes because filter water quality typically improved to filter effluent turbidities < 0.1 NTU and particle concentrations < 5-10 particles/mL (such as those observed during stable operation) during the first thirty minutes of operation after backwashing. Samples were collected at 5, 10, 15, 20, and 25 minutes after seeding commenced. Sample collection was designed so that it was essentially continuous, resulting in composite samples representing five-minute intervals. The ripening experiments and seeding conditions are summarized in Table 6.4.

Table 6.4

Summary of Ripening Experiments at the Ottawa Pilot Plant

Experiment	Date	*C. parvum*	*B. subtilis*	Blue Spheres	Yellow Spheres
Ripening	10/27/98	✓	✓		
	11/03/98	✓	✓		
	11/10/98	✓	✓		

6.2.2.2 Results

Filter effluent turbidity and particle counts, *C. parvum* and *B. subtilis* removals by filtration, and total particle reductions during ripening at Ottawa are summarized in Table 6.5. The general filter performance data and detailed instantaneous turbidity and particle

data are available in Appendix D (Tables D.4 and D.5 respectively). The detailed instantaneous *C. parvum* and *B. subtilis* data are also in Appendix D (Table D.6).

Filter effluent turbidity, total particle counts, and microorganism concentrations varied throughout the ripening period. Peak filter effluent turbidity and particle counts during ripening at Ottawa ranged from 0.41 to 0.69 NTU and 91 to 840 particles/mL respectively. The turbidity and particle response on November 10, 1998 is shown in Figure 6.7. This figure depicts a sharp filter effluent turbidity and particle peak approximately 5 to 10 minutes after the filter returned to service following backwashing. The seeding period and sampling times are noted in Figure 6.7 and indicate that microorganism seeding occurred during the turbidity and particle spikes associated with ripening while sampling captured most of the spikes, which lasted less than 30 minutes.

The filter effluent turbidity and particle counts peaked at approximately 0.67 NTU and 840 particles/mL during the November 10, 1998 experiment (Figure 6.7). On October 27, 1998, the filter effluent turbidity and particle counts peaked at 0.69 NTU and 174 particles/mL respectively; they peaked at 0.41 NTU and 91 particles/mL during the November 3, 1998 experiment. Although the magnitude of spikes varied somewhat between experiments, the duration of the ripening period was generally similar between the three experiments. The instantaneous turbidity and particle data (which did not necessarily indicate the peak turbidity and particle concentrations that occurred during ripening) are available in Appendix D (Table D.5).

Table 6.5

Filter Performance During Ripening at Ottawa

Date	Log_{10} Removal (mean ± standard deviation)				Log Red.*	Effluent Concentration	
	C. parvum	*B. subtilis*	Blue Spheres	Yellow Spheres	Particles ≥ 2μm	Particles (#/mL)	Turbidity (NTU)
10/27/98	5.0 ± 0.67	1.4 ±0.84	---	---	3.1 ± 0.48	9 ± 12	0.07 ± 0.02
11/03/98	5.1 ± 0.73	2.1 ±0.69	---	---	3.0 ± 0.46	21 ± 22	0.17 ± 0.09
11/10/98	5.0 ± 0.75	2.9 ±1.93	---	---	2.6 ± 0.67	36 ± 61	0.16 ± 0.05
Overall	**5.1 ± 0.66**	**2.1 ± 1.36**	**---**	**---**	**2.9 ± 0.55**	**22 ± 37**	**0.13 ± 0.07**

*Log reduction of particles through treatment process (plant influent to filter effluent).

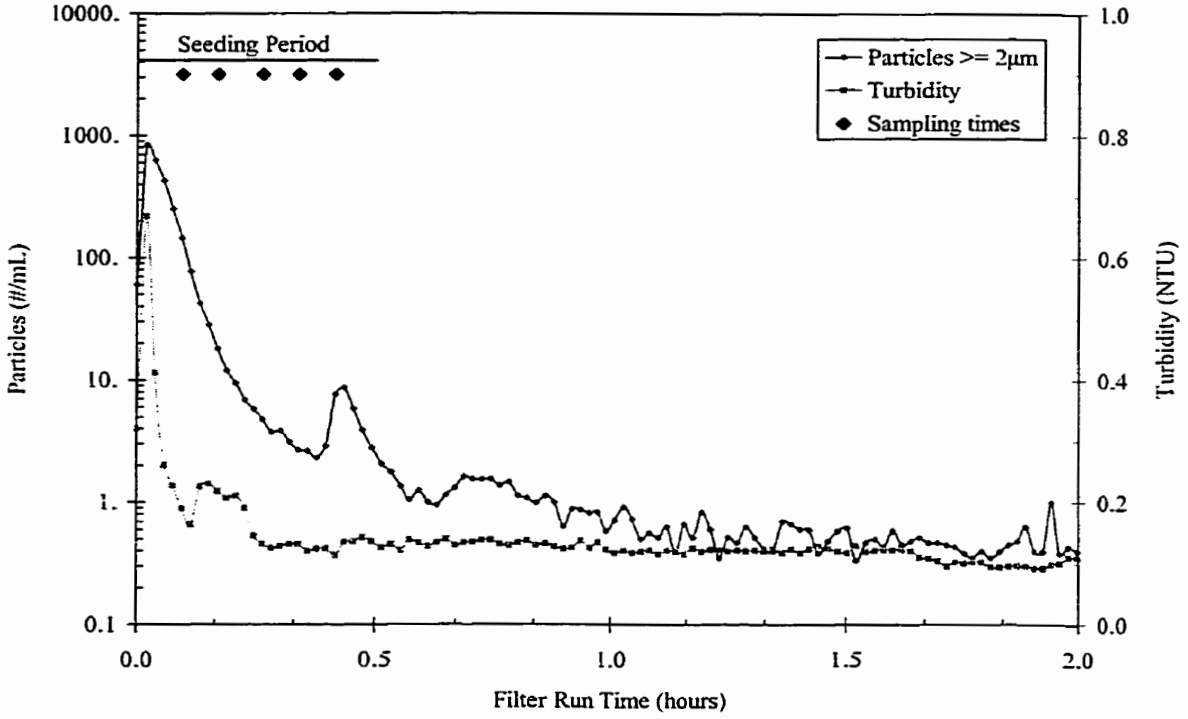


Figure 6.7 Filter effluent turbidity and particle concentration during November 10, 1998 ripening experiment at Ottawa.

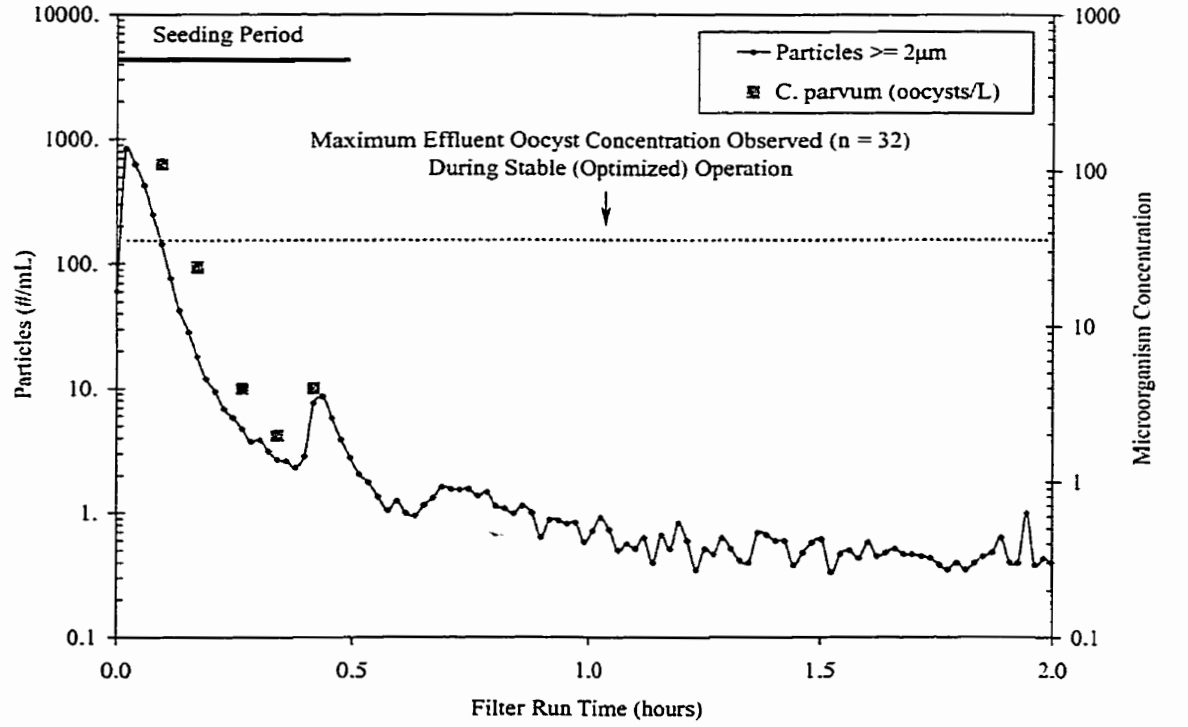


Figure 6.8 Filter effluent particle and *C. parvum* concentrations during November 10, 1998 ripening experiment at Ottawa.

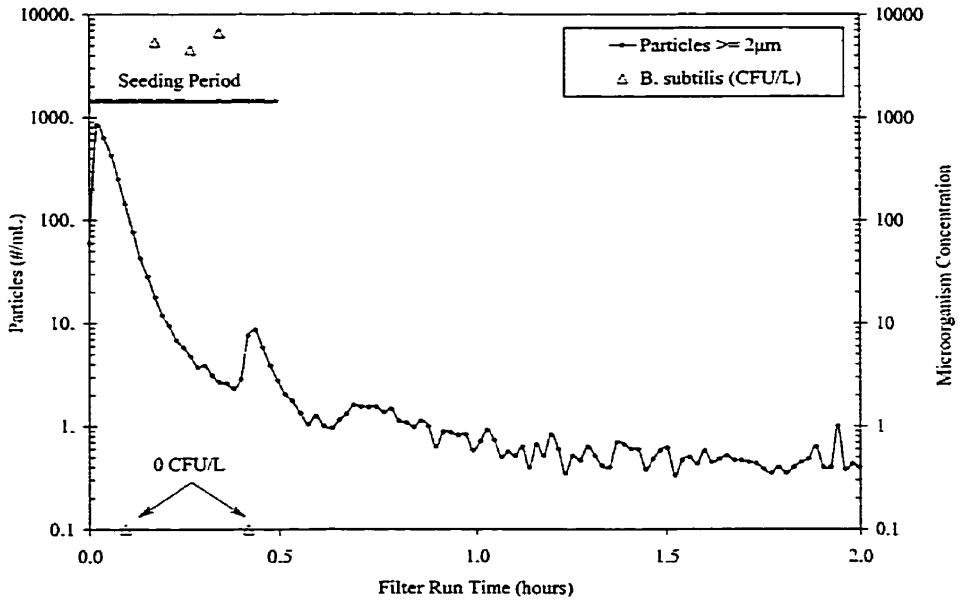


Figure 6.9 Filter effluent particle and *B. subtilis* concentrations during November 10, 1998 ripening experiment at Ottawa.

C. parvum passage through the Ottawa filter during ripening appeared to have a dynamic pattern similar to that of turbidity and particles. Consistent with the moderately elevated turbidity and particle counts, more oocysts were detected in the filter effluent during ripening relative to stable operation. Filter effluent *C. parvum* and particle concentrations during the November 10, 1998 experiment are shown in Figure 6.8. This figure indicates that the moderate spike in filter effluent oocyst concentration that occurred at 5 minutes was concurrent with the spikes in turbidity and particles (Figure 6.7). The 110 oocyst/L spike during ripening on November 10, 1998 accompanied the highest instantaneous particle concentration during microorganism sampling (145 particles/mL) and one of the highest turbidity values (0.19 NTU) experienced during the ripening experiments.

During the October 27, 1998 experiment the highest filter effluent total particle counts during sampling were considerably lower (31 particles/mL) than on November 10, with a generally comparable turbidity (0.09 NTU). Accordingly, the peak filter effluent *C. parvum* concentration was lower (82 oocysts/L). The lowest peak filter effluent oocyst concentration during ripening at Ottawa (62 oocysts/L) occurred during the

November 3, 1998 experiment when the highest filter effluent particle counts and turbidity during sampling were 58 particles/mL and 0.32 NTU respectively. *B. subtilis* passage through the filter was somewhat consistent with the *C. parvum* data, however, trends in filter effluent spore concentration spikes were not necessarily commensurate with the *C. parvum*, particle, and turbidity spikes during the ripening experiments (Figure 6.9). The detailed *C. parvum* and *B. subtilis* data are in Appendix D (Table D.6).

As during stable operation, *B. subtilis* removals by filtration were lower than *C. parvum* removals (Table 6.5) and were not indicative of the filter's ability to remove *C. parvum* (Figure 6.10). In this case, the slope of the best fit line by linear regression describing the relationship between oocyst and spore removals was negative, suggesting higher oocyst removals corresponding to lower spore removals – an unlikely scenario in reality. Of course, reductions in total particle counts (≥ 2μm) were also lower than *C. parvum* removals during ripening (Table 6.5). They did, however, appear to be somewhat similar to oocyst removals during these experiments, as indicated by the R^2 of 0.76 (Figure 6.10).

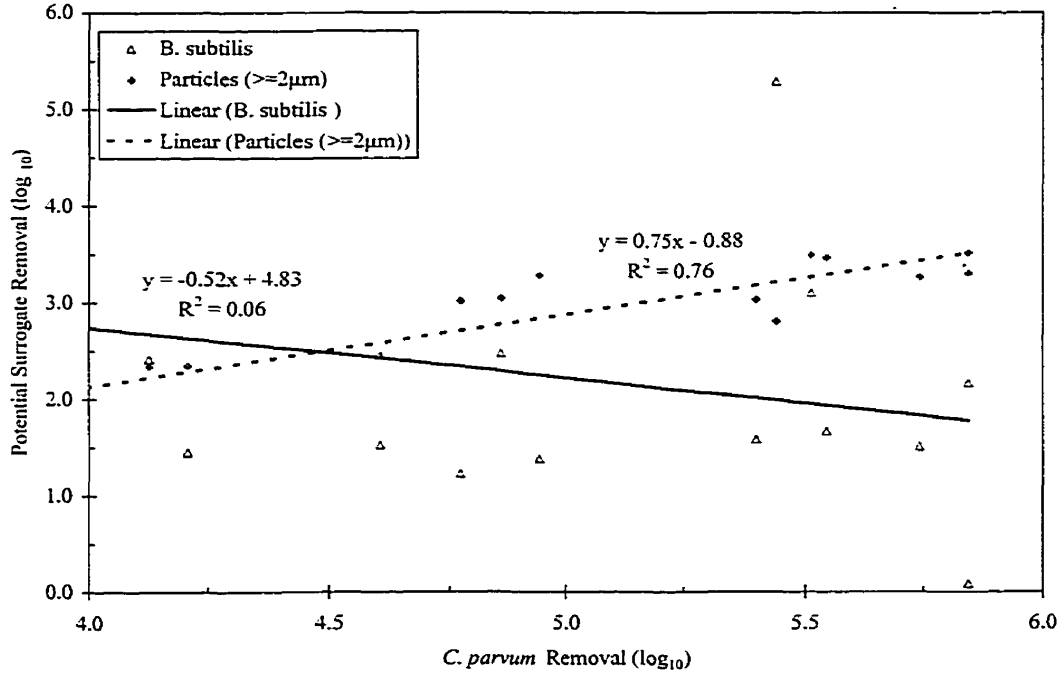


Figure 6.10 Relationship between *C. parvum* and *B. subtilis* removals by filtration and total particle (≥ 2 μm) reductions through the plant during ripening at Ottawa.

6.2.2.3 Statistical Analysis

Confidence intervals for *C. parvum* removals during filter ripening were calculated using the method described in Chapter 4. The endpoints of the 95% confidence intervals for oocyst removal are summarized in Table 6.6. During each experiment, the *C. parvum* removal at the beginning of the ripening period, corresponding to the highest level of filter effluent turbidity and particles, was significantly lower (Table 6.6, α=0.05) than the removals observed during the subsequent samples when filtered water quality was improving (*i.e.*, turbidity was decreasing). After ripening, the water quality and *C. parvum* removals improved and were consistent with those observed during the stable operation experiments (Table 6.3). Overall confidence intervals for *C. parvum* removal could not be calculated due to the dynamic changes in attachment efficiency within the filter during this period. The endpoints of the range of *C. parvum* removals observed during ripening and adjusted to incorporate analytical uncertainty are 3.7- and 7.2-log, the higher endpoint being affected by the length of the sampling period (a longer sampling approaches or extends into stable operating conditions). The removal ranges determined for the individual experiments are summarized in Table 6.6.

Table 6.6

95% Confidence Intervals and *C. parvum* Removal Ranges

During Ripening at Ottawa

Experiment	Date	Sample Time	CI_{lower} (log_{10})	CI_{upper} (log_{10})	R_{lower} (log_{10})	R_{upper} (log_{10})
Ripening	10/27/98	t = 5	**3.93**	4.21	**3.93**	**7.17**
		t = 10	4.36	4.87		
		t = 15	4.49	5.08		
		t = 20	5.01	6.33		
		t = 25	5.11	**7.17**		
	11/03/98	t = 5	**3.99**	4.30	**3.99**	**7.17**
		t = 10	n/a	n/a		
		t = 15	4.42	4.98		
		t = 20	5.11	**7.17**		
		t = 25	4.97	6.29		
	11/10/98	t = 5	**3.74**	3.98	**3.74**	**7.07**
		t = 10	4.20	4.69		
		t = 15	4.86	6.18		
		t = 20	5.01	**7.07**		
		t = 25	4.89	6.22		

6.2.2.4 Discussion

Relative to stable operation, moderately higher filter effluent *C. parvum* and *B. subtilis* concentrations were observed during ripening (Table C.6). These data suggested lesser or weaker and more transient attachment of these microorganisms during ripening than during stable filter operation. The filter microorganism concentration trends were generally consistent with spikes in filter effluent turbidity and particle counts. Although the instantaneous turbidity and particle count data (Table C.5) were generally indicative of filter performance, the actual values were not quantitative indicators of *C. parvum* passage through the filters. While raw water total particle counts and filter influent oocyst concentrations were relatively constant during the experimental period, filter effluent total particle counts or turbidity values were not necessarily correlated with specific filter effluent *C. parvum* concentrations (Figure 6.10). There appeared to be some relationship between the reduction of total particles through the treatment process and *C. parvum* removals by filtration during filter ripening (Figure 6.10). It should be recalled, however, that the particle reductions are instantaneous concentrations that continuously changed during the *C. parvum* sampling period as a result of dynamic raw and filter effluent water quality at the time of sampling. Since the detention time between the raw water and filter influent locations was approximately three hours, the particle reductions were not necessarily indicative of those that occurred during sampling. The raw water data were not adjusted to account for detention time since substantial differences in raw water quality did not occur during these, or any of the other experiments. Potential surrogates for *C. parvum* removal during filtration are further discussed in Chapter 7.

During the first few minutes of ripening, the removals of *C. parvum* were lower than during stable filter operation. The range of *C. parvum* removals during ripening is large because, in addition to incorporating analytical uncertainty associated with oocyst recovery, the statistical comparisons and overall *C. parvum* removal ranges included all of the removals observed during the sampling periods. The twenty-five minute sampling period (thirty-minute seeding period) at Ottawa included the very transient spike in particle counts and turbidity as well as periods of improved filter effluent turbidity and

particle concentrations. If the ripening period were defined differently (*e.g.*, as the period when filter effluent turbidities were 0.2 NTU or higher) and did not include the last few samples collected when water quality had substantially improved, the overall *C. parvum* removal ranges during ripening would have been smaller (Table 6.6).

In general, these experiments suggested a brief and minimal-to-moderate increase in *C. parvum* passage through filters concurrent with spikes in filter effluent turbidity and particle counts during ripening. Overall, the *C. parvum* removals during ripening were approximately 0.5-log lower than during stable operation, consistent with Patania *et al.* (1995), Hall *et al.* (1995), and Swaim *et al.* (1996) who demonstrated oocyst removals during ripening that were 0.5 to 1.0-log lower than during stable operation. These results were generally consistent with the findings of LeChevallier *et al.* (1991), which failed to demonstrate statistically different oocyst removals between ripening and stable filter operation.

6.2.3 Breakthrough

Both non-attachment and detachment occur during breakthrough conditions (Ginn *et al.*, 1992; Moran *et al.*, 1993b). As particle detachment and non-attachment increase, increasing pathogen passage through filters would also be expected. To assess the effect of end-of-run and breakthrough conditions on the removal of *C. parvum*, *B. subtilis*, and polystyrene microspheres by filters, several experiments were conducted at Ottawa. End-of-run, early breakthrough, and late breakthrough filtration conditions were investigated.

6.2.3.1 Experimental Design

End-of-run operation was defined as the period during which subtle changes in filter effluent turbidity and particle counts were noticed and increased to approximately 0.1 NTU of filter effluent turbidity. Early breakthrough filtration was defined as the period when filter effluent turbidities were between approximately 0.1 and 0.3 NTU. Though non-optimal due to changing filter effluent turbidity and particle counts, both of these operating conditions were at the upper limit of compliance with the IESWTR filter effluent turbidity requirement of 0.3 NTU or less in greater than 95% of measured

samples. The late breakthrough experiments investigated *C. parvum* and surrogate removal when filters were operated for a period shortly after reaching effluent turbidities of approximately 0.3 NTU (just out of compliance with the IESWTR).

Jar-coagulated *C. parvum* were seeded into the filter influent for one hour during these experiments; samples were collected at 15, 30, 45, and 55 minutes after the start of seeding. *B. subtilis* spores and polystyrene microspheres were also seeded during some of these experiments. When microsphere seeding was included, yellow and blue polystyrene microspheres were sequentially seeded into the filter influent.

The seeding of blue microspheres was planned for a one-hour period of stable operation approximately five hours prior to the onset of breakthrough (when filter effluent turbidities started noticeably changing). However, it was very difficult to predict when turbidity breakthrough would occur at the end of a filter cycle, so blue microsphere seeding occurred anywhere from one to five hours before the onset of turbidity breakthrough. This was done to elucidate mechanistic behavior during early breakthrough (detachment versus non-attachment) by accumulating the spheres on the filter just before the filter effluent quality started substantially deteriorating.

Yellow microspheres, when included, were always seeded concurrently with the *C. parvum* oocysts during the one-hour seeding period when filter effluent turbidities were deteriorating. The *C. parvum* and yellow microsphere concentrations in the filter effluents were considered indicative of either non-attachment or very poor, transient attachment and subsequent detachment. It should be noted that these experiments were not designed to conclusively determine whether non-capture or release of previously deposited oocysts was the dominant mechanism of oocyst passage into filter effluents during breakthrough. Rather, they were designed to determine the effects of filter operation on pathogen passage through filters while providing some insight into the mechanistic behavior of pathogen passage through filters during breakthrough.

B. subtilis spores were typically seeded concurrently with the *C. parvum* oocysts; the only exceptions to this were two early breakthrough experiments (December 20 and 22, 1999). During these experiments, jar-coagulated *B. subtilis* spores were seeded for one

hour and then jar-coagulated *C. parvum* oocysts were seeded for one hour as breakthrough commenced. Samples were only collected at 15, 30, 45, and 55 minutes after the start of oocyst seeding. The staggered seeding was planned to help elucidate mechanistic behavior during early breakthrough by accumulating *B. subtilis* spores on the filter, just before the filter effluent quality started substantially deteriorating. As with the blue microspheres, if spores were subsequently detected in the filter effluent when influent concentrations were low (ideally near 0 cfu/L), they would be indicative of detachment. The breakthrough experiments and seeding conditions are summarized in Table 6.7.

Table 6.7

Summary of Breakthrough Experiments at the Ottawa Pilot Plant

Experiment	Date	*C. parvum*	*B. subtilis*	Blue Spheres	Yellow Spheres
End-of-Run	01/21/99	✓	✓		
	03/08/00	✓		✓*	✓
	03/09/00	✓		✓*	✓
Early Breakthrough	03/01/00	✓		✓*	✓
	03/03/00	✓		✓*	✓
	03/04/00	✓		✓*	✓
Late Breakthrough	11/25/98	✓	✓		
	12/09/98	✓	✓		
	01/13/99	✓	✓		
	12/20/99	✓	✓*		✓
	12/22/99	✓	✓*		✓

*Seeded before *C. parvum* to elucidate mechanistic behavior.

6.2.3.2 Results

Filter effluent turbidity and particle counts, C. *parvum*, *B. subtilis*, and polystyrene microsphere removals, and total particle reductions during end-of-run, early breakthrough, and late breakthrough at Ottawa are summarized in Table 6.8. The general filter performance data and detailed instantaneous turbidity and particle data are available in Appendix D (Tables D.4 and D.5 respectively). The detailed instantaneous *C. parvum*, *B. subtilis*, and microsphere data are also available in Appendix D (Table D.6 and D.7 respectively).

Table 6.8

Filter Performance During End-of-Run and Breakthrough at Ottawa

Date	Log_{10} Removal (mean ± standard deviation)				Log_{10} Red.*	Effluent Concentration	
	C. parvum	*B. subtilis*	Blue Spheres	Yellow Spheres	Particles ≥ 2μm	Particles (#/mL)	Turbidity (NTU)
End-of-Run							
01/21/99	3.0 ± 0.52	2.2 ± 0.21	---	---	3.3 ± 0.18	1.8 ± 0.7	0.06 ± 0.01
03/08/00	2.3 ± 0.53	---		2.3 ± 0.49	3.3 ± 0.08	4.5 ± 0.81	0.09 ± 0.03
03/09/00	2.7 ± 0.44	---		2.6 ± 0.42	3.4 ± 0.05	3.2 ± 0.38	0.05 ± 0.01
Overall	**2.7 ± 0.54**	**—**		**2.4 ± 0.46**	**3.4 ± 0.12**	**3.2 ± 1.29**	**0.06 ± 0.02**
Early Breakthrough							
03/01/00	2.2 ± 0.50	---		2.2 ± 0.44	3.1 ± 0.11	6.8 ± 1.82	0.22 ± 0.04
03/03/00	2.2 ± 0.34	---		2.1 ± 0.42	3.4 ± 0.06	5.8 ± 0.74	0.15 ± 0.04
03/04/00	2.1 ± 0.33	---		2.1 ± 0.23	3.3 ± 0.06	6.3 ± 0.84	0.12 ± 0.03
Overall	**2.1 ± 0.36**	---		**2.1 ± 0.34**	**3.3 ± 0.11**	**6.3 ± 1.22**	**0.17 ± 0.06**
Late Breakthrough							
11/25/98	1.8 ± 0.03	1.2 ± 0.16	---	---	1.5 ± 0.14	249 ± 74	0.94 ± 0.09
12/09/98	1.7 ± 0.06	1.0 ± 0.09	---	---	1.7 ± 0.05	140 ± 16	0.50 ± 0.04
01/13/99	1.5 ± 0.68	1.3 ± 0.18	---	---	3.0 ± 0.05	4 ± 0.4	0.61 ± 0.15
12/20/99	1.5 ± 0.20	0.5 ± 0.35	---	1.5 ± 0.23	2.5 ± 0.46	32 ± 36	0.28 ± 0.03
12/22/99	1.4 ± 0.05	0.9 ± 0.08	---	1.7 ± 0.26	**	**	0.29 ± 0.02
Overall	**1.6 ± 0.32**	**1.0 ± 0.34**	**—**	**1.6 ± 0.25**	**2.2 ± 0.67**	**106 ± 107**	**0.52 ± 0.26**

*Log reduction of particles through treatment process (plant influent to filter effluent).
**Data not available.

Of the end-of-run and breakthrough investigations, the largest deterioration in oocyst, spore, and microsphere removals was expected during late breakthrough filtration when filter effluent turbidities were high (~0.3 NTU). Two experiments investigating *C. parvum* and microsphere removal during late breakthrough were performed. Three other late breakthrough experiments investigated *C. parvum* and *B. subtilis* removal by filtration. The filter effluent turbidity and total particle counts were typically ~0.25-0.3 NTU at the start of these experiments. The filter effluent turbidity, seeding period, and sampling points during one of the late breakthrough experiments investigating *C. parvum* and polystyrene microsphere removal by filtration are presented in Figure 6.11; unfortunately, total particle counts were not available during this experiment.

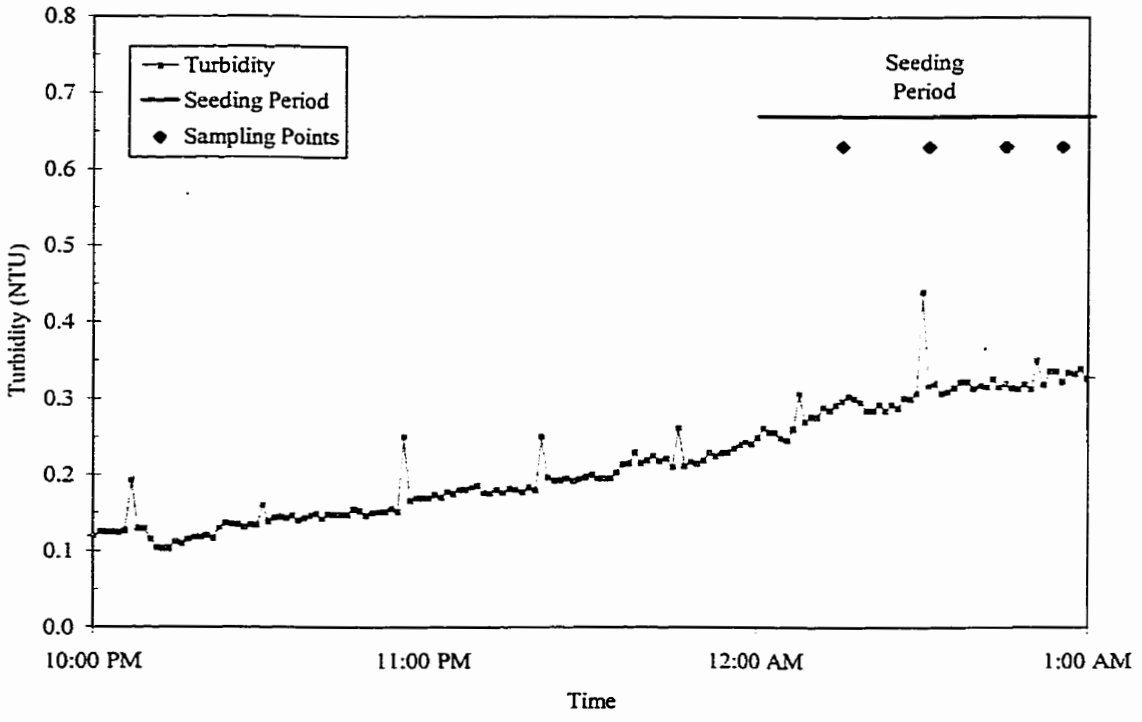


Figure 6.11. Filter effluent turbidity during December 22, 1999 late breakthrough experiment at Ottawa.

The elevated filter effluent turbidities during late breakthrough at Ottawa were accompanied by high filter effluent *C. parvum* and microsphere concentrations relative to those obtained during the stable operation experiments. *C. parvum* during the late breakthrough experiments ranged from 1.3 to 1.8-log, with a mean oocyst removal of 1.4-log. Microsphere removals during late breakthrough ranged from 1.3 to 2.0-log, with a mean microsphere removal of 1.8-log. Relative to stable operation, these experiments clearly demonstrated a >3-log reduction in *C. parvum* and microsphere removals during late breakthrough. The filter influent oocyst and microsphere concentrations during these experiments were similar, ~6.9 × 10^5 oocysts/L and ~6.8 × 10^5 microspheres/L on average. Both *C. parvum* and microspheres were found in all of the filter effluent samples during the late breakthrough experiments (Figure 6.12). Detailed microorganism and microsphere concentration and removal data are provided in Appendix D (Table D.6 and Table D.7, respectively).

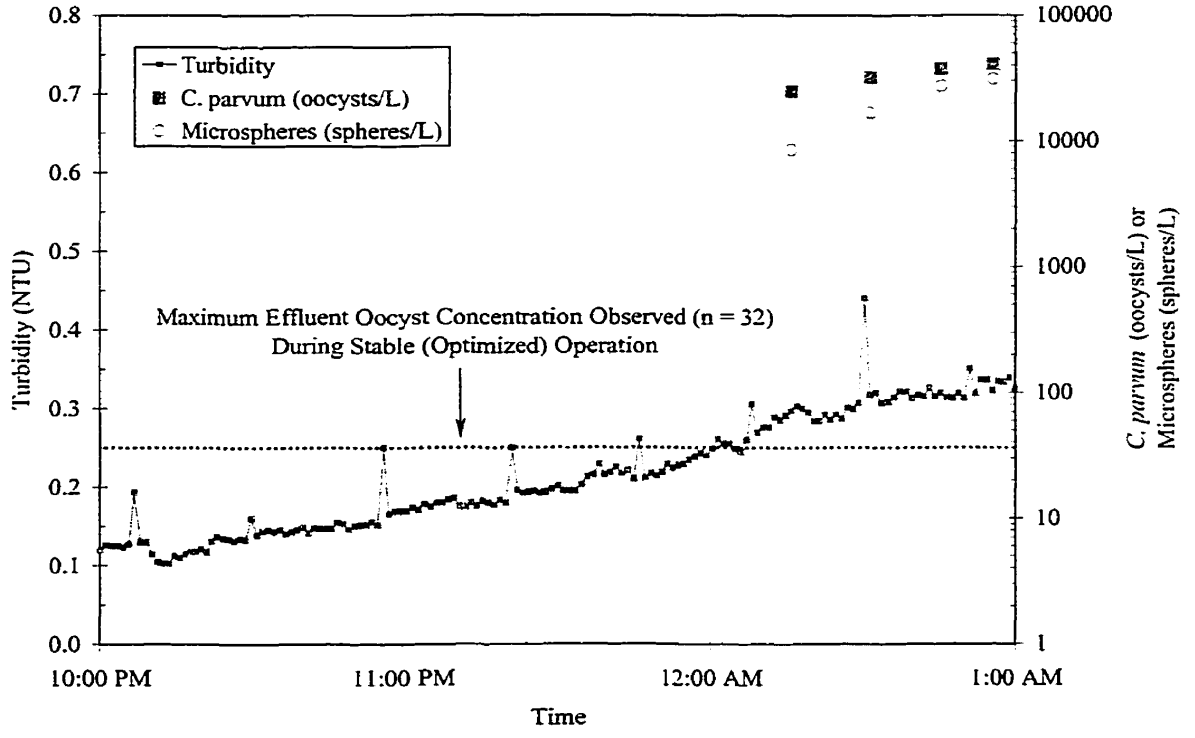


Figure 6.12. Filter effluent turbidity, *C. parvum*, and microsphere concentrations during December 22, 1999 late breakthrough experiment at Ottawa.

The elevated filter effluent particle counts and turbidities during late breakthrough at Ottawa were accompanied by high filter effluent aerobic spore concentrations relative to those obtained during the stable operation experiments. *B. subtilis* removals by the pilot filter ranged from 0.1 to 1.5-log during the late breakthrough experiments, with a mean spore removal of 1.0-log. Relative to stable operation, these experiments clearly demonstrated an ~3-log reduction in *B. subtilis* removals during late breakthrough. This result was similar to the decrease in *C. parvum* removals during this period relative to stable operation. Filter effluent *B. subtilis* spore concentrations were similar to the *C. parvum* concentrations; similarly, spores were detected in all of the filter effluent samples during the late breakthrough experiments (Figure 6.13). Detailed microorganism concentration and removal data are provided in Appendix D (Table D.6).

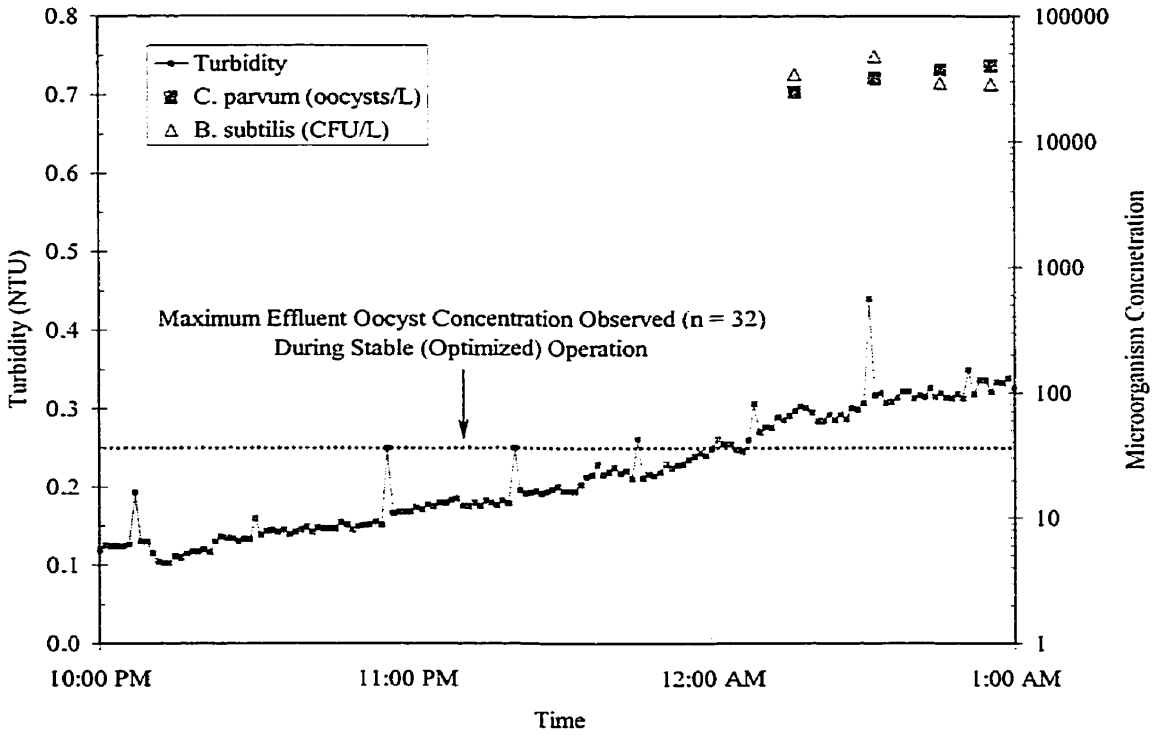


Figure 6.13. Filter effluent particle counts, *C. parvum*, and *B. subtilis* concentrations during December 22, 1999 late breakthrough experiment at Ottawa.

The early breakthrough filtration performance data demonstrated a very dynamic period during which filter effluent turbidity and total particle counts changed considerably at the Ottawa pilot plant. Three experiments investigating *C. parvum* and microsphere removal during early breakthrough were performed. The filter effluent turbidity was low (~0.04 – 0.08 NTU) at the start of these experiments and increased to approximately 0.2 NTU by the end of the experiments, a level still in compliance with the IESWTR requirements for filter effluent turbidity. The filter effluent turbidity and particle concentrations during one of the early breakthrough experiments (March 3, 2000) are presented in Figure 6.14.

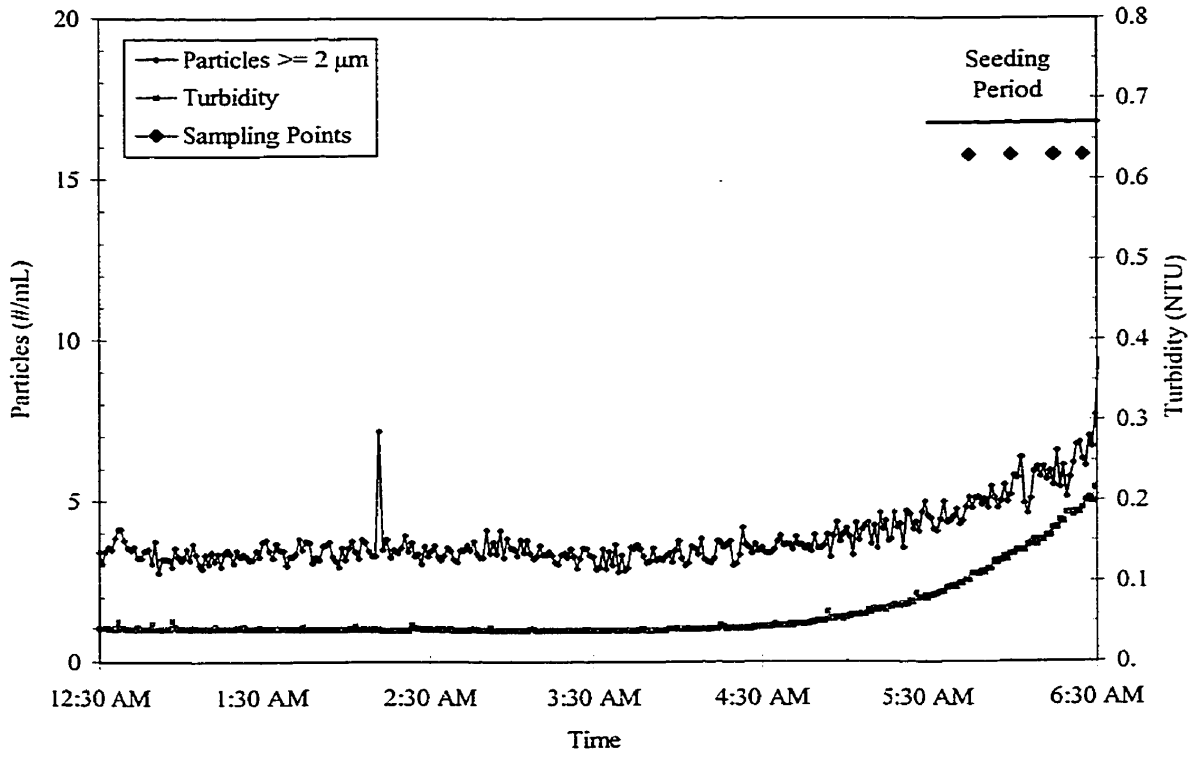


Figure 6.14. Filter effluent turbidity and particle concentration during March 3, 2000 early breakthrough experiment at Ottawa.

The increased filter effluent turbidities and particle concentrations during early breakthrough were also accompanied by increased filter effluent *C. parvum* and microsphere concentrations relative to those obtained during stable operation. *C. parvum* removals by the pilot filter during early breakthrough ranged from 1.7 to 2.8-log, with a mean oocyst removal of 2.1-log. Microsphere removals during early breakthrough also ranged from 1.7 to 2.8-log, with a mean microsphere removal of 2.1-log. The observed ranges of *C. parvum* and microsphere removals were >2-log lower than those observed during stable operation. The filter influent oocyst and microsphere concentrations during the early breakthrough experiments were similar and averaged ~6.6×10^5 oocysts/L and ~5.7×10^5 microspheres/L respectively. *C. parvum* oocysts and microspheres were found in all of the filter effluent samples during these experiments (Figure 6.15).

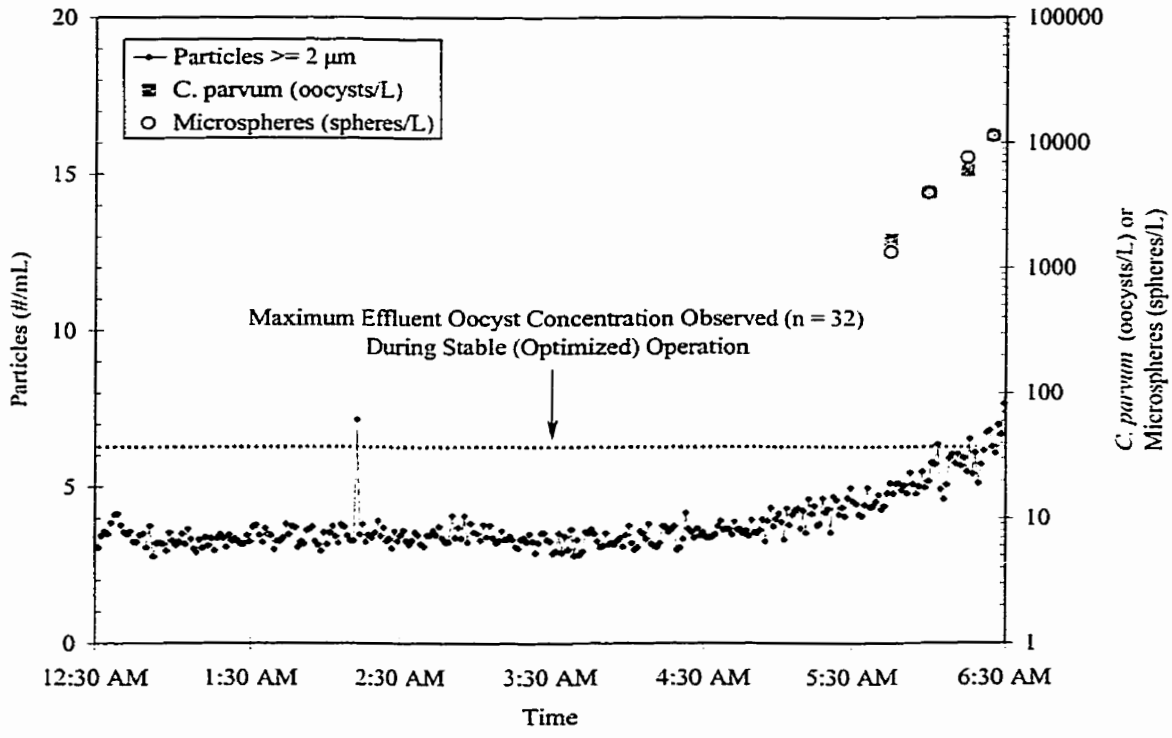


Figure 6.15. Filter particle, *C. parvum*, and microsphere concentrations during March 3, 2000 early breakthrough experiment at Ottawa.

Considerable deterioration in *C. parvum* and polystyrene microsphere removal during end-of-run filtration when filter effluent turbidities were increasing but still below 0.1 NTU was not expected given other data in the literature (Patania *et al.*, 1995; Baudin and Laîné, 1998). Two experiments investigating *C. parvum* and microsphere removal during end-of-run filtration were performed. The filter effluent turbidity was low (~0.04 NTU) at the start of these experiments and increased to approximately 0.13 NTU by the end of the seeding period in the first experiment and 0.06 NTU at the end of the seeding period in the second experiment. The filter effluent turbidity and particle concentration data from the second (March 9, 2000) of three end-of-run experiments are presented in Figure 6.16.

Although the filter effluent turbidities and particle concentrations increased only slightly during the end-of-run experiments at Ottawa, they were accompanied by considerably elevated filter effluent *C. parvum* and microsphere concentrations relative to those obtained during stable operation. *C. parvum* removals by the pilot filter during end-of-run filtration ranged from 1.8 to 3.3-log, with a mean oocyst removal of 2.5-log. Microsphere removals during early breakthrough ranged from 1.8 to 3.1-log, with a mean microsphere removal of 2.4-log. The filter influent oocyst and microsphere concentrations during the end-of-run experiments were similar, ~6.8×10^5 oocysts/L and ~5.6×10^5 microspheres/L on average. Both *C. parvum* oocysts and microspheres were found in all of the filter effluent samples during the end-of-run experiments (Figure 6.17).

An increase in filter effluent *B. subtilis* was also observed during the end-of-run filtration experiments. Filter effluent particle counts and *C. parvum* and *B. subtilis* removal data for the January 21, 1999 experiment are presented in Figure 6.18. These data demonstrated that the increase in filter effluent spores during the experimental period was generally consistent with the increase in filter effluent oocyst concentrations. The March 8 and 9, 2000 data were generally consistent with the January 21, 1999 data and indicated considerable passage of oocysts through the filter during end-of-run conditions.

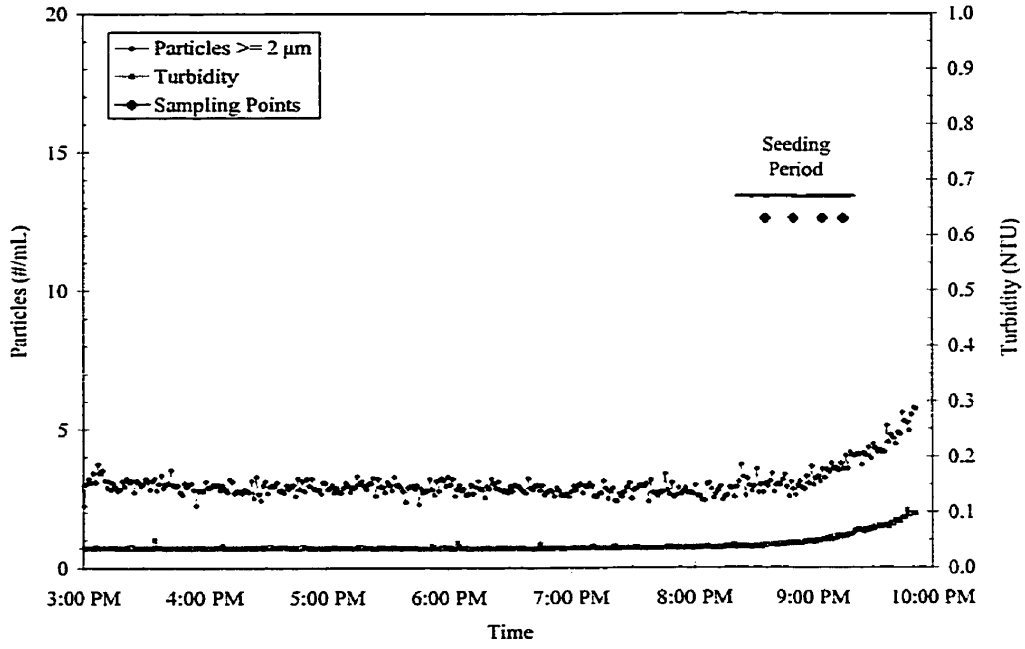


Figure 6.16. Filter effluent turbidity and particle concentration during March 9, 2000 end-of-run experiment at Ottawa.

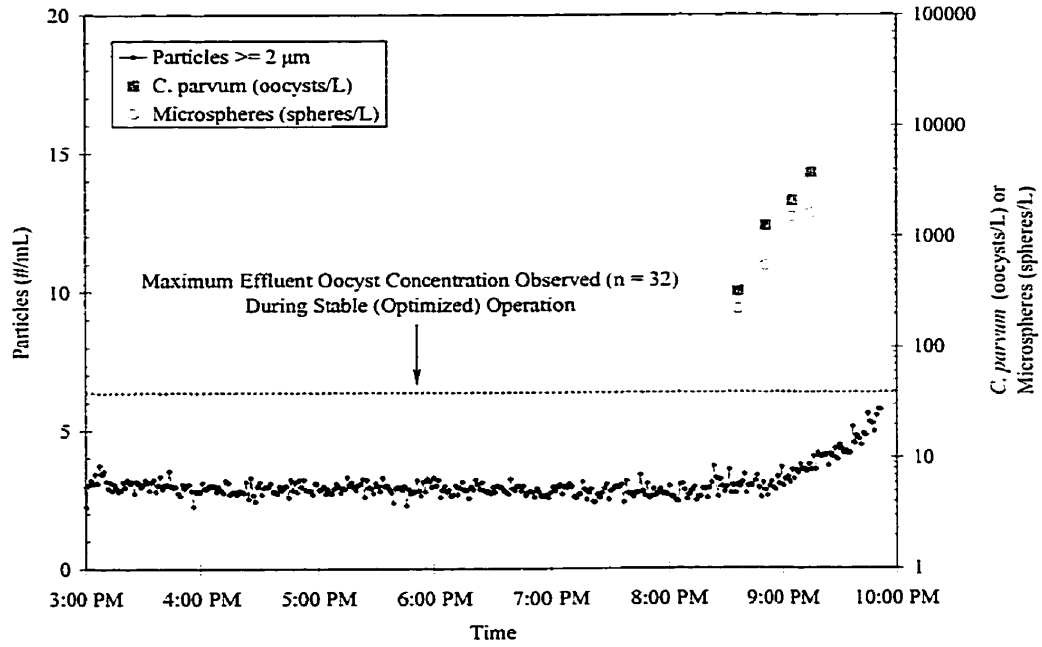


Figure 6.17. Filter effluent particle, *C. parvum*, and microsphere concentrations during March 9, 2000 end-of-run experiment at Ottawa.

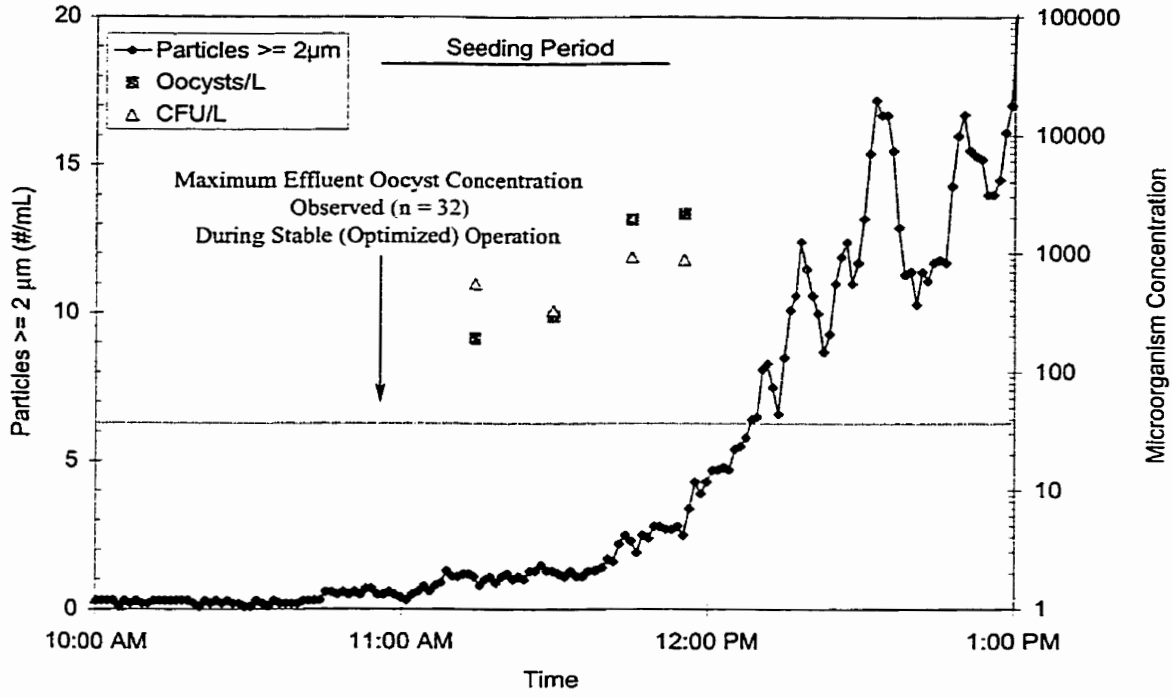


Figure 6.18 Filter effluent particle, *C. parvum*, and *B. subtilis* concentrations during January 21, 1999 end-of-run experiment at Ottawa.

The *C. parvum*, *B. subtilis*, and polystyrene microsphere removals and total particle reductions through the treatment process are summarized in a box-and-whisker plot (Figure 6.19). This plot includes the entire range of removals observed during the end-of-run and breakthrough experimental periods (defined in Section 6.2.3.1). Removals during stable operation are also included for comparison. These data clearly indicated severe deterioration in oocyst, spore, and microsphere removals during end-of-run (< 0.1 NTU filter effluent turbidity), early breakthrough (0.1 – 0.3 NTU filter effluent turbidity), and late breakthrough (> 0.3 NTU filter effluent turbidity) filtration. The deterioration in total particle reduction through the treatment process did not appear as severe as the deterioration in oocyst, spore, and microsphere removals. Not surprisingly, microorganism and microsphere removals generally continued to decrease as filter effluent turbidities and total particle concentrations increased during these successive operating periods.

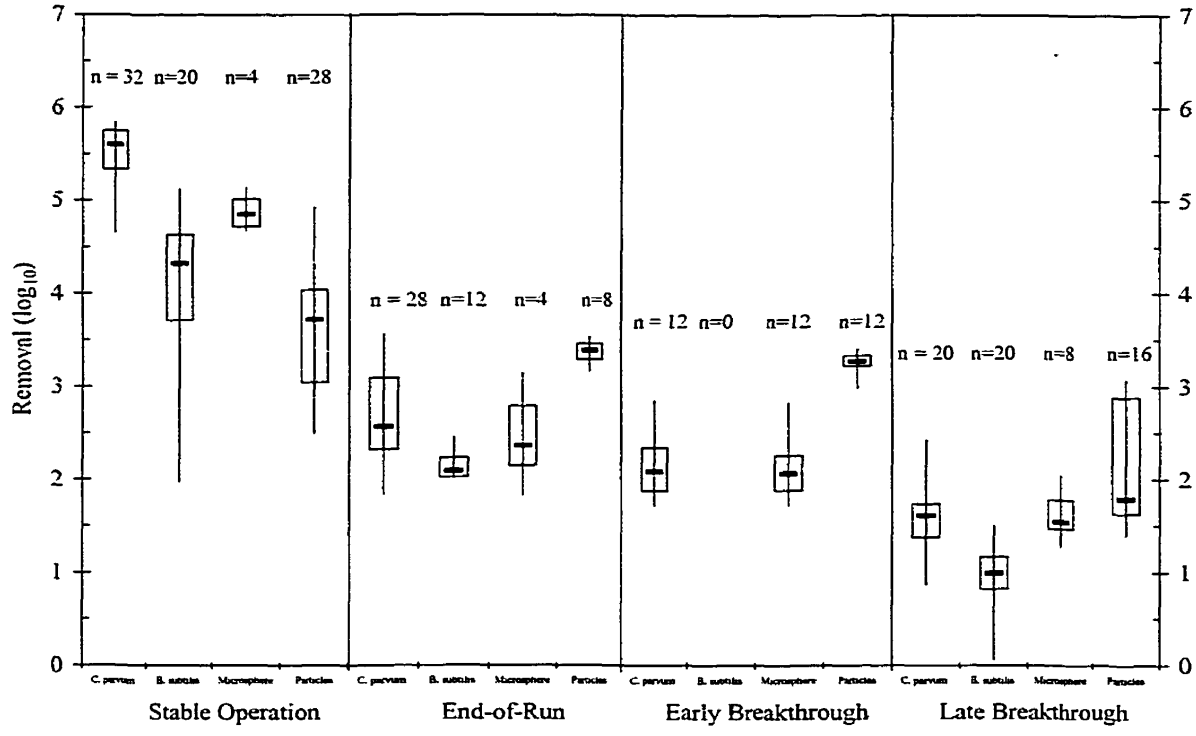


Figure 6.19. Box-and-whisker plot of *C. parvum*, *B. subtilis*, microsphere removals by filtration and total particle (≥ 2μm) reductions by the plant during stable operation, end-of-run, and breakthrough at Ottawa.

The box-and-whisker plot indicates considerable similarity between *C. parvum* oocyst and polystyrene microsphere removals during the range of end-of-run and breakthrough operational conditions investigated (Figure 6.19). As indicated in Figure 6.20, the relationship between oocyst and microsphere removals by the pilot filter was linear with a coefficient of determination (R^2) of 0.92. Although oocyst and microsphere removals during stable operation (Figure 6.5) were not as similar as those obtained during the end-of-run and breakthrough conditions, the data in Figure 6.19 and Figure 6.20 suggest that polystyrene microsphere removals were good, and under some circumstances (stable operation), conservative indicators of *C. parvum* removals by filtration. Further investigations are necessary to determine if this relationship is consistent during stable operation and other non-optimal operating periods. Potential surrogates for *C. parvum* removal during filtration will be discussed in further detail in Chapter 7.

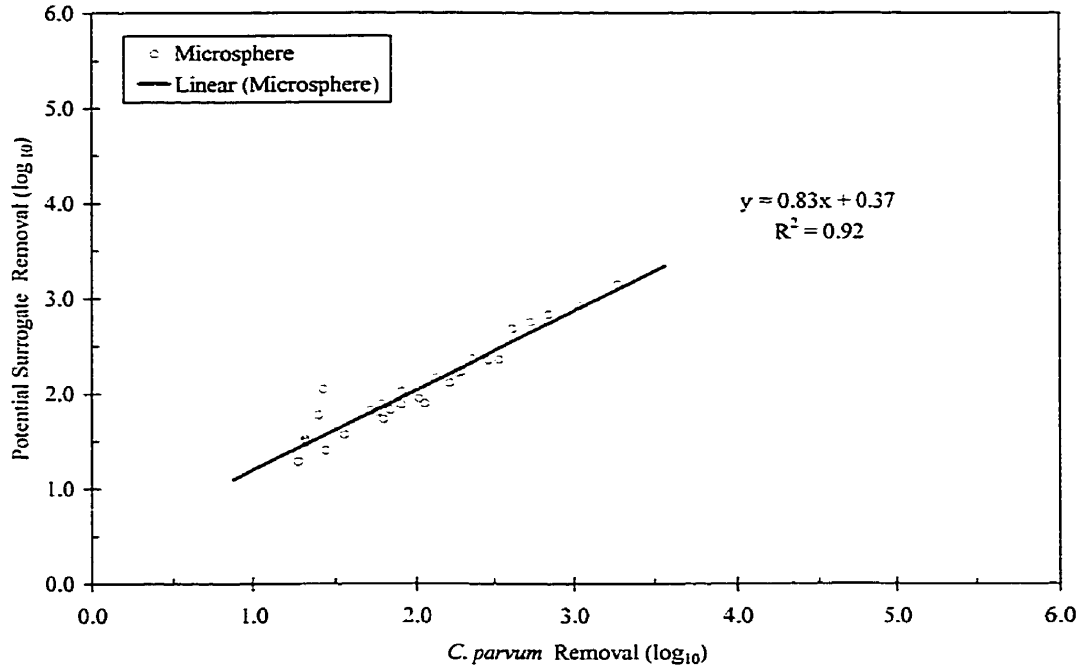


Figure 6.20. Relationship between *C. parvum* and microsphere removals by filtration during end-of-run, early breakthrough, and late breakthrough at Ottawa.

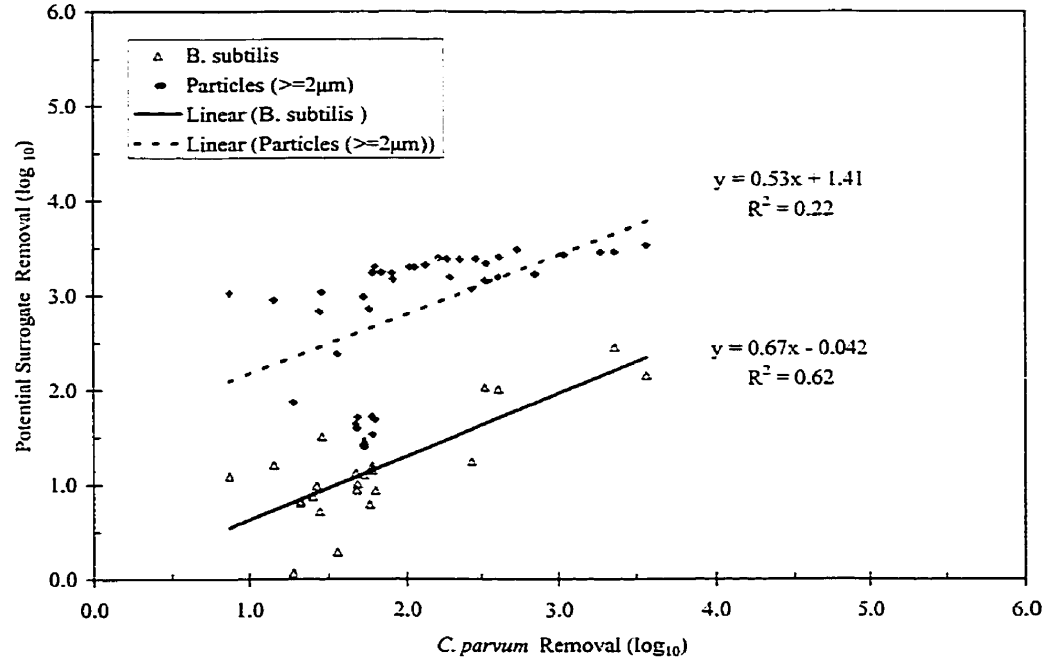


Figure 6.21. Relationship between *C. parvum* and *B. subtilis* removals by filtration and total particle (≥ 2µm) reduction through the plant during end-of-run, early breakthrough, and late breakthrough at Ottawa.

B. subtilis spore removals and total particle reductions during end-of-run, early breakthrough, and late breakthrough were compared to *C. parvum* removals in Figure 6.21. These data indicated some correlation between *C. parvum* and *B. subtilis* removals. Least-squares linear regression indicated a coefficient of determination (R^2) of 0.62. The relationship between *C. parvum* removal by filtration and total particle reductions through the treatment process was considerably weaker, with a coefficient of determination (R^2) of only 0.22. As mentioned above, a more detailed discussion of these relationships is provided in Chapter 7.

The end-of-run and breakthrough experiments also included the seeding of blue microspheres to elucidate the mechanisms associated with *C. parvum* and polystyrene microsphere passage through the filter during this operational period. The blue microspheres were seeded several hours (typically ~3 to 5 hours) before the end-of-run and breakthrough conditions were anticipated and before the seeding of oocysts and yellow microspheres. Ideally, filter influent concentrations of blue microspheres near 0 spheres/L were targeted during the end-of-run and breakthrough conditions so that filter effluent concentrations could be attributed to detachment. Although the filter influent concentrations of blue microspheres steadily decreased after seeding, the desired low concentrations of blue microspheres were rarely achieved during these experiments (Table C.7), due to non-plug-flow conditions in the standing water above the filter media. Some examples of possible detachment occurred during the March 1 and March 4, 2000 early breakthrough experiments when filter effluent sphere concentrations exceeded filter influent concentrations. However, regardless of influent concentration, the filter effluent concentrations of yellow microspheres were higher than the filter effluent concentrations of blue microspheres, typically by one order of magnitude or more (Table C.7). This result suggested that the passage of oocysts through the filter during end-of-run and breakthrough filtration was largely a function of non-attachment or weak, transient attachment and subsequent detachment.

6.2.3.3 Statistical Analysis

Confidence intervals for *C. parvum* log removals for the individual samples during end-of-run, early breakthrough, and late breakthrough were calculated using the method described in Chapter 4. The endpoints of the 95% confidence intervals for oocyst removal are summarized in Table 6.9. A comparison of the individual confidence intervals for *C. parvum* removal during end-of-run, early breakthrough, or late breakthrough at Ottawa, relative to those obtained during stable operation, demonstrated that the lower removals during these conditions were significantly different from those obtained during stable operation (Table 6.3 and Table 6.9, α=0.05).

As during other dynamic operating periods, the overall confidence intervals for end-of-run and breakthrough operating conditions could not be calculated. During these conditions, the attachment efficiency of the filter was likely decreasing (or detachment increasing), resulting in the observed deterioration in treated water (filter effluent) quality. Therefore, the individual data could not be pooled, as they were not true replicates, precluding the calculation of the overall confidence intervals.

The endpoints of the range are the lowest and highest endpoints of the individual 95% confidence intervals for *C. parvum* removal observed during the given operating period. The *C. parvum* removal range was 2.0- to 3.6-log during end-of-run operation, 1.7- to 2.9-log during early-breakthrough operation, and 0.001- to 2.0-log during late breakthrough operation. The range for the individual experiments is summarized in Table 6.9. In general, these data demonstrated a clear deterioration in *C. parvum* removal as water quality deteriorated (*i.e.*, filter effluent turbidity was increasing) as the filter cycle progressed.

Table 6.9

95% Confidence Intervals and *C. parvum* Removal Ranges During End-of-Run, Early Breakthrough, and Late Breakthrough at Ottawa

Experiment	Date	Sample Times	CI_{lower} (log_{10})	CI_{upper} (log_{10})	R_{lower} (log_{10})	R_{upper} (log_{10})
End-of-Run	01/21/99	t = 15	3.42	**3.60**	**2.48**	**3.60**
		t = 30	3.24	3.39		
		t = 45	2.56	2.62		
		t = 55	**2.48**	2.54		
	03/08/00	t = 15	2.83	**3.13**	**1.94**	**3.13**
		t = 30	2.24	2.39		
		t = 45	**1.94**	2.05		
		t = 55	2.48	2.54		
	03/09/00	t = 15	2.91	**3.33**	**2.16**	**3.33**
		t = 30	2.57	2.79		
		t = 45	2.33	2.51		
		t = 55	**2.16**	2.29		
Early Breakthrough	03/01/00	t = 15	2.66	**2.93**	**1.66**	**2.93**
		t = 30	2.17	2.31		
		t = 45	1.83	1.94		
		t = 55	**1.66**	1.75		
	03/03/00	t = 15	2.47	**2.67**	**1.73**	**2.67**
		t = 30	2.11	2.24		
		t = 45	1.97	2.08		
		t = 55	**1.73**	1.82		
	03/04/00	t = 15	2.40	**2.58**	**1.71**	**2.58**
		t = 30	2.03	2.15		
		t = 45	1.83	1.94		
		t = 55	**1.71**	1.81		
Late Breakthrough	11/25/98	t = 15	1.76	1.78	**1.00**	**1.79**
		t = 30	**1.00**	1.73		
		t = 45	1.71	1.73		
		t = 55	1.76	**1.79**		
	12/09/98	t = 15	1.66	1.68	**1.66**	**2.00**
		t = 30	1.78	1.80		
		t = 45	1.67	**2.00**		
		t = 55	**1.66**	1.68		
	01/13/99	t = 15	2.39	**2.44**	**0.001**	**2.44**
		t = 30	1.44	2.00		
		t = 45	**0.001**	0.88		
		t = 55	1.15	2.00		

Table 6.9

95% Confidence Intervals and *C. parvum* Removal Ranges During End-of-Run, Early Breakthrough, and Late Breakthrough at Ottawa (Continued)

Experiment	Date	Sample Times	CI_{lower} (log_{10})	CI_{upper} (log_{10})	R_{lower} (log_{10})	R_{upper} (log_{10})
	12/20/99	t = 15	1.69	**1.79**	**1.24**	**1.79**
		t = 30	1.40	1.47		
		t = 45	1.50	1.58		
		t = 55	**1.24**	1.30		
	12/22/99	t = 15	1.38	**1.45**	**1.28**	**1.45**
		t = 30	1.36	1.42		
		t = 45	**1.28**	1.34		
		t = 55	1.29	1.35		

6.2.3.4 Discussion

The data collected during this research indicated that end-of-run, early breakthrough, and late breakthrough filtration all represent operating periods where *C. parvum* removals can be severely compromised relative to those obtained during optimized filtration (Figure 6.19). During stable filtration, the pilot-scale dual-media filter approximately achieved >5-log removal of both oocysts and microspheres. During end-of-run operation when filter effluent turbidities demonstrated the first signs of increasing (but were still below 0.1 NTU), *C. parvum* removal decreased to approximately 2-3-log; oocyst and microsphere removals decreased even more during early and late breakthrough.

The early and late breakthrough findings were in general agreement with earlier findings that demonstrated that turbidity breakthrough could be accompanied by considerable passage of *Giardia* cysts (Logsdon *et al.*, 1981a). The early breakthrough results were also different from those obtained during other investigations of *Giardia* and *Cryptosporidium* passage through filters during breakthrough when effluent turbidities increased from 0.1 NTU to 0.2 NTU or higher. While *Giardia* removal was approximately 0.5-log lower during breakthrough, no difference between *C. parvum* removals during stable operation and breakthrough was observed (Patania *et al.*, 1995). Nonetheless, *C. parvum* oocysts were clearly present in the filter effluents during these experiments and at high concentrations. It is possible that other factors such as chemical

pretreatment, which is critical for optimizing *C. parvum* removal by filtration (Patania *et al.*, 1995), may impact the degree of pathogen passage that occurs during early breakthrough filtration, resulting in differences between studies, such as the one observed between the present study and that of Patania *et al.* (1995). Commensurate with other studies (Patania *et al.*, 1995; Nieminski and Ongerth, 1995), the elevated filter effluent *C. parvum* concentrations during end-of-run and breakthrough filtration were loosely correlated with increasing filter effluent particle counts and turbidity. This study supported previous studies that suggested that oocyst-sized microsphere removals may be good surrogates for *C. parvum* removal by filtration (Swertfeger *et al.*, 1998). A good linear fit between oocyst and microsphere removals was presented in Figure 6.17.

As previously discussed, the microsphere findings are particularly important because no reliable quantitative surrogates for the removal of *C. parvum* during water treatment exist at this time. The microspheres offer several advantages for use over oocysts in treatment process evaluations such as those reported in this study. The microspheres cost substantially less than oocysts, do not require antibody staining, do not pose the public health threats of *C. parvum* (although they could not necessarily be introduced into full-scale plants), are resilient during treatment, and may possibly lend themselves to automated enumeration. As was shown above, the microspheres also appear to be removed at levels that are comparable to oocyst removals (or slightly lower in the case of stable operation), suggesting that they are generally conservative surrogates that can be effectively used for investigating *C. parvum* removal in treatment evaluations.

The severe reduction in *C. parvum* removal during the end-of-run and breakthrough experiments relative to the optimized filtration experiments should be considered in the context of the experimental conditions. Since filter influent *C. parvum* concentrations are not typically anywhere near the ~10^5 oocysts/L influent concentrations used during these experiments, the removal data collected during this investigation should not be used to quantitatively predict differences in oocyst removals at various points in the filter cycle in full-scale plants.

The end-of-run and early breakthrough filtration data are noteworthy because they clearly indicated a severe deterioration in *C. parvum* removal by filtration during operating conditions in compliance with the 0.3 NTU IESWTR filter effluent turbidity requirement. From an operational perspective, these data might challenge the appropriateness of an upper turbidity limit of 0.3 NTU for all points in the filter cycle. The data suggest that placing filters out of service earlier in the filter cycle (perhaps when effluent turbidities are near 0.1 NTU) may be a desirable strategy for maximizing pathogen removal.

The high *C. parvum* and yellow microsphere concentrations that were found in the filter effluents after only fifteen minutes of seeding suggested that the primary mechanism of oocyst passage through the filter during end-of-run and breakthrough filtration was either non-attachment or weak, transient attachment and subsequent detachment. The relatively low filter effluent concentrations of blue microspheres also supported this conclusion. Though far from incontrovertible, this conclusion is in general agreement with other studies that suggested non-attachment was an important mechanism of particle passage through filters during breakthrough operation (Ginn *et al.*, 1992; Moran *et al.*, 1993b).

6.2.4 Coagulant Effects

Delivering the optimal coagulant dosage during water treatment can be challenging because the residence time for water in treatment plants is relatively short. In locations where influent water quality can change rapidly, similar rapid responses in coagulant dosing are not always easily attained. Even though the treatment plant may be capable of producing low-turbidity water when high-turbidity water consistently enters the plant, the speed of the raw water quality changes relative to the coagulation response may lead to increased particle and potentially pathogen passage through the treatment process.

Adequate chemical pretreatment has been repeatedly shown to be a critical step in maintaining good particle removal during filtration (O'Melia, 1985; Vaidyanathan and Tien, 1988; Tobiason and O'Melia, 1988). Several studies have suggested the importance of coagulation processes for improving filter removal efficiencies of pathogens (Logsdon *et al.*, 1981a; Ongerth and Pecoraro, 1995; Patania *et al.*, 1995). The importance of coagulation for *C. parvum* removal by filters was also demonstrated by the

bench-scale investigations (Chapter 5) that indicated >5-log removal of oocysts during optimized treatment while only approximately 1-log removal of oocysts during coagulation failure. Other studies have yielded similar results (Charles *et al.*, 1995; Swertfeger *et al.*, 1998).

6.2.4.1 Experimental Design

As described in Chapter 3, the coagulation regime applied at Ottawa utilizes a high alum dose with silicate addition for combined TOC and particle removal. To investigate the effect of coagulation on *C. parvum* removal by filtration, two basic types of experiments were performed: no coagulation and sub-optimal coagulation. Coagulation effects on *C. parvum* removal by filtration were then assessed by comparing these conditions to optimal coagulation conditions (stable operation experiments).

The no coagulant experiments were conducted to estimate the worst-case condition of coagulant failure. Coagulants were removed from the treatment process in several different combinations. These combinations were as follows:

1. ***No Coagulant – Extended Duration.*** No coagulant was used in either the pilot plant or the jar-coagulation apparatus for three filter runs prior to the experiment, simulating a long-term coagulant failure. Coagulant aid (silicate) was maintained at its' optimum dosage.

2. ***No Coagulant – Short Duration.*** No coagulant was used in the pilot plant or the jar-coagulation apparatus, simulating a short-term coagulant failure. Coagulant aid (silicate) was maintained at its' optimum dosage. During these experiments, the primary coagulant (alum) pump was turned off for a short period of time prior to seeding the microorganisms. Filters are typically already conditioned during such short-term failures with substantial amounts of floc present in the filters that can act as effective collectors for some period of time. Comparing data from this experiment to those obtained during the no coagulant – extended duration experiments revealed the effects of filter conditioning with optimally coagulated water prior to coagulation failure.

3. ***No Coagulant in the Pilot Plant.*** These experiments were identical to the no coagulant – short duration experiments with the exception that although no coagulant was fed to the pilot plant, it was added to the jar-coagulation apparatus. Coagulant aid (silicate) was maintained at its' optimum dosage in both the pilot plant and the jar-coagulation apparatus. This experiment investigated coagulant effects associated with the addition of jar-coagulated microorganisms to the filter influent.

4. ***No Coagulant in the Jar.*** This experiment was identical to the stable operation experiments with the exception that no coagulant was added to the jar-coagulation apparatus. Coagulant aid (silicate) was maintained at its' optimum dosage in both the pilot plant and the jar-coagulation apparatus. Like the previous experiment (no coagulant in the pilot plant), this experiment investigated potential filter aid effects associated with the relatively high filter influent concentration of coagulant that resulted from the addition of jar-coagulated microorganisms at the filter influent location and the relative role of filter conditioning.

5. ***No Silicate (Coagulant Aid).*** In this experiment the activated silica feed was discontinued for two filter cycles prior to the experiment. Normal alum coagulation was maintained in the pilot plant and jar-coagulation apparatus, simulating an extended duration coagulant aid failure. This experiment was conducted to investigate the role of activated silica (as compared to alum) in the coagulation/filtration process at Ottawa.

C. parvum oocysts were seeded into the filter influent for one hour during these experiments. Samples were collected at 15, 30, 45, and 55 minutes after the start of seeding. *B. subtilis* spores were also seeded into the filter influent during all of these experiments except the no coagulant–extended duration experiment, during which yellow polystyrene microspheres were seeded.

In addition to the no coagulant experiments, sub-optimal coagulation experiments were conducted to determine the effects of changing coagulation conditions (without a change in raw water quality) on pathogen passage. The experiments simulated a 40 to 60 percent underfeed in coagulant dosage from the optimum. The silicate concentration did not change from the optimum concentration during these experiments. Filter effluent turbidities of 0.2 to 0.3 NTU, which were still in compliance with IESWTR requirements, were targeted for the sub-optimal coagulation experiments. It was difficult to predict how long it would take the filters to respond to the coagulant underfeed; therefore, during some experiments the target filter effluent turbidity was exceeded.

Jar-coagulated *C. parvum* oocysts were seeded into the filter influent for one hour during the sub-optimal coagulation experiments. Samples were collected at 15, 30, 45, and 55 minutes after the start of seeding. *B. subtilis* spores and polystyrene microspheres were also seeded during some of these experiments. During the one experiment that included microsphere seeding, both blue and yellow polystyrene microspheres were seeded into the filter influent.

As during the breakthrough experiments, the seeding of blue microspheres was planned for a one-hour period of stable operation approximately five hours prior to the period of deteriorated filter effluent turbidities resulting from sub-optimal coagulation. Since it was difficult to predict exactly when the effects of sub-optimal coagulation would be observed in the filters, the seeding of the blue microspheres was actually conducted four hours prior to the deterioration in filter effluent turbidities that resulted from the coagulant underfeed. As during the breakthrough experiments, the goal of seeding the blue microspheres was to help elucidate mechanistic behavior during sub-optimal coagulation (detachment versus non-attachment) by accumulating the spheres on the filter, just before the filter effluent quality started substantially deteriorating. Yellow microspheres were seeded concurrently with the *C. parvum* oocysts, during the one-hour seeding period when filter effluent turbidities were deteriorating. The *C. parvum* and yellow microsphere concentrations in the filter effluents were considered indicative of either non-attachment or very poor, transient attachment and subsequent detachment. The coagulant effects experiments and seeding conditions are summarized in Table 6.10.

Table 6.10

Summary of Coagulant Effects Experiments at the Ottawa Pilot Plant

Experiment	Date	*C. parvum*	*B. subtilis*	Blue Spheres	Yellow Spheres
No Coagulant Extended Duration	12/13/99	✓	✓		✓
No Coagulant	02/09/99	✓	✓		
Short Duration	08/04/99	✓	✓		
No Coagulant in	06/29/99	✓	✓		
Plant	07/13/99	✓	✓		
No Coagulant in Jar	08/18/99	✓	✓		
No Silicate	12/17/99	✓	✓		✓
Sub-Optimal	02/18/99	✓	✓		
Coagulation	03/23/99	✓	✓		
(~50% underfeed)	05/04/99	✓	✓		
	03/10/00	✓	✓	✓	✓

6.2.4.2 Results

Filter effluent turbidity and total particle counts, *C. parvum*, *B. subtilis*, and polystyrene microsphere removals, and particle reductions through the treatment process during the no coagulant and sub-optimal coagulation experiments at Ottawa are summarized in Table 6.11. The general filter performance data and detailed instantaneous turbidity and particle data are available in Appendix D (Tables D.4 and D.5 respectively). The detailed instantaneous *C. parvum* and *B. subtilis* and microsphere data are also available in Appendix D (Table D.6 and D.7, respectively).

Table 6.11

Filter Performance During No Coagulant and Sub-Optimal Coagulation Experiments at Ottawa

Date	Log_{10} Removal (mean ± standard deviation)				Log_{10} Red.*	Effluent Concentration	
	C. parvum	*B. subtilis*	Blue Spheres	Yellow Spheres	Particles ≥ 2 μm	Particles (#/mL)	Turbidity (NTU)
No Coagulant – Extended Duration							
12/13/99	**0.3 ± 0.02**	**0.2 ± 0.19**	—	**0.2 ± 0.04**	**0.3 ± 0.03**	**5172 ± 266**	**2.27 ± 0.00**
No Coagulant – Short Duration							
02/09/99	2.1 ± 0.33	0.4 ± 0.29	—	—	1.6 ± 0.13	206 ± 50	0.76 ± 0.06
08/04/99	3.1 ± 0.20	0.2 ± 0.22	—	—	0.8 ± 0.03	565 ± 35	0.56 ± 0.01
Overall	**2.6 ± 0.56**	**0.3 ± 0.28**	—	—	**1.2 ± 0.39**	**385 ± 196**	**0.66 ± 0.12**
No Coagulant in Plant							
06/29/99	4.8 ± 0.02	2.5 ± 0.12	—	—	1.1 ± 0.02	438 ± 25	0.74 ± 0.02
07/13/99	5.0 ± 0.03	2.2 ± 0.18	—	—	1.1 ± 0.03	498 ± 34	0.69 ± 0.03
Overall	**4.9 ± 0.12**	**2.4 ± 0.22**	—	—	**1.1 ± 0.03**	**468 ± 43**	**0.71 ± 0.04**
No Coagulant in Jar							
08/18/99	**5.6 ± 0.03**	**3.2 ± 0.11**	—	—	**4.1 ± 0.01**	**0.4 ± 0.0**	**0.03 ± 0.00**
No Silicate							
12/17/99	**5.0 ± 0.39**	**3.5 ± 0.11**	—	**3.9 ± 0.25**	**3.6 ± 0.09**	**3.6 ± 0.8**	**0.03 ± 0.00**
Sub-Optimal Coagulation (50% underfeed)							
02/18/99	1.5 ± 0.24	0.9 ± 0.13	—	—	0.8 ± 0.02	588 ± 23	0.98 ± 0.00
03/23/99	3.3 ± 0.42	1.7 ± 0.25	—	—	1.7 ± 0.02	71 ± 3.7	0.93 ± 0.04
05/04/99	5.3 ± 0.17	0.9 ± 0.04	—	—	2.4 ± 0.04	21 ± 2.1	0.16 ± 0.03
03/10/00	4.3 ± 0.13	—			1.8 ± 0.05	134 ± 13	0.28 ± 0.06
Overall	**3.6 ± 1.48**	**1.2 ± 0.43**			**1.7 ± 0.61**	**204 ± 233**	**0.59 ± 0.38**

*Log reduction of particles through treatment process (plant influent to filter effluent).

The box-and-whisker plots in Figure 6.22 through Figure 6.24 summarize the respective *C. parvum* and *B. subtilis* removals by filtration and the total particle reductions through the treatment process during the various no coagulant and sub-optimal coagulation experiments. As would be expected, the largest deterioration in oocyst, spore, and particle reductions (relative to stable operation) was observed during the no coagulant–extended duration experiment when filter effluent turbidities were high (~2.2 NTU) and approximately equal to settled water turbidities. Almost no *C. parvum* or *B. subtilis* removal or particle reduction was observed during this experiment, emphasizing the importance of good chemical pretreatment for the removal of pathogens by filtration.

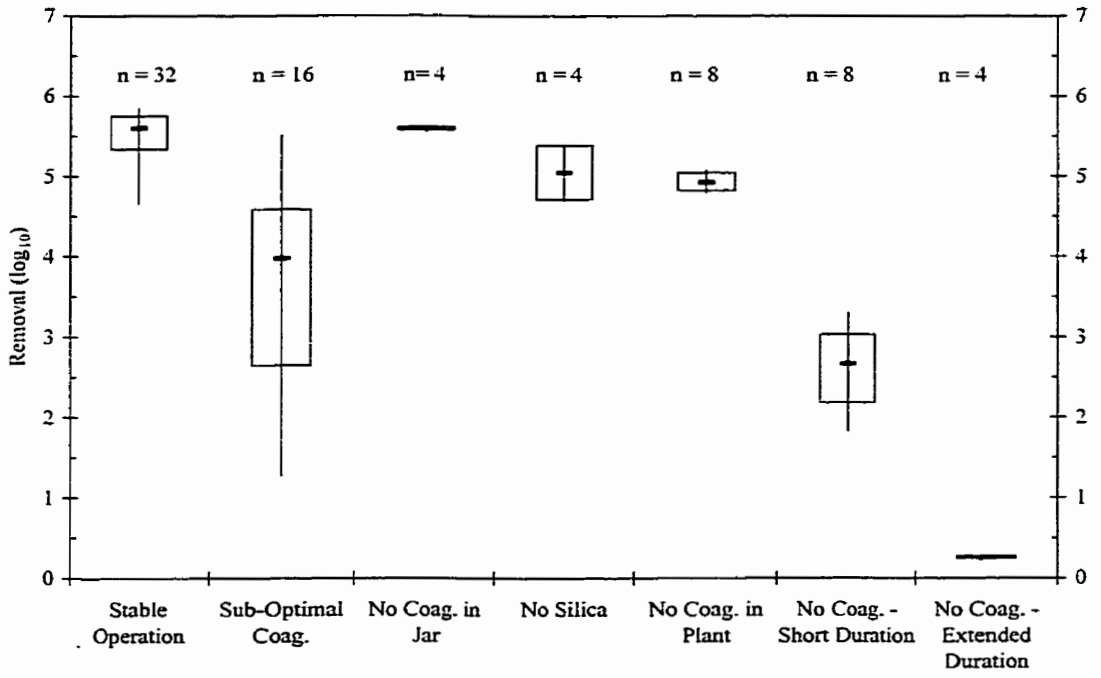


Figure 6.22 Box-and-whisker plot of *C. parvum* removals by filtration during the no coagulant and sub-optimal coagulation experiments at Ottawa.

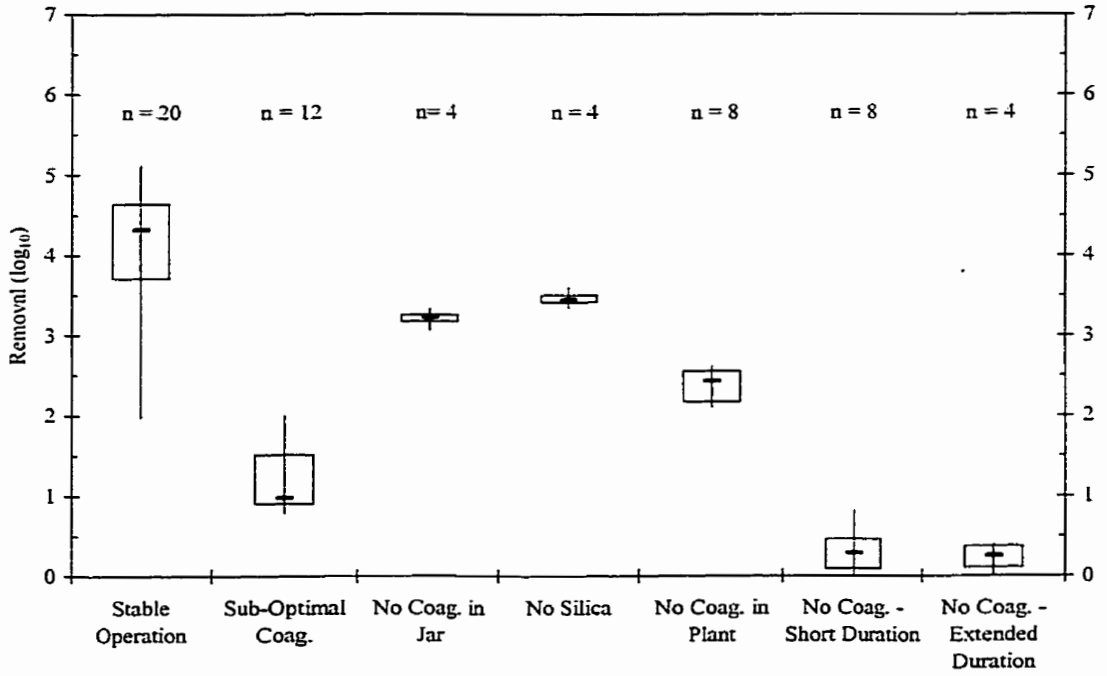


Figure 6.23 Box-and-whisker plot of *B. subtilis* removals by filtration during the no coagulant and sub-optimal coagulation experiments at Ottawa.

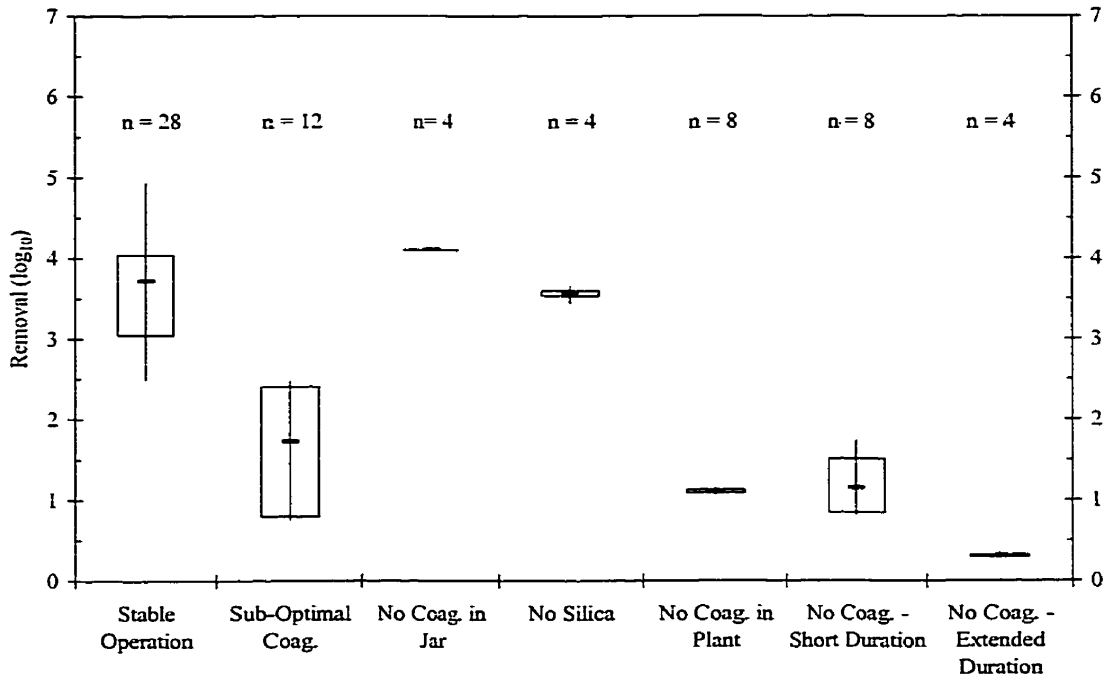


Figure 6.24 Box-and-whisker plot of total particle (≥ 2μm) reductions through the plant during the no coagulant and sub-optimal coagulation experiments at Ottawa.

Although filter effluent turbidities were also high (~0.6 to 0.8 NTU) during the no coagulant–short duration experiments, the deterioration in oocyst and spore removals by filtration and total particle reductions through the treatment process (relative to stable operation) was considerably less than during the no coagulant–extended duration experiment. More specifically, when coagulant was absent for only a short duration (several hours prior to and during seeding), the filter still managed to achieve ~2.7-log removal *C. parvum*. This level of oocyst removal represented an ~3-log reduction relative to stable operation (based on median removals during the experiments). The respective ~4-log and ~2-log decreases in *B. subtilis* removals and total particle reductions through the treatment process were generally consistent with this trend.

The absence of coagulant in the pilot plant and absence of coagulant during jar-coagulation experiments (respectively labeled "no coagulant in plant" and "no coagulant in jar") investigated the relative role of coagulant in oocyst coagulation and general filter performance. As indicated by Figure 6.22 and Figure 6.23, the *C. parvum* and *B. subtilis*

removals during these experiments were both in the range observed during stable operation. The absence of coagulant in the pilot plant had a somewhat more noticeable, though still modest effect on *C. parvum* and *B. subtilis* removals by filtration.

Unlike the *C. parvum* and *B. subtilis* removals, particle reductions were considerably lower during the experiments during which coagulant was absent from the plant, but not the jar (Figure 6.24). This result appeared consistent with differences in filter effluent turbidity and particles. During the no coagulant in the jar experiments, filter effluent turbidity and particles were consistently 0.03 NTU and 0.4 particles/mL respectively; however, during the no coagulant in the plant experiments filter effluent turbidities ranged from 0.65-0.76 NTU and 403-536 particles/mL respectively (Table C.5).

The absence of activated silica during both pilot plant and jar coagulation appeared to have no effect on the removal of *C. parvum* or *B. subtilis* by filtration, or the total particle ($\geq$ 2μm) reductions through the plant. As demonstrated by Figure 6.22 through Figure 6.24, the microorganism removals and particle reductions during the no silicate experiments were in the range of those observed during stable operation. This result was also consistent with the low filter effluent turbidities and particle counts, which were 0.03 NTU and <5 particles/mL respectively during the no silicate experiment (Table C.5).

The sub-optimal coagulation experiments (40-60% decrease in coagulant concentration) demonstrated varied effects on *C. parvum* removals by filtration, which ranged from 1.3 to 5.5-log. *C. parvum* removals during sub-optimal operating conditions ranged from comparable to or up to 3-log lower than those achieved during stable operation. It should be noted, however, that *C. parvum* removals during sub-optimal coagulation when filter effluent turbidities were below 0.3 NTU (May 4, 1999 and March 10, 2000) were within ~1-log of the removals achieved during stable operation. *B. subtilis* removals by filtration and total particle reductions ($\geq$2 μm) through the treatment process similarly varied, respectively ranging from 0.8 to 2.0-log and 0.8 to 2.5-log during sub-optimal coagulation conditions. This range of microorganism removals and particle reductions may be in part explained by the wide range of filter effluent turbidities (0.13-0.98 NTU) encountered during these experiments.

During the one sub-optimal coagulation experiment performed with microspheres (March 10, 2000), *C. parvum* removals ranged from 4.1 to >4.4-log. Similarly, the polystyrene microsphere removals ranged from 3.8 to 4.4-log. Relative to the other sub-optimal coagulation experiments, the filter effluent turbidity was low (~0.17 NTU) at the start of this experiment and increased to approximately 0.36 NTU by the end of the seeding period. Consistent with the turbidity data, total filter effluent particle concentrations (≥2 μm) ranged from 109 to 151 particles/mL during the experiment. The filter effluent *C. parvum*, microsphere, and particle concentrations during the March 10, 2000 sub-optimal coagulation experiment are presented in Figure 6.25. This figure similar removals of *C. parvum* oocysts and polystyrene microspheres.

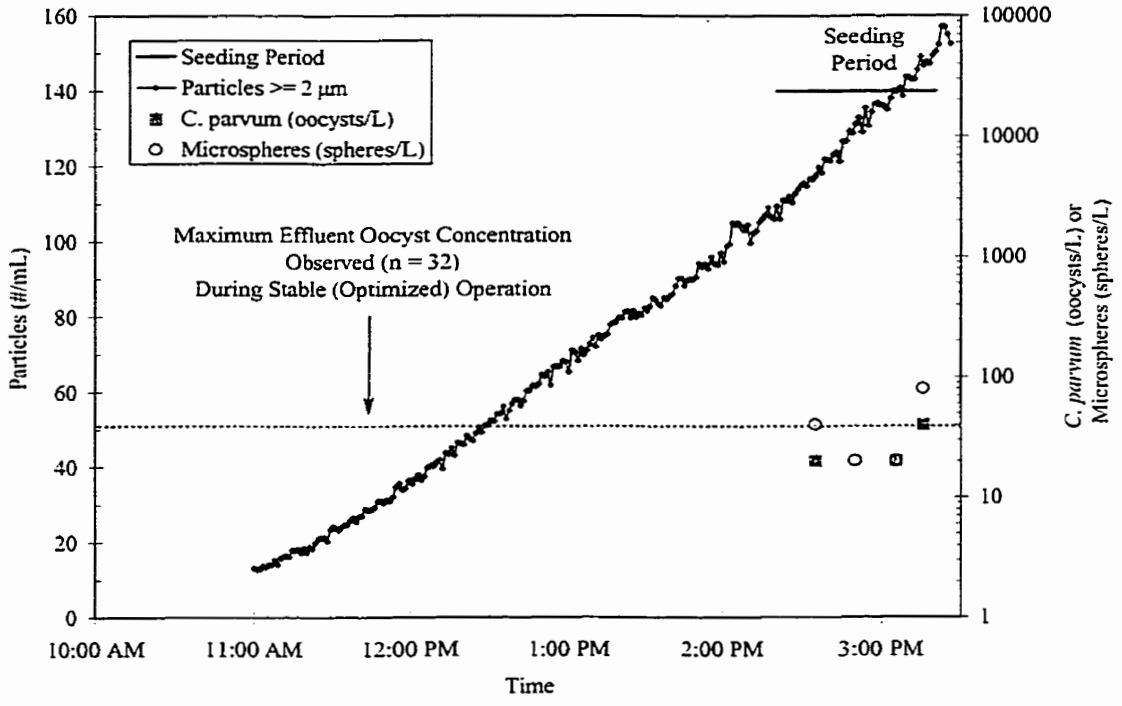


Figure 6.25 Filter effluent particle, *C. parvum*, and microsphere concentrations during March 10, 2000 sub-optimal coagulation experiment at Ottawa.

The relationship between oocyst and microsphere removals by the pilot filter during the no coagulant and sub-optimal coagulation experiments was fairly linear, as indicated in Figure 6.26, with a coefficient of determination (R^2) of 0.95. This relationship must be regarded as provisional, however, because data are only available for the two extremes. Nonetheless, the data in Figure 6.22 and Figure 6.26 provide additional support for the hypothesis that oocysts-sized polystyrene microsphere removals are reasonable indicators of *C. parvum* removal by filtration. Further investigations are necessary to determine if this relationship is consistent during stable operation and other non-optimal operating periods. Potential surrogates for *C. parvum* removal during filtration will be discussed in further detail in Chapter 7.

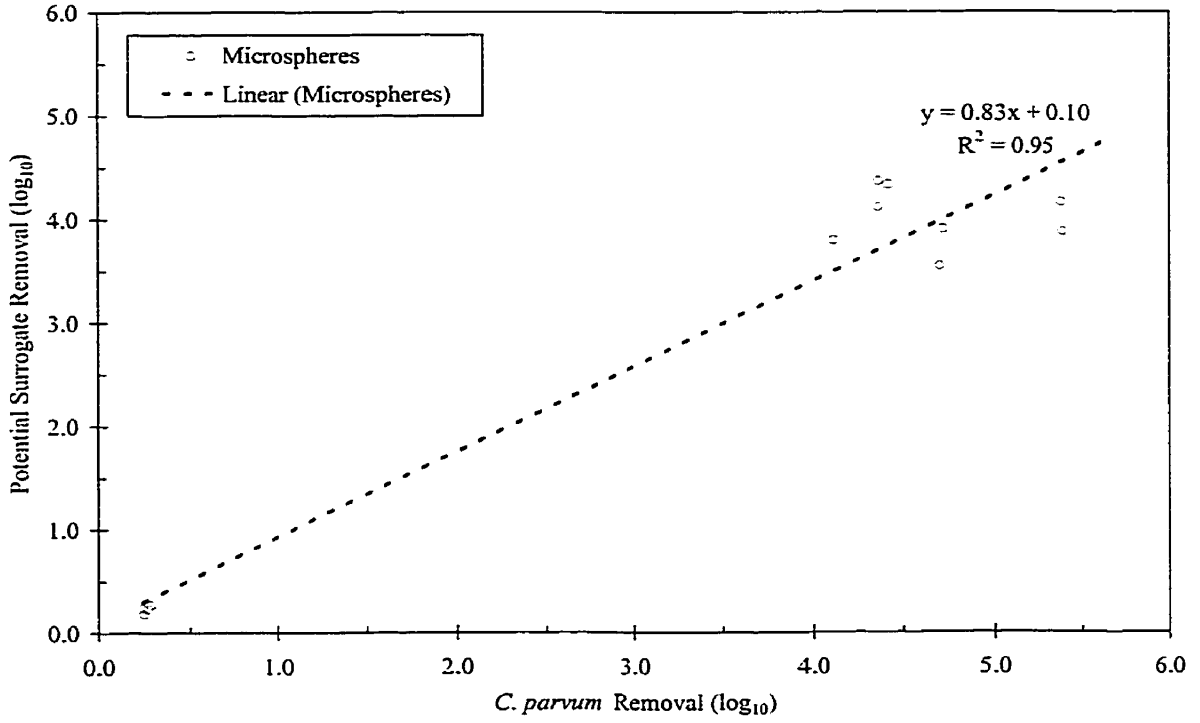


Figure 6.26. Relationship between *C. parvum* and microsphere removals by filtration during the no coagulant-extended duration, no silicate, and sub-optimal coagulation experiments at Ottawa.

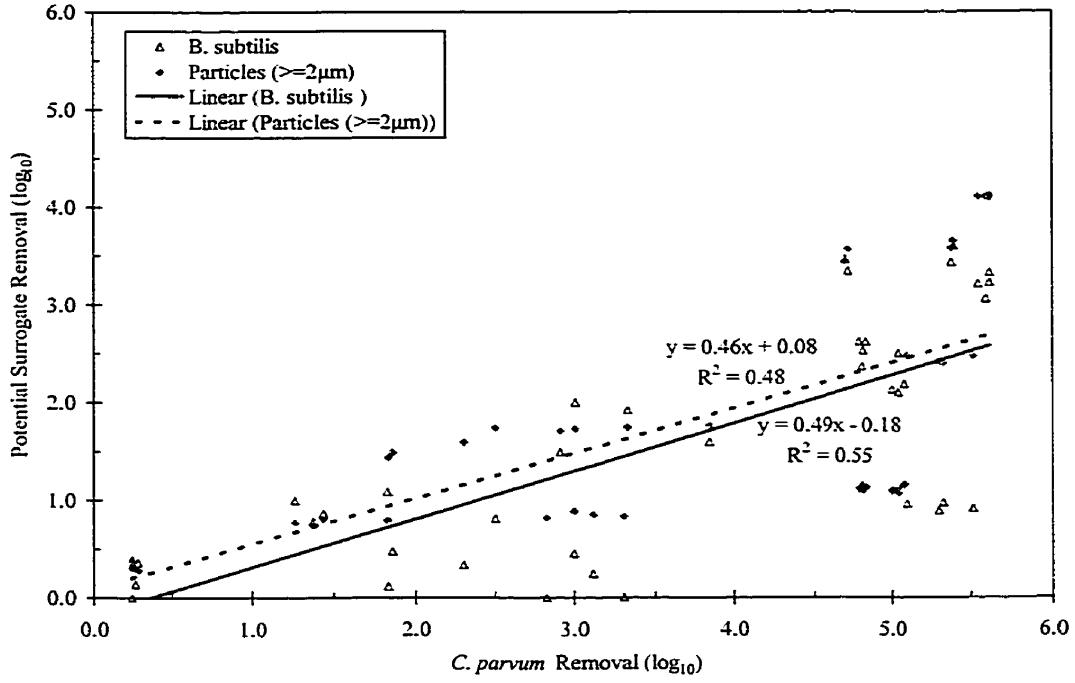


Figure 6.27. Relationship between *C. parvum* and *B. subtilis* removals by filtration and total particle (≥ 2µm) reductions through the plant during all no coagulant and sub-optimal coagulation experiments at Ottawa.

B. subtilis spore removals by filtration and total particle reductions during the no coagulant and sub-optimal coagulation experiments were compared to *C. parvum* removals in Figure 6.27. These data indicated some correlation between *C. parvum* and *B. subtilis* removals. Linear regression indicated a coefficient of determination (R^2) of 0.55. The relationship between *C. parvum* removals by filtration and total particle reductions was considerably weaker, with a coefficient of determination (R^2) of only 0.48. Further discussion of these relationships is provided in Chapter 7.

To elucidate the mechanisms associated with *C. parvum* passage through filters, the March 10, 2000 sub-optimal coagulation experiment included the seeding of blue microspheres approximately 5.5 hours before sub-optimal coagulation conditions affected filter performance. The filter influent concentrations of these microspheres would ideally be near 0 spheres/L during sample collection, so that effluent concentrations could be attributed to detachment; however, these desired concentrations were not achieved due to non-plug-flow conditions in the standing water above the filter media (Table C.7).

6.2.4.3 Statistical Analysis

Confidence intervals for the individual *C. parvum* removals during coagulant effects experiments were calculated using the method described in Chapter 4. The endpoints of the 95% confidence intervals for oocyst removal are summarized in Table 6.12. The importance of coagulation for filtration is clear from these data.

Comparison of the 95% confidence intervals for stable operation (Table 6.3) and the no coagulant (short and extended duration) experiments indicated that the lower *C. parvum* removals observed during the no coagulant experiments were significantly different from those observed during stable operation (Table 6.12, α=0.05). Relative to stable operation, the no coagulant in plant, no coagulant in jar, and no silicate experiments failed to demonstrated significant differences in *C. parvum* removal (Table 6.3 and Table 6.12, α=0.05). Sub-optimal coagulation yielded a large range of *C. parvum* removals that were likely related to the range of filter effluent turbidities during these experiments.

Data from the no coagulant-extended duration, no coagulant in the jar, no coagulant in the plant, and no silicate experiments could be pooled (whereas the other conditions could not be pooled due to continuing changes in settled water turbidities). The resulting overall 95% confidence intervals were 0.25- to 0.28-log, 5.1- to 6.0-log, 4.7- to 5.2-log, and 4.6- to 5.2-log respectively. The overall removals the no coagulant in the jar experiment failed to demonstrate significant differences in *C. parvum* removal relative to stable operation, whereas the no coagulant in the plant, no silicate, and no coagulant-extended duration experiments demonstrated *C. parvum* removals significantly different from those obtained during stable operation.

The overall 95% confidence intervals for microsphere removals during the no coagulant-extended duration and no silicate experiments were 3.7- to 3.8-log and 0.22- to 0.25-log respectively. The microsphere removals during the no coagulant-extended duration experiment were not significantly different from *C. parvum* removals (α=0.05) during that experiment, whereas microsphere removals during the no silicate experiment were significantly different from the *C. parvum* removals (α=0.05). The overall role of microspheres as surrogates for *C. parvum* removal by filtration is addressed in Chapter 7.

Table 6.12

95% Confidence Intervals and *C. parvum* Removal Ranges During the No Coagulant and Sub-Optimal Coagulation Experiments at Ottawa

Experiment	Date	Sample Time	CI_{lower} (log_{10})	CI_{upper} (log_{10})	R_{lower} (log_{10})	R_{upper} (log_{10})
No Coagulant-Extended Duration	12/13/99	t = 15	0.22	0.27	**0.22**	**0.31**
		t = 30	0.25	0.30		
		t = 45	**0.22**	0.26		
		t = 55	0.26	**0.31**		
No Coagulant-Short Duration	02/09/99	t = 15	2.40	**2.53**	**1.77**	**2.53**
		t = 30	2.21	2.32		
		t = 45	1.80	1.87		
		t = 55	**1.77**	1.85		
	08/04/99	t = 15	2.77	3.12	**2.21**	**3.36**
		t = 30	**2.21**	2.32		
		t = 45	2.96	**3.36**		
		t = 55	2.64	2.91		
No Coagulant in Plant	06/29/99	t = 15	4.31	**6.39**	**4.16**	**6.39**
		t = 30	**4.16**	6.23		
		t = 45	4.23	6.30		
		t = 55	4.20	6.25		
	07/13/99	t = 15	**4.52**	5.84	**4.52**	**6.65**
		t = 30	4.53	6.59		
		t = 45	4.55	6.61		
		t = 55	4.59	**6.65**		
No Coagulant in Jar	08/18/99	t = 15	4.87	6.91	**4.82**	**6.94**
		t = 30	**4.82**	6.89		
		t = 45	4.89	6.94		
		t = 55	4.87	**6.94**		
No Silicate	12/17/99	t = 15	4.67	6.73	**4.22**	**6.73**
		t = 30	4.67	**6.73**		
		t = 45	**4.22**	5.26		
		t = 55	4.24	5.13		
Sub-Optimal Coagulation	02/18/99	t = 15	1.78	**1.84**	**1.24**	**1.84**
		t = 30	1.41	1.44		
		t = 45	**1.24**	1.27		
		t = 55	1.36	1.39		
	03/23/99	t = 15	3.51	**3.89**	**2.82**	**3.89**
		t = 30	3.17	3.38		
		t = 45	2.89	3.04		
		t = 55	**2.82**	2.94		
	05/04/99	t = 15	4.79	**6.85**	**4.58**	**6.85**
		t = 30	**4.58**	5.61		
		t = 45	4.76	6.08		
		t = 55	4.78	6.11		
	03/10/00	t = 15	3.63	5.69	**3.58**	**5.73**
		t = 30	3.63	5.69		
		t = 45	3.67	5.73		
		t = 55	3.58	4.91		

6.2.4.4 Discussion

As indicated by Figure 6.22 and Figure 6.23, the *C. parvum* and *B. subtilis* removals during the no coagulant in jar experiment were both in the ranges observed during stable operation. This result may suggest that the presence of aluminum hydrolysis species and destabilized particles in the filter played a greater role in pathogen removal than coagulation of the pathogens themselves. Consistent with this hypothesis, the absence of coagulant only in the pilot plant only had a somewhat more noticeable, though still modest effect on *C. parvum* and *B. subtilis* removals by filtration. The same trend was not observed with total particle reductions through the treatment process. Relative to stable operation, particle reductions did not change considerably during the no coagulant in the jar experiment; however, they decreased dramatically during the no coagulant in the plant experiments (Figure 6.24).

The absence of activated silica during both pilot plant and jar coagulation appeared to have no effect on the removal of *C. parvum* and *B. subtilis* by filtration and total particle ($\geq$ 2μm) reductions through the plant. This result demonstrated that the silica did not play an important role in pathogen removal during filtration. Rather, given other performance data, the role of silica was likely as a settling aid that helped to reduce settled water turbidities and provide for longer filter cycles. During this experiment, total particle reduction through the treatment process was similar to that observed during the stable operation experiments (Figure 6.24).

The sub-optimal coagulation experiments (40-60% decrease in coagulant concentration) demonstrated varied effects on *C. parvum* removals, which ranged from 1.3 to 5.5-log. *C. parvum* removals during sub-optimal coagulation conditions were therefore anywhere from comparable to >3-log lower than those achieved during stable operation. The *C. parvum* removals during sub-optimal coagulation when filter effluent turbidities were below 0.3 NTU were within 1-log of the removals achieved during stable operation, whereas the poorer removal were associated with considerably higher filter effluent turbidities (~ 0.8 – 0.9 NTU). The *C. parvum* removal capacity of the filter decreased perhaps as non-optimally coagulated particles deposited within the filter, subsequently resulting in less favorable attachment (removal) conditions and poorer effluent quality.

The overall and long term effects of poor coagulation were revealed in the no coagulant-short duration and no coagulation-extended duration experiments, which yielded substantially lower removals of *C. parvum* relative to stable operation. Other studies have demonstrated that the relative effects of poor coagulation can be source-water and/or coagulation regime specific, although the general trends are similar (Huck *et al.*, 2001). Therefore, care should be taken when extrapolating such results to other source waters or coagulation regimes.

The results of the various coagulation investigations clearly indicated that substantial deterioration of *C. parvum* removals by filtration could occur if chemical pretreatment was not adequately optimized and maintained. Moreover, substantial deterioration in *C. parvum* removals even occurred at filter effluent turbidities below 0.3 NTU, when filters were in compliance with the requirements of the IESWTR. The no coagulant in the plant, no coagulant in the jar, and long- and short-term coagulation failure experiments also suggested that filter conditioning (at least at utilities such as Ottawa where a relatively high alum dose is used) might play a role in maintaining *C. parvum* removal during short-term coagulation failures.

6.2.5 Hydraulic Step

Hydraulic conditions can significantly impact the quality of filter effluents. It is generally recognized that filter performance is adversely affected by non-steady flow (Trussell *et al.*, 1980). Several studies have concluded that different filtration rates do not necessarily adversely impact protozoan removal by filters (Al-Ani *et al.*, 1986; Horn *et al.*, 1988). Fitzpatrick *et al.* (1999) demonstrated that large and sudden changes in flow dramatically deteriorated particle removal by filters while smaller changes that were implemented gradually did not always increase particle counts; it is possible that similar relationships could exist between *C. parvum* removal by filters and changes in flow.

6.2.5.1 Experimental Design

Experiments evaluating the effect of hydraulic changes were performed to assess the effect of filtration rate changes on the removal of *C. parvum* and *B. subtilis*. Hydraulic

steps were imposed during stable (optimized) operating conditions. Each of the hydraulic step experiments consisted of a 25% increase in filtration rate over less than one minute and was achieved by opening the filter effluent valves. The higher rate was maintained throughout the remainder of the filter cycle. The experiments were designed to represent a scenario that results in increased flow to the filters, such as when one filter is put out of service resulting in increased flow rates through the other filters that remain in operation. The experimental design tested the hypothesis that microorganisms accumulate within the filter during stable operation and then are released following a sudden increase in hydraulic loading.

During the hydraulic step experiments, the microorganisms were seeded in to the filter influent for five hours, which was an extended period of time relative to the other experimental conditions investigated. The seeding occurred during stable filtration conditions with the presumption that microorganisms would accumulate in the filter during this period. The hydraulic step was imposed immediately after the seeding period. The goal of the hydraulic step experiments was to yield information regarding the detachment of microorganisms; one way of achieving this was by seeding the filter with microorganisms and then initiating the hydraulic step when filter influent microorganism concentrations were low (ideally near 0 microorganisms/L).

Samples were collected prior to, during, and after the hydraulic step. The flow increase occurred at a time of 300 minutes (at the end of the five-hour seeding period). Samples were collected at 280 and 295 minutes (prior to the increase in hydraulic loading) to confirm that the filter was removing microorganisms at levels comparable to those achieved during the stable filter operation experiments. A 5-minute composite sample was collected at 300 minutes to collect what passed through the filter as the hydraulic step occurred. Samples were also collected at 5 minute intervals after the hydraulic step was imposed (305, 310, 315 minutes) to assess any subsequent effects on water quality. Additional samples were collected during the second and third experiments (320 and 360 minutes) to determine if microorganism removals returned to baseline levels (*i.e.*, those achieved during stable filter operation) after the change in flow rate. The hydraulic step experiments and seeding conditions are summarized in Table 6.13.

Table 6.13

Summary of Hydraulic Step Experiments at the Ottawa Pilot Plant

Experiment	Date	*C. parvum*	*B. subtilis*	Blue Spheres	Yellow Spheres
Hydraulic Step	06/07/99	✓	✓		
	06/15/99	✓	✓		
	06/21/99	✓	✓		

6.2.5.2 Results

The hydraulic step experiments were conducted in Ottawa on June 7, 15, and 22, 1999. Even though the same protocol was followed during each experiment, the resulting impact on water quality differed between each of the experiments. Filter effluent turbidity and particle counts, *C. parvum* and *B. subtilis* removals by filtration, and total particle reductions through the treatment process during the 25% increase in flow hydraulic step experiments are summarized in Table 6.14. The general filter performance data and detailed instantaneous turbidity and particle data are available in Appendix D (Tables D.4 and D.5 respectively). The detailed instantaneous *C. parvum* and *B. subtilis* data are also available in Appendix D (Table D.6).

Table 6.14

Filter Performance During Hydraulic Steps at Ottawa

Date	Log_{10} Removal (mean ± standard deviation)				Log_{10} Red.*	Effluent Concentration	
	C. parvum	*B. subtilis*	Blue Spheres	Yellow Spheres	Particles ≥ 2µm	Particles (#/mL)	Turbidity (NTU)
06/07/99	0.2 ± 1.28	0.7 ± 0.51	---	---	2.0 ± 1.01	156 ± 117	0.24 ± 0.11
06/15/99	4.0 ± 0.58	2.4 ± 0.42	---	---	2.4 ± 0.95	99 ± 140	0.05 ± 0.02
06/21/99	2.7 ± 0.78	1.8 ± 0.69	---	---	---	---	---
Overall	**2.5 ± 1.74**	**1.7 ± 0.87**	**---**	**---**	**2.2 ± 0.93**	**125 ± 126**	**0.14 ± 0.12**

*Log reduction of particles through treatment process (plant influent to filter effluent).

Both filter effluent particle counts and turbidity considerably increased for a period of approximately 30 minutes (Figure 6.28) following the flow increase on June 7. The filter effluent turbidity during stable filter operation portion prior to the hydraulic step was ~0.06 NTU and the filter effluent particle concentration (≥ 2 μm) was ~0.5 particles/mL. As a result of the hydraulic step, the filter effluent turbidity and particle concentration temporarily increased up to 0.37 NTU and 297 particles/mL respectively. During the course of that filter cycle, neither the filter effluent turbidity nor effluent particle concentration returned to the baseline levels that had been achieved prior to the hydraulic step (Figure 6.28). Generally consistent with the stable operation experiments, filter effluent concentrations of *C. parvum* and *B. subtilis* were relatively low (respectively <10 oocysts/L and <100 cfu/L) during the stable operation portion of the filter cycle prior to the hydraulic step. The increase in filter effluent turbidity and particle concentration (Figure 6.28) was commensurate with an increase in filter effluent microorganisms, with effluent concentrations of *C. parvum* reaching 4412 oocysts/L and *B. subtilis* reaching 2000 cfu/L (Figure 6.29). Detailed turbidity, particle, and microorganism concentration and removal data are available in Appendix D.

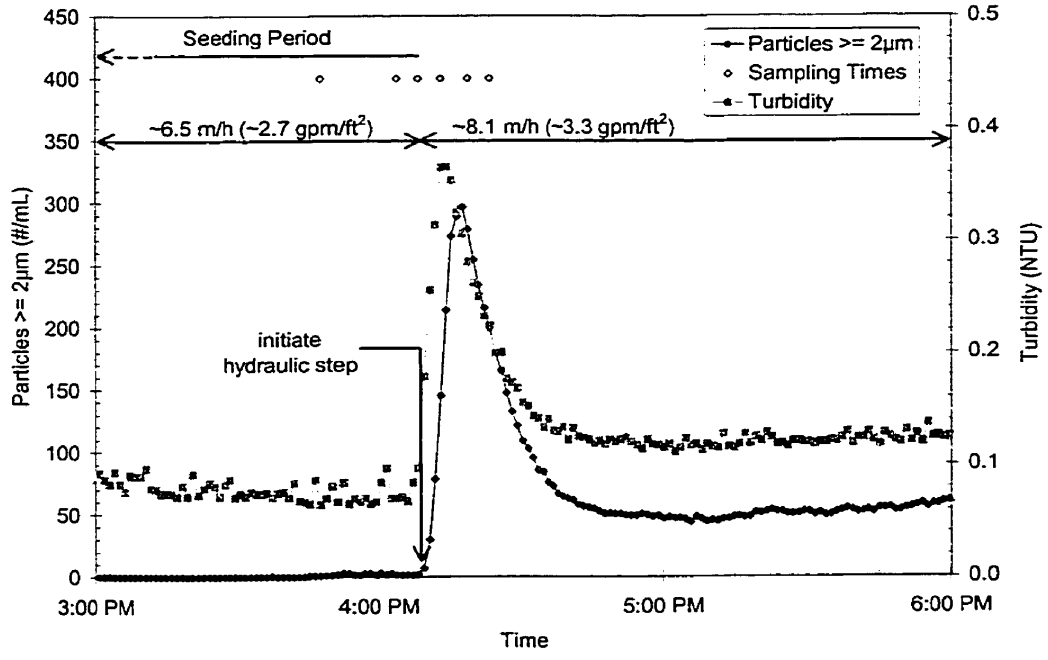


Figure 6.28 Turbidity and particle response of filter during hydraulic step experiment on June 7, 1999 at Ottawa pilot plant.

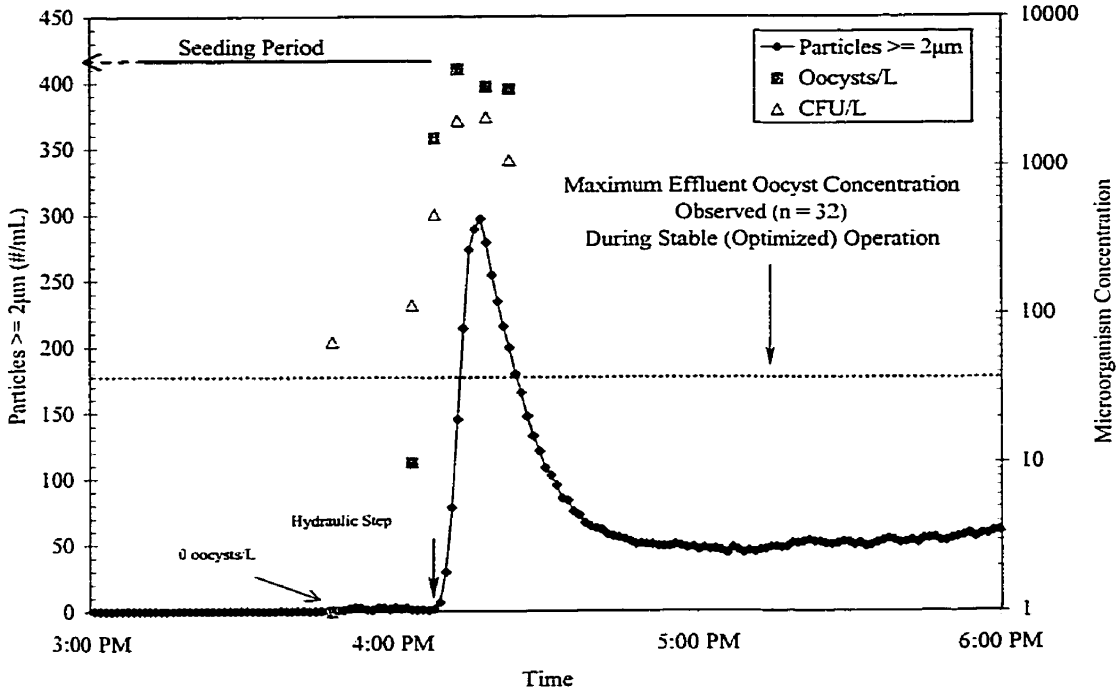


Figure 6.29 Particle and microorganism response of filter during hydraulic step experiment on June 7, 1999 at Ottawa pilot plant.

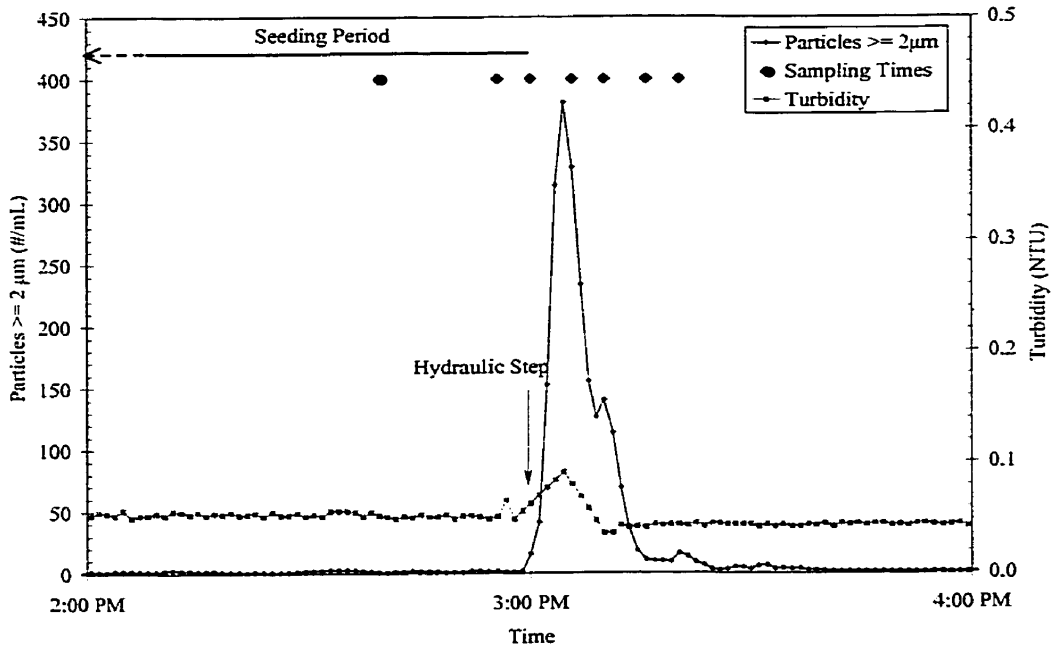


Figure 6.30 Turbidity and particle response of filter during hydraulic step experiment on June 15, 1999 at Ottawa pilot plant.

The hydraulic step had a very different impact on filter effluent water quality on June 15. The filter effluent turbidity only slightly increased whereas the particle concentration peaked considerably (Figure 6.30). The filter effluent turbidity during stable filter operation prior to the hydraulic step was approximately 0.05 NTU and the filter effluent particle concentration (≥ 2 μm) was approximately 1.0 particle/mL. As a result of the hydraulic step the filter effluent turbidity and particle concentration temporarily increased to 0.09 NTU and 381 particles/mL respectively. Unlike the June 7 experiment, both the filter effluent turbidity and particle concentration returned to the baseline levels that were being achieved prior to the hydraulic step (Figure 6.30). Despite the considerable increase in filter effluent particle concentration (over a period of approximately 30 minutes), no appreciable changes in filter effluent *C. parvum* and *B. subtilis* concentrations relative to the stable operation period of the filter cycle were observed (Figure 6.31). While relatively low concentrations of *B. subtilis* spores were found in the filter effluent during the stable and hydraulic step portions of the experiment (< 500 cfu/L), almost no *C. parvum* oocysts were detected.

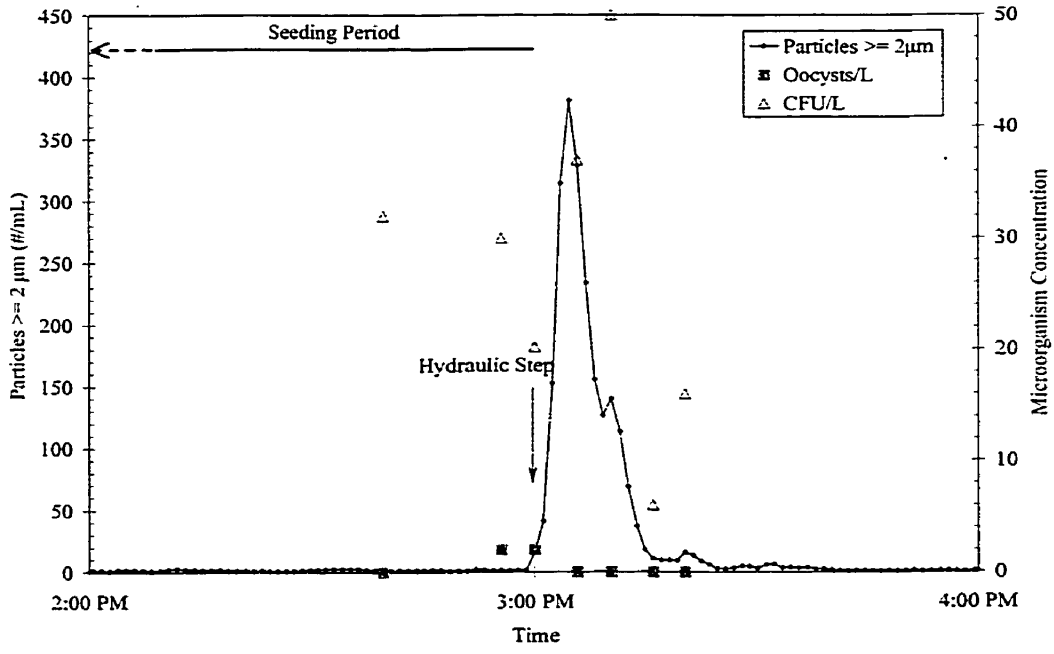


Figure 6.31 Particle and microorganism response of filter during hydraulic step experiment on June 15, 1999 at Ottawa pilot plant.

A third hydraulic step experiment was performed on June 22. Filter effluent turbidity and particle data were not available during this experiment due to difficulties with the data acquisition system. These problems made it impossible to exactly pinpoint when the hydraulic step (and associated sampling) occurred. Filter effluent turbidity and particle data collected prior to the experiment indicated that the filter was likely operating at less than optimal conditions just prior to the hydraulic step; the effluent particle counts were slightly above what was typically observed at the Ottawa pilot plant during stable filter operation. A slight increase in filter effluent oocyst concentrations was observed as a result of the hydraulic step, however, the filter effluent spore concentrations did not demonstrate this trend. Although the filter effluent oocyst concentrations were slightly elevated as a result of the hydraulic step, the increase was not as dramatic as that which occurred during the June 7 experiment.

Confidence intervals for the removal of *C. parvum* during the hydraulic step experiments were not calculated. This was because the confidence intervals could be somewhat misleading because the filter influent concentrations of the microorganisms were decreasing as sampling occurred. *C. parvum* removals based on filter influent and effluent pairs of data would not account for any oocysts already accumulated on the filters during the seeding period.

6.2.5.3 Discussion

One hydraulic step experiment at Ottawa (June 15) did not appear to yield substantial increases in filter effluent microorganism concentrations (*C. parvum* and *B. subtilis*) between the stable operation and hydraulic step portions of the experiments. These data suggest that little detachment of microorganisms occurred as a result of 25% increase in flow hydraulic steps. Considerable changes in filter effluent *C. parvum* concentrations occurred during the other two of the hydraulic step experiments at Ottawa (June 7 and 22). The presence of oocysts in the filter effluent samples during these experiments was likely due to at least a moderate amount of detachment from the filters; this was particularly evident in the June 7 data where the filter effluent oocysts were higher than

the influent concentrations. Further mechanistic investigations would be necessary to appropriately address this speculation however.

Overall, the traditional performance data (filter effluent turbidity and particle concentration) proved to be good indicators of treatment performance (Figure 6.28 to Figure 6.31). The June 7 and 15 hydraulic step experiments underscored the conclusion that filter effluent turbidity and particle counts were not directly indicative of microorganism passage through filters. A higher peak particle concentration occurred during the June 15 (Figure 6.30) experiment than during the June 7 experiment (Figure 6.28). In contrast, the filter effluent microorganism concentration was dramatically higher on June 7 (Figure 6.29) than on June 15 (Figure 6.31). Moreover, the filter effluent particle spike on June 15 was not accompanied by an increase in effluent oocyst concentration, emphasizing that increases in performance measures such as particle counts are not necessarily directly indicative of microorganism passage through filters.

Filter effluent particle concentration, turbidity, oocyst and spore concentrations increased for a short period of time during two hydraulic step experiments at Ottawa. Even though the increase in effluent microorganism concentrations was temporary, the number of oocysts that passed through the filter was substantial (given that a high number of oocysts were seeded into the filter during the experiment). This result was consistent with the findings of Cleasby *et al.* (1963), Tuepker and Buescher (1968), and Fitzpatrick *et al.* (1999) who showed that sudden, large flow rate changes cause deterioration of filtered water quality. These findings were also consistent with those of Logsdon *et al.* (1981) who similarly demonstrated that increases in *Giardia* passage through filters could be expected as a result of hydraulic changes.

The June 15 hydraulic step experiment demonstrated that a 25% increase in flow could leave filter effluent *C. parvum* and *B. subtilis* concentrations and turbidity essentially unaffected while moderate spikes in filter effluent particle concentrations ($\geq$ 2 μm) occurred. The difference between these results and those from other hydraulic step experiments that resulted in considerable passage of microorganisms might be explained in part by the work of Cleasby *et al.* (1963), which demonstrated that particle passage

through filters following a disturbance was dependent on the composition of the filter influent. These data suggest that the balance between attachment and detachment forces may be variable at a given location and result in performance from no risk to a high risk of microorganism release from a filter as a result of hydraulic changes. These results further indicate that it may be possible to optimize or at least identify the factors that affect these forces so that the potentially severe effects of hydraulic changes (such as those observed during the June 7 experiment) can be minimized.

It is difficult to speculate on a cause for the differences in filter effluent water quality between the hydraulic step experiments. The hydraulic step experiments were performed within a week of each other (essentially no variation in water temperature), at approximately 25 to 30 hours into the filter cycle, and with comparable raw water and filter effluent quality and coagulation conditions prior to imposing the hydraulic step. Differences in oocyst and spore removals relative to stable operation were at least partly affected by the lower (relative to stable operation) filter influent oocyst concentrations that resulted from the extended seeding period during the hydraulic step experiments.

It was expected that the microorganism removals during the stable operation portion of the hydraulic step experiments might be lower than those observed during the stable operation experiments. This was because of already low effluent microorganism concentrations (often near detection limits at Ottawa) during the stable operation experiments and lower influent concentrations (compared to stable operation) associated with the longer seeding period during the hydraulic step experiments. Although the filter effluent *C. parvum* concentrations were often lower during the hydraulic step experiments than during the stable operation, the decrease in filter effluent oocyst concentrations was not directly proportional to the decrease in influent concentrations. These data emphasized the need to further study the relationship between filtration studies using high seeded microorganism concentrations as opposed to indigenous microorganism concentrations.

6.3 University of Waterloo (UW) Pilot Plant Investigations

A limited number of pilot-scale *C. parvum*, *B. subtilis*, and polystyrene microsphere removal investigations were conducted at the University of Waterloo (UW) Pilot Plant in Waterloo, Ontario, Canada. The UW pilot plant was operated in direct filtration mode with in-line flocculation (contact filtration). It treated synthetic raw water comprised of dechlorinated tap water with kaolinite-induced turbidity (~1.5 NTU at filter influent). The coagulation regime consisted of a relatively low alum dosage (~5 mg/L) for particle removal. The raw water was coagulated in-line and then filtered by both dual- and tri-media filters. The rationale and specific seeding conditions for each of the experiments were summarized in Chapter 3 and are elaborated upon below.

6.3.1 Stable (Optimized) Operation

Stable operation experiments were performed in duplicate to determine the *C. parvum*, *B. subtilis*, and polystyrene microsphere removals achieved by the pilot-scale filters under optimal operating conditions at UW. The stable operation experiments were conducted in duplicate and represented optimized pretreatment and filtration conditions (*i.e.*, no perturbations or upsets). As at Ottawa, the seeding and sampling were conducted after at least four hours of filter operation, during the early to middle portion of the filter cycle.

6.3.1.1 Experimental Design

Four stable operation experiments were conducted at UW (two investigated dual-media and two investigated tri-media). The use of both media types allowed for direct comparison and investigation of potential improvements in pathogen removal that might be associated with tri-media filtration. Jar-coagulated *C. parvum*, *B. subtilis*, and yellow, oocyst-sized polystyrene microspheres were seeded into the filter influent for one hour during these experiments. Samples were collected at 20, 40, and 55 minutes after the start of seeding. Filter effluent turbidities of <0.1 NTU were targeted during these experiments. The stable operation experiments and seeding conditions are summarized in Table 6.15.

Table 6.15

Summary of Stable Operation Experiments at the UW Pilot Plant

Experiment	Date	*C. parvum*	*B. subtilis*	Blue Spheres	Yellow Spheres
Stable Operation	11/23/99	✓	✓		✓
Dual-Media	11/24/99	✓	✓		✓
Stable Operation	11/28/99	✓	✓		✓
Tri-Media	11/28/99	✓	✓		✓

6.3.1.2 Results

The filter effluent turbidity and particle counts were consistently low (< 0.1 NTU and < ~25 particles/mL, respectively) during the stable operation experiments at the UW pilot plant. Though excellent filter effluent quality water was produced during the stable operation experiments at UW, filter effluent particle concentrations and turbidities were somewhat less consistent between the duplicate experiments in each filter type than between the replicate experiments at Ottawa. Filter effluent turbidity and particle counts and *C. parvum*, *B. subtilis*, and polystyrene microsphere removals by filtration during stable operation at UW are summarized in Table 6.16. The stable operation performance of the UW filters was generally consistent with that observed at Ottawa; therefore, filter effluent turbidity, particle concentrations, and microorganism concentration data are not exhaustively discussed. The general filter performance data and detailed instantaneous turbidity and particle data are available in Appendix D (Tables D.10 and D.11 respectively). The detailed instantaneous *C. parvum*, *B. subtilis*, and microsphere data are also available in Appendix D (Table D.12).

As summarized in Table 6.16, excellent *C. parvum* removals were achieved by the dual- and tri-media filters during stable operation. The >5-log removals were generally consistent between the replicate experiments for a given media type. *C. parvum* removals by the dual-media filter during stable (optimized) operation ranged from 4.7 to

5.4-log, with a mean oocyst removal of 5.0-log (6 samples in total). *C. parvum* removals by tri-media filtration were somewhat higher, ranging from 4.9 to 5.7-log, with a mean oocyst removal of 5.3-log (6 samples in total). Given these data, tri-media filters did not appear to offer appreciable advantages in *C. parvum* removal relative to dual-media filters. Since these experiments were only performed in duplicate, further experiments would better elucidate this relationship.

Though more variable than *C. parvum* passage through filtration between the replicate tri-media experiments, *B. subtilis* spore passage through the UW filters appeared to be consistent with the *C. parvum* data. *B. subtilis* removal by dual-media filtration during stable operation ranged from 2.9 to 3.3-log, with a mean removal of 3.1-log (6 samples in total). The tri-media filter removed 3.3 to 4.8-log of B. *subtilis*, with a mean spore removal of 3.9-log (6 samples in total). These data were generally consistent with the *C. parvum* removal data that indicated slightly better oocyst removals by the tri-media filter. The confidence intervals for these data are in Appendix D (Table D.15).

Table 6.16

Filter Performance During Stable Operation at UW

Date	Log_{10} Removal (mean ± standard deviation)					Log_{10} Red.*	Effluent Concentration
	C. parvum	*B. subtilis*	Blue Spheres	Yellow Spheres	Particles ≥ 2μm	Particles (#/mL)	Turbidity (NTU)
11/23/99	5.0 ± 0.25	3.2 ± 0.09	—	4.8 ± 0.29	—	19.2 ± 4.2	0.07 + 0.00
11/24/99	5.1 ± 0.26	3.1 ± 0.13	—	4.4 ± 0.31	—	2.7 ± 0.4	0.06 + 0.03
Dual-Med. Overall	**5.0 ± 0.23**	**3.1 ± 0.13**	—	**4.6 ± 0.35**	—	**10.9 ± 9.4**	**0.06 ± 0.02**
11/28/99	5.1 ± 0.12	3.4 ± 0.09	—	4.6 ± 0.16	—	6.9 ± 0.9	0.05 + 0.00
11/28/99	5.6 ± 0.03	4.4 ± 0.30	—	6.0 ± 0.20	—	1.6 ± 0.8	0.03 + 0.00
Tri-Media Overall	**5.3 ± 0.31**	**3.9 ± 0.57**	—	**5.3 ± 0.78**	—	**4.2 ± 3.0**	**0.04 ± 0.01**

*Log reduction of particles through treatment process (plant influent to filter effluent).

Tri-media filtration also appeared to provide slightly better removals of polystyrene microspheres compared to those achieved by dual-media filtration. Microsphere removals by the dual-media filter during stable operation ranged from 4.1 to 5.1-log, with a mean microsphere removal of 4.6-log (6 samples in total). Microsphere removals by tri-media filtration were somewhat higher, ranging from 4.4 to 6.1-log, with a mean microsphere removal of 5.3-log (6 samples in total). The confidence intervals for these data are in Appendix D (Table D.15).

B. subtilis and polystyrene microsphere removals by both dual- and tri-media filtration were generally lower than those of *C. parvum*. A comparison of *B. subtilis* and microsphere removals as potential surrogates for *C. parvum* removals by filtration is provided in Figure 6.32. Total particle reductions through the treatment process could not be examined because raw water particle concentrations were not measured due to equipment limitations (only one particle counter was available). These data demonstrated that both *B. subtilis* spore and polystyrene microsphere removals were somewhat indicative of *C. parvum* removals by dual- and tri-media filtration at UW. Least-squares linear regression of the *B. subtilis* and microsphere removal data yielded coefficients of determination (R^2) of 0.75 and 0.80 respectively. The relationship between *C. parvum* removals by filtration during stable operation and *B. subtilis* and microsphere removals at UW yielded somewhat different conclusions than the Ottawa data. It is possible that differences in filtration regime (contact filtration as compared to conventional treatment) or coagulation regime might affect how well removals of potential surrogates correlate with *C. parvum* removals. As mentioned previously, potential surrogates for *C. parvum* removal by filtration will be discussed in greater detail in Chapter 7.

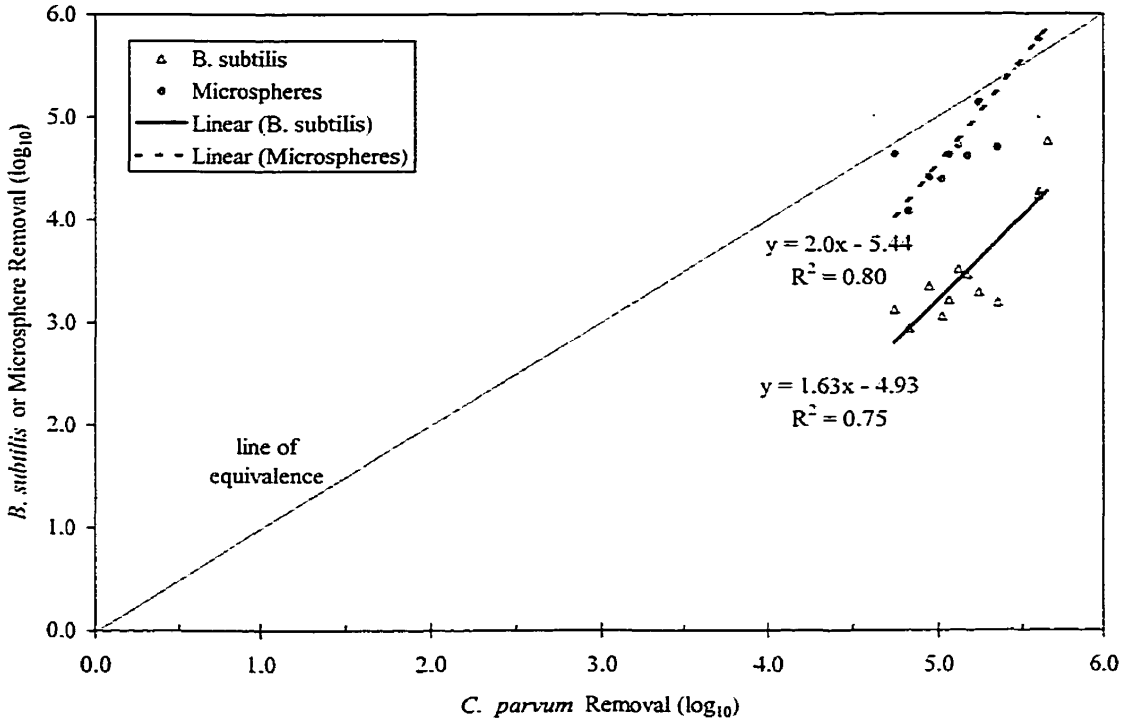


Figure 6.32 Relationship between *C. parvum*, *B. subtilis*, and microsphere removals by the pilot-scale dual- and tri-media filters during stable operation at UW.

6.3.1.3 Statistical Analysis

Summarized in Table 6.17, the endpoints of the 95% confidence intervals for the individual *C. parvum* log removals by dual- and tri-media filters during stable operation in were calculated using the method described in Chapter 4. All of these confidence intervals overlap, failing to demonstrate statistically significant differences between the data collected during stable operation in a filter of a given media type or between media types (Table 6.17, α=0.05). This result suggests that, for the raw water investigated, tri-media filters did not offer appreciable advantages in *C. parvum* removal during stable operating conditions. The endpoints of the range indicate the lowest and highest *C. parvum* removals observed during the operating period and incorporate analytical uncertainty. The *C. parvum* removal ranges for the dual- and tri-media filters during stable operation were 4.2- to 6.7-log and 4.4- to 7.0-log respectively. The range for the individual experiments is summarized in Table 6.17.

Table 6.17

95% Confidence Intervals and *C. parvum* Removal Ranges

During Stable Operation at UW

Experiment	Date	Sample Time	CI_{lower} (log_{10})	CI_{upper} (log_{10})	R_{lower} (log_{10})	R_{upper} (log_{10})
Stable - Dual-Media	11/23/99	20	4.5	5.6	**4.2**	**6.7**
		40	**4.2**	4.9		
		55	4.7	**6.0**		
Stable - Dual-Media	11/24/99	20	**4.3**	5.3	**4.3**	**6.7**
		40	4.6	**6.7**		
		55	4.5	5.8		
Stable - Tri-Media	11/28/99	20	4.6	**5.9**	**4.4**	**5.9**
		40	**4.4**	5.5		
		55	4.6	5.9		
Stable - Tri-Media	11/28/99	20	4.9	**7.0**	**4.9**	**7.0**
		40	**4.9**	6.9		
		55	4.9	7.0		

6.3.1.4 Discussion

Similar to the more numerous experiments at Ottawa, the stable filter operation experiments at UW demonstrated that *C. parvum* removals of >5-log could be achieved by both dual- and tri-media filtration. Commensurate with the bench-scale findings from Chapter 5, tri-media filtration provided marginal, but not statistically significant advantages in *C. parvum* removal relative to dual-media filtration. These results are in general agreement with *C. parvum* removal data that have been reported in the literature (Patania *et al.*, 1995; Fox *et al.*, 1998). They provided the baseline removals to which the other operating conditions (hydraulic step) at UW were compared.

6.3.2 Hydraulic Step

As described in Chapter 2, hydraulic conditions can significantly impact the quality of filter effluents (Trussell *et al.*, 1980). The pilot-scale experiments conducted at Ottawa and described in Section 6.2.5.2 investigated the effects of hydraulic steps on *C. parvum* and *B. subtilis* removal by dual-media filtration. They failed to conclusively demonstrate

whether or not considerable deterioration in either particle or pathogen removal could be expected as a result of a sudden, 25% increase in flow to the pilot-scale filter. As a result, further hydraulic step experiments were conducted at the UW pilot plant.

6.3.2.1 Experimental Design

Experiments evaluating the effect of hydraulic changes were performed to assess the effect of filtration rate changes on the removal of *C. parvum*, *B. subtilis*, and polystyrene microspheres. These experiments were conducted with both dual- and tri-media filters to investigate whether tri-media filter designs could potentially mitigate pathogen passage through filters operating during hydraulic step conditions. As during the Ottawa experiments, hydraulic steps were imposed during stable (optimized) operating conditions. Each of the hydraulic step experiments consisted of a 25% increase in the filtration rate over less than one minute, which was achieved by opening the filter effluent valves. The higher rate was maintained throughout the remainder of the filter cycle.

During the hydraulic step experiments, the *C. parvum* oocysts and yellow microspheres were seeded in the filter influent for one hour prior to the initiation of the hydraulic step. The seeding occurred during stable filtration conditions with the presumption that microorganisms would accumulate in the filter during this period. The hydraulic step was imposed immediately after the seeding period. *B. subtilis* spores were seeded during the one hour period that began fifteen minutes prior to the initiation of the hydraulic step. The goal of this experimental design was to yield information regarding the detachment and non-attachment (or weak attachment and subsequent detachment) of microorganisms.

Samples were collected prior to, during, and after the hydraulic step. The flow increase occurred at a time labeled 0 minutes (when seeding of *C. parvum* and microspheres ended and fifteen minutes into the seeding of *B. subtilis*). Samples were collected at 15 minutes (prior to the change in hydraulic loading) to confirm that the filter was removing microorganisms at levels comparable to those achieved during the stable filter operation experiments. A sample was collected at 0 minutes to collect what passed through the filters as the hydraulic step occurred. Samples were also collected at 10-minute intervals

after the hydraulic step (10 and 20 minutes) to determine any subsequent effects on water quality. The dual- and tri-media hydraulic step experiments were each conducted in duplicate. The hydraulic step experiments and seeding conditions are summarized in Table 6.18.

6.3.2.2 Results

The hydraulic step experiments were conducted at UW on December 10 and 11, 1999, and January 15 and 16, 2000. Filter effluent turbidity and particle counts and *C. parvum* and *B. subtilis* removals during the 25% increase in flow hydraulic step experiments are summarized in Table 6.19. The summarized data include all of the time points (-15, 0, 10, and 20 minutes) except for t = -60 minutes, because these samples were only collected during the dual-media experiments; their inclusion would make it difficult to compare the dual- and tri-media filtration data. The general filter performance data and detailed instantaneous turbidity and particle data are available in Appendix D (Tables D.10 and D.11 respectively). The detailed instantaneous *C. parvum*, *B. subtilis*, and microsphere data are also available in Appendix D (Table D.12).

Table 6.18

Summary of Hydraulic Step Experiments at the UW Pilot Plant

Experiment	Date	*C. parvum*	*B. subtilis*	Blue Spheres	Yellow Spheres
Hydraulic Step	12/10/99	✓	✓		✓
Dual-Media	12/11/99	✓	✓		✓
Hydraulic Step	01/15/00	✓	✓		✓
Tri-Media	01/16/00	✓	✓		✓

Table 6.19

Filter Performance During Hydraulic Steps at UW

Date	Log_{10} Removal (mean ± standard deviation)				Log_{10} Red.*	Effluent Concentration	
	C. parvum	*B. subtilis*	Blue Spheres	Yellow Spheres	Particles ≥ 2μm	Particles (#/mL)	Turbidity (NTU)
12/10/99	4.6 ± 0.69	1.8 ± 0.35	—	4.4 ± 0.62	—	16 ± 10	0.05 ± 0.00
12/11/99	4.3 ± 0.82	2.5 ± 0.41	—	3.1 ± 0.79	—	25 ± 11	0.06 ± 0.00
Dual-Med. Overall	**4.4 ± 0.71**	**2.1 ± 0.51**	—	**3.8 ± 0.96**	—	**21 ± 11**	**0.05 ± 0.01**
01/15/00	4.2 ± 0.57	1.9 ± 0.33	—	4.3 ± 0.53	—	47 ± 30	0.06 ± 0.01
01/16/00	4.4 ± 0.73	2.2 ± 0.23	—	4.6 ± 0.53	—	42 ± 28	0.06 ± 0.01
Tri-Media Overall	**4.3 ± 0.62**	**2.1 ± 0.31**	—	**4.5 ± 0.52**	—	**44 ± 27**	**0.06 ± 0.01**

*Log reduction of particles through treatment process (plant influent to filter effluent).

The hydraulic step experiments at UW yielded results similar to those observed for the June 15, 1999 hydraulic step experiment at Ottawa (Figure 6.30 and Figure 6.31). The filter effluent turbidity did not appreciably increase whereas the particle concentration peaked slightly. The filter effluent turbidity during stable filter operation prior to, during, and after the hydraulic step was approximately 0.05 NTU. The dual- and tri-media filter effluent particle concentrations (≥ 2 μm) during stable operation (prior to the hydraulic step) were approximately 10-20 particles/mL. As a result of the hydraulic step the filter effluent particle concentrations temporarily increased to 40-75 particles/mL, with slightly higher peaks observed in the tri-media filter effluent. The filter effluent particle concentrations returned to the baseline levels achieved prior to the hydraulic step. Small, but appreciable changes in filter effluent *C. parvum* concentrations relative to the stable operation period of the filter cycle were observed concurrent to the moderate increase in filter effluent particles. Filter effluent *B. subtilis* spore concentrations were also elevated, relative to those observed during stable operation, as a result of the hydraulic steps; however, it should be recalled that *C. parvum* and *B. subtilis* were not concurrently seeded during these experiments at UW.

Although *C. parvum* removal data can be somewhat misleading since filter influent concentrations were decreasing during sampling, the relative removal of oocysts and microspheres could still be compared since they were concurrently seeded. The relative removal of *C. parvum* oocysts and polystyrene microspheres during the hydraulic step experiments at UW is presented in Figure 6.33. This figure demonstrates some correlation between oocyst and oocyst-sized microsphere removals by filtration. Least-squares linear regression of the microsphere removal data yielded coefficients of determination (R^2) of 0.65 and 0.91 for dual- and tri-media filtration respectively. Combination of all of the microsphere data resulted in a slightly lower coefficient of determination (R^2) of 0.52. These relationships will be further examined in Chapter 7.

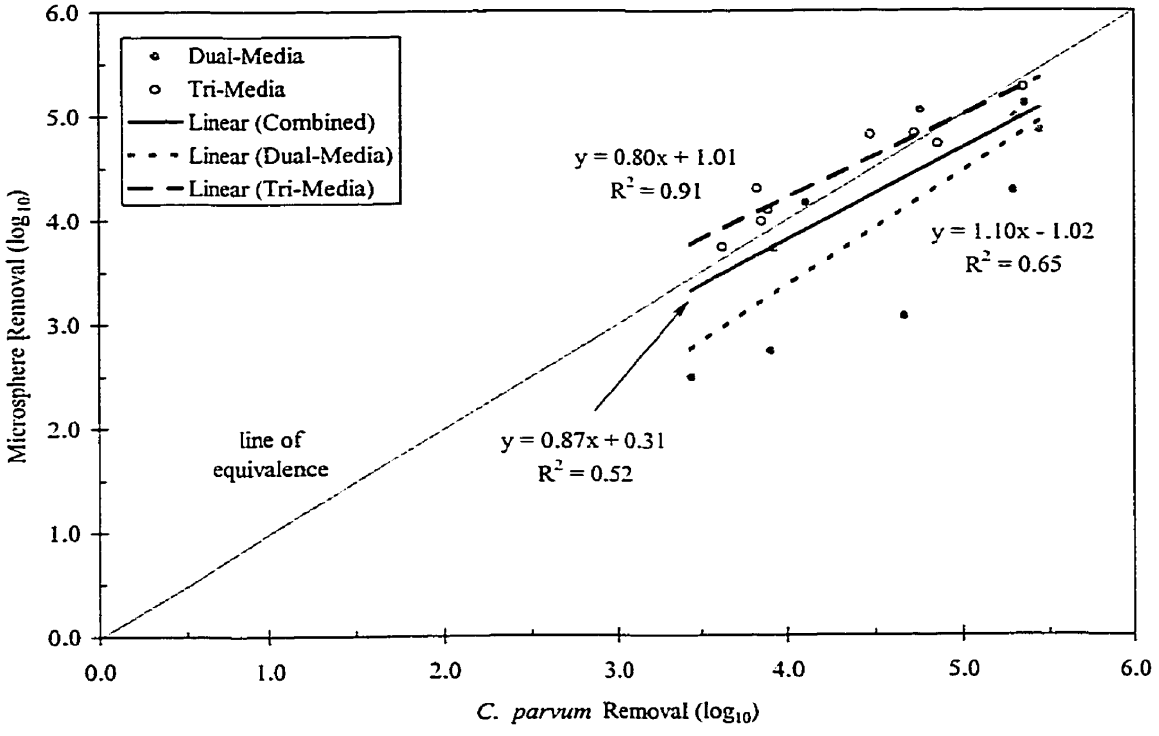


Figure 6.33 Relationship between *C. parvum* and microsphere removals by the pilot-scale dual- and tri-media filters during hydraulic steps at UW.

6.3.2.3 Discussion

In general, it is difficult to make mechanistic conclusions from the UW pilot data. The presence of oocysts in the filter influent during the imposition of the hydraulic step, albeit at low concentrations, made it difficult to clearly determine whether or not the presence of oocysts in the filter effluent resulted from detachment or non-attachment. Although it was difficult to elucidate mechanistic information from the hydraulic step experiments, the filter influent *C. parvum* and microsphere concentrations were decreasing while filter effluent concentrations were increasing as a result of the imposed hydraulic steps. These data suggested the possibility of moderate amount of detachment from the filters, however, they were not as convincing as the June 7, 1999 Ottawa data which included filter effluent oocysts concentrations that were higher than influent concentrations. Conversely, the *B. subtilis* effluent concentrations were somewhat higher than during stable operation, suggesting some non-attachment or weak attachment and subsequent detachment of spores within the filter.

The traditional performance data (filter effluent turbidity and particle concentration) proved to be good indicators of treatment efficiency. While no increase in filter effluent turbidities was observed, a noticeable increase in total filter effluent particle counts signaled a moderate increase in filter effluent *C. parvum, B. subtilis* and polystyrene microsphere concentrations. Although the filter effluent microorganism and microsphere concentrations were slightly higher than during stable operation, this deterioration in finished water quality was typically brief.

These experiments did demonstrate that a 25% increase in flow through dual- and tri-media filters would not necessarily result in dramatically increased filter effluent pathogen concentrations. As mentioned earlier, the UW results and their relationship with the variable results observed at Ottawa might be explained in part by the work of Cleasby *et al.* (1963) which demonstrated that particle passage through filters following a disturbance was dependent on the composition of the filter influent. These results further indicate that it may be possible to optimize or at least identify the factors that affect these forces so that the potentially severe effects of hydraulic can be minimized.

6.4 WINDSOR PILOT PLANT INVESTIGATIONS

A limited number of pilot-scale *C. parvum* and *B. subtilis* removal investigations were conducted at the Windsor Pilot Plant in Windsor, Ontario, Canada. As described in Chapter 3, the Ottawa and Windsor pilot plants are virtually identical in design and construction. Operated in a conventional mode with dual-media filtration, the Windsor pilot plant also employed a relatively high coagulant dose, but for a different raw water quality than Ottawa. At Windsor, the raw water TOC was considerably low and the alkalinity and turbidity (which could spike as high as 350 NTU during the spring) were higher than at Ottawa. The rationale and specific seeding conditions for each of the Windsor experiments were summarized in Chapter 3 and are elaborated upon below.

6.4.1 Stable (Optimized) Operation

Stable operation experiments were performed in triplicate to determine the best *C. parvum* and *B. subtilis* removals that could be achieved by the pilot-scale, dual-media filter at optimal operating conditions at Windsor. As at Ottawa and UW, the seeding and sampling were conducted during the early to middle portion of the filter cycle, after at least four hours of filter operation. Though the experiments were planned without pre-ozonation, pre-ozonation did occur during one experiment.

6.4.1.1 Experimental Design

Three stable operation experiments were conducted at Windsor. Jar-coagulated *C. parvum* oocysts and *B. subtilis* spores were seeded into the filter influent for one hour during these experiments. Samples were collected at 20, 40, and 55 minutes after the start of seeding. Filter effluent turbidities of <0.1 NTU were targeted during these experiments. The stable operation experiments and seeding conditions are summarized in Table 6.20.

Table 6.20

Summary of Stable Operation Experiments at the Windsor Pilot Plant

Experiment	Date	*C. parvum*	*B. subtilis*	Blue Spheres	Yellow Spheres
Stable Operation	10/14/99*	✓	✓		
	10/21/99	✓	✓		
	10/22/99	✓	✓		

*Stable operation with pre-ozonation.

6.4.1.2 Results

Filter effluent turbidity and particle counts were consistently low (<0.1 NTU and <~20 particles/mL respectively) during the stable operation experiments at Windsor. Unfortunately, on-line data logging of filter effluent turbidities was not available during these experiments. Filter effluent particle counts, *C. parvum* and *B. subtilis* removals by filtration, and total particle (≥ 2μm) reductions through the treatment process during stable operation at Windsor are summarized in Table 6.21. The general filter performance data and detailed instantaneous particle data are presented in Appendix D (Tables D.13 and D.14 respectively). The detailed instantaneous *C. parvum* and *B. subtilis* data are also available in Appendix D (Table D.15).

Table 6.21

Filter Performance During Stable Operation at Windsor

Date	Log_{10} Removal (mean ± standard deviation)				Log_{10} Red.*	Effluent Concentration	
	C. parvum	*B. subtilis*	Blue Spheres	Yellow Spheres	Particles ≥ 2μm	Particles (#/mL)	Turbidity (NTU)
10/14/99**	4.2 ± 0.49	3.9 ± 0.29	---	---	1.6 ± 0.10	13 ± 2.4	---
10/21/99	4.2 ± 0.24	3.8 ± 0.53	---	---	1.5 ± 0.03	15 ± 1.2	---
10/22/99	4.1 ± 0.04	3.5 ± 0.16	---	---	1.6 ± 0.04	14 ± 1.3	---
Overall	**4.2 ± 0.28**	**3.7 ± 0.36**	**---**	**---**	**1.6 ± 0.06**	**14 ± 1.6**	**---**

*Log reduction of particles through treatment process (plant influent to filter effluent).
**Experiment performed with pre-ozonation.

Traditional performance measures (turbidity and total particle counts) and filter effluent microorganism and microsphere concentrations were relatively consistent during the stable operation investigations and between replicate experiments. Filter effluent particle counts ranged from 13-16 particles/mL during stable operation at Windsor. The filter effluent particle counts were generally consistent between the replicate experiments.

C. parvum removals during stable (optimized) operation ranged from 3.8 to 4.7-log, with a mean oocyst removal of 4.2-log (9 samples in total). *B. subtilis* removals ranged from 3.3 to 4.4-log, with a mean removal of 3.7-log (9 samples in total). These data were summarized in Table 6.21. Although there was some variation in removals calculated on the basis of individual influent-effluent sample pairs of microorganisms, the calculated removals during a given experiment were fairly reproducible as indicated by the relatively low standard deviations (Table 6.21). The overall standard deviations were also relatively low, demonstrating good reproducibility between the replicate experiments. The confidence intervals for these data are in Appendix D (Table D.16).

B. subtilis removals by filtration were generally lower than *C. parvum* removals. A comparison of *C. parvum* and *B. subtilis* removals during stable operation did not indicate a clear relationship between oocyst and spore removals (Figure 6.34). The best fit line from least squares linear regression yielded a coefficient of determination (R^2) of only 0.18. While spore removals were lower than oocysts removals, the lack of a relationship between oocyst and spore removals suggested that *B. subtilis* removal data were not indicative of the filter's ability to remove *C. parvum*.

Similar results were observed with total particle (≥2 μm) reductions through the plant. A comparison of *C. parvum* removals and total particle reductions through the treatment process during stable operation did not indicate a clear relationship between oocyst removals and particle reductions (Figure 6.34). The best-fit line from least squares linear regression yielded a coefficient of determination (R^2) of only 0.21. As demonstrated and noted during previous experiments, these data suggested that total particle reductions through the treatment process are not adequate surrogates for *C. parvum* removals during filtration.

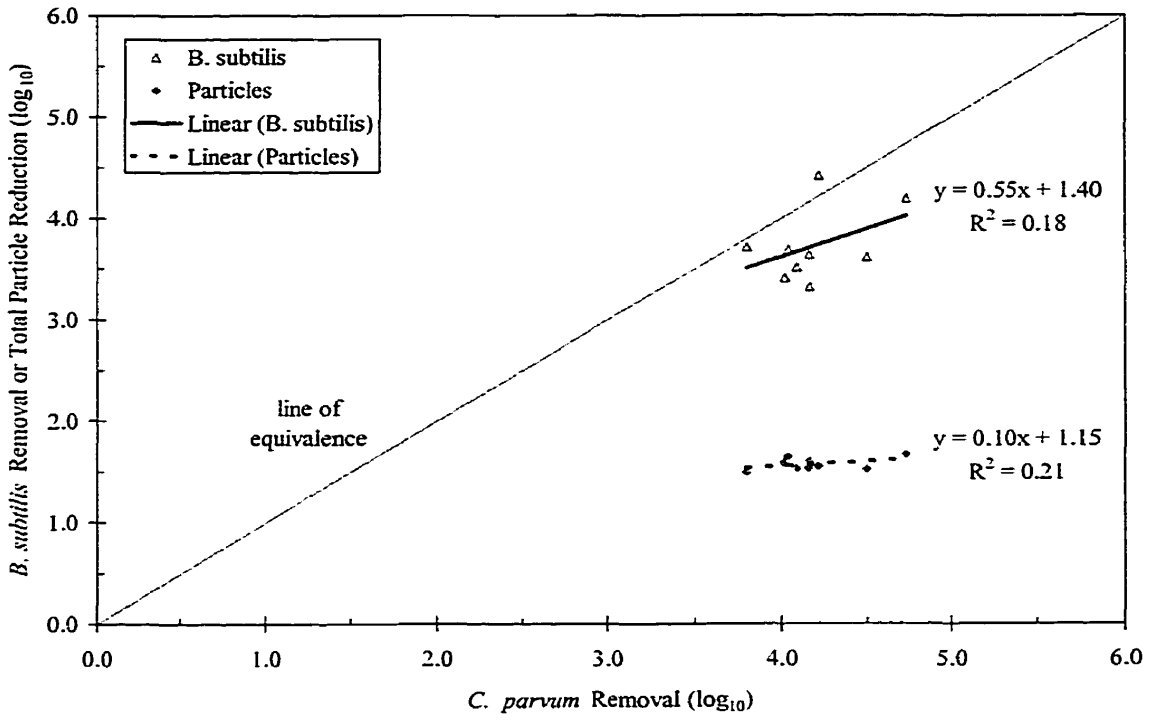


Figure 6.34 Relationship between *C. parvum* and *B. subtilis* removals by filtration and total particle (≥2 μm) reductions through the plant at Windsor.

6.4.1.3 Statistical Analysis

Confidence intervals for the individual *C. parvum* removals during stable operation were calculated using the method described in Chapter 4, and therefore incorporated analytical recovery and uncertainty of recovery. The endpoints of the 95% confidence intervals for oocyst removal are summarized in Table 6.22. All of these confidence intervals overlap, thereby failing to demonstrate statistically significant differences between the data collected during the stable operation experiments (Table 6.22, α=0.05). The stable operation with pre-ozonation experiment did demonstrate significant differences in *C. parvum* removals between the first and third replicate samples (Table 6.22, α=0.05); more data would be necessary to determine if this difference was due to experimental drift or some other phenomenon.

A comparison of the confidence intervals for the stable operation and stable operation with pre-ozonation experiments generally failed to demonstrate statistically significant differences between *C. parvum* removals during these periods (Table 6.22, α=0.05). The exception to this was the third sample (t = 55 minutes). Overall, the *C. parvum* removal range was 3.6- to 5.0-log during stable operation and 3.6- to 5.2-log during stable operation with pre-ozonation. The range for the individual experiments is summarized in Table 6.22. These results generally suggested that, for the operational conditions investigated, pre-ozonation did not substantially affect *C. parvum* removals by filtration. As mentioned previously, more data would be necessary to better support this conclusion.

6.4.2 Discussion

The stable filter operation experiments demonstrated that *C. parvum* removals of >4-log could be consistently achieved by the dual-media pilot filter at Windsor. This result was important because it demonstrated that a pilot plant essentially identical in construction to the one at Ottawa provided ~1-log lower *C. parvum* removal at optimal operating conditions, when treating a different source water. The higher filter effluent particle counts during stable (optimized) operation at Windsor also underscored the difference in overall performance and particle reduction by filtration.

The Windsor data also demonstrated that pre-ozonation appeared to have little impact on *C. parvum* removal by filtration. This experiment was only performed once and provided only three data points. No operational explanation was found to justify why the *C. parvum* removal data were so variable during this operating condition (relative to other experiments at Windsor, Ottawa, and UW). A more thorough investigation with replicate experiments would be necessary to fully investigate the effects of pre-ozonation on *C. parvum* removal by filtration.

Table 6.22

95% Confidence Intervals and *C. parvum* Removal Ranges

During Stable Operation at Windsor.

Experiment	Date	Sample Time	CI_{lower} (log_{10})	CI_{upper} (log_{10})	R_{lower} (log_{10})	R_{upper} (log_{10})
Stable Operation with Pre-Ozonation	10/14/99	20	**3.6**	3.9	3.6	5.2
		40	3.7	4.1		
		55	4.3	**5.2**		
Stable Operation	10/21/99	20	4.1	**4.9**	3.6	4.9
		40	3.8	4.4		
		55	**3.6**	4.1		
Stable Operation	10/22/99	20	**3.7**	4.2	3.7	4.3
		40	3.8	4.3		
		55	3.7	**4.3**		

6.4.3 Rate Effects

As mentioned previously, Cleasby *et al.* (1963) and Tuepker and Buescher (1968) showed that large flow rate changes cause deterioration of filtered water quality by the detachment of previously retained particles. These relationships supported observations that declining rate filters may provide better performance than constant rate filters (Hudson, 1959; DiBernardo and Cleasby, 1980); however, subsequent experiments by Hilmoe and Cleasby (1986) found no significant differences between declining rate and constant rate filters. The authors speculated that the previously reported poorer effluent quality achieved by constant rate filtration might have been caused by the constant rate control system used by DiBernardo and Cleasby (1980), which might have inadvertently resulted in continuous flow rate fluctuations or surges. If declining rate filtration provides filter performance advantages over constant rate filtration, it may also provide an operational strategy for mitigating *C. parvum* passage through filtration.

6.4.3.1 Experimental Design

Four rate effects experiments were conducted at Windsor (two investigating constant rate filtration and two investigating declining rate filtration). These experiments involved side-by-side comparisons of two dual-media filters, one operating in a constant rate mode

and the other operating in a declining rate mode. The experiments were performed approximately halfway into the filter cycle (~20 hours into a ~40-hour cycle). Laboratory limitations (sample processing) precluded these experiments from being conducted with both oocysts and spores. Only jar-coagulated *B. subtilis* spores were seeded into the filter influent for one hour during these experiments. Samples were collected at 20, 40, and 55 minutes after the start of seeding. Filter effluent turbidities of <0.1 NTU, essentially representing stable operation, were targeted during these experiments. The rate effects experiments and seeding conditions are summarized in Table 6.23.

Table 6.23

Summary of Rate Effects Experiments at the Windsor Pilot Plant

Experiment	Date	*C. parvum*	*B. subtilis*	Blue Spheres	Yellow Spheres
Stable Operation	03/10/99		✓		
Constant Rate	03/22/99		✓		
Stable Operation	03/10/99		✓		
Declining Rate	03/22/99		✓		

6.4.3.2 Results

As during the stable operation experiments, on-line data logging of filter effluent turbidities was not available during these experiments. Filter effluent particle counts, *B. subtilis* removals, and total particle reductions through the treatment process during the rate effects experiments at Windsor are summarized in Table 6.24. The general filter performance data and detailed instantaneous particle data are available in Appendix D (Tables D.13 and D.14 respectively). The detailed instantaneous *B. subtilis* data are also available in Appendix D (Table D.15).

The March 10, 1999 data were disregarded because chemical pretreatment and filter operation were not optimized during these experiments. This result was underscored by

the elevated filter effluent particle concentrations (>2000 particles/mL) in both of the dual-media filters (operating at constant and declining rate modes). The filter effluent particle concentrations during stable operation at Windsor were typically less than ~250 particles/mL during the time of year during which the rate effects experiments were performed. Stable operation conditions were achieved by the constant and declining rate filters during the March 22, 1999 experiments.

Table 6.24

Filter Performance During Rate Effects Experiments at Windsor

Date	Log_{10} Removal (mean ± standard deviation)				Log_{10} Red.*	Effluent Concentration	
	C. parvum	*B. subtilis*	Blue Spheres	Yellow Spheres	Particles ≥ 2μm	Particles (#/mL)	Turbidity (NTU)
Stable Operation – Constant Rate Filtration							
03/10/99**	---	0.8 ± 0.08	---	---	0.5 ± 0.02	2159 ± 29	---
03/22/99	---	**3.2 ± 0.42**	---	---	**1.6 ± 0.03**	**244 ± 6**	---
Stable Operation – Declining Rate Filtration							
03/10/99**	---	2.5 ± 0.49	---	---	0.4 ± 0.01	2742 ± 107	---
03/22/99	---	**3.1 ± 0.54**	---	---	**1.8 ± 0.04**	**136 ± 5**	---

*Log reduction of particles through treatment process (plant influent to filter effluent).
**Filter operation was not optimized during the 03/10/99 experiments and data were not evaluated.

6.4.3.3 Discussion

Though each experiment was only performed once, the *B. subtilis* spore removals by constant and declining rate filtration were essentially the same. This result demonstrated that declining rate filtration did not offer an obvious advantage over constant rate filtration for *B. subtilis* removal, and likely *C. parvum* removal, at the operating conditions investigated. Further experimentation would be necessary to conclusively determine any potential differences in *C. parvum* removal that might result from operating filters in constant or declining rate modes.

The observed spore removals were similar to the removals obtained during the stable operation experiments (that were performed in a constant rate filtration mode). The data were slightly more variable during the present experiments, however, with slightly lower mean removals. Given that the filter performance changed seasonally, as indicated by the filter effluent particle concentrations (Table 6.21 and Table 6.24), it was difficult to further compare the results between the stable operation and rate effects experiments.

Chapter 7
Integrated *C. parvum* and Potential Surrogate Data

7.1 Overall *C. parvum* Removal

The main focus of this research was to investigate *C. parvum* removal during various portions of the filter cycle and during operational periods when turbidity and particle removal processes are challenged. The majority of these investigations were conducted at the Ottawa pilot plant. *C. parvum* removals during all of the operational conditions investigated at Ottawa (except hydraulic steps) are summarized in Figure 7.1.

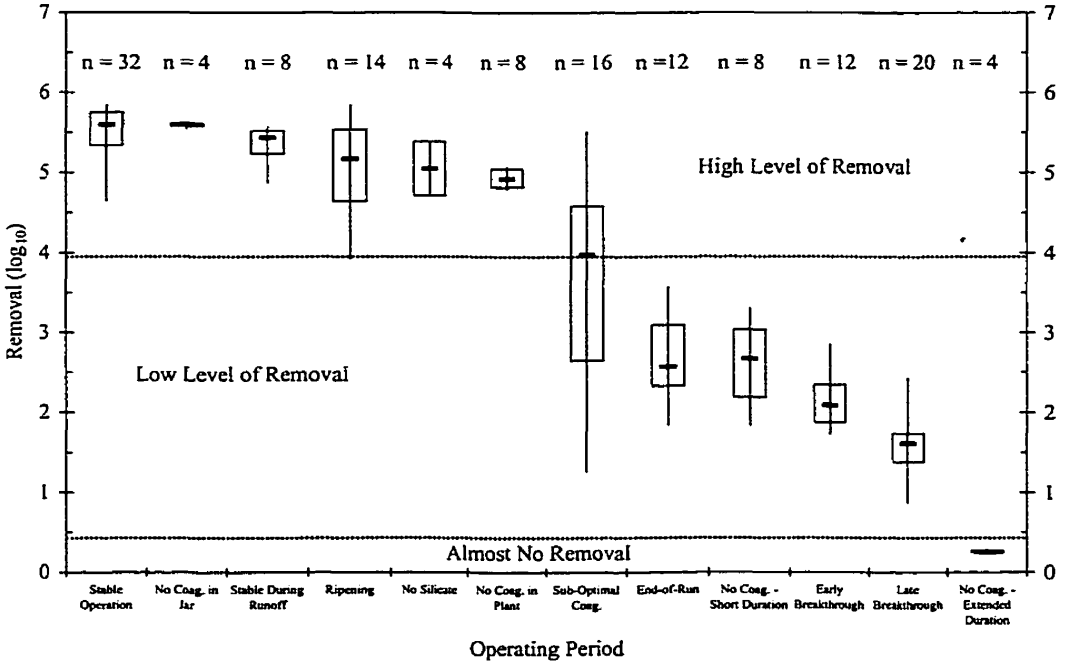


Figure 7.1 Box-and-whisker plot of *C. parvum* removals by filtration during all operating periods (except hydraulic steps) investigated at Ottawa.

The *C. parvum* removal data in Figure 7.1 generally indicated three levels of removal: high, low, and almost no removal. High removals were in the range of those observed during stable operation and were generally >4-log. Oocyst removals during the stable operation, no coagulant in the jar, ripening, no silicate, and no coagulant in the plant experiments were high. The end-of-run, no coagulant-short duration, early breakthrough, and late breakthrough conditions resulted in low removals of *C. parvum* by filtration. These low removals were typically <3-log, a marked decrease relative to stable operation during which *C. parvum* removals were typically >5-log. Sub-optimal coagulation conditions spanned the high and low *C. parvum* removal range, while the no coagulant-extended duration conditions essentially precluded *C. parvum* removal by filtration. The hydraulic step experiments were not included in Figure 7.1 because the experiments were conducted in a manner that resulted in slowly decreasing filter influent *C. parvum* concentrations during the implementation of the hydraulic step conditions.

From Figure 7.1, it can be concluded that the granular media filters best remove *C. parvum* from waters that are well-coagulated. The Ottawa pilot plant generally maintained good oocyst removals during brief periods of non-optimal coagulation; however, extended periods of poor coagulation almost completely prevented oocyst removal. Relative to stable filter operation, *C. parvum* removals were slightly lower during the ripening portion of the filter cycle. End-of-run and breakthrough operation resulted in dramatically lower oocyst removals relative to stable operation, suggesting that the later portion of the filter cycle can be particularly vulnerable in terms of maintaining pathogen removal.

Using the statistical framework developed in Chapter 4 to account for analytical recovery and uncertainty associated with recovery, the range of *C. parvum* removals during each experiment was calculated. The replicate data from the each of the stable operation, no coagulant in the jar, stable operation during runoff, no silicate, no coagulant in the plant, and no coagulant–extended duration experiments were pooled to calculate overall 95% confidence intervals for each of these conditions. As described in Chapters 4 and 6, this was possible because no obvious changes were occurring in the independent operational variables (*e.g.*, settled water turbidity, pretreatment conditions, filter loading rates, etc.)

during these experiments. For the remaining experiments (sub-optimal coagulation, end-of-run operation, no coagulant–short duration, early breakthrough, and late breakthrough) the range of *C. parvum* removals during each experiment was calculated based on the lowest and highest endpoints of the highest posterior density (HPD) regions calculated from each of the individual probability density functions (pdfs) for *C. parvum* removal (each pdf was based on one influent-effluent pair). This "adjusted" range of *C. parvum* removals at Ottawa is depicted in Figure 7.2, with the operating conditions listed in the same order as in Figure 7.1.

The *C. parvum* removal data in Figure 7.2 indicate the same general trends as the data in Figure 7.1. In the case of the data collected during dynamic (changing) operating conditions which precluded the pooling of data, however, the ranges of removals are generally larger due to the uncertainty associated with the analytical method for concentrating and enumerating *C. parvum* oocysts from water. While the unadjusted minimum and maximum removals from these experiments spanned a relatively small range of approximately 1-log (Figure 7.1), the adjusted data (Figure 7.2) indicated a substantially larger range of removals that could be expected (approximately 2-log) given the uncertainty associated with the analytical method used to concentrate and enumerate the *C. parvum* oocysts.

It should be noted that the analytical method used during this thesis research and to generate the range of *C. parvum* removals in Figure 7.2 was consistent and reliable relative to others that have been reported in the literature (Appendix A). For similar filter influent and effluent *C. parvum* data collected using a less reliable method, these ranges would be larger. This result underscores the importance of assessing the reliability of available *C. parvum* data in the context of the analytical methods used during data collection and enumeration, particularly when non-detects or very low oocyst counts are observed.

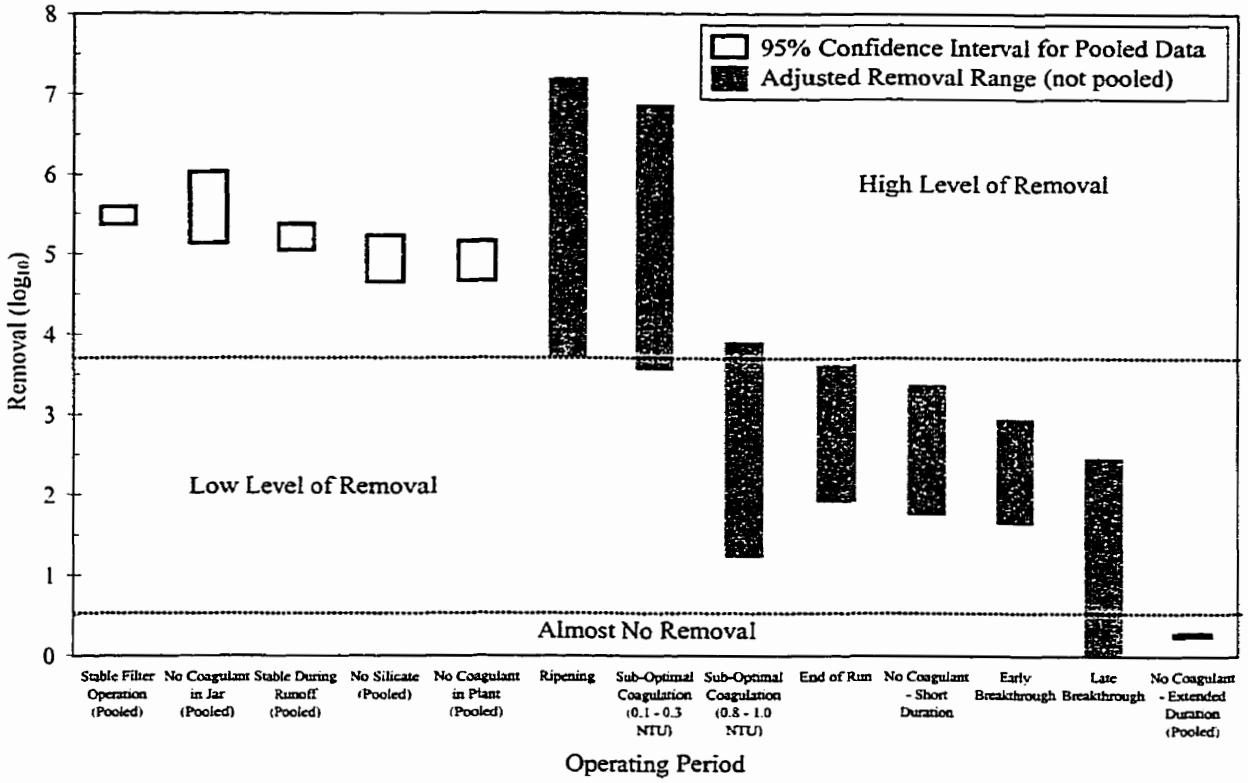


Figure 7.2 95% Confidence intervals and adjusted ranges of *C. parvum* removals by filtration during all operating periods (except hydraulic steps) investigated at Ottawa.

Although the data in Figure 7.1 and Figure 7.2 present log removals calculated from the seeding experiments, these results should be considered in terms of the relative differences in pathogen removals during the different operating conditions. As discussed in previous chapters of this thesis, the actual log removals of *C. parvum* that can be obtained by full-scale filtration processes are limited by the influent oocyst concentrations. Furthermore, *C. parvum* removals can be source-water and treatment design specific, as demonstrated in Figure 7.3, which depicts *C. parvum* removals by dual-media filters during stable operation at the Ottawa, Windsor, and UW pilot plants.

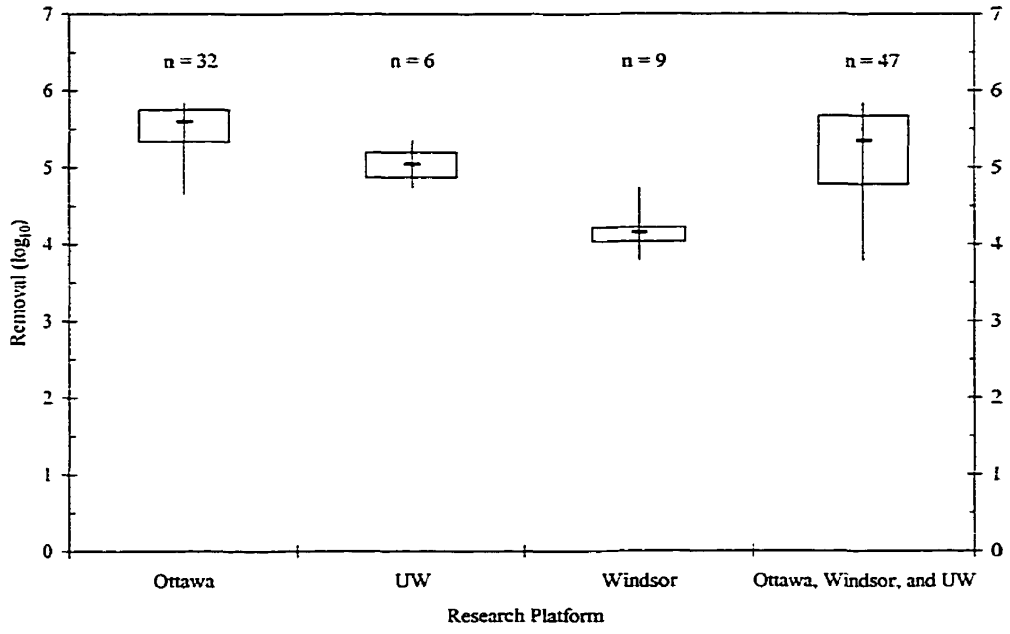


Figure 7.3 Box-and-whisker plot of *C. parvum* removals by dual-media filters during stable operation at Ottawa, Windsor, and UW research platforms.

Figure 7.4 also depicts the *C. parvum* removals by dual-media filters during stable operation at the various research platforms, however, the data from each location were pooled and the 95% confidence intervals were calculated. They were 5.4- to 5.6-log at Ottawa, 4.7- to 5.2-log at UW, and 4.0- to 4.1-log at Windsor. The confidence intervals account for analytical recovery and uncertainty of recovery. Figure 7.4 further emphasizes that the reliability of *C. parvum* data will considerably increase with higher counts per sample or with pooling of replicate data. These data also demonstrate that similar filtration schemes may offer different levels of *C. parvum* removal. None of the 95% confidence intervals in this figure overlap, demonstrating that the *C. parvum* removals at the three research platforms were significantly different from one another (α=0.05). A comparison of the Ottawa and Windsor *C. parvum* removals is interesting because the pilot plants are essentially identical in construction, but treat different raw waters (described in Chapter 3). The significant differences in oocyst removal during stable operation at these two plants suggest that the *C. parvum* removal capacity of granular media filters may be source water specific; this may be associated with the necessity of different pretreatment strategies for source waters of various quality.

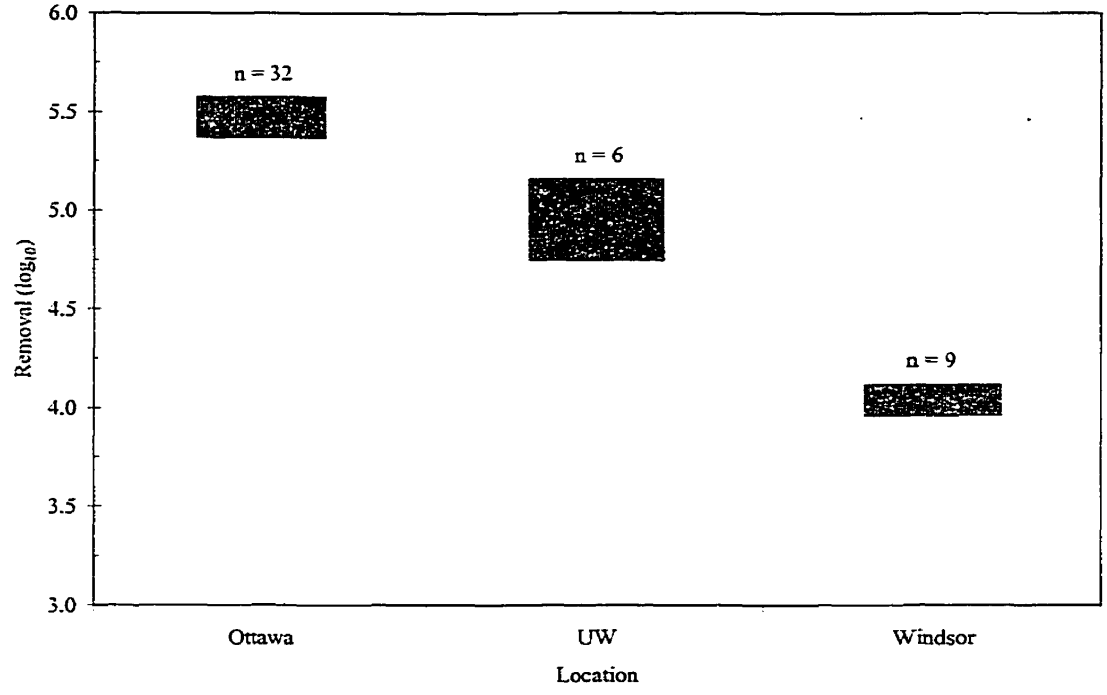


Figure 7.4 95% confidence intervals for *C. parvum* removals by dual-media filters during stable operation (pooled data) at Ottawa, Windsor, and UW.

7.2 OVERALL ASSESSMENT FOR POTENTIAL SURROGATES FOR *C. PARVUM*

B. subtilis and polystyrene microsphere removals and total particle (≥2 μm) reductions through the treatment process were investigated during several periods of operational challenge to investigate potential surrogates for *C. parvum* removal by filtration. The experiments were performed concurrently with the *C. parvum* investigations, most of which were conducted at the Ottawa pilot plant. The *B. subtilis* and microsphere removals by filtration and the total particle (≥2 μm) reductions through the treatment process during all of the operational conditions investigated at Ottawa (except hydraulic steps) are summarized in Figure 7.5 through Figure 7.7 respectively. Comparison of these figures to the *C. parvum* removal data in Figure 7.1 suggests that overall, neither the spore removals nor the particle reductions are reasonable, quantitative surrogates for *C. parvum* removal. The microsphere removal data corresponded closely to the *C. parvum* removal trends. These data are limited, however, since they were only obtained during some of the operating investigated during this research.

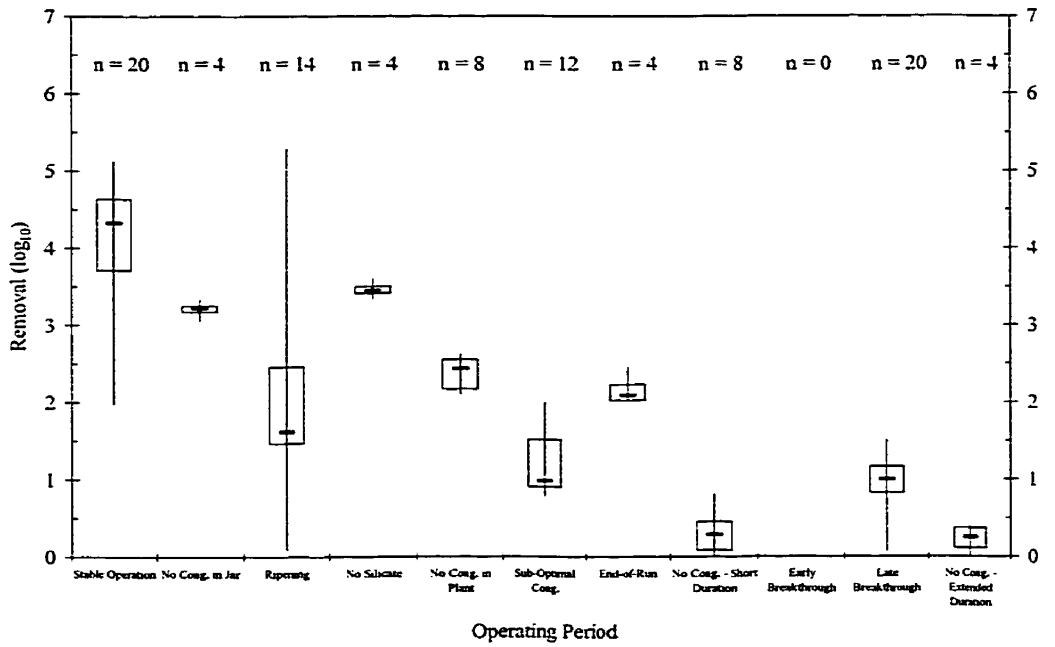


Figure 7.5 Box-and-whisker plot of *B. subtilis* removals by filtration during all operating periods (except hydraulic steps) investigated at Ottawa.

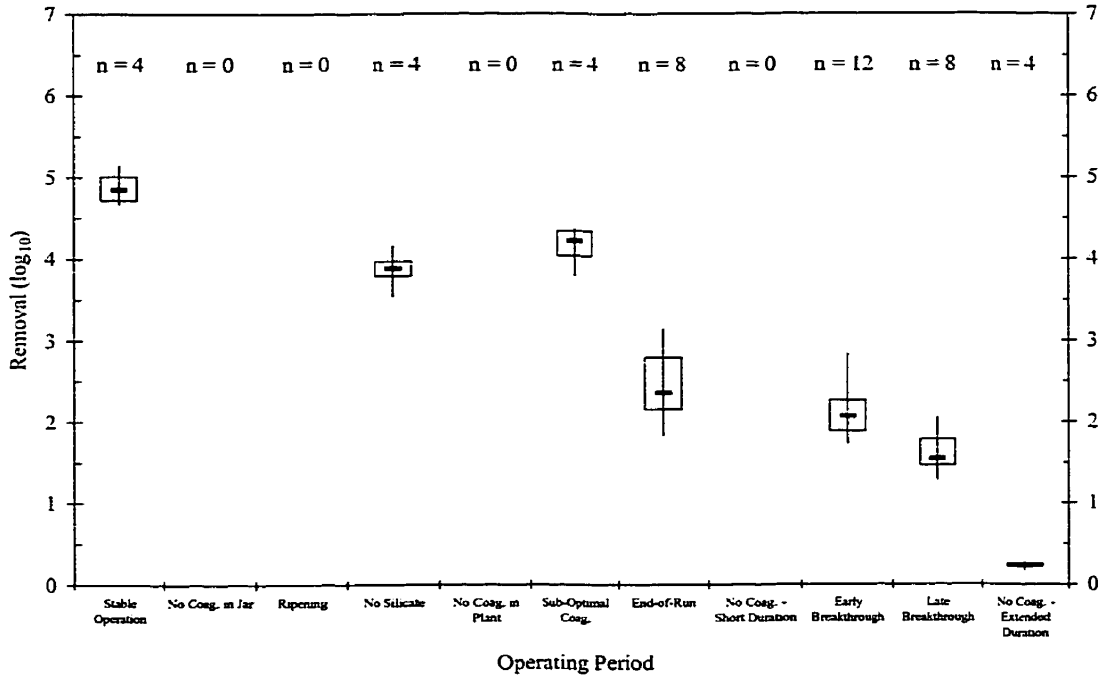


Figure 7.6 Box-and-whisker plot of polystyrene microsphere removals by filtration during all operating periods (except hydraulic steps) investigated at Ottawa.

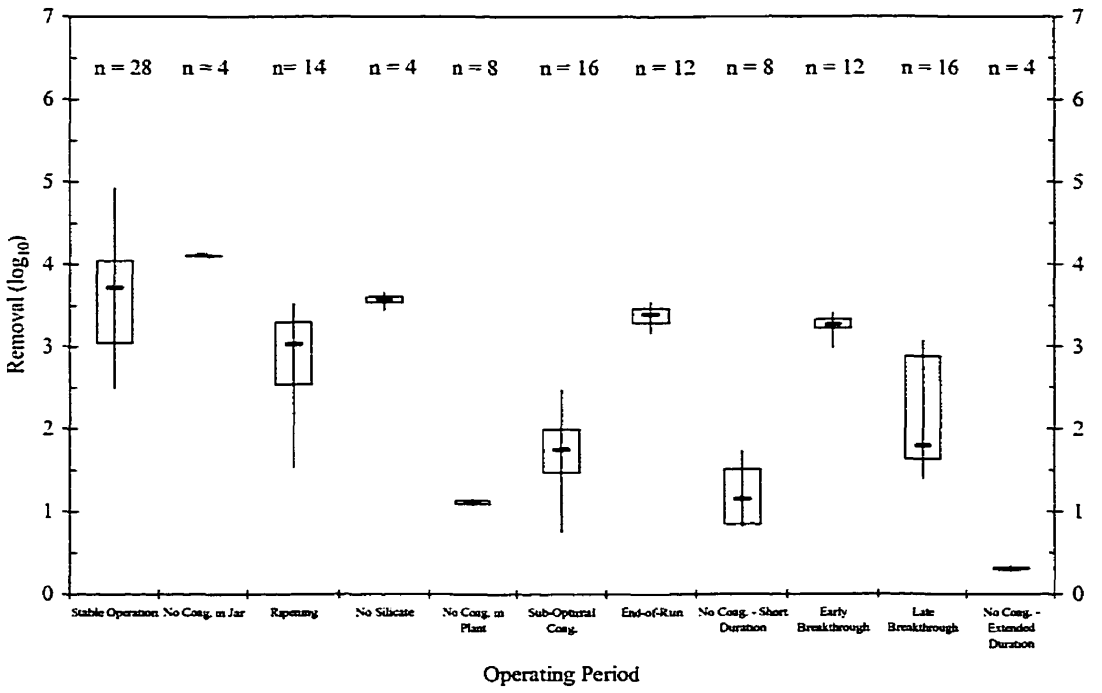


Figure 7.7 Box-and-whisker plot of total particle (≥2 μm) reductions through the plant during all operating periods (except hydraulic steps) investigated at Ottawa.

The *C. parvum*, *B. subtilis*, and polystyrene microsphere removals by filtration and the total particle (≥ 2μm) reductions from the Ottawa and UW pilot experiments were combined to assess the potential surrogates for *C. parvum* removal by filtration. Some variation in the removals calculated on the basis of individual influent-effluent sample pairs was observed for all of these parameters. In general, the calculated removals during a given experiment were fairly reproducible as indicated by the relatively low standard deviations for the replicate samples; the overall standard deviations were also low, demonstrating good reproducibility between the replicate experiments (Appendix D).

B. subtilis and polystyrene microsphere removals and total particle (≥ 2μm) reductions through the treatment process are presented relative to *C. parvum* removals by filtration in Figure 7.8 through Figure 7.12. These figures respectively correspond to the overall removals at Ottawa (all conditions investigated at Ottawa), overall removals at stable oepration at UW), and combined removals (all conditions investigated at Ottawa,

Windsor, and UW). Least squares linear regression was used to assess the relationships between *C. parvum* and potential surrogate removals in all cases.

Figure 7.8 and Figure 7.12 demonstrate that *B. subtilis* removals by filtration were generally lower than removals of *C. parvum*. However, these figures also demonstrate that these removals were not always lower than *C. parvum* removals, as there are some points on each figure that correspond to spore removals that were higher than oocyst removals. The coefficients of determination (R^2) for the best fit linear regression models were 0.48 and 0.47 for the Ottawa and combined Ottawa, Windsor, and UW data sets respectively, suggesting that spores of *B. subtilis* were not adequate quantitative surrogates for *C. parvum* removal by filtration. This result was not surprising given that *B. subtilis* spores are ~1 μm in size, which is at the minimum transport efficiency for particle removal by filtration. It was also consistent with other studies reported in the literature (*e.g.*, Lytle *et al.*, 1996; Nieminski and Bellamy, 1998; Swertfeger *et al.*, 1999). Non-linear relationships (*e.g.*, exponential, logarithmic, *etc.*) between *C. parvum* and *B. subtilis* removals by filtration were not expected and were not suggested by the data.

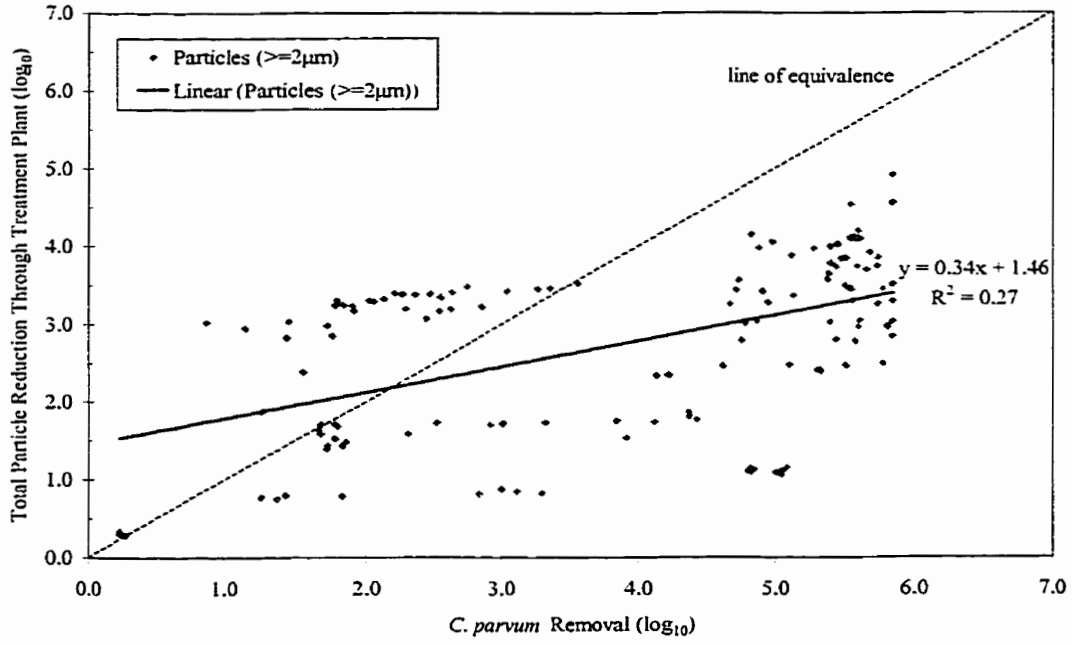


Figure 7.8 Relationship between *C. parvum* removals by filtration and total particle (≥2 μm) reductions through the plant during all operational periods investigated at Ottawa.

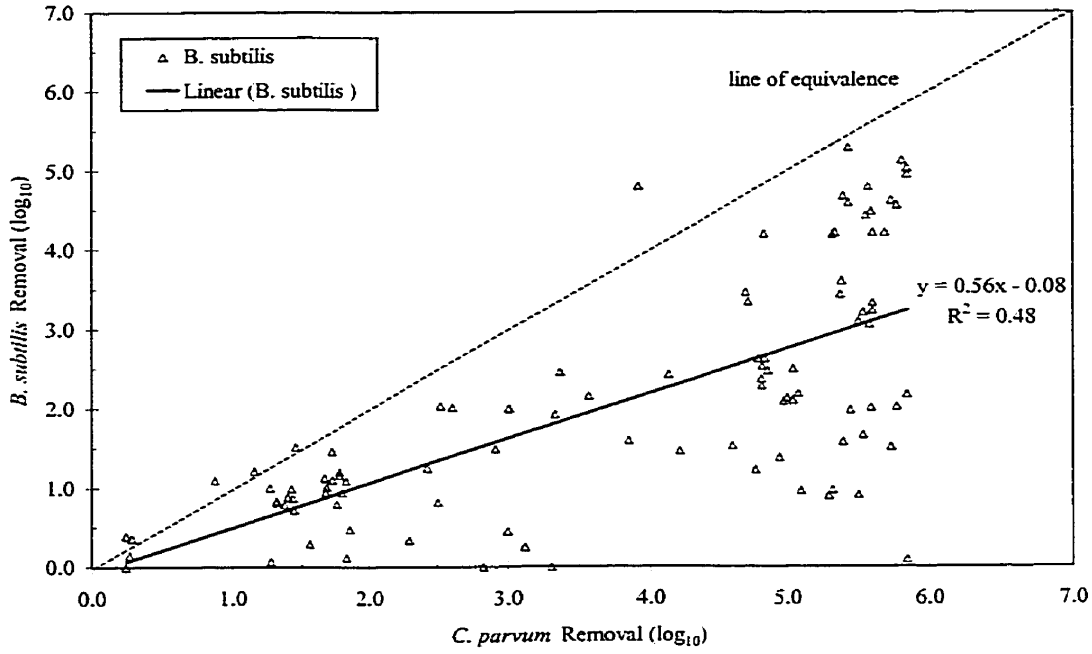


Figure 7.9 Relationship between *C. parvum* and *B. subtilis* removals by filtration during all operational periods investigated at Ottawa.

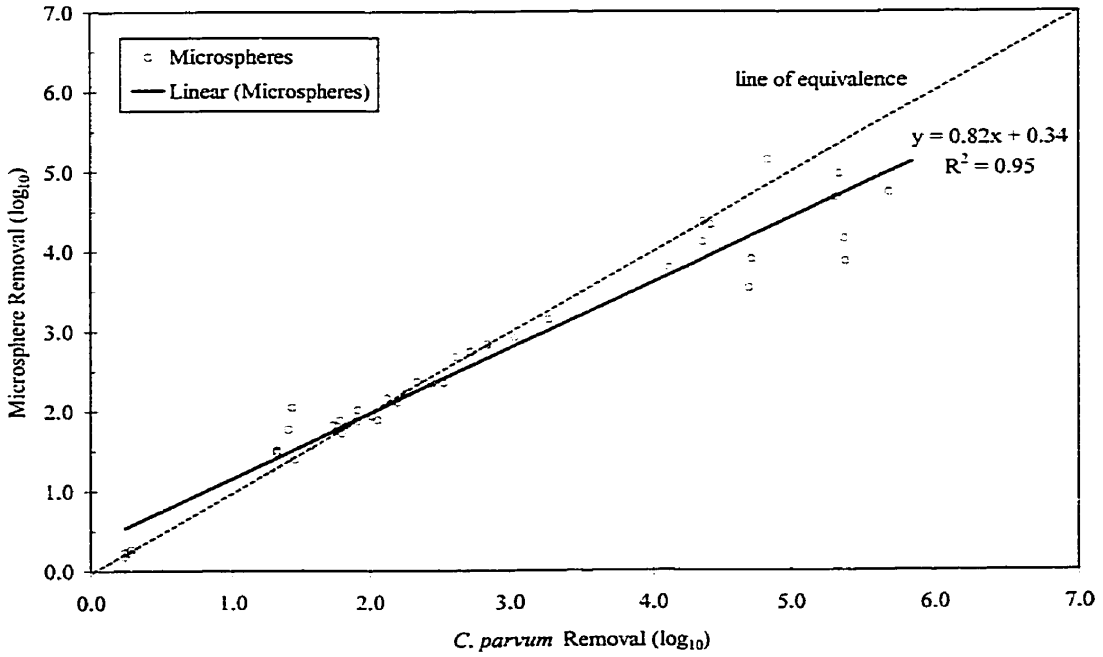


Figure 7.10 Relationship between *C. parvum* and polystyrene microsphere removals by filtration during all operational periods investigated at Ottawa.

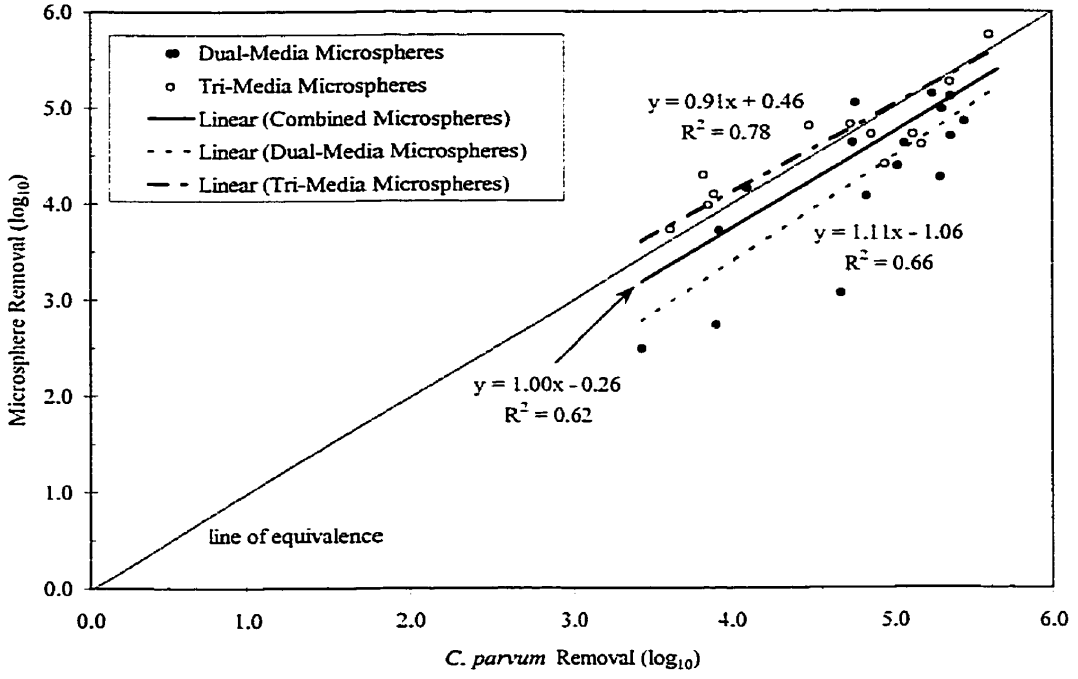


Figure 7.11 Relationship between *C. parvum* and polystyrene microsphere removals by filtration during all operational periods investigated at UW.

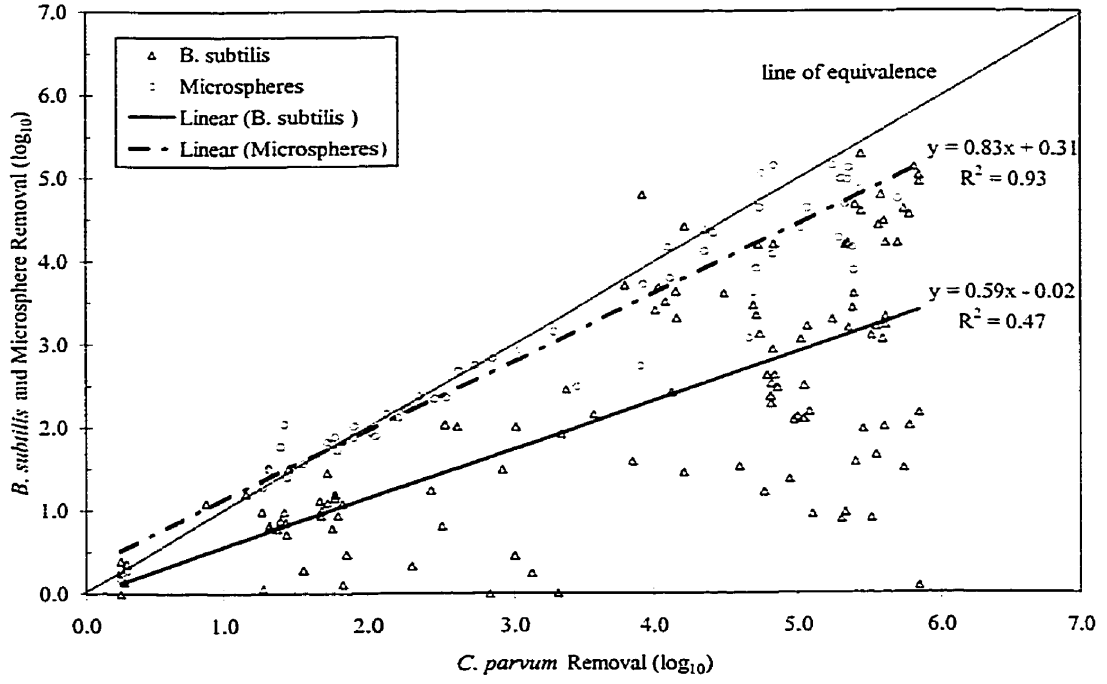


Figure 7.12 Relationship between *C. parvum*, *B. subtilis*, and polystyrene microsphere removals by filtration during all operational periods investigated at Ottawa, Windsor, and UW.

Although it might be tempting to suggest that *B. subtilis* removal provided a conservative indication of a filter's ability to remove *C. parvum*, this statement is not completely accurate because in some instances spore removals were higher than oocyst removals. While it is true that spore removals were generally lower than oocyst removals, the lack of a reproducible or consistent relationship between oocyst and spore removals suggested that *B. subtilis* removals were not indicative of the filter's ability to remove *C. parvum*. A more accurate and conservative conclusion would be that spore removals were generally indicative of treatment performance, but not necessarily *C. parvum* removal. This conclusion is also supported by other studies presented in the literature (*e.g.*, Lytle *et al.*, 1996; Nieminski and Bellamy, 1998; Swertfeger *et al.*, 1999).

As mentioned in Chapter 6, total particle (≥ 2 μm) reductions through the treatment process were calculated based on raw water rather than filter influent values. Filter influent particle concentrations could not be measured due to floc breakage and accumulation on and in the particle counter sensor chamber. The coefficient of determination for the best fit linear regression model was 0.27 for the Ottawa data set, suggesting that particle removals were inadequate surrogates for *C. parvum* removal by filtration. This result was also consistent with studies previously reported in the literature (LeChevallier *et al.*, 1991c; Nieminski and Ongerth, 1995; Swaim *et al.*, 1996). The lack of a linear relationship between *C. parvum* removals by filtration and total particle reductions through the treatment process was not surprising given that the nature and distribution of particles change as they pass through coagulation, flocculation, sedimentation, and filtration processes. Non-linear relationships between *C. parvum* removals by filtration and total particle reductions through the treatment process were expected and demonstrated by the data.

As with *B. subtilis*, the particle removals tended to be lower than *C. parvum* removals at Ottawa and were not necessarily indicative of the filter's ability to remove *C. parvum*. Overall, particle removals were generally indicative of treatment performance, but were not quantitatively associated with *C. parvum* removal. The hydraulic step experiments at Ottawa underscored this point by demonstrating a substantial increase in filter effluent

particle concentrations that was not accompanied by an increase in effluent oocyst concentrations.

As shown in Figure 7.8 and Figure 7.12, of the potential surrogates investigated, oocyst-sized polystyrene microsphere removals were closest to *C. parvum* removals by filtration. The coefficients of determination for the best fit linear regression models were 0.95, 0.78, 0.66, and 0.93 for the respective Ottawa, UW dual-media, UW tri-media, and combined Ottawa and UW (dual-media) data sets, suggesting that polystyrene microsphere removals may likely be appropriate surrogates for *C. parvum* removal by filtration. The UW dual- and tri-media filtration data suggested that the relationships are system specific. The poorer relationships between microsphere and oocyst removals at UW relative to those observed at Ottawa also suggested that coagulation (*e.g.*, low coagulant dose for particle removal vs. high coagulant dose for combined TOC and particle removal) or filtration regime (*e.g.*, conventional vs. in-line filtration) may also impact the reliability of polystyrene microspheres as surrogates for *C. parvum* removal. Although these relationships must still be determined, the excellent correlation coefficients at Ottawa and the slightly lower correlation coefficients based on more limited data from UW suggest that polystyrene microsphere removals should continue to be investigated as surrogates for *C. parvum* removal by filtration.

Chapter 8
Conclusions, Implications, and Recommendations

8.1 Conclusions

The key conclusions from this thesis research are presented below.

1. The Gibbs sampler may be effectively used to incorporate analytical uncertainty into confidence intervals on removals of discrete particles such as *C. parvum* by drinking water treatment processes such as granular media filtration.

2. Removals of formalin-inactivated *C. parvum* oocysts were similar to those of viable oocysts during stable operation, ripening, and coagulation failure in both dual- and tri-media filters, suggesting that formalin-inactivated oocysts are good surrogates for viable oocysts.

3. *C. parvum* removals were not different in dual- and tri-media filters during stable operation, ripening, hydraulic step, and coagulation failure conditions. These results suggest that, for the water matrices studied, tri-media filtration does not provide superior *C. parvum* removal in comparison to dual-media filtration.

4. During optimal operating conditions (filter effluent turbidities and cumulative particles <0.1 NTU and <25 particles/mL respectively), >4.5-log removal of *C. parvum* could be achieved by filtration. At two of the three pilot plants investigated, >5-log removal of *C. parvum* was achieved, even at temperatures as low as 1°C and during spring runoff conditions.

5. *C. parvum* removals by filtration were moderately lower (by ~0.5- to 1-log) during ripening than during stable operation. These differences were minor likely due to the short duration of the ripening period.

6. *C. parvum* removals deteriorated substantially (by 3- to 4-log) during end-of-run and early breakthrough filtration relative to stable operation, even at filter effluent turbidities below 0.1 NTU. This result suggested that filter operation during breakthrough, as measured by turbidity or even particle counts, should be avoided.

7. During the coagulation failure conditions investigated at UW (low coagulant dose for particle removal), *C. parvum* removal by dual- and tri-media filters was significantly compromised, with a >4-log decrease in *C. parvum* removal relative to stable operation. Coagulant failure at Ottawa (high coagulant dose for TOC and particle removal) resulted in a >3-log decrease in *C. parvum* removal relative to stable operation. The lack of coagulant over a short duration of several hours resulted in this deterioration of *C. parvum* removal capacity; however, when the coagulation failure conditions persisted for several filter cycles, the absence of coagulant resulted in almost no *C. parvum* removal by filtration.

8. Sub-optimal coagulation conditions resulted in considerably deteriorated *C. parvum* removals by filtration, even at filter effluent turbidities below 0.3 NTU.

9. Relatively rapid changes in hydraulic loading (hydraulic steps) demonstrated varied effects on *C. parvum* removal by filtration. In most cases, little to no deterioration in filter effluent *C. parvum* concentrations occurred. Turbidity monitoring proved more useful than particle counting in gauging the effects of hydraulic steps on *C. parvum* passage through filters. These events should be investigated further to better define how and when they impact pathogen passage.

10. Oocyst-sized polystyrene microspheres appeared to be reasonable surrogates for *C. parvum* removal by filtration during several operating conditions; however, they should continue to be evaluated relative to oocysts to better define the limits of their applicability as surrogates.

11. As expected, based on filtration theory and research literature, *B. subtilis* spore removals and total particle reductions through the treatment process were indicative of treatment performance, but were inadequate as quantitative surrogates for *C. parvum* removal by filtration.

12. Increasing the number of oocysts in a given sample (*e.g.*, by increasing the processed sample volume) can considerably increase data reliability when the observed number of counts is low (*e.g.*, below 10 oocysts).

8.2 IMPLICATIONS

This research demonstrated that excellent removal of *C. parvum* oocysts can be achieved by granular media filtration processes during optimized treatment conditions. The magnitude of *C. parvum* removal was somewhat site- and source water-specific. A particularly important finding was that, in some cases, poor *C. parvum* removal was observed when otherwise excellent filtered water qualities of <0.1 NTU were achieved (during the end-of-run experiments). Similarly, relatively large spikes in filter effluent particle counts (during the hydraulic step experiments) were not necessarily indicative of deterioration in *C. parvum* removal by filtration. Nonetheless, this research has demonstrated the validity of the water treatment industry's general approach of minimizing filter effluent turbidity and particle concentrations for maximizing *C. parvum* removal by granular media filtration. The experimental results clearly support the importance and usefulness of monitoring the effluent turbidity and particle counts of individual filters.

It was shown that *C. parvum* removal by granular media filtration varies during different periods of the filter cycle and sub-optimal performance events that can occur during typical water treatment conditions. These differences can be site- and source-water specific, making it difficult to extrapolate data from one study to another. Furthermore, the difficulty in identifying vulnerable periods of operation is compounded by this investigation's demonstration that the relative potential for pathogen passage during operation during a given event or period of the filter cycle may be very different than that

during another event or period of the filter cycle, even though the filter effluent water quality measured by turbidity or particle counts may be exactly the same. Despite site-specific details affecting the magnitude of *C. parvum* removals, this research has indicated that general trends in *C. parvum* removal by granular media filtration can be associated with specific design and operating conditions. The use of the developed statistical framework for assessing the reliability of *C. parvum* removal data made it easier to compare data from different operating periods and research platforms. The statistical approach also emphasized that the reliability of *C. parvum* concentration and removal data can be considerably increased when higher counts (*e.g.*, more than 10 oocysts in a given sample) are obtained (*e.g.*, by processing larger sample volumes).

Finally, this research demonstrated that the use of polystyrene microspheres as a surrogate for *C. parvum* removal by filtration may provide utilities with a cost-effective and safe tool for performing pilot-scale investigations to better gauge the *C. parvum* removal capacity of their specific systems. This tool may be particularly useful in the context of the USEPA's Long-Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) and other such regulations aimed at *C. parvum* removal from drinking water because they utilize a treatment technology approach for compliance, rather than the implementation of a stringent filter effluent monitoring requirement.

8.3 RECOMMENDATIONS

8.3.1 Water Treatment Plant Operations and Management

The following recommendations for maximizing *C. parvum* removal by water treatment operations and management are based on the conclusions of this thesis research.

1. Utilize filter to waste capacity during ripening if possible.

2. Use a turbidimeter or particle counter as a relative indicator of filter performance during ripening.

3. Minimize the duration of the filter effluent turbidity and particle spikes associated with ripening by optimizing backwashing and pretreatment (*i.e.*, coagulation) conditions.

4. Use particle counters to monitor filter effluents and to signal end-of-run operation and early breakthrough.

5. Consider the relative cost implications of extended filter cycles versus maintaining water quality by terminating filter cycles when end-of-run is signaled (*e.g.*, noticeable, steady rise in particle counts).

6. Clearly specify filter backwash criteria (maximum run time, filter effluent turbidity, head loss, *etc.*).

7. Maintain optimized chemical pretreatment by performing jar tests and pilot studies to maximize turbidity and particle removal by coagulation and filtration.

8. Recognize that source water quality changes may result in sub-optimal coagulation and subsequent sub-optimal filtration.

9. Respond to sub-optimal coagulation conditions by adjusting coagulant dosing to optimal levels as quickly as possible.

10. Minimize the effects of changes in hydraulic loading on filter performance by implementing changes gradually or by adjusting plant operations to minimize hydraulic changes.

11. Do not rely on particle removals or *B. subtilis* removals as surrogates for *C. parvum* removal by filtration.

12. Utilize the statistical framework developed in this research, or a comparable approach, to assess the reliability of *C. parvum* removal data.

13. Utilize particle counts and turbidity measurements as indicators of treatment efficiency. Recognize that in some cases, *C. parvum* passage through filters can increase without dramatic increase in either of these parameters. Conversely, recognize that turbidity and/or particle counts can increase substantially without resulting in comparable increases in *C. parvum* passage through filters. Generally, however, increases in filter effluent particles and turbidity tend to signal increases in the potential for *C. parvum* to pass through filters.

8.3.2 Water Treatment Research

This research demonstrated that various operating periods and conditions during granular media filtration could result in very different levels of *C. parvum* removal. These results were obtained by performing bench- and pilot-scale seeding studies utilizing very high concentration of formalin-inactivated *C. parvum* oocysts that were introduced into filter influents. Several critical questions have arisen from this research and are summarized in the following list of research recommendations.

1. Determine if removals achieved during studies involving seeding at high influent *C. parvum* concentrations are representative of those achieved with lower *C. parvum* concentrations (such as those that occur indigenously).

2. Determine if jar-coagulation of *C. parvum* oocysts is representative of coagulation at the rapid mix.

3. Determine the relative impact of the presence of surfactant (to prevent oocyst aggregation) in *C. parvum* samples used during seeding studies on coagulation and removal by filtration.

4. Investigate the potential for the coagulation-flocculation-sedimentation process to result in a specific sub-population of oocysts that subsequently enters disinfection and/or filtration processes.

5. Further investigate the deterioration of *C. parvum* removal during end-of-run operation to determine when it begins and how consistently it occurs.

6. Investigate the relative impact of coagulation regime and filter conditioning on *C. parvum* removal by filtration.

7. Elucidate the impact of hydraulic steps on *C. parvum* through filters, investigating different changes in hydraulic loading and implementation of the changes at various periods during the filter cycle.

8. Develop universal (non site-specific) methods for evaluating the robustness of *C. parvum* removals by filtration. The statistical framework developed in this thesis, or something comparable, should be used to assess the reliability of *C. parvum* removal data if applicable.

9. Continue to investigate polystyrene microspheres as surrogates for *C. parvum* removal, potentially integrating their use into combined disinfection-removal studies.

10. Base conclusions on *C. parvum* removal data that are present in reliably countable numbers (*e.g.*, more than 10 oocysts in a given sample or at least 10 observations).

Appendix A
Analytical Method Development: *C. parvum*

A.1 Objective

To date, one of the challenges associated with the detection of *C. parvum* has been poor and highly variable oocyst recovery. To complete the research objectives stated in Chapter 1, it was necessary to reliably concentrate and enumerate *C. parvum* oocysts from water. State-of-the-art analytical methods reported in the literature were considered. A method was then selected, implemented, and optimized to consistently yield high recoveries of *C. parvum* from waters representative of those studied during the present research. Method selection was based on the following criteria:

1. The method had to be relatively reliable (high recoveries with low variability).
2. The method had to allow for concurrent identification of *C. parvum* and polystyrene microspheres.
3. The method had to be readily implemented, without requiring extensively trained personnel.
4. The method did not *necessarily* have to discern between viable and non-viable *C. parvum*.
5. The method had to be relatively inexpensive, without requiring exorbitantly expensive equipment.

A.2 AVAILABLE METHODS: ADVANTAGES AND LIMITATIONS

Environmental detection and enumeration methods for *Cryptosporidium* oocysts were originally developed from those developed for *Giardia*. One of the greatest challenges associated with the detection of *C. parvum* has typically been poor and highly variable oocyst recovery (*e.g.*, Clancy *et al.*, 1994). This is in part because oocysts typically require concentration from large samples of water prior to detection. It has generally been recommended that at least 100L of source waters and $\geq$1000L of finished waters be concentrated prior to enumeration of indigenous oocyst concentrations (Jakubowski *et al.*, 1996).

The method proposed by the American Society for Testing and Materials (ASTM) (1993) involved passing large water volumes of water through a polypropylene yarn-wound cartridge filter followed by an immunofluorescence assay (IFA)-Percoll-sucrose gradient protocol and epifluorescence microscopy. The literature strongly and frequently indicates that the ASTM method is inadequate, however (*e.g.*, Hargy *et al.*, 1996; Vesey and Slade, 1991) and often results in highly variable and typically low oocyst recoveries (*e.g.*, Hargy *et al.*, 1996). LeChevallier *et al.* (1995) examined the ASTM method to determine when major losses occurred. The results revealed that the centrifugation and clarification steps could each result in losses as high as 30%. Further difficulties with the ASTM method can result from the presence of algae because numerous species of algae can fluoresce and result in false positive counts (Rodgers *et al.*, 1995). Although the United States EPA's new Method 1622 has preliminary indications of recoveries >70% (Clancy *et al.*, 1997), more data are necessary for a thorough evaluation of the method.

Due to poor analytical recoveries and low indigenous *C. parvum* concentrations, experimental evaluations of oocyst removal typically involve seeding with concentrations considerably higher than those typically present in raw water. Seeding with high oocyst concentrations ensures reliably countable (*i.e.*, non-zero) concentrations in process effluents, thereby allowing for removal calculations. Subtle differences in methodology can affect recoveries and data interpretation, however. For example, Musial *et al.* (1987) showed that the overall efficiency of recovery of an ASTM-like protocol could decrease as oocyst number present in the sample decreased; they also demonstrated that processing

samples at higher flow rates could also decrease recoveries. Ensuring that spiked oocyst concentrations are accurately measured prior to spiking adds to the consistency of recoveries (Straub *et al.*, 1996). Regardless of the analytical methodology used, it is important to evaluate a range of possible or expected oocyst concentrations from all waters investigated (*e.g.*, filter influents and effluents from all raw water sources) so that recovery can be appropriately described. As described above, implementing methods consistently will allow for better data interpretation, particularly when trying to incorporate the uncertainty associated with recovery into descriptions of *C. parvum* or other pathogen removal by treatment processes (as was done in Chapter 4). Similarly, comparisons between studies should be evaluated with caution because oocyst recovery is often very sensitive to seeded concentration (*e.g.*, Straub *et al.*, 1996; Musial *et al.*, 1987), quality of oocysts used (Dawson *et al.*, 1993), sample volume processed (*e.g.*, Nieminski *et al.*, 1995), and water characteristics such as turbidity (*e.g.*, Nieminski *et al.*, 1995).

Several alternatives to the ASTM protocol have been examined (*e.g.*, Nieminski *et al.* 1995; Whitmore and Carrington, 1993; Vesey *et al.*, 1993a). Table A.1 includes a concise summary of the more common methods used in detecting *C. parvum* from water. Observations relevant to the present study are included, however, only the methods that can be readily applied are listed. Those methods in the process of being developed (*e.g.*, PCR, flow cytometry, etc.) are discussed in detail elsewhere in the literature (*e.g.*, Jakubowski *et al.*, 1997; Vesey *et al.*, 1991).

Table A.1

Summary of Common Methods Used in Detecting *Cryptosporidium*

Method	Method Description	Typical Recovery (%)	Some Key References	Observations Relevant to Present Study
EPA Method 1622	**Concentration:**			
	vortex flow filtration	>70	Clancy *et al.*, 1997	no further information currently available
	polysulfone capsule	72	Clancy *et al.*, 1997	no further information currently available
	PCTE membrane disk	96	Clancy *et al.*, 1997	no further information currently available
	Purification:			
	immunomagnetic separation		Rossomondo *et al.*, 1994	Good recovery requires optimal bead concentration.
		67-83	Bukhari *et al.*, 1998	>62% recovery at turbidities of 5000 NTU.
		35 ± 15	Clancy *et al.*, 1999	Recent studies reported much lower recoveries originally reported/expected.
	Enumeration:			
	Immunofluorescence Assay (IFA)		*same as for ASTM*	*same as for ASTM*
	DAPI/PI staining		Dowd and Pillai, 1997	Stain in solution prevents background fluorescence.
			Clancy *et al.*, 1997	Concurrent enumeration and viability measurement.
ASTM	**Concentration:**			
	polypropylene yarn-wound cartridge	1-60	LeChevallier *et al.*, 1995	Centrifugation and clarification result in losses as high as 30%.
			LeChevallier *et al.*,1990; 1991 a,b	Recovery is greatly impacted by amount and type of debris present in the water.
	Purification:			
	flotation on Percoll-sucrose gradient		Rodgers *et al.*, 1995	Algae can result in false-positives.
		7	Hargy *et al.*, 1996	Highly variable, typically low recoveries.

Table A.1

Summary of Common Methods Used in Detecting *Cryptosporidium* (Continued)

Method	Method Description	Typical Recovery (%)	Some Key References	Observations Relevant to Present Study
	Enumeration:			
	Immunofluorescence Assay (IFA)		Jakubowski *et al.*, 1996	Evaluate >100L raw water, >1000L filter effluent.
			Musial *et al.*, 1987	Higher flow rates can decrease recoveries.
			Straub *et al.*, 1996	Accurate measurement of spike dose increases consistency of recoveries.
			Straub *et al.*, 1996	Recovery is sensitive to seeded concentration.
		25	Dawson *et al.*, 1993	Recovery is sensitive to quality of oocysts.
		<10	Nieminski *et al.*, 1995	Recovery is sensitive to sample volume and turbidity.
ASTM Variations	**Concentration:**			
	polycarbonate membrane	< 10	Nieminski *et al.*, 1995	Method suffers from high standard deviations. Increased turbidity decreases recovery regardless of use of membrane/cartridge).
			Kfir *et al.*, 1995	Cartridge filters can sometimes yield slightly better recoveries than membranes due to the ability to process larger sample volumes.
	Cross-Flow Membrane Filtration	37-86	Whitmore & Carrington, 1993	Rapid membrane deterioration due to fouling.
	Sand Column Filtration	2-98	Whitmore & Carrington, 1993	Recovery varies largely with loading rate.
	Continuous Flow Centrifugation	1-31	Whitmore & Carrington, 1993	Cannot process large sample volumes.
	Vortex-Flow Filtration	26-37	Whitmore & Carrington, 1993	Recoveries were fairly consistent.
	Non-Polypropylene Cartridge Filtration	<10	Whitmore & Carrington, 1993	Better removal achieved with ASTM method.

Table A.1

Summary of Common Methods Used in Detecting *Cryptosporidium* (Continued)

	Purification:			
	$CaCO_3$ Flocculation	69-79	Vesey *et al.*, 1993a	May not be applicable for samples that do not have much particulate matter.
Membrane Dissolution	Cellulose acetate filtration followed by dilution of filter in acetone.	61-87	Aldom & Chagla, 1995	Small coefficient of variation.

A.3 ORIGINAL METHOD OF YATES *ET AL.* (1997)

The *C. parvum* analytical protocol described by Yates *et al.* (1997) was employed because of its relative ease of implementation and typically consistent recoveries that averaged between 30 and 50% (Yates, 1997). This recovery range is comparable to those presented for ASTM-like methods in Table A.1. This method was designed for use during seeding studies (*i.e.*, it was not developed for studying indigenous oocyst concentrations) and involved processing filter influent and effluent samples volumes of ≤ 300 mL. Processing small sample volumes (of less than 1 L) required seeding the filter influent location with very high oocyst concentrations (~10^5 oocysts/L); however, it also eliminated the need for a purification step, thereby increasing oocyst recovery. This method was selected as a starting point because it met all of the selection criteria discussed above (unlike U.S. EPA Method 1622 at the time the research commenced).

The *C. parvum* analytical method of Yates *et al.* (1997) utilized filter housings (Swinnex; Millipore Canada Ltd., Nepean, ON.) containing pre-wetted, 25 mm, 0.45 µm cellulose acetate filters (VWR Canlab, Missisauga, ON.). The filter housings were connected to disposable syringes. Approximately 2 mL of 1% bovine serum albumin (BSA) were passed through the filters and then the water samples were passed through the filters. The syringes then were rinsed with a few milliliters of a buffered detergent solution (1× phosphate buffered saline [PBS] with final concentrations of: 0.1% sodium dodecyl sulphate, 0.1% Tween 80, and 0.01% Sigma Antifoam A and final pH of 7.4) to help maximize oocyst recovery. An additional 2 mL of 1% BSA were then passed through the filters. The membrane filters were then removed from the filter housings and were placed on top of 25 mm, 8.0µm nitrocellulose support membranes (Millipore Canada Ltd., Nepean, ON) on a manifold (Hoefer Scientific, San Francisco, CA.); weights held the membranes in place. The sample concentration steps are summarized in Figure A.1. A standard immunofluorescence assay (IFA) was then used to stain the oocysts (USEPA, 1996). Presumptive microscopic analysis for *C. parvum* enumeration was performed using epifluorescence microscopy at 400× magnification (Nikon Labophot 2A, Nikon Canada Inc., Toronto).

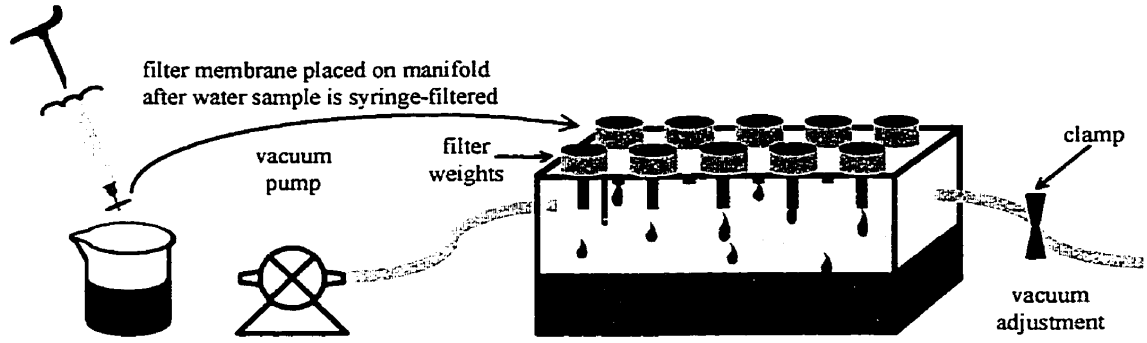


Figure A.1 *C. parvum* analytical method of Yates *et al.* (1997)

A.4 *C. PARVUM* INACTIVATION AND PRESERVATION

Samples of *C. parvum* were preserved in a penicillin/streptomycin solution because recent research has indicated that oocysts stored in this preservative may more closely represent oocyst behavior in the natural environment (Li *et al.* 1997). The oocysts were obtained from a commercial laboratory (Waterborne, Inc., New Orleans, LA. or University of Arizona, Department of Veterinary Science, Tucson, AZ.). Vials of ~10^8 oocysts were obtained; they were inactivated with 5% formalin (final concentration) for *C. parvum* in 1X PBS with 0.01% Tween 20 to prevent oocyst clumping. All microorganism stocks were refrigerated at 4°C in the dark until use.

A.5 ENUMERATION OF *C. PARVUM* STOCK SUSPENSION

The *C. parvum* stock suspension was briefly vortexed and a small portion of the suspension (< 100 µL in total) was removed to enumerate the oocyst concentration. The stock concentration was determined by averaging five replicate counts with a hemocytometer (Petroff-Hausser Bacterial Counting Chamber, Hausser Scientific Corporation, Horsham, PA). The entire grid (1 mm^2) was used in the enumeration process (Nikon Labophot 2A, Nikon Canada Inc., Toronto).

A.6 METHOD OPTIMIZATION

Several potential modifications to the *C. parvum* concentration and enumeration method described by Yates *et al.*, (1997) were investigated. The investigated modifications included the use of a different type of filter membrane, the use of direct vacuum filtration rather than syringe filtration, the role of the buffered surfactant rinse in maximizing oocyst recovery, and the importance of sample handling strategies such as vigorous shaking prior to oocyst concentration. The results and implications of each set of optimization experiments are discussed below.

A.6.1 Membrane Type (Polycarbonate vs. Cellulose Acetate)

Polycarbonate membranes were compared to cellulose acetate membranes of similar pore size (~0.4 μm). As indicated in Table A.1, polycarbonate membranes have been investigated in other studies in the context of ASTM-like protocols (*e.g.*, Nieminski *et al.*, 1995; Kfir *et al.*, 1995). They are desirable because they do not fold and bubble on microscope slides as much as cellulose acetate membranes. The disadvantage of their use is that because they are somewhat thereby precluding confirmation of internal structures with Nomarski differential interference contrast (DIC) microscopy. Given the high concentrations of *C. parvum* oocysts and the epifluorescence microscopy used during this research, identification of *C. parvum* oocysts was not difficult. Since only presumptive microscopic analyses were performed during this research (*i.e.*, no confirmation of internal structures), the relative opacity of the membranes was not of concern.

Recovery studies using Milli Q™ water and cellulose acetate (0.45 μm) or polycarbonate (0.4 μm) membranes were performed using concentrations of formalin-inactivated *C. parvum* that would be expected at the filter influent and effluent locations during the pilot-scale experiments completed during this thesis research. All of the pipettes, syringes, and glassware were rinsed with a few milliliters of a buffered detergent solution (1× phosphate buffered saline [PBS] with final concentrations of: 0.1% sodium dodecyl sulphate, 0.1% Tween 80, and 0.01% Sigma Antifoam A and final pH of 7.4) before and after coming into contact with the water samples. This rinse was also passed through the membrane filters to ensure higher recoveries. The cellulose acetate and polycarbonate

membrane data are presented in Table A.2 and Table A.3 respectively. A comparison of the recovery data failed to demonstrate statistical differences between cellulose acetate and polycarbonate membranes used in conjunction with either syringe or manifold filtration (two sided t-test, α=0.05, n_1= n_2=15 for each comparison, Table A.4).

A.6.2 Direct Vacuum Filtration (vs. Syringe Filtration)

Direct vacuum filtration was compared to syringe filtration to minimize oocyst losses, allow for easier and faster handling, and to ensure constant pressure on the filter membranes. Although passing less than ~50 mL through a membrane with a syringe is not difficult, passing larger volumes requires the syringe to be refilled multiple times. As the filter membrane clogs, it becomes increasingly difficult to apply the pressure necessary to pass the water through the filter; this can potentially lead to large surges in pressure that might damage the membrane. A manifold provides constant vacuum consistently for all samples and allows for concurrent processing of multiple samples.

Recovery studies using MilliQ™ water and syringe or direct vacuum (manifold) filtration were conducted in conjunction with the cellulose acetate and polycarbonate membrane investigations described in Section A.6.1. These recovery experiments were also performed using concentrations of formalin-inactivated *C. parvum* that would be expected at the filter influent and effluent locations during the pilot-scale experiments completed during this thesis research (Chapter 6). All of the pipettes, syringes, and glassware were rinsed with a few milliliters of a buffered detergent solution (described in Section A.6.1) before and after coming into contact with the water samples. This rinse was also passed through the membrane filters to ensure higher recoveries. The syringe and direct vacuum filtration data are summarized in Table A.2 and Table A.3 for cellulose acetate and polycarbonate membranes respectively. A comparison of the recovery data demonstrated statistically different recoveries with both types of membranes when direct vacuum filtration, as compared to syringe filtration, was used (two sided t-test, α=0.05, n_1= n_2=15 for each comparison, Table A.5). This result clearly demonstrated that the higher recoveries achieved with direct vacuum filtration were significantly different from those achieved with syringe filtration.

Table A.2

C. parvum Recovery from Cellulose Acetate Membranes

Concentration Method	Processed Volume	Seeded (Oocysts/L)	# Counted	*C. parvum* Oocysts/L	Recovery (%)
Cellulose Acetate + Syringe Filtration	2.5	1.0 E+6	1444	5.8 E+5	58
			1665	6.7 E+5	67
			1352	5.4 E+5	54
			1559	6.2 E+5	62
			478	1.9 E+5	19
			1370	5.5 E+5	55
			1828	7.3 E+5	73
			1346	5.4 E+5	54
			1537	6.1 E+5	61
			1663	6.7 E+5	67
				Average:	**57**
				Std. dev.:	**15**
				Coeff. Var.:	**26**
	500	1000	233	466	47
			362	724	72
			209	418	42
			357	714	71
			415	830	83
				Average:	**63**
				Std. dev.:	**18**
				Coeff. Var.:	**28**
Cellulose Acetate + Manifold Filtration	2.5	1.0 E+6	1528	6.1 E+5	61
			1586	6.3 E+5	63
			1896	7.6 E+5	76
			2021	8.1 E+5	81
			2223	8.9 E+5	89
			1874	7.5 E+5	75
			2249	9.0 E+5	90
			2091	8.4 E+5	84
			1776	7.1 E+5	71
			2358	9.4 E+5	94
				Average:	**78**
				Std. dev.:	**11**
				Coeff. Var.:	**14**
	500	1000	319	638	64
			405	810	81
			407	814	81
			308	616	62
			372	744	74
				Average:	**72**
				Std. dev.:	**9**
				Coeff. Var.:	**13**

Table A.3

C. parvum Recovery from Polycarbonate Membranes

Concentration Method	Processed Volume	Seeded Conc. (Oocysts/L)	# Counted	*C. parvum* Oocysts/L	Recovery (%)
Polycarbonate + Syringe Filtration	2.5	1.0 E+6	2219	8.9 E+5	89
			1269	5.1 E+5	51
			1593	6.4 E+5	64
			1298	5.2 E+5	52
			1534	6.1 E+5	61
			1333	5.3 E+5	53
			1601	6.4 E+5	64
			1843	7.4 E+5	74
			1650	6.6 E+5	66
			1432	5.7 E+5	57
				Average:	**63**
				Std. dev.:	**12**
				Coeff. Var.:	**18**
	500	1000	252	504	50
			224	448	45
			312	624	62
			383	766	77
			385	770	77
				Average:	**62**
				Std. dev.:	**15**
				Coeff. Var.:	**24**
Polycarbonate + Manifold Filtration	2.5	1.0 E+6	1898	7.6 E+5	76
			1902	7.6 E+5	76
			2117	8.5 E+5	85
			1744	7.0 E+5	70
			1620	6.5 E+5	65
			2214	8.9 E+5	89
			1971	7.9 E+5	79
			2213	8.9 E+5	89
			1860	7.4 E+5	74
			1772	7.1 E+5	71
				Average:	**77**
				Std. dev.:	**8**
				Coeff. Var.:	**10**
	500	1000	329	658	66
			364	728	73
			288	576	58
			375	750	75
			404	808	81
				Average:	**70**
				Std. dev.:	**9**
				Coeff. Var.:	**13**

Table A.4

Statistical Analysis of Cellulose Acetate and Polycarbonate Membranes

F-Test Two-Sample for Variances

	CA Syringe	*PC Syringe*
Mean	58.99	62.81
Variance	239.32	147.25
Observations	15	15
df	14	14
F	1.63	
P(F<=f) one-tail	0.19	
F Critical one-tail	2.48	

t-Test: Two-Sample Assuming Equal Variances

	CA Syringe	*PC Syringe*
Mean	58.99	62.81
Variance	239.32	147.25
Observations	15	15
Pooled Variance	193.29	
Hypothesized Mean Difference	0	
df	28	
t Stat	**-0.75**	
P(T<=t) one-tail	0.23	
t Critical one-tail	1.70	
P(T<=t) two-tail	0.46	
t Critical two-tail	**2.05**	

F-Test Two-Sample for Variances

	CA Manifold	*PC Manifold*
Mean	76.42	74.96
Variance	113.89	75.13
Observations	15	15
df	14	14
F	1.52	
P(F<=f) one-tail	0.22	
F Critical one-tail	2.48	

t-Test: Two-Sample Assuming Equal Variances

	CA Manifold	*PC Manifold*
Mean	76.42	74.96
Variance	113.89	75.13
Observations	15	15
Pooled Variance	94.51	
Hypothesized Mean Difference	0	
df	28	
t Stat	**0.41**	
P(T<=t) one-tail	0.34	
t Critical one-tail	1.70	
P(T<=t) two-tail	0.68	
t Critical two-tail	**2.05**	

[CA] Cellulose Acetate.
[PC] Polycarbonate.

Table A.5

Statistical Analysis of Direct Vacuum (Manifold) and Syringe Filtration

F-Test Two-Sample for Variances

	CA Syringe	*CA Manifold*
Mean	58.99	76.42
Variance	239.32	113.89
Observations	15	15
df	14	14
F	2.10	
P(F<=f) one-tail	0.09	
F Critical one-tail	2.48	

t-Test: Two-Sample Assuming Equal Variances

	CA Syringe	*CA Manifold*
Mean	58.99	76.42
Variance	239.32	113.89
Observations	15	15
Pooled Variance	176.60	
Hypothesized Mean Difference	0	
df	28	
t Stat	**-3.59**	
P(T<=t) one-tail	0.00	
t Critical one-tail	1.70	
P(T<=t) two-tail	0.00	
t Critical two-tail	**2.05**	

F-Test Two-Sample for Variances

	PC Syringe	*PC Manifold*
Mean	62.81	74.96
Variance	147.25	75.13
Observations	15	15
df	14	14
F	1.96	
P(F<=f) one-tail	0.11	
F Critical one-tail	2.48	

t-Test: Two-Sample Assuming Equal Variances

	PC Syringe	*PC Manifold*
Mean	62.81	74.96
Variance	147.25	75.13
Observations	15	15
Pooled Variance	111.19	
Hypothesized Mean Difference	0	
df	28	
t Stat	**-3.16**	
P(T<=t) one-tail	0.00	
t Critical one-tail	1.70	
P(T<=t) two-tail	0.00	
t Critical two-tail	**2.05**	

[CA] Cellulose Acetate.

[PC] Polycarbonate.

A.7 OPTIMIZED *C. PARVUM* METHOD PROTOCOL

The findings from the method optimization experiments described in the previous section (Section A.6) were applied to the method of Yates *et al.* (1997) to yield an optimized *C. parvum* analytical protocol. The key results that were integrated into the *C. parvum* analytical method from the method optimization experiments were:

1. the use of 0.40 μm polycarbonate membranes because they do not warp as readily as cellulose acetate membranes (thereby resulting in more easily obtained and reliable counts) and

2. the use of direct vacuum filtration rather than syringe filtration because it is easier, faster, and results in less sample loss (higher recovery).

The optimized *C. parvum* concentration and enumeration protocol is presented Table A.6. This protocol was employed for all of the *C. parvum* analyses performed during this thesis research.

Table A.6

C. parvum Concentration and Enumeration Protocol

1. Autoclave the support membranes and filter membranes in 1X PBS.

↓

2. Prepare Primary and Secondary stains according to the protocol provided by the Hydrofluor™ Combo Kit (1/10 volume primary or secondary stain, 1/10 volume goat serum, 8/10 volume 1X PBS). Store prepared stains in a dark place due to their light sensitivity.

↓

3. Open manifold ports and connect the manifold to the vacuum pump. Adjust pressure release valve to obtain a vacuum of 5 inches of mercury. Close manifold ports and turn off vacuum pump.

↓

4. With flamed forceps add the support membranes and then the filter membranes to the manifold. Place weights on the membranes. (Note of where each sample will be filtered.)

↓

5. Filter 2 mL of 1% BSA through each membrane.

↓

6. Rinse graduated cylinder (larger sample volumes) or pipette (smaller sample volumes) with buffered surfactant solution and discard excess solution

↓

7. Shake samples bottles to mix the contents. Add appropriate amounts of filter influent and filter effluent to each pre-rinsed, labeled graduated cylinder or pipette.

↓

8. Carefully add each sample to the appropriate manifold and filter. (Add 1X PBS to keep the membrane moist when filtering is completed.)

↓

9. Rinse each graduated cylinder with buffered surfactant solution, retaining the solution. Filter through the appropriate membrane.

↓

10. Rinse a small test tube with eluting solution and discard excess solution. Add 50µL of the positive control to the test tube and add ~5 mL of 1X PBS; filter through appropriate membrane. Rinse test tube with eluting solution and filter the retained solution.

↓

11. Filter 2 mL of 1X PBS for the negative control.

Table A.6

C. parvum Concentration and Enumeration Protocol (Continued)

12. After filtering all the samples, filter 2 mL of 1% BSA through each membrane.

↓

13. After all of the BSA has been filtered, turn off vacuum. Close all ports and add 500µL of the prepared Primary Stain to each membrane. Let stand, covered (light sensitive), for 25 minutes. (At this time the slides can be labeled and placed on the slide warmer at 37°C.)

↓

14. After 25 minutes, turn on the vacuum and open the ports to allow the Primary Stain to filter through.

↓

15. Rinse each membrane 5 times with approximately 1 mL of 1X PBS.

↓

16. Turn off vacuum. Close all ports and add 500µL of the prepared Secondary Stain to each membrane. Let stand, covered, for 25 minutes. (At this time, add 2 drops of 2% DABCO/glycerol to each slide.)

↓

17. After 25 minutes, turn on the vacuum and open the ports to allow the Secondary Stain to drain through.

↓

18. Rinse each membrane 5 times with approximately 1 mL of 1X PBS.

↓

19. Add approximately 1 mL Ethanol series in the following order: 10%, 20%, 40% 80%, and 90.2%. Allow each volume to completely drain before adding the next volume.

↓

20. Remove the filter membranes from the manifolds and transfer them to the labeled slides, using flamed forceps between transfers. Allow the filters to remain on the slide warmer for 5 minutes.

↓

21. Add 1 drop of 2% DABCO/glycerol on top of each membrane.

↓

22. Place a cover slip on each membrane and let stand on the warmer for 5 minutes.

↓

23. Remove air bubbles and excess glycerol with cotton swabs. With clear fingernail polish in a syringe, seal around the cover slip. Store slides at 4°C in the dark. Enumerate at 400× magnification.

A.8 METHOD RECOVERY IN DIFFERENT WATER TYPES

Once the optimized protocol for *C. parvum* concentration and analysis was established, it was necessary to evaluate the recovery of *C. parvum* oocysts from the various water matrices encountered during this thesis research. Recovery experiments were performed on the following water matrices:

1. Treated Ottawa River Water
2. Treated UW Synthetic Water (1.5 NTU)
3. Treated UW Synthetic Water (3.5 NTU)

Recovery of *C. parvum* from filter influent and effluent samples from each of the water matrices was investigated. The *C. parvum* recovery data for the Ottawa, UW 1.5 NTU, and UW 3.5 NTU waters are available in Table A.7, Table A.8, and Table A.9 respectively. A single factor analysis of variance (ANOVA) was used (Table A.10) to determine if there were significant differences in *C. parvum* recovery from the various water matrices studied when the optimized *C. parvum* analytical method (Section A.7) was used. These results indicate that the ANOVA analysis failed to demonstrate significant differences between *C. parvum* recoveries from filter influent and effluent waters from any of the water matrices investigated during this thesis research (α=0.05).

Ideally, treated water from the Windsor Pilot Plant (filter influent and effluent) would also have been included in these recovery investigations. Given the small number of experiments performed at Windsor (relative to Ottawa and UW) and time commitment necessary to complete the recovery studies (while experiments were concurrently being performed in Ottawa), recovery experiments were not performed on the water from Windsor. If further *C. parvum* seeding investigations were planned for Windsor, recovery experiments would be warranted so that accurate statistical analyses of oocyst removal by the treatment process could be performed. Given the consistent *C. parvum* recoveries from the other water matrices investigated (discussed above), it was assumed that the oocyst recoveries from Windsor filter influent and effluent would also be consistent with these recoveries.

Table A.7

C. parvum Recovery from Ottawa Water

Date	Sample Location	Processed Volume (mL)	Seeded Concentration (Oocysts/L)	# Counted (Oocysts)	*C. parvum* Measured Oocysts/L	Recovery (%)
5/11/99	Filter influent*	100	1.0 E+6	7.0	7.0 E+5	70 est.
			average from 50 fields of view	7.2	7.2 E+5	72 est.
				8.5	8.5 E+5	85 est.
				8.2	8.2 E+5	82 est.
				7.1	7.1 E+5	71 est.
					Average:	**76 est.**
					Std. Dev.:	**7 est.**
					Coeff.	**9 est.**
5/12/99	Filter effluent	500	1000	357	714	71
				366	732	73
				431	862	86
				378	756	76
				345	690	69
					Average:	**75**
					Std. Dev.:	**7**
					Coeff.	**9**
5/18/99	Filter effluent	500	100	36	72	72
				42	84	84
				35	70	70
				38	76	76
				37	74	74
					Average:	**75**
					Std. Dev.:	**5**
					Coeff.	**7**

*Estimated (est.) by field counts @ 400× using Equation 3.1. (Nikon Labophot 2A, Nikon Canada Inc., Toronto, ON)

Table A.8

C. parvum Recovery from UW Water (1.5 NTU)

Date	Sample Location	Processed Volume (mL)	Seeded Concentration (Oocysts/L)	# Counted (Oocysts)	*C. parvum* Measured (Oocysts/L)	Recovery (%)
4/4/00	Filter influent	2.5	1.0E+05	128	5.1 E+4	51
				134	5.4 E+4	54
				177	7.1 E+4	71
				130	5.2 E+4	52
				164	6.6 E+4	66
				158	6.3 E+4	63
				195	7.8 E+4	78
				141	5.6 E+4	56
				183	7.3 E+4	73
4/19/00	Filter influent	2.5	1.0E+05	175	7.0 E+4	70
				205	8.2 E+4	82
				167	6.7 E+4	67
				217	8.7 E+4	87
				202	8.1 E+4	81
					Average:	**68**
					Std. Dev.:	**12**
					Coeff. Var.:	**17**
4/6/00	Filter effluent	500	1000	358	716	72
				394	788	79
				420	840	84
				396	792	79
				405	810	81
				377	754	75
				362	724	72
				341	682	68
				408	816	82
				362	724	72
					Average:	**76**
					Std. Dev.:	**5**
					Coeff. Var.:	**7**
4/7/00	Filter effluent	500	100	32	64	64
				27	54	54
				29	58	58
				35	70	70
				40	80	80
				39	78	78
				44	88	88
				37	74	74
				39	78	78
				39	78	78
					Average:	**72**
					Std. Dev.:	**11**
					Coeff. Var.:	**15**

Table A.9

C. parvum Recovery from UW Water (3.5 NTU)

Date	Sample Location	Processed Volume (mL)	Seeded Concentration (Oocysts/L)	# Counted (Oocysts)	*C. parvum* Measured (Oocysts/L)	Recovery (%)
	Filter influent*	100	1.0 E+6	8.3	8.3 E+5	83 est.
				6.9	6.9 E+5	69 est.
			average from 50 fields of view	7.1	7.1 E+5	71 est.
				6.5	6.5 E+5	65 est.
				9.0	9.0 E+5	90 est.
					Average:	**76 est.**
					Std. Dev.:	**10 est.**
					Coeff.	**14 est.**
	Filter influent	5	1.0 E+5	433	8.7 E+4	87
				395	7.9 E+4	79
				317	6.3 E+4	63
				299	6.0 E+4	60
				386	7.7 E+4	77
					Average:	**73**
					Std. Dev.:	**11**
					Coeff.	**15**
	Filter effluent	500	1000	366	732	73
				411	822	82
				276	552	55
				417	834	83
				354	708	71
				368	***Average:***	**73**
					Std. Dev.:	**11**
					Coeff.	**16**
	Filter effluent	500	100	30	60	60
				42	84	84
				35	70	70
				31	62	62
				44	88	88
					Average:	**73**
					Std. Dev.:	**13**
					Coeff.	**17**

*Estimated (est.) by field counts @ 400× using Equation 3.1. (Nikon Labophot 2A, Nikon Canada Inc., Toronto, ON)

Table A.10

ANOVA Analysis of *C. parvum* Recovery from Various Water Matrices

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Ottawa FI	5	380.00	76.00	48.50
Ottawa FE	10	751.40	75.14	32.76
UW 1.5 FI	14	950.40	67.89	135.57
UW 1.5 FE	20	1486.60	74.33	71.22
UW 3.5 FI	10	742.00	74.20	195.07
UW 3.5 FE	10	742.00	74.20	122.62

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	517.72	5	103.54	**1.01**	0.42	**2.36**
Within Groups	6463.52	63	102.60			
Total	6981.24	68				

A.9 EFFECT OF COAGULANT ON RECOVERY

One additional set of recovery experiments was performed using Ottawa filter influent and effluent water with a dose of 30 mg/L alum ($Al_2(SO_4)_3 \cdot 18H_2O$). This experiment was performed to ensure that the presence of high doses of coagulant, as used at Ottawa during the jar coagulation of *C. parvum* oocysts prior to seeding (Chapter 3), did not affect the recovery of *C. parvum* oocysts from the water (*e.g.*, perhaps by causing additional aggregation). The *C. parvum* recovery data for Ottawa water with 30 mg/L alum addition are summarized in Table A.11. A single factor analysis of variance (ANOVA) was again used (Table A.12) to determine if there were significant differences in *C. parvum* recovery from the various water matrices studied when the optimized *C. parvum* analytical method (Section A.7) was used. These results indicate that the ANOVA analysis failed to demonstrate significant differences between *C. parvum* recoveries from filter influent and effluent waters from any of the water matrices including the Ottawa water dosed with high concentrations of alum (α=0.05).

Table A.11

C. parvum Recovery from Ottawa Water with 30 mg/L Alum ($Al_2(SO_4)_3 \cdot 18H_2O$)

Date	Sample Location	Processed Volume (mL)	Seeded Concentration (Oocysts/L)	# Counted (Oocysts)	*C. parvum* Measured (Oocysts/L)	Recovery (%)
6/2/99	Filter influent	10	1.0 E+6	6992	7.0 E+5	70
				8019	8.0 E+5	80
				5888	5.9 E+5	59
				7583	7.6 E+5	76
				7961	8.0 E+5	80
					Average:	**73**
					Std. Dev.:	**9**
					Coeff.	**12**
6/2/99	Filter effluent	500	1000	419	838	84
				302	604	60
				295	590	59
				375	750	75
				401	802	80
					Average:	**72**
					Std. Dev.:	**11**
					Coeff.	**16**
6/2/99	Filter effluent	500	100	32	64	64
				39	78	78
				38	76	76
				41	82	82
				28	56	56
					Average:	**71**
					Std. Dev.:	**11**
					Coeff.	**15**

Table A.12

ANOVA Analysis of *C. parvum* Recovery from Various Water Matrices Including Ottawa Water with a High Coagulant Dose

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Ottawa-Alum FI	5	364.43	72.89	78.05
Ottawa-Alum FE	10	714.40	71.44	109.77
Ottawa FI	5	380.00	76.00	48.50
Ottawa FE	10	751.40	75.14	32.76
UW 1.5 FI	14	950.40	67.89	135.57
UW 1.5 FE	20	1486.60	74.33	71.22
UW 3.5 FI	10	742.00	74.20	195.07
UW 3.5 FE	10	742.00	74.20	122.62

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	545.55	7	77.94	**0.76**	0.62	**2.13**
Within Groups	7763.62	76	102.15			
Total	8309.17	83				

Appendix B
C. parvum Quality Assurance and Quality Control Data

B.1 Method Blanks (Negative Controls)

Method blanks (negative controls) were included almost every time samples were processed for *C. parvum*; they were not processed on the few occasions where space on the manifold was limited. The method blank consisted of a filtered 2-mL sample of phosphate buffered saline (PBS) at pH 7.4. This control came into contact with all of the reagents used to process the *C. parvum* oocysts from the water samples. The inclusion of this control during all (or most) of the *C. parvum* analyses ensured that the processed water samples were not contaminated with an outside source of oocysts during processing.

The method blank data for the Ottawa, Windsor, and UW experiments are available in Table B.1, Table B.2, and Table B.3 respectively. These tables indicate that negative controls were processed for all but two experiments at each of the pilot plants; an "x" in the negative control column indicates that no oocysts were found in control sample. As indicated in Table B.1 through Table B.3, no oocysts were found in any of the 50 processed negative control samples, thereby suggesting no contaminant source of oocysts associated with sample processing.

B.2 POSITIVE CONTROLS

Positive controls were included almost every time samples were processed for *C. parvum*; they were not processed on the few occasions where space on the manifold was limited. The positive control sample consisted of a filtered 50-µL sample of formalinized stool containing *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts preserved with 10% formalin. This control came into contact with all of the reagents used to process the *C. parvum* oocysts from the water samples. The inclusion of this control during all (or most) of the *C. parvum* analyses ensured that the identification method was reliable for staining oocysts (and cysts). Well-stained oocysts (and cysts) were expected from every positive control sample; if they were not found, the oocyst counts from water samples processed at the same time as the positive control would have to be considered inconclusive due to inadequate staining.

The positive control data for the Ottawa, Windsor, and UW experiments are available in Table B.1, Table B.2, and Table B.3 respectively. These tables indicate that positive controls were processed for all but 1 experiment at Ottawa, 2 experiments at Windsor, and four experiments at UW. Although positive controls were not associated with 7 experiments, the experiments represented only 4 sample processing occasions. A "✓" in the positive control column indicates that oocysts (and cysts) were found in the control samples. As indicated in Table B.1 through Table B.3, oocysts were found in all of the 49 processed positive control samples, suggesting reliable staining of oocysts. These data support the argument that samples in which little or no oocysts were found truly had low concentrations of oocysts rather than low counts due to poor staining methods.

B.3 FILTER INFLUENT AND EFFLUENT NEGATIVE CONTROLS

Filter influent and effluent negative controls were collected during almost every experiment. Filter influent negative controls were collected to ensure that no substantial outside source of oocysts affected the *C. parvum* removal data; such a source was highly unlikely because seeded oocyst concentrations were typically 10^5-10^6 oocysts/L during the pilot-scale experiments. Although filter influent negative controls could also be

processed to determine background concentrations of cysts and oocysts in the filter influent, it would be somewhat misleading to suggest that the controls collected during this research would adequately serve that purpose. The filter influent negative control volumes that were processed were typically 0.1 to 1 L; while inappropriate for determining background concentrations of indigenous oocysts (typically >100 - 1000 L are required) these volumes were adequate for ensuring that background levels of oocysts did not substantially impact the seeded oocyst concentrations.

Filter effluent negative controls were collected and processed in 1-L volumes to ensure that the *C. parvum* removal data were not affected by background concentrations of oocysts (either indigenous or from previous experiments) exiting the filters. The samples were processed in 1-L volumes because the experiments were designed with seeded concentrations aimed at ensuring that oocysts would be found in reliably countable concentrations in 1-L samples of filter effluents.

It was originally planned that the collection of filter influent and effluent controls would alternate between the influent and effluent locations during successive experiments. After March 1999, and during most of the pilot-scale experiments, negative controls were collected at both the filter influent and effluent locations to better assure minimal effects, if any, from outside sources of oocysts.

The filter influent and effluent negative control data for the Ottawa, Windsor, and UW experiments are available in Table B.1, Table B.2, and Table B.3 respectively. These tables indicate that filter influent negative controls were processed for all but 11 experiments at Ottawa (due to the original alternating sampling scheme) and 2 experiments at UW. Filter effluent controls were processed for all but 12 experiments at Ottawa (again, primarily due to the original alternating sampling scheme) and 2 experiments at UW. An "x" in the filter influent and effluent negative control columns indicates that no oocysts (or cysts) were found in control samples. As indicated in Table B.1 through Table B.3, no oocysts were found in any of the 43 processed filter influent control samples or 42 processed filter effluent negative control samples, suggesting no substantial outside sources of oocysts (either indigenous or from previous experiments).

Table B.1

Methodological Positive and Negative Control and Filter Influent and Effluent Control Results for Pilot-Scale Experiments at Ottawa

Experiment Date	Experimental Conditions	Processing Date	Positive Control*	Negative Control§	Filter Influent Control§	Filter Effluent Control§
8/6/98	Stable Filter Operation (Shakedown #1)	8/7/98	✓	×	NS	×
9/9/98	Stable Filter Operation	9/10/98	✓	×	×	NS
9/22/98	Stable Filter Operation	9/23/98	✓	×	NS	×
10/6/98	Stable Filter Operation	10/7/98	✓	×	×	NS
10/27/98	Ripening	10/28/98	✓	×	NS	×
11/3/98	Ripening	11/4/98	✓	×	×	NS
11/10/98	Ripening	11/11/98	✓	×	NS	×
11/25/98	Breakthrough	11/26/98	✓	×	×	NS
12/9/98	Breakthrough	12/10/98	✓	×	NS	×
1/13/99	Breakthrough	1/15/99	✓	×	×	NS
1/21/99	Onset of Breakthrough	1/22/99	✓	×	NS	×
2/9/99	No Coagulants in Plant, No Coagulants in Jar	2/10/99	✓	×	×	NS
2/18/99	Sub-optimal Coagulation	2/19/99	✓	×	NS	×
3/9/99	Stable Filter Operation	3/10/99	✓	×	×	NS
3/23/99	Sub-optimal Coagulation	3/24/99	✓	×	NS	×
4/8/99	Stable Filter Operation During Runoff	4/9/99	✓	×	×	×
4/13/99	Stable Filter Operation During Runoff	4/12/99	✓	×	×	×
5/4/99	Sub-optimal Coagulation	5/5/99	✓	×	×	×
5/31/99	Stable Filter Operation	6/1/99	✓	×	×	NS
6/7/99	Hydraulic step	6/8/99	✓	×	×	NS
6/15/99	Hydraulic step	6/23/99	✓	×	×	×
6/22/99	Hydraulic step	6/23/99	✓	×	×	×
6/29/99	No Coagulants in Plant	7/5/99	✓	NP	×	×
7/13/99	No Coagulants in Plant	7/14/99	✓	×	×	×
7/20/99	Stable - Seeded at Rapid Mix	7/21/99	NP	NP	×	×
7/27/99	Stable Filter Operation	7/29/99	✓	×	×	×
8/4/99	No Coagulants in Plant, No Coagulants in Jar	8/5/99	✓	×	×	×
8/18/99	Stable Filter Operation, No Coagulants in Jar	8/19/99	✓	×	×	×
8/24/99	No Coagulants, No Media	8/27/99	✓	×	×	×
12/13/99	No Coagulants Since Backwash	12/14/99	✓	×	×	×
12/17/99	No Silicate in Plant/Jar	12/18/99	✓	×	×	×
12/20/99	Onset of Breakthrough	12/21/99	✓	×	NS	NS
12/22/99	Onset of Breakthrough	12/23/99	✓	×	NS	NS
1/19/00	Stable Filter Operation	1/20/00	✓	×	×	×
2/29/00	Onset of Breakthrough	3/16/00	✓	×	×	×
3/1/00	Onset of Breakthrough	3/13/00	✓	×	×	×
3/3/00	Onset of Breakthrough	3/8/00 3/9/00	✓	×	×	×
3/4/00	Onset of Breakthrough	3/8/00 3/9/00	✓	×	NS	NS
3/8/00	End-of-Run	3/13/00	✓	×	×	×
3/9/00	End-of-Run	3/14/00 3/15/00	✓	×	×	×
3/10/00	Sub-optimal Coagulation	3/16/00 3/17/00	✓	×	×	×

* A check mark (✓) indicates the presence of oocysts in the sample.
§ An × indicates that no oocysts were found in the sample.
NS Not sampled.
NP Not processed.

Table B.2

Methodological Positive and Negative Control and Filter Influent and Effluent Control Results for Pilot-Scale Experiments at Windsor

Experiment Date	Experimental Conditions	Processing Date	Positive Control*	Negative Control§	Filter Influent Control§	Filter Effluent Control§
3/10/99	Stable - Constant Rate Filtration	3/11/99	✓	×	×	×
"	Stable - Declining Rate Filtration	3/11/99	✓	×	×	×
3/22/99	Stable - Constant Rate Filtration	3/23/99	✓	×	×	×
"	Stable - Declining Rate Filtration	3/23/99	✓	×	×	×
10/14/99	Stable Operation with Pre-ozonation	10/15/99	✓	×	×	×
10/21/99	Stable Operation	10/23/99	NP	NP	×	×
10/22/99	Stable Operation	10/23/99	NP	NP	×	×

* A check mark (✓) indicates the presence of oocysts in the sample.
§ An × indicates that no oocysts were found in the sample.
NP Not processed.

Table B.3

Methodological Positive and Negative Control and Filter Influent and Effluent Control Results for Pilot-Scale Experiments at UW

Experiment Date	Experimental Conditions	Processing Date	Positive Control*	Negative Control§	Filter Influent Control§	Filter Effluent Control§
11/23/99	Stable Operation - Dual-media	11/25/99	✓	×	NS	NS
11/24/99	"	11/25/99	✓	×	NS	NS
11/28/99	Stable Operation - Tri-media	11/29/99	NP	×	×	×
11/28/99	"	11/29/99	NP	×	×	×
12/10/99	Hydraulic Step - Dual-media	12/12/99	NP	NP	×	×
12/11/99	"	12/12/99	NP	NP	×	×
1/15/00	Hydraulic Step - Tri-media	1/17/00	✓	×	×	×
1/16/00	"	1/17/00	✓	×	×	×

* A check mark (✓) indicates the presence of oocysts in the sample.
§ An × indicates that no oocysts were found in the sample.
NS Not sampled.
NP Not processed.

Appendix C
Analytical Recovery of *C. parvum* and Microspheres

Table C.1

Calculation of Beta Parameters *a* and *b* for *C. parvum* Recovery from Ottawa Filter Influent

Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(*p*)	Var(*p*)	E(*p*)/Var(p)	E(*p*)2/Var(p)	1/Var(*p*)	Number of trials
1.0E+06	0.1	0.5	500	351	351	149	0.70	4.176 E-4	1.681 E+3	1.180 E+3	2.395 E+3	
1.0E+06	0.1	0.5	500	360	360	140	0.72	4.024 E-4	1.789 E+3	1.288 E+3	2.485 E+3	
1.0E+06	0.1	0.5	500	427	427	73	0.85	2.489 E-4	3.432 E+3	2.931 E+3	4.018 E+3	
1.0E+06	0.1	0.5	500	409	409	91	0.82	2.972 E-4	2.753 E+3	2.252 E+3	3.365 E+3	
1.0E+06	0.1	0.5	500	355	355	145	0.71	4.110 E-4	1.728 E+3	1.227 E+3	2.433 E+3	
						sum			1.138 E+4	8.877 E+3	1.470 E+4	5

pooled mean recovery 0.7745
pooled variance 0.0045
pooled standard deviation 0.0674

a 28.96
b 8.43

Table C.2

Calculation of Beta Parameters *a* and *b* for *C. parvum* Recovery from Ottawa Filter Effluent

Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(*p*)	Var(*p*)	E(*p*)/Var(p)	E(*p*)2/Var(p)	1/Var(*p*)	Number of trials
1000	0.5	100	500	357	357	143	0.71	4.076 E-4	1.752 E+3	1.251 E+3	2.453 E+3	
1000	0.5	100	500	366	366	134	0.73	3.916 E-4	1.869 E+3	1.368 E+3	2.554 E+3	
1000	0.5	100	500	431	431	69	0.86	2.374 E-4	3.630 E+3	3.129 E+3	4.212 E+3	
1000	0.5	100	500	378	378	122	0.76	3.682 E-4	2.053 E+3	1.552 E+3	2.716 E+3	
1000	0.5	100	500	345	345	155	0.69	4.269 E-4	1.616 E+3	1.115 E+3	2.342 E+3	
100	0.5	100	50	36	36	14	0.72	3.953 E-3	1.821 E+2	1.311 E+2	2.530 E+2	
100	0.5	100	50	42	42	8	0.84	2.635 E-3	3.188 E+2	2.678 E+2	3.795 E+2	
100	0.5	100	50	35	35	15	0.70	4.118 E-3	1.700 E+2	1.190 E+2	2.429 E+2	
100	0.5	100	50	38	38	12	0.76	3.576 E-3	2.125 E+2	1.615 E+2	2.796 E+2	
100	0.5	100	50	37	37	13	0.74	3.773 E-3	1.962 E+2	1.452 E+2	2.651 E+2	
						sum			1.200 E+4	9.241 E+3	1.570 E+4	10

pooled mean recovery 0.7645
pooled variance 0.0048
pooled standard deviation 0.0696

a 27.65
b 8.52

Table C.3

Calculation of Beta Parameters *a* and *b* for *C. parvum* Recovery from Ottawa Water (Influent and Effluent)

Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(p)	Var(p)	E(p)/Var(p)	E(p)2/Var(p)	1/Var(p)	Number of trials
1.0E+06	0.1	0.5	500	351	351	149	0.70	4.176 E-4	1.681 E+3	1.180 E+3	2.395 E+3	
1.0E+06	0.1	0.5	500	360	360	140	0.72	4.024 E-4	1.789 E+3	1.288 E+3	2.485 E+3	
1.0E+06	0.1	0.5	500	427	427	73	0.85	2.489 E-4	3.432 E+3	2.931 E+3	4.018 E+3	
1.0E+06	0.1	0.5	500	409	409	91	0.82	2.972 E-4	2.753 E+3	2.252 E+3	3.365 E+3	
1.0E+06	0.1	0.5	500	355	355	145	0.71	4.110 E-4	1.728 E+3	1.227 E+3	2.433 E+3	
1000	0.5	100	500	357	357	143	0.71	4.076 E-4	1.752 E+3	1.251 E+3	2.453 E+3	
1000	0.5	100	500	366	366	134	0.73	3.916 E-4	1.869 E+3	1.368 E+3	2.554 E+3	
1000	0.5	100	500	431	431	69	0.86	2.374 E-4	3.630 E+3	3.129 E+3	4.212 E+3	
1000	0.5	100	500	378	378	122	0.76	3.682 E-4	2.053 E+3	1.552 E+3	2.716 E+3	
1000	0.5	100	500	345	345	155	0.69	4.269 E-4	1.616 E+3	1.115 E+3	2.342 E+3	
100	0.5	100	50	36	36	14	0.72	3.953 E-3	1.821 E+2	1.311 E+2	2.530 E+2	
100	0.5	100	50	42	42	8	0.84	2.635 E-3	3.188 E+2	2.678 E+2	3.795 E+2	
100	0.5	100	50	35	35	15	0.70	4.118 E-3	1.700 E+2	1.190 E+2	2.429 E+2	
100	0.5	100	50	38	38	12	0.76	3.576 E-3	2.125 E+2	1.615 E+2	2.796 E+2	
100	0.5	100	50	37	37	13	0.74	3.773 E-3	1.962 E+2	1.452 E+2	2.651 E+2	
						sum			2.338 E+4	1.812 E+4	3.039 E+4	15

pooled mean recovery 0.7693

pooled variance 0.0047

pooled standard deviation 0.0687

a 28.12

b 8.43

Table C.4

Calculation of Beta Parameters *a* and *b* for *C. parvum* Recovery from Ottawa Filter Influent with 40 mg/L Alum

Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(*p*)	Var(*p*)	E(*p*)/Var(p)	E(*p*)2/Var(p)	1/Var(*p*)	Number of trials
1.0E+06	0.01	100	1.0E+04	6992	6992	3008	0.70	2.103 E-5	3.325 E+4	2.325 E+4	4.755 E+4	
1.0E+06	0.01	100	1.0E+04	8019	8019	1981	0.80	1.588 E-5	5.048 E+4	4.048 E+4	6.296 E+4	
1.0E+06	0.01	100	1.0E+04	5888	5888	4112	0.59	2.421 E-5	2.432 E+4	1.432 E+4	4.131 E+4	
1.0E+06	0.01	100	1.0E+04	7583	7583	2417	0.76	1.833 E-5	4.138 E+4	3.138 E+4	5.457 E+4	
1.0E+06	0.01	100	1.0E+04	7961	7961	2039	0.80	1.623 E-5	4.905 E+4	3.905 E+4	6.161 E+4	
						sum			1.985 E+5	1.485 E+5	2.680 E+5	5

pooled mean recovery 0.7406
pooled variance 0.0055
pooled standard deviation 0.0744

a 24.99
b 8.75

Table C.5

Calculation of Beta Parameters *a* and *b* for *C. parvum* Recovery from Ottawa Filter Effluent with 40 mg/L Alum

Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(*p*)	Var(*p*)	E(*p*)/Var(p)	E(*p*)2/Var(p)	1/Var(*p*)	Number of trials
1000	0.5	100	500	419	419	81	0.84	2.710 E-4	3.093 E+3	2.592 E+3	3.690 E+3	
1000	0.5	100	500	302	302	198	0.60	4.774 E-4	1.265 E+3	7.642 E+2	2.095 E+3	
1000	0.5	100	500	295	295	205	0.59	4.828 E-4	1.222 E+3	7.210 E+2	2.071 E+3	
1000	0.5	100	500	375	375	125	0.75	3.743 E-4	2.004 E+3	1.503 E+3	2.672 E+3	
1000	0.5	100	500	401	401	99	0.80	3.170 E-4	2.530 E+3	2.029 E+3	3.155 E+3	
100	0.5	100	50	32	32	18	0.64	4.518 E-3	1.417 E+2	9.067 E+1	2.214 E+2	
100	0.5	100	50	39	39	11	0.78	3.365 E-3	2.318 E+2	1.808 E+2	2.972 E+2	
100	0.5	100	50	38	38	12	0.76	3.576 E-3	2.125 E+2	1.615 E+2	2.796 E+2	
100	0.5	100	50	41	41	9	0.82	2.894 E-3	2.833 E+2	2.323 E+2	3.455 E+2	
100	0.5	100	50	28	28	22	0.56	4.831 E-3	1.159 E+2	6.491 E+1	2.070 E+2	
						sum			1.110 E+4	8.339 E+3	1.503 E+4	10

pooled mean recovery	0.7383
pooled variance	0.0103
pooled standard deviation	0.1015
a	**13.11**
b	**4.65**

Table C.6

Calculation of Beta Parameters *a* and *b* for *C. parvum* Recovery from Ottawa Water (Influent and Effluent) with 40 mg/L Alum

Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(*p*)	Var(*p*)	E(*p*)/Var(p)	E(*p*)2/Var(p)	1/Var(*p*)	Number of trials
1.0E+06	0.1	0.5	500	351	351	149	0.70	4.176 E-4	1.681 E+3	1.180 E+3	2.395 E+3	
1.0E+06	0.1	0.5	500	360	360	140	0.72	4.024 E-4	1.789 E+3	1.288 E+3	2.485 E+3	
1.0E+06	0.1	0.5	500	427	427	73	0.85	2.489 E-4	3.432 E+3	2.931 E+3	4.018 E+3	
1.0E+06	0.1	0.5	500	409	409	91	0.82	2.972 E-4	2.753 E+3	2.252 E+3	3.365 E+3	
1.0E+06	0.1	0.5	500	355	355	145	0.71	4.110 E-4	1.728 E+3	1.227 E+3	2.433 E+3	
1000	0.5	100	500	357	357	143	0.71	4.076 E-4	1.752 E+3	1.251 E+3	2.453 E+3	
1000	0.5	100	500	366	366	134	0.73	3.916 E-4	1.869 E+3	1.368 E+3	2.554 E+3	
1000	0.5	100	500	431	431	69	0.86	2.374 E-4	3.630 E+3	3.129 E+3	4.212 E+3	
1000	0.5	100	500	378	378	122	0.76	3.682 E-4	2.053 E+3	1.552 E+3	2.716 E+3	
1000	0.5	100	500	345	345	155	0.69	4.269 E-4	1.616 E+3	1.115 E+3	2.342 E+3	
100	0.5	100	50	36	36	14	0.72	3.953 E-3	1.821 E+2	1.311 E+2	2.530 E+2	
100	0.5	100	50	42	42	8	0.84	2.635 E-3	3.188 E+2	2.678 E+2	3.795 E+2	
100	0.5	100	50	35	35	15	0.70	4.118 E-3	1.700 E+2	1.190 E+2	2.429 E+2	
100	0.5	100	50	38	38	12	0.76	3.576 E-3	2.125 E+2	1.615 E+2	2.796 E+2	
100	0.5	100	50	37	37	13	0.74	3.773 E-3	1.962 E+2	1.452 E+2	2.651 E+2	
						sum			2.338 E+4	1.812 E+4	3.039 E+4	15

pooled mean recovery 0.7693

pooled variance 0.0047

pooled standard deviation 0.0687

a 28.12

b 8.43

Table C.7

Calculation of Overall Beta Parameters *a* and *b* for *C. parvum* Recovery from Ottawa Water (All Influent and Effluents)

Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(*p*)	Var(*p*)	E(*p*)/Var(p)	E(*p*)2/Var(p)	1/Var(*p*)	Number of trials
1.0E+06	0.1	0.5	500	351	351	149	0.70	4.176 E-4	1.681 E+3	1.180 E+3	2.395 E+3	
1.0E+06	0.1	0.5	500	360	360	140	0.72	4.024 E-4	1.789 E+3	1.288 E+3	2.485 E+3	
1.0E+06	0.1	0.5	500	427	427	73	0.85	2.489 E-4	3.432 E+3	2.931 E+3	4.018 E+3	
1.0E+06	0.1	0.5	500	409	409	91	0.82	2.972 E-4	2.753 E+3	2.252 E+3	3.365 E+3	
1.0E+06	0.1	0.5	500	355	355	145	0.71	4.110 E-4	1.728 E+3	1.227 E+3	2.433 E+3	
1.0E+06	0.01	100	10000	6992	6992	3008	0.70	2.103 E-5	3.325 E+4	2.325 E+4	4.755 E+4	
1.0E+06	0.01	100	10000	8019	8019	1981	0.80	1.588 E-5	5.048 E+4	4.048 E+4	6.296 E+4	
1.0E+06	0.01	100	10000	5888	5888	4112	0.59	2.421 E-5	2.432 E+4	1.432 E+4	4.131 E+4	
1.0E+06	0.01	100	10000	7583	7583	2417	0.76	1.833 E-5	4.138 E+4	3.138 E+4	5.457 E+4	
1.0E+06	0.01	100	10000	7961	7961	2039	0.80	1.623 E-5	4.905 E+4	3.905 E+4	6.161 E+4	
1000	0.5	100	500	357	357	143	0.71	4.076 E-4	1.752 E+3	1.251 E+3	2.453 E+3	
1000	0.5	100	500	366	366	134	0.73	3.916 E-4	1.869 E+3	1.368 E+3	2.554 E+3	
1000	0.5	100	500	431	431	69	0.86	2.374 E-4	3.630 E+3	3.129 E+3	4.212 E+3	
1000	0.5	100	500	378	378	122	0.76	3.682 E-4	2.053 E+3	1.552 E+3	2.716 E+3	
1000	0.5	100	500	345	345	155	0.69	4.269 E-4	1.616 E+3	1.115 E+3	2.342 E+3	
1000	0.5	100	500	419	419	81	0.84	2.710 E-4	3.093 E+3	2.592 E+3	3.690 E+3	
1000	0.5	100	500	302	302	198	0.60	4.774 E-4	1.265 E+3	7.642 E+2	2.095 E+3	
1000	0.5	100	500	295	295	205	0.59	4.828 E-4	1.222 E+3	7.210 E+2	2.071 E+3	
1000	0.5	100	500	375	375	125	0.75	3.743 E-4	2.004 E+3	1.503 E+3	2.672 E+3	
1000	0.5	100	500	401	401	99	0.80	3.170 E-4	2.530 E+3	2.029 E+3	3.155 E+3	
100	0.5	100	50	36	36	14	0.72	3.953 E-3	1.821 E+2	1.311 E+2	2.530 E+2	
100	0.5	100	50	42	42	8	0.84	2.635 E-3	3.188 E+2	2.678 E+2	3.795 E+2	
100	0.5	100	50	35	35	15	0.70	4.118 E-3	1.700 E+2	1.190 E+2	2.429 E+2	
100	0.5	100	50	38	38	12	0.76	3.576 E-3	2.125 E+2	1.615 E+2	2.796 E+2	
100	0.5	100	50	37	37	13	0.74	3.773 E-3	1.962 E+2	1.452 E+2	2.651 E+2	
100	0.5	100	50	32	32	18	0.64	4.518 E-3	1.417 E+2	9.067 E+1	2.214 E+2	
100	0.5	100	50	39	39	11	0.78	3.365 E-3	2.318 E+2	1.808 E+2	2.972 E+2	
100	0.5	100	50	38	38	12	0.76	3.576 E-3	2.125 E+2	1.615 E+2	2.796 E+2	
100	0.5	100	50	41	41	9	0.82	2.894 E-3	2.833 E+2	2.323 E+2	3.455 E+2	
100	0.5	100	50	28	28	22	0.56	4.831 E-3	1.159 E+2	6.491 E+1	2.070 E+2	
						sum			2.330 E+5	1.749 E+5	3.134 E+5	30

pooled mean recovery	0.7433
pooled variance	0.0058
pooled standard deviation	0.0759
a	**23.91**
b	**8.26**

Table C.8

Calculation of Beta Parameters *a* and *b* for *C. parvum* Recovery from UW (1.5 NTU) Filter Influent

Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(p)	Var(p)	E(p)/Var(p)	E(p)2/Var(p)	1/Var(p)
1.0E+06	0.0025	100	2500	1280	1.3E+03	1.2E+03	0.51	9.990 E-5	5.125 E+3	2.624 E+3	1.001 E+4
1.0E+06	0.0025	100	2500	1340	1.3E+03	1.2E+03	0.54	9.944 E-5	5.390 E+3	2.889 E+3	1.006 E+4
1.0E+06	0.0025	100	2500	1770	1.8E+03	7.3E+02	0.71	8.266 E-5	8.565 E+3	6.064 E+3	1.210 E+4
1.0E+06	0.0025	100	2500	1300	1.3E+03	1.2E+03	0.52	9.980 E-5	5.210 E+3	2.709 E+3	1.002 E+4
1.0E+06	0.0025	100	2500	1640	1.6E+03	8.6E+02	0.66	9.023 E-5	7.270 E+3	4.769 E+3	1.108 E+4
1.0E+06	0.0025	100	2500	1580	1.6E+03	9.2E+02	0.63	9.299 E-5	6.796 E+3	4.295 E+3	1.075 E+4
1.0E+06	0.0025	100	2500	1950	2.0E+03	5.5E+02	0.78	6.861 E-5	1.137 E+4	8.867 E+3	1.457 E+4
1.0E+06	0.0025	100	2500	1410	1.4E+03	1.1E+03	0.56	9.832 E-5	5.736 E+3	3.235 E+3	1.017 E+4
1.0E+06	0.0025	100	2500	1830	1.8E+03	6.7E+02	0.73	7.844 E-5	9.332 E+3	6.831 E+3	1.275 E+4
1.0E+06	0.0025	100	2500	1750	1.8E+03	7.5E+02	0.70	8.397 E-5	8.337 E+3	5.836 E+3	1.191 E+4
1.0E+06	0.0025	100	2500	2050	2.1E+03	4.5E+02	0.82	5.902 E-5	1.389 E+4	1.139 E+4	1.694 E+4
1.0E+06	0.0025	100	2500	1670	1.7E+03	8.3E+02	0.67	8.867 E-5	7.533 E+3	5.032 E+3	1.128 E+4
1.0E+06	0.0025	100	2500	2170	2.2E+03	3.3E+02	0.87	4.581 E-5	1.895 E+4	1.645 E+4	2.183 E+4
1.0E+06	0.0025	100	2500	2020	2.0E+03	4.8E+02	0.81	6.203 E-5	1.303 E+4	1.053 E+4	1.612 E+4
						sum			1.265 E+5	9.152 E+4	1.796 E+5
						pooled mean recovery	0.7045				
						pooled variance	0.0133				
						pooled standard deviation	0.1152				
						a	**10.34**				
						b	**4.34**				

Table C.9

Calculation of Beta Parameters *a* and *b* for *C. parvum* Recovery from UW (1.5 NTU) Filter Effluent

Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(*p*)	Var(*p*)	E(*p*)/Var(p)	E(*p*)2/Var(p)	1/Var(*p*)
1000	0.5	100	500	358	358	142	0.72	4.059 E-4	1.764 E+3	1.263 E+3	2.464 E+3
1000	0.5	100	500	394	394	106	0.79	3.334 E-4	2.363 E+3	1.862 E+3	2.999 E+3
1000	0.5	100	500	420	420	80	0.84	2.683 E-4	3.131 E+3	2.630 E+3	3.728 E+3
1000	0.5	100	500	396	396	104	0.79	3.288 E-4	2.409 E+3	1.908 E+3	3.041 E+3
1000	0.5	100	500	405	405	95	0.81	3.072 E-4	2.637 E+3	2.136 E+3	3.255 E+3
1000	0.5	100	500	377	377	123	0.75	3.702 E-4	2.037 E+3	1.536 E+3	2.701 E+3
1000	0.5	100	500	362	362	138	0.72	3.989 E-4	1.815 E+3	1.314 E+3	2.507 E+3
1000	0.5	100	500	341	341	159	0.68	4.329 E-4	1.575 E+3	1.074 E+3	2.310 E+3
1000	0.5	100	500	408	408	92	0.82	2.997 E-4	2.723 E+3	2.222 E+3	3.337 E+3
1000	0.5	100	500	362	362	138	0.72	3.989 E-4	1.815 E+3	1.314 E+3	2.507 E+3
100	0.5	100	50	32	32	18	0.64	4.518 E-3	1.417 E+2	9.067 E+1	2.214 E+2
100	0.5	100	50	27	27	23	0.54	4.871 E-3	1.109 E+2	5.987 E+1	2.053 E+2
100	0.5	100	50	29	29	21	0.58	4.776 E-3	1.214 E+2	7.043 E+1	2.094 E+2
100	0.5	100	50	35	35	15	0.70	4.118 E-3	1.700 E+2	1.190 E+2	2.429 E+2
100	0.5	100	50	40	40	10	0.80	3.137 E-3	2.550 E+2	2.040 E+2	3.188 E+2
100	0.5	100	50	39	39	11	0.78	3.365 E-3	2.318 E+2	1.808 E+2	2.972 E+2
100	0.5	100	50	44	44	6	0.88	2.071 E-3	4.250 E+2	3.740 E+2	4.830 E+2
100	0.5	100	50	37	37	13	0.74	3.773 E-3	1.962 E+2	1.452 E+2	2.651 E+2
100	0.5	100	50	39	39	11	0.78	3.365 E-3	2.318 E+2	1.808 E+2	2.972 E+2
100	0.5	100	50	39	39	11	0.78	3.365 E-3	2.318 E+2	1.808 E+2	2.972 E+2
						sum			2.438 E+4	1.886 E+4	3.169 E+4

pooled mean recovery	0.7696
pooled variance	0.0038
pooled standard deviation	0.0613
a	**35.52**
b	**10.64**

Table C.10

Calculation of Beta Parameters *a* and *b* for *C. parvum* Recovery from UW (1.5 NTU) Water (Influent and Effluent)

Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(*p*)	Var(*p*)	E(*p*)/Var(p)	E(*p*)2/Var(p)	1/Var(*p*)
1.0E+06	0.0025	100	2500	1280	1280	1220	0.51	9.990 E-5	5.125 E+3	2.624 E+3	1.001 E+4
1.0E+06	0.0025	100	2500	1340	1340	1160	0.54	9.944 E-5	5.390 E+3	2.889 E+3	1.006 E+4
1.0E+06	0.0025	100	2500	1770	1770	730	0.71	8.266 E-5	8.565 E+3	6.064 E+3	1.210 E+4
1.0E+06	0.0025	100	2500	1300	1300	1200	0.52	9.980 E-5	5.210 E+3	2.709 E+3	1.002 E+4
1.0E+06	0.0025	100	2500	1640	1640	860	0.66	9.023 E-5	7.270 E+3	4.769 E+3	1.108 E+4
1.0E+06	0.0025	100	2500	1580	1580	920	0.63	9.299 E-5	6.796 E+3	4.295 E+3	1.075 E+4
1.0E+06	0.0025	100	2500	1950	1950	550	0.78	6.861 E-5	1.137 E+4	8.867 E+3	1.457 E+4
1.0E+06	0.0025	100	2500	1410	1410	1090	0.56	9.832 E-5	5.736 E+3	3.235 E+3	1.017 E+4
1.0E+06	0.0025	100	2500	1830	1830	670	0.73	7.844 E-5	9.332 E+3	6.831 E+3	1.275 E+4
1.0E+06	0.0025	100	2500	1750	1750	750	0.70	8.397 E-5	8.337 E+3	5.836 E+3	1.191 E+4
1.0E+06	0.0025	100	2500	2050	2050	450	0.82	5.902 E-5	1.389 E+4	1.139 E+4	1.694 E+4
1.0E+06	0.0025	100	2500	1670	1670	830	0.67	8.867 E-5	7.533 E+3	5.032 E+3	1.128 E+4
1.0E+06	0.0025	100	2500	2170	2170	330	0.87	4.581 E-5	1.895 E+4	1.645 E+4	2.183 E+4
1.0E+06	0.0025	100	2500	2020	2020	480	0.81	6.203 E-5	1.303 E+4	1.053 E+4	1.612 E+4
1000	0.5	100	500	358	358	142	0.72	4.059 E-4	1.764 E+3	1.263 E+3	2.464 E+3
1000	0.5	100	500	394	394	106	0.79	3.334 E-4	2.363 E+3	1.862 E+3	2.999 E+3
1000	0.5	100	500	420	420	80	0.84	2.683 E-4	3.131 E+3	2.630 E+3	3.728 E+3
1000	0.5	100	500	396	396	104	0.79	3.288 E-4	2.409 E+3	1.908 E+3	3.041 E+3
1000	0.5	100	500	405	405	95	0.81	3.072 E-4	2.637 E+3	2.136 E+3	3.255 E+3
1000	0.5	100	500	377	377	123	0.75	3.702 E-4	2.037 E+3	1.536 E+3	2.701 E+3
1000	0.5	100	500	362	362	138	0.72	3.989 E-4	1.815 E+3	1.314 E+3	2.507 E+3
1000	0.5	100	500	341	341	159	0.68	4.329 E-4	1.575 E+3	1.074 E+3	2.310 E+3
1000	0.5	100	500	408	408	92	0.82	2.997 E-4	2.723 E+3	2.222 E+3	3.337 E+3
1000	0.5	100	500	362	362	138	0.72	3.989 E-4	1.815 E+3	1.314 E+3	2.507 E+3
100	0.5	100	50	32	32	18	0.64	4.518 E-3	1.417 E+2	9.067 E+1	2.214 E+2
100	0.5	100	50	27	27	23	0.54	4.871 E-3	1.109 E+2	5.987 E+1	2.053 E+2
100	0.5	100	50	29	29	21	0.58	4.776 E-3	1.214 E+2	7.043 E+1	2.094 E+2
100	0.5	100	50	35	35	15	0.70	4.118 E-3	1.700 E+2	1.190 E+2	2.429 E+2
100	0.5	100	50	40	40	10	0.80	3.137 E-3	2.550 E+2	2.040 E+2	3.188 E+2
100	0.5	100	50	39	39	11	0.78	3.365 E-3	2.318 E+2	1.808 E+2	2.972 E+2
100	0.5	100	50	44	44	6	0.88	2.071 E-3	4.250 E+2	3.740 E+2	4.830 E+2
100	0.5	100	50	37	37	13	0.74	3.773 E-3	1.962 E+2	1.452 E+2	2.651 E+2
100	0.5	100	50	39	39	11	0.78	3.365 E-3	2.318 E+2	1.808 E+2	2.972 E+2
100	0.5	100	50	39	39	11	0.78	3.365 E-3	2.318 E+2	1.808 E+2	2.972 E+2
						sum			1.509 E+5	1.104 E+5	2.113 E+5

pooled mean recovery	0.7143
pooled variance	0.0124
pooled standard deviation	0.1113
a	**11.05**
b	**4.42**

Table C.11

Calculation of Beta Parameters *a* and *b* for *C. parvum* Recovery from UW (3.5 NTU) Filter Influent

Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(*p*)	Var(*p*)	E(*p*)/Var(p)	E(*p*)2/Var(p)	1/Var(*p*)
1.0E+06	0.1	0.5	500	415	4.2E+02	8.5E+01	0.83	2.816 E-4	2,947 E+3	2,446 E+3	3.551 E+3
1.0E+06	0.1	0.5	500	347	3.5E+02	1.5E+02	0.69	4.239 E-4	1.637 E+3	1.136 E+3	2.359 E+3
1.0E+06	0.1	0.5	500	353	3.5E+02	1.5E+02	0.71	4.143 E-4	1.704 E+3	1.203 E+3	2.414 E+3
1.0E+06	0.1	0.5	500	326	3.3E+02	1.7E+02	0.65	4.529 E-4	1.440 E+3	9.387 E+2	2.208 E+3
1.0E+06	0.1	0.5	500	452	4.5E+02	4.8E+01	0.90	1.732 E-4	5.219 E+3	4.718 E+3	5.773 E+3
1.0E+06	0.005	100	5000	4330	4.3E+03	6.7E+02	0.87	2.320 E-5	3.732 E+4	3.232 E+4	4.310 E+4
1.0E+06	0.005	100	5000	3950	4.0E+03	1.1E+03	0.79	3.317 E-5	2.381 E+4	1.881 E+4	3.014 E+4
1.0E+06	0.005	100	5000	3170	3.2E+03	1.8E+03	0.63	4.640 E-5	1.366 E+4	8.663 E+3	2.155 E+4
1.0E+06	0.005	100	5000	2990	3.0E+03	2.0E+03	0.60	4.807 E-5	1.244 E+4	7.439 E+3	2.080 E+4
1.0E+06	0.005	100	5000	3860	3.9E+03	1.1E+03	0.77	3.520 E-5	2.193 E+4	1.693 E+4	2.841 E+4
						sum			1.221 E+5	9.461 E+4	1.603 E+5

pooled mean recovery	0.7618
pooled variance	0.0099
pooled standard deviation	0.0997
a	**13.15**
b	**4.11**

Table C.12

Calculation of Beta Parameters *a* and *b* for *C. parvum* Recovery from UW (3.5 NTU) Filter Effluent

Seeded Concentration (oocysts/L.)	Sample Volume (L.)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(*p*)	Var(*p*)	E(*p*)/Var(p)	E(*p*)2/Var(p)	1/Var(*p*)
1000	0.5	100	500	366	366	134	0.73	3.916 E-4	1.869 E+3	1.368 E+3	2.554 E+3
1000	0.5	100	500	411	411	89	0.82	2.920 E-4	2.815 E+3	2.314 E+3	3.424 E+3
1000	0.5	100	500	276	276	224	0.55	4.936 E-4	1.118 E+3	6.173 E+2	2.026 E+3
1000	0.5	100	500	417	417	83	0.83	2.763 E-4	3.018 E+3	2.517 E+3	3.619 E+3
1000	0.5	100	500	354	354	146	0.71	4.126 E-4	1.716 E+3	1.215 E+3	2.423 E+3
100	0.5	100	50	30	30	20	0.60	4.706 E-3	1.275 E+2	7.650 E+1	2.125 E+2
100	0.5	100	50	42	42	8	0.84	2.635 E-3	3.188 E+2	2.678 E+2	3.795 E+2
100	0.5	100	50	35	35	15	0.70	4.118 E-3	1.700 E+2	1.190 E+2	2.429 E+2
100	0.5	100	50	31	31	19	0.62	4.620 E-3	1.342 E+2	8.321 E+1	2.165 E+2
100	0.5	100	50	44	44	6	0.88	2.071 E-3	4.250 E+2	3.740 E+2	4.830 E+2
						sum			1.171 E+4	8.952 E+3	1.558 E+4

pooled mean recovery	0.7517
pooled variance	0.0101
pooled standard deviation	0.1007
a	**13.08**
b	**4.32**

Table C.13

Calculation of Beta Parameters *a* and *b* for *C. parvum* Recovery from UW (3.5 NTU) Water (Influent and Effluent)

Seeded Concentration (oocysts/L)	Sample Volume (L)	Volume Processed (%)	Seeded Number of Oocysts	Observed Number of Oocysts	x	y	E(*p*)	Var(*p*)	E(*p*)/Var(p)	E(*p*)2/Var(p)	1/Var(*p*)
1.0E+06	0.1	0.5	500	415	415	85	0.83	2.816 E-4	2.947 E+3	2.446 E+3	3.551 E+3
1.0E+06	0.1	0.5	500	347	347	153	0.69	4.239 E-4	1.637 E+3	1.136 E+3	2.359 E+3
1.0E+06	0.1	0.5	500	353	353	147	0.71	4.143 E-4	1.704 E+3	1.203 E+3	2.414 E+3
1.0E+06	0.1	0.5	500	326	326	174	0.65	4.529 E-4	1.440 E+3	9.387 E+2	2.208 E+3
1.0E+06	0.1	0.5	500	452	452	48	0.90	1.732 E-4	5.219 E+3	4.718 E+3	5.773 E+3
1.0E+06	0.005	100	5000	4330	4330	670	0.87	2.320 E-5	3.732 E+4	3.232 E+4	4.310 E+4
1.0E+06	0.005	100	5000	3950	3950	1050	0.79	3.317 E-5	2.381 E+4	1.881 E+4	3.014 E+4
1.0E+06	0.005	100	5000	3170	3170	1830	0.63	4.640 E-5	1.366 E+4	8.663 E+3	2.155 E+4
1.0E+06	0.005	100	5000	2990	2990	2010	0.60	4.807 E-5	1.244 E+4	7.439 E+3	2.080 E+4
1.0E+06	0.005	100	5000	3860	3860	1140	0.77	3.520 E-5	2.193 E+4	1.693 E+4	2.841 E+4
1000	0.5	100	500	366	366	134	0.73	3.916 E-4	1.869 E+3	1.368 E+3	2.554 E+3
1000	0.5	100	500	411	411	89	0.82	2.920 E-4	2.815 E+3	2.314 E+3	3.424 E+3
1000	0.5	100	500	276	276	224	0.55	4.936 E-4	1.118 E+3	6.173 E+2	2.026 E+3
1000	0.5	100	500	417	417	83	0.83	2.763 E-4	3.018 E+3	2.517 E+3	3.619 E+3
1000	0.5	100	500	354	354	146	0.71	4.126 E-4	1.716 E+3	1.215 E+3	2.423 E+3
100	0.5	100	50	30	30	20	0.60	4.706 E-3	1.275 E+2	7.650 E+1	2.125 E+2
100	0.5	100	50	42	42	8	0.84	2.635 E-3	3.188 E+2	2.678 E+2	3.795 E+2
100	0.5	100	50	35	35	15	0.70	4.118 E-3	1.700 E+2	1.190 E+2	2.429 E+2
100	0.5	100	50	31	31	19	0.62	4.620 E-3	1.342 E+2	8.321 E+1	2.165 E+2
100	0.5	100	50	44	44	6	0.88	2.071 E-3	4.250 E+2	3.740 E+2	4.830 E+2
						sum			1.338 E+5	1.036 E+5	1.759 E+5

pooled mean recovery	0.7609
pooled variance	0.0100
pooled standard deviation	0.0998
a	**13.13**
b	**4.13**

Appendix D
Detailed *C. parvum*, *B. subtilis*, and Microsphere Data

Table D.1

C. parvum Removal Data from Bench-Scale Experiments

Date	Type of Experiment	Seed Duration at Sampling (min)	Dual-Media *C. parvum* FI (oocysts/L)	Dual-Media *C. parvum* FE (oocysts/L)	Dual-Media Log Removal (-)	Tri-Media *C. parvum* FI (oocysts/L)	Tri-Media *C. parvum* FE (oocysts/L)	Tri-Media Log Removal (-)
	Stable Operation	10	5.52 E+5	6	5.0	5.86 E+5	5	5.1
	Inactivated *C. parvum*	25	5.66 E+5	3	5.3	6.22 E+5	1	5.8
		40	4.84 E+5	5	5.0	5.98 E+5	2	5.5
		10	4.79 E+5	1	5.7	4.49 E+5	2	5.4
		25	5.52 E+5	10	4.7	5.34 E+5	6	4.9
		40	5.58 E+5	2	5.4	5.20 E+5	5	5.0
		10	5.03 E+5	7	4.9	5.52 E+5	2	5.4
		25	4.66 E+5	2	5.4	5.08 E+5	2	5.4
		40	4.87 E+5	2	5.4	5.85 E+5	1	5.8
	Stable Operation	10	5.36 E+5	3	5.3	5.69 E+5	1	5.8
	Viable *C. parvum*	25	4.77 E+5	7	4.8	4.51 E+5	1	5.7
		40	5.78 E+5	5	5.1	4.80 E+5	1	5.7
		10	4.91 E+5	2	5.4	3.69 E+5	8	4.7
		25	4.64 E+5	1	5.7	3.56 E+5	2	5.3
		40	4.64 E+5	10	4.7	3.98 E+5	9	4.6
		10	3.82 E+5	7	4.7	3.27 E+5	2	5.2
		25	3.97 E+5	1	5.6	3.68 E+5	2	5.3
		40	4.10 E+5	3	5.1	3.28 E+5	4	4.9
	Ripening	5	3.68 E+5	5	4.9	4.26 E+5	3	5.2
	Inactivated *C. parvum*	10	3.94 E+5	11	4.6	4.47 E+5	5	5.0
		15	3.51 E+5	32	4.0	4.61 E+5	14	4.5
		20	3.77 E+5	10	4.6	4.57 E+5	8	4.8
		5	4.11 E+5	3	5.1	3.79 E+5	2	5.3
		10	3.88 E+5	9	4.6	3.89 E+5	2	5.3
		15	4.04 E+5	43	4.0	3.60 E+5	22	4.2
		20	3.66 E+5	12	4.5	3.70 E+5	11	4.5
		5	4.24 E+5	4	5.0	5.53 E+5	3	5.3
		10	4.24 E+5	16	4.4	5.69 E+5	5	5.1
		15	4.53 E+5	37	4.1	5.26 E+5	30	4.2
		20	4.25 E+5	10	4.6	5.34 E+5	11	4.7
	Ripening	5	5.53 E+5	2	5.4	4.01 E+5	2	5.3
	Viable *C. parvum*	10	4.91 E+5	7	4.8	4.78 E+5	7	4.8
		15	5.32 E+5	56	4.0	4.25 E+5	16	4.4
		20	5.58 E+5	8	4.8	4.57 E+5	1	5.7
		5	4.26 E+5	4	5.0	—	—	—
		10	4.69 E+5	9	4.7	—	—	—
		15	4.64 E+5	25	4.3	—	—	—
		20	4.44 E+5	7	4.8	—	—	—
		5	5.98 E+5	5	5.1	—	—	—
		10	5.75 E+5	8	4.9	—	—	—
		15	6.00 E+5	44	4.1	—	—	—
		20	5.50 E+5	38	4.2	—	—	—

Table D.1

C. parvum Removal Data from Bench-Scale Experiments (Continued)

Date	Type of Experiment	Seed Duration at Sampling (min)	Dual-Media *C. parvum* FI (oocysts/L)	Dual-Media *C. parvum* FE (oocysts/L)	Dual-Media Log Removal (-)	Tri-Media *C. parvum* FI (oocysts/L)	Tri-Media *C. parvum* FE (oocysts/L)	Tri-Media Log Removal (-)
	Coagulation Failure	10	5.05 E+5	4.80 E+4	1.0	5.24 E+5	2.90 E+4	1.3
	Inactivated *C. parvum*	25	5.31 E+5	7.80 E+4	0.8	5.87 E+5	6.60 E+4	0.9
		40	5.85 E+5	1.10 E+5	0.7	5.80 E+5	1.00 E+5	0.8
		10	4.21 E+5	6.70 E+4	0.8	4.84 E+5	4.20 E+4	1.1
		25	4.64 E+5	1.00 E+5	0.7	5.04 E+5	5.60 E+4	1.0
		40	4.60 E+5	1.30 E+5	0.5	4.38 E+5	1.10 E+5	0.6
		10	5.96 E+5	3.00 E+4	1.3	5.44 E+5	4.20 E+4	1.1
		25	6.21 E+5	8.50 E+4	0.9	5.52 E+5	9.30 E+4	0.8
		40	5.62 E+5	1.20 E+5	0.7	6.21 E+5	1.30 E+5	0.7
	Coagulation Failure	10	5.38 E+5	4.30 E+4	1.1	3.75 E+5	5.80 E+4	0.8
	Viable *C. parvum*	25	5.50 E+5	9.20 E+4	0.8	3.12 E+5	9.80 E+4	0.5
		40	4.48 E+5	1.30 E+5	0.5	3.87 E+5	1.30 E+5	0.5
		10	5.78 E+5	6.20 E+4	1.0	3.71 E+5	1.20 E+3	2.5
		25	5.91 E+5	1.50 E+5	0.6	4.00 E+5	7.90 E+3	1.7
		40	5.09 E+5	2.10 E+5	0.4	4.20 E+5	6.30 E+3	1.8
		10	4.62 E+5	2.70 E+4	1.2	3.75 E+5	3.90 E+4	1.0
		25	4.55 E+5	1.50 E+5	0.5	4.00 E+5	6.50 E+4	0.8
		40	4.94 E+5	2.00 E+5	0.4	3.96 E+5	8.80 E+4	0.7

Table D.2

Calculation of $C_{theoretical}$ for Bench-Scale Experiments

Experiment	Dual-Media			Tri-media							
	$C_{theoretical}$ (oocysts/L)	C_{FI} (oocysts/L)	$C_{FI}/C_{theoretical}$	$C_{theoretical}$ (oocysts/L)	C_{FI} (oocysts/L)	$C_{FI}/C_{theoretical}$	$V_{spike,dual}$ (mL)	$C_{spike,dual}$ (oocysts/mL)	$V_{spike,tri}$ (mL)	$C_{spike,tri}$ (oocysts/mL)	V_{seed} (mL)
STABLE - Inactivated 1	7.14E+05	5.52E+05	0.77	7.14E+05	5.86E+05	0.82	0.1	1.07E+08	0.1	1.07E+08	1500
	7.14E+05	5.66E+05	0.79	7.14E+05	6.22E+05	0.87	0.1	1.07E+08	0.1	1.07E+08	1500
	7.14E+05	4.84E+05	0.68	7.14E+05	5.98E+05	0.84	0.1	1.07E+08	0.1	1.07E+08	1500
STABLE - Inactivated 2	7.14E+05	4.79E+05	0.67	7.14E+05	4.49E+05	0.63	0.1	1.07E+08	0.1	1.07E+08	1500
	7.14E+05	5.52E+05	0.77	7.14E+05	5.34E+05	0.75	0.1	1.07E+08	0.1	1.07E+08	1500
	7.14E+05	5.58E+05	0.78	7.14E+05	5.20E+05	0.73	0.1	1.07E+08	0.1	1.07E+08	1500
STABLE - Inactivated 3	7.14E+05	5.03E+05	0.70	7.14E+05	5.52E+05	0.77	0.1	1.07E+08	0.1	1.07E+08	1500
	7.14E+05	4.66E+05	0.65	7.14E+05	5.08E+05	0.71	0.1	1.07E+08	0.1	1.07E+08	1500
	7.14E+05	4.87E+05	0.68	7.14E+05	5.85E+05	0.82	0.1	1.07E+08	0.1	1.07E+08	1500
STABLE - Viable 1	6.90E+05	5.36E+05	0.78	6.27E+05	5.69E+05	0.91	0.1	1.04E+08	0.1	9.41E+07	1500
	6.90E+05	4.77E+05	0.69	6.27E+05	4.51E+05	0.72	0.1	1.04E+08	0.1	9.41E+07	1500
	6.90E+05	5.78E+05	0.84	6.27E+05	4.80E+05	0.76	0.1	1.04E+08	0.1	9.41E+07	1500
STABLE - Viable 2	6.90E+05	4.91E+05	0.71	6.27E+05	3.69E+05	0.59	0.1	1.04E+08	0.1	9.41E+07	1500
	6.90E+05	4.64E+05	0.67	6.27E+05	3.56E+05	0.57	0.1	1.04E+08	0.1	9.41E+07	1500
	6.90E+05	4.64E+05	0.67	6.27E+05	3.98E+05	0.64	0.1	1.04E+08	0.1	9.41E+07	1500
STABLE - Viable 3	6.90E+05	3.82E+05	0.55	6.27E+05	3.27E+05	0.52	0.1	1.04E+08	0.1	9.41E+07	1500
	6.90E+05	3.97E+05	0.57	6.27E+05	3.68E+05	0.59	0.1	1.04E+08	0.1	9.41E+07	1500
	6.90E+05	4.10E+05	0.59	6.27E+05	3.28E+05	0.52	0.1	1.04E+08	0.1	9.41E+07	1500
RIPENING - Inactivated 1	5.61E+05	3.68E+05	0.66	5.61E+05	4.26E+05	0.76	0.05	8.42E+07	0.05	8.42E+07	750
	5.61E+05	3.94E+05	0.70	5.61E+05	4.47E+05	0.80	0.05	8.42E+07	0.05	8.42E+07	750
	5.61E+05	3.51E+05	0.63	5.61E+05	4.61E+05	0.82	0.05	8.42E+07	0.05	8.42E+07	750
	5.61E+05	3.77E+05	0.67	5.61E+05	4.57E+05	0.81	0.05	8.42E+07	0.05	8.42E+07	750
RIPENING - Inactivated 2	5.61E+05	4.11E+05	0.73	5.61E+05	3.79E+05	0.68	0.05	8.42E+07	0.05	8.42E+07	750
	5.61E+05	3.88E+05	0.69	5.61E+05	3.89E+05	0.69	0.05	8.42E+07	0.05	8.42E+07	750
	5.61E+05	4.04E+05	0.72	5.61E+05	3.60E+05	0.64	0.05	8.42E+07	0.05	8.42E+07	750
	5.61E+05	3.66E+05	0.65	5.61E+05	3.70E+05	0.66	0.05	8.42E+07	0.05	8.42E+07	750

Table D.2

Calculation of Ctheoretical for Bench-Scale Experiments (Continued)

Experiment	Dual-Media			Tri-media								
	$C_{theoretical}$ (oocysts/L)	C_{FI} (oocysts/L)	$C_{FI}/C_{theoretical}$	$C_{theoretical}$ (oocysts/L)	C_{FI} (oocysts/L)	$C_{FI}/C_{theoretical}$	$V_{spike,dual}$ (mL)	$C_{spike,dual}$ (oocysts/mL)	$V_{spike,tri}$ (mL)	$C_{spike,tri}$ (oocysts/mL)	V_{seed} (mL)
RIPENING - Inactivated 3	5.61E+05	4.24E+05	0.76	6.98E+05	5.53E+05	0.79	0.05	8.42E+07	0.05	1.05E+08	750
	5.61E+05	4.24E+05	0.76	6.98E+05	5.69E+05	0.82	0.05	8.42E+07	0.05	1.05E+08	750
	5.61E+05	4.53E+05	0.81	6.98E+05	5.26E+05	0.75	0.05	8.42E+07	0.05	1.05E+08	750
	5.61E+05	4.25E+05	0.76	6.98E+05	5.34E+05	0.77	0.05	8.42E+07	0.05	1.05E+08	750
RIPENING - Viable 1	6.90E+05	5.53E+05	0.80	6.27E+05	4.01E+05	0.64	0.05	1.04E+08	0.05	9.41E+07	750
	6.90E+05	4.91E+05	0.71	6.27E+05	4.78E+05	0.76	0.05	1.04E+08	0.05	9.41E+07	750
	6.90E+05	5.32E+05	0.77	6.27E+05	4.25E+05	0.68	0.05	1.04E+08	0.05	9.41E+07	750
	6.90E+05	5.58E+05	0.81	6.27E+05	4.57E+05	0.73	0.05	1.04E+08	0.05	9.41E+07	750
RIPENING - Viable 2	6.90E+05	4.26E+05	0.62	---	---	---	0.05	1.04E+08	---	---	750
	6.90E+05	4.69E+05	0.68	---	---	---	0.05	1.04E+08	---	---	750
	6.90E+05	4.64E+05	0.67	---	---	---	0.05	1.04E+08	---	---	750
	6.90E+05	4.44E+05	0.64	---	---	---	0.05	1.04E+08	---	---	750
RIPENING - Viable 3	6.90E+05	5.98E+05	0.87	---	---	---	0.05	1.04E+08	---	---	750
	6.90E+05	5.75E+05	0.83	---	---	---	0.05	1.04E+08	---	---	750
	6.90E+05	6.00E+05	0.87	---	---	---	0.05	1.04E+08	---	---	750
	6.90E+05	5.50E+05	0.80	---	---	---	0.05	1.04E+08	---	---	750
CF - Inactivated 1	7.14E+05	5.05E+05	0.71	6.98E+05	5.24E+05	0.75	0.1	1.07E+08	0.1	1.05E+08	1500
	7.14E+05	5.31E+05	0.74	6.98E+05	5.87E+05	0.84	0.1	1.07E+08	0.1	1.05E+08	1500
	7.14E+05	5.85E+05	0.82	6.98E+05	5.80E+05	0.83	0.1	1.07E+08	0.1	1.05E+08	1500
CF - Inactivated 2	7.14E+05	4.21E+05	0.59	6.98E+05	4.84E+05	0.69	0.1	1.07E+08	0.1	1.05E+08	1500
	7.14E+05	4.64E+05	0.65	6.98E+05	5.04E+05	0.72	0.1	1.07E+08	0.1	1.05E+08	1500
	7.14E+05	4.60E+05	0.64	6.98E+05	4.38E+05	0.63	0.1	1.07E+08	0.1	1.05E+08	1500
CF - Inactivated 3	7.14E+05	5.96E+05	0.83	6.98E+05	5.44E+05	0.78	0.1	1.07E+08	0.1	1.05E+08	1500
	7.14E+05	6.21E+05	0.87	6.98E+05	5.52E+05	0.79	0.1	1.07E+08	0.1	1.05E+08	1500
	7.14E+05	5.62E+05	0.79	6.98E+05	6.21E+05	0.89	0.1	1.07E+08	0.1	1.05E+08	1500

Table D.2

Calculation of Ctheoretical for Bench-Scale Experiments (Continued)

Experiment	Dual-Media			Tri-media							
	$C_{theroretical}$ (oocysts/L)	C_{FI} (oocysts/L)	$C_{FI}/C_{theroretical}$	$C_{theroretical}$ (oocysts/L)	C_{FI} (oocysts/L)	$C_{FI}/C_{theroretical}$	$V_{spike,dual}$ (mL)	$C_{spike,dual}$ (oocysts/mL)	$V_{spike,tri}$ (mL)	$C_{spike,tri}$ (oocysts/mL)	V_{seed} (mL)
CF - Viable 1	6.90E+05	5.38E+05	0.78	6.27E+05	3.75E+05	0.60	0.1	1.04E+08	0.1	9.41E+07	1500
	6.90E+05	5.50E+05	0.80	6.27E+05	3.12E+05	0.50	0.1	1.04E+08	0.1	9.41E+07	1500
	6.90E+05	4.48E+05	0.65	6.27E+05	3.87E+05	0.62	0.1	1.04E+08	0.1	9.41E+07	1500
CF - Viable 2	6.90E+05	5.78E+05	0.84	6.27E+05	3.71E+05	0.59	0.1	1.04E+08	0.1	9.41E+07	1500
	6.90E+05	5.91E+05	0.86	6.27E+05	4.00E+05	0.64	0.1	1.04E+08	0.1	9.41E+07	1500
	6.90E+05	5.09E+05	0.74	6.27E+05	4.20E+05	0.67	0.1	1.04E+08	0.1	9.41E+07	1500
CF - Viable 3	6.90E+05	4.62E+05	0.67	6.27E+05	3.75E+05	0.60	0.1	1.04E+08	0.1	9.41E+07	1500
	6.90E+05	4.55E+05	0.66	6.27E+05	4.00E+05	0.64	0.1	1.04E+08	0.1	9.41E+07	1500
	6.90E+05	4.94E+05	0.72	6.27E+05	3.96E+05	0.63	0.1	1.04E+08	0.1	9.41E+07	1500

Table D.3

Experimental Schedule

Day	Date	Test Condition	*C. parvum*	*B. subtilis*	Comments
T	7/21/98	Lab Recovery Experiment	y	n	UW Pilot plant water.
R	7/23/98	Lab Recovery Experiment	y	n	UW Pilot plant water.
W	7/29/98	Lab Recovery Experiment	y	n	UW Pilot plant water.
R	7/32/98	Lab Recovery Experiment	y	n	UW Pilot plant water.
R	8/6/98	Stable Filter Operation - Ottawa	y	n	
W	9/9/98	Stable Filter Operation - Ottawa	y	n	*Bacillus* method being validated - incubator problem.
T	9/22/98	Stable Filter Operation - Ottawa	y	n	*Bacillus* method being validated - incubator problem.
T	10/6/98	Stable Filter Operation - Ottawa	y	y	
W	10/21/98	Ripening - Ottawa	y	y	Pump failure at start of experiment.
T	10/27/98	Ripening - Ottawa	y	y	
T	11/3/98	Ripening - Ottawa	y	y	
T	11/10/98	Ripening - Ottawa	y	y	
W	11/25/98	Late Breakthrough - Ottawa	y	y	Rapid increase in turbidity - sampling at 0.8 NTU.
W	12/9/98	Late Breakthrough - Ottawa	y	y	Breakthrough occurred at 2 am - sampling at 0.5 NTU.
W	1/13/99	Late Breakthrough - Ottawa	y	y	
R	1/21/99	End-of-Run - Ottawa	y	y	
T	2/9/99	No Coagulant - Short Duration - Ottawa	y	y	
R	2/18/99	Sub-optimal Coagulation - Ottawa	y	y	Spore pump failure for a few minutes at T = 30.
T	3/9/99	Stable Filter Operation - Ottawa	y	y	
W	3/10/99	Rate Effects - Windsor	n	y	
M	3/22/99	Rate Effects - Windsor	n	y	
T	3/23/99	Sub-optimal Coagulation - Ottawa	y	y	

Table D.3

Experimental Schedule (Continued)

Day	Date	Test Condition	*C. parvum*	*B. subtilis*	Comments
R	4/8/99	Stable Filter Operation During Runoff - Ottawa	y	n	
T	4/13/99	Stable Filter Operation During Runoff - Ottawa	y	n	
T	4/20/99	---	---	---	Spring runoff
T	4/27/99	---	---	---	Spring runoff
T	5/4/99	Sub-optimal Coagulation - Ottawa	y	y	
T	5/11/99	Lab Recovery Experiment	y	n	Ottawa pilot plant water.
W	5/12/99	Lab Recovery Experiment	y	n	Ottawa pilot plant water.
T	5/18/99	Lab Recovery Experiment	y	n	Ottawa pilot plant water.
R	5/20/99	Lab Recovery Experiment	y	n	Ottawa pilot plant water.
M	5/31/99	Stable Filter Operation - Ottawa	y	y	
M	6/7/99	Hydraulic Step - Ottawa	y	y	Seeded for 5 hours prior to hydraulic step.
M	6/14/99	Lab Recovery Experiment	y	n	MWD pilot plant water.
T	6/15/99	Hydraulic Step - Ottawa	y	y	Seeded for 5 hours prior to hydraulic step.
T	6/22/99	Hydraulic Step - Ottawa	y	y	Seeded for 5 hours prior to hydraulic step.
T	6/29/99	No Coagulant in Plant - Ottawa	y	y	
T	7/13/99	No Coagulant in Plant - Ottawa	y	y	
T	7/20/99	Stable - Seeded at Rapid Mix - Ottawa	y	y	
T	7/27/99	Stable Filter Operation - Ottawa	y	y	
W	8/4/99	No Coagulant - Short Duration - Ottawa	y	y	
W	8/18/99	No Coagulans in Jar - Ottawa	y	y	
T	8/24/99	No Coagulants, No Media - Ottawa	y	y	
R	10/14/99	Stable Filter Operation - Windsor	y	y	
F	10/21/99	Stable Filter Operation - Windsor	y	y	
R	10/22/99	Stable Filter Operation - Windsor	y	y	

Table D.3

Experimental Schedule (Continued)

Day	Date	Test Condition	*C. parvum*	*B. subtilis*	Comments
T	11/23/99	Stable Filter Operation - UW	y	y	
W	11/24/99	Stable Filter Operation - UW	y	y	
Sun	11/28/99	Stable Filter Operation - UW	y	y	
F	12/10/99	Hydraulic Step - UW	y	y	
S	12/11/99	Hydraulic Step - UW	y	y	
M	12/13/99	No Coagulant - Extended Duration - Ottawa	y	y	
F	12/17/99	No Silicate - Ottawa	y	y	
M	12/20/99	Late Breakthrough - Ottawa	y	y	
W	12/22/99	Late Breakthrough - Ottawa	y	y	
S	1/15/00	Hydraulic Step - UW	y	y	
Sun	1/16/00	Hydraulic Step - UW	y	y	
W	1/19/00	Stable Filter Operation - Ottawa	y	y	
	2/29/00	Early Breakthrough - Ottawa	y	n	Hydraulic surge occurred during experiment.
	3/1/00	Early Breakthrough - Ottawa	y	n	
	3/3/00	Early Breakthrough - Ottawa	y	n	
	3/4/00	Early Breakthrough - Ottawa	y	n	
	3/8/00	End-of-Run - Ottawa	y	n	
	3/9/00	End-of-Run - Ottawa	y	n	
	3/10/00	Sub-optimal Coagulation - Ottawa	y	n	
T	4/4/00	Lab Recovery Experiment	y	n	UW pilot plant water.
R	4/6/00	Lab Recovery Experiment	y	n	UW pilot plant water.
F	4/7/00	Lab Recovery Experiment	y	n	UW pilot plant water.
W	4/19/00	Lab Recovery Experiment	y	n	UW pilot plant water.

Table D.4

Filter Performance at Ottawa

Date	Type of Run	Raw Water Temperature During Seeding (°C)	Settled Water Temperature During Seeding (°C)	Rate of Headloss During Entire Filter Cycle (Δft/hr)	Turbidity During Seeding				Particle Counts >= 2 µm (#/mL) During Seeding			
					Median PI	Median FE	Log Reduction** Median	Log Reduction** 95th Percentile	Median PI	Median FE	Log Reduction** Median	Log Reduction** 95th Percentile
8/6/98	Stable Operation	23.5	23.5	0.085	1.04	0.02	1.7	1.7	4896	2.3	3.3	3.5
9/9/98	"	19.8	20.1	0.092	1.08	0.02	1.7	1.7	5043	0.9	3.8	3.9
9/22/98	"	17.9	16.9	*	1.17	0.03	1.6	1.7	5324	7.8	2.8	3.0
10/6/98	"	14.5	14.5	0.059	2.14	0.02	2.0	2.0	7251	0.2	4.5	4.8
3/9/99	"	3.3	2.3	0.095	2.35	0.03	1.9	1.9	4533	0.3	4.2	4.3
5/31/99	"	21.0	18.7	0.044	2.54	0.03	1.9	1.9	5665	0.9	3.8	3.9
7/27/99	"	25.6	24.7	0.044	1.00	0.04	1.4	1.8	4427	4.8	3.0	3.3
1/19/00	"	2.7	1.6	0.086	*	0.03	*	*	*	4.1	*	*
4/8/99	Stable Operation During Runoff	2.2	3.1	0.069	8.53	0.04	2.4	2.4	14185	1.2	4.1	4.2
4/13/99	"	4.3	3.8	0.059	13.22	0.03	2.6	2.6	15882	2.2	3.8	4.0
7/20/99	Stable Operation - Seeded at Rapid Mix	23.8	22.7	0.051	1.39	0.08	1.3	1.5	4958	5.5	3.0	3.0
10/27/98	Filter Ripening	11.6	12.0	0.067	2.42	0.07	1.6	1.7	6691	2.7	3.4	3.7
11/3/98	"	8.4	8.8	0.078	5.56	0.14	1.6	1.8	12695	11	3.1	3.6
11/10/98	"	9.0	9.4	0.044	1.93	0.14	1.1	1.2	5029	7.3	2.8	3.3
1/21/99	End-of-Run	1.2	3.7	0.096	1.47	0.05	1.5	1.5	3723	1.3	3.5	3.8
3/8/00	"	0.9	1.1	0.108	3.93	0.08	1.7	1.9	9714	3.8	3.4	3.5
3/9/00	"	1.0	2.6	0.096	3.54	0.05	1.9	2.0	8659	3.1	3.4	3.5
3/1/00	Early Breakthrough	0.9	1.0	*	3.78	0.21	1.3	1.4	9393	6.0	3.2	3.3
3/3/00	"	0.6	1.0	0.072	5.91	0.13	1.6	1.8	12892	5.1	3.4	3.5
3/4/00	"	1.0	0.9	0.101	5.49	0.11	1.7	1.9	12201	6.0	3.3	3.4
11/25/98	Late Breakthrough	6.1	6.4	0.062	2.97	0.98	0.5	0.6	7695	247	1.5	1.7
12/9/98	"	5.3	5.6	0.044	2.37	0.51	0.7	0.7	6537	136	1.7	1.8
1/13/99	"	1.3	3.7	0.083	1.52	0.55	0.4	0.6	3905	4.2	3.0	3.1
12/20/99	"	2.3	3.3	0.127	4.53	0.26	1.1	1.2	6252	9	2.8	2.9
12/22/99	"	1.7	2.1	0.098	4.32	0.25	1.2	1.6	*	*	*	*

* Data are not available.

** Reduction through entire treatment plant.

Table D.4

Filter Performance at Ottawa (Continued)

Date	Type of Run	Raw Water Temperature During Seeding (°C)	Settled Water Temperature During Seeding (°C)	Rate of Headloss During Entire Filter Cycle (Δft/hr)	Turbidity During Seeding				Particle Counts >= 2 µm (#/mL) During Seeding			
					Median PI	Median FE	Log Reduction** Median	Log Reduction** 95th Percentile	Median PI	Median FE	Log Reduction** Median	Log Reduction** 95th Percentile
8/24/99	No Coagulants, No Media	*	*	*	*	*	*	*	*	*	*	*
12/13/99	No Coagulant - Extended Duration	3.7	6.1	0.013	4.11	2.27	0.3	0.3	10271	5117	0.3	0.3
2/9/99	No Coagulant - Short Duration	2.2	2.8	0.111	2.52	0.74	0.5	0.6	7416	186	1.6	1.7
8/4/99	"	24.4	23.6	0.019	1.28	0.55	0.4	0.4	3967	554	0.9	0.9
6/29/99	No Coagulant in Plant	23.7	22.7	0.014	1.66	0.74	0.4	0.4	5725	432	1.1	1.2
7/13/99	"	21.8	21.1	0.021	1.77	0.68	0.4	0.5	6361	500	1.1	1.2
8/18/99	No Coagulant in Jar	21.8	21.0	0.051	1.07	0.03	1.6	1.6	5110	0.4	4.1	4.3
12/17/99	No Silicate	3.5	4.1	0.057	4.58	0.03	2.2	2.2	12485	3.3	3.6	3.6
2/18/99	Sub-Optimal Coagulation	2.6	2.1	0.150	2.05	0.98	0.3	0.3	3583	584	0.8	0.8
3/23/99	"	1.4	3.8	0.113	2.71	0.90	0.5	0.5	3740	71	1.7	1.7
5/4/99	"	11.5	11.6	0.139	2.54	0.15	1.2	1.3	5829	19	2.5	2.5
3/10/99		1.2	1.3	0.098	3.44	0.26	1.1	1.3	8411	129	1.8	1.9
6/7/99	Hydraulic Step	22.0	20.0	0.051	2.09	0.09	1.4	1.5	5853	0.2	4.4	4.7
6/15/99	"	21.2	20.2	0.055	1.81	0.06	1.5	1.6	5740	1.4	3.6	3.8
6/22/99	"	*	*	*	*	*	*	*	*	*	*	*

* Data are not available.

** Reduction through entire treatment plant.

Table D.5

Turbidity and Total Particle Data from Ottawa

Date	Type of Experiment	Seed Duration at Sampling (min)	Turbidity (NTU)			Particles >=2 μm (#/mL)		
			PI	FE	Log Reduction*	PI	FE	Log Reduction*
8/6/98	Stable Filter Operation	15	1.04	0.02	1.72	4966	1.9	3.42
		30	1.04	0.02	1.72	4949	8.0	2.79
		45	1.04	0.02	1.72	4868	2.7	3.26
		55	1.04	0.02	1.72	4915	2.1	3.37
9/9/98	Stable Filter Operation	15	1.10	0.02	1.74	5031	0.9	3.75
		30	1.08	0.02	1.73	5024	0.9	3.75
		45	1.08	0.02	1.73	5026	0.6	3.92
		55	1.08	0.02	1.73	5008	1.0	3.70
9/22/98	Stable Filter Operation	15	1.17	0.04	1.47	5317	17	2.50
		30	1.17	0.03	1.59	5329	7.5	2.85
		45	1.17	0.03	1.59	5255	5.6	2.97
		55	1.17	0.03	1.59	5225	4.8	3.04
10/6/98	Stable Filter Operation	15	2.14	0.02	2.03	7382	0.2	4.57
		30	2.14	0.02	2.03	8381	0.1	4.92
		45	2.19	0.02	2.04	7258	0.2	4.56
		55	2.18	0.02	2.04	7021	0.2	4.55
3/9/99	Stable Filter Operation	15	2.35	0.03	1.89	4818	0.3	4.21
		30	2.35	0.03	1.89	4583	0.4	4.06
		45	2.35	0.03	1.89	4278	0.3	4.15
		55	2.35	0.03	1.89	4291	0.4	4.03
5/31/99	Stable Filter Operation	15	2.59	0.04	1.81	5757	0.8	3.86
		30	2.54	0.03	1.93	5548	0.9	3.79
		45	2.54	0.03	1.93	5543	1.0	3.74
		55	2.53	0.03	1.93	5662	2.0	3.45
7/27/99	Stable Filter Operation	15	1.00	0.05	1.30	4472	7.3	2.79
		30	1.00	0.04	1.40	4325	4.6	2.97
		45	1.35	0.04	1.53	8342	4.1	3.31
		55	1.24	0.04	1.49	4796	4.3	3.05
1/19/00	Stable Filter Operation	15	-	0.02	-	-	4.4	-
		30	-	0.03	-	-	5.3	-
		45	-	0.03	-	-	5.3	-
		55	-	0.03	-	-	4.3	-
4/8/99	Runoff	15	8.52	0.04	2.33	14258	1.5	3.98
		30	8.61	0.03	2.46	14097	1.5	3.97
		45	8.56	0.04	2.33	14591	1.1	4.12
		55	8.52	0.04	2.33	14628	1.1	4.12
4/13/99	Runoff	15	13.20	0.03	2.64	15836	2.2	3.86
		30	13.23	0.03	2.64	15865	2.3	3.84
		45	13.22	0.03	2.64	15905	2.1	3.88
		55	13.22	0.03	2.64	15867	1.6	4.00

* Reduction through entire treatment plant.

Table D.5

Turbidity and Total Particle Data from Ottawa (Continued)

Date	Type of Experiment	Seed Duration at Sampling (min)	Turbidity (NTU)			Particles >=2 µm (#/mL)		
			PI	FE	Log Reduction*	PI	FE	Log Reduction*
7/20/99	Stable Filter Operation seeded at rapid mix	150	1.40	0.08	1.24	4973	5.4	2.96
		165	1.39	0.07	1.30	4801	4.4	3.04
		180	1.39	0.06	1.36	4983	4.9	3.01
		195	1.39	0.06	1.36	4861	5.1	2.98
		210	1.39	0.05	1.44	4846	4.6	3.02
		225	1.39	0.05	1.44	5162	5.0	3.01
		240	1.37	0.05	1.44	4898	5.6	2.94
		255	1.36	0.05	1.43	4968	6.9	2.86
		270	1.34	0.05	1.43	5093	9.2	2.74
10/27/98	Filter Ripening	5	2.42	0.09	1.43	6663	31	2.34
		10	2.42	0.08	1.48	6566	6.4	3.01
		15	2.43	0.07	1.54	6646	3.5	3.28
		20	2.42	0.05	1.68	6590	2.3	3.46
		25	2.42	0.06	1.61	6836	2.1	3.51
11/3/98	Filter Ripening	5	5.61	0.32	1.24	12739	58	2.34
		10	5.59	0.19	1.47	12808	26	2.69
		15	5.56	0.14	1.60	12702	12	3.04
		20	5.56	0.09	1.79	12383	6.2	3.30
		25	5.52	0.10	1.74	12757	4.1	3.49
11/10/98	Filter Ripening	5	1.93	0.19	1.01	4989	145	1.54
		10	1.93	0.22	0.94	5142	18	2.46
		15	1.93	0.13	1.17	5081	4.8	3.02
		20	1.92	0.13	1.17	4960	2.7	3.26
		25	1.92	0.11	1.24	4864	7.7	2.80
1/21/99	End-of-Run	15	1.47	0.05	1.47	3746	1.1	3.53
		30	1.47	0.05	1.47	3788	1.3	3.46
		45	1.47	0.06	1.39	3641	2.3	3.20
		55	1.47	0.06	1.39	3669	2.5	3.17
3/8/00	End-of-Run	-165	4.03	0.04	2.06	10552	3.5	3.48
		-155	4.03	0.03	2.07	9804	3.2	3.48
		-90	3.97	0.04	2.05	9825	2.9	3.53
		15	3.93	0.06	1.81	9893	3.7	3.43
		30	3.93	0.08	1.69	9726	4.0	3.38
		45	3.93	0.10	1.59	9553	4.8	3.30
		55	3.93	0.12	1.52	9707	5.5	3.25
3/9/00	End-of-Run	-365	3.61	0.04	1.99	8721	2.8	3.50
		-355	3.61	0.04	1.99	8882	3.2	3.44
		-290	3.56	0.04	1.99	8775	3.0	3.46
		-230	3.69	0.04	2.00	9340	2.7	3.54
		-170	3.56	0.04	2.00	8896	3.0	3.47
		15	3.52	0.04	1.93	8682	3.0	3.46
		30	3.52	0.05	1.88	8724	2.8	3.49
		45	3.56	0.05	1.85	8751	3.6	3.39
		55	3.56	0.06	1.80	8751	3.6	3.39

* Reduction through entire treatment plant.

Table D.5

Turbidity and Total Particle Data from Ottawa (Continued)

Date	Type of Experiment	Seed Duration at Sampling (min)	Turbidity (NTU)			Particles >=2 µm (#/mL)		
			PI	FE	Log Reduction*	PI	FE	Log Reduction*
3/1/00	Early Breakthrough	-345	3.28	0.04	1.88	7842	3.90	3.30
		-335	3.36	0.04	1.90	7936	2.45	3.51
		-300	3.40	0.04	1.92	8297	2.31	3.55
		-270	3.37	0.04	1.91	8098	2.61	3.49
		-240	3.49	0.04	1.93	8799	2.28	3.59
		-210	3.53	0.04	1.92	8877	2.54	3.54
		15	3.70	0.18	1.31	9454	5.5	3.23
		30	3.78	0.21	1.26	9516	6.1	3.20
		45	3.78	0.25	1.18	9616	6.4	3.17
		55	3.78	0.26	1.16	9223	9.6	2.98
3/3/00	Early Breakthrough	-240	5.74	0.04	2.15	12759	3.7	3.53
		-230	5.70	0.04	2.14	12908	3.3	3.60
		-195	5.77	0.04	2.17	13338	3.3	3.61
		-165	5.74	0.04	2.16	13225	3.7	3.55
		-105	5.75	0.04	2.17	12794	3.7	3.54
		15	5.91	0.10	1.76	13096	5.1	3.41
		30	5.87	0.13	1.64	12848	5.2	3.39
		45	5.91	0.17	1.55	12945	6.5	3.30
		55	5.87	0.19	1.49	12667	6.3	3.30
3/4/00	Early Breakthrough	-385	5.53	0.04	2.15	12123	3.3	3.57
		-375	5.57	0.04	2.13	12364	4.4	3.45
		-310	5.57	0.04	2.15	12544	4.1	3.49
		-250	5.53	0.04	2.12	12362	4.1	3.48
		-190	5.49	0.04	2.15	12325	4.0	3.49
		15	5.49	0.09	1.79	12135	5.5	3.35
		30	5.49	0.11	1.71	12148	5.7	3.33
		45	5.49	0.14	1.60	12195	7.1	3.23
		55	5.45	0.15	1.55	12260	7.0	3.24
11/25/98	Late Breakthrough	15	2.97	0.80	0.57	7726	147	1.72
		30	2.97	0.98	0.48	7744	311	1.40
		45	3.08	0.98	0.50	8097	295	1.44
		55	3.08	0.98	0.50	8216	243	1.53
12/9/98	Late Breakthrough	15	2.38	0.50	0.68	6419	147	1.64
		30	2.38	0.47	0.70	6387	131	1.69
		45	2.36	0.48	0.69	6334	124	1.71
		55	2.35	0.55	0.63	6233	158	1.60
1/13/99	Late Breakthrough	15	1.52	0.65	0.37	3891	3.3	3.07
		30	1.52	0.40	0.58	3999	3.7	3.03
		45	1.52	0.62	0.39	3829	3.6	3.03
		55	1.52	0.76	0.30	3860	4.3	2.95
12/20/99	Late Breakthrough	15	4.48	0.26	1.23	6216	8.7	2.86
		30	4.57	0.26	1.24	6115	9.1	2.83
		45	4.48	0.26	1.23	6128	25	2.38
		55	4.45	0.32	1.14	6243	84	1.87

* Reduction through entire treatment plant.

Table D.5

Turbidity and Total Particle Data from Ottawa (Continued)

Date	Type of Experiment	Seed Duration at Sampling (min)	Turbidity (NTU)			Particles >=2 µm (#/mL)		
			PI	FE	Log Reduction*	PI	FE	Log Reduction*
12/22/99	Late Breakthrough	15	4.27	0.25	1.23	-	-	-
		30	4.32	0.30	1.16	-	-	-
		45	4.32	0.30	1.16	-	-	-
		55	4.31	0.30	1.16	-	-	-
8/24/99	No Coagulants, No Media	15	-	-	-	-	-	-
		30	-	-	-	-	-	-
		45	-	-	-	-	-	-
		55	-	-	-	-	-	-
12/13/99	No Coagulant - Extended Duration	15	4.07	2.27	0.25	1.1E+04	4815	0.35
		30	4.07	2.27	0.25	1.0E+04	5172	0.30
		45	4.07	2.27	0.25	1.1E+04	5250	0.31
		55	4.24	2.27	0.27	1.0E+04	5452	0.28
2/9/99	No Coagulant - Short Duration	15	2.61	0.69	0.58	7748	143	1.73
		30	2.52	0.74	0.53	7336	189	1.59
		45	2.45	0.79	0.49	7218	237	1.48
		55	2.41	0.83	0.46	6862	254	1.43
8/4/99	No Coagulant - Short Duration	15	1.28	0.58	0.34	3978	521	0.88
		30	1.28	0.55	0.37	3941	555	0.85
		45	1.28	0.55	0.37	3940	586	0.83
		55	1.24	0.55	0.35	3932	599	0.82
6/29/99	No Coagulant in Plant	15	1.66	0.72	0.36	5645	403	1.15
		30	1.66	0.74	0.35	5643	438	1.11
		45	1.66	0.75	0.35	5906	447	1.12
		55	1.64	0.76	0.33	5736	462	1.09
7/13/99	No Coagulant in Plant	15	1.78	0.65	0.44	6424	453	1.15
		30	1.77	0.68	0.42	6378	500	1.11
		45	1.75	0.69	0.40	6265	505	1.09
		55	1.75	0.72	0.39	6281	536	1.07
8/18/99	No Coagulant in Jar	15	1.07	0.03	1.55	5093	0.4	4.10
		30	1.07	0.03	1.55	5110	0.4	4.11
		45	1.07	0.03	1.55	5174	0.4	4.11
		55	1.07	0.03	1.55	4999	0.4	4.10
12/17/99	No Silicate	15	4.53	0.03	2.18	1.2E+04	3.3	3.58
		30	4.58	0.03	2.18	1.3E+04	2.8	3.65
		45	4.92	0.03	2.21	1.3E+04	3.6	3.56
		55	4.79	0.03	2.20	1.3E+04	4.6	3.44

* Reduction through entire treatment plant.

Table D.5

Turbidity and Total Particle Data from Ottawa (Continued)

Date	Type of Experiment	Seed Duration at Sampling (min)	Turbidity (NTU)			Particles >=2 μm (#/mL)		
			PI	FE	Log Reduction*	PI	FE	Log Reduction*
2/18/99	Sub-Optimal Coagulation (52% reduction to 20 mg/L)	15	2.05	0.98	0.32	3631	589	0.79
		30	2.05	0.98	0.32	3515	557	0.80
		45	2.03	0.98	0.32	3503	595	0.77
		55	2.03	0.98	0.32	3479	612	0.75
3/23/99	Sub-Optimal Coagulation (47% reduction to 20 mg/L)	15	2.71	0.89	0.48	3776	66	1.76
		30	2.71	0.90	0.48	3876	71	1.74
		45	2.71	0.95	0.46	3815	72	1.72
		55	2.79	0.98	0.45	3799	75	1.71
5/4/99	Sub-Optimal Coagulation (49% reduction to 22 mg/L)	15	2.54	0.13	1.29	5818	20	2.47
		30	2.54	0.14	1.26	5793	19	2.48
		45	2.54	0.17	1.17	5774	22	2.41
		55	2.54	0.19	1.13	6007	24	2.40
3/10/00	Sub-Optimal Coagulation (49% reduction to 20 mg/L)	-245	3.48	0.04	1.95	8618	7	3.09
		-235	3.48	0.04	1.94	8580	8	3.02
		-200	3.48	0.04	1.91	8327	13	2.80
		-170	3.48	0.05	1.84	8580	24	2.55
		-140	3.48	0.06	1.76	8625	36	2.38
		-65	3.48	0.10	1.55	8366	78	2.03
		-35	3.48	0.12	1.48	8274	88	1.97
		15	3.44	0.21	1.21	8539	118	1.86
		30	3.44	0.26	1.12	8514	131	1.81
		45	3.44	0.31	1.05	8205	140	1.77
		55	3.44	0.34	1.00	8095	147	1.74
6/7/99	Hydraulic Step (25% increase in flow)	280	2.10	0.06	1.54	5816	0.5	4.07
		295	2.10	0.07	1.48	5593	2.9	3.29
		300	2.10	0.10	1.32	5802	1.9	3.48
		305	2.10	0.37	0.75	5859	145.2	1.61
		310	2.10	0.28	0.88	5957	278.9	1.33
		315	2.09	0.22	0.98	5880	199.4	1.47
6/15/99	Hydraulic Step (25% increase in flow)	280	1.79	0.05	1.54	5835	0.9	3.81
		295	1.80	0.05	1.55	5749	1.5	3.58
		300	1.80	0.06	1.45	5863	15.7	2.57
		305	1.80	0.08	1.35	5728	328.5	1.24
		310	1.80	0.04	1.70	5851	140.0	1.62
		315	1.80	0.04	1.64	5791	10.8	2.73
		320	1.80	0.05	1.60	6083	1.4	3.62
6/22/99	Hydraulic Step (25% increase in flow)	280	-	-	-	-	-	-
		295	-	-	-	-	-	-
		300	-	-	-	-	-	-
		305	-	-	-	-	-	-
		310	-	-	-	-	-	-
		315	-	-	-	-	-	-
		320	-	-	-	-	-	-
		360	-	-	-	-	-	-

* Reduction through entire treatment plant.

Table D.6

Microorganism Data from Ottawa

Date	Type of Experiment	Seed Duration at Sampling (min)	*C. parvum* (oocysts/L)			*B. subtilis* (CFU/L)		
			FI	FE	Log Removal	FI	FE	Log Removal
8/6/98	Stable Filter Operation *†	15	1.6E+5	0	> 4.9	-	-	-
		30	1.1E+5	0	> 4.7	-	-	-
		45	1.8E+5	4	4.7	-	-	-
		55	2.7E+5	2	5.1	-	-	-
9/9/98	Stable Filter Operation *†	15	1.1E+6	0	> 5.7	-	-	-
		30	7.9E+5	0	> 5.6	-	-	-
		45	9.7E+5	2	5.7	-	-	-
		55	9.2E+5	0	> 5.7	-	-	-
9/22/98	Stable Filter Operation *†	15	1.2E+6	0	> 5.8	1.0E+5	*1.0E+3*	2.0 est.
		30	1.4E+6	0	> 5.8	8.8E+4	*0.0E+0*	> 4.9 est.
		45	1.3E+6	0	> 5.8	1.3E+5	*0.0E+0*	> 5.1 est.
		55	1.4E+6	2	5.8	1.1E+5	*0.0E+0*	> 5.0 est.
10/6/98	Stable Filter Operation *†	15	1.4E+6	0	> 5.8	-	-	-
		30	1.4E+6	0	> 5.8	-	-	-
		45	1.4E+6	2	5.8	-	-	-
		55	1.4E+6	4	5.5	-	-	-
3/9/99	Stable Filter Operation	15	1.6E+6	4	5.6	7.9E+4	*7.8E+2*	2.0 est.
		30	1.7E+6	18	5.0	8.2E+4	*6.7E+2*	2.1 est.
		45	1.7E+6	26	4.8	8.5E+4	*4.4E+2*	2.3 est.
		55	1.7E+6	6	5.5	9.5E+4	*1.0E+3*	2.0 est.
5/31/99	Stable Filter Operation †	15	1.1E+6	2	5.7	4.1E+6	9.8E+1	4.6
		30	1.0E+6	4	5.4	5.7E+6	1.2E+2	4.7
		45	1.1E+6	4	5.4	4.7E+6	1.2E+2	4.6
		55	1.2E+6	0	> 5.8	5.4E+6	1.5E+2	4.6
7/27/99	Stable Filter Operation †	15	7.6E+5	0	> 5.6	3.1E+5	*5.0E+0*	4.8 est.
		30	8.0E+5	0	> 5.6	2.7E+5	*9.0E+0*	4.5 est.
		45	7.3E+5	0	> 5.6	2.7E+5	*1.0E+1*	4.4 est.
		55	8.2E+5	0	> 5.6	2.3E+5	*1.4E+1*	4.2 est.
1/19/00	Stable Filter Operation	15	4.2E+5	2	5.3	9.9E+5	6.4E+1	4.2
		30	4.4E+5	2	5.3	1.1E+6	6.6E+1	4.2
		45	5.0E+5	1	5.7	1.0E+6	6.4E+1	4.2
		55	4.9E+5	7	4.8	8.8E+5	5.6E+1	4.2
4/8/99	Stable Filter Operation During Runoff	15	1.2E+6	16	4.9	-	-	-
		30	1.5E+6	8	5.3	-	-	-
		45***	1.5E+6	4	5.6	-	-	-
		55	1.4E+6	4	5.5	-	-	-
4/13/99	Stable Filter Operation During Runoff	15	1.3E+6	4	5.5	-	-	-
		30	1.2E+6	4	5.5	-	-	-
		45	1.3E+6	10	5.1	-	-	-
		55	1.5E+6	6	5.4	-	-	-

Table D.6

Microorganism Data from Ottawa (Continued)

Date	Type of Experiment	Seed Duration at Sampling (min)	*C. parvum* (oocysts/L)			*B. subtilis* (CFU/L)		
			FI	FE	Log Removal	FI	FE	Log Removal
7/20/99	Stable Filter Operation [†] seeded at rapid mix	150	30	0	> 1.2	5.0E+2	4.2E+1	1.1
		165	710	0	> 2.6	3.3E+2	3.5E+1	1.0
		180	300	0	> 2.2	6.1E+2	2.2E+1	1.4
		195	210	0	> 2.0	4.7E+2	1.9E+1	1.4
		210	120	0	> 1.8	5.7E+2	1.5E+1	1.6
		225	90	0	> 1.7	4.1E+2	1.5E+1	1.4
		240	10	0	> 0.7	3.3E+2	9.0E+0	1.6
		255	0	0	> 0.0	0.0E+0	1.7E+1	-1.2
		270	0	0	> 0.0	4.7E+2	7.0E+0	1.8
10/27/98	Filter Ripening	5	1.1E+6	82	4.1	*1.5E+5*	*5.6E+2*	2.4 est.
		10	1.3E+6	22	4.8	*1.5E+5*	9.0E+3	1.2 est.
		15	1.4E+6	16	4.9	*1.8E+5*	7.6E+3	1.4 est.
		20	1.4E+6	4	5.5	2.0E+5	4.2E+3	1.7 est.
		25	1.4E+6	2	5.8	*1.0E+4*	8.2E+3	0.1 est.
11/3/98	Filter Ripening	5	1.0E+6	62	4.2	*6.5E+4*	2.3E+3	1.5 est.
		10	1.3E+6	-	-	*7.5E+4*	2.3E+3	1.5 est.
		15	1.3E+6	18	4.9	*1.7E+5*	*5.6E+2*	2.5 est.
		20	1.4E+6	2	5.8	2.2E+5	*1.5E+3*	2.2 est.
		25	1.3E+6	4	5.5	2.1E+5	*1.7E+2*	3.1 est.
11/10/98	Filter Ripening	5	9.1E+5	110	3.9	*6.3E+4*	*0.0E+0*	> 4.8 est.
		10	9.6E+5	24	4.6	1.8E+5	5.4E+3	1.5
		15	1.0E+6	4	5.4	1.7E+5	4.5E+3	1.6
		20	1.1E+6	2	5.7	2.1E+5	6.6E+3	1.5
		25	1.1E+6	4	5.4	1.9E+5	*0.0E+0*	> 5.3 est.
1/21/99	End-of-Run [•]	15	7.1E+5	194	3.6	7.9E+4	*5.6E+2*	2.2 est.
		30	6.8E+5	298	3.4	9.5E+4	*3.3E+2*	2.5 est.
		45	7.9E+5	1948	2.6	9.7E+4	*9.4E+2*	2.0 est.
		55	7.2E+5	2162	2.5	9.5E+4	*8.9E+2*	2.0 est.
3/8/00	End-of-Run	15	7.8E+5	720	3.0	-	-	-
		30	7.2E+5	3220	2.3	-	-	-
		45	7.4E+5	7140	2.0	-	-	-
		55	6.6E+5	9620	1.8	-	-	-
3/9/00	End-of-Run	15	5.9E+5	320	3.3	-	-	-
		30	6.8E+5	1260	2.7	-	-	-
		45	6.0E+5	2100	2.5	-	-	-
		55	6.8E+5	3760	2.3	-	-	-
3/1/00	Early Breakthrough	15	6.2E+5	880	2.8	-	-	-
		30	6.9E+5	3660	2.3	-	-	-
		45	5.7E+5	7080	1.9	-	-	-
		55	6.3E+5	11920	1.7	-	-	-
3/3/00	Early Breakthrough	15	7.0E+5	1700	2.6	-	-	-
		30	6.4E+5	4020	2.2	-	-	-
		45	6.8E+5	6040	2.1	-	-	-
		55	7.2E+5	11660	1.8	-	-	-
3/4/00	Early Breakthrough	15	6.7E+5	1960	2.5	-	-	-
		30	6.9E+5	5240	2.1	-	-	-
		45	6.2E+5	7740	1.9	-	-	-
		55	6.3E+5	10500	1.8	-	-	-

Table D.6

Microorganism Data from Ottawa (Continued)

Date	Type of Experiment	Seed Duration at Sampling (min)	*C. parvum* (oocysts/L)			*B. subtilis* (CFU/L)		
			FI	FE	Log Removal	FI	FE	Log Removal
11/25/98	Late Breakthrough	15	8.1E+5	1.4E+4	1.8	*8.5E+4*	5.4E+3	1.2 est.
		30	9.4E+5	1.8E+4	1.7	*1.5E+5*	5.2E+3	1.5 est.
		45	9.0E+5	1.7E+4	1.7	*9.5E+4*	7.6E+3	1.1 est.
		55	9.5E+5	1.6E+4	1.8	*1.3E+5*	9.1E+3	1.2 est.
12/9/98	Late Breakthrough	15[††]	9.7E+5	2.1E+4	1.7	4.2E+4	3.2E+3	1.1
		30[††]	9.6E+5	1.5E+4	1.8	3.1E+4	3.6E+3	0.9
		45[††]	8.5E+5	1.8E+4	1.7	6.9E+4	6.8E+3	1.0
		55[††]	9.2E+5	1.9E+4	1.7	6.8E+4	7.8E+3	0.9
1/13/99	Late Breakthrough	15	6.6E+5	2.5E+3	2.4	3.1E+4	*1.8E+3*	1.2 est.
		30[††]	5.3E+5	1.9E+4	1.5	3.5E+4	*1.1E+3*	1.5 est.
		45[††]	5.5E+5	7.4E+4	0.9	2.4E+4	2.0E+3	1.1 est.
		55[††]	5.4E+5	3.8E+4	1.2	3.2E+4	2.0E+3	1.2 est.
12/20/99	Late Breakthrough	15	5.7E+5	1.0E+4	1.8	4.8E+5	7.8E+4	0.8
		30	6.1E+5	2.2E+4	1.4	3.7E+5	7.2E+4	0.7
		45	6.0E+5	1.7E+4	1.6	8.2E+4	4.2E+4	0.3
		55	6.4E+5	3.4E+4	1.3	6.7E+4	5.7E+4	0.1
12/22/99	Late Breakthrough	15	6.6E+5	2.5E+4	1.4	3.3E+5	3.4E+4	1.0
		30	8.0E+5	3.2E+4	1.4	3.6E+5	4.8E+4	0.9
		45	7.8E+5	3.8E+4	1.3	1.9E+5	2.9E+4	0.8
		55	8.5E+5	4.1E+4	1.3	1.9E+5	2.9E+4	0.8
8/24/99	No Coagulants, No Media	15	2.9E+5	1.7E+05	0.23	2.9E+5	1.9E+5	0.18
		30	3.3E+5	3.5E+05	-0.03	4.4E+5	3.4E+5	0.11
		45	3.7E+5	2.8E+05	0.12	5.0E+5	4.1E+5	0.09
		55	3.4E+5	3.3E+05	0.01	4.7E+5	2.9E+5	0.21
12/13/99	No Coagulant - Extended Duration[•]	15	4.2E+5	2.4E+05	0.2	1.4E+6	1.4E+6	0.00
		30	4.8E+5	2.6E+05	0.3	1.8E+6	1.3E+6	0.14
		45	4.2E+5	2.4E+05	0.2	4.2E+6	1.7E+6	0.39
		55	4.8E+5	2.5E+05	0.3	3.9E+6	1.7E+6	0.36
2/9/99	No Coagulant - Short Duration[•]	15	5.8E+5	1820	2.5	6.7E+4	1.0E+4	0.81
		30	5.4E+5	2740	2.3	7.6E+4	3.6E+4	0.33
		45	4.3E+5	5990	1.9	1.0E+5	3.4E+4	0.47
		55	4.2E+5	6180	1.8	7.1E+4	5.4E+4	0.12
8/4/99	No Coagulant - Short Duration[•]	15	2.4E+5	240	3.0	3.1E+5	1.1E+5	0.45
		30	2.6E+5	200	3.1	3.7E+5	2.1E+5	0.25
		45	3.8E+5	190	3.3	3.2E+5	3.2E+5	0.00
		55	2.9E+5	430	2.8	3.2E+5	3.2E+5	0.00
6/29/99	No Coagulant in Plant[§]	15	6.5E+5	0	> 4.8	1.3E+5	5.6E+2	2.4
		30	6.2E+5	0	> 4.8	3.2E+5	7.7E+2	2.6
		45	6.9E+5	0	> 4.8	3.4E+5	8.2E+2	2.6
		55	6.6E+5	0	> 4.8	3.1E+5	9.2E+2	2.5
7/13/99	No Coagulant in Plant[§]	15	1.2E+6	0	> 5.1	7.8E+4	5.1E+2	2.2
		30	1.1E+6	0	> 5.0	2.2E+5	7.0E+2	2.5
		45	1.0E+6	0	> 5.0	5.9E+4	4.4E+2	2.1
		55	1.1E+6	0	> 5.0	7.9E+4	6.3E+2	2.1

Table D.6

Microorganism Data from Ottawa (Continued)

Date	Type of Experiment	Seed Duration at Sampling (min)	*C. parvum* (oocysts/L)			*B. subtilis* (CFU/L)		
			FI	FE	Log Removal	FI	FE	Log Removal
8/18/99	No Coagulant in Jar [†]	15	7.8E+5	0	> 5.6	6.3E+4	5.5E+1	3.1
		30	7.0E+5	0	> 5.5	7.0E+4	4.3E+1	3.2
		45	8.2E+5	0	> 5.6	6.1E+4	3.6E+1	3.2
		55	8.2E+5	0	> 5.6	6.6E+4	3.1E+1	3.3
12/17/99	No Silicate [†]	15	4.8E+5	2	5.4	7.6E+5	2.8E+2	3.4
		30	4.9E+5	0	> 5.4	1.2E+6	3.0E+2	3.6
		45	4.7E+5	9	4.7	1.1E+6	5.0E+2	3.3
		55	4.0E+5	8	4.7	1.1E+6	3.8E+2	3.5
2/18/99	Sub-optimal Coagulation [*]	15	4.5E+5	6680	1.8	6.4E+4	5.3E+3	1.1
	(52% reduction to 20 mg/L)	30	5.2E+5	19093	1.4	6.7E+4	9.2E+3	0.9
		45	5.3E+5	28747	1.3	7.1E+4	7.2E+3	1.0
		55	6.2E+5	26047	1.4	6.5E+4	1.1E+4	0.8
3/23/99	Sub-optimal Coagulation	15	1.4E+6	200	3.8	3.4E+4	*8.9E+2*	1.6 est.
	(47% reduction to 20 mg/L)	30	1.5E+6	710	3.3	5.7E+4	*6.8E+2*	1.9 est.
		45	1.6E+6	1570	3.0	5.2E+4	*5.3E+2*	2.0 est.
		55	2.1E+6	2550	2.9	4.2E+4	1.4E+3	1.5 est.
5/4/99	Sub-optimal Coagulation	15	1.3E+6	4	5.5	7.1E+3	8.8E+2	0.9
	(49% reduction to 22 mg/L)	30	1.5E+6	12	5.1	8.3E+3	9.2E+2	1.0
		45	1.6E+6	8	5.3	7.6E+3	9.7E+2	0.9
		55	1.7E+6	8	5.3	7.0E+3	7.5E+2	1.0
3/10/00	Sub-optimal Coagulation [†††]	15	4.5E+5	20	4.4	-	-	-
	(49% reduction to 20 mg/L)	30	4.6E+5	0	> 4.4	-	-	-
		45	5.2E+5	20	4.4	-	-	-
		55	5.2E+5	40	4.1	-	-	-
6/7/99	Hydraulic Step [‡]	280	2.0E+5	0	> 5.0	4.6E+4	*6.5E+1*	2.8 est.
	(25% increase in flow)	295	2.2E+5	10	4.3	4.6E+4	*1.2E+2*	2.6 est.
		300	2.0E+5	1522	2.1	1.3E+4	4.7E+2	1.4
		305	2.1E+3	4412	-0.3	8.2E+3	2.0E+3	0.6
		310	1.3E+3	3384	-0.4	5.3E+3	2.1E+3	0.4
		315	9.0E+2	3270	-0.6	2.4E+3	1.1E+3	0.3
6/15/99	Hydraulic Step [†]	280	1.8E+5	0	> 4.9	4.0E+4	*3.2E+1*	3.1 est.
	(25% increase in flow)	295	2.0E+5	2	5.0	4.4E+4	*3.0E+1*	3.2 est.
		300	1.9E+5	2	5.0	2.4E+4	*2.0E+1*	3.1 est.
		305	3.0E+4	0	> 4.2	7.6E+3	3.7E+1	2.3
		310	1.8E+4	0	> 3.9	5.1E+3	5.0E+1	2.0
		315	7.9E+3	0	> 3.6	2.3E+3	*6.0E+0*	2.6 est.
		320	6.9E+3	0	> 3.5	2.4E+3	*1.6E+1*	2.2 est.
6/22/99	Hydraulic Step	280	2.0E+5	4	4.7	2.6E+4	*3.6E+1*	2.9 est.
	(25% increase in flow)	295	2.2E+5	28	3.9	2.6E+4	*1.9E+1*	3.1 est.
		300	2.1E+5	14	4.2	9.1E+3	*2.2E+1*	2.6 est.
		305	3.8E+4	48	2.9	4.7E+3	*1.7E+1*	2.4 est.
		310	1.6E+4	76	2.3	1.1E+3	4.6E+1	1.4 est.
		315	6.4E+3	50	2.1	1.1E+3	*2.0E+1*	1.7 est.
		320	5.0E+3	22	2.4	1.0E+3	*1.4E+1*	1.9 est.
		360	3.7E+3	22	2.2	4.4E+2	7.8E+1	0.8

Note: All counts in italics are estimates (est.) because they are not in the statistically valid range of 20-200 counts per plate.

[*] Filter influent *Cryptosporidium* data estimated by strip counts at 400X.

[†] Non-detects (values of 0) were treated as 2 oocysts/L.

[‡] Non-detects (values of 0) were treated as 10 oocysts/L.

[**] Pump failure (for a few minutes) accounts for decreased concentration.

[††] Filter effluent *Cryptosporidium* data estimated by strip counts at 400X.

[‡‡] Substantial clumping was observed in filter influent *Cryptosporidium* samples.

[***] Some sample loss occurred during processing.

[†††] Non-detects (values of 0) were treated as 20 oocysts/L.

Table D.7

Microsphere Data from Ottawa

Date	Type of Experiment	Seed Duration at Sampling (min)	Yellow Microspheres (spheres/L)			Blue Microspheres (spheres/L)		
			FI	FE	Log Removal	FI	FE	Log Removal
1/19/00	Stable Filter Operation	15	6.1E+5	13	4.7	-	-	-
		30	5.6E+5	6	5.0	-	-	-
		45	6.5E+5	12	4.7	-	-	-
		55	7.9E+5	6	5.1	-	-	-
3/8/00	End-of-Run	-165	-	-	-	9.4E+5	78	4.1
		-155	-	-	-	9.9E+5	80	4.1
		-90	-	-	-	0.0E+0	172	n/a
		15	7.9E+5	960	2.9	1.6E+4	620	1.4
		30	8.5E+5	3620	2.4	6000	340	1.2
		45	8.8E+5	1.0E+4	1.9	2000	760	0.4
		55	7.6E+5	1.1E+4	1.8	4000	740	0.7
3/9/00	End-of-Run	-365	-	-	-	7.9E+5	16	4.7
		-355	-	-	-	9.0E+5	23	4.6
		-290	-	-	-	2.5E+5	19	4.1
		-230	-	-	-	5.6E+3	3	3.3
		-170	-	-	-	9.0E+2	2	2.7
		15	3.1E+5	220	3.1	7.0E+2	5	2.1
		30	3.0E+5	540	2.7	8.0E+2	40	n/a
		45	3.3E+5	1500	2.3	0.0E+0	20	n/a
		55	2.7E+5	1620	2.2	8.0E+2	220	n/a
3/1/00	Early Breakthrough	-345	-	-	-	8.2E+5	6	5.1
		-335	-	-	-	7.9E+5	7	5.1
		-300	-	-	-	2.0E+5	11	4.3
		-270	-	-	-	4.9E+4	18	3.4
		-240	-	-	-	3.4E+4	7	3.7
		-210	-	-	-	2.2E+4	12	3.3
		15	6.0E+5	880	2.8	800	880	n/a
		30	6.3E+5	3700	2.2	400	1000	n/a
		45	6.5E+5	6240	2.0	0	980	n/a
		55	6.2E+5	9320	1.8	0	1000	n/a
3/3/00	Early Breakthrough	-240	-	-	-	8.3E+5	13	4.8
		-230	-	-	-	7.7E+5	10	4.9
		-195	-	-	-	1.2E+5	20	3.8
		-165	-	-	-	5.1E+4	27	3.3
		-105	-	-	-	1.6E+4	79	2.3
		15	6.4E+5	1340	2.7	8400	660	1.1
		30	5.2E+5	3980	2.1	6000	680	0.9
		45	6.0E+5	7640	1.9	6400	640	1.0
		55	6.0E+5	1.1E+4	1.7	7200	1000	0.9
3/4/00	Early Breakthrough	-385	-	-	-	7.5E+5	16	4.7
		-375	-	-	-	7.8E+5	9	4.9
		-310	-	-	-	1.8E+5	8	4.4
		-250	-	-	-	1.7E+4	9	3.3
		-190	-	-	-	6.1E+3	6	3.0
		15	4.7E+5	2060	2.4	0	220	n/a
		30	5.5E+5	3820	2.2	800	420	0.3
		45	4.7E+5	6200	1.9	400	460	n/a
		55	5.5E+5	7100	1.9	400	540	n/a

Table D.7

Microsphere Data from Ottawa (Continued)

Date	Type of Experiment	Seed Duration at Sampling (min)	Yellow Microspheres (spheres/L)			Blue Microspheres (spheres/L)		
			FI	FE	Log Removal	FI	FE	Log Removal
12/20/99	Late Breakthrough	15	4.3E+5	6680	1.8	-	-	-
		30	3.9E+5	1.6E+4	1.4	-	-	-
		45	4.1E+5	1.1E+4	1.6	-	-	-
		55	4.2E+5	2.2E+4	1.3	-	-	-
12/22/99	Late Breakthrough	15	9.5E+5	8.5E+3	2.0	-	-	-
		30	9.9E+5	1.7E+4	1.8	-	-	-
		45	8.9E+5	2.7E+4	1.5	-	-	-
		55	9.5E+5	3.1E+4	1.5	-	-	-
12/13/99	No Coagulant - Extended Duration *	15	3.4E+5	2.3E+05	0.2	-	-	-
		30	3.9E+5	2.3E+05	0.2	-	-	-
		45	3.8E+5	2.2E+05	0.2	-	-	-
		55	3.8E+5	2.0E+05	0.3	-	-	-
12/17/99	No Silicate	15	7.5E+5	52	4.2	-	-	-
		30	7.3E+5	98	3.9	-	-	-
		45	6.1E+5	77	3.9	-	-	-
		55	5.9E+5	170	3.5	-	-	-
3/10/00	Sub-optimal Coagulation	-245	-	-	-	6.7E+5	2	5.5
	(49% reduction to 20 mg/L)	-235	-	-	-	5.7E+5	1	5.8
		-200	-	-	-	9.9E+4	0	> 5.0
		-170	-	-	-	6.8E+4	0	> 3.7
		-140	-	-	-	3.3E+4	0	> 3.5
		-65	-	-	-	9.5E+3	0	> 2.8
		-35	-	-	-	3.0E+3	0	> 2.3
		15	5.2E+5	40	4.1	0.0E+0	20	n/a
		30	4.6E+5	20	4.4	4.0E+2	0	> 1.3
		45	4.3E+5	20	4.3	0.0E+0	40	n/a
		55	4.9E+5	80	3.8	4.0E+2	0	> 1.3

* Filter influent microsphere data estimated by enumerating 50 random fields of view at 400X (Nikon Labophot 2A, Nikon Canada Inc., Toronto, ON).

Table D.8

Microorganism and Microsphere Removal (Filtration) and Total Particle Reduction (Plant) - Summary from Ottawa

Date	Experiment	Log Removal (Mean ± 1 Standard Deviation)***				Filter Effluent (Mean ± 1 Standard Deviation)	
		C. parvum	*B. subtilis*	Particles	Yellow Microspheres	Particles (#/mL)	Turbidity (NTU)
8/6/98	Stable Filter Operation	4.9 ± 0.21	*	3.2 ± 0.29	*	3.7 ± 2.9	0.02 ± 0.00
9/9/98		5.7 ± 0.06	*	3.8 ± 0.10	*	0.9 ± 0.2	0.02 ± 0.00
9/22/98		5.8 ± 0.03	4.3 * 1.51	2.8 ± 0.24	*	8.7 ± 5.6	0.03 ± 0.00
10/6/98		5.8 ± 0.15	*	4.6 ± 0.18	*	0.2 ± 0.1	0.02 ± 0.00
3/9/99		5.2 ± 0.38	2.1 ± 0.14	4.1 ± 0.10	*	0.4 ± 0.1	0.03 ± 0.00
5/31/99		5.6 ± 0.20	4.6 ± 0.05	3.7 ± 0.18	*	1.2 ± 0.6	0.03 ± 0.00
7/27/99		5.6 ± 0.02	4.5 ± 0.24	3.0 ± 0.22	*	5.1 ± 1.5	0.04 ± 0.00
1/19/00		5.3 ± 0.36	4.2 ± 0.01	*	4.9 ± 0.22	4.8 ± 0.6	0.03 ± 0.00
	Overall	*5.5 ± 0.37*	*3.9 ± 1.13*	*3.62 ± 0.63*	*	*3.11 ± 3.5*	*0.03 ± 0.01*
4/8/99	Stable Operation During Runoff	5.3 ± 0.32	*	4.0 ± 0.09	*	1.3 ± 0.2	0.04 ± 0.00
4/13/99		5.4 ± 0.18	*	3.9 ± 0.07	*	2.1 ± 0.3	0.03 ± 0.00
	Overall	*5.3 ± 0.24*	*	*4.0 ± 0.11*	*	*1.7 ± 0.5*	*0.03 ± 0.01*
7/20/99	Stable - Seeded at Rapid Mix	1.3 ± 0.93	1.1 ± 0.92	3.0 ± 0.10	*	5.7 ± 1.51	0.1 ± 0.01
10/27/98	Filter Ripening	5.0 ± 0.67	1.4 ± 0.84	3.1 ± 0.48	*	9 ± 12	0.07 ± 0.02
11/3/98		5.1 ± 0.73	2.1 ± 0.69	3.0 ± 0.46	*	21 ± 22	0.17 ± 0.09
11/10/98		5.0 ± 0.75	2.9 ± 1.93	2.6 ± 0.67	*	36 ± 61	0.16 ± 0.05
	Overall	*5.1 ± 0.66*	*2.1 ± 1.36*	*2.9 ± 0.55*	*	*22 ± 37*	*0.13 ± 0.07*

* Not applicable.

** Only data after the initiation of the hydraulic step are summarized.

*** Log reduction of total particles through treatment plant..

Table D.8

Microorganism and Microsphere Removal (Filtration) and Total Particle Reduction (Plant) - Summary from Ottawa (Continued)

Date	Experiment	Log Removal (Mean ± 1 Standard Deviation)				Filter Effluent (Mean ± 1 Standard Deviation)	
		C. parvum	*B. subtilis*	Particles	Yellow Microspheres	Particles (#/mL)	Turbidity (NTU)
1/21/99	End-of-Run	3.0 ± 0.52	2.2 ± 0.21	3.3 ± 0.18	*	1.8 ± 0.70	0.06 ± 0.01
3/8/00		2.3 ± 0.53	*	3.3 ± 0.08	2.3 ± 0.49	4.5 ± 0.81	0.09 ± 0.03
3/9/00		2.7 ± 0.44	*	3.4 ± 0.05	2.6 ± 0.42	3.2 ± 0.38	0.05 ± 0.01
	Overall	*2.7 ± 0.54*	*	*3.4 ± 0.12*	*2.4 ± 0.46*	*3.2 ± 1.29*	*0.06 ± 0.02*
3/1/00	Early Breakthrough	2.2 ± 0.50	*	3.1 ± 0.11	2.2 ± 0.44	6.9 ± 1.82	0.22 ± 0.04
3/3/00		2.2 ± 0.34	*	3.4 ± 0.06	2.1 ± 0.42	5.8 ± 0.74	0.15 ± 0.04
3/4/00		2.1 ± 0.33	*	3.3 ± 0.06	2.1 ± 0.23	6.3 ± 0.84	0.12 ± 0.03
	Overall	*2.1 ± 0.36*	*	*3.3 ± 0.11*	*2.1 ± 0.34*	*6.3 ± 1.22*	*0.17 ± 0.06*
11/25/98	Late Breakthrough	1.8 ± 0.03	1.2 ± 0.16	1.5 ± 0.14	*	249 ± 74	0.94 ± 0.09
12/9/98		1.7 ± 0.06	1.0 ± 0.09	1.7 ± 0.05	*	140 ± 16	0.50 ± 0.04
1/13/99		1.5 ± 0.68	1.3 ± 0.18	3.0 ± 0.05	*	4 ± 0.4	0.61 ± 0.15
12/20/99		1.5 ± 0.20	0.5 ± 0.35	2.5 ± 0.46	1.5 ± 0.23	32 ± 36	0.28 ± 0.03
12/22/99		1.4 ± 0.05	0.9 ± 0.08	*	1.7 ± 0.26	*	0.29 ± 0.02
	Overall	*1.6 ± 0.32*	*1.0 ± 0.34*	*2.2 ± 0.67*	*1.6 ± 0.25*	*106 ± 107*	*0.52 ± 0.26*
8/24/99	No Coagulants, No Media	0.09 ± 0.12	0.15 ± 0.06	*	*	*	*
12/13/99	No Coagulant - Extended Duration	0.3 ± 0.02	0.2 ± 0.19	0.3 ± 0.03	0.2 ± 0.04	5172 ± 266	2.27 ± 0.00
2/9/99	No Coagulant - Short Duration	2.1 ± 0.33	0.4 ± 0.29	1.6 ± 0.13	*	206 ± 50	0.76 ± 0.06
8/4/99		3.1 ± 0.20	0.2 ± 0.22	0.8 ± 0.03	*	565 ± 35	0.56 ± 0.01
	Overall	*2.6 ± 0.56*	*0.3 ± 0.28*	*1.2 ± 0.39*	*	*385 ± 196*	*0.66 ± 0.12*

* Not applicable.

** Only data after the initiation of the hydraulic step are summarized.

*** Log reduction of total particles through treatment plant,.

Table D.8

Microorganism and Microsphere Removal (Filtration) and Total Particle Reduction (Plant) - Summary from Ottawa (Continued)

Date	Experiment	Log Removal (Mean ± 1 Standard Deviation)***				Filter Effluent (Mean ± 1 Standard Deviation)	
		C. parvum	*B. subtilis*	Particles	Yellow Microspheres	Particles (#/mL)	Turbidity (NTU)
6/29/99	No Coagulant in Plant	4.8 ± 0.02	2.5 ± 0.12	1.1 ± 0.02	*	438 ± 25	0.74 ± 0.02
7/13/99		5.0 ± 0.03	2.2 ± 0.18	1.1 ± 0.03	*	498 ± 34	0.69 ± 0.03
	Overall	*4.9 ± 0.12*	*2.4 ± 0.22*	*1.1 ± 0.03*	*	*468 ± 43*	*0.71 ± 0.04*
8/18/99	No Coagulant in Jar	5.6 ± 0.03	3.2 ± 0.11	4.1 ± 0.01	*	0.4 ± 0.00	0.03 ± 0.00
12/17/99	No Silicate	5.0 ± 0.39	3.5 ± 0.11	3.6 ± 0.09	3.9 ± 0.25	3.6 ± 0.76	0.03 ± 0.00
2/18/99	Sub-Optimal Coagulation	1.5 ± 0.24	0.9 ± 0.13	0.8 ± 0.02	*	588 ± 23	0.98 ± 0.00
3/23/99		3.3 ± 0.42	1.7 ± 0.25	1.7 ± 0.02	*	71 ± 4	0.93 ± 0.04
5/4/99		5.3 ± 0.17	0.9 ± 0.04	2.4 ± 0.04	*	21 ± 2	0.16 ± 0.03
3/10/00		4.3 ± 0.13	*	1.8 ± 0.05	4.1 ± 0.26	134 ± 13	0.28 ± 0.06
	Overall	*3.6 ± 1.48*	*1.2 ± 0.43*	*1.7 ± 0.61*	*	*204 ± 233*	*0.59 ± 0.38*
6/7/99	Hydraulic Step**	0.2 ± 1.28	0.7 ± 0.51	2.0 ± 1.01	*	156 ± 117	0.24 ± 0.11
6/15/99		4.0 ± 0.58	2.4 ± 0.42	2.4 ± 0.95	*	99 ± 140	0.05 ± 0.02
6/22/99		2.7 ± 0.78	1.8 ± 0.69	*	*	*	*
	Overall	*2.5 ± 1.74*	*1.7 ± 0.87*	*2.2 ± 0.93*	*	*125 ± 126*	*0.14 ± 0.12*

* Not applicable.

** Only data after the initiation of the hydraulic step are summarized.

*** Log reduction of total particles through treatment plant..

Table D.9

Filter Performance at Windsor

Date	Type of Run	Raw Water Temperature During Seeding (°C)	Rate of Headloss During Entire Filter Cycle (Δft/hr)	Turbidity During Seeding				Particle Counts >= 2 µm (#/mL) During Seeding			
				Median PI	Median FE	Log Reduction** Median	Log Reduction** 95th Percentile	Median PI	Median FE	Log Reduction** Median	Log Reduction** 95th Percentile
3/10/99	Stable - Constant Rate Filtration	1.7	0.0028	*	*	*	*	7765	2163	0.6	0.6
"	Stable - Declining Rate Filtration	1.7	0.0018	*	*	*	*	7765	2774	0.4	0.5
3/22/99	Stable - Constant Rate Filtration	4.0	0.0027	*	*	*	*	8811	240	1.6	1.6
"	Stable - Declining Rate Filtration	4.0	0.0028	*	*	*	*	8811	137	1.8	1.9
10/14/99	Stable Operation with Pre-ozonation	14.5	0.0015	26.2	0.10	2.7	3.1	504	12	1.6	1.7
10/21/99	Stable Operation	12.5	0.0016	5.6	0.11	1.7	1.9	525	13	1.6	1.7
10/22/99	Stable Operation	13.0	0.0015	13.7	0.10	2.2	2.4	500	13	106	1.6

* Data are not available.

** Reduction through entire treatment plant.

Table D.10

Total Particle Reduction (Plant) Data from Windsor

Date	Type of Run	Seed Time (minutes)	Particles >=2 µm (#/mL)		
			PI	FE	Log Reduction*
3/10/99	Stable Filter Operation	15	7362	2120	0.5
	constant rate filter	30	7765	2165	0.6
		45	7200	2163	0.5
		55	7918	2190	0.6
"	Stable Filter Operation	15	7882	2872	0.4
	declining rate filter	30	7362	2620	0.4
		45	7877	2701	0.5
		55	7765	2774	0.4
3/22/99	Stable Filter Operation	15	8811	239	1.6
	constant rate filter	30	9639	252	1.6
		45	8156	247	1.5
		55	8843	240	1.6
"	Stable Filter Operation	15	8811	129	1.8
	declining rate filter	30	9639	136	1.9
		45	8156	139	1.8
		55	8843	140	1.8
10/14/99	Stable Filter Operation	20	493	16	1.5
	with pre-ozonation	40	539	12	1.6
		55	539	12	1.7
10/21/99	Stable Filter Operation	20	527	16	1.5
		40	519	15	1.5
		55	514	14	1.6
10/22/99	Stable Filter Operation	20	491	14	1.5
		40	533	16	1.5
		55	524	13	1.6

* Reduction through entire treatment plant.

Table D.11

Microorganism Data from Windsor

Date	Experiment	Seed Time (minutes)	*Cryptosporidium parvum*			*Bacillus subtilis*		
			FI (#/L)	FE (#/L)	Log Removal	FI (CFU/L)	FE (CFU/L)	Log Removal
3/10/99	Stable Filter Operation constant rate filter	15	---	---	---	9.1E+03	1280	0.8
		30	---	---	---	7.6E+03	1440	0.7
		45	---	---	---	6.6E+03	1180	0.7
		55	---	---	---	8.7E+03	1100	0.9
"	Stable Filter Operation declining rate filter	15	---	---	---	6.8E+03	*10*	2.8 est.
		30	---	---	---	1.1E+03	*18*	1.8 est.
		45	---	---	---	3.7E+03	*11*	2.5 est.
		55	---	---	---	1.1E+04	*16*	2.8 est.
3/22/99	Stable Filter Operation constant rate filter	15	---	---	---	*5.0E+03*	*1*	3.7 est.
		30	---	---	---	*6.7E+03*	*3*	3.3 est.
		45	---	---	---	*5.6E+03*	*7*	2.9 est.
		55	---	---	---	*1.0E+04*	*16*	2.8 est.
"	Stable Filter Operation declining rate filter	15	---	---	---	3.8E+04	6	3.8 est.
		30	---	---	---	*1.1E+04*	*5*	3.3 est.
		45	---	---	---	*5.6E+03*	*14*	2.6 est.
		55	---	---	---	*8.3E+03*	*13*	2.8 est.
10/14/99	Stable Filter Operation with pre-ozonation	20	2.4E+5	38	3.8	5.7E+05	112	3.7
		40	2.2E+5	20	4.0	6.0E+05	124	3.7
		55	2.2E+5	4	4.7	5.6E+05	*36*	4.2 est.
10/21/99	Stable Filter Operation	20	1.9E+5	6	4.5	3.6E+05	88	3.6
		40	1.6E+5	10	4.2	3.4E+06	134	4.4
		55	1.7E+5	16	4.0	3.3E+05	130	3.4
10/22/99	Stable Filter Operation	20	2.1E+5	17	4.1	3.7E+5	115	3.5
		40	2.3E+5	16	4.2	2.8E+5	66	3.6
		55	2.0E+5	14	4.2	3.5E+5	170	3.3

Note: All concentrations in italics are estimates (est.) because the counts were not in the statistically valid range of 20-200 counts per plate.

Table D.12

Filter Performance at UW

Date	Type of Run	Turbidity During Seeding				Particle Counts >= 2 µm (#/mL)
		Median PI	Median FE	Log Reduction* Median	Log Reduction* 95th Percentile	Median FE
11/23/99	Stable Operation - Dual-media	1.30	0.07	1.3	1.3	19.3
11/24/99	"	1.33	0.04	1.5	1.6	3.0
11/28/99	Stable Operation - Tri-media	1.45	0.05	1.5	1.5	7.6
11/28/99	"	1.44	0.03	1.6	1.7	1.4
12/10/99	Hydraulic Step - Dual-media	1.61	0.05	1.5	1.6	10.7
12/11/99	"	1.48	0.06	1.4	1.4	13.7
1/15/00	Hydraulic Step - Tri-media	1.51	0.05	1.5	1.6	11.1
1/16/00	"	1.39	0.05	1.4	1.5	9.7

* Reduction through entire treatment plant.

Table D.13

Turbidity and Total Particle Data from UW

Date	Type of Run	Seed Time (minutes)	Turbidity			Particle Counts >= 2 µm (#/mL)
			PI	FE	Log Reduction*	FE
11/23/99	Stable Filter Operation	20	1.37	0.07	1.3	23.5
	dual-media	40	1.30	0.07	1.3	19.0
		55	1.29	0.06	1.3	15.1
11/24/99	Stable Filter Operation	20	1.33	0.04	1.5	3.1
	dual-media	40	1.56	0.10	1.2	2.4
		55	1.26	0.03	1.6	2.6
11/28/99	Stable Filter Operation	20	1.38	0.05	1.4	7.6
	tri-media	40	1.51	0.05	1.5	7.1
		55	1.61	0.05	1.5	5.9
11/28/99	Stable Filter Operation	20	1.45	0.03	1.6	1.1
	tri-media	40	1.55	0.03	1.7	2.5
		55	1.44	0.03	1.6	1.1
12/10/99	Hydraulic Step	-15	1.59	0.04	1.6	10.6
	25% flow increase	0	1.62	0.04	1.6	31.3
	dual-media	10	1.59	0.05	1.5	11.3
		20	1.62	0.05	1.5	10.9
12/11/99	Hydraulic Step	-15	1.48	0.06	1.4	13.8
	25% flow increase	0	1.55	0.06	1.4	40.4
	dual-media	10	1.48	0.06	1.4	20.4
		20	1.53	0.06	1.4	26.1
1/15/00	Hydraulic Step	-15	1.51	0.05	1.5	11.5
	25% flow increase	0	1.50	0.06	1.4	73.2
	tri-media	10	1.51	0.06	1.4	69.3
		20	1.53	0.05	1.5	33.0
1/16/00	Hydraulic Step	-15	1.34	0.06	1.3	9.6
	25% flow increase	0	1.41	0.06	1.4	64.2
	tri-media	10	1.38	0.05	1.4	66.1
		20	1.38	0.06	1.4	26.8

* Reduction through entire treatment plant.

Table D.14

Microorganism and Microsphere Data from UW

Date	Experiment	Seed Time (minutes)	*C. parvum* FI (#/L)	*C. parvum* FE (#/L)	*C. parvum* Log Removal	*B. subtilis* FI (CFU/L)	*B. subtilis* FE (CFU/L)	*B. subtilis* Log Removal	Microspheres FI (spheres/L)	Microspheres FE (spheres/L)	Microspheres Log Removal
11/23/99	Stable Filter Operation	20	3.5E+5	3	5.1	5.8E+5	3.6E+2	3.2	1.3E+6	32	4.6
	dual-media	40	3.3E+5	6	4.7	4.6E+5	3.5E+2	3.1	1.2E+6	29	4.6
		55	3.5E+5	2	5.2	5.6E+5	2.9E+2	3.3	1.4E+6	10	5.1
11/24/99	Stable Filter Operation	20	2.0E+5	3	4.8	3.9E+5	4.6E+2	2.9	8.9E+5	75	4.1
	dual-media	40	2.2E+5	0*	5.4	1.1E+6	7.0E+2	3.2	1.2E+6	25	4.7
		55	2.1E+5	2	5.0	9.5E+5	8.4E+2	3.1	1.2E+6	49	4.4
11/28/99	Stable Filter Operation	20	3.0E+5	2	5.2	7.2E+5	2.5E+2	3.5	1.3E+6	31	4.6
	tri-media	40	2.6E+5	3	4.9	8.1E+5	3.6E+2	3.3	1.2E+6	47	4.4
		55	2.6E+5	2	5.1	1.0E+6	3.2E+2	3.5	1.2E+6	22	4.7
11/28/99	Stable Filter Operation	20	4.5E+5	0*	5.7	3.3E+6	5.8E+1	4.8	1.2E+6	1	6.1
	tri-media	40	4.0E+5	0*	5.6	1.2E+6	6.2E+1	4.3	1.3E+6	1	6.1
		55	4.0E+5	0*	5.6	9.8E+5	5.8E+1	4.2	1.1E+6	2	5.8
12/10/99	Hydraulic Step	-60	2.2E+5	1	5.4	---	---	---	2.6E+5	2	5.1
	25% flow increase	-15	2.7E+5	0*	5.4	---	---	---	2.1E+5	3	4.9
	dual-media	0	2.3E+5	4	4.8	2.6E+5	9.6E+3	1.4	2.2E+5	2	5.0
		10	5.0E+4	4	4.1	7.3E+5	1.1E+4	1.8	8.7E+4	6	4.2
		20	1.7E+4	0**	3.9	1.5E+6	1.1E+4	2.1	2.1E+4	4	3.7
12/11/99	Hydraulic Step	-60	2.0E+5	1	5.3	---	---	---	2.9E+5	3	5.0
	25% flow increase	-15	1.9E+5	1	5.3	---	---	---	3.2E+5	17	4.3
	dual-media	0	1.9E+5	4	4.7	4.3E+5	3.8E+3	2.1	2.8E+5	244	3.1
		10	3.2E+4	4	3.9	1.1E+6	3.3E+3	2.5	5.4E+4	100	2.7
		20	5.5E+3	0**	3.4	1.7E+6	2.3E+3	2.9	1.2E+4	40	2.5
1/15/00	Hydraulic Step	-15	2.1E+5	3	4.9	---	---	---	3.0E+5	6	4.7
	25% flow increase	0	2.2E+5	8	4.5	1.2E+6	6.8E+3	2.2	3.2E+5	5	4.8
	tri-media	10	6.0E+4	14	3.6	9.6E+5	1.1E+4	1.9	6.2E+4	11	3.7
		20	2.8E+4	4	3.9	2.5E+5	6.6E+3	1.6	3.8E+4	4	4.0
1/16/00	Hydraulic Step	-15	4.5E+5	2	5.3	---	---	---	3.7E+5	2	5.3
	25% flow increase	0	4.3E+5	8	4.7	6.1E+5	5.0E+3	2.1	4.0E+5	6	4.8
	tri-media	10	1.3E+5	20	3.8	8.4E+5	6.0E+3	2.1	1.7E+5	9	4.3
		20	3.4E+4	4	3.9	8.9E+5	2.8E+3	2.5	8.3E+4	7	4.1

* Non-detects (values of 0) were treated as 1 oocysts/L.

** Non-detects (values of 0) were treated as 2 oocysts/L.

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