Removal of MS2 Bacteriophage, *Cryptosporidium*, *Giardia* and Turbidity by Pilot-Scale Multistage Slow Sand Filtration

by

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Author's Declaration

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

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Abstract

This research aimed to address the knowledge gaps in the literature regarding the removal of waterborne pathogens (viruses and protozoa) by modified multistage slow sand filtration. In the current study, two pilot-scale multistage slow sand filtration systems were operated continuously for over two years. The pilot systems treated agricultural- and urban-impacted raw river water of variable quality with turbidity peaks over 300 NTU and seasonal cold temperatures <2°C.

The first system (Pilot 1) consisted of two independent trains that included pre-ozonation, shallow-bed upflow gravel roughing filtration, and shallow-bed slow sand filtration. Pilot 1 was a pilot-scale version of an innovative, commercially available full-scale system. The second system (Pilot 2) included a full-depth upflow gravel roughing filter, a full-depth slow sand filter, and a second shallow-depth slow sand filter in series. The SSFs of both pilots were operated at high hydraulic loading rates (typically 0.4 m/h) at the upper limit of the literature recommended range (0.05 to 0.4 m/h).

Both pilot systems provided excellent turbidity removal despite the high filtration rates. Effluent turbidity of all multistage SSF pilot systems were within the regulated effluent limits in Ontario for full-scale SSFs (below 1 NTU at least 95% of the time and never exceeded 3 NTU), despite raw water turbidity peaks over 100 NTU. The roughing filters contributed to approximately 60-80% of the full-train turbidity removal, compared to and 20-40% for the slow sand filters. On average, the second slow sand filter in pilot 2 provided almost no additional turbidity removal. The slow sand filter run lengths were short because of frequent high raw water turbidity, with about 50-80% of the runs in the range of 1-3 weeks. To prevent excessive SSF clogging and maintenance, filtration rates should be decreased during periods of high turbidity.

Seven *Cryptosporidium* and *Giardia* challenge tests were conducted on the slow sand filters of both pilot systems at varying filtration rates (0.4 or 0.8 m/h), temperatures (2 to 25°C), and biological maturities (4 to 20 months). Removal of oocysts and cysts were good regardless of sand depth, hydraulic loading rate, and water temperature in the ranges tested. Average removals in the SSFs ranged from 2.6 to >4.4 logs for *Cryptosporidium* oocysts and ranged from >3.8 to >4.5 logs for *Giardia* cysts. This was consistent with findings in the literature, where oocyst and cyst removals of >4 logs have been reported. *Cryptosporidium* oocyst removals improved with

increased biological maturity of the slow sand filters. At a water temperature of 2°C, average removal of oocysts and cysts were 3.9 and >4.5 logs, respectively, in a biologically mature SSF. Doubling the filtration rate from 0.4 to 0.8 m/h led to a marginal decrease in oocyst removals. Sand depths in the range tested (37-100 cm) had no major impact on oocyst and cyst removals, likely because they are removed primarily in the upper section of slow sand filter beds by straining. In general, good oocyst and cyst removals can be achieved using shallower slow sand filter bed depths and higher filtration rates than recommended in the literature.

There are very few studies in the literature that quantify virus removal by slow sand filtration, especially at high filtration rates and shallow bed depths. There are no studies that report virus removal by slow sand filtration below 10°C. As such, 16 MS2 bacteriophage challenge tests were conducted at varying water temperatures (<2 to >20°C) and filtration rates (0.1 vs. 0.4 m/h) between February and June 2006 on biologically mature slow sand filters with varying bed depths (40 vs. 90 cm). Biologically mature roughing filters were also seeded with MS2.

Average MS2 removals ranged from 0.2 to 2.2 logs in the SSFs and 0.1 to 0.2 logs in the RFs under all conditions tested. Virus removal by slow sand filtration was strongly dependant on hydraulic loading rate, sand depth, and water temperature. Virus removal was greater at a sand depth of 90 cm vs. 40 cm, at an HLR of 0.1 m/h vs. 0.4 m/h, and at warm (20-24°C) vs. cold (<2-10°C) water temperatures when sufficient warm water acclimation time was provided. Increased sand depth likely increased MS2 removal because of greater detention time for predation and greater contact opportunities for attachment to sand grains and biofilms. A lower HLR would also increase MS2 removal by increasing detention time, in addition to decreasing shear and promoting attachment to filter media and biofilms. Greater MS2 removal at warmer water temperatures was attributed to improved biological activity in the filters. Schmutzdecke scraping was found to have only a minor and short-term effect on MS2 removals.

Virus removal can be optimized by providing deep SSF beds and operating at low filtration rates. Virus removal may be impaired in cold water, which could affect the viability of using SSF/MSF at northern climates if communities do not use disinfection or oxidation. As a stand-alone process, slow sand filtration (with or without roughing filtration) may not provide complete virus removal and should be combined with other treatment processes such as disinfection and oxidation to protect human health.

Acknowledgements

Dedication

This thesis is dedicated to my parents Leo and Cheryl DeLoyde, whose love and encouragement gave me the opportunities to pursue my dreams.

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1.0 Introduction

Slow sand filtration is a robust and sustainable water treatment technology for small communities in developed and developing countries. It is a 200-year-old technology used worldwide to treat microbially impacted surface waters by biological, physio-chemical, and physical removal mechanisms. Slow sand filtration has experienced resurgence in the past two decades in response to new regulations that target emerging pathogens that are highly resistant to conventional (chlorine) disinfection. Under certain conditions, slow sand filtration can meet or exceed regulated targets for effluent turbidity and the removal of microorganisms such as *Cryptosporidium*, *Giardia*, and bacteria (Cleary, 2005; Amy *et al*., 2006; Ellis, 1985).

Slow sand filtration is ideal for small communities because it is simple to operate, does not require chemical pretreatment, functions over a wide range of influent water quality without the need for process adjustment, requires minimal maintenance, and is cost-effective to build and operate. Indeed, Wegelin (1988) stated "no other single water treatment process can improve the physical, chemical, and bacteriological water quality of surface water better than slow sand filtration."

However, single stage slow sand filtration has a number of disadvantages, including frequent filter clogging during extended periods of high raw water turbidity, large land requirement, and low removal of dissolved organic disinfection byproduct precursors. To overcome these challenges, multistage slow sand filtration is emerging as an effective treatment technology. Multistage slow sand filtration is a multi-barrier treatment approach that includes treatment processes before or after slow sand filtration. In particular, pre-filtration using gravel "roughing" filters effectively removes influent turbidity peaks and algae, thereby increasing filter run lengths, decreasing maintenance requirements, and broadening the range of raw water qualities that are suitable for treatment by slow sand filtration (Collins *et al*., 2005). Roughing filters do not contribute to removal of disinfection byproduct precursor material. Multiple slow sand filters in series and pre-ozonation have also been employed in multistage slow sand filtration.

1.1 Problem Statement and Research Needs

There is a significant knowledge gap regarding the effect of process and design modifications on the performance and operation of multistage slow sand filtration. Process modifications include pre-treatment using ozonation or roughing filtration and multiple slow sand filters in series. Design alternatives include shallower media depths and higher filtration rates.

If the regulated treatment targets could be achieved, shallow filter beds and high hydraulic loading rates could lead to less-expensive and smaller-footprint multistage slow sand filtration installations. There exists a strong need for the operational boundaries of multistage slow sand filtration to be researched so that the treatment processes can be optimized for maximum pathogen and turbidity removal while minimizing the cost and operational burdens on small communities.

One important research need is to quantify pathogen removals by multistage slow sand filtration under various process configurations (pretreatment, shallow bed depths) and operating conditions (high filtration rates, cold water temperatures, high influent turbidity, etc.). Quantification of pathogen removals at very cold water temperatures could provide support for the use of multistage slow sand filtration in small communities in northern countries such as Canada and the northern United States.

Of particular importance is the need to quantify the removal and inactivation of enteric viruses that would likely be present in sewage-contaminated surface water. Viruses are typically difficult to remove by filtration because their small size precludes straining and their negative surface charge impairs attachment to granular media. In areas of the world (rural regions of developing countries, for example) where slow sand filtration would be an appropriate drinking water treatment technology to help meet the World Heath Organization's Millennium Development Goals of improved health and access to safe drinking water, disinfectant chemicals are likely not used in drinking water treatment. In these areas viruses could pose a serious health threat if they were to pass through to the slow sand filter effluent. Despite the need, there are very few studies

in the literature that report on virus removal by slow sand filtration. Furthermore, there are no studies in the literature on virus removal by slow sand filtration at water temperatures below 5°C. Therefore, there is a significant knowledge gap and need to quantify virus removal by modified multistage slow sand filtration under various operating conditions.

Another important research need is to quantify the removal of pathogens such as *Cryptosporidium* oocysts and *Giardia* cysts by modified multistage slow sand filtration if it is to be used as a stand-alone treatment technology.

1.2 Objectives and Approach

To address the knowledge gaps in the literature related to the removal of pathogens (with an emphasis on viruses) by slow sand filtration and to determine the operational and treatment performance of modified-design multistage slow sand filtration systems, the current study had multiple objectives:

1. Determine if slow sand filtration can eliminate viruses sufficiently to provide safe drinking water when used as a stand-alone water treatment system without disinfection (as would be the case in some areas of developing countries). The approach was to quantify the removal of viruses at varying water temperatures (2-10°C vs. >20°C), filtration rates (0.1 vs. 0.4 m/h), and bed depths (literature recommended vs. shallow). The effect of slow sand filter scraping was tested. Virus removal by roughing filtration and a second slow sand filter in series were investigated. MS2 bacteriophage were used as surrogates for human enteric viruses.

2. Quantify the removal of *Cryptosporidium* oocysts and *Giardia* cysts by two biologically mature slow sand filters in series (pilot 2) for comparison to results from a previous challenge test when the filters were less mature. The results of the seven challenge tests on the two pilot systems in this and previous studies were analyzed to determine oocyst and cyst removals over a range of filtration rates, bed depths, water temperatures, and filter maturities.

3. Operate two pilot-scale multistage slow sand filtration systems continuously at a high filtration rate (0.4 m/h), even during challenging periods of high raw water turbidity and cold water temperatures, to determine the effect on turbidity removal and filter run lengths.

4. Monitor turbidity removal by each treatment unit in the multistage slow sand filtration systems in order to evaluate the effectiveness of roughing filtration for turbidity removal. Full train turbidity removals were also quantified for comparison with regulated effluent turbidity targets for full-scale slow sand filters.

1.3 Thesis Organization

In Chapter 2, a review of studies in the literature provides an introduction to slow sand filtration, focusing particularly on the removal of *Cryptosporidium* oocysts, *Giardia* cysts, and viruses. Factors that impact removals are presented.

Details of the pilot filtration systems, experimental methods and materials are presented in Chapter 3. Chapter 4 includes analyses of raw and effluent turbidities over the study period, in addition to turbidity removals achieved by the pilot filters.

Results of the *Cryptosporidium* and *Giardia* challenge tests are discussed in Chapter 5. Results of the MS2 challenge tests are detailed in Chapter 6. Conclusions and recommendations are presented in Chapter 7 along with a discussion of the significance of results in this study.

2.0 Literature Review

2.1 Slow Sand Filtration

Slow sand filtration was invented in 1804 in Paisley, Scotland. The current design was introduced in 1829 by James Simpson to treat surface water from the Thames River for the Chelsea Water Company in London, England (Barrett *et al*., 1991). Slow sand filtration is still in use today around the world, particularly in Europe. In North America, rapid rate filtration gained popularity over SSF in the early 1900s because of its ability to treat variable turbidity raw water, higher filtration rates, and smaller footprint per volume treated (Ellis, 1985).

In the last two decades, new waterbourne microbial pathogens such as *Cryptosporidium* and *Giardia* have been accounted for in new drinking water regulations (USEPA, 2006). Since *Cryptosporidium* is highly resistant to conventional (chlorine) disinfection, water utilities have had to explore treatment options such as chemically assisted granular media filtration, UV irradiation, ozonation, and membranes. These treatment technologies are particularly expensive and operationally complex for small, rural communities. Globally, there are approximately 3.3 billion people living in rural areas (United Nations, 2005). In 2001, it was reported that 30% (9 million) of Canada's population lived in rural areas with population densities less than 150 people/km^2 (Agriculture and Agri-Food Canada, 2002). Slow sand filtration is an appropriate surface water treatment technology for small communities with populations less than 1000 to 2000 people with an upper limit of approximately 5000 people (AWWA, 1991).

Small rural communities typically have operators with limited technical skills for process control and limited funds for capital and operating costs. Therefore, small communities require a water treatment technology that is simple and cost effective to operate and can achieve regulated performance targets. Visscher (1990) reported that the operation and maintenance costs of a slow sand filtration plant were five times less than a conventional treatment plant. It is for these reasons that slow sand filtration installations and research have experienced resurgence in the past two decades.

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2.1.1 Design Parameters

In slow sand filtration, water flows downwards through a bed of fine sand. Unlike rapid rate filtration, SSF does not require pre-treatment chemicals (which can be expensive for small rural communities to purchase, transport and store), and does not employ water- and energy-intensive backwashing. SSF is capable of operating under a wide range of raw water conditions (with the exception of extended high turbidity events) without the need for process adjustment by operators, provided that the system is designed and operated properly. A summary of design criteria is presented in Table 2-1.

Table 2-1: Slow sand filtration design parameters

Design Parameter	Recommended Value	
	SSF*	RF[+]
Hydraulic loading rate (m/h)	0.1-0.4	0.3-1.5
Bed depth (m)	1.0-1.3 (initial)	0.9-1.2
	0.6 (minimum**)	
Effective grain size (mm)	0.15-0.35	4-18[++]
Media uniformity coefficient	<2-3	n.s.
Depth of underdrain gravel (m)	0.3-0.5	n.s.
Height of supernatant (m)	1.0-1.5	0.1
Empty bed contact time (h)	3-10	n.s.
Filter bed area[+] (m^2)	5-200	n.s.
Filter run length (days) [+++]	30->300	30->300
Influent turbidity (NTU)	<10 (peaks up to 50)	10-150

* Sources: Huisman & Wood (1974), Visscher (1990), Galvis *et al.* (1992 and 2002), Ellis (1985), Amy *et al.* (2006), AWWA (1991)
** SSF resanded once minimum sand depth is reached
[+] Design criteria for upflow RF; Sources: Wegelin (1996), Galvis *et al.* (1998), AWWA (1991)
[++] Design based on three layers with diameters 12-18 mm (layer 1, 0.2-0.3 m deep), 8-12 mm (layer 2, 0.15-0.2 m deep), and 4-8 mm (layer 3, 0.15-0.2 m deep) ranging from coarse to fine in direction of flow
[+++] SSF should be cleaned after 6 to 12 months even if terminal head loss has not been reached; RF cleaning frequency much shorter than filter run length (i.e. weekly/monthly cleaning)
n.s. – not specified

As shown in Table 2-1, effective grain size of the SSF sand should be 0.15 to 0.35 mm in order to avoid impairment of filter performance if the grain size were too large and rapid clogging if it were too small (Visscher, 1990; AWWA, 1991). Furthermore, to prevent filter clogging and

excessive maintenance, it is recommended that SSFs treat surface waters with turbidity less than 10 NTU, with peaks of up to 50 NTU for one to two days (Galvis *et al*., 1992; AWWA, 1991).

SSFs are operated in the downflow mode at filtration rates in the range of 0.1 to 0.4 m/h (Table 2-1). Filtration rates at the low end of this range may be required during periods of high raw water turbidity to prevent rapid head loss development and filter clogging. Visscher (1990) reported that filtration rate could be increased to 0.3 m/h for short periods of one or two days without undue filter clogging and could be operated at even higher rates up to 0.6 m/h if water quality were very good.

Since SSFs are operated in the downflow mode, a skin of organic matter, algae, microorganisms, and extracellular material accumulates on the top of the SSF bed in a layer known as the "schmutzdecke" layer (German for "dirty" layer) (Ellis, 1985; AWWA, 1991). Since the schmutzdecke layer is biologically active and can be quite dense with biofilms and accumulated material, most removal of organic and inorganic material occurs in this layer by biological, physical, and physio-chemical mechanisms (Ellis, 1985). The predominant organisms living in slow sand filters include aerobic bacteria, algae, protozoa, rotifers, worms, and other eucaryotes (Duncan, 1988; Eighmy *et al*., 1993; AWWA, 1991).

The greatest pressure drop or head loss occurs in the schmutzdecke layer. Once head loss exceeds the acceptable limit and the height of supernatant water reaches the overflow, the SSF is drained so that the water level is a few inches below the top of the sand bed. The top 2 cm of schmutzdecke are then scraped and the filter is refilled with water from the bottom up to prevent entrapment of air bubbles. An alternative cleaning method known as harrowing has also been proposed (Eighmy and Collins, 1988). Once the sand depth declines to approximately 0.6 m due to multiple filter scrapings over time, the SSF should be resanded (Table 2-1).

Roughing filters serve as sedimentation basins that are biologically active (Wegelin, 1996; Schulz and Okun, 1984; AWWA, 1991). Various roughing filter designs can be used, including horizontal, upflow, and downflow configurations. For upflow roughing filters, the recommended

depth of gravel is 0.9 to 1.2 m (Table 2-1), typically consisting of three layers of gravel graded from coarse at the bottom to fine at the top.

Roughing filtration pre-treatment is recommended for raw waters with turbidity ranging from 10 NTU (Bernardo, 1988) to 150 NTU (Wegelin, 1996). Roughing filters are cleaned by gravity draining whereby approximately 2.5 meters of head (between the water level and drain) is provided so that drainage velocities are in the range of 40 to 60 m/h (Galvis *et al*., 1998; Wegelin, 1996). To prevent breakthrough of solids and SSF clogging, roughing filters should be cleaned once per week during periods of high raw water turbidity and every one or two months during periods of low turbidity (Wegelin, 1996).

2.1.2 Treatment Capabilities

The treatment performance of slow sand and roughing filtration has been reported for a variety of water quality parameters, including the removal of bacteria, turbidity, organics, and microbial pathogens (Table 2-2).

Table 2-2: Multistage slow sand filtration treatment performance

Parameter	Removal Performance	
	SSF*	RF[+]
Turbidity	<1 NTU	50-90%
Coliform and enteric bacteria	1-3 logs	80-99%
Enteric viruses	1-4 logs	
Giardia cysts	2->4 logs	
Cryptosporidium oocysts	>4 logs	
Algae		30-80%
Dissolved organic carbon (DOC)	5-40%	15%
Biodegradable DOC	40-80%	
UV absorbance (254 nm)	5-35%	
Colour	30%	20-50%
THM precursors	<20-35%	
Iron/Manganese	30-90%	50%

* Sources: Amy *et al*. (2006), Galvis *et al*. (1998 and 2002), Ellis (1985), AWWA (1991)
+ Sources: Wegelin (1996 and 1988); Wegelin and Schertenleib (1993); Barett *et al*. (1991)

The treatment performances shown in Table 2-2 can be impaired by high filtration rates and cold water temperatures, which leads to decreased microbial activity (Huisman and Wood, 1974;

Schuler *et al*., 1988; Ellis, 1985). Treatment performance can also be impaired by low dissolved oxygen concentrations, which should remain above 3 mg/L (Ellis, 1985) and not drop below 0.5 mg/L (Visscher *et al*., 1987).

While the ability of slow sand filtration to remove dissolved organics and colour is relatively poor (Ellis, 1985), of particular concern to human health is the removal of disinfectant-resistant microorganisms such as *Cryptosporidium* oocysts and the removal of viruses (in cases where no or limited disinfection may be provided) because they are typically difficult to remove by filtration because of their small size and negative charge.

2.2 *Cryptosporidium* and *Giardia*

Cryptosporidium and *Giardia* are protozoan parasites of concern in drinking water treatment. *Cryptosporidium* oocysts are spherical and 4 to 6 μm in diameter, compared to the larger, ovoid *Giardia* cysts that are 8 to 14 μm long by 7 to 10 μm wide (Health Canada, 2004b). Oocysts and cysts are environmentally resistant and fairly ubiquitous in surface waters as a result of contamination by human and animal feces.

2.2.1 Cryptosporidiosis

In light of an infectious dose ranging from 30 to 100 oocysts (DuPont *et al*., 1995; Meinhardt *et al*., 1996), there is concern over source water contamination from agricultural runoff, wastewater treatment plant effluents, and land application of manure or sewage sludge (Table 2-3).

In agreement with the data in Table 2-3, Wallis *et al*. (1995) showed that 11% of 162 raw sewage samples across Canada contained 1 to 120 oocysts/L. Bukhari *et al*. (1997) reported that 26% of treated wastewater effluents sampled in the UK contained up to 60 oocysts/L. It has also been shown that infected individuals can excrete up to 10^{10} oocysts/g feces (Smith and Rose, 1998). Therefore, it is not surprising that oocyst contamination of surface water is commonly reported.

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Table 2-3: Sources of *Cryptosporidium* oocysts

	Cryptosporidium Concentration (oocysts/L)	Source
Dairy Farm Runoff	up to 3600	Graczyk *et al*. (2000)
Raw Sewage	800 to 5000	Madore *et al*. (1987)
Wastewater Effluent	0.02 to 4000	Smith and Rose (1998), Chauret *et al*. (1999)
Manure	100 to 400 oocysts/g	Madore *et al*. (1987), Zuckerman *et al*. (1997)
Sewage Sludge	<40 oocysts/g	Chauret *et al*. (1999)

Cryptosporidium and *Giardia* have been detected in the Grand River, which was used as the raw water source in the current study. Van Dyke *et al*. (2006) reported that *Cryptosporidium* and *Giardia* were detected in 93% and 100%, respectively, of 25 samples taken from the Grand River at the Mannheim intake over a one year period (refer to Section 3.1 of this thesis). The geometric mean concentrations were 19 oocysts/100 L (maximum 186 oocysts/100L) and 56 cysts/100 L (maximum 486 cysts/100 L) (Van Dyke *et al*., 2006). They reported that *Cryptosporidium* concentrations (but not *Giardia*) were correlated with precipitation and turbidity peaks.

Dorner *et al*. (2007) reported that *Cryptosporidium* and *Giardia* were detected in 14% (n=79) and 38% (n=89), respectively, of samples taken from the Grand River at the Mannheim intake. The median *Giardia* concentration of positive samples was 92 cysts/100 L (Dorner *et al*., 2007), which is similar to findings by Van Dyke *et al*. (2006). Dorner *et al*. (2004) summarized the potential sources of pathogens in the Grand River as being from (i) urban runoff, including treated and untreated wastewater discharge, (ii) agricultural runoff, (iii) livestock, and (iv) wildlife.

In another study where samples were collected at the Mannheim intake on the Grand River, LeChevallier *et al*. (2000) detected *Cryptosporidium* and *Giardia* in 51.6% and 35.5% of samples (n=99), respectively. They reported that the majority of protozoan detections occurred between October and April, likely attributable to precipitation and snowmelt events in the fall and spring months.

Cryptosporidium and *Giardia* were found in 98% of surface waters sampled by LeChevallier and Norton (1995) and in 12% of groundwaters sampled by Hancock *et al*. (1998). Rose (1988) detected *Cryptosporidium* oocysts in 77% of rivers and 75% of lakes sampled, including the detection of oocysts (likely from wild animals) in 83% of "pristine" surface waters sampled that had no known contact with human sewage or livestock manure. From a study of 25 water utilities in the United States, 11% of raw water samples were shown to contain oocysts (Arora *et al*., 2001). Zuckerman *et al*. (1997) reported concentrations of 0.001 to 500 oocysts/L in surface waters and 0.3 to 1.1 oocysts/L in drinking water source reservoirs.

Of particular concern is the detection of *Cryptosporidium* oocysts in treated drinking water. In a comprehensive study of 82 surface water treatment plants in 14 states, Aboytes *et al*. (2004) detected infectious *Cryptosporidium* oocysts in finished water samples from 27% of the utilities. They noted that 70% of oocyst-containing samples had been filtered and had turbidities less than 0.1 NTU. LeChevallier *et al*. (1991a) detected oocysts in 27% of 83 filtered water samples collected in the northeastern United States. Similarly, Rose (1988) found oocysts in 28% of treated drinking water samples.

Although oocysts have been shown to survive up to 18 months in water at 4°C (AWWA, 1988), the viability of oocysts in water can be low (Health Canada, 2004b; Smith *et al.*, 1993). LeChevallier *et al*. (1991a) reported that 21 of 23 oocysts recovered from treated water samples were non-viable and therefore non-infectious.

Cryptosporidiosis can be potentially fatal for young children, the elderly, pregnant women, and immunocompromized individuals (Ford, 1999; Hoxie *et al*., 1997; Daniel, 1996; O'Donoghue, 1995). Transmission of the protozoan parasites can occur via the fecal-oral route and by the ingestion of contaminated food or water. Stomach acids and bile salts destabilize the oocyst or cyst walls and permit the release of sporozoites and trophozoites, respectively, that go on to infect cells in the intestinal tract (O'Donoghue, 1995). Cryptosporidiosis can persist for 7 to 14

days and can cause watery diarrhea, nausea, vomiting, fever, and abdominal cramping (Hoxie *et al*., 1997).

An astounding 250 to 500 million *Cryptosporidium* infections occur each year in Asia, Africa, and Latin America (Current and Garcia, 1991). In the United States, nearly 500,000 people have been infected with cryptosporidiosis by waterbourne transmission since 1984 (Craun *et al*., 1998), most of them during a 1993 waterbourne outbreak in Milwaukee in which 403,000 people were infected. However, the occurrence of cryptosporidiosis is suspected of being vastly underreported (Hunter *et al*., 2001; Tillett *et al*., 1998). Ford (1999) estimated that 420,000 cases of cryptosporidiosis occur annually in the United States. One study has shown that 58% of 800 adolescents ranging in age from 14 to 21 were seropositive for *C. parvum* and had therefore been infected in the past (Ford, 1999). There is currently no treatment for cryptosporidiosis, although more than 120 drugs have been tested (Health Canada, 2004b).

2.2.2 Giardiasis

Giardia is the most commonly reported intestinal parasite in the world, with over 5000 cases of giardiasis reported in Canada in 1999 alone (Health Canada, 2004b). The infectious dose is as low as 1 to 10 cysts and infection causes severe diarrhea that persists for 2 to 3 weeks (Health Canada, 2004b). Other mammals such as beaver and muskrat can be infected by human-source *Giardia* (e.g. via sewage discharge to surface waters) and can subsequently excrete additional cysts to the water (Health Canada, 2004b). Infection by *Giardia* is especially prevalent in areas with small water treatment systems that treat cold and low-turbidity surface waters (Health Canada, 2004b; Fogel *et al*., 1993; Bellamy *et al*., 1985a).

In Canada, *Giardia* cysts are commonly found in sewage and surface waters and occasionally in treated drinking water (Health Canada, 2004b). In a Canada-wide study by Wallis *et al*. (1995), *Giardia* cysts were detected in 56.2% of 162 raw sewage samples (1-88000 cysts/L) and in 10% of raw and treated drinking water samples (0.001-2 cysts/L). A study in the northeastern United States by LeChevallier *et al*. (1991a and 1991b) found *Giardia* cysts in 81% of 83 raw water

samples (0.05 to 242 cysts/L) and in 17% of 83 filtered drinking water samples (0.003 to 0.6 cysts/L).

Although cysts have been reported to survive up to 2.8 months in river water at <10°C and ~1 month in lake water at 15-20°C, cysts in surface waters may often be dead (Health Canada, 2004b). LeChevallier *et al*. (1991a) showed that 40 of the 46 cysts isolated from treated water samples that tested positive were non-viable.

2.2.3 Regulations and Slow Sand Filtration Credits

Cryptosporidium oocysts are highly resistant to disinfection by chlorination and chloramination at typical concentrations and contact times used in water treatment (Health Canada, 2004b). As such, regulations have increasingly focused on the removal and inactivation of *Cryptosporidium* oocysts and *Giardia* cysts as target organisms in surface water treatment. While ozonation and UV disinfection are effective for inactivating oocysts and cysts, filtration is still the primary barrier to remove oocysts (and to some extent cysts) at many water treatment plants, especially in small communities. Conventional treatment (coagulation, flocculation, sedimentation, and filtration) and slow sand filtration are discussed below.

The US EPA's Long-term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) requires water utilities treating surface water or ground water under the direct influence of surface water (GWUDI) to achieve varying *Cryptosporidium* oocyst removals based on source water contamination (Table 2-4) (USEPA, 2006).

The reductions required by the LT2ESWTR (Table 2-4) are more stringent than the 1989 Surface Water Treatment Rule (SWTR) and 1998 Interim Enhanced Surface Water Treatment Rule (IESWTR). The SWTR and IESWTR required at least 3 logs of *Giardia* cyst and 2 log *Cryptosporidium* oocyst removal/inactivation, respectively. The IESWTR's focus on oocyst removal was necessary because *Cryptosporidium* oocysts are more difficult to remove/inactivate than *Giardia* cysts. Therefore, the 3 log *Giardia* cyst removal required under the SWTR may not

have protected systems with heavily contaminated source water. The LT2ESWTR (Table 2-4) is a further extension to ensure that heavily contaminated source waters are adequately treated.

Table 2-4: LT2ESWTR *Cryptosporidium* regulations

Bin No.	Surface Water or GWUDI Contamination* (oocysts/L)	Total Required *Cryptosporidium* Reduction (logs)
1	<0.075	3
2	>0.075 but <1.0	4
3	>1.0 but <3.0	5
4	>3.0	5.5

Source: USEPA (2006)
* as demonstrated by source monitoring
Note: systems serving <10,000 people monitor *E. coli* instead of *Cryptosporidium*

Under the LT2ESWTR, a 3 log *Cryptosporidium* oocyst treatment credit is given for single stage slow sand filtration when used as the primary filtration step (USEPA, 2006). As proof of the effectiveness of slow sand filtration as a treatment technology, it is given the same oocyst treatment credit as conventional treatment involving coagulation/flocculation, clarification, and granular media filtration (USEPA, 2006).

Regulations set forth by the Ontario Ministry of Environment (MOE) require that drinking water systems that treat surface water or ground water under the direct influence of surface water (GWUDI) provide a minimum removal and/or inactivation of 2 logs for *Cryptosporidium* oocysts, 3 logs for *Giardia* cysts, and 4 logs for viruses (MOE, 2006). Higher removals may be required depending on source water contamination and at least 0.5 logs of *Giardia* cyst reduction must come from disinfection. Slow sand filters are given a 2 log *Giardia* cyst removal credit, which is the similar to the 2.5 log *Giardia* cyst removal credit given to conventional treatment systems (MOE, 2006).

Although slow sand filters are credited with a 3 log oocyst removal (USEPA, 2006) and 2 log oocyst and 3 log cyst removals (MOE, 2006), studies in the literature have demonstrated much greater removals depending on water and operating conditions, as discussed in the next section.

2.3 *Cryptosporidium* and *Giardia* Removal by Slow Sand Filtration

Numerous studies have demonstrated that slow sand filters can acheive >4 logs of *Cryptosporidium* oocyst removal (Dullemont *et al.*, 2006; Amy *et al.*, 2006; Hijnen *et al.*, 2004; Timms *et al.*, 1995; Schuler *et al.*, 1991) and from 2 to >4 logs of *Giardia* cyst removal (Schuler *et al.*, 1991; Bellamy *et al.*, 1985a; Pyper *et al.*, 1985). Several factors influence the removal of oocysts and cysts during slow sand filtration (Table 2-5).

Table 2-5: Influence of selected factors on oocyst and cyst removal by SSF

Factor	Influence
Biological maturity	Important factor; improved removals as filter bed matures Range tested: virgin bed to >1 year old (Bellamy *et al.*, 1985a; Schuler *et al.*, 1991)
Hydraulic loading rate	Not of major importance; slight decrease in removal with higher HLRs Range tested: 0.04 to 0.4 m/h (Bellamy *et al.*, 1985a; Schuler *et al.*, 1991; Timms *et al.*, 1995; Heller and Brito, 2006)
Sand depth	Not of major importance; most removal in top of sand bed Range tested: 0.5 to 1.5 m (Timms *et al.*, 1995; Heller and Brito, 2006; Dullemont *et al.*, 2006; Logan *et al.*, 2001)
Sand grain size	Variable influence; large grain size and uniformity coefficient could impair removal Range tested: 0.13 to 0.62 mm effective size; UC up to 3.5 (Bellamy *et al.*, 1985b; Fogel *et al.*, 1993; Kohne, 2002)
Water Temperature	Negligible influence; removals may improve slightly at warmer temperatures due to increased inactivation Range tested: 1 to 23°C (Bellamy *et al.*, 1985a; Pyper, 1985; Fogel *et al.*, 1993)
Influent concentration	No effect (Schuler *et al.*, 1991; Heller and Brito, 2006) Range tested: 10^2 to 10^3 (oo)cysts/L

In general, studies in the literature demonstrate that removal of oocysts and cysts improves with increased biological maturity of the SSF, the presence of a well-developed schmutzdecke layer, lower HLRs, more uniform sand grain size, and warmer water temperatures. Good removals can be achieved at sand depths below design recommendations. Oocyst and cyst seeding concentration has no effect on removals. A review of findings in the literature is presented below.

2.3.1 Biological Maturity and Schmutzdecke Layer

The biological maturity of the SSF bed, which increases over a finite time period but is not directly measurable, has a major influence on oocyst and cyst removals. It has been consistently shown that removal increases as filter maturity increases.

Bellamy *et al.* (1985a) reported that a new SSF with virgin sand and virgin support gravel seeded with *Giardia* cysts achieved 2.1 logs removal, compared to >4.6 logs removal in an 80-week-old SSF with mature sand and gravel beds. In another experiment, Bellamy *et al.* (1985a) showed that cyst removal in a SSF with virgin sand and mature (67-week-old) support gravel was >5.0 logs. However, this may simply prove that physical straining in the virgin sand bed was important and does not necessarily indicate that cysts were removed by biological mechanisms in the mature gravel layer Note that removals were reported as ~100% when no cysts were recovered in the effluent (log removals were calculated by the current author based on detection limits reported by Bellamy *et al*, 1985a). These tests suggested that SSFs with mature biological populations are capable of removing cysts to below the detection limit and that even immature SSFs can remove >2 logs of cysts.

Bellamy *et al.* (1985a) also reported that schmutzdecke age had no effect on *Giardia* cyst removals in mature SSFs. They conducted a set of 15 experiments in which a SSF that had been in operation from 26 to 70 weeks was seeded with *Giardia* cysts immediately after scraping (i.e. no schmutzdecke layer). Removals ranged from >2.7 to >4.9 logs (calculated by current author) in ten experiments without a schmutzdecke layer when the SSF was over 41 weeks old. However, cysts were detected in the effluent at concentrations ranging from 0.002 to 5.4 cysts/L

during the first four tests when the SSF was less than 41 weeks old without a schmutzdecke layer. This demonstrated that the biologically mature SSFs were able to completely remove cysts at the influent concentrations tested, regardless of the presence of a schmutzdecke layer (Bellamy *et al*, 1985a).

In a final set of 25 experiments on a 29- to 68-week-old SSF, Bellamy *et al*. (1985a) showed that schmutzdecke age had no effect when a SSF was mature. For example, the SSF achieved >3.6 log cyst removal at 57 weeks maturity with a 5-week-old schmutzdecke, but only removed 2.8 logs when the filter was less mature (39 weeks old) but had a 12-week-old schmutzdecke (log removals calculated by current author). Therefore, Bellamy *et al*. (1985a) concluded that SSF biological maturity had a major influence on cyst removal, but schmutzdecke age did not, especially in a mature filter. They also noted that influent *Giardia* concentrations in the range of 50 to ~5000 cysts/L had no effect on removals under any of the conditions studied. It should be noted that these influent concentrations are small but were used to represent the highest expected cyst concentrations in surface waters (Bellamy *et al*., 1985a).

Schuler *et al*. (1991) found that removal of *Giardia* cysts was 2.8 to 3.7 logs in an immature SSF that had been operated in cold water for two months, compared to >4 log cyst and oocyst removals in mature SSFs during 17 additional tests conducted after four to 12 months of operation. The increased removals observed during tests conducted at later dates was attributed to increased biological maturity of the SSFs (Schuler *et al*., 1991)

2.3.2 Hydraulic Loading Rate

Although increasing HLR would be expected to increase pore velocity, increase detachment, and decrease adsorption of particles to SSF media, it has only a marginal (if any) negative effect on cyst and oocyst removal in SSFs.

Bellamy *et al*. (1985a) operated pilot SSFs for 16 months and reported average *Giardia* cyst removals of 4.0 logs at 0.04 m/h, 4.2 logs at 0.12 m/h, and 3.7 logs at 0.4 m/h based on >200 analyses. While the general trend appears to indicate that removals decreased slightly at higher

HLRs, the three averages were not statistically different and were consistently high. Similarly, Schuler *et al*. (1991) demonstrated consistently high (>4 log) oocyst and cyst removals in a mature SSF operated at 0.15 or 0.4 m/h. Timms *et al*. (1995) reported that oocyst removals were >4 logs and that increasing the SSF HLR from 0.3 to 0.4 m/h had no adverse effect. Therefore, HLR typical of slow sand filtration has a slight, if any, influence on cyst and oocyst removals.

Heller and Brito (2006) reported complete oocyst removals in a 75 cm deep SSF at HLRs of 0.125 and 0.25 m/h. Although oocysts did not breakthrough into the effluentat either HLR, there were more oocysts at each depth tested at the higher HLR, indicating more oocysts had penetrated deeper into the SSF at the higher HLR.

2.3.3 Sand Depth and Grain Size

Due to their relatively large size, oocysts and cysts can be removed by straining and attachment to biofilms in the upper portion of SSF beds. Timms *et al*. (1995) reported >4 log oocyst removal in a 50 cm deep SSF, all of which occurred in the top 2.5 cm. Fox *et al*. (1984) found that removal of 7 to 12 µm sized particles occurred almost entirely in the top 7 cm. Heller and Brito (2006) demonstrated that the first 15 cm of a SSF contained the highest number of retained oocysts, that removal of oocysts was always >80% in the first 45 cm, and that no oocysts penetrated below 60 cm. Dullemont *et al*. (2006) showed that the 5.3 log oocyst removal occurred predominantly in the top 10 cm of the SSF bed. Logan *et al*. (2001) also showed >2 logs removal of *C. parvum* oocysts were removed in the top 10 cm of biologically active intermittent sand filters.

The effect of sand grain size on protozoan cyst removal is variable. Bellamy *et al*. (1985b) showed that *Giardia* cyst removal by SSF was consistently high for grain sizes ranging from 0.13 to 0.62 mm effective size (ES). Kohne (2002) cited a 1996 report by Thames Water Utilities that showed consistently high oocyst removals (4 to 6 logs) in SSFs containing sand with 0.29 to 0.33 mm ES. In a study by Fogel *et al*. (1993), poor removals of cysts and oocysts (1.2 and 0.3 logs, respectively) were attributed to large pore spaces in a SSF sand with a high uniformity coefficient (UC 3.3-3.8; ES 0.2-0.3). Pore sizes were reported as being large enough to permit

passage of oocysts for sand with a d_{50} of 0.31 mm and UC 2.1 (Logan *et al.*, 2001) and for 0.33 mm glass beads (Tufenkji and Elimelech, 2005).

2.3.4 Water Temperature

Although warmer water temperature is known to substantially enhance biological activity in SSFs, temperature was reported as having a negligible influence on oocyst and cyst removal in the literature.

Bellamy *et al.* (1985a) seeded pilot SSFs operated at water temperatures of 15 or 5°C and found that there was no discernable difference in *Giardia* cyst removal at the different temperatures (generally >3 log). However, since temperatures were reduced from 15 to 5°C only one day prior to each test, the filters' biological populations were likely not altered and these results may not have reflected cyst removal at prolonged cold temperatures. In follow-up experiments, Bellamy *et al*. (1985b) showed that *Giardia* cyst removal was complete (~100%) for SSFs operated long-term at either 17°C or 5°C. From this they concluded that temperature in the range studied had no effect. However, different removals may have been observed if higher influent concentrations had been employed so that some cysts could be recovered in the effluent. Temperature was, however, cited as an important factor determining the rate of biological maturation of SSFs (Bellamy *et al,* 1985a).

Pyper (1985) reported *Giardia* cyst removal by SSF decreased slightly at colder water temperatures. They found that removals ranged from 3.7 to 4.0 logs at 7 to 21°C, but dropped to 2.2 to 3.0 logs at cold temperatures <1°C. Fogel *et al*. (1993) presented limited data showing poor removals of *Giardia* cysts (1.2 logs) and *Cryptosporidium* oocysts (0.3 logs) in a SSF operated at very cold temperatures (<1°C). While the very cold temperatures and decreased biological activity may have contributed to the poor removals, they cited the high uniformity coefficient of the sand as the primary cause (UC 3.3-3.8; ES 0.2-0.3). In a rapid biological sand filter (0.45 mm ES, 6.3 m/h), Swertfeger *et al.* (1999) found that average *Giardia* cyst removal was better at 23 vs. 14°C (>4.4 vs. 2.7 logs) while removal of *Cryptosporidium* oocysts remained unchanged (2.7 vs. 2.8 logs). They postulated that the increased cyst removal was possibly due to

greater cyst disintegration or inactivation in warmer water, or perhaps due to changes in water characteristics such as microorganism populations.

King *et al*. (2005) argued that oocyst inactivation increases at warmer water temperatures because the finite carbohydrate (amylopectin) reserves in the oocyst are used up more quickly. When the energy reserve falls below a critical level, sporozoites lack sufficient energy to break free of the oocysts and commence infection (King *et al*., 2005).

2.3.5 Mechanisms of Removal

Oocysts and cysts can be removed by granular media filters by physical (straining), physiochemical (adsorption), and biological (predation) mechanisms. Mechanisms that facilitate entrapment in the filter bed will be discussed, followed by biological mechanisms that serve to degrade or inactivate the protozoan parasites.

2.3.5.1 Physical Straining and Adsorption

Physical straining of oocysts and cysts has been commonly cited as a primary removal mechanism because of their relatively large size compared to the sand media (Dullemont *et al*., 2006; Hijnen *et al*., 2005; Tufenkji *et al*., 2004; Hijnen *et al*., 2004). In addition to straining, physiochemical removal via adsorption is important for the removal of protozoan oo/cysts by SSF (Hijnen *et al*., 2004). Both attractive and repulsive forces influence adsorption.

Cryptosporidium oocysts have a negative surface charge, with a point of zero charge at a pH of 3 to 3.5 (Considine *et al.*, 2002; Tufenkji *et al*., 2004). Therefore, at natural water pH, oocysts and sand grains are negatively charged and experience electrostatic repulsion. In agreement with the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, which models adsorption based on the sum of attractive van der Waals forces and repulsive electrostatic forces, higher ionic strengths compacts the double layers, allowing oocysts to approach sand grains more closely (Considine *et al.*, 2002). This double layer compression and shorter separation distance leads to improved adsorption to filter media (Tufenkji and Elimelech, 2005; Tufenkji *et al*., 2004). However, studies have shown that the DLVO theory under predicts the repulsive forces and thus over

predicts attachment. Non-DLVO forces such as steric repulsion are likely present between oocysts and sand grains (Considine *et al.*, 2002).

The wall of a *Cryptosporidium* oocyst is 40 to 50 nm thick and consists of an inner and outer layer with a thin lipid layer in between. The outer wall is 5 to 10 nm thick and thought to consist primarily of acidic glycoproteins, which include ionizable carboxylic acid groups that give the oocyst its surface charge (Byrd and Walz, 2005; Considine *et al.*, 2002). However, these surface proteins anchored to the surface are thought to extend into solution because of repulsion between surface ionizable groups. This gives rise to a brush-like, or "hairy" layer on the oocyst surface (Byrd and Walz, 2005; Considine *et al.*, 2002). The proteins that make up the hairy layer impart steric repulsion between the oocyst and sand surface, thereby impairing adsorption (Byrd and Walz, 2005; Kunzar and Elimelech, 2005; Considine *et al.*, 2002). The steric repulsion, characterized as large and long-range by Byrd and Walz (2005), exits in addition to the weaker electrostatic repulsion forces.

Despite the steric repulsion forces, Considine *et al.* (2002) demonstrated that surface proteins occasionally adsorb irreversibly to the sand grains, thereby preventing oocyst detachment. This protein tethering can extend several tens of nanometers out from the oocyst to the grain surface. Tufenkji and Elimelech (2005) also showed that protein tethering causes irreversible attachment, as evidenced by elution experiments in which oocysts previously retained in a column of glass beads were slowly and incompletely released, compared to rapid release of retained microspheres. That some oocysts were irreversibly retained in the filter was attributed to the adsorption of oocyst surface proteins to the collectors.

Although some attachment can occur by protein tethering, overall oocyst removal can be improved by eliminating the hairy layer. Kunzar and Elimelech (2005) concluded that adsorption of formalin- and heat-inactivated oocysts to granular media was enhanced compared to viable oocysts because the inactivation treatments disrupted surface proteins, thereby reducing steric repulsion forces. They found that formalin inactivation of oocysts caused the proteins in the hairy layer to cross-link instead of extending into solution. Likewise, heat inactivation of oocysts

caused the denaturation of the surface proteins. It should be noted that formalin or heat inactivation of oocysts was not shown to affect their electrostatic properties since zeta potential remained unchanged compared to viable oocysts (Kunzar and Elimelech, 2005; Considine *et al.*, 2002). Emelko (2003) also reported that formalin-inactivated oocysts are good surrogates of viable oocysts in filtration experiments.

2.3.5.2 Biological Mechanisms and Predation

Biological removal mechanisms play an important role for the elimination of protozoan parasites once they are entrapped or are residing in SSFs. Dullemont *et al.* (2006) seeded a SSF with *C. parvum* oocysts for 100 days and reported that extraction from sand cores revealed that nearly all of the retained oocysts had disappeared over time. After 180 days, 1.6% of the total seeded oocysts were recovered in the top 30 cm of the sand bed, and after 252 days only 0.2% could be recovered. They attributed the elimination of oocysts retained in the SSF to degradation and predation.

Similarly, Heller and Brito (2006) reported that sand cores showed no accumulation of *Cryptosporidium* oocysts in SSF beds during seeding experiments, even though good removal was achieved. They suggested a biological control mechanism such as predation or degradation was at work inside the SSF. Others have suggested that higher inactivation of oocysts at warmer temperatures is due to biological mechanisms such as predation by larger organisms that exist in larger numbers and are more active at warmer temperatures (King *et al.,* 2005; Chauret *et al.,* 1998). In fact, numerous studies have shown that protozoa and rotifers prey upon oocysts.

Stott *et al.* (2003) showed that free living ciliated protozoa, rotifers, and an amoeba common to aquatic environments ingested up to 2 oocysts per individual after a 1 hour exposure to a prey density of 200 oocysts. They demonstrated that the mean rate of oocyst ingestion by the cilate *Paramecium caudatum* increased from 0.6 to ~70 oocysts/cell/hour as prey density increased from 10^1 to 10^3 oocysts. The ciliated protozoa were shown to ingest more oocysts than the rotifers or amoeba included in the experiment. This correlates well with the study by Fayer *et al.* (2000), who found that all six genera of rotifers tested preyed upon *C. parvum* oocysts and

ingested up to 25 oocysts per rotifer when exposed to a prey density of 10^4 oocysts over an unspecified period of time. Stott *et al*. (2001) tested four species of free-living ciliated protozoa and reported that the mean ingestion rate varied by species and ranged from 4 to 170 oocysts/cell/hour at a prey density of 10^3 oocysts. At this rate, they estimated that a typical population of ciliated protozoa in a constructed wetland would be capable of removing up to 5000 oocysts/mL every hour (Stott *et al*., 2001).

Despite the documented predation of oocysts by ciliated protozoa and rotifers, the fate of ingested oocysts is unknown. The studies did not determine if oocysts were digested or inactivated after ingestion, but did find that oocysts of unknown viability were excreted in boluses by rotifers and in fecal pellets by protozoa (Stott *et al*., 2003; Fayer *et al*., 2000). Therefore, oocyst predation may implicate protozoa and rotifers in the transmission of *Cryptosporidium* in aquatic environments (Stott *et al*., 2003). Fortunately in the case of slow sand filtration, neither the predatory protozoa or rotifers or aggregated excreted oocysts would likely pass through to the filter effluent.

2.4 Viruses

As detection methods have improved over the last two decades, more types of viruses have been discovered and more waterborne illnesses attributed to viral contamination. Viruses are responsible for 80% of disease outbreaks for which the microorganisms responsible were identified (Ryan *et al*., 2002).

2.4.1 Virus Morphology

Viruses range in size from 25 nm (e.g. poliovirus) to 200 nm (e.g. smallpox) (Madigan, 2003). Viruses can be classified by the host they infect: bacteria, animal cells (insects and mammals), and plant cells. All contain genetic information in the form of nucleic acid (single- or double-stranded DNA or RNA) inside a protective protein coat called a capsid (Madigan, 2003). Viruses that infect animal cells commonly have a lipid bilayer around the capsid (Madigan, 2003).

If the nucleic acid is damaged (by disinfectant chemicals, extreme pH, UV irradiation, drying, etc.), the virus loses its ability to infect a host. The outer surface of the virus protein coat

contains ionizable functional groups such as carboxyl and amino groups, which cause the virus to be positively or negatively charged, depending on the water pH and ionic strength of the water (Madigan, 2003; Schijven *et al.*, 2000). The pH at which the virus has no net charge is called the isoelectric pH (IEP) and varies among different virus types and even among different strains of the same virus type (Harvey *et al.*, 2004).

2.4.2 Waterbourne Human Enteric Viruses

Enteric viruses infect host cells in the gastrointestinal tract of humans and animals. More than 140 different enteric viruses have been documented to infect humans, and many cannot be cultured (AWWA, 1999). Enteric viruses are transmitted by the faecal-oral route (e.g. contaminated water, food, hands, etc.) and cause nausea, vomiting, and diarrhea that can lead to death in severe cases (Health Canada, 2004a). Numerous waterborne enteric virus outbreaks occur worldwide, including the United States and Canada (Table 2-6).

Table 2-6: Waterborne enteric virus outbreaks in the United States and Canada

	Time Period	Number of Reported Waterborne Viral Outbreaks*	Number of Confirmed Cases of Viral Illness	Enteric Virus Responsible**
USA	1980-1994	28	11,200	Noroviruses
Canada	1974-1995	21	1,400	Hepatitis A Rotaviruses

Source: Health Canada (2004a)
* Many waterborne outbreaks of unknown etiology were also reported; waterborne outbreaks reported for public and private water supplies.
** Both US and Canadian outbreaks

Infected individuals can excrete over one billion infectious viruses per gram of feces (Health Canada, 2004a). Enteric viruses can survive for several months in surface water and have been reported to remain infectious for up to 2 years in 10°C groundwater (Health Canada, 2004a). Furthermore, they can survive some types of drinking water treatment, including filtration and inadequate disinfection, and have been detected in treated drinking water samples that were free of coliform bacteria (Health Canada, 2004a). Therefore, although the presence of *E. coli* in water

can indicate faecal contamination, the absence of *E. coli* does not guarantee that the more resistant enteric viruses are absent. Waterbourne enteric viruses that cause illness in humans include enteroviruses, noroviruses, and rotaviruses.

2.4.2.1 Enteroviruses

Enteroviruses are one of the most common causes of human viral infections worldwide and are estimated to cause 30 million infections in the United States each year, although most infections are unreported (due to absent or self-limiting symptoms) or may have unidentified etiology (WHO, 2006). Enteroviruses are a large group of approximately 69 virus species that infect humans, including poliovirus types 1 to 3, hepatitis viruses types A and E, coxsackievirus types B1 to B6, echovirus types 1 to 33, and other ungrouped enteroviruses (WHO, 2005). They are 20 to 30 nm in diameter, non-enveloped, single-stranded RNA viruses with icosahedral symmetry that are resistant to inactivation in the environment and disinfection (Health Canada, 2004a; WHO, 2006).

Transmission is by person-to-person contact, inhalation of airborne viruses, and consumption of contaminated water (WHO, 2005). Once ingested, enteroviruses can survive transit through the stomach to the lower intestinal tract and cause illness after a one to two week incubation period. Although illness is usually mild and self-limiting, serious illness can include passage into the bloodstream, organ damage, paralysis, meningitis, foot-and-mouth disease, and neonatal multi-organ failure (WHO, 2005).

Of the five hepatitis viruses, only Hepatitis A (HAV) and E (HEV) are enteric and can be transmitted to humans via the fecal-oral route. Hepatitis viruses belong to the enterovirus group and are about 27 nm in diameter (Health Canada, 2004a). Both HAV and HEV are highly infectious, leading to fever, fatigue, nausea and abdominal pain about 30 days after exposure (WHO, 2006). Liver damage causing death occurs in 2% of people infected with HAV (WHO, 2006). For HEV, the fatality rate is 0.1-1%, except in pregnant women, for whom the fatality rate can approach 20% (Health Canada, 2004a). Although HEV has caused a vast number of very large outbreaks worldwide, no outbreaks are reported to have occurred in North America.

2.4.2.2 Noroviruses

Noroviruses (also known as Norwalk-like viruses) have been widely associated with waterborne and foodborne disease (Montgomery, 2005) and may be responsible for more than 50% of epidemic nonbacterial gastroenteritis in the United States (Redman *et al.*, 1997). Illness occurs within 24 to 48 hours and can last for a few days, although virus shedding can continue for up to two weeks after infection (Montgomery, 2005). Symptoms include nausea, vomiting, diarrhea, and abdominal cramps. Noroviruses are environmentally resistant and may survive chlorination, freezing, and heating (Health Canada, 2004a; WHO, 2006). They are non-enveloped, spherical, single-stranded RNA icosahedral viruses 27 to 32 nm in diameter (Health Canada, 2004a).

2.4.2.3 Rotaviruses

Rotaviruses are the single most important cause of infant death in the world (WHO, 2005). They are the leading cause of severe diarrhea among children and 3.5 million infections occur each year in the United States (Health Canada, 2004a). It is estimated that rotaviruses cause 30 to 50% of all cases of severe diarrheal disease in humans (Montgomery, 2005). Although person-to-person contact and inhalation are the primary transmission routes, exposure to contaminated water and food are also significant (WHO, 2006). After an incubation period of one to two days, illness and excretion of up to 10^{11} viruses per gram of feces lasts for about 8 days (Health Canada, 2004a).

Rotaviruses are double-stranded RNA viruses approximately 70 nm in diameter that have a triple-layered protein coat and are wheel-shaped (hence the name "rota") (Health Canada, 2004a). They are important in drinking water because evidence suggests that rotaviruses are more resistant to disinfection than other enteric viruses (WHO, 2006).

2.4.3 Bacteriophage

Viruses that infect bacteria are called bacteriophage, a word derived from the Greek word "phagein" ("to eat"). Bacteriophage (phage) are harmless to humans as they cannot infect human cells because only host bacteria contain the receptor sites needed for virus attachment and infection. They are valuable as models or surrogates of some human viruses because of their similar shape, size, transport in the environment, ease of measurement, and lack of health risks

(Schijven *et al*., 2000). Phage can be prepared in high concentration stock solutions up to 10^{12} viruses per mL and enumeration is rapid, inexpensive, and reliable.

2.4.3.1 Bacteriophage as Surrogates for Human Enteric Viruses

Bacteriophage are typically removed less than human enteric viruses during passage through granular media (Table 2-7). Therefore, bacteriophage are good conservative model viruses (Schijven *et al*., 2000).

Table 2-7: Comparative removal of viruses in filtration studies

Study Author	Experimental Conditions	Comparative Virus Removal*
Bales *et al*. (1993)	pH 7, silica	Polio > MS2
Bales *et al*. (1993)	pH 5, silica	MS2 > polio
Funderberg *et al*. (1981)	8 soils	Polio > φX174
Kinoshita *et al*. (1993)	3 sands, pH 5.7-8	PRD1 > MS2
Powelson *et al*. (1993)	sand, pH 7.2	PRD1 > MS2
Bales *et al*. (1991)	silica, pH 5.5	PRD1 > MS2
Jin *et al*. (1997)	sand	φX174 > MS2
DeBorde *et al*. (1998)	sand, pH 6 to 7	φX174 > MS2
Schijven *et al*. (1999)	sand, pH 7.5	MS2 = PRD2
Dowd *et al*. (1998)	sand, pH 7.1	MS2 > PRD1 > φX174
Penrod *et al*. (1996)	sand, pH 5 to 7	Norwalk virus > MS2
Redman *et al*. (1997)	sand, pH 5 to 8	Norwalk virus > MS2
Sobsey *et al*. (1995)	muck 5°C	hep A > echo 1 > polio 1 > MS2
DeBorde *et al*. (1999)	sand-gravel, pH 7	polio 1 > φ174 > PRD1 > MS2
Bradford *et al*. (1993)		polio 1 > rotavirus > MS2
Farrah *et al*. (1993)	sand, Fe-oxide	polio 1 = coxsackie > MS2
Herbold *et al*. (1991)	coarse sand	rotavirus > φX174 > MS2
Goyal and Gerba (1979)	sand-clay	rotavirus > coxsackie > MS2

* All are human enteric viruses, except bacteriophages MS2, φX174 and PRD1

In Table 2-7, bacteriophage MS2, φX174, and PRD1 were generally removed less than human enteric viruses (polioviruses, echoviruses, coxsackieviruses, rotaviruses, noroviruses, and hepatitis A) under a variety of experimental conditions. A good model virus must adsorb less and be inactivated less (i.e. survive longer) than other viruses. Generally, MS2 meets the

requirements of low removal and long survival and is therefore a good conservative model virus (Schijven *et al*., 2000).

2.4.3.2 MS2

MS2 is an icosahedral phage 26 nm in diameter that contains single-stranded RNA inside a protein coat (Madigan, 2003). MS2 is widely used as a model virus and has an isoelectric pH (IEP) of 3.9, meaning that the surface charge is positive at a water pH below 3.9 and negative above pH 3.9 (Schijven *et al*., 2000). Therefore, MS2 has a strong negative charge at a natural water pH of 7. The IEP of MS2 is lower than many other human enteric viruses and bacteriophage (Table 2-8).

Table 2-8: Comparison of virus properties

	Size (nm)	Shape	Lipid Envelope	Nucleic Acid	Isoelectric pH (IEP)
Human Enteric Viruses					
Enteroviruses:					
Polio					6.6
Coxsackie	20-30	Icosahedral	No	ss RNA	5-6
Echo					5-6.4
Hepatitis A & E					
Noroviruses	27-32	Circular	No	ss RNA	5*
Rotaviruses	70	Wheel	Yes	ds RNA	-
Bacteriophage					
MS2	26	Icosahedral	No	ss RNA	3.9
φX174	25	Icosahedral	No	ss DNA	6.6
PRD1	62	Icosahedral	Yes	ds DNA	3-4

Adapted from Gerba (1984)
* from Redman *et al.* (1997)

The low IEP and negative surface charge causes MS2 to be repelled from granular media surfaces, which commonly have a negative charge at pH 7 (Gerba, 1984). This results in poor attachment of MS2 during drinking water filtration or flow through groundwater aquifers (Table 2-7). Studies have shown that the attachment of MS2 to granular media is less than most other viruses, making it a good conservative model for the removal of human viruses (Bales *et al*., 1993; DeBorde *et al*., 1999; Goyal and Gerba, 1979).

MS2 is part of a larger group called male specific F-RNA bacteriophage (Madigan, 2003). This group only attaches to the fertility (F) sex pili of "male" strains of host bacteria (Madigan, 2003). In the case of *E. coli*, fertility pili are produced only at temperatures above 30ºC (Grabow, 2001). Therefore, replication of F-RNA phage such as MS2 occurs exclusively in the intestinal tract of warm-blooded animals where host *E. coli*. are present and temperatures are high enough for the host to form pili (Grabow, 2001).

F-RNA male-specific coliphage such as MS2 are among the best models/surrogates for enteric viruses such as polioviruses because (i) they both reproduce in the intestinal tract, (ii) they both fail to multiply in the environment, (iii) they are similar in size and structure, and (iv) F-RNA phage are more resistant to water treatment and disinfection (Grabow, 2001; Schijven *et al*., 2000).

It has been recommended that other coliphage be co-injected with MS2 in filtration and groundwater studies in order to cover a wider range of virus transport and removal behaviours. Schijven *et al*. (2000) recommend that φX174, which has an IEP of 6.6 and therefore only a slight negative charge at natural water pH, and PRD1, which has a large diameter (62 nm), be co-injected as a cocktail with MS2. The use of multiple bacteriophage with a broader spectrum of properties would serve as a better model system for human enteric viruses (Schijven *et al*., 2000). For example, PRD1 is a better surrogate than MS2 for some larger human enteric viruses such as adenoviruses (80 nm) and rotaviruses (70 nm) (Harvey *et al*., 2004).

2.5 Virus Removal and Inactivation by Slow Sand Filtration

Slow sand filtration employs both biological processes (predation and inactivation by microbial enzymes) and physio-chemical processes (adsorption/attachment to sand grains and biofilms) in the removal and inactivation of viruses. Note that the terms adsorption and attachment are used in the sections below. Although it may not be appropriate to use the term adsorption instead of attachment for colloid/virus transport, the term adorption has been used below if it was used in the literature sources cited. Published literature reviews have credited slow sand filtration with enteric virus removals of 2 to 4 logs (Amy *et al*., 2006) and <1 to 3 logs (Rachwal *et al*., 1996).

While attachment to sand grains and biofilms contributes to virus removal and inactivation, some have reported that elimination of viruses by biological processes may be an equally important process during slow sand filtration (McConnell *et al*., 1984; Poynter and Slade, 1977; Slade, 1978) (Table 2-9).

Table 2-9: Mechanisms that contribute to virus removal/inactivation during slow sand filtration

Removal Mechanism	Influence
Predation	Filter feeding protozoa and bacteria can ingest viruses (Kim and Unno, 1996; Cliver and Herrman, 1972)
Biological activity	Increased biological activity and longer residence time in non-sterile water leads to increased virus inactivation (Poynter and Slade, 1977; Elliott *et al*., 2006)
Bacteria and aerobic microorganisms	Bacteria and aerobic microorganisms can inactivate viruses (Deng and Cliver, 1995; Hurst *et al*., 1980; Jansons *et al*., 1989; Quanrud *et al*., 2003)
Bacterial enzymes	Bacterial enzymes can inactivate viruses (Cliver and Herrmann, 1972; Deng and Cliver, 1992; Nasser *et al*., 2002; Ward *et al*., 1986)
Attachment to biofilms	Viruses can be entrapped in or adsorbed onto biofilms (Storey and Ashbolt, 2001 and 2003; Wheeler *et al*., 1988)
Adsorption/attachment to granular media	Viruses undergo reversible adsorption/attachment; long-term detachment has been observed after seeding stops (Schijven *et al*., 1999, 2001, 2002, 2003; Dullemont *et al*., 2006; Hijnen *et al*., 2004; Dizer *et al*., 2004)

In general, viruses can be removed or inactivated in a SSF bed by biological removal mechanisms such as predation and antagonism by other microorganisms and substances they produce. Important physical removal mechanisms include attachment to filter media and biofilms. Several factors influence the removal/inactivation of viruses during slow sand filtration (Table 2-10).

Table 2-10: Influence of selected factors on virus removal and inactivation by SSF

Factor	Influence
Hydraulic loading rate	Major importance; lower HLRs promote greater virus removals due to increased residence time in the SSF Range tested: 0.05-0.5 m/h (Poynter and Slade, 1977; Wang *et al*., 1981; Lance *et al*., 1982)
Water temperature	Major importance; warmer temperatures increase virus removals due to increased biological activity Range tested: 10-20°C (Poynter and Slade, 1977; Dullemont *et al*., 2006)
Sand depth	Greater virus removals observed for SSFs with deeper beds, likely due to increased residence time in the SSF and increased opportunities for attachment Range tested: >30 cm (Poynter and Slade, 1977; Slade, 1978; Graham *et al*., 1996)
Biological maturity	Virus removal increases with increased biological maturity (Dizer *et al*., 2004; Windle-Taylor, 1969; Poynter and Slade, 1977; Wheeler *et al*., 1988)
Schmutzdecke scraping	No major effect on virus removals; SSF draining >24 h for cleaning may desiccate microbes in the bed (Hijnen *et al*., 2004; Dullemont *et al*., 2006; Slade, 1978; McConnell *et al*., 1984; Poynter and Slade, 1977; Ellis, 1985)

Studies in the literature demonstrate that virus removal improves with increased sand depth, lower hydraulic loading rates. Warmer water temperatures and increased biological maturity of the SSF also improve virus removal by facilitating both biological and physical removal mechanisms. Schmutzdecke scraping was not reported to have a major effect on virus removal. In general, literature recommended bed depths (~1m) and hydraulic loading rates (0.1-0.4 m/h) should be employed to optimize virus removal by SSF, especially in cold water conditions (when biological activity would be low) or if the SSF is not sufficiently mature. A review of findings in the literature is presented below.

2.5.1 Predation

During their seeding experiments in pilot slow sand filters (SSFs), Poynter and Slade (1977) found that the inactivation rates of poliovirus-1 in non-sterile raw water were much greater than

in raw water that had been sterilized by passage through a membrane. Furthermore, inactivation was highest at warm water temperatures and varied at different times of the year. The high poliovirus inactivation in non-sterile water and at warm temperatures was attributed to biological activity in the water (Poynter and Slade, 1977), particularly due to predation by protozoa living in the SSF water (Poynter, 1966; Windle-Taylor, 1969). Windle-Taylor (1969) observed poliovirus-1 inactivation of 75% per day at 4 to 5°C and near complete removal in 24 hours at 20°C, which he attributed to predation by protozoa.

Elliott *et al*. (2006) demonstrated that removals of *E. coli*, bacteriophage MS2 and PRD1, and a human enteric virus (echovirus type 12) by intermittent household scale "biosand" slow sand filters increased with greater residence time in the filters. The water retained in the filter beds was allowed to sit idle overnight, thus providing long residence times for the seeded microorganisms to be eliminated by predation or attachment to biofilms and media grains. Removals increased as the filters became biologically mature, further suggesting biological mechanisms of removal (Elliott *et al*., 2006).

Lloyd (1996) reported that protozoa and rotifers are the numerically dominant microfauna in slow sand filters and that protozoan ciliate suspension feeders are largely confined to the top 5 to 10 cm of the sand bed.

It has also been shown that filter-feeding microbes can ingest and inactivate viruses. In a study by Kim and Unno (1996), filter feeding protozoa and rotifers were shown to consume and inactivate viruses. Polioviruses (10^4 PFU/mL) were inoculated into separate flasks of 27°C wastewater containing bacterial flocs, dispersed bacteria, and either filter feeding protozoa (*T. pyriformis*), filter feeding metazoa (*P. erythrophthalma*), or detritus feeding protozoa (*A. hemprichi*). Poliovirus concentrations decreased 50-60% during the first hour of contact with the filter feeders, then decreased up to 80-90% after an additional 6 hours. The initial rapid decrease of poliovirus concentration was attributed to adsorption to bacterial flocs (and to a lesser extent bacteria cells), whereas the later gradual decrease was attributed to predation by the filter feeders (Kim and Unno, 1996). The filter feeders likely consumed free viruses from the liquid phase as

well as those adsorbed to the surface of bacteria cells. The detritus feeding protozoa were less effective predators because the liquid-phase viruses first had to adsorb to flocs or bacterial cells before they could be ingested (Kim and Unno, 1996). They characterized virus adsorption to the surface of bacteria cells as reversible but did not report if bacteria could inactivate viruses.

Cliver and Herrman (1972) demonstrated that bacteria can inactivate viruses by using them as substrate. They inoculated coxsackievirus A9 with tracer labeled ^{32}P (in its nucleic acid) and ^{14}C-leucine (in its protein coat) into a solution of *P. aeruginosa* bacteria. Over time, Cliver and Herrman (1972) detected the viral ^{14}C-leucine in the cell components of the living bacteria, which suggested that virus protein coats may have served as a substrate for bacterial growth. However, viral labeled ^{32}P was not detected in the living bacteria, which suggested that viral nucleic acid was not broken down enough to permit phosphorus uptake (Cliver and Herrman, 1972).

Deng and Cliver (1995) found that hepatitis A viruses were inactivated by bacteria found in sewage. Hurst *et al*. (1980) found that aerobic microorganisms inactivated poliovirus but anaerobic microorganisms did not. Jansons *et al*. (1989) showed that increased groundwater dissolved oxygen concentrations led to increased virus inactivation because of enhanced microbial activity or direct oxidation of virus capsid components. Quanrud *et al*. (2003) demonstrated that killing aerobic microbes significantly decreased poliovirus and coliphage removal during flow through a sand column.

2.5.2 Inactivation by Bacterial Enzymes

It has also been shown that bacteria can inactivate viruses with extracellular enzymes. Cliver and Herrmann (1972) demonstrated that out of seven bacterial species tested, only two (*B. subtilis* and *P. aeruginosa*) inactivated coxsackievirus A9. They inferred that inactivation was due to hydrolysis of the viral protein coat by enzymes and other substances produced by the bacteria and not by adsorption to the bacterial cells (Cliver and Herrmann, 1972). Similarly, in the study by Deng and Cliver (1992), inactivation of poliovirus-1 in sewage was partly attributed to the virucidal effects of proteolytic enzymes produced by bacteria.

The study by Nasser *et al*. (2002) demonstrated that the inactivation of four viruses by bacterial enzymes depended strongly on virus type. In the first experiment, viruses were incubated for 30 minutes in pure bacterial enzymes (pronase or elastase). Both enzymes are capable of cleaving components of viral protein coats, thereby exposing viral nucleic acid to the destructive effects of nuclease enzymes and other substances (Nasser *et al*., 2002). They found that the protease enzyme caused 90% inactivation of coxsackievirus A9 but had no effect on the survival of hepatitis A, poliovirus-1, or MS2 phage. Cliver and Herrmann (1972) also found that pronase and seven other enzymes inactivated coxsackievirus A9 but not poliovirus-1. The elastase enzyme caused 99% inactivation of coxsackievirus A9, but failed to inactivate poliovirus-1 or MS2 (Nasser *et al*., 2002). Since all the viruses tested had protein coats and single-stranded RNA nucleic acid, Nasser *et al*. (2002) concluded that the coxsackievirus A9 was more susceptible to inactivation by the bacterial enzymes because the enzymes destroyed some component of its protein coat that the other viruses did not have. That different viruses have different components on the surface of their protein coats is not surprising since viruses are known to have different isoelectric points, which arise from various functional groups on the protein coat (Nasser *et al*., 2002). This suggests that virus inactivation by bacterial enzymes during slow sand filtration likely differs according to virus type.

In support of the findings of Nasser *et al*. (2002), Ward *et al*. (1986) showed that inactivation of radioactively labeled echovirus-12 in surface water was caused by the cleavage of capsid proteins by short-lived dispersed enzymes or bacteria-associated enzymes, followed by the breakdown of viral RNA by nuclease enzymes. Since no labeled virus components were found inside living bacteria, they hypothesized that virus inactivation occurred outside bacterial cells, likely during physical contact with enzymes on bacterial surfaces (Ward *et al*., 1986). They also showed that 22 of 27 bacterial strains isolated from a surface water caused 1 to >3 log inactivation of four enteric viruses (poliovirus-1, coxsackievirus B5, rotavirus SA-11, and echovirus-12) after 24 hours of exposure. Virus inactivation was shown to increase at higher temperatures, which was attributed to increased bacterial populations and not heat-induced inactivation (Ward *et al*., 1986). When bacteria were inactivated or removed (by heat, UV,

hypochlorous acid, or membrane filtration), no virus inactivation was observed. This demonstrated that microorganisms in non-sterile freshwater are capable of inactivating viruses, although the magnitude of inactivation depends on virus type (Ward *et al*., 1986).

It has also been shown that substances produced by microorganisms such as humic acids, tannins, phenolics, and ascorbic acid can serve as oxidizing or reducing agents that inactivate viruses (Melnick and Gerba, 1980).

In a second experiment by Nasser *et al*. (2002), viruses were exposed to different extracellular substances, including enzymes, produced by Gram negative bacteria (*Pseudomonas aeruginosa*) in order to simulate the overall antiviral effects of microbial activity. They observed a 99% inactivation of coxsackievirus A9 and hepatitis A, compared to no inactivation of poliovirus-1 or MS2 after ~7 hours of exposure. They proposed that hepatitis A was likely inactivated by substances other than enzymes produced by *P. aeruginosa* (Nasser *et al*., 2002; Cliver and Herrmann, 1972).

2.5.3 Attachment to Biofilms

Since viruses have been shown to accumulate in distribution system biofilms, it is likely that viruses can also attach to biofilms present in slow sand filters. Biofilms have many sorption sites capable of accumulating inorganic and organic particles, including biocolloidal enteric viruses (Storey and Ashbolt, 2003). Biofilms contain many sites for adsorption/attachment of particles, particularly extracellular polymeric substances (EPS), which are a web-like matrix of polysaccharides, proteins, and lipids (Flemming, 1995). Since biofilms generally have a porous, low-density structure when hydraulic shear is low and a smooth, patchy, and dense structure when shear is high, low flow rates will promote the development of porous biofilms that offer more attachment sites for particles (van Loosdrecht *et al*., 1995).

In biofims, bacteria were found to be localized in microcolonies surrounded by dense EPS matrix material (Storey and Ashbolt, 2003). In areas of lower density matrix material, biofilms were

perforated with pores and channels 20 to 100 μm in diameter which emptied into large bacteria-free voids up to 250 μm in diameter (Storey and Ashbolt, 2003).

Storey and Ashbolt (2001 and 2003) showed that bacteriophage MS2, φX147, and B40-8 attached to distribution system biofilms at concentrations equaling 1% of the bulk water concentration. The viruses were observed throughout the full depth of the biofilms and were found to penetrate both biofilm macropores (i.e. accumulate in voids) and micropores (i.e. accumulate in bacterial microcolonies) (Storey and Ashbolt, 2003).

A fraction of the bacteriophage incorporated in the biofilms remained infectious throughout the 30 day experiment, highlighting the potential for virus accumulation in biofilms over time (Storey and Ashbolt, 2003). Modeling showed that virus inactivation in the biofilms occurred rapidly at first, followed by a constant slow inactivation rate that could allow some viruses (including MS2 phage) to remain infectious for over 100 days (Storey and Ashbolt, 2003). Furthermore, the location of infectious viruses inside biofilms could protect them from disinfectants such as chlorine. It may be possible for infectious viruses that persist in biofilms to re-enter the bulk water individually or associated with sloughed biofilm (Storey and Ashbolt, 2001 and 2003). Therefore, biofilms could serve as a continuous source of viral contamination in drinking water distribution systems (Storey and Ashbolt, 2001). It is reasonable to extend theses findings to slow sand filters, where adsorption of viruses to biofilms is expected to occur throughout the full bed depth or, likely to a greater extent, in the schmutzdecke layer.

Wheeler *et al.* (1988) stated that adsorption to biomass or biofilms, microbial predation, and adsorption to non-biological surfaces are the primary mechanisms of virus elimination by slow sand filtration. They hypothesized that adsorption of viruses to biomass/biofilms is likely the most important removal mechanism because biofilms are porous and contain microbial extracellular polymers (for example, peptidoglycans and lipopolysaccharides) that serve as binding sites for virus entrapment (Wheeler *et al.*, 1988). They emphasized that the presence of microbial biomass and biofilms are at least as important for virus removal as it is for bacteria removal (Wheeler *et al.*, 1988).

2.5.4 SSF Biological Maturity

A recent study by Dizer *et al*. (2004) found that coliphage removal by a SSF increased as the filter matured. Removal of Coliphage 138 was 0.4 logs in the first experiment when a schmutzdecke layer was absent and 2 logs in the second experiment after two months of ripening when a schmutzdecke had developed.

Similarly, Windle-Taylor (1969) found that while poliovirus-1 was effectively removed by a mature SSF, the viruses were not removed by clean sterile sand in a SSF. Poynter and Slade (1977) suggested that the biomass in mature SSFs is responsible for the increased removal of viruses and bacteria compared to immature filters. They also noted that warm water conditions accelerated SSF maturation, as would be expected.

A study by Wheeler *et al*. (1988) demonstrated that the schmutzdecke layer and biological maturity of SSFs were responsible for virus removal. In one experiment, Wheeler *et al*. (1988) showed that a biologically mature SSF with a schmutzdecke layer removed simian rotavirus SA11 concentrations by 1 log, compared to no removal in an acid washed sand filter or a clean (sterile) sand filter for the same detention time. This implies an important role of microorganisms in the schmutzdecke and filter bed for virus removal.

In addition, a mature SSF would be expected to have improved physical filtration capacity due to accumulation of material in the sand bed, including extracellular polymeric substances produced by the resident microorganisms and other organic and inorganic matter.

2.5.5 Schmutzdecke Scraping

In the highly biologically active schmutzdecke layer (and, to a lesser extent, deeper in the sand bed), adsorption to biomass or biofilms, microbial predation, and adsorption to non-biological surfaces are considered the primary mechanisms of bacteria and virus elimination by slow sand filtration (Wheeler *et al*., 1988).

Lloyd (1996) noted that protozoa and rotifers are the numerically dominant microfauna in slow sand filters and that protozoan ciliate suspension feeders are largely confined to the top 5 to 10 cm of the sand bed. Lloyd (1996) found when filter beds were drained for cleaning, rapid desiccation of these ciliate protozoa can occur and lead to reduced predation and impaired filter performance (microorganism breakthrough) upon startup.

Sanchez et al. (2006) found that scraping of pilot SSFs reduced protozoa populations on the surface of the sand bed from approximately 32,000 to 0 protozoa/cm^2, from 66,000 to 0 protozoa/cm^2, and from 30,000 to 3,700 protozoa/cm^2 in three different experiments. The impact of SSF cleaning on protozoa was less severe when less sand was scraped off. Protozoa populations returned to pre-scraping levels after 68 to 320 hours of operation (Sanchez et al., 2006).

However, the studies by Lloyd (1996) and Sanchez et al. (2006) did not actually test the effect of schmutzdecke scraping on slow sand filter performance as measured by virus and bacteria removal. Numerous other studies have shown that scraping the schmutzdecke layer has a negligible effect on virus removals (Hijnen et al., 2004; Dullemont et al., 2006; Slade, 1978; McConnell et al., 1984; Poynter and Slade, 1977).

Hijnen et al. (2004) reported that schmutzdecke scraping had a marginal effect on MS2 removal by two pilot scale SSFs. Schmutzdecke scraping occurred between tests A and B and again between tests C and D (Table 2-11). As shown in Table 2-11, the MS2 removal by filter 1 (tests A and B) decreased after the schmutzdecke was scraped, which may have been partly due to a decrease in biological activity in the filter due to a drop in temperature (Dullemont et al., 2006). The same was found in filter 2, where schmutzdecke scraping between tests C and D had no major impact on MS2 removal but did cause a 1 to 2 log decrease in *E. coli* WR1 removal.

Table 2-11: MS2 removal after schmutzdecke scraping

Filter	Test #	Water Temperature (°C)	Schmutzdecke Age (days)	MS2 Removal (logs)		E. coli WR1 Removal (logs)
				Low C_{in}*	High C_{in}*	Low C_{in}
1	A	14	553	-	3.5	-
	B	10	12	1.7	1.8	2.1
2	C	10	81	1.8	2.2	3.9
	D	10	4	1.7	1.9	2.0

Adapted from Hijnen *et al*. (2004) and Dullemont *et al.* (2006)
* Low and high influent concentrations were approximately $5x10^2$ and $5x10^5$ PFU/mL, respectively; removal of MS2 and *E. coli* WR1 did not depend on the seeding concentration (Dullemont *et al.*, 2006); seeding duration was 24 hours
Notes: SSFs 1.5 m sand depth, 0.3 mm diameter effective size, operated at 0.3 m/h

Based on the results in Table 2-11, Hijnen *et al*. (2004) concluded that, in addition to biological activity, both straining and adsorption are significant removal mechanisms in SSFs controlling the elimination of microorganisms larger than viruses. Therefore, they argue that schmutzdecke scraping did not effect phage removal because they are too small to be strained by the schmutzdecke (Hijnen *et al*., 2004).

In a follow-up study, Dullemont *et al*. (2006) also found schmutzdecke scraping decreased MS2 removal in a pilot SSF seeded with MS2 and *E. coli* (Table 2-12). As shown in Table 2-12, while schmutzdecke scraping (between tests G and H) caused MS2 removal to decrease by 0.6 logs, *E. coli* removal decreased by ~2 logs due to less efficient straining (Dullemont *et al*., 2006). Test I shows that the schmutzdecke was restored in 53 days, as evidenced by the return of *E. coli* removal to pre-scraping (test G) levels (Dullemont *et al*., 2006). Slade (1978) also found that while schmutzdecke scraping had no effect on the removal of viruses (poliovirus-1), scraping led to decreased bacteria removals.

Table 2-12: MS2 removal after schmutzdecke scraping in warm water

	Test #	Water Temperature (°C)	Schmutzdecke Age (days)	MS2 Removal (logs)	*E. coli* WR1 Removal (logs)
Filter 4*	G	13	137	3.4	4.9
	H	14	4	2.8	3.1
	I	16	53	3.9	5.6

Adapted from Dullemont *et al.* (2006)
* Filter 4 with 1.5 m depth of 0.5 mm diameter sand operated at 0.3 m/h
Notes: Influent concentrations were approximately 5×10^2 PFU/mL; seeding duration was 24 hours

Poynter and Slade (1977) found that scraping of the top 2.5 cm of schmutzdecke from pilot SSFs had a negligible effect on poliovirus-1 removals for short-duration cleaning. The short duration filter cleanings lasting only a few hours caused minor decreases (0 to 0.3 logs) in poliovirus-1 removal. However, after the filters were drained for 24 hours (without schmutzdecke scraping), poliovirus-1 removals dropped by ~1.1 logs. Poynter and Slade (1977) concluded that SSF performance for microorganism removal was impacted more negatively by the duration of SSF draining than by the scraping of the schmutzdecke.

Ellis (1985) reported that SSF draining causes significant death of microorganisms living in the filters and a major removal in biological activity once the filters are put back in operation. In addition, an exposed sand surface creates highly aerobic conditions with no nutrients for microorganisms to consume, which leads to the consumption of extracellular materials as a food source (Ellis, 1985). The loss of this extracellular material leads to reduced biomass and biofilms in the filter bed and subsequent washout of microorganisms when the filter is put back in operation (Ellis, 1985). Ellis (1985) and Visscher (1990) recommended draining full-scale SSFs for a maximum of 24 hours.

2.5.6 Hydraulic Loading Rate

Poynter and Slade (1977) demonstrated that greater virus removal was achieved at an HLR of 0.2 m/h compared to 0.5 m/h over a one year study period (Table 2-13).

Table 2-13: Poliovirus removal by slow sand filtration

Season (1974)	Water Temperature (°C)	SSF Number	Hydraulic Loading Rate (m/h)	Poliovirus 1 Removal (logs)	Difference in Removal between HLRs (logs)
Winter	5 –8	1	0.2	2.5	
		2	0.5	1.8	0.7
Spring	9 – 18	1	0.2	3.7	
		2	0.5	2.9	0.8
Summer	16 - 18	1	0.2	4.5	
		2	0.4	2.9	1.6
Fall	9 - 16	1	0.2	3.3	
		2	0.4	2.4	0.9
Annual			0.2	3.5	
Average			0.4 – 0.5	2.5	1.0

Adapted from Poynter and Slade (1977)
* Slow sand filter 2 was the control filter operated at 0.2 m/h; both filters had 60cm sand depth

As shown in Table 2-13, poliovirus removal was 0.7 to 1.7 logs (average 1.0 logs) greater at an HLR of 0.2 m/h compared to 0.4-0.5 m/h, depending on water temperature. Poynter and Slade (1977) concluded that even though higher HLRs led to less efficient virus and bacteria removal, satisfactory removal of viruses and bacteria can be obtained at high HLRs and water temperatures as low as 5°C. They also noted that HLRs on the high end of typical could be safely used, especially at warmer water temperatures when the SSF biological activity and thus ability to remove viruses is at its highest (Poynter and Slade, 1977). Virus removal did drop to less than 2 logs at 0.5 m/h in cold water (Table 2-13).

Poynter and Slade (1977) also tested the impact of suddenly doubling HLR from 0.2 m/h to 0.4 m/h over a presumed period of several hours (although not specifically mentioned). They measured removals one week before and up to three weeks after the HLR increase and found that the mature SSF quickly recovered to original poliovirus-1 removals (Table 2-14). They concluded that mature SSFs can respond quickly to large, sudden changes in HLR and do not need long acclimation times at new flow rates (Poynter and Slade, 1977).

Table 2-14: Effect of doubling HLR on poliovirus removal by SSF

Week*	HLR (m/h)	Log Removal**			
		Poliovirus 1	Bacteriophage T7	*E. coli*	Coliform Bacteria
1	0.2	2.9	3.5	2.1	2.5
2	0.4	2.3	2.2	1.5	1.7
3	0.4	2.7	3.3	1.6	1.5
4	0.4	3.0	3.4	3.0	2.1

Adapted from Poynter and Slade (1977)
* Week 1 (19-25 Nov.), 2 (26-30 Nov.), 3 (3-9 Dec.) and 4 (10-16 Dec.) 1974
** Results from slow sand filter No.2 control filter, which had been operated at 0.2 m/h for one year; water temperature 9 - 11°C

Wang *et al*. (1981) seeded columns of four different sandy soils with poliovirus-1 and echovirus-1 and found that removal of both viruses decreased as the HLR increased. Linear regression analysis revealed a significant (r^2=0.83) negative correlation between virus removal and HLR ranging from 0.01 to 0.13 m/h. However, when HLR was further increased to 0.56 m/h, no significant relationship was found between virus removal and an HLR of 0.56 m/h (Wang *et al*., 1981).

An experiment by Lance *et al*. (1982) observed breakthrough of echovirus-1 from a 240 cm long column of coarse sand at HLRs of 0.05 and 0.1 m/h. At a lower HLR of 0.025 m/h, no viruses were detected below a depth of 160 cm (i.e. no viruses were detected in the effluent of the column). This demonstrated that increased HLR led to deeper penetration of viruses into the sand bed and eventual breakthrough into the column effluent.

2.5.7 Water Temperature

Numerous studies have demonstrated that higher water temperatures can lead to higher inactivation of viruses such as MS2, poliovirus-1, echovirus, bacteriophage PRD1, and hepatitis A (Yates *et al*., 1990; Yates and Gerba, 1985; Hurst *et al*., 1980; Yates *et al*., 1985; Yahya *et al*., 1993b; Sobsey *et al*., 1995). Nasser and Oman (1999) seeded viruses into various water samples and found that inactivation of hepatitis A virus and poliovirus-1 was greater at high (20-30°C) vs. low temperatures (4-10°C). They hypothesized that this was due to greater microbial activity at the higher temperatures (Nasser and Oman, 1999).

Poynter and Slade (1977) found that poliovirus removal by SSF consistently increased with increasing water temperature over a four year study period. They attributed the increased virus removals at higher temperatures to increased biological activity. Increased activity of microorganisms living in SSFs that prey on or inactivate viruses was likely the cause of the higher virus removals at higher temperatures.

An approximate 2 log increase in MS2 and *E. coli* removals were observed in warm water SSF experiments (13 to 16°C, Table 2-12) by Dullemont *et al*. (2006) compared to the experiments at cooler temperatures (10°C, Table 2-11). These enhanced microorganism removals were attributed to increased biological activity in the SSFs at warmer temperatures (Dullemont *et al*., 2006).

In a study by Yahya *et al*. (1993a), slow sand filtration reduced MS2 and PRD1 phage by 2 and 3 logs, respectively. The two slow sand filters (operated in parallel for 14 months at 0.3 m/h) had sand depths of 120 cm and surface areas of 50 ft^2 each with influent phage concentrations of 10^5 PFU/mL. Although not reported, water temperatures were likely warm because the experimental site was in Arizona.

2.5.8 Sand Bed Depth

During the 1971 study by Poynter and Slade (1974), it was determined that SSF performance was enhanced by greater sand depths and that greater sand depth could be used to offset the negative impact of high HLRs. In the study, Poynter and Slade found that average poliovirus removals were 0.5 to 1.0 logs greater in a 60 cm deep SSF compared to a 30 cm deep SSF. Slade (1978) found less poliovirus-1 removals in full-scale SSFs with bed depths of 30 and 45 cm compared to those reported by Poynter and Slade (1977) for 60 cm deep pilot-scale SSFs. The lower removals were attributed to the shallower sand depths (Slade, 1978).

In a study by Graham *et al*. (1996), virus removal was found to increase with greater SSF sand depth (Table 2-15).

Table 2-15: Effect of sand depth on poliovirus and bacteriophage removals

	Sand Depth (cm)	Poliovirus-1 Removal (logs)	Bacteriophage Removal (logs)
SSF A	20	3.2	1.9
SSF B	30	4.0	2.9
SSF C	50	>4.0	3.5

Notes: Slow sand filter; sand effective diameter 0.30 mm, uniformity coefficient 2.1; hydraulic loading rate 0.15 m/h; bacteriophage of *Serratia marsescens*

In the study by McConnell *et al*. (1984), the majority of sand-associated infectious reoviruses were detected in the top 42 cm of the SSF columns. Most (>65%) of the radio-labelled ^{125}I-reoviruses were also removed in the top 35 cm of the SSF bed. These results showed that microorganism removals were greatest in the upper portion of the SSFs (McConnell *et al*., 1984).

Although the study by Wang *et al*. (1981) did not involve SSFs, experiments on soil columns (without schmutzdecke) showed that removal of both poliovirus-1 and echovirus-1 was significantly greater in the upper 17 cm of the soil columns. Similarly, Wheeler *et al*. (1988) found the largest bacteriophage removal occurred in the top 10 cm of a biologically mature SSF, which was attributed to the high biological activity in the upper section of the SSF.

2.5.9 Adsorption

The adsorption of viruses to sand grains is typically poor due to the negative surface charges that both exhibit at natural water pH (Schijven *et al*., 2000). Adsorption can be reversible or irreversible (Montgomery, 2005). The most significant force preventing virus adsorption to a grain surface is electrostatic repulsion.

The Derjaguin-Landau-Verwey-Overbeek (DLVO) theory predicts that as like-charged particles get closer together, repulsive energy increases and creates an energy potential barrier that must be overcome in order for adsorption to occur (Schijven *et al*., 2000). If the repulsive barrier is overcome, then the proximity of the two particles allows van der Waals attraction to dominate. The result is that rapid, strong and irreversible adsorption will occur in the primary minimum (Schijven *et al*., 2000). The DLVO theory also states that a weakly attractive secondary

minimum exists at further separation distances, but adsorption in the secondary minimum is rapid and reversible (Schijven *et al*., 2000). Therefore, viruses would be removed permanently during filtration if irreversible adsorption in the primary minimum occurred, while viruses adsorbed in the secondary minimum would desorb and be washed out of the filter over time (Schijven *et al*., 2002).

In practice, the adsorption of negatively-charged viruses onto negatively-charged silica sand in a SSF bed would predominantly involve reversible adsorption to the secondary minimum, which is supported by observations of virus desorption and washout over extended periods of time following seeding experiments (Schijven *et al*., 1999; Schijven *et al*., 2001; Schijven *et al*., 2002; Schijven *et al*., 2003; Dullemont *et al*., 2006; Hijnen *et al*., 2004; Dizer *et al*., 2004).

Irreversible adsorption of viruses to filter media would be more likely to occur on areas of the grain surface where patches of high IEP material (such as iron or aluminum hydroxides) induces favourable adsorption of viruses. Silica sand and other granular media with positively charged patches of metal oxides such as iron, aluminum, and manganese have been shown to have good adsorption capacity for viruses (Schijven *et al*., 2000; Farrah and Preston, 1993).

In general, however, sandy and organic soils are poor adsorbers of viruses, while clay and metal hydroxide soils are good adsorbers (Keswick *et al*., 1980; Burge *et al*., 1978; Herbold-Pasche *et al*., 1991; Sobsey *et al*., 1995 and 1980; Meschke and Sobsey, 1998 and 2003). Clay has a good capacity to adsorb viruses because it has edges that include positively charged metal ions or metal oxides with high IEPs in the range of 8 to 9 (Harvey *et al*., 2004; Gerba, 1984; Vilker *et al*., 1983).

The DLVO theory predicts that increasing ionic strength and decreasing pH would lead to a removal in double layer thickness, reduced repulsive energy barrier, and increased adsorption to the primary minimum (Schijven *et al*., 2000). Numerous batch, column and field studies have demonstrated that decreased pH leads to improved virus adsorption to granular media (Penrod *et*

al., 1996; Redman *et al*., 1997; Bales *et al*., 1991) and that increased pH leads to detachment of previously adsorbed viruses (Bales *et al*., 1995).

It has also been demonstrated that increased ionic strength leads to increased virus adsorption to granular media (Goyal and Gerba, 1979; Bales *et al.,*1991; Lance *et al*., 1982). Multivalent cations have been shown to improve the adsorption rate of more negatively charged viruses (Schijven *et al*., 2001; Redman *et al*., 1999; Gerba, 1984; Lance *et al*., 1984; Mix, 1974; Harvey *et al*., 2004). If ionic strength is decreased, previously adsorbed viruses can detach due to double layer expansion and increased repulsion between virus and media (Bales *et al*., 1993; Lance *et al*., 1982; Funderburg *et al*., 1981).

In summary, the validity of any single model bacteriophage to predict the adsorption of enteric viruses must take into consideration the pH, ionic strength, and filter media type. The removal and inactivation of viruses by slow sand filtration depends on both physio-chemical (adsorption) and biological (predation) mechanisms.

2.6 Summary of Relevant Findings and Knowledge Gaps in the Literature

Important findings regarding the removal of *Cryptosporidium* and *Giardia* by slow sand filtration included the following:

- *Cryptosporidium* and *Giardia* were found in 14 to 100% of samples taken from the Grand River at the Mannheim intake at concentrations ranging from 20 to 100 (oo)cysts/100 L. The intake was the location where raw water was obtained for the current study.
- The USEPA gives slow sand filtration a 3 log *Cryptosporidium* oocyst treatment credit. The Ontario Ministry of Environment gives slow sand filtration a 2 log *Giardia* cyst removal credit. These regulatory credits are less than removals demonstrated in the literature.
- Studies in the literature show that removals of *Cryptosporidium* oocysts and *Giardia* cysts in biologically mature slow sand filters are typically >4 logs.
- Removal of (oo)cysts improved as SSFs became more biologically mature. As SSFs mature, the accumulation of microorganisms, biofilms, and (in)organic matter in the filter bed contribute to (oo)cyst removal by physical (and, to a lesser extent, biological) mechanisms.

- Factors that did not have a major influence on (oo)cyst removals included hydraulic loading rate (0.04-0.4 m/h), sand depth (0.5-1.5m), sand grain size (0.1-0.6 mm effective size and uniformity coefficient up to 3.5), and water temperature (1-23°C).

Knowledge gaps currently exist in the literature regarding how the removal of (oo)cysts in slow sand filters is influenced by the following parameters:

- Multiple SSFs in series
- Hydraulic loading rates at or above 0.4 m/h
- Extended duration cold water temperatures
- High influent concentrations greater than 10^3 (oo)cysts/L

Relevant findings in the literature that relate to virus removal by slow sand filtration include the following:

- Virus removals generally range from <1 to 4 logs, depending on multiple SSF variables.
- Viruses are removed/inactivated by biological mechanisms (predation by filter feeding protozoa and bacteria, inactivation by bacterial enzymes, antagonism from microorganisms in the SSF bed, etc.) and physical mechanisms (adsorption to filter media and attachment to biofilms).
- Virus removals increased with lower hydraulic loading rates, deeper sand depths, warmer water temperatures, and increased biological maturity.
- In general, bed depths in the upper range (~1m) and hydraulic loading rates in the lower range (0.1-0.4 m/h) of those recommended in the literature should be employed to optimize virus removal by SSF, especially in cold water conditions (when biological activity would be low) or if the SSF is not sufficiently mature.
- Schmutzdecke scraping was not reported to have a major effect on virus removal.

There are significant gaps in the literature regarding virus removal by slow sand filtration, including the following:

- Limited number of studies on virus removal by slow sand filtration and the effects of process variables.
- Few long-term studies that investigate the influence of the major factors that affect virus removal by SSF (hydraulic loading rate, sand depth, water temperature).
- No studies on virus removal by SSF below 10°C.
- No studies on virus removal in roughing filters.
- Few (or no) studies on virus removals in SSFs with amended filter media such as sandwich layers of granular activated carbon, iron-oxide coated sand, zero-valent iron beads, positively-charged zeolites, etc.

3.0 Materials and Methods

Two pilot-scale multistage slow sand filter systems were used in this study. The pilot systems were operated for approximately 2.5 years, during which time frequent filter performance and water quality measurements were taken. Additionally, numerous *Cryptosporidium*, *Giardia*, and MS2 bacteriophage challenge tests were performed throughout the study period.

3.1 Multistage Slow Sand Filters Pilot Systems

The pilot-scale multistage slow sand filter systems were located inside the Region of Waterloo's Lowlift Pump Station building (Kitchener, Ontario, Canada), which serves as the surface water intake for the Region's Mannheim Water Treatment Plant (Figure 3-1 and Figure 3-2). The Mannheim plant blends treated Grand River water (20%) with groundwater (80%) and services Kitchener-Waterloo and adjacent smaller communities.

Figure 3-1: Dam on the Grand River at the Mannheim intake

Figure 3-2: Lowlift pumping station and Mannheim intake

The Grand River watershed covers approximately 7000 km^2, is the largest watershed in southern Ontario, and includes a population of ~800,000 people that is projected to increase by 37% over the next 20 years (Dorner *et al*., 2007). The river is heavily impacted by agricultural and urban pollution, with 80% of the watershed used for agriculture and livestock and 20% occupied by urban centers with a net population of 500,000 people (Dorner *et al*., 2007). The Grand River receives secondary and tertiary treated wastewater from 26 sewage treatment plants (servicing 680,000 people) (Dorner *et al*., 2007), nine of which were upstream of the Mannheim intake where raw water was obtained for this study. Water quality in the Grand River is highly variable, with average turbidity of 30 NTU and recorded peaks up to 500 NTU (LeChevallier *et al*., 2002). An overview of Grand River water quality was presented by Cleary (2005).

The air temperature in the pump station building was maintained at 10°C during winter months and allowed to follow seasonal temperature changes during spring, summer and fall. The building air temperature was increased to 35°C from May 11 to June 21, 2006, during a series of warm water virus experiments. The Lowlift Pump Station takes in raw water from the Grand

50

River via an intake pipe located upstream of a dam. The raw water passes through a traveling screen to remove debris and passes through a wet well before being pumped to on-site reservoirs and then to the Mannheim plant. Pilots 1 and 2 received raw water pumped directly from the wet well.

3.1.1 Design Parameters

Pilot 1 included pre-ozonation, shallow-bed roughing filtration, and shallow-bed slow sand filtration in series. Pilot 2 included roughing filtration and two slow sand filters in series.

A comparison of design parameters used for pilots 1 and 2 to those recommended in the literature is presented in Table 3-1. As can be seen, the roughing filters (RF) of pilot 1 were designed with shallower bed depths of finer media than recommended in the literature and included granular activated carbon (GAC) caps. The pilot 1 slow sand filters (SSF) also had shallow bed depths and were operated at high hydraulic loading rates (HLRs). The RFs of pilot 2 had deep bed depths in accordance with recommendations in the literature, although roughing filter B (RFB) employed finer-than-recommended media. Lastly, Table 3-1 shows that pilot 2 slow sand filter 1 (SSF1) had a 1 m deep sand bed, whereas slow sand filter 2 (SSF2) had a shallower 0.5 m sand bed. Both pilots are described in further detail below.

Table 3-1: Roughing filter and slow sand filter design parameters

	Literature	Pilot 1	Pilot 2[+]	
		RF	RFA	RFB
Roughing Filters				
Gravel depth (m)				
Total depth	0.9 - 1.2	0.6	1.2	1.2
Top layer	0.15 - 0.2	0.3	0.3	0.55
Middle layer	0.15 - 0.2	0.2	0.4	0.55
Bottom layer	0.2 - 0.3	0.1	0.5	0.1
Media diameter (mm)				
Top layer	4 - 8	0.85 - 1.2 (GAC)	4.8 – 9.5	1.5 – 3.2
Middle layer	8 - 12	2.4 - 3.4	9.5-12.7	4.8-9.5
Bottom layer	12 - 18	8 – 12.5	12.7 – 19.1	9.5 – 12.7
HLR (m/h)*	0.3 - 1.5	0.37 - 3.0	0.47-1.35	
Slow Sand Filters		SSF	SSF1	SSF2
Sand ES (mm)*	0.15 - 0.35	0.35	0.37	0.37
UC*	below 2-3	1.7	1.7	1.7
Bed depth (m)	0.7 - 1.2	0.45	1.0	0.5
HLR (m/h)*	0.05 - 0.4	0.1 - 0.8	0.2-0.6	0.2-0.6
D/d ratio[++]	>50[++]	900	800	400

 * Hydraulic loading rate (HLR); Effective size (ES); Uniformity coefficient (UC)
[+] Roughing filter A (RFA) & B (RFB); Slow sand filter 1 (SSF1) & 2 (SSF2)
++ Column diameter (D) over media diameter (d) (Lang *et al*., 1993)
Adapted from Cleary (2005); Sources: Wegelin (1996); Galvis *et al.,* (1998); Huisman and Wood (1974)

3.1.2 Pilot 1

Pilot 1 was provided to the University of Waterloo by MS Filter Inc. (Newmarket, Ontario, Canada) and is a pilot-scale version of their commercially available full-scale multistage filtration system. It was housed in a portable trailer (Figure 3-3) and was commissioned in October 2003 and operated continuously for over 2.5 years until it was decommissioned in June 2006. Pilot 1 consisted of two identical trains (trains 1 and 2) that received the same raw water but were operated independently (Figure 3-4).

Figure 3-3: Pilot 1 trailer (Cleary, 2005)
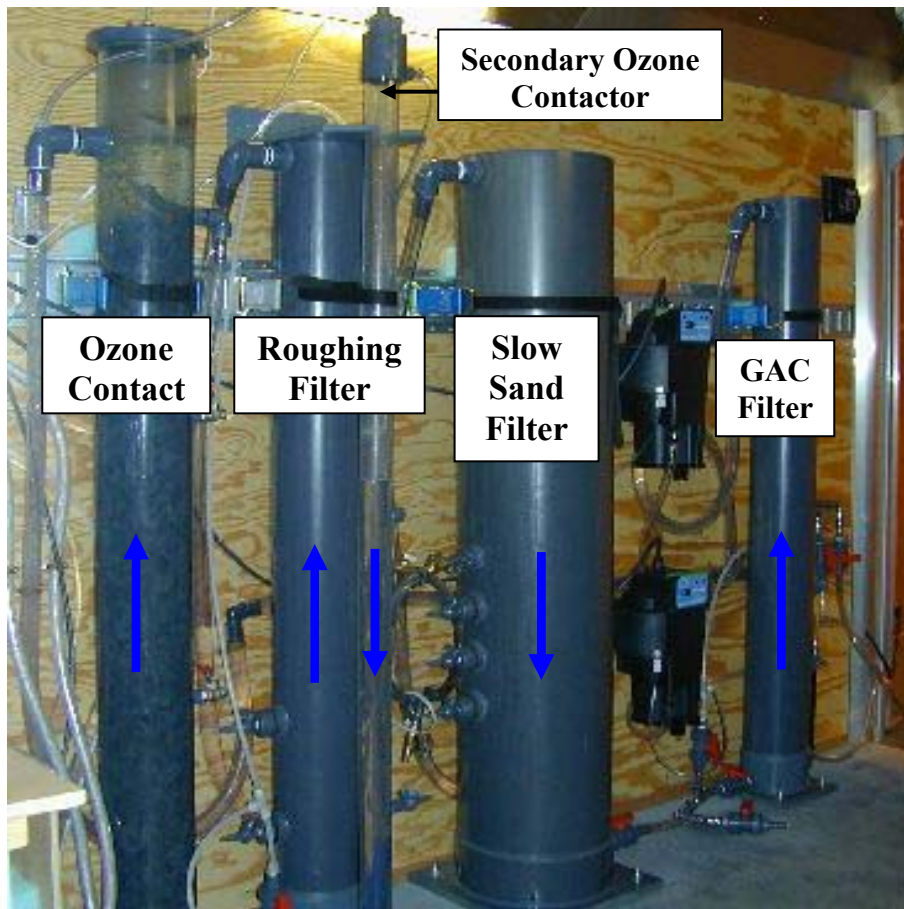
Figure 3-4: Pilot 1 treatment train photograph (Cleary, 2005)

Each train of pilot 1 included an upflow pre-ozonation column, a downflow plug flow secondary ozone contact column, an upflow shallow-bed RF that included relatively fine gravel and a GAC cap, a downflow shallow-bed SSF, and a GAC filter in series. The GAC filter was not used in experiments reported here. A detailed schematic of Pilot 1 is provided in Figure 3-5.

As shown in Figure 3-5, raw Grand River water was pumped to the pilot 1 trailer at a pressure of 20 to 50 psi and split to provide a flow of approximately 7-8 L/min to the upflow ozone columns of trains 1 and 2. Flow was controlled by Fabco variable area rotameters (model F44376) with polysulfone bodies and stainless steel floats (Fabco Plastics Wholesale Ltd., Maple, ON). Pre-ozonation was used to remove colour and to oxidize biodegradable dissolved organic carbon into smaller, more biodegradable constituents.

Ozone was produced onsite by two AZCOZON SNOA-4 ozone generators (AZCO Industries Ltd., Surrey, BC) and supplied to the raw water via a venturi injector on a partial vacuum at an unmeasured dose of approximately 8 mg/L. Since the transfer efficiency was estimated to be 25 to 50% depending on bubble size and water temperature, the applied ozone dose was approximately 2 to 4 mg/L. The ozone column was filled with many small pieces of PVC that were curved and perforated to promote bubble diffusion. Excess ozone was collected from the top of the column and vented. Because the ozone dose could not be regulated, it was necessary to turn it off for extended periods during winter months due to off-gassing associated issues.

Most of the ozonated supernatant in the ozone column was discharged into the wet well, while a small portion was pumped to the top of the secondary contact column using a Masterflex® L/S EasyLoad peristaltic pump with Masterflex® Norprene tubing (Cole-Parmer, Vernon Hills, IL). Flow was controlled by adjusting the peristaltic pump speed. Flow was controlled by Fabco variable area rotameters (model F44376) with polysulfone bodies and PVC floats (Fabco Plastics Wholesale Ltd., Maple, ON).

Figure 3-5: Pilot 1 schematic (Cleary, 2005)

The downflow, plug flow secondary contact column was used to provide additional contact time and allow ozone residual to dissipate before entering the roughing filter. It was assumed that no ozone residual reached the roughing filter based on measurements by Cleary (2005) and others. If ozone residual were to have reached the roughing filter, it would have been quickly consumed by the large quantity of solids accumulated at the bottom of the filter.

Water flowed by gravity to the bottom of the shallow-bed upflow roughing filter (Figure 3-5). The purpose of the RF was to provide turbidity and particulate removal via sedimentation in order to extend the filter run length of the downstream slow sand filter. The RF contained 30 cm of relatively fine gravel under a 30 cm granular activated carbon cap. This design was based on optimization studies by MS Filter Inc. that showed good turbidity removals despite shallower bed depths than recommended in the literature. Shallower bed depths were aimed at reducing costs to purchase media for full-scale systems. The GAC cap was used to prevent ozone from reaching the downstream slow sand filter if a residual was present, in addition to providing a good substrate for microbial and biofilm attachment. The RF column diameter was selected to meet desired flow and filtration rates.

The slow sand filter (SSF) had a maximum sand depth of 45 cm, which was below recommendations in the literature (~100 cm) (Figure 3-5 and Table 3-1). After multiple filter cleanings sand depth was as low as 30 cm. The SSF of pilot 1 was intended for continuous operation at a hydraulic loading rate (HLR) of 0.4 m/h, which is at the high end of the typical design range of 0.05 to 0.4 m/h. At full-scale, the SSF could also be periodically operated at an HLR of 0.8 m/h when one of the two trains normally operated at 0.4 m/h is offline for maintenance. These high HLRs aimed at reducing the footprint and cost of full-scale systems sold by MS Filter Inc. Sand with an effective size (ES) at the upper end of the design range (0.35 mm) was used to prevent excessive head loss at these high HLRs (Table 3-1). The ratios of the column diameter (D) to sand diameter (d) ranged from 400 to 900 (Table 3-1), which were above the minimum D/d ratio of 50 recommended by Lang *et al*. (1993) to ensure that pilot filters accurately represent the filtration performance of full-scale filters (headloss buildup, effluent turbidity, effluent particle counts).

The SSF effluent tubing rose to a height of 5 cm above the sand surface to prevent draining during periods of no flow and included an air vent to prevent siphoning. Although a portion of the train 1 and 2 SSF effluents passed through the online effluent turbidity meters, all SSF effluent water was discharged to the wet well. The GAC filter, used for additional colour and organics removal, was offline for the duration of this study.

Of the sampling ports shown in Figure 3-5, only port 0 (raw water), port 1 (ozonated water), port 2* (SSF influent), and port 3 (SSF effluent) were used in this study. Due to problems with sediment released from the secondary ozone contactor when taking samples from port 1, sampling port 1* was installed in the peristaltic pump tubing to allow sampling of the post-ozonated water.

Water depth in the secondary ozone contactor was measured to quantify head loss buildup in the RF over time. Water depth was also measured in the RF and SSF columns, which permitted the quantification of head loss buildup in the SSF over time. All columns, valves, and sampling ports were made of PVC while process tubing was either Teflon® coated or laboratory-grade clear tubing. The exterior of the PVC columns were wrapped in insulation to prevent changes in water temperature and shield them from interior lighting in order to prevent algal growth.

3.1.3 Pilot 2

Pilot 2 was designed and constructed by Cleary (2005) and commissioned in December 2003. It operated continuously for 2.5 years until decommissioning in June 2006. Pilot 2 included a head tank, two gravity-fed RFs in parallel, followed by two SSFs in series (Figure 3-6 and Figure 3-7). Raw water was pumped into the head tank on the second floor of the building and water level was kept constant by a v-notch weir located 1.3 m above the water level in the roughing filters. Raw water in the head tank was split between the two RFs in parallel, referred to as RFA and RFB. Water flow to the RFs was controlled by valves and measured by Kobold KSK flow meters with polyvinylidene fluoride (PVDF) bodies and floats (Kobold Instruments Canada Inc., Pointe-Claire, QC).

The upflow RFA was designed according to literature recommended bed depth (1.2 m) and media sizes (Figure 3-6 and Table 3-1) (Wegelin, 1996; Collins *et al.*, 1994). The upflow RFB was designed by Cleary (2005) with about half the bed depth comprised of gravel smaller than the minimum recommended diameter of 4 mm. With finer media, RFB was expected to have improved filtration efficiency (i.e. better particulate removal). Cleary (2005) reported that RFB was superior to RFA for coliform bacteria and turbidity removal, which was confirmed in this study for turbidity removal. Although RFB did achieve better turbidity removal than RFA, RFB became permanently clogged on two occasions and required the media to be removed and washed. Typically, RFB effluent was diverted back to the wet well whereas RFA effluent was piped into the influent of the first SSF.

The first SSF in series (SSF1) had a 1.0 m bed depth in accordance with literature recommendations (Figure 3-6). Sand grain size was 0.37 mm ES with a uniformity coefficient of 1.7. The SSF1 was operated at an HLR of 0.4 m/h with its effluent being diverted through an online turbidity meter or piped to the influent of SSF2. Flow was controlled by opening or closing valves. The column diameter was selected to be similar to that of the SSF columns of pilot 1 in order to achieve a similar HLR at a given flow rate.
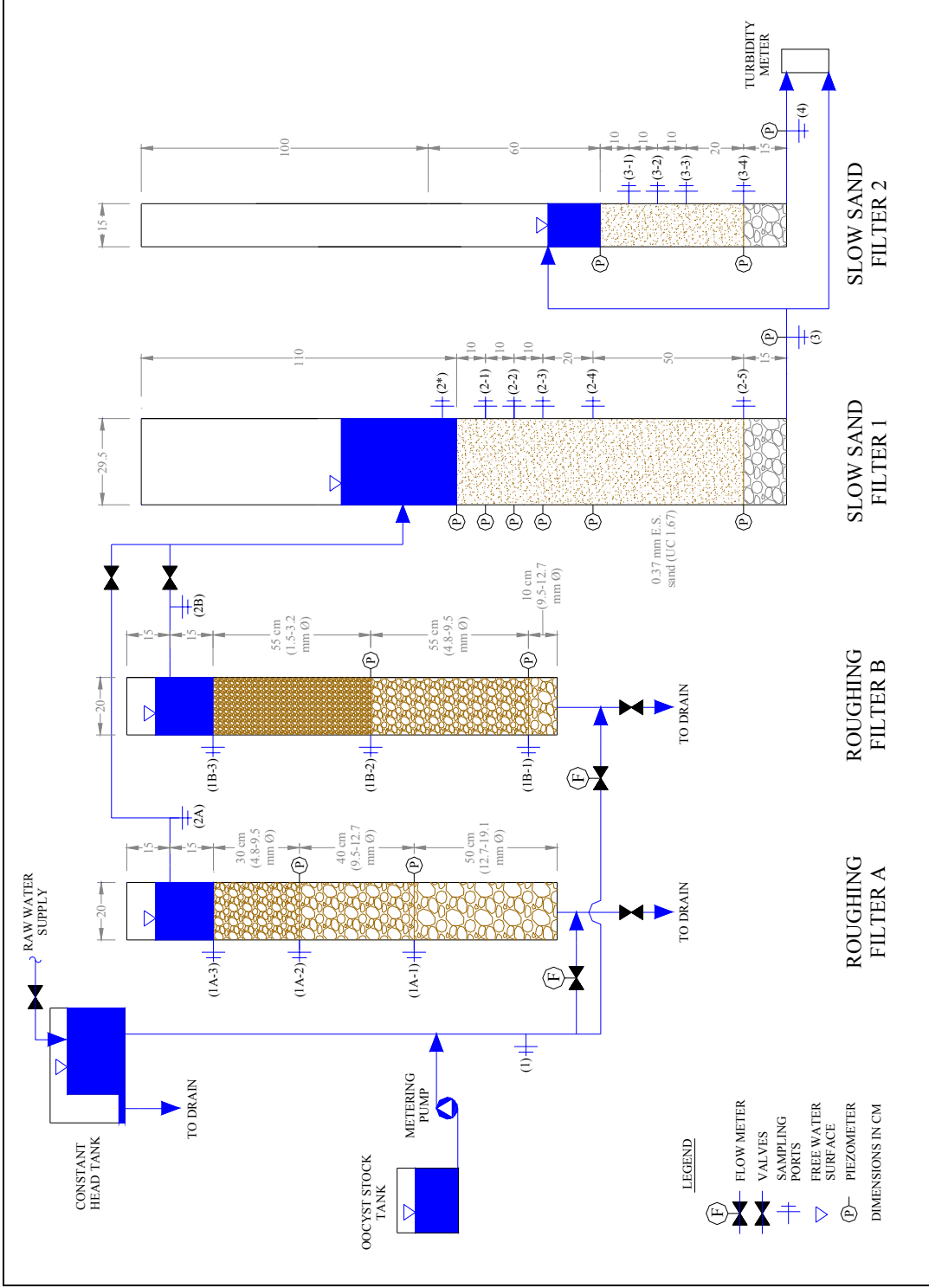
Figure 3-6: Pilot 2 schematic (Cleary, 2005)
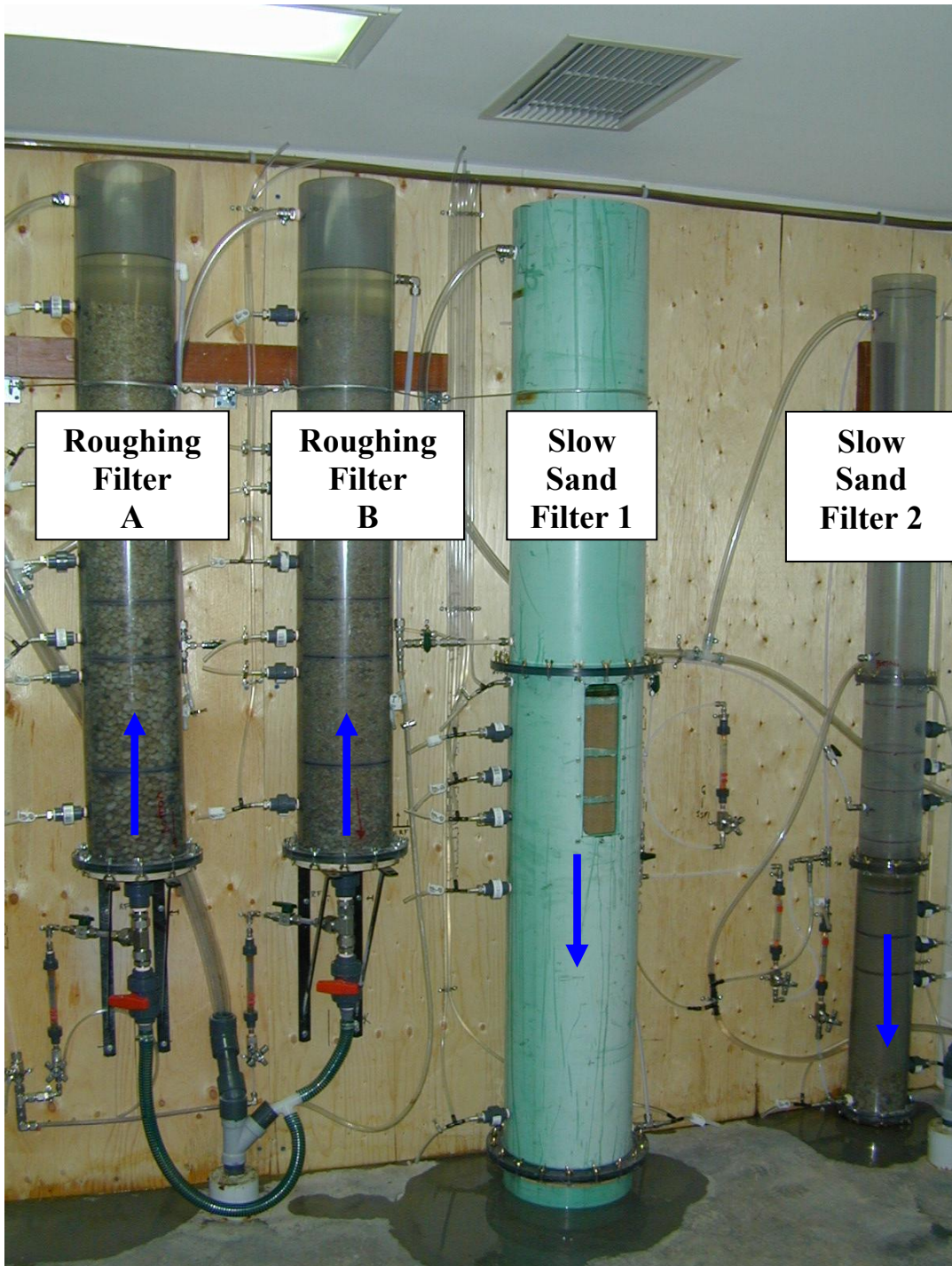
Figure 3-7: Pilot 2 photograph (Cleary, 2005)

A second SSF was included in pilot 2 based on a design by Daniel Urfer-Frund (Urfer-Frund, 1998), who recommended a second SSF in series for added robustness especially during challenging periods of high turbidity, high hydraulic loading rates, and low temperatures. For financial reasons, SSF2 was a smaller diameter column with a maximum sand depth of 0.5 m (Figure 3-6). SSF2 was operated at a target HLR of 0.4 m/h, although this was sometimes difficult to regulate because of the gravity-fed nature of pilot 2. SSF2 effluent flowed through an online turbidity meter and then discharged into the wet well. When filters were seeded with *Cryptosporidium*, *Giardia*, or MS2 bacteriophage, effluents were diverted to a waste holding tank instead of to the wet well. The holding tank was periodically pumped out and the wastewater was hauled to the Kitchener wastewater treatment plant for disposal.

In this study, pilot 2 water was typically sampled from port 1 (raw water), port 2A (RFA effluent), port 2B (RFB effluent), port 2* (SSF1 influent), port 3 (SSF1 effluent), and port 4 (SSF2 effluent). Head loss was recorded by measuring the water level in numerous piezometer tubes located at various depths in the RFs and SSFs (Figure 3-6).

All pilot 2 filter columns were made of clear PVC and were covered with polyfoam insulation to shield them from interior lighting. All pilot 2 piping was Teflon® tubing (Johnston Industrial Products, Toronto, ON) with stainless steel Swagelok® fittings and valves (Swagelok, Solon, OH). Sample ports and piezometers were made of Tygon® R-3603 tubing (Saint-Gobain Performance Plastics, Akron, OH) with nylon or polypropylene fittings.

3.1.4 Filter Cleaning and Resanding

In pilot 1, the upflow RFs were cleaned by pumping raw, non-ozonated water into a port at the bottom of the column. The objective of RF backwashing was to achieve 30 to 40% expansion of the GAC media in order to wash out accumulated solids. The RF outlet valve was left open but the SSF inlet valve was closed. The RFs were backwashed for approximately 15 minutes and care was taken not to wash out any GAC. RF backwashing was done when RF headloss was high, as indicated by water depth measurements in the secondary ozone contactor.

In pilot 2, the upflow RFA and RFB were cleaned by closing the effluent valve, allowing the water level in the columns to rise to the overflow tube (approximately 1.4 m total water head), then rapidly opening the drain valve at the bottom of the columns so that the water flushed out retained solids. The columns were refilled and flushed a second time. RF cleaning was performed occasionally to prevent breakthrough of solids to the downstream SSFs.

All SSFs were cleaned in the typical fashion by allowing water to drain to a couple inches below the sand surface, scraping off approximately 2 cm of sand, and refilling the filter with RF effluent. Although backfilling SSFs after cleaning is recommended, it was not possible in pilot 1 or 2. SSF cleaning, also called scraping, was performed when head loss had increased such that the water in the SSF columns had reached the overflow piping.

When sand depth became unacceptably low after numerous SSF cleanings (~30 cm for pilot 1 and ~60 cm for pilot 2 SSF1), virgin clean sand was added. First, the top 1 inch of existing mature sand was removed and placed in a bucket. After a second layer (~2 inches deep) of existing mature sand was placed in another bucket, washed virgin sand was added to the SSF column and backwashed to remove fines. After backwashing, the second layer of mature sand was placed on top of the virgin sand, and then the first layer of mature sand was placed on the top of the bed. This method is recommended in the literature to prevent excessive disruption to the biological populations in the top of the SSF bed (Barrett *et al.*, 1991).

3.1.5 Chloride Tracer Tests

Chloride tracer tests were performed to determine hydraulic detention times of the SSFs and RFs. Chloride was selected as a tracer because it is conservative, non-reactive, and short-term exposures should not negatively impact the filters' biological community. The stock solution for each chloride tracer test was prepared by mixing 92 g NaCl into 1 L of deionized water. The chloride tracer stock solution had a chloride concentration of 55,800 mg/L and conductivity of 88.6 micro Siemens per centimeter (μS/cm). The chloride stock was applied as a continuous (step-dose) injection at 1 mL/min into the effluent of the upstream filter using a peristaltic pump with Teflon® tubing. After mixing and dilution, the influent chloride concentrations were

approximately 117 mg/L (as calculated by $C_{influent} = [Q_{stock} \times C_{stock}]/Q_{influent} = [0.001\text{L/min} \times 55800\text{ mg/L}]/0.0501\text{ L/min} = 117\text{ mg/L}$).

Each filter was tested independently to avoid any confounding effects of testing multiple filters in series. A calibration curve was generated to confirm that it was acceptable to use conductivity as a surrogate measurement for chloride concentration (Figure A-1, Appendix A). As described in Section 3.2 conductivity was measured using a HACH CO150 conductivity meter (Hach Company, Loveland, CO). Note that this instrument was only calibrated using a basic method (Section 3.2 because the goal of the tracer tests was to determine relative changes in conductivity during the tracer test, not exact conductivity valves.

Chloride tracer tests were performed on the pilot 1 train 1 SSF at 0.4 m/h (Figure A-2) and RF at 1.5 m/h (Figure A-4), in addition to the pilot 2 SSF1 at 0.4 m/h (Figure A-3) and RFA at 0.95 m/h (Figure A-5) (Appendix A). Although the hydraulic loading rates for each filter were different, the flow rate was 0.5 L/min for all tracer tests. Conductivity measurements were taken before chloride injection, during injection, and after the injection pump was stopped. Therefore, background and steady state conductivities were recorded. From the results in Figure A-2 to Figure A-5 (Appendix A), hydraulic detention times (HDTs) were determined as the time it took for conductivity to drop to half way between the steady state and background values after the chloride injection pump was stopped (Table 3-2).

Table 3-2: Chloride tracer test results and HDT determination

Pilot	Filter	Conductivity (µS/cm)			HDT or t_{50} (min)
		Background	**Steady state**	**Target***	
1**	RF	743	1066	905	21
	SSF	750	1050	900	82
2***	RFA	411	685	548	30
	SSF1	535	795	665	119

Notes: flow 0.5 L/min for all chloride tracer tests
* Target conductivity is the midpoint between background and steady state conductivities; for determination of t_{50}
* Pilot 1 train 1

The t_{50} time represents the time it takes for 50% of the chloride tracer to pass through the filter. The t_{50} time was selected as an estimate of HDT (Table 3-2) because it is more conservative than the t_{10} time (i.e. time for 10% of the tracer to pass through the filter, which is typically used for microbial inactivation estimates). The t_{50} time is also more representative than the t_{90} time. For comparison with the HDTs determined from tracer tests (Table 3-2), theoretical HDTs were also calculated based on ideal conditions (Table 3-3).

In Table 3-3, the total theoretical HDTs represented the time for microorganisms sampled from the water column at the influent sampling port to travel to the effluent sampling port. The theoretical HDTs were compared to the HDTs determined from chloride tracer tests (Table 3-4 and Table 3-5).

Table 3-3: Theoretical hydraulic detention times of SSFs

Parameter	Pilot 1 Trains 1 & 2		Pilot 2 (0.4 m/h)	
	0.1 m/h	**0.4 m/h**	**SSF1**	**SSF2**
Area (m^2)	0.078	0.078	0.068	0.018
Flow (m^3/min)	0.00012	0.0005	0.0005	0.00012
Depth (m)				
Sand*	0.38	0.38	0.92	0.44
Underdrain gravel	0.20	0.20	0.15	0.15
Total	0.58	0.58	1.07	0.59
Theoretical HDT of media (min)**	163	41	65	40
Depth water between sand and influent sample port (m)	0.12	0.12	0.10	0.15[++]
HDT of water between sand and influent sample port (min)[+]	78	19	14	22
Total theoretical HDT (min) = HDT of media + HDT of water	241	60	79	62

* depth of sand during the MS2 challenge tests
** assuming a porosity of 0.45; also assumed by Cleary (2005)
[+] microorganisms sampled from the water column at the influent sampling port would be expected to travel down to the media surface in the times listed
[++] depth of water above the sand surface; there is no influent sampling port on the SSF2 column

Table 3-4: Comparison of HDTs for SSFs

	Pilot 1 Trains 1 & 2		Pilot 2 (0.4 m/h)	
	0.1 m/h	0.4 m/h	SSF1	SSF2
HDT from chloride tracer tests (min)[+]	n.d.	82	119	n.d.
HDT from chloride tracer tests by Cleary (2005) (min)[++]	n.d.	50	80	45
Theoretical HDT (min)*	241	60	79	62
HDT used in challenge tests (min)	240	60	120	60

[+] from Table 3-2; No chloride tracer test on SSF of pilot 1 train 2
[++] No chloride tracer test on SSF of pilot 1 train 2
* from Table 3-3
n.d. – not determined

Table 3-5: Comparison of HDTs for RFs

	Pilot 1 Train 1 RF (1.5 m/h)	Pilot 2 RFA (0.95 m/h)
HDT from chloride tracer tests (min)	21	30
HDT from chloride tracer tests by Cleary (2005) (min)	15	20
HDT used in challenge tests (min)	20	30

There are two explanations for the shorter HDTs determined from the chloride tracer tests by Cleary (2005) (Table 3-4 and Table 3-5). First, Cleary assumed the HDTs of the filters were the elapsed time between when the chloride injection pump was stopped and when the effluent conductivity started to decrease. In this study, the HDTs were assumed to be the same as the t_{50} times, which were the time it took for the effluent conductivity to drop to the midpoint between steady state and background conductivity after the pump was stopped.

The second reason for the differences between the HDTs determined by chloride tracer tests in this study and the Cleary (2005) study is that the depths of water above the sand surface in this

study were much higher. During the tracer tests on the SSF of pilot 1 train 1 at 0.4 m/h, the depth of water above the sand surface was 51 cm in this study but was only 7.5 cm in the study by Cleary (2005). More water above the sand surface would mean that it would take longer to see a drop in effluent conductivity after the tracer injection pump was stopped (i.e. more time for the chloride in the column of water above the SSF bed to be washed through).

As a result, the HDTs determined from the chloride tracer tests in this study were greater than those determined by Cleary (2005) because this study assumed HDTs equaled the t_{50} times and there was more water above the filters.

As shown in Table 3-4, a one hour detention time was used for the pilot 1 SSFs during the pathogen challenge tests conducted at 0.4 m/h. The train 2 SSF was assumed to have the same HDT as train 1. For pilot 1 SSFs operated at 0.1 m/h, it was assumed that the HDT would be four times greater (i.e. 4 hours) than at 0.4 m/h. For the SSF1 and SSF2 of pilot 2 operated at 0.4 m/h, HDTs of 2 hours and 1 hour were used as the lag time between corresponding influent and effluent samples during challenge tests.

It is important to note that the assumptions used to select HDTs for use in MS2 challenge tests were not expected to have a major impact on calculated MS2 log removals. This is because the influent MS2 concentrations were at steady state during sampling.

3.2 Water Quality Measurements

Turbidity of the raw water and the SSF effluents of pilot 1 and pilot 2 were monitored continuously (every 15 minutes) using HACH 1720D turbidity meters and dataloggers (Hach Company, Loveland, CO). The online turbidity data were not smoothed. However, it should be noted that pilot-scale systems are particularly susceptible to disturbances that would not affect full-scale systems. As such, a detailed log was kept during this study to determine when turbidity fluctuations were caused by operator or process disturbances and not by filter performance. Online turbidity data were removed from the data sets (prior to summarizing into tables in Chapter 5.0) when artificial turbidity increases resulted from operator disturbances such as

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cleaning online turbidity meters, flushing influent lines, and sampling. Data were also removed for time periods when no flow was reaching the online meters due to air lock in the influent tubes or when no flow was passing through the pilot filters (for example, during power outages or shutdowns). No data were removed when turbidity fluctuations were associated with SSF scraping, RF cleaning, or in response to high raw water turbidity events. The Region of Waterloo also operated and maintained a HACH 1720D online turbidity meter to measure raw water turbidity, but these data were only used in this study for onsite comparison and were not downloaded or presented in this thesis.

In order to determine the turbidity removal by individual treatment units, handheld turbidity measurements were taken using a portable HACH 2100P meter (Hach Company, Loveland, CO). For both trains of pilot 1, handheld turbidity measurements were taken from the raw water port 0, secondary ozone contactor port 1, RF effluent port 2*, and SSF effluent port 3 (Figure 3-5). For pilot 2, handheld turbidity measurements were taken from the raw water port 1, RFA effluent port 2A, RFB effluent port 2B, SSF1 effluent port 3, and SSF2 effluent port 4 (Figure 3-6). At locations where continuous online turbidity measurements were being taken, the handheld were compared to online values to ensure the values were approximately equal. Also for quality control, turbidity of a deionized water blank was taken using the handheld meter each day sampling occurred. Blank turbidity was always between 0.03 and 0.08 NTU, indicating that the handheld unit functioned as well as could be expected.

Dissolved oxygen and water temperature measurements were taken using a portable Orion 835 meter (Thermo Electron Corporation, Wyman, MA). During the MS2 bacteriophage challenge tests, hardness and conductivity were measured using a HACH model HA-DT hardness meter with digital titrator and HACH CO150 conductivity meter, respectively (Hach Company, Loveland, CO). The conductivity meter was calibrated on-site before and after water sampling using a single-point measurement of a stock potassium chloride and ultrapure water solution with a known conductivity of 1411μS/cm (VWR Traceable Conductivity Calibration Standard, VWR International Ltd. of Canada, Mississauga, ON). At the University of Waterloo, the pH of water

samples was measured using an Orion 710A pH/ISE meter (Thermo Electron Corporation, Wyman, MA), which was calibrated daily.

3.3 *Cryptosporidium* and *Giardia* Challenge Tests

Seven *Cryptosporidium* and *Giardia* challenge tests were performed between April 2004 and August 2005 on the slow sand filters of pilot 1 (train 1) and pilot 2. The purpose of these tests was to quantify oocyst and cyst removal in the SSFs under different operating and water quality conditions. In challenge tests 1 to 3, only oocysts were injected (Cleary, 2005). Oocysts and cysts were simultaneously injected into the slow sand filter influent during challenge tests 4 to 7. The experimental procedures are described below, including feedstock preparation, sampling, sample processing, and enumeration.

3.3.1 *Cryptosporidium* and *Giardia* Feedstock

Inactivated *Cryptosporidium parvum* oocysts (calf Iowa isolate) and *Giardia muris* cysts (Roberts-Thompson strain isolated from mice) were purchased from a commercial laboratory (Waterborne, Inc., New Orleans, LA). *C. parvum* oocysts were provided in vials containing 10^9 oocysts in 50 mL of 5% formalin and 1x phosphate buffered saline (PBS) with 0.01% polyoxyethylene sorbitan monolaureate (Tween 20, J.T. Baker Chemical Co., Philadelphia, PA) to prevent clumping. *G. muris* cysts were supplied in vials containing 10^8 cysts in 10 mL 5% formalin/PBS solution with 0.01% Tween 20. The oocysts and cysts were provided in a clean, purified form.

It has been reported that formalin inactivated oocysts are good surrogates for viable oocysts (Emelko, 2003) and that formalin inactivation does not alter the electrostatic properties (i.e. zeta potential) of oocysts (Kunzar and Elimelech, 2005; Considine *et al.*, 2002; Butkus *et al.*, 2003). However, Kunzar and Elimelech (2005) found that adsorption of formalin- and heat-inactivated oocysts to granular media was enhanced compared to viable oocysts because the inactivation treatments disrupted surface proteins, thereby reducing steric repulsion forces. For *Giardia*, Schuler *et al.* (1991) reported that although *G. muris* and *G. lamblia* do not behave identically in all cases, they do share many physical characteristics.

The target SSF influent *Cryptosporidium* and *Giardia* concentrations were 10^6 oocysts/L and 10^5 cysts/L, respectively. To achieve this, the target *Cryptosporidium* and *Giardia* feedstock concentration was 4.6×10^8 oocysts/L and 9.1×10^7 cysts/L. A feedstock solution was prepared on the day of each challenge test by adding 12 mL of vortexed *C. parvum* oocyst stock and 4.5 mL of vortexed *G. muris* cyst stock to 500 mL of raw Grand River water. For each challenge test, feedstock concentration was quantified by placing a small volume (<100 μL) of feedstock onto a hemacytometer (Petroff-Hausser Bacterial Counting Chamber, Hausser Scientific Corporation, Horsham, PA) and counted using light microscopy (Ziess Axioscope 2, Empix Imaging, Mississauga, ON). Three to five replicate feedstock counts were performed for each challenge test, except test 7 in which no hemacytometer counting was performed.

3.3.2 Slow Sand Filter Seeding

Feedstock was seeded into the filters continuously for six hours during each challenge test. Using a peristaltic pump and Teflon® tubing, the continuously-stirred oocyst and cyst feedstock was injected into the RF effluent tubing to allow mixing before reaching the SSF columns. Feedstock was injected at a rate of 1 mL/min for a SSF HLR of 0.4 m/h and 2 mL/min for an HLR of 0.8 m/h. The target SSF influent concentrations were 10^6 oocysts/L and 10^5 cysts/L. The target influent concentration of *G. muris* cysts was lower due to the prohibitive cost of purchasing additional cysts.

3.3.3 Sampling Protocol

To measure the removal of cysts or oocysts, samples must be taken that are representative of time of travel through the filter media bed (called the hydraulic detention time, HDT). The HDTs in Table 3-6 were assumed based on the results of chloride tracer tests performed by Cleary (2005) and in this study (Section 3.1.5). These HDTs were used as the lag time between corresponding influent and effluent samples. For example, if the HDT is one hour, then the influent sample taken at time zero would correspond to an effluent sample taken at a time of 1 hour.

Table 3-6: SSF hydraulic detention times

HLR (m/h)	Pilot	HDT (h)	Test No.
0.4	1 (Train 1)	1	2, 4, 6
	2 (SSF1)	2	1, 7
	2 (SSF2)	1	1, 7
0.8	1 (Train 1)	0.5	3, 5

Notes: Hydraulic loading rate (HLR); hydraulic detention time (HDT)

For pilots 1 and 2, influent samples were collected from sample port 2*, located approximately 5 cm above the sand surface (Figure 3-5 and Figure 3-6). Influent samples were collected before seeding (0-hour sample) and up to 8 hours after the start of seeding. Note that the SSFs were seeded for 6 hours, after which time the injection pump was stopped. Influent samples were collected in 250 mL Wheaton® glass bottles rinsed in buffered detergent to prevent attachment of oocysts or cysts to the bottle. The buffered detergent contained 100 mL 1% sodium dodecyl sulfate, 100 mL 1% Tween 80 (J.T. Baker Chemical Co., Philadelphia, PA), 100 mL of sterile OmniPur® 10x PBS (EMD Chemicals Inc., Merck KGaA, Darmstadt, Germany), 0.1 mL Sigma Antifoam A (Sigma-Aldrich, St. Louis, MO), and 700 mL deionized water.

Effluent samples were taken from sampling port 3 before seeding and up to 9 hours after the start of seeding. For pilot 2, effluent samples from SSF2 were taken from port 4. Effluent samples were collected in 1 L Wheaton glass bottles rinsed in buffered detergent. All influent and effluent samples were refrigerated on-site at 4°C immediately after collection. Samples were transported in coolers with ice packs to the University of Waterloo and then refrigerated at 4°C. Samples were processed and enumerated within three weeks, except test 7 where enumeration took place at a later date (Section 6.0).

Before, during, and after each challenge test, measurements of influent and effluent turbidity (handheld and online), dissolved oxygen, temperature, water depth above the filter, and head loss were recorded.

3.3.4 Sample Processing

Influent and effluent samples were processed and enumerated at the University of Waterloo by Shawn Cleary (tests 1 to 3) (Cleary, 2005), Souleymane Ndiongue (tests 4 to 6), Jeff DeLoyde (tests 6 and 7), and Kyle Tabor (test 7).

Samples were filtered through 25 mm 0.4 μm Nuclepore® polycarbonate track-etched membranes (Whatman Inc., Florham Park, NJ). The 0.4 μm membranes were placed on top of 8.0 μm nitrocellulose support membranes (Millipore Corporation, Billerica, MA) on a vacuum manifold (Hoefer Scientific, San Francisco, CA) operated at 125 mm of mercury. Stainless steel weights rinsed in buffered detergent were used to hold the membranes in place.

Before the samples were filtered through the membranes, each membrane was wetted with 1x PBS and placed on the vacuum manifold. The 1xPBS solution was prepared by mixing 100 mL of sterile OmniPur® 10x PBS (EMD Chemicals Inc., Merck KGaA, Darmstadt, Germany) and 900 mL Milli-Q® ultrapure water (Millipore Corporation, Billerica, MA). When processing each sample, 2 mL of 1% bovine serum albumen (BSA) was filtered through the membranes, followed by the sample volume, then by another 2 mL of 1% BSA. To prevent drying of the membranes, 1x PBS was added as needed.

To obtain between 20 and 1000 oocysts on each membrane, 1 to 2 mL of each influent sample was filtered through the membranes, except for the 0-hour and 8-hour influent samples where 50 to 200 mL was filtered. Effluent samples were processed by filtering 200 to 250 mL of each sample through the membranes, except in test 1 where 400 to 500 mL was filtered. The sample volume to be filtered through the membranes was first transferred from the sample bottle to plastic graduated cylinders rinsed in buffered detergent. Due to time limitations, influent samples were processed the day after effluent samples.

3.3.5 Immunofluorescence Assay & Enumeration

The immunofluorescence assay (IFA) microscopy and direct antibody staining was carried out using the Hydrofluor™ Combo *Giardia* and *Cryptosporidium* Immunofluorescence Detection Kit (Strategic Diagnostics, Inc., Newark, DE).

Although the Hydrofluor™ Kit is not approved for use with the USEPA Method 1623 for the detection of *Cryptosporidium* and *Giardia* (USEPA, 2005), the Hydrofluor™ kit was used in studies at the University of Waterloo (Emelko *et al*., 2003; Emelko, 2003; Watling, 2004; Cleary, 2005) because of the bright fluorescence of the stain and because it could be used with the direct filtration method that was employed, as described in Section 3.3.4 of this thesis. The USEPA Method 1623 requires immunomagnetic separation (IMS), which was deemed to be an unnecessary step for clean water applications with high concentrations of cysts and oocysts such as seeding experiments with filter effluents (Emelko, 2006). The direct filtration method and Hydrofluor™ staining method used in this study has been reported to provide mean oocyst recovery of 74% with a coefficient of variation of 16% (Emelko *et al*., 2003).

After the samples were filtered through the membranes (refer to Section 3.3.4), primary and secondary stains included in the Hydrofluor™ kit were added to the membranes. In accordance with the Hydrofluor™ kit instructions, 500 μL of a primary stain was first added to each membrane used to filter samples. The vacuum manifold was off and the membranes sat covered for 25 minutes after the stain was applied. The membranes were rinsed with 10 mL of 1x PBS and then 500 μL of a secondary stain was added. The membranes sat covered for another 25 minutes and were rinsed with 10 mL 1x PBS.

For quality control, negative and positive controls were included on each day of processing. Negative controls were used to confirm that no cross-contamination had occurred during processing since they were processed identically except that no sample was added (i.e. 1x PBS and 1% BSA only). For the positive control, 50 μL of a formalinized stool containing oocysts and cysts from the Hydrofluor™ Kit was stained to verify that staining yielded adequate

detection. For all challenge tests, oocysts or cysts were never detected on the negative control slides and were always detected on the positive control slides.

During staining, glass microscope slides were warmed to 37°C and one drop of DABCO-glycerol mounting medium, made by mixing 100 mL glycerol and 2 mL 1,4 diazabicyclo [2.2.2] octane (DABCO, Sigma-Aldrich, St. Louis, MO), was added to the surface of the slide. The membranes were lifted using sterilized forceps and placed on the warmed microscope slides on top of the drop of oil. A drop of DABCO-glycerol was then added to the top of the membrane and a glass cover slip was placed over the membrane. The glass cover slip was sealed to the glass microscope slide using clear nail polish. Once the nail polish had cooled and hardened, the slides were refrigerated at 4°C in the dark until they were counted.

The slides were then counted using UV light microscopy at a magnification 400x (Ziess Axioscope 2, Empix Imaging, Mississauga, ON). As mentioned previously, the slides were enumerated within three weeks, except in test 7. The number of oocysts and cysts on each slide were recorded and the concentrations calculated by dividing the count by the volume of sample processed. Percent removals were determined by calculating $[1-(C_{influent} / C_{effluent}) \times 100]$, where $C_{influent}$ and $C_{effluent}$ represent the oocyst or cyst concentrations in oocysts/L or cysts/L for corresponding influent and effluent samples. Note that recovery studies were not done and count data and calculated removals presented in this thesis were not adjusted to account for recovery rate. The recovery rate was not deemed to be of critical importance because it was reasonably assumed that the recovery rate was the same for both influent and effluent samples.

For each test, average percent removal was determined by calculating the arithmetic mean of percent removals calculated for corresponding influent and effluent samples. Throughout this thesis, the terms "average" is used synonymously with "arithmetic mean." Standard deviations were also calculated. The average percent removal was transformed into an average log removal by calculating $[-Log_{10}(1-average/100]$. Note that it would have been incorrect to calculate

average removals for each test (by taking the arithmetic mean of the log removals for corresponding influent and effluent samples) because log removal is not a linear function.

When no oocysts or cysts were recovered from one or more effluent samples (i.e. complete removal was achieved), removals were calculated based on an effluent concentration of 1 oocyst/L or cyst/L. This assumption was conservative and reasonable considering that influent concentrations were orders of magnitude greater (approximately 10^6 oocysts/L and 10^5 cysts/L). In cases where complete removal was achieved and an effluent concentration of 1 oocyst/L or cyst/L was assumed, the calculated removals were labeled with a "greater than" sign to indicate that removals were at least, and possibly greater than, the value shown. Greater removals may have been calculated if higher concentrations of oocysts or cysts had been seeded into the SSF influent so that some could be recovered in the effluent. For tests in which one or more removal was labeled with a "greater than" sign, these removals were included when calculating average removal. Average removals calculated in this manner were labeled with a "greater than" sign to indicate that mean removals were at least, and possibly greater than, the value shown. It was not possible to calculate standard deviations for averages with "greater than" signs.

Statistical analyses using the removal data were not carried out because it was beyond the scope of this thesis to determine if the microbial data were normally distributed (an assumption used when performing t-tests). Although the Central Limit Theorem states that while data may not be normally distributed, means typically are. However, the small number of data points (n = 5) used to calculate averages for each test was assumed to prevent the use of the Central Limit Theorem for these experimental data.

3.4 MS2 Bacteriophage Challenge Tests

A total of 16 challenge tests were conducted using MS2 bacteriophage (phage) between February and June 2006. The purpose was to quantify MS2 removal in slow sand and roughing filters under different conditions. The sections below detail the microorganisms, materials, sampling protocol and processing methods used in the MS2 challenge tests.

3.4.1 Host Bacteria

The host bacteria was *E. coli* HFr (ATCC 15597), which is a male specific F+ bacteria producing sex pili during logarithmic growth. The sex pili contain the receptor sites for MS2 bacteriophage, thus allowing for attachment of the virus and infection of the host bacteria. MS2 cannot infect humans or any other organism without the receptor site.

The *E. coli* HFr was acquired from the American Type Culture Collection (ATCC, Manassas, VA) and stored at –20°C. Some *E. coli* HFr were taken from the freezer and streaked onto a plate of nutrient agar using a sterile swab. The streaked plate was incubated overnight at 35 °C in a sealed plastic container. The *E. coli* HFr were re-streaked and re-incubated several times before being used in the MS2 experiments. Streaked agar plates were stored at room temperature in a sealed plastic container. Thereafter, the *E. coli* HFr plates were re-streaked onto fresh agar plates two to three times per week to maintain viability. If the *E. coli* HFr became resistant to infection by MS2 (which occurred four times, likely because of sub-optimal nutrient or temperature conditions and a subsequent failure to produce sex pili), new *E. coli* HFr were taken from the freezer stock.

3.4.2 High Titer MS2 Stock

The source of MS2 bacteriophage for this study was a high concentration ($\sim 10^{12}$ plaque forming units (PFU) per mL) titer made by staff at the University of Waterloo in February 2005. This high titer was stock was made using MS2 obtained from the American Type Culture Collection (ATCC 15597-B1) and stored at –70°C at the University of Waterloo.

In order to make a new high titer MS2 stock, the following procedure was followed. Tryptone-Yeast-Glucose-Broth (TYGB), described in 3.4.5 , was inoculated with *E. coli* HFr and incubated overnight at 35°C. The following day, fresh broth was inoculated with 1 mL of the overnight *E. coli* HFr culture. The newly inoculated broth was shaken at 150 rpm in a 35°C incubator while measurements were taken using a spectrophotometer at 600 nm (UV-Vis model 8453, Hewlett-Packard, Palo Alto, CA). When an absorbance of 0.3 was reached, which

indicated a peak bacteria concentration of ~3x10^8 cells/mL, 0.1 mL of the old (February 2005) MS2 stock was added, followed by overnight incubation at 35°C. The next day, the infected culture was centrifuged at 10,000 rpm for 15 minutes to remove the *E. coli* cells. The high titer MS2 supernatant was decanted into a sterile bottle and refrigerated at 4°C. The MS2 titer had a concentration of approximately 10^{11} PFU/mL. The MS2 titer was prepared on October 18, 2005 and used for all MS2 challenge tests in this study.

3.4.3 Quality Control Stock

The purpose of the quality control (QC) stock was to have a MS2 stock of known concentration that yielded a known number of plaques at a given dilution. The QC stock was plated each time samples were plated to ensure that the method and materials employed were capable of yielding the expected number of plaques. The QC stock was prepared by adding 200 µL of the high titer MS2 stock to 200 mL of phosphate buffered water (PBW). The QC stock had an approximate concentration of 10^6 PFU/mL and yielded 20 to 30 plaques at a 10^5 dilution. This QC stock was used from January to June 2006. A second QC stock, referred to as QC-2, was prepared on May 17, 2006 and yielded 15 to 25 plaques at a 10^7 dilution.

3.4.4 MS2 Spike Preparation

For each test, a MS2 "spike" bottle was prepared, which contained 4 L of PBW and 2 mL of the high titer MS2 stock in a sterile bottle with a stir bar. At a 10^6 dilution, the plaque counts were approximately 50 to 100. Therefore, the concentration of the MS2 spikes ranged from of 5x10^7 to 1x10^8 PFU/mL. A total of nine MS2 spikes were prepared, all of which were used for experiments within 1 to 14 days after being made except for the February 23 spike, which was used 26 days after it was made for test 6. The spike bottles were stored at 4°C and the MS2 concentrations were approximately the same before and after experiments.

3.4.5 Materials

A number of materials were needed for the MS2 challenge tests, including glucose, agar, broth, and phosphate buffered water. A glucose solution was prepared as an ingredient for agar and broth. Glucose was made by mixing 20 mL of deionized water (DI), 1 g of D-glucose (Sigma-

Aldrich, St. Louis, MO), and 0.3 g of CaCl$_2$-2H$_2$O (EMD Chemicals Inc., Merck KGaA, Darmstadt, Germany). For sterilization, glucose was passed through a 0.22 μm polycarbonate membrane (Millipore Corporation, Billerica, MA) and stored in a separate, sterile glass bottle at 4°C.

Tryptone-Yeast-Glucose-Agar (TYGA) was made by combining 1 L DI, 10 g of Bacto^{TM} Tryptone (BD, Franklin Lakes, NJ), 10 g of granulated agar (BD, Franklin Lakes, NJ), 1 g of yeast extract (BD, Franklin Lakes, NJ), and 8 g of NaCl (EMD Chemicals Inc., Merck KGaA, Darmstadt, Germany). The pH of the solution was measured and confirmed to be 7.0 ± 0.2. After mixing and pH measurement, the agar bottle, clean 30 mL screw-cap glass test tubes, and a dispenser were autoclaved for 15 minutes at 121°C. After autoclaving, 20 mL of glucose was added and the agar was stirred for five minutes. Approximately 20 mL of agar was aseptically dispensed into each 30 mL screw-cap test tube. Agar was refrigerated at 4°C and used within one month, although it could have been stored for up to three months. For quality control, three agar tubes were plated on the day of agar preparation. One had *E. coli* and MS2 QC stock added to confirm that plaques developed, one had only *E. coli* to confirm a bacterial lawn developed, and one contained only agar to ensure it was not contaminated.

Tryptone-Yeast-Glucose-Broth (TYGB) was used as the liquid growth medium for *E. coli* HFr. TYGB preparation procedure and ingredients were the same as for the TYGA, except that no agar was added. The broth was stored at 4°C and used within one month, although it could have been stored for up to three months. For quality control, a small volume of new broth was inoculated with *E. coli* HFr, incubated overnight at 35°C, and used when plating the QC stock to ensure good lawn formation and good plaque counts.

Phosphate buffered water (PBW) was needed for serial dilutions of MS2 samples. PBW was made by mixing 1 L DI, 1.25 mL of KH$_2$PO$_4$ solution, and 5 mL of MgCl$_2$-6H$_2$O solution. The KH$_2$PO$_4$ solution was made from 34g KH$_2$PO$_4$ (EMD Chemicals Inc., Merck KGaA, Darmstadt, Germany) in 1 L DI. The MgCl$_2$-6H$_2$O solution was made from 81.1g MgCl$_2$-6H$_2$O (EMD

Chemicals Inc., Merck KGaA, Darmstadt, Germany) in 1 L DI. The PBW was confirmed to have a pH of 7.0 ± 0.2 and was autoclaved at 121°C for 15 minutes. PBW was dispensed into sterile test tubes in 9.0 and 9.9 mL aliquots and refrigerated at 4°C. The PBW aliquots were used for serial dilutions.

3.4.6 Filter Seeding

MS2 spike bottles were transported 25 minutes from the University of Waterloo to the Lowlift Pump Station in coolers at 4°C, then refrigerated on-site at 4°C. During seeding, spike bottles were continuously stirred and situated in an insulated box filled with ice packs. The ice packs were replaced daily.

Almost all of the 16 MS2 challenge tests involved seeding of the slow sand filters of pilots 1 and 2, except tests 3 and 6, which involved seeding of roughing filters. A peristaltic pump with Teflon® tubing was used to inject the MS2 bacteriophage into the filters. For the tests on the SSFs, MS2 was seeded into the RF effluent piping to allow mixing before entering the SSF columns above the sand beds. For test 3 on the RF of pilot 1 train 1, MS2 was seeded into the top of the secondary ozone contact column. For test 6 on the RFA of pilot 2, MS2 was seeded into the outlet of the constant head tank. MS2 was seeded into the filters at 1 mL/min and influent concentrations were approximately 10^4 to 10^5 PFU/mL.

3.4.7 Sampling Protocol

Samples were aseptically collected in sterile clear polypropylene 60 mL centrifuge tubes (Biologix Research Company, Lenexa, KS). Samples were refrigerated on-site at 4°C immediately after collection. Sample tubes and MS2 spike bottles were transported in a cooler with ice at 4°C to the University of Waterloo, where they were refrigerated at 4°C.

SSF influent and effluent samples were taken from sampling ports 2* and 3, respectively (Figure 3-5 and Figure 3-6). For the SSFs of pilot 1, sampling port 2* was located approximately 10 cm above the sand surface. For pilot 2 SSF1, sampling port 2* was located 15 cm above the sand surface. The effluent samples from pilot 2 SSF2 were taken from sampling port 4. Hydraulic

detention times (HDTs) were used as the lag time between the collection of corresponding influent and effluent samples. For example, a one hour HDT would indicate that effluent samples would be collected one hour after the corresponding influent samples. The corresponding influent and effluent samples were used to calculate log removals of MS2. The HDTs are summarized in Table 3-7.

Table 3-7: Hydraulic detention times used for MS2 challenge tests

HLR (m/h)	HDT (h)				
	Pilot 1		Pilot 2		
	SSF	RF	SSF1	SSF2	RFA
0.1	4	-	-	-	-
0.4	1^{+}	-	2	1	-
0.95	-	-	-	-	0.5
1.5	-	0.33	-	-	-

Notes: Hydraulic loading rate (HLR); hydraulic detention time (HDT)
[+] Pilot 1 trains 1 and 2

Most often, seeding commenced between 8 and 10 pm the night before the first day of sampling. Sampling took place in the morning and early afternoon of the subsequent days of the test. This procedure was followed because the 12 hours between the start of seeding and the start of sampling allowed the influent MS2 concentration to reach steady state. Therefore, during sampling, the MS2 concentration in the filter influent remained approximately constant. As detailed in Section 6.0 of this thesis, the SSF influent MS2 concentrations during tests 1 and 2 at 0.4 m/h reached steady state within 5 to 6 hours after the start of seeding. For the SSF tests run at 0.1 m/h, steady state had already been reached by the time sampling began approximately 12 hours after the start of seeding. During RF challenge tests 3 and 6, steady state influent MS2 concentrations were reached by the time sampling began 1 to 2 hours after the start of seeding.

3.4.8 Sample Processing

All sample processing was performed at the University of Waterloo. Effluent samples were processed before and kept separate from influent samples. Samples that contained high MS2

concentrations were serial diluted using PBW. Following the serial dilutions, the sample bottles and dilution test tubes were refrigerated.

Test tubes containing agar were boiled to melt the agar and placed in a 55°C warm water bath. To plate the serial diluted samples, the test tubes of molten agar were placed in a test tube rack. The agar test tube caps were unscrewed but left on to prevent contamination. The day before plating samples, 150 mL of broth had been inoculated with *E. coli* HFr and incubated overnight at 35°C. This broth was used the following day for plating.

Approximately 1 mL of *E. coli* HFr inoculated broth was added to each test tube of molten agar using a sterile pipette. The test tubes containing the PBW and serial diluted samples were gently vortexed and 1 mL of each serial diluted sample was injected into the molten agar. When no serial dilution was required, 1 mL of the sample was taken directly from the sample bottle and injected into the test tube of molten agar. The screw caps were tightened, the test tubes were gently inverted three times, and the contents were poured into sterile petri dishes. As described, this method employs the single layer agar technique. The agar was allowed to cool with the lid ajar for a maximum of 30 minutes, after which time the petri dishes were inverted and incubated overnight in a sealed plastic container at 35°C. All plating was done in triplicate for quality control. Plates were removed from the incubator after 18 to 24 hours and either enumerated immediately or placed in the refrigerator at 4°C for counting at a later date.

Quality control plates were plated at the same time as samples. Negative controls that contained only agar and *E. coli* were plated before, during, and after sample plating to ensure that an *E. coli* lawn would grow and that no plaques were present as a result of MS2 contamination of labware. The quality control (QC) stock was plated in triplicate on the same day as sample plating and using the same *E. coli* inoculated broth to verify that a known number of plaques would form. In addition, the QC stock was plated with 1 mL of raw water from the sampling day to ensure that the raw water did not interfere with plaque counts. For quality control, all materials (glucose, agar, broth, and PBW) were checked for contamination and quality on the day they were made.

The lab bench and equipment were sterilized before, during, and after sample processing. Clean glassware was used and autoclaved as needed.

3.4.9 Plaque Counting and Calculation of Removals

Enumeration was performed using a Quebec colony counter (American Optical Corp., Buffalo, NY) and plaques were visible as clear spots in the bacterial lawn. A black permanent marker was used to label each plaque that had been counted while the number of plaques was recorded using a handheld counter. Plaque counts were only considered valid if the count was in the range of 20 to 200 plaques per plate. This was because counts lower than 15 or 20 plaques would present uncertainty and counts above 200 would be subject to counting error because of overlapping plaques. Therefore, if plaque counts were not in the range of 20 to 200, the samples were replated using a different dilution.

MS2 concentrations were calculated by multiplying the plaque counts by the dilution factor. For example, if 80 plaques were counted for a 10^3 diluted sample, then the MS2 concentration of that sample would be 80×10^3 PFU/mL because 1 mL of sample was processed.

For each test, average percent removal was determined by calculating the arithmetic mean of percent removals calculated for corresponding influent and effluent samples. Throughout this thesis, the terms "average" is used synonymously with "arithmetic mean." Standard deviations were also calculated. The average percent removal was transformed into an average log removal by calculating $[-Log_{10}(1-average/100]$. Note that it would have been incorrect to calculate average removals for each test (by taking the arithmetic mean of the log removals for corresponding influent and effluent samples) because log removal is not a linear function.

Statistical analyses were used to compare the average of one test to another when the number of data points for each test were approximately ≥ 7 because it was assumed that means are typically normally distributed according to the Central Limit Theorem. Only challenge tests conducted on pilot 1 (trains 1 and 2) had $n \geq 7$, except for tests 4 and 15. A modified t-test known as the Smith-Satherwaite approximation was used to compare averages for challenge tests. The Smith-

Satherwaite approximation assumes normal distribution and unequal and unknown population variances and is appropriate when n is less than approximately 30. This test was used for comparing average MS2 removals because the variances of the averages were unknown, and an F-test for testing homogeneity of the variances would have had only very low power as a result of the small number of data points. The Smith-Satherwaite approximation was calculated using the following equations:

$$T = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \qquad\qquad df' = \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)^2}{\frac{\left(\frac{s_1^2}{n_1}\right)^2}{n_1 - 1} + \frac{\left(\frac{s_2^2}{n_2}\right)^2}{n_2 - 1}}$$

where T is the observed t-statistic; df' is the degrees of freedom; X_1, S_1 and n_1 are the sample mean, variance and sample size of the first test being compared, respectively; X_2, S_2 and n_2 are the sample mean, variance and sample size of the second test being compared, respectively. For this two sided test, the critical t-statistic was $t_{\alpha/2,\ df'}$ (where α was the significace level) and the null hypothesis was that the population means of the two tests were equal ($\mu_1 = \mu_2$). When $T_{observed}$ was greater than $t_{critical}$ the null hypothesis was rejected, which indicated that the means were statistically different. When $T_{observed}$ was less than $t_{critical}$ rejection of the null hypothesis failed, which indicated that the means were not statistically different. Although it may be imperfect to assume a normal distribution and use a t-test, based on the Central Limit Theorem the means of replicate performance measurements at each test condition should be normally distributed. Since it was these mean performance values that were being compared, the use of a t-test was reasonable. However, since each performance measurement (i.e. at a given sampling time) was based on averaged microbial counts (which is standard practice), the true variance of the means would be underestimated. The implication of this is that the true reported significance level for the t-test is somewhat different than that reported. A standard approach for the analysis of microbial data in investigations such as the present one is not in use.

4.0 Multistage System Performance

The pilot-scale multistage slow sand filter systems were operated continuously for approximately 2.5 years, during which time online turbidity measurements were collected every 15 minutes. Other system performance data were collected two to three times per week, including head loss measurements, dissolved oxygen (DO) concentrations, and water temperature. Turbidity data were analyzed for comparison to regulatory limits with respect to compliance and to determine percent turbidity removal. Head loss data were analyzed to determine filter run lengths. In addition, temperature and DO data were used as potential indicators of biological activity in the filters. The current study includes data collected from September 2004 to June 2006. Data from October 2003 to August 2004 were presented by Cleary (2005).

4.1 Turbidity

Online turbidity was monitored in pilot plants 1 and 2 in the 2004 to 2006 study periods. Turbidity monitoring was important to assess the ability of the plants to meet the regulatory effluent requirements and in order to maintain filter performance at typical full-scale efficiencies for pathogen spiking tests. Raw and SSF effluent turbidities for pilot 1 from 2004 to 2006 are presented in Figure 4-1 to Figure 4-4. For pilot 2, SSF1 and SSF2 online effluent turbidities were recorded for most of 2005 (Figure 4-5). Frequency analyses of raw and effluent online turbidities are presented in Table 4-1 and Table 4-2, respectively.

Table 4-1: Pilot 1 raw water turbidity frequency analysis

Year	% Occurrence of Raw Water Turbidity in Stated NTU Ranges				n
	<5	5-≤10	10-≤50	50-100	
2004	61.7	34.3	3.9	0.1	4427
2005	61.3	24.6	13.7	0.4	30942
2006	35.6	32.8	28.7	2.9	15655
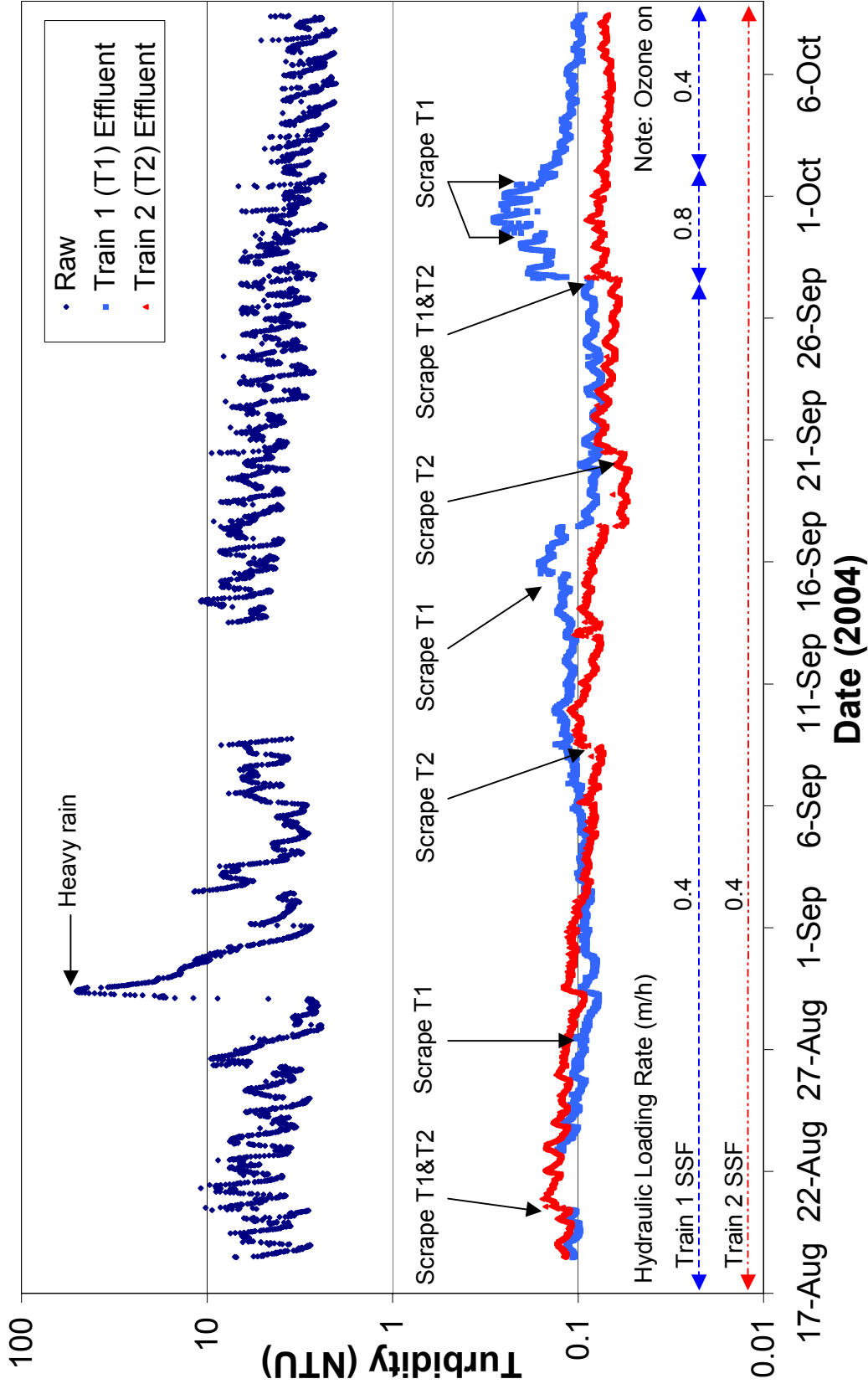
Figure 4-1: Pilot 1 2004 turbidity
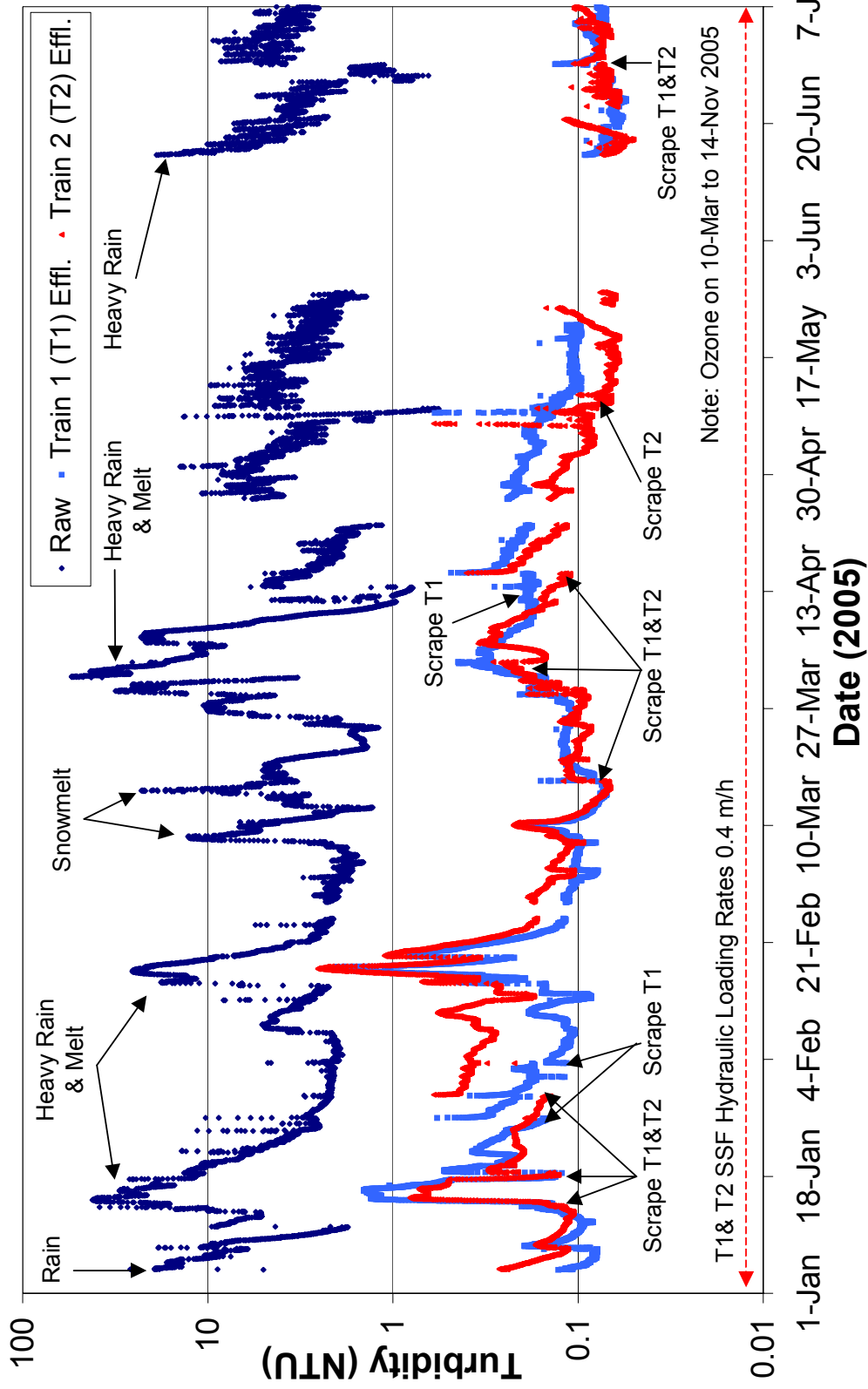
Figure 4-2: Pilot 1 2005 turbidity (January to June)
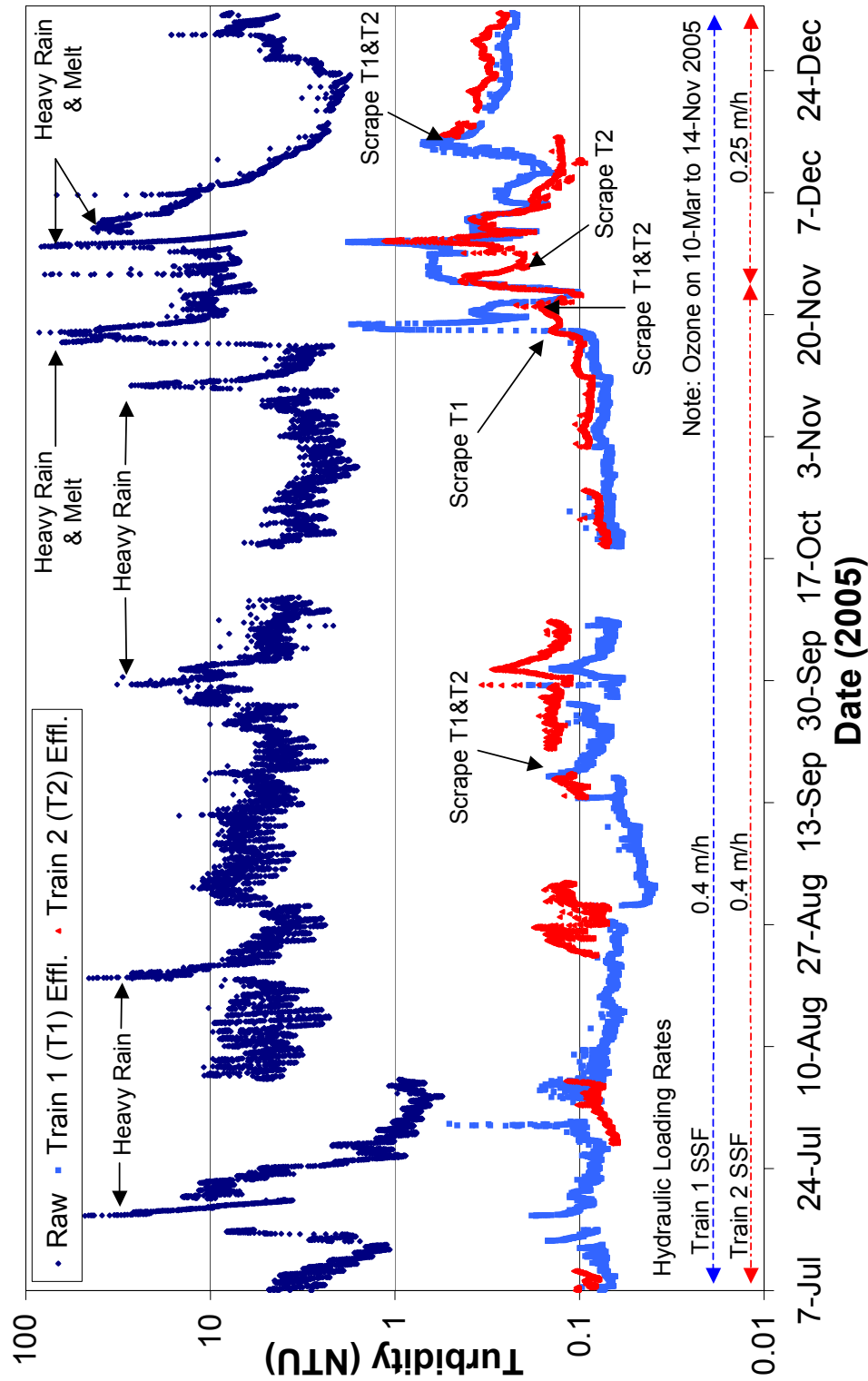
Figure 4-3: Pilot 1 2005 turbidity (July to December)

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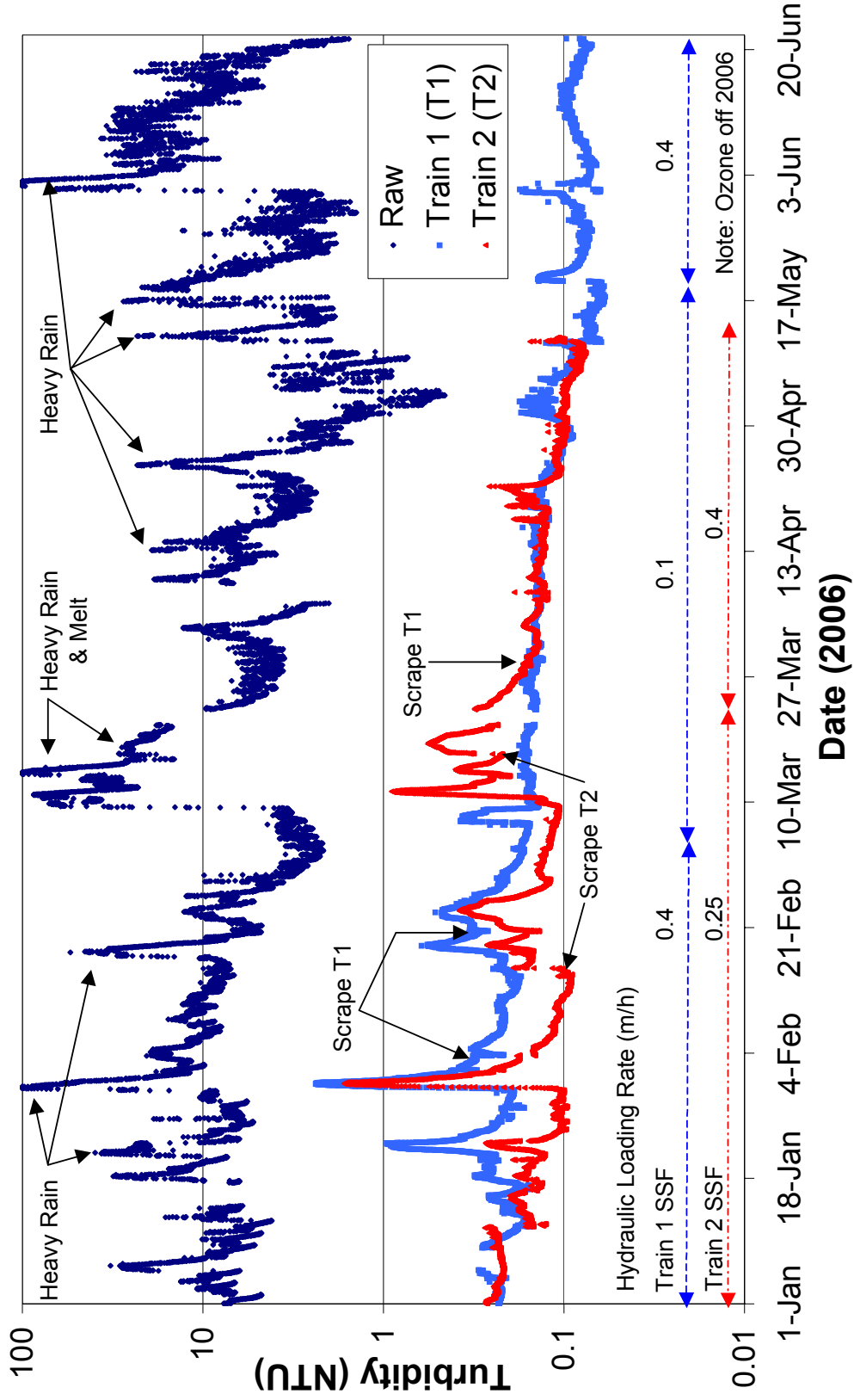
Figure 4-4: Pilot 1 2006 turbidity
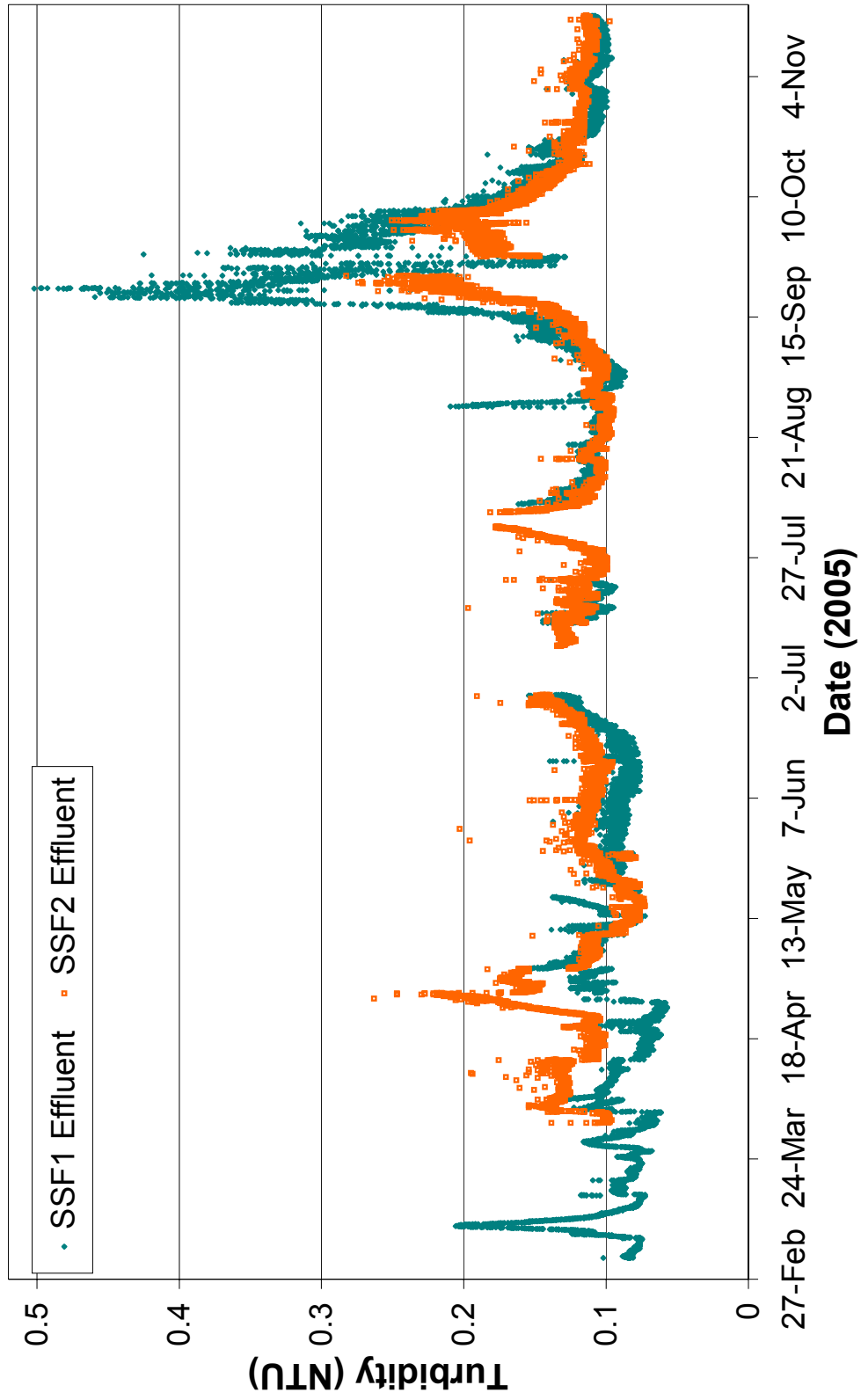
Figure 4-5: Pilot 2 2005 turbidity

The data in Table 4-1 show that pilot 1 raw water turbidities were less than 10 NTU approximately 85 to 95% of the time during the 2004 and 2005 study periods. In comparison, the 2006 study period had the greatest proportion of high raw water turbidities. Examination of actual data points, however, showed that 2005 and 2006 had approximately the same number (~4500) of turbidity measurements above 10 NTU. High raw water turbidities resulted from runoff during heavy rainfall and/or snowmelt events. The variable raw water turbidities, combined with water temperatures that ranged from 1 to 30°C, provided challenging operating conditions for the multistage slow sand filter systems.

Table 4-2: Slow sand filter effluent turbidities from online data set

Year	% Occurrence of SSF Effluent Water Turbidity in Stated NTU Ranges					n
	<0.1	0.1-≤0.3	0.3-≤1	1-≤3	≥3	
Pilot 1 Train 1						
2004	49.8	50.2	0	0	0	4648
2005	47.8	42.6	8.3	1.3	0	29549
2006	27.2	62.9	9.3	0.6	0	15730
Pilot 1 Train 2						
2004	71.7	28.3	0	0	0	4872
2005	32.4	51.8	15.2	0.6	0	29676
2006	10.9	81.5	7.1	0.5	0	12346
Pilot 2 SSF1						
2005	39.3	57.1	3.6	0	0	21528
Pilot 2 SSF2						
2005	10.3	89.7	0	0	0	20240

Despite the sometimes-elevated raw water turbidities, cold temperatures, and hydraulic loading rates at the high end of typical practice or above, the multistage slow sand filtration systems consistently achieved turbidity levels that met federal guidelines and Ontario regulations (Table 4-2). Federal guidelines in Canada and regulations in the province of Ontario, Canada, state that slow sand filtration systems shall provide treated water with turbidity less than or equal to 1.0 NTU in at least 95% of the measurements and shall not exceed 3.0 NTU at any time (Health Canada, 2003; MOE, 2006). As shown in Table 4-2, effluent turbidities were below 1.0 NTU

more than 98% of the time and never exceeded 3 NTU for both pilots during the entire study period. This excellent performance demonstrates the robustness of the tested multistage slow sand filtration systems, even under challenging conditions.

Although the online tubidity data set was not smoothed, it should be noted that pilot-scale systems are particularly susceptible to disturbances that would not affect full-scale systems. As such, a detailed log was kept during this study to determine when turbidity fluctuations were caused by operator or process disturbances and not by filter performance. Readings were removed from the data sets (prior to summarizing into tables in this chapter) when artificial turbidity increases resulted from operator disturbances such as cleaning online turbidity meters, flushing influent lines, and sampling. Data were also removed for time periods when no flow was reaching the online meters due to air lock in the influent tubes or when no flow was passing through the pilot filters (for example, during power outages or shutdowns). No data were removed when turbidity fluctuations were associated with SSF scraping, RF cleaning, or in response to high raw water turbidity events.

4.1.1 Turbidity Removal

Turbidity removals were calculated from data recorded by the online turbidimeters and measurements from the handheld turbidity meter.

4.1.1.1 Online Turbidity Data

For pilot 1, full-train turbidity removals were calculated for the 2004 to 2006 study periods by comparing simultaneous raw and effluent turbidities (Table 4-3).

Table 4-3: Pilot 1 full-train turbidity removals

Year	Average Turbidity Removal ± Standard Deviation (%)	
	Train 1*	Train 2**
2004	97.3 ± 1.4 (n = 4187)	97.9 ± 0.8 (n = 4409)
2005	96.0 ± 3.9 (n = 29465)	95.1 ± 4.6 (n = 29560)
2006	97.0 ± 2.7 (n = 14840)	97.1 ± 2.4 (n = 11544)

Note: calculated using data from online turbidimeters
* Log removals: 1.6±0.01 (2004), 1.4±0.02 (2005), 1.5±0.01 (2006)
** Log removals: 1.7±0.00 (2004), 1.3±0.02 (2005), 1.5±0.01 (2006)

As shown in Table 4-3, turbidity removals for pilot 1 (trains 1 and 2) were consistently high and ranged from 95 to 97%.

For pilot 2, full-train turbidity removals were not calculated using the online data set because pilot 2 raw water turbidity was not explicitly monitored (only pilot 1 raw water turbidity was measured continuously). However, when online effluent turbidities for 2005 were compared, it was calculated that SSF2 provided an average additional turbidity removal of –8% (n = 18299). This indicated that, on average, SSF2 provided no additional turbidity removal.

When the average effluent turbidities (and not turbidity removals) were analyzed, average online effluent turbidity was 0.12 ± 0.06 NTU for SSF1 (n = 21527) and 0.12 ± 0.03 NTU for SSF2 (n = 20240). Therefore, the online data set shows that SSF1 and SSF2 effluent turbidities were the same (at the 0.1% significance level) and that SSF2 provided no measurable turbidity removal. However, the second slow sand filter in series in pilot 2 may serve a valuable function to the system as an extra barrier to prevent pathogen breakthrough and ensure robustness (see Chapters 5 and 6 of this thesis).

4.1.1.2 Handheld Turbidity Data

In order to compare the contribution of the RF and SSF of each train to overall turbidity removal, turbidity was periodically measured using a handheld turbidity meter. For quality control,

handheld turbidity measurements for the raw water and SSF effluents were compared to online turbidity measurements to confirm the values were approximately equal. The turbidity of a deionized water blank was taken using the handheld meter each day sampling occurred ensure the unit was functioning. Handheld turbidity measurements were made for samples from the raw water line, secondary ozone contactors, RF effluents (SSF influents), and SSF effluents (Table 4-4).

Table 4-4: Contribution of each treatment unit to full-train turbidity removal

Treatment Unit	Average Contribution to Full-Train Turbidity Removal (%)		
	Pilot 1		Pilot 2***
	Train 1*	Train 2**	
RF	76 ± 17 (n = 95)	75 ± 21 (n = 82)	RFA 60 ± 16 (n=140) RFB 87 ± 7 (n = 59)
SSF	21 ± 16 (n = 94)	23 ± 20 (n = 82)	SSF1 37 ± 16 (n= 128) SSF2 0.2 ± 2.4 (n= 83)
Full Train	97 ± 3 (n = 95)	97 ± 3 (n = 84)	96 ± 4 (n = 88)

Note: calculated using data from handheld turbidity meter for 2004 to 2006
* Log removals: 0.6±0.08 (RF), 0.1±0.08 (SSF), 1.5±0.01 (full train)
** Log removals: 0.6±0.10 (RF), 0.1±0.10 (SSF), 1.5±0.01 (full train)
*** Log removals: 0.4±0.08 (RFA), 0.9±0.03 (RFB), 0.2±0.08 (SSF1), 0.00±0.01 (SSF2), 1.4±0.02 (full train)

Table 4-4 shows that average full-train turbidity removals for pilot 1 (trains 1 and 2) in the 2004 to 2006 study periods were 97 ± 3%, which compared well with average turbidity removals of 95-97% calculated from the online data set (Table 4-3).

Using the handheld measurements, it was determined that the RFs and SSFs of pilot 1 contributed to approximately 75-76% and 21-23% of overall (full-train) turbidity removal, respectively (Table 4-4). Therefore, in general, the pilot 1 RFs contributed to three-quarters of full-train turbidity removal, compared to one-quarter for the SSFs. Note that, as would be expected, the ozone contactors of pilot 1 were found to contribute very little (<2%) to full-train turbidity removal.

For pilot 2, the average full train turbidity removal was calculated to be 96 ± 4% by comparing raw and SSF2 effluent turbidities measured using the handheld meter (Table 4-4). This average was similar to the average pilot 1 full-train removals of 95-97% calculated using online data (Table 4-3).

Using the pilot 2 handheld data, it was calculated that, on average, nearly two-thirds (60%) of pilot 2 full-train turbidity removal occurred in RFA, over one-third (37%) occurred in SSF1, and very little additional removal was achieved in SSF2 (0.2%) (Table 4-4). Average turbidity removal was greater in RFB (87%) than RFA (60%), which was likely the result of the finer media and greater filtration efficiency in RFB. However, RFB became clogged on two occasions during this study and was dismantled so the media could be cleaned. To preserve the operation performance of RFs and prevent excessive clogging, literature recommended media sizes should be used.

In summary, the RFs of pilots 1 and 2 provided the majority of turbidity removal, while the SSFs were shown to provide one-quarter to one-third of full-train turbidity removal.

4.1.2 Effect of Filter Cleaning

In this pilot-scale study, SSF scraping generally caused minimal effluent turbidity increases of 0.02 to 0.1 NTU. Effluent turbidities typically returned to pre-scraping levels within 12 hours. This effect was expected and, at full scale, SSF effluents would be directed to waste following scraping until water quality targets (typically effluent turbidity and bacterial concentrations) were met.

However, greater-magnitude and longer-duration increases in effluent turbidities were observed following SSF scraping when raw water turbidity was high (i.e. during rain and snowmelt events), especially when temperatures were cold and HLRs were high. Therefore, SSF scraping should be avoided during challenging operational periods in order to minimize negative impacts on treatment performance. To avoid rapid head loss development and the need for filter scraping, HLRs should be decreased to below 0.4 m/h during extended periods of high raw water turbidity.

One example of rapid head loss due to a high HLR and filter scraping occurred in September 2004 (Figure 4-1). The turbidity removal of pilot 1 train 1 was negatively impacted when the SSF was operated at 0.8 m/h and scraped three times in five days. Although the SSFs of both trains 1 and 2 were scraped on September 27, only the effluent turbidity of train 1 increased because only the train 1 SSF had its HLR increased from 0.4 to 0.8 m/h (0.5 to 1.0 L/min). As shown in Figure 4-1, the train 1 SSF effluent turbidity increased from approximately 0.1 to 0.2 NTU after the first scraping, then increased to 0.3 NTU after the second scraping. Effluent turbidity actually decreased following the third SSF scraping, likely because the HLR was decreased to 0.4 m/h. Therefore, the combination of filter scraping and a very high HLR (0.8 m/h) impaired train 1 performance, whereas filter scraping during operation at a literature-recommended upper HLR limit (0.4 m/h) had no major impact.

4.1.3 Effect of Rain and Snowmelt Events

In general, the pilot systems were able to produce good effluent turbidity during short periods of high raw water turbidity that occurred, for example, during summer rainstorms. However, during extended periods of high influent turbidity caused by long duration rain and snowmelt events in the spring and fall, effluent turbidities increased measurably for several days.

The highest raw water turbidity in the 2004 study period (Figure 4-1) was approximately 50 NTU due to a summer rainstorm, compared to 85 NTU in November 2005 due to heavy extended rainfall (Figure 4-3). In the winter of 2006, raw water turbidities reached over 100 NTU on two occasions due to heavy rainfall and snowmelt (Figure 4-4). In June 2006 torrential rainfall produced raw turbidity of 360 NTU. The response of the pilot 1 multistage filtration systems to rain and snowmelt events in 2004 to 2006 are discussed below.

4.1.3.1 2004

In the 2004 study period, there were two very large rainstorms (Aug. 29 and Sept. 9) that dropped over 100 mm of rain each. However, these rainfall events did not cause a major increase in pilot 1 effluent turbidities because the challenging conditions lasted only for a short time (Figure 4-1). Both storms only caused small (~0.02 NTU) increases in effluent turbidities that

persisted for less than two days. This demonstrates that the tested multistage SSF systems operated at 0.4 m/h were robust and performed well during short-duration challenging conditions of high raw water turbidity.

4.1.3.2 2005

The 2005 turbidity data showed that long duration, high raw water turbidity events could temporarily disrupt system performance (Figure 4-2 and Figure 4-3). From January 13 to 15, 2005, a combination of heavy rainfall, snowmelt, SSF scraping (trains 1 and 2 on Jan. 13), high HLRs (0.4 m/h), and cold water temperatures resulted in effluent turbidity increases of 1.2 NTU (train 1) and 0.6 NTU (train 2). Effluent turbidities returned to pre-rainfall levels after four days. Train 1 effluent turbidity was twice as high as train 2 because the train 1 peristaltic pump had failed on January 12, 2005, thereby exposing the sand surface and disrupting the system.

A similarly challenging combination of heavy rainfall and snowmelt from February 15 to 17, 2005, resulted in raw water turbidity of 25 NTU. Pilot 1 effluent turbidities increased by 2 NTU and returned to pre-rainfall levels after three days. Another example of long-duration rain and melt conditions occurred from March 29 to April 7, 2005, which caused elevated effluent turbidities over a long time period (Figure 4-2).

In the fall of 2005 (Nov. 15-19, 25-30; Dec. 2-3), snowfall alternated with rain and snowmelt conditions, which caused high raw water turbidity spikes and long-duration increases in effluent turbidities (Figure 4-3). In response to the high solids loading and rapid head loss development, the pilot 1 SSFs needed to be scraped during this challenging time period (train 1 on Nov. 17 and 21; train 2 on Nov. 21 and 28, 2005). In general, filter scraping during challenging high turbidity events should be avoided and instead HLR should be reduced in order to prevent rapid increases in head loss.

4.1.3.3 2006

In 2006, a number of rainfall events (Jan. 21, 29-30; Feb. 17) and rain/snowmelt events (Feb. 22-23; Mar. 9-13, 16) resulted in high raw water turbidity and corresponding high effluent turbidities (Figure 4-4).

The effect of HLR on pilot 1 effluent turbidity was measurable during January and February 2006, during which time train 2 effluent turbidities increased less and for shorter durations than train 1 during rain/snowmelt events because train 2 was operated at 0.25 m/h compared to 0.4 m/h for train 1. Similarly, in March 2006, train 1 had a lower HLR and lower turbidity increase after rain and melt events compared to train 2. Therefore, during challenging periods of high raw water turbidity, SSFs should be operated at lower hydraulic loading rates to reduce turbidity breakthrough.

It should also be noted that the effect of scraping pilot 1 SSFs (train 1 on Feb. 3, Feb. 20 and Mar. 29; train 2 on Feb. 15 and Mar. 16) was difficult to determine since rain events often occurred shortly after scraping. However, as a general rule, SSF scraping should be avoided during challenging periods of high raw water turbidity.

4.2 Filter Run Length

Slow sand filter run lengths were determined by measuring the depths of water above the sand surfaces. In each SSF column, head loss increased over time until the water reached the overflow outlet near the top of the columns. Once terminal head loss was reached and the SSFs overflowed, the SSFs were cleaned by scraping off approximately 2 cm of sand from the top of the bed.

Over the study period (from January 2004 to June 2006), most filter runs ranged from 1 to 3 weeks in length before terminal head loss was reached (Figure 4-6). However, the SSF filter runs were occasionally ended even if terminal head loss had not been reached. For example, filter runs were ended when the filters were resanded or scraped in preparation for pathogen challenge tests. Figure 4-7 shows all filter runs, including those ended by terminal head loss and those ended for other reasons. Most filter runs were 1 to 3 weeks in length, regardless of whether terminal head loss was reached or not (Table 4-5).

As shown in Figure 4-7 and Table 4-5, a total of 46 filter runs were recorded between January 2004 and June 2006 for the SSF of pilot 1 train 1. Of those, 78% (36 filter runs) were 1 to 3 weeks long and 22% (10 filter runs) were 4 to 12 weeks long. Filter runs were ended with or without terminal head loss having been reached. When only filter runs that ended because of terminal head loss were considered, the proportion of filter run lengths less than or greater than 3 weeks (79% and 21%, respectively) was practically unchanged (Table 4-5).
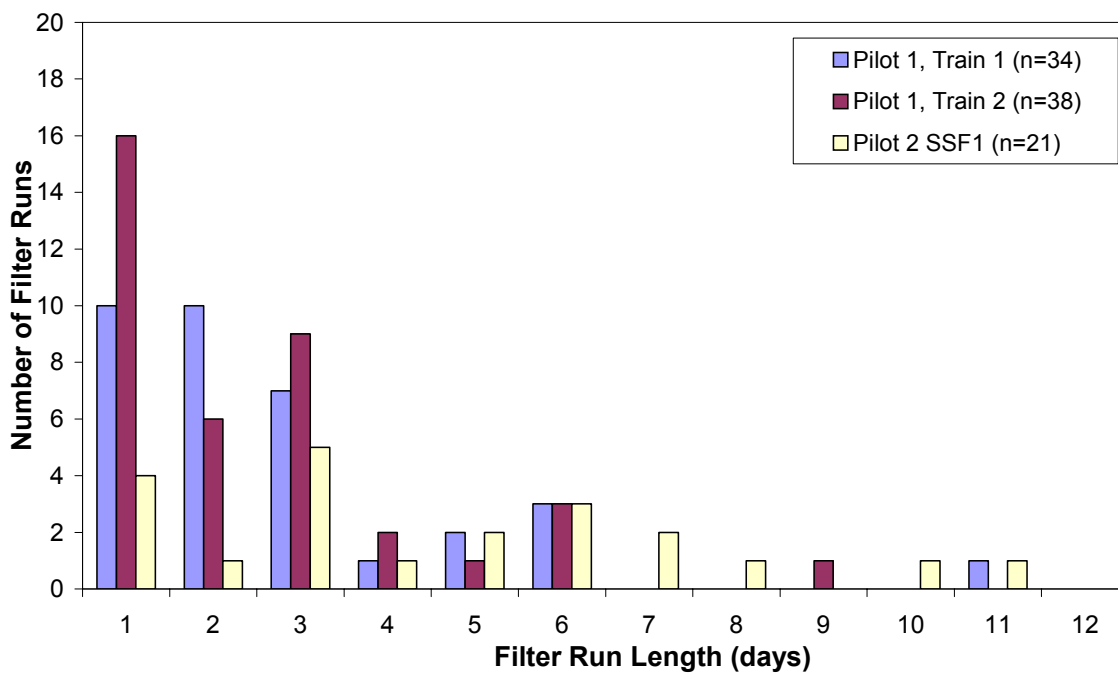
Figure 4-6: SSF run lengths based on terminal head loss
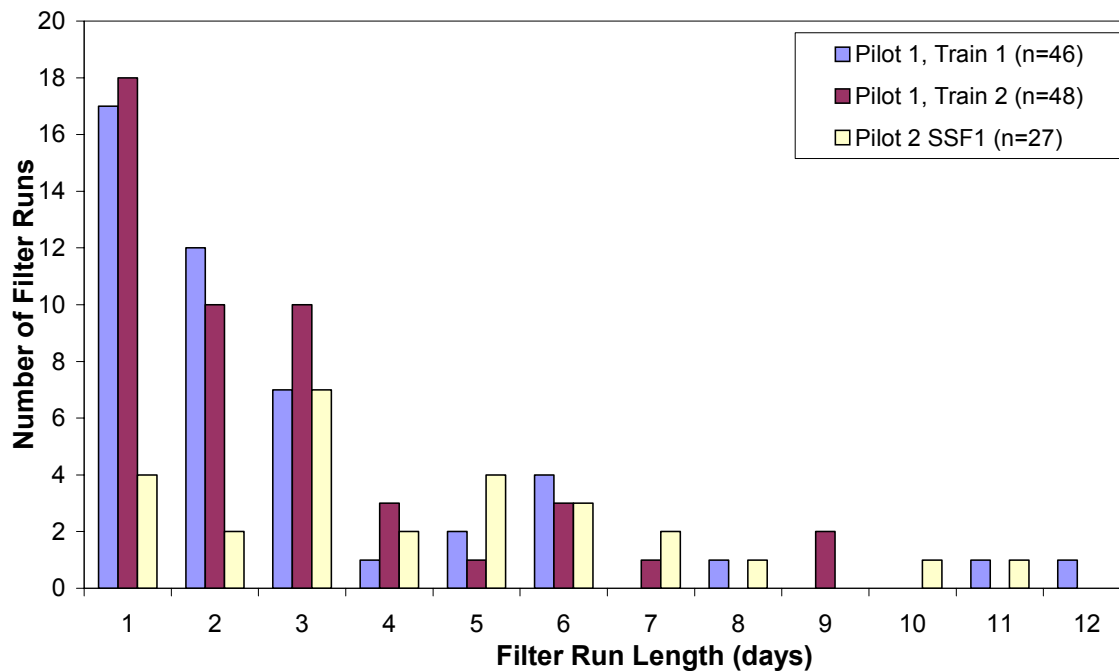
Figure 4-7: All SSF run lengths

Table 4-5: Filter run lengths

| Pilot | Filter Run Termination* | No. Filter Runs (%)** | | |
		1-3 weeks	4-12 weeks	Total
Train 1	Terminal HL	27 (79)	7 (21)	34
	All	36 (78)	10 (22)	46
Train 2	Terminal HL	31 (82)	7 (18)	38
	All	38 (79)	10 (21)	48
Pilot 2 SSF1	Terminal HL	10 (48)	11 (52)	21
	All	13 (48)	14 (52)	27

* Terminal HL - filter runs terminated because terminal head loss (HL) reached; All – includes all filter runs, even those where terminal HL had not been reached
** Filter run lengths recorded over the study period from January 2004 to June 2006

The pilot 1 train 1 filter run lengths correlated well with those for the pilot 1 train 2 SSF, for which a total of 48 filter runs were recorded between January 2004 and April 2006. Of those, 79% (38 filter runs) were 1 to 3 weeks long and 21% (10 filter runs) were 4 to 9 weeks long (Table 4-5 and Figure 4-7). For the train 2 SSF, no filter runs were longer than 9 weeks.

For the pilot 2 SSF1, a total of 27 filter runs were recorded between March 2004 and June 2006. Approximately half were 1 to 3 weeks long and half were 4 to 11 weeks long (Table 4-5). The filter run lengths for the SSF2 of pilot 2 were not included in the analysis because it was only scraped twice during the study period for reasons other than terminal head loss. Note that terminal head loss (i.e. SSF2 overflow) was never reached, likely due to the low solids loading. Therefore, if the SSF2 scrapings are disregarded and it is assumed that it would not have reached terminal head loss during the study period, the SSF2 filter run length would have been over 900 days long.

The short filter runs can be attributed to the pilot-scale nature of the system and occasional high raw water turbidity. A graphical depiction of filter run lengths, including vertical lines showing the dates when SSFs were scraped, illustrates that filter runs were shortest during the spring and fall when rainfall and snowmelt resulted in high raw water turbidity (Figure B-6 to Figure B-8 in Appendix B). Longer filter runs were experienced in the summer when raw water turbidities were low. For reference, the dates when the SSFs overflowed and were scraped are provided in Table B-1 to Table B-6 (Appendix B).

4.3 Water Temperature

Water temperatures were measured for the influent and effluent of each treatment unit of pilot 1 train 1 (Figure C-9), train 2 (Figure C-10), and pilot 2 (Figure C-11) (Appendix C). During the winter months, ambient air temperature inside the pump station was set at approximately 10°C to prevent water in the pilot filters from warming up excessively. During the summer months, the building was not cooled since it had no cooling system. It should be noted that from May to June 2006, the building air temperature was raised to 35°C to warm the water in the pilot filters during MS2 challenge tests 10 to 15 (see Chapter 6.0 of this thesis).

Figure C-9 to Figure C-11 (Appendix C) show that water temperature followed a seasonal pattern and did not increase to a large extent as water flowed through the pilot systems. In Figure C-9 for pilot 1 train 1, the average temperature increase was 0.6°C through the ozone contactor (between ports 0 and 1), 0.3°C through the RF (between ports 1 and 2), and 0.3° through the SSF

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(between ports 2 and 3). From May to June 2006 when the building air temperature was 35°C, water temperature increases averaged 2.1°C, 0.9°C, and 1.1°C through the ozone contactor, RF, and SSF, respectively.

In Figure C-10 for pilot 1 train 2, the average temperature increase was 0.7°C through the ozone contactor (between ports 0 and 1), 0.4°C through the RF (between ports 1 and 2), and 0.2°C through the SSF (between ports 2 and 3). Average temperature increases were similar in pilot 2 (Figure C-11), increasing 0.7°C through RFA (between ports 1 and 2A or 1 and 2*), 0.5°C through RFB (between ports 1 and 2B), 0.5°C through SSF1 (between ports 2A or 2* and 3), and 0.7°C through SSF2 (between ports 3 and 4).

The temperature increases through the pilot filters likely did not have a major impact on system performance but may have slightly shifted seasonal temperature variations by a small amount. However, during May and June 2006 when the building air temperature was raised to 35°C, treatment performance may have been improved due to increased biological activity in the filters.

4.4 Dissolved Oxygen

Dissolved oxygen (DO) concentration was measured in the influent and effluent of each treatment unit of the two pilot systems. DO concentrations showed the expected seasonal pattern of increasing DO with decreasing water temperature. In biological filters, DO is consumed by respiring microorganisms. By comparing the DO concentrations before and after each treatment unit, it was possible to determine the consumption of DO in each unit at a given point in time. An overall average DO consumption was calculated for each filter for the entire study period (Table 4-6).

As expected, the pilot 1 ozone contactors increased DO concentrations (Table 4-6). It appears that DO consumption in the RFs were greater than in the SSFs, which may indicate greater biological activity in the RFs. The RFs were upstream of the SSFs and therefore had higher concentrations of nutrients, organics, and dissolved oxygen to promote biological activity. Also, the RFs contained media with larger grain sizes than the sand in the SSFs, which would to some

extent have allowed microbial colonization and biofilm development throughout the entire depth of the RF beds. In contrast, although the SSF beds were also biologically active throughout the entire bed depth, most of the microbial populations would have been concentrated in the schmutzdecke layer on or near the top of the sand bed.

Table 4-6: Dissolved oxygen consumption in pilots 1 and 2

Pilot		Average Dissolved Oxygen Consumption (mg/L)			
		<10°C	10-20°C	>20°C	Overall***
Ozone Contactor*					
1	Train 1	-0.3	-0.7	-0.9	-0.6 ± 1.1
	Train 2	-0.3	-1.2	-0.9	-0.7 ± 1.1
Roughing Filter					
1	Train 1	2.2	3.0	2.5	2.4 ± 1.1
	Train 2	2.0	3.6	2.2	2.4 ± 1.0
2	RFA	1.6	1.4	1.3	1.5 ± 0.9
	RFB	2.0	0.7	0.9	1.3 ± 1.0
Slow Sand Filter					
1	Train 1	1.4	1.5	1.3	1.4 ± 0.7
	Train 2	1.4	1.7	1.3	1.5 ± 1.5
2	SSF1	1.3	1.4	1.2	1.3 ± 0.9
	SSF2**	-0.2	-0.8	-1.0	-0.5 ± 0.7

* Negative dissolved oxygen consumption indicates that dissolved oxygen increased through the ozone contactor
** In the influent of the pilot 2 SSF2, DO concentration increased because the inlet of SSF2 was usually above the water level in the column, which led to cascading influent water and re-aeration.
*** Number of observations (n): n = 83 for pilot 1 train 1; n = 69 for pilot 1 train 2; n = 81 for pilot 2 RFA, SSF1 and SSF2; n = 54 for pilot 2 RFB

The DO consumption in the pilot 1 RFs was greater than in pilot 2 RFA and RFB, despite the deeper bed depths of the pilot 2 RFs (Table 4-6). This was attributed to pre-ozonation in pilot 1, which promoted microbial growth by increasing DO concentrations and breaking down organic matter into more biodegradable fractions. The granular activated carbon (GAC) layer in the RFs of pilot 1 provided increased surface area for microbial attachment compared to the gravel in the pilot 2 RFs.

The overall average DO consumption in the RFs of pilot 1 were similar (Table 4-6). The greatest average DO consumption in these RFs was at water temperatures ranging from 10 to 20°C. At water temperatures greater than 20°C, biological activity (and hence DO consumption) may have been limited by low influent DO concentrations. Below 10°C, biological activity was likely limited by cold water temperatures.

For RFA and RFB of pilot 2, the greatest average DO consumption was at temperatures below 10°C (Table 4-6). RFB had lower average DO consumption than RFA, which suggests RFB had lower biological activity.

The pilot 1 SSFs and the pilot 2 SSF1 showed greatest average DO consumption at water temperatures of 10 to 20°C (Table 4-6). The average DO consumptions in these filters were approximately the same, despite SSF1 having had approximately twice the bed depth compared to the pilot 1 SSFs.

4.5 Concluding Remarks and Summary of Findings

Turbidity results were as follows:

- Effluent turbidity of all multistage SSF pilot systems were within the regulated effluent limits in Ontario for full-scale SSFs (below 1 NTU at least 95% of the time and never exceeded 3 NTU), despite measured raw water turbidity peaks over 300 NTU.
- Average full-train turbidity removals were 95-97% for pilot 1 (trains 1 and 2) and 96% for pilot 2.
- On average, the pilot 1 RFs and SSFs contributed to three-quarters (75-76%) and one-quarter (21-23%) of full-train turbidity removal, respectively. Minimal turbidity removal (<2%) was achieved through the ozone contact column, as expected.
- The average contribution of the pilot 2 SSF1 to full-train turbidity removal was much higher (37%) than SSF2 (0.2%). The average online effluent turbidity of SSF1 (0.12 ± 0.06 NTU) and SSF2 (0.12 ± 0.03 NTU) were approximately the same, which indicates that SSF2 provided no additional turbidity removal.

- On average, pilot 2 RFB contributed more to full-train turbidity removal (87%) compared to RFA (60%), likely due to the finer media and greater filtration efficiency in RFB.
- RFB became clogged on two occasions, which required it to be dismantled and cleaned. Literature recommended media sizes should be used when constructing RFs to avoid excessive head loss and clogging.

Filter run and SSF scraping findings included the following:

- Most SSF filter runs were short (1-3 weeks long), especially during spring and fall when rainfall and snowmelt events resulted in extended periods of high raw water turbidity. The longest filter runs were in the summer when raw turbidity was low.
- The short filter runs can be attributed to the pilot-scale nature of the system and occasional high raw water turbidity.
- For pilot 1 (trains 1 and 2), 80% of SSF filter runs were 1-3 weeks long and 20% were 4-12 weeks long.
- For pilot 2 SSF1, 50% of filter runs were 1-3 weeks long and 50% were 4-12 weeks long. SSF2 was only scraped twice in the study period because solids loading and head loss development were low.
- SSF scraping generally caused minimal effluent turbidity increases of 0.02 to 0.1 NTU. Effluent turbidities typically returned to pre-scraping levels within 12 hours of scraping.
- Large and long-duration increases in effluent turbidities were observed following SSF scraping when raw water turbidity was high, especially when temperatures were cold and HLRs were high. SSF scraping should be avoided during challenging operational periods.
- To avoid rapid head loss development and the need for filter scraping, HLRs should be decreased to below 0.4 m/h during extended periods of high raw water turbidity.
- Effluent turbidities increased measurably for several days during extended periods of high influent turbidity caused by long duration rain and snowmelt events in the spring and fall.

Results from the analysis of dissolved oxygen data included the following:

- Dissolved oxygen consumption in the RFs was greater than in the SSFs, which suggested that the RFs had greater biological populations and activities.
- DO consumption in the pilot 1 RFs was greater than in pilot 2 RFA and RFB, likely because of pre-ozonation and thus greater biodegradable organics fraction in pilot 1.
- Average DO consumption was similar in the pilot 1 RFs. In pilot 2, average DO consumption was higher in RFA than in the parallel RFB.
- The pilot 1 SSFs and the pilot 2 SSF1 showed greatest average DO consumption at water temperatures of 10 to 20°C (Table 4-6).
- Average DO consumption in the pilot 1 SSFs and pilot 2 SSF1 was approximately the same, despite the deeper bed of SSF1.
- The fact that sand depth was not an important factor determining DO consumption suggested that microbial populations were concentrated in the top of the sand bed.

5.0 *Cryptosporidium* and *Giardia* Challenge Test Results

Seven *Cryptosporidium* and *Giardia* challenge tests were performed between April 2004 and

August 2005 (Table 5-1). Each of the seven challenge tests listed in Table 5-1 are discussed in

Appendix E, including detailed test results and quality control procedures. Water quality data are

presented in Appendix D. The purpose of these challenge tests was to quantify oocyst and cyst

removal by slow sand filtration at different hydraulic loading rates (HLRs), water temperatures,

sand depths, and biological maturities.

Table 5-1: Overview of *Cryptosporidium* and *Giardia* challenge tests

Test Number*	Date	Pilot**	Hydraulic Loading Rate+ (m/h)	Protozoa++
1	28-Apr-04	2	0.4	C
2	31-May-04	1	0.4	C
3	7-Jun-04	1	0.8	C
4	26-Aug-04	1	0.4	C & G
5	30-Sep-04	1	0.8	C & G
6	2-Feb-05	1	0.4	C & G
7	9-Aug-05	2	0.4	C & G

* Challenge tests were carried out by Shawn Cleary (tests 1 to 3; Cleary, 2005), S. Ndiongue (tests 4
  and 5), S. Ndiongue and J. DeLoyde (test 6), and J. DeLoyde and K. Tabor (test 7)
** Pilot 1 train 1 (1) and pilot 2 (2), commissioned in Oct. and Dec. 2003, respectively
+ Only slow sand filters were seeded
++ Formalin inactivated *Cryptosporidium parvum* oocysts (C) and *Giardia muris* cysts (G)
Note: refer to Section 3.3 and Appendix E of this thesis for methods and results, respectively

As shown in Table 5-1, only *Cryptosporidium* oocysts were seeded into the SSF influents during

challenge tests 1 to 3 (Cleary, 2005). The SSFs of pilots 1 and 2 were relatively biologically

immature at the time of challenge tests 1 to 3 because the filters had only been in operation for 4

to 7 months in relatively cold water following commissioning in the fall of 2003. In order to

determine removals in more biologically mature SSFs, challenge tests 4 to 7 were conducted at

later dates (Table 5-1). *Cryptosporidium* oocysts and *Giardia* cysts were seeded simultaneously

into the SSF influents during challenge tests 4 to 7. Influent concentrations for *Cryptosporidium*

and *Giardia* were 10^6 oocysts/L and 10^5 cysts/L, respectively.

Most tests were conducted at a SSF hydraulic loading rate (HLR) of 0.4 m/h in order to determine oocyst and cyst removals at an HLR at the upper limit of that recommended in the literature. Tests 3 and 6 were conducted at an HLR of 0.8 m/h to simulate the situation where one of two trains would be offline for maintenance and all flow would be directed to one filter train. The SSFs of pilots 1 and 2 were tested in different seasons in order to determine the influence of water temperature and sand depth.

To measure the removal of cysts or oocysts, samples were collected from the SSF influent and effluent sampling ports (refer to Section 3.3 and Appendix E of this thesis). The effluent samples were collected 0.5 to 2 hours after the influent samples (depending on the hydraulic loading rate and SSF being tested) to account for the hydraulic detention time of water flowing through the filters. The samples were filtered onto membranes, antibody stains were applied, and the oocysts and cysts were enumerated by immunofluorescence assay (IFA) microscopy. Where no oocysts or cysts were recoved from one or more effluent samples, an effluent concentration of 1 oocyst/L or cyst/L was used for removal calculations. Percent removals were determined by calculating $[1-(C_{influent} / C_{effluent}) \times 100]$, where $C_{influent}$ and $C_{effluent}$ represent the oocyst or cyst concentrations in oocysts/L or cysts/L for corresponding influent and effluent samples. Note that recovery studies were not done and count data and calculated removals were not adjusted to account for recovery rate. For each test, average percent removal was determined by calculating the arithmetic mean of removals calculated for corresponding influent and effluent samples test (Appendix E). Throughout this thesis, the terms "average" has been used synonymously with "arithmetic mean." The average percent removal was transformed into an average log removal by calculating $[-Log_{10}(1-average/100]$. A summary of the results of all *Cryptosporidium* and *Giardia* challenge tests is presented below along with an analysis of important factors that influenced removals.

5.1 Summary and Analysis of Results

A summary of average *Cryptosporidium* oocyst and *Giardia* cyst removals for challenge tests 2 to 6 conducted on the SSF of pilot 1 train 1 are presented in Figure 5-1 and Table 5-2. The

average *Cryptosporidium* oocyst removals ranged from 2.6 to >4.4 logs. The average *Giardia* cyst removals were >3.8 to >4.5 logs.

The "greater than" sign on some of the calculated averages indicates that for a given challenge test, no oocysts or cysts were recovered from one or more effluent samples (i.e. complete removal was achieved). In such cases, removals were calculated based on an effluent concentration of 1 oocyst/L or cyst/L, which was reasonable considering that influent concentrations were approximately 10^6 oocysts/L and 10^5 cysts/L. The calculated removals were labeled with a "greater than" sign to indicate that removals were at least (and possibly greater than) the value shown (Appendix E). Greater removals may have been calculated if higher concentrations of oocysts and cysts had been seeded into the SSF influent so that some could be recovered in the effluent. It was not possible to calculate standard deviations for averages with "greater than" signs (Figure 5-1 and Table 5-2).

A summary of average *Cryptosporidium* oocyst and *Giardia* cyst removals for challenge tests 1 and 7 conducted at an HLR of 0.4 m/h on the SSF1 and SSF2 of pilot 2 are presented in Figure 5-2 and Table 5-3.

In general, it can be concluded that oocyst removal improved as the SSFs became biologically mature over time, that a mature SSF can achieve good oocyst and cyst removal even in cold water, and that oocyst removals were better at a HLR of 0.4 m/h compared to 0.8 m/h. It also appears that sand depth does not have a major impact on oocyst or cyst removal. Lastly, results for the SSF2 of pilot 2 are not representative because influent concentrations were too low to accurately quantify removal. SSF2 would need to be seeded separately with high concentrations of oocysts and cysts in order to quantify removal. These finding are discussed more thoroughly in the sections below.
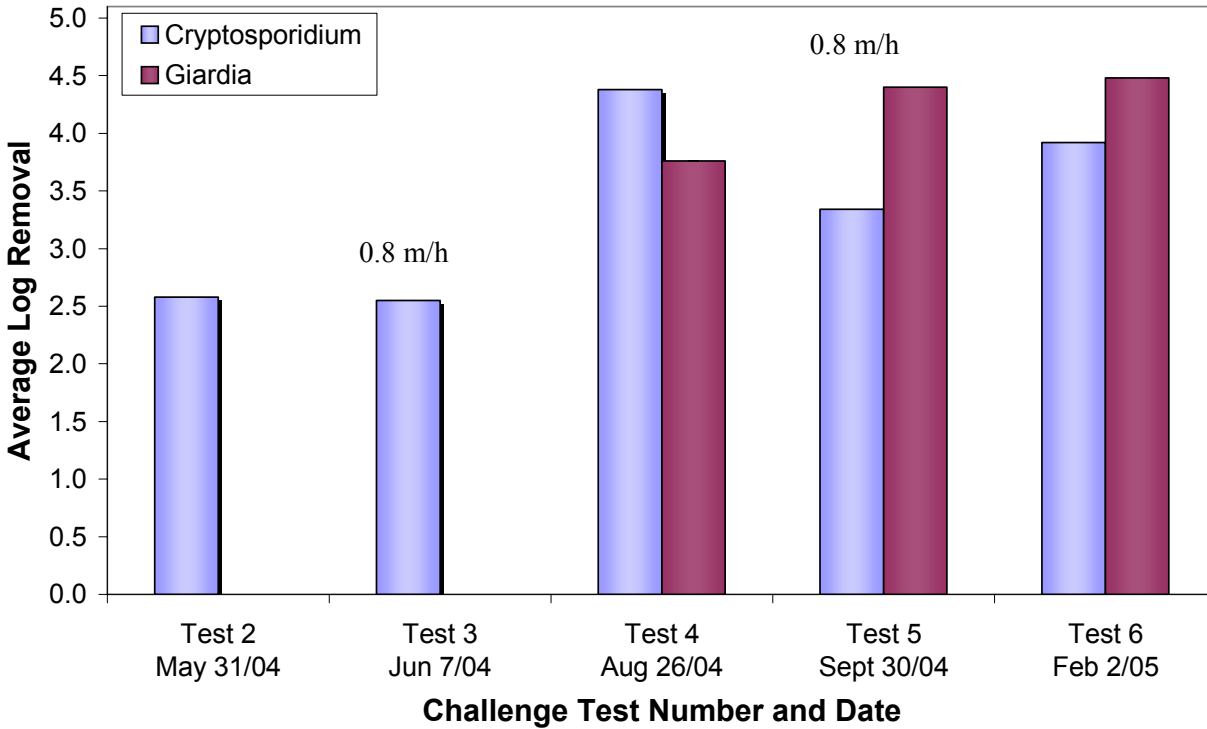
Figure 5-1: Pilot 1 train 1 SSF *Cryptosporidium* and *Giardia* average removals

Table 5-2: Summary of average removals for pilot 1 train 1 SSF

Test No.	Date	Average % Removal ± Standard Deviation* (logs)	
		Cryptosporidium oocysts	*Giardia* cysts***
2	31-May-04	99.73 ± 0.21 (2.6)	n.s.
3[+]	7-Jun-04	99.72 ± 0.23 (2.6)	n.s.
4	26-Aug-04	>99.996 (>4.4)**	> 99.98 (>3.76)
5[+]	30-Sept-04	99.95 ± 0.05 (3.3)	>99.996 (>4.40)
6[++]	2-Feb-05	99.988 ± 0.01 (3.9)	>99.997 (>4.48)

* Five samples (n=5) for each average log removal calculation (Appendix E)
** Greater than sign used because no oocysts detected in 2 effluent samples (Appendix E)
*** Greater than sign used because no cysts detected in 3 of the test 4 effluent samples, 4 of the test 5 effluent samples, and all 5 of the test 6 effluent samples (Appendix E)
[+] 0.8 m/h; all other tests at 0.4 m/h
[++] Cold influent water temperature (~2°C) at time of test
n.s. – cysts not seeded into SSF
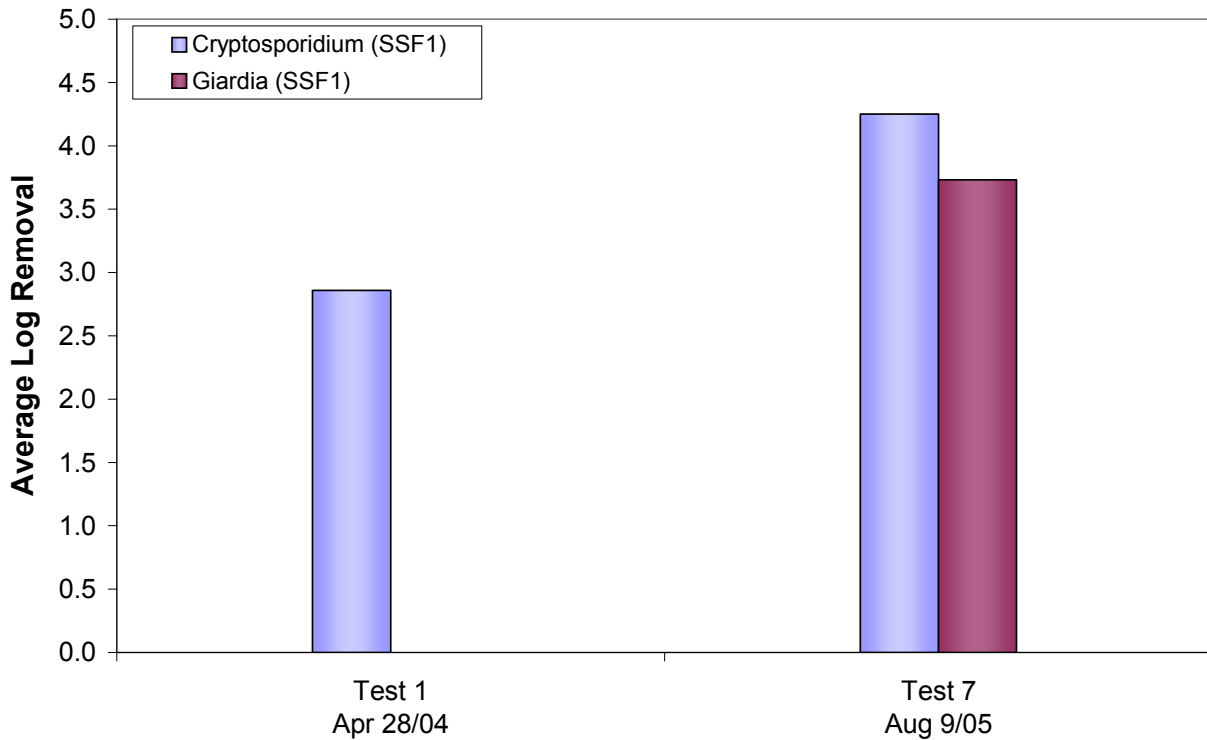
Figure 5-2: Average *Cryptosporidium* and *Giardia* removals in Pilot 2 SSF1

Table 5-3: Summary of average removals for pilot 2

		Average Removal ± Standard Deviation (logs)	
		Cryptosporidium oocysts	*Giardia* cysts
Test 1*	SSF1	99.86 ± 0.09 (2.9)	n.s.
28-Apr-04	SSF2	69.5 ± 38.5 (0.5)	n.s.
Test 7**	SSF1	>99.994 (>4.3)	>99.98 (>3.7)
9-Aug-05	SSF2	>-351 (>-0.7)	>-520 (>-0.8)

* n=4 for SSF1 and n=3 for SSF2 average log removal calculation (Appendix E); Sand depths: 100 cm (SSF1) and 50 cm (SSF2) (Appendix D)

** n=5 for each average log removal calculation (Appendix E); Greater than sign used because no cysts detected in 1 of each SSF1 and SSF2 test 1 effluent samples, 1 of the test 7 SSF1 effluent samples, and 3 of the test 7 SSF2 effluent samples (Appendix E); Sand depths: 100 cm (SSF1) and 44 cm (SSF2) (Appendix D)

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5.1.1 SSF Biological Maturity

Results of the challenge tests demonstrated that oocyst removals improved as the SSFs became biologically mature, which is consistent with findings in the literature (Bellamy *et al.*, 1985a; Schuler *et al.*, 1991). In a biologically mature filter, there would be greater biofilm coverage and build-up of organics and solids on the surface of the SSF and in the pore spaces. These factors would be expected to lead to increased oocyst and cyst removals by physical removal mechanisms. Microbial populations (bacteria, protozoa, rotifers, etc.) would also be higher in mature SSFs, which could improve oocyst and cyst removals by biological mechanisms. However, due to their relatively large size, physical straining and entrapment of (oo)cysts in a mature SSF would likely contribute more to removal than would biological mechanisms.

Comparing the 0.4 m/h challenge tests for pilot 1(Table 5-2) shows that average oocyst removals increased from 2.6 logs in test 2 (31-May-04) to >4.4 logs in test 4 (26-Aug-04) when the SSF was more biologically mature with greater biofilm coverage and solids accumulation in the pores of the filter media. Similarly, the pilot 1 challenge test results at an HLR of 0.8 m/h showed that average oocyst removals were greater in test 5 (3.3 logs, 30-Sept-04) compared to test 3 (2.6 logs, 7-Jun-04). Note that standard statistical analyses (e.g. T-tests based on the assumption of normally distributed data) were not undertaken due to the low number of data points (n = 5) used to calculate average removals for each challenge test. More advanced statistical analyses were beyond the scope of this research.

A similar trend of increasing *Cryptosporidium* removal with increasing maturity was observed in pilot 2 (Table 5-3). Oocyst removals in the SSF1 of pilot 2 increased from 2.9 logs in test 1 (28-Apr-04) to >4.3 logs in test 7 (9-Aug-05), which was attributed to increased biologically maturity and improved physical removal in the SSF bed.

The effect of biological maturity on average *Giardia* cyst removals in test 4 (>3.8 logs), test 5 (>4.4 logs), and test 6 (>4.5 logs) can not be evaluated because of the "greater than" signs, which indicate that no cysts were recovered in some effluent samples. If more cysts had been seeded into the SSF influent, then some cysts may have been recovered in the effluent and greater

removals calculated for comparison. It can only be concluded that average cyst removals in tests 4 to 6 were excellent.

5.1.2 Cold Water Temperatures

Very good *Cryptosporidium* oocyst and *Giardia* cyst removals were observed during test 6 in the biologically mature SSF of pilot 1 train 1, even at cold water temperatures of ~2°C (Table 5-2). In agreement with this finding, temperature was also reported as having no major influence on oocyst and cyst removal by slow sand filtration in the literature (Bellamy *et al.,* 1985a and 1985b; Pyper, 1985; Fogel *et al.*, 1993; Swertfeger *et al.,* 1999).

At the time of the cold water challenge test 6 in February 2005, the SSF of pilot 1 train 1 was considered to be biologically mature because it had been in continuous operation for approximately 15 months since the start-up of pilot 1 in October 2003. The mature SSF achieved good oocyst and cyst removals even at very cold water temperatures (1.4 to 2.5°C) and correspondingly low biological activity. Therefore, the removal of the oocysts and cysts in the mature SSF, which had well developed biofilms and deposits of organic material, was likely achieved by physical removal mechanisms such as straining.

Average removals of oocysts and cysts in the cold water challenge test 6 were similarly good in comparison to challenge tests 4 and 5 (Table 5-2), although the latter were conducted at much warmer water temperatures in the range of 17 to 23°C. Average oocyst removals were >4.4 logs in test 4, 3.3 logs in test 5, and 3.9 logs in test 6. Average *Giardia* cyst removals were >3.8 logs in test 4, >4.4 logs in test 5, and >4.5 logs in test 6. As demonstrated, cold water temperatures appeared to have no major effect on removals in the mature SSF.

5.1.3 Hydraulic Loading Rate

Cryptosporidium oocyst removals were slightly better at the lower HLR of 0.4 m/h compared to 0.8 m/h for the pilot 1 train 1 SSF (Table 5-2). Studies in the literature also reported a slight (if any) improvement in oocyst and cyst removals at lower HLRs (Bellamy *et al.,*1985a; Schuler *et al.*, 1991; Timms *et al.*, 1995).

The following analysis compares tests that were conducted at approximately the same filter maturity but at different HLRs (tests 2 and 3 were conducted 1 week apart; tests 4 and 5 were conducted 5 weeks apart). Average oocyst removal was approximately the same in test 2 (2.58 logs at 0.4 m/h) compared to test 3 (2.55 logs at 0.8 m/h). Average oocyst removal was greater in test 4 (>4.4 logs at 0.4 m/h) than test 5 (3.3 logs at 0.8 m/h). Average *Giardia* cyst removals were similarly good in test 4 (>3.8 logs) and test 5 (>4.4 logs).

Therefore, it can be concluded that for this filter configuration, oocyst removals were 0.03 to >1.1 logs higher at an HLR of 0.4 m/h compared to 0.8 m/h. Lower HLRs likely improved the removal of oocysts and cysts by reducing hydraulic shear, interstitial pore velocities, and the depth to which these particles penetrated into the bed. It is interesting to note that good removals were achieved at both HLRs.

5.1.4 Sand Depth

Since oocyst and cyst removals were similar in the SSFs of pilot 1 and 2, it can be concluded that the additional sand depth in the SSF1 of pilot 2 had no major effect on removals. This is consistent with findings in the literature that show oocyst and cyst removals occur primarily in the top 10 cm of SSF beds (Dullemont *et al*., 2006; Heller and Brito, 2006; Logan *et al*., 2001; Timms *et al*., 1995; Fox *et al*., 1984).

For pilot 2, SSF1 and SSF2 sand depths were 100 cm and 44 to 50 cm, respectively. For the pilot 1 train 1 SSF, sand depths ranged from 37 to 44 cm. At an HLR of 0.4 m/h, average oocyst removals ranged from 2.6 to >4.4 logs for the SSF of pilot 1 train 1 (Table 5-2) and ranged from 2.9 to >4.3 for the SSF1 of pilot 2 (Table 5-3). Likewise, average *Giardia* cyst removal ranged from >3.8 to >4.5 logs for pilot 1 and was >3.7 logs for the SSF1 of pilot 2 in test 7.

Sand depth likely had no major influence because oocysts and cysts are removed primarily by straining in the upper portion of the SSF bed. Therefore, it would appear that good oocyst and cyst removals can be achieved using shallower SSF bed depths than recommended in the literature. However, for full scale SSFs continuously exposed to (oo)cysts, a deeper bed's ability

to prevent breakthrough by storing (oo)cysts might be important, depending on the relative influent concentrations and rate of degradation (inactivation) of (oo)cysts retained in the SSF bed.

5.1.5 Second SSF in Series

In this study, the second SSF in series in pilot 2 appeared to provide minimal, if any, additional oocyst or cyst removal (Table 5-3). However, the low (and sometimes negative) removals calculated for the SSF2 of pilot 2 were likely not a result of poor removal in the filter, but were a result of the low SSF2 influent concentrations that were in the range of the detection limit (Table E-23 and Table E-25, Appendix E). In tests 1 and 7, the concentration of oocysts and cysts in the SSF1 effluent (which served as the SSF2 influent) were too low to permit quantification of removal through SSF2. SSF1 influent concentrations were approximately 10^6 oocysts/L and 10^5 cysts/L, compared to SSF1 effluent (i.e. SSF2 influent) concentrations ranging from 0-400 oocysts/L and 0-20 cysts/L. Therefore, because SSF2 influent concentrations were too low, the removals in SSF2 for tests 1 and 7 are unrepresentative and cannot be used to draw conclusions. In order to accurately quantify log removals in SSF2, high concentrations of oocysts and cysts should be seeded directly into the influent of SSF2.

5.2 Concluding Remarks and Summary of Findings

Five challenge tests were conducted on the SSF of pilot 1 train 1 between May 2004 and February 2005. HLRs were 0.4 or 0.8 m/h, water temperature ranged from 2 to 23°C, and sand depth ranged from 37 to 43 cm. Two challenge tests were conducted on both SSFs of pilot 2, one in April 2004 and the other in August 2005. HLR was 0.4 m/h, water temperature ranged from 10 to ~25°C, and sand depth was 100 cm in SSF1 and ranged from 44 to 50 cm in SSF2. A number of important findings can be drawn from this study:

- In general, all SSFs provided excellent removal of *Cryptosporidium* oocysts and *Giardia* cysts regardless of sand depth, hydraulic loading rate, and water temperature in the ranges tested.
- It would appear that good oocyst and cyst removals can be achieved using shallower SSF bed depths and higher HLRs than recommended in the literature.

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- Sand depths in the range tested had no major impact on oocyst and cyst removals in SSFs. Average *Cryptosporidium* removals were similar in the SSF of pilot 1 train 1 (2.6 to >4.4 logs) and in the SSF1 of pilot 2 (2.9 to >4.3 logs). Average *Giardia* removals were similar in the SSF of pilot 1 train 1 (>3.8 to >4.5 logs) and in the SSF1 of pilot 2 (>3.7 logs). Sand depth was likely not an important factor because (oo)cysts are removed primarily by physical straining in the top of the SSF beds.

- At cold water temperatures (~2°C in test 6), good removals of oocysts (3.9 logs) and cysts (>4.5 logs) were achieved in the biologically mature SSF of pilot 1 train 1, which had been in operation for 15 months.

- Results show that sand depth is not an important factor and that good removals can be achieved in a mature SSF even at ~2°C when biological activity is low, both of which suggest that physical (not biological) removal mechanisms predominate.

- Oocyst removals increased as SSF biological maturity increased over time, likely because of improved straining and entrapment due to greater biofilm coverage and accumulation of solids in the pore spaces of the filter media.

- Oocyst removals were slightly better at an HLR of 0.4 m/h compared to 0.8 m/h. Average oocyst removals in the SSF of pilot 1 train 1 were higher at 0.4 m/h (2.6 logs in test 2; >4.4 logs in test 4) compared to 0.8 m/h (2.6 logs in test 3; 3.3 logs in test 5).

- *Giardia* cyst removals in different tests were excellent, but could not be compared since the calculated averages had "greater than" signs because some effluent samples had zero cyst recoveries (i.e. complete removals). Therefore, *Giardia* removals were at least, and possibly greater than, the calculated average values which ranged from >3.7 to >4.5 logs.

- In the SSF2 of pilot 2, average *Cryptosporidium* removals ranged from >-0.7 to 0.5 logs and *Giardia* removals averaged >-0.8 logs. This was likely a result of low influent oocyst and cyst concentrations in the range of 0-400 (oo)cysts/L entering SSF2 via the SSF1 effluent, which were orders of magnitude lower than SSF1 influent concentrations. SSF2 should be seeded independently to more accurately quantify oocyst and cyst removal.

- The average removals demonstrated in this study were often greater than the removal credits given to slow sand filtration by regulatory agencies (3 logs oocyst removal – USEPA, 2006; 2 logs cyst removal – MOE, 2006).

6.0 MS2 Bacteriophage Challenge Test Results

A total of 16 MS2 bacteriophage challenge tests were conducted between February and June 2006. Water quality data for the MS2 challenge tests are included in Appendix F, plaque counts and removal calculations are presented in Appendix G, details of individual challenge tests are discussed in Appendix H, and quality control data are included in Appendix I.

The objectives of the MS2 challenge tests were to quantify MS2 removals by multistage slow sand filtration and determine the effects of hydraulic loading rate (HLR), water temperature, sand depth, and scraping of the schmutzdecke layer (Table 6-1).

Table 6-1: Experimental variables for MS2 challenge tests

Treatment Unit*	Pilot	Hydraulic Loading Rate (m/h)	Water Temperature**	Test Number
SSF	1	0.4	Cold	1, 2, 8***
			Warm	12, 13, 15
		0.1	Cold	4, 5, 7a, 7b
			Warm	10, 11
	2	0.4	Cold	9
			Warm	14
RF	1	1.5	Cold	3
	2	0.95	Cold	6

* Slow sand filter (SSF) and roughing filter (RF)
** Cold influent water temperatures 2 to 5°C (tests 1, 2, 3 and 6) and 6 to 10°C (tests 4, 5, 7a, 7b, 8 and 9); Warm influent water temperatures >20°C (tests 10 to 15) (Appendix F)
*** All pilot 1 tests on train 1, except test 8 on train 2

As shown in Table 6-1, challenge tests were conducted on both pilot systems at HLRs of 0.1 and 0.4 m/h for the slow sand filters and at HLRs of 0.95 and 1.5 m/h for the roughing filters. Water temperatures ranged from 2 to 25°C.

The MS2 challenge tests conducted on the SSF of pilot 1 train 1 were replicated as follows: tests 1 and 2 at 0.4 m/h in cold water; tests 4, 5 and 7a/b at 0.1 m/h in cold water; tests 10 and 11 at 0.1 m/h in warm water; and tests 12, 13 and 15 at 0.4 m/h in warm water. MS2 challenge tests 3,

6, 8, 9, and 14 were not replicated. Note that factorial experimental design was not employed (to determine interaction effects) as it was not feasible to control all parameters on the pilot systems because they treated naturally variable raw river water.

MS2 were seeded into the influent of the slow sand filters or roughing filters of pilots 1 or 2, depending on the experiment (Table 6-1). MS2 seeding typically continued for 12 hours before the start of sampling to allow the MS2 concentrations to reach steady state of 10^4 to 10^5 PFU/mL (Appendix G). Effluent samples were collected 0.3 to 4 hours after their corresponding influent samples, depending on the hydraulic loading rate of the SSF or RF being tested, to account for the hydraulic detention time of water flowing through the filters (refer to Section 3.4 of this thesis). Average (arithmetic mean) removals were calculated for each test. The average removal of each test were statistically compared to each other using a modified t-test only if n $\geq$ 7 (refer to Section 3.4.9

A summary of results for the MS2 challenge tests is presented below along with an analysis of important factors that influenced MS2 removals. More detailed information on individual experiments can be found in Appendix F to Appendix I.

6.1 Experimental Conditions

The experimental conditions for MS2 challenge tests 1 to 15 are listed in Table 6-2. The MS2 challenge tests in Table 6-2 are listed in chronological order according to the date they were performed. For the cold water tests 1 to 9, the 0.4 m/h slow sand filter tests (tests 1 and 2) were carried out first, followed by the 0.1 m/h tests (tests 4, 5, and 7). The two roughing filter tests (tests 3 and 6) were also carried out in this same time period. Since test 7 involved nearly four days of MS2 seeding, other cold water tests were carried out at the same time on pilot 1 train 2 (test 8) and pilot 2 (test 9). The last day of cold water experimentation (test 7b) was conducted on March 31, 2006, and the first warm water experiment (test 10) was conducted on May 15, 2006 (Appendix F).

As shown in Table 6-2, the temperature of the filter influent was always 1 to 5°C greater than the raw water because, as water passed through system piping and the upstream roughing filter, it was warmed by the air in the building that housed the pilot filters. During the cold water MS2 challenge tests 1 to 9 from February to March 2006, the air temperature in the building was maintained at 10°C to minimize warming of water as it passed through the filters and associated plumbing. Conversely, during the warm water MS2 challenge tests 10 to 15 from May to June 2006, the air temperature in the building was maintained at 35°C to promote warming of the water in order to increase the biological activity in the filters.

Table 6-2: Detailed experimental conditions for MS2 challenge tests

Test No.	Pilot[++]	HLR* (m/h)	Influent Water Temperature (°C)***		Sand Depth (cm)
			Raw	Influent	
1		0.4	2	4	40
2		0.4	1	3	38
3[+]		1.5	2	3	
4		0.1	3	7	38
5		0.1	2	6	38
6[+]	P2	0.95	5	5	
7a		0.1	7	9	38
7b		0.1	9	10	36
8	P1/T2	0.4	7	8	36
9	P2	0.4	7	8	92/44**
10		0.1	16	21	36
11		0.1	17	22	36
12		0.4	21	23	36
13		0.4	20	22	36
14	P2	0.4	22	22	88/44**
15		0.4	22	24	36

[+] All tests on slow sand filters except tests 3 and 6 which were conducted on roughing filters
[++] All tests on pilot 1 train 1 except as noted for pilot 1 train 2 (P1/T2) & Pilot 2 (P2)
* Hydraulic loading rate (HLR) of the filter
** First value is sand depth of SSF1, second value is sand depth of SSF2 (Appendix F)
*** Approximate temperature of raw water and filter influent water (Appendix F)

The raw water temperature was below 5°C for tests 1 to 6 (Table 6-2), which were carried out between February 14 and March 21, 2006 (Table 6-3). Tests 7, 8, and 9 were conducted from March 27 to 31, 2006, during which time the first warm days of spring occurred. As a result, the raw water temperatures increased to between 6 and 10°C. Nevertheless, tests 7 to 9 were considered to be cold water experiments because it had taken the raw water less than a week to warm up from less than 5°C to between 6 and 10°C (Appendix F).

The slow sand filter of pilot 1 train 1 was only scraped twice during the period of MS2 spiking, once after test 2 and again between tests 7a and 7b (Table 6-2 and Appendix H). The sand depth of the pilot 1 train 1 SSF dropped due to scraping from 40 to 36 cm over the test period, which was approximately the same as the SSF of pilot 1 train 2 (36 cm, test 8) and pilot 2 SSF2 (44 cm, tests 9 and 14). Only the SSF1 of pilot 2 had a considerably deeper sand bed, which was 92 cm deep in test 9 and 88 cm deep in test 14 (Table 6-2).

A summary of the hydraulic loading rates, sampling times, MS2 spike concentrations, and MS2 seeding durations for the challenge tests is presented in Table 6-3. As shown in Table 6-3, a flow rate of 0.5 L/min corresponded to an HLR of 0.4 m/h for the SSFs of pilot 1 trains 1 and 2. For pilot 2, a flow rate of 0.5 L/min yielded an HLR of 0.4 m/h for SSF1. Due to the smaller column diameter of SSF2, only a fraction of the SSF1 effluent flow (0.11 L/min) was diverted into SSF2 to yield an HLR of 0.4 m/h (refer to Section 3.1).

In addition to sampling during filter seeding, samples were taken at time zero before seeding began and up to a few weeks after seeding terminated (Table 6-3). The duration of MS2 seeding into the filters ranged from 3 to over 87 hours. The preparation dates of the MS2 spikes are listed in Table 6-3 along with the MS2 concentration in the spike bottles before and after each test. MS2 concentrations in the spike bottles remained stable throughout all tests.

Table 6-3: Hydraulic conditions, sampling times and MS2 seeding durations for spiking tests

Test No.	Test Details*	Flow (L/min)	HDT (h)**	Sampling Times (2006)	MS2 Spike Preparation Date	Concentration (PFU/mL)*** Before	After	MS2 Seeding Start Time	End Time	Duration (h)	Influent Water Temp. (°C)
1	P1/T1 SSF 0.4m/h	0.5	1	0-7h (14-Feb) 22-27h (15-Feb)	27-Jan-06	8.0×10^7	6.8×10^7	14-Feb 09:00	15-Feb 12:00	27	4
2	P1/T1 SSF 0.4m/h	0.5	1	0-7h (27-Feb) 24-28h (28-Feb)	23-Feb-06	7.7×10^7	7.7×10^7	27-Feb 08:00	28-Feb 12:00	28	3
3	P1/T1 RF 1.5m/h	0.5	0.33	0-180 min (6-Mar)	23-Feb-06	7.7×10^7	7.7×10^7	6-Mar 19:00	6-Mar 22:00	3	3
4	P1/T1 SSF 0.1m/h	0.125	4	11-15h (9-Mar)	23-Feb-06	7.7×10^7	8.4×10^7	8-Mar 21:15	9-Mar 12:15	15	7
5	P1/T1 SSF 0.1m/h	0.125	4	10.5-15.5h (20-Mar) 38-43h (21-Mar)	15-Mar-06	-	1.0×10^8	19-Mar 21:15	21-Mar 16:15	43	6
6	P2 RFA 0.95m/h	0.5	0.5	2-3.5h (21-Mar)	23-Feb-06	7.7×10^7	8.4×10^7	21-Mar 13:20	21-Mar 16:50	3.5	5
7a	P1/T1 SSF 0.1m/h	0.125	4	11.5-24h (28-Mar) 35.5-40.25h (29-Mar)	27-Mar-06	-	8.3×10^7	27-Mar 20:45	29-Mar 13:00	40.25	9
7b	P1/T1 SSF 0.1m/h	0.125	4	59.75-64.5h (30-Mar) 82.5-87.25h (31-Mar)	28-Mar-06	-	8.7×10^7	29-Mar 13:30	31-Mar 12:00	87.25	10
8	P1/T2 SSF 0.4m/h	0.5	1	11.5-15.5h (29-Mar)	28-Mar-06	-	8.7×10^7	28-Mar 21:00	29-Mar 12:30	15.5	8
9	P2 SSF1-2 0.4m/h	0.5+ 0.11++	2h+ 1h++	19.5-24h (30-Mar)	28-Mar-06	-	8.7×10^7	29-Mar 13:00	30-Mar 13:00	24	8
10	P1/T1 SSF 0.1m/h	0.125	4	10.25-15h (15-May) 34.5-39.25h (16-May)	12-May-06	-	8.4×10^7	14-May 21:30	16-May 12:45	39.25	21
11	P1/T1 SSF 0.1m/h	0.125	4	11.25-16h (18-May) 34.75-39.5h (19-May)	17-May-06	1.1×10^8	1.2×10^8	17-May 21:45	19-May 13:15	39.5	22
12	P1/T1 SSF 0.4m/h	0.5	1	11.5-15.5h (2-Jun)	24-May-06	7.4×10^7	6.3×10^7	1-Jun 20:15	2-Jun 12:30	16.25	23
13	P1/T1 SSF 0.4m/h	0.5	1	12-16h (5-Jun)	24-May-06	7.4×10^7	6.3×10^7	4-Jun 20:15	5-Jun 12:15	16	22
14	P2 SSF1-2 0.4m/h	0.5+ 0.11++	2h+ 1h++	11.5-16h (5-Jun)	01-Jun-06	7.1×10^7	5.6×10^7	4-Jun 20:45	5-Jun 12:45	16	22
15	P1/T1 SSF 0.4m/h	0.5	1	16.75-18.75h (8-Jun)	24-May-06	6.3×10^7	3.6×10^7	7-Jun 15:30	8-Jun 10:15	18.75	24

* Pilot 1 (P1), train 1 (T1), train 2 (T2); pilot 2 (P2), slow sand filter 1 (SSF1), slow sand filter 2 (SSF2); roughing filter (RF)
** Hydraulic detention time (HDT); *** MS2 concentration in spike bottle before and after challenge test; + SSF; ++ SSF2

6.2 Summary of Average MS2 Removals

A summary of average MS2 removals is presented in Figure 6-1. The removal of MS2 phage refers to the removal of MS2 by physical and biological mechanisms such as adsorption to sand grains, entrapment in biofilms, predation, inactivation, etc. Average removals are also included in Table 6-4 for the pilot 1 SSF tests, Table 6-5 for the pilot 2 SSF tests, and Table 6-6 for the roughing filter tests.

Table 6-4: Average MS2 removals for pilot 1 SSF tests

Test No.	Pilot 1** SSF HLR (m/h)	Average % MS2 Removal ± % Standard Deviation (log removal)*		n
Cold Water Tests[++]				
4	0.1	99.3	(2.2)	1[+]
5	0.1	96.9 ± 0.7	(1.5)	9
7a	0.1	99.3 ± 0.1	(2.2)	8
7b	0.1	98.3 ± 0.7	(1.8)	8
1	0.4	58.9 ± 14.1	(0.4)	15
2	0.4	39.7 ± 13.7	(0.2)	14
8	0.4	66.6 ± 2.8	(0.5)	7
Warm Water Tests[+++]				
10	0.1	99.2 ± 0.2	(2.1)	8
11	0.1	99.1 ± 0.2	(2.0)	8
12	0.4	85.9 ± 1.9	(0.9)	7
13	0.4	90.5 ± 0.8	(1.0)	7
15	0.4	94.5 ± 1.9	(1.3)	5

* Detailed results in Appendix G
** All tests on slow sand filter (SSF) of pilot 1 train 1 except test 8 on pilot 1 train 2; hydraulic loading rate (HLR)
[+] Due to sampling error (discussed in Appendix H)
[++] Cold influent water temperatures 2 to 5°C (tests 1 and 2) and 6 to 10°C (tests 4, 5, 7a, 7b, and 8) (Appendix F)
[+++] Warm influent water temperatures >20°C (tests 10 to 15) (Appendix F)
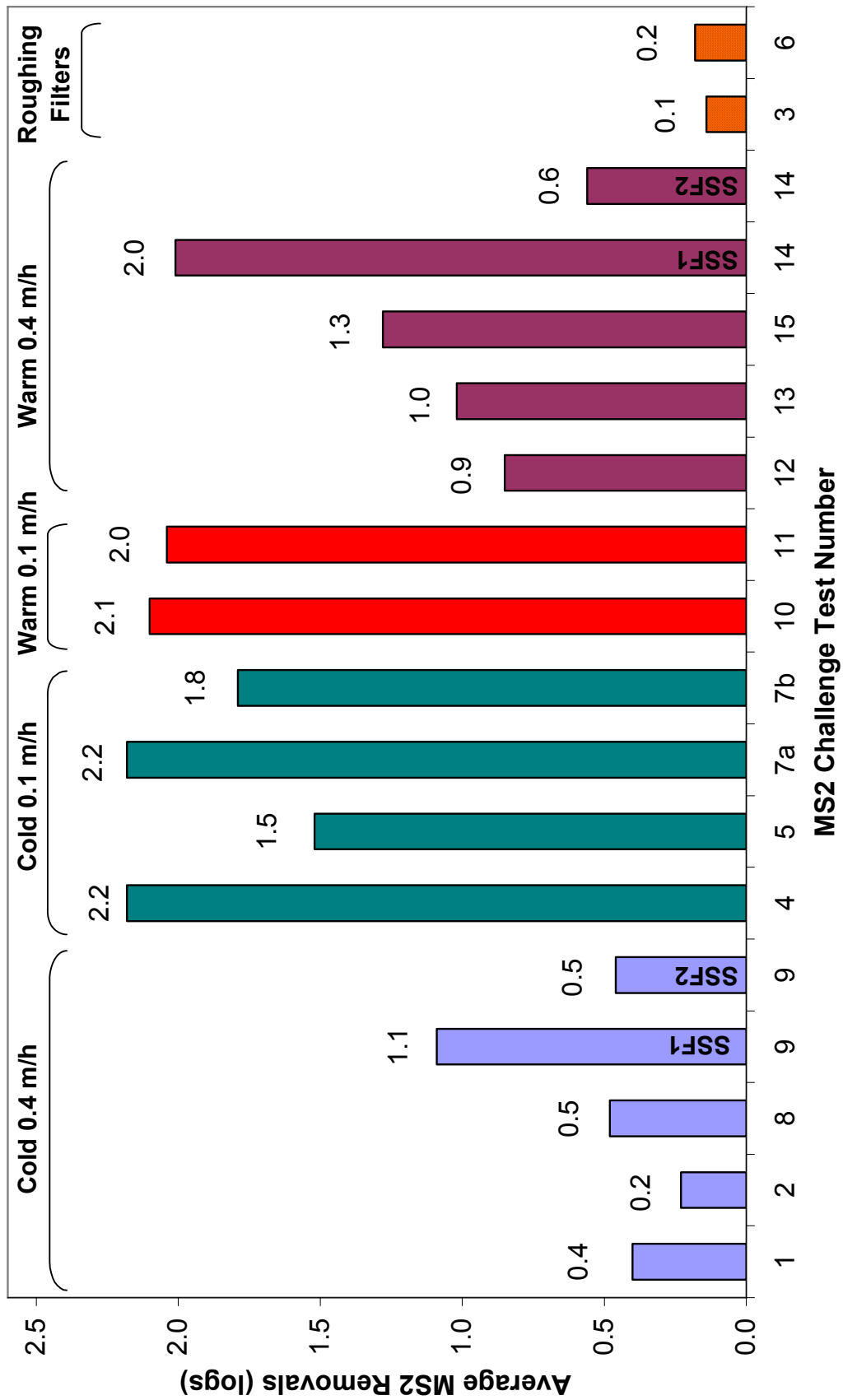
Figure 6-1: Summary of average MS2 removals

Table 6-5: Average MS2 removals through two in-series SSF in pilot 2 at 0.4 m/h HLR

Test No.		Average % MS2 Removal ± % Standard Deviation (log removal)*	n
Cold Water Test*			
9	SSF1 Effluent	91.8 ± 0.9 (1.1)	6
	SSF2 Effluent	65.1 ± 6.4 (0.5)	4
	Total (SSF1+SSF2)	97.2 ± 0.8 (1.6)	
Warm Water Test**			
14	SSF1 Effluent	99.0 ± 0.1 (2.0)	6
	SSF2 Effluent	72.2 ± 4.9 (0.6)	6
	Total (SSF1+SSF2)	99.7 ± 0.1 (2.6)	

* Detailed results in Appendix G
** Cold influent water temperatures 6 to 10°C (test 9) (Appendix F)
*** Warm influent water temperatures >20°C (test 14) (Appendix F)

Table 6-6: Average MS2 removals for roughing filter tests

Test No.	Roughing Filter** HLR (m/h)	Average % MS2 Removal ± % Standard Deviation (log removal)*	n
Cold Water Test*			
3	1.5	28.0 ± 5.2 (0.1)	6
6	0.95	32.1 ± 14.1 (0.2)	5

* Detailed results in Appendix G
** Test 3 on RF of Pilot 1 Train 1; Test 6 on RFA of pilot 2
*** Cold influent water temperatures 2 to 5°C (Appendix F)

In general, MS2 removals were greater at an HLR of 0.1 m/h compared to 0.4 m/h. MS2 removals were greater in warm water compared to cold water (provided that sufficient warm water acclimation time was provided). The results of tests 7a/b demonstrated that schmutzdecke scraping had only a minor impact on MS2 removals. The deeper sand bed of SSF1 led to dramatically higher MS2 removals compared to the shallow-bed SSFs. In pilot 2, the second SSF in series provided additional MS2 removals. MS2 removals by the two roughing filters tested were minimal. The factors influencing MS2 removals are discussed in detail below.

6.3 Factors Influencing MS2 Removal by SSF

Both biological and physiochemical mechanisms act to remove or inactivate viruses in slow sand filters. The primary biological removal mechanisms include predation by microorganims, such as bacteria and filter feeding protozoa, and attachment to biofilms and biomass. Although viruses are too small to be removed by physical straining, physiochemical adsorption/attachment to filter media is an important removal mechanism under favourable conditions. Note that although it may be more appropriate to use the term attachment instead of adsorption for colloid/virus transport, the term adorption has been used below if it was used in the literature sources cited.

In this study, a number of factors such as hydraulic loading rate, water temperature, the presence or absence of the schmutzdecke layer, and sand depth were varied to determine their influence on the removal or inactivation of MS2 bacteriophage. An analysis of each factor is presented below.

6.3.1 Hydraulic Loading Rate

In this study, removal of MS2 by slow sand filtration increased with decreasing hydraulic loading rate. For the cold water tests, average MS2 removals were significantly greater (at the 1% significance level) for tests conducted at an HLR of 0.1 m/h compared to 0.4 m/h (Table 6-7).

For the cold water SSF tests (Table 6-7), average removals at an HLR of 0.4 m/h ranged from 0.2 to 0.5 logs. Average removals were significantly greater for cold water tests conducted at 0.1 m/h (ranging from 1.5 to 2.2 logs) compared to 0.4 m/h (0.2 to 0.5 logs). The greater MS2 removals at the lower HLR were likely the result of longer detention times in the SSFs, which would have provided more time for removal of MS2 by biological mechanisms such as predation and inactivation by microbial enzymes. In addition, lower HLRs would have decreased hydraulic shear, thereby permitting greater transport/diffusion of virus-sized particles to media and biofilm surfaces for attachment. Lower shear would also cause less detachment of attached phage and provide greater opportunity for detached viruses to re-attach lower in the filter.

Table 6-7: MS2 removals by SSF at different HLRs in cold water

Hydraulic Loading Rate (m/h)	Test No.	Average MS2 Removal (Logs)
0.1	4	2.2
	5	1.5
	7a	2.2
	7b	1.8
0.4	1	0.4
	2	0.2
	8*	0.5
	9*	0.5 (SSF2)**

* All tests on the SSF of pilot 1 train 1 except test 8 on pilot 1 train 2 & test 9 on pilot 2
** SSF2 test 9 included because sand depth was comparable to pilot 1 SSFs (Table 6-2)

For the warm water experiments, average MS2 removals at an HLR of 0.1 m/h in test 10 (2.0 logs) and test 11 (2.1 logs) were significantly greater (at the 1% significance level) than the average removals in warm water tests conducted at 0.4 m/h, which ranged from 0.9 to 1.3 logs (Table 6-8).

Table 6-8: MS2 removals by SSF at different HLRs in warm water

Hydraulic Loading Rate (m/h)	Test No.	Average MS2 Removal (Logs)
0.1	10	2.1
	11	2.0
0.4	12	0.9
	13	1.0
	15	1.3

Note: all tests on the SSF of Pilot 1 Train 1

Studies have shown that removal of polioviruses and echoviruses improved when HLRs were decreased in slow sand filters (Poynter and Slade, 1977) and in sand columns (Wang *et al*., 1981; Lance *et al*., 1982). Van Loosdrecht *et al*. (1995) found that low filtration rates promoted the growth of porous and low-density biofilms, which could be expected to provide numerous sites for entrapment of particles such as viruses. However, since the time required for biofilm porosity

to change following a change in HLR was not stated, this may not have been an operative mechanism influencing virus removals during challenge test 4, which was conducted three days after the HLR was decreased from 0.4 to 0.1 m/h (Table 6-3 and Appendix H).

In summary, this study demonstrated that decreasing the HLR from 0.4 to 0.1 m/h led to significant increases in MS2 removals by the pilot 1 train 1 SSF. This is consistent with findings in the literature. Therefore, it can be concluded that virus removal can be optimized by operating SSFs at low HLRs, especially during periods of cold water temperatures.

6.3.2 Water Temperature

Studies in the literature have shown that virus removals in slow sand filters improve as water temperatures increase due to enhanced biological activity (Nasser and Oman, 1999; Poynter and Slade, 1977).

However, the MS2 removals in the 0.1 m/h warm water tests were similar to those observed in the 0.1 m/h cold water tests, with an average increase of 0.1 logs in warm vs. cold water (Table 6-9). Average MS2 removals were significantly lower (at the 1% level) in the cold water test 5 compared to the warm water tests 10 and 11. Although cold water test 7a average removal was not significantly different than test 10 (at the 5% level), it was significantly greater (at the 5% level) compared to test 11. Cold water test 7b average removal was significantly less than test 10 (at the 1% level) and test 11 (at the 5% level). Test 4 could not be used for statistical analysis because there was only one data point.

While the results in Table 6-9 appears to suggest that removals did not improve despite the warmer water temperatures, the likely reason why the cold and warm water 0.1 m/h tests were not different was because insufficient warm water acclimation time had been provided before the start of test 10.

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Table 6-9: MS2 removals by SSF at 0.1 m/h in warm vs. cold water

Water Temperature	Test No.	Average MS2 Removal (Logs)
Cold (2 to 10°C)	4	2.2
	5	1.5
	7a	2.2
	7b	1.8
Warm (>20°C)	10	2.1
	11	2.0

Notes: all tests on SSF of pilot 1 train 1; Influent water temp 6-10°C (tests 4, 5, 7a, and 7b) and 20-22°C (tests 10 and 11) (Appendix F)

In agreement of findings in the literature, results from the warm water tests at 0.4 m/h showed the benefits of greater warm water acclimation time. MS2 removals were significantly higher (at the 1% level) in each of the 0.4 m/h warm water tests compared to each of the 0.4 m/h cold water tests (Table 6-10). This was attributed to the fact that the 0.4 m/h warm water tests were performed after providing sufficient warm water acclimation time for biological activity in the SSF to increase sufficiently to cause greater MS2 removals (Table 6-11).

Table 6-10: MS2 removals by SSF at 0.4 m/h in warm vs. cold water

Water Temperature*	Test No.**	Average MS2 Removal (Logs)
Cold (2-10°C)	1	0.4
	2	0.2
	8*	0.5
Warm (20-23°C)	12	0.9
	13	1.0
	15	1.3

* Influent water temp 2-4°C (tests 1 and 2), 7-8°C (test 8), and 22-24°C (tests 12, 13 and 15) (Appendix F)
** All tests on SSF of pilot 1 train 1, except test 8 on pilot 1 train 2

Table 6-10 shows that MS2 removals in tests 12, 13 and 15 increased in each subsequent test even though the tests were each conducted only three days apart. Average removals in tests 12,

13 and 15 were significantly different (at the 1% significance level) from each other and from each of the 0.4 m/h cold water test averages. This suggests that the biological activity in the SSF was rapidly increasing at the time of the 0.4 m/h warm water tests. Even higher MS2 removals may have been observed if further challenge tests had been undertaken after providing more warm water acclimation time. These results demonstrate the importance of warm water acclimation time on SSF biological activity and MS2 removals.

Table 6-11: Warm water test dates

Warm Water Test No.	Test Date (2006)	Hydraulic Loading Rate (m/h)	Raw Water Temperature
10	15-16 May	0.1	16 - 17°C
11	18-19 May		
12	2-June	0.4	20 - 22°C
13	5-June		
14	5-June		
15	8-June		

Note: air temperature in building increased from 15 to 35°C on May 11, 2006

The dates listed in Table 6-11 illustrate that the 0.4 m/h warm water tests were conducted two to three weeks after the 0.1 m/h warm water tests, by which time the SSF was likely more biologically active. The raw water temperatures during tests 12 to 15 (20-22°C) were higher than during tests 10 and 11 (16-17°C), even though the SSF influent water temperatures for tests 10 to 15 were similar (20-23°C) because the air temperature in the building was maintained at 35°C after May 11. This meant that between the 0.1 and 0.4 m/h warm water tests, the SSFs had a few weeks of extra time to acclimate at warmer raw water temperatures and develop extra biomass. Therefore, the improved MS2 removals in the warm water 0.4 m/h tests (compared to the 0.4 m/h cold water tests) were likely due to enhanced biological removal mechanisms in the filters, in addition to more developed biofilms for virus attachment.

At warm water temperatures, biological populations and activity would be expected to be greater compared to cold temperatures. Viruses can be removed in several ways including predation by

bacteria (Cliver and Herrmann, 1972) and filter feeding protozoa (Kim and Unno, 1996; Windle-Taylor, 1969). Furthermore, virus inactivation can be caused by damage to their protein coats from enzymes and other virucidal substances produced by bacteria (Nasser *et al.*, 2002; Ward *et al.*, 1986; Melnick and Gerba, 1980; Cliver and Herrmann, 1972). Increased microbial activity at warmer temperatures would also promote biofilm growth, which would be expected to increase virus attachment because of their porous and sticky nature (Storey and Ashbolt, 2003; van Loosdrecht *et al.*, 1995).

Heat-induced inactivation of MS2 was not found to be an important removal mechanism in this study because greater MS2 removals were only observed in tests 12 to 15 and not in tests 10 and 11, despite similar temperatures. If it had been important, MS2 removals would have been greater in all warm water tests (tests 10 to 15) compared to the cold water tests. However, this was not the case.

Similar to the pilot 1 SSF challenge test results, MS2 removals in the pilot 2 SSFs increased with increased water temperature (Table 6-12).

Table 6-12: MS2 removals by pilot 2 SSFs at 0.4 m/h in warm vs. cold water

Water Temperature	Test No.	Average MS2 Removal (Logs)
Cold (6-10°C)	9	1.1 (SSF1) 0.5 (SSF2)
Warm (22-23°C)	14	2.0 (SSF1) 0.6 (SSF2)

Notes: Tests on SSF1 and SSF2 of pilot 2; HLR 0.4 m/h

The average MS2 removals listed in Table 6-12 for the SSF1 of pilot 2 were greater in the warm water test 14 compared to the cold water test 9. The improved MS2 removals in the warm water test were likely due to extra microbial predators and antiviral enzymes in the filters, in addition

to more extensive biofilms for virus attachment. Tests 9 and 14 could not be compared statistically due to the low number of data points (n = 6) (refer to Section 3.4.9).

The importance of a biological mechanism for MS2 removal is supported by the fact that the average MS2 removals in the pilot 2 SSF2 were only 0.1 logs higher in warm water compared to cold water (Table 6-12). The biomass in SSF2 was expected to be lower than in SSF1 because of the limited nutrients and organics entering SSF2 via the SSF1 effluent. Compared to SSF1, the lower solids loading in SSF2 likely yielded lower biomass and schmutzdecke development and thus less improvement in MS2 removal in warm water conditions.

In the pilot 2 SSF tests, additional increases in MS2 removals in SSF1 and SSF2 may have been observed if further tests had been conducted after providing more warm water acclimation time. As previously mentioned, warmer water temperatures did lead to increased MS2 removals in some pilot 1 SSF tests, provided that enough time was given for the filters to acclimate to warm water conditions.

These results suggest that even greater MS2 removals may have been observed if additional warm water tests had been conducted on pilots 1 and 2 at later dates in the summer when microbial populations, biological activity, and biofilm coverage had increased further. In light of the impaired virus removal observed at cold water temperatures, SSFs should be operated at lower HLRs during the cold winter and spring months.

6.3.3 Schmutzdecke Scraping

In general, schmutzdecke scraping had no major or long-term effect on MS2 removals. The scraping of the schmutzdecke layer between tests 7a and 7b resulted in a 0.3 to 0.5 log decrease in average MS2 removals (Table 6-13).

The day following SSF schmutzdecke scraping, the average MS2 removal was 0.5 logs less than the average removal of test 7a (Table 6-13). On the second day after scraping, the biological community in the SSF had already partially recovered, as evidenced by an average MS2 removal

that was only 0.3 logs below the test 7a average. The average removal in test 7a was significantly greater (at the 1% significance level) than average removal in test 7b after schmutzdecke scraping (Table 6-13). These finding agree well with the practice of running full-scale SSF effluent to waste for a few days following schmutzdecke scraping (AWWA, 1991). This ripening period allows the biological community and treatment performance in SSFs to recover.

Table 6-13: Effect of schmutzdecke scraping after test 7a

Test No.	Schmutzdecke Age (days)	Average MS2 Removal (Logs)
7a	35	2.2 (28-Mar and 29-Mar)
7b	1	1.6 (30-Mar) 1.9 (31-Mar) 1.8 (overall)

* Percent decrease compared to test 7a average removal of 2.18 logs
Notes: Tests 7a/b on SSF of pilot 1 train 1 at 0.1 m/h in cold water; Schmutzdecke scraped on 29-Mar-06 after test 7a

In this study, schmutzdecke scraping had no major long-term effect on MS2 removals, which is in agreement with the findings of Dullemont *et al*. (2006), Hijnen *et al*. (2004), McConnell *et al*. (1984), Poynter and Slade (1977), and Slade (1978). It is interesting to note that while Hijnen *et al*. (2004) and Dullemont *et al*. (2006) found that schmutzdecke scraping had no major effect on MS2 removal, they reported that scraping did dramatically reduce *E. coli* removal. They suggested that scraping the schmutzdecke reduced the physical straining removal mechanism, which is important for larger organisms such as bacteria and protozoan (oo)cysts, but not for small particles such as viruses. This is in agreement with the findings of Slade (1978).

The minor decrease in average MS2 removal following schmutzdecke scraping observed in the study may be attributed to the loss of predatory microorganisms. Wheeler *et al*. (1988) demonstrated that the most significant bacteriophage removals occurred in the top of mature SSF beds due to abundant biological activity. Protozoan ciliate suspension feeders primarily inhabit

the top of SSFs and can be rapidly desiccated when filters are drained during scraping, which leads to reduced predation of viruses (Lloyd, 1996). Ellis (1985) and Sanchez *et al*. (2006) reported that schmutzdecke scraping vastly reduces populations of bacteria and protozoa, both of which are known to prey on viruses (Kim and Unno, 1996; Cliver and Herrmann, 1972).

Poynter and Slade (1977) observed that the duration of filter draining had a more deleterious effect on biological activity than the actual scraping of the SSF, likely due to desiccation. Ellis (1985) reported that prolonged SSF draining leads to reduced biofilm coverage (because microorganisms can utilize some of the extracellular polymeric substances in biofilms as substrate when other substrates are not available) and subsequent washout of bacteria and other microorganisms after filter operation is restarted. Desiccation of the biofilm itself could also be the cause of microorganism washout after prolonged cleaning.

One possible reason why filter cleaning had no major impact on MS2 removals in the current study is because the pilot scale SSFs were only drained for a short period of time (~30 minutes). Since full scale SSFs can be drained for days during filter cleaning, the loss of biomass/biofilms and predatory microorganisms at full scale may be more important than observed in this pilot scale study.

In addition to the loss of predatory microorganisms, SSF draining and schmutzdecke scraping can lead to biofilm loss and impairment of virus removal. The schmutzdecke layer is a thick cake of porous, sticky biofilm material that is ideal for virus adsorption (Storey and Ashbolt, 2001 and 2003). Furthermore, bacteria living in biofilms can produce enzymes and other substances that inactivate viruses (Nasser *et al*., 2002; Ward *et al*., 1986; Melnick and Gerba, 1980; Cliver and Herrmann, 1972). Wheeler *et al*. (1988) cited adsorption to biomass/biofilms and microbial predation as the primary biological mechanisms of virus elimination. Based on the findings of the above-mentioned literature, scraping the schmutzdecke layer removes biomass and biofilms, which could reduce virus attachment and inactivation.

Aside from test 7, where the effect of schmutzdecke scraping was explicitly tested, analysis of the effect of schmutzdecke age during the other MS2 challenge tests was somewhat inconclusive but generally indicated that removals increased with increasing schmutzdecke age (Table 6-14).

Table 6-14: MS2 removals at different schmutzdecke ages

Conditions	Test*	Schmutzdecke Age (days)	Average MS2 Removal (logs)
Cold, 0.1 m/h	4	17	2.2
	5	28	1.5
	7a	35	2.2
Cold, 0.4 m/h	2	7	0.2
	1	11	0.4
	8	12	0.5
Warm, 0.1 m/h	10	47	2.1
	11	50	2.0
Warm, 0.4 m/h	12	64	0.9
	13	67	1.0
	15	70	1.3

* All tests on the SSF of Pilot 1 Train 1, except Test 8 on Train 2; Tests 9 and 14 on Pilot 2 not included because of deep sand bed of SSF1 and long schmutzdecke age of SSF2

Comparing the cold water 0.1 m/h tests (Table 6-14, the average removal in test 5 was significantly less (at the 1% level) than the average removal in test 7a (which had a larger schmutzdecke age), although this does not necessarily imply a cause-and-effect.. Similarly, when comparing the cold water 0.4 m/h tests in Table 6-14, the test with the smallest schmutzdecke age (7 days, test 2) corresponded to the lowest MS2 removal (although this does not necessarily imply a cause-and-effect). Test 2 average removal was significantly less than removals in tests 1 and 8 (at the 1% level).

Comparing the warm water 0.1 m/h tests 10 and 11, schmutzdecke ages were similar and average MS2 removals were not significantly different (at the 5% level). For the warm water 0.4 m/h tests, average removals in tests 12, 13 and 15 were statistically different from each other at the

1% level. Therefore, increased schmutzdecke age corresponded to increased MS2 removals. Other factors such as water temperature should also be considered when interpreting this data.

6.3.4 Sand Depth

In tests 9 and 14 on pilot 2, the SSF1 had a 87-92 cm deep sand bed, which was approximately twice as deep as the other SSF beds (36-40 cm) (Table 6-15). The deeper sand bed of SSF1 resulted in MS2 removals that were 0.6 to 0.9 logs higher compared to removals in the shallower SSFs tested (Table 6-15).

Table 6-15: Sand depth and MS2 removals for pilot 2 SSFs

	Challenge Test 9		Challenge Test 14	
	Sand Depth (cm)	Average MS2 Removal (Logs)	Sand Depth (cm)	Average MS2 Removal (Logs)
SSF1	92	1.1	87	2.0
SSF2	44	0.5	44	0.6

Note: HLR 0.4 m/h; Test 9 in cold water (<10°C); Test 14 in warm water (>20°C); Pilot 1 sand depth 36-40 cm (train 1) and 36 cm (train 2)

In test 9, the average MS2 removal in SSF1 was greater than removals in SSF2 (Table 6-15) and other 0.4 m/h cold water tests 1, 2 and 8 on the SSFs of pilot 1 (Table 6-4). The superior removal in SSF1 was attributed to the sand depth in SSF1, which was approximately twice as much compared to the other SSFs tested.

Similarly, in test 14, the average MS2 removal in SSF1 was greater than removals in SSF2 (Table 6-15) and other 0.4 m/h warm water tests 12, 13 and 15 on the SSF of pilot 1 train 1 (Table 6-4). The greater removal in SSF1 was again attributed to the much deeper bed depth. Therefore, in both cold and warm water conditions, the double sand depth in SSF1 (compared to the sand depths of the other SSFs tested) corresponded to greater average MS2 removals.

Increased SSF sand depth likely resulted in greater virus removals because of greater detention time in the SSFs. For a given filtration rate, doubling the sand depth would double the empty bed and actual contact time. Greater detention time in the SSF would allow more time for virus removal by predation and inactivation by bacterial enzymes. Increased sand depth would also provide more contact opportunities for virus attachment to sand grains and biofilms. Furthermore, a deeper sand bed would provide additional opportunities for viruses that detached higher in the bed to re-adsorb. Studies in the literature have shown that virus removal increases with increased SSF bed depth (Graham *et al.*, 1996; Slade, 1978; Poynter and Slade, 1977; Windle-Taylor, 1969).

At full scale, employing literature recommended bed depths and low HLRs, particularly at warm water temperatures, would maximize the removal of viruses by SSF.

6.4 Multistage Slow Sand Filtration

The contribution of roughing filtration for MS2 removal in pilot 1 and pilot 2 was determined, as was the contribution of the second slow sand filter in series in pilot 2. MS2 removal by ozonation in pilot 1 was not tested because ozone is known to inactivate viruses very effectively.

6.4.1 Second Slow Sand Filter in Series

The results of tests 9 and 14 demonstrated that the deep-bed SSF1 of pilot 2 followed by the shallow-bed SSF2 in series provided superior MS2 removals compared to any single SSF tested (Table 6-4 and Table 6-5).

In the cold water test 9, the total average MS2 removal of 1.6 logs was calculated by combining the average removals in SSF1 (1.1 logs) and SSF2 (0.5 logs). This combined MS2 removal was greater than average removals for any single SSF tested under similar conditions (tests 1, 2, 8, and 9 SSF1 and SSF2 in cold water at 0.4 m/h). As mentioned previously, tests 9 and 14 could not be compared statistically due to the low number of data points (n = 6) (refer to Section 3.4.9 .

In the warm water test 14, the total average MS2 removal of 2.6 logs was calculated by combining the average removals in SSF1 (2.0 logs) and SSF2 (0.6 logs). This combined MS2 removal was greater than average removals for any single SSF tested under similar conditions (tests 12, 13, 14 SSF1 and SSF2, and 15 in warm water at 0.4 m/h). Therefore, as expected, the two slow sand filters in series provided an effective multi-barrier approach that reduced MS2 concentrations more than any single SSF tested. Another multi-barrier approach tested in this study was roughing filtration as a pretreatment technology upstream of the SSFs.

6.4.2 Roughing Filters

The roughing filters of pilot 1 (train 1) and pilot 2 provided very little MS2 removal in the cold water conditions tested. Average MS2 removals were 0.1 logs in test 3 at 1.5 m/h and 0.2 logs in test 6 at 0.95 m/h (Table 6-6). Tests 3 and 6 could not be compared statistically due to the low number of data points (n = 5 to 6).

Although the roughing filters had been in continuous operation for over two years and were biologically mature, MS2 removals were low under the experimental conditions tested. This was likely due to increased shear from the relatively high HLRs (0.95 and 1.5 m/h), low biological activity from the cold water temperatures at the time of tests 3 and 6, large media size, and relatively large void spaces in the gravel bed. The short hydraulic detention times (20 to 30 minutes) arising from high HLRs likely impaired the removal of MS2 by biological mechanisms such as attachment to biofilms, predation by microbes, and inactivation by virucidal enzymes.

Although higher MS2 removals may have been observed if the roughing filters had been tested at lower HLRs and in warm water conditions, the MS2 removals would likely have been low compared to removals in SSFs under similar conditions. Therefore, as expected, roughing filtration has been shown to provide little MS2 removal in this study when operated at high HLRs in cold water.

6.5 Long-Term Detachment of MS2

Long-term detachment of MS2 from the slow sand filters was observed from 40 hours to 260 hours following the termination of MS2 seeding in the challenge tests (Table 6-16). In Table 6-16, the trend was for MS2 concentrations in effluent samples to decrease over time and eventually reach zero. The long-term shedding of MS2 from the filters implied that some attachment of phage to filter media was reversible. Adsorption of MS2 and PRD1 to quartz was shown to be reversible in the studies by Bales *et al*. (1991 and 1993) and Kinoshita *et al*. (1993).

Reversible attachment to media grains can be characterized by fast attachment and slow detachment (Schijven *et al*., 2000). Long-term detachment of viruses including MS2 due to reversible attachment has been observed in other studies employing slow sand filters (Dullemont *et al*., 2006; Hijnen *et al*., 2004; Dizer *et al*., 2004) and sand columns (Schijven and Simunek, 2002; Schijven *et al*., 1999; Hijnen *et al*., 2005; Bales *et al*., 1993).

Table 6-16: Long-term detachment of MS2

Test No.	MS2 Effluent Concentration during Seeding (PFU/mL)	Time Seeding Stopped (h)*	Effluent Sample Time (h)*	Average MS2 Concentration (PFU/mL)
1	10^4	27	144	20
			147	29
			245.5	23
			311	12
2	10^4	28	102.5	119
			179	49
3	10^5	3	n.d	n.d.
4	10^3	15	108	20
			180	14
			264	6
5	10^4	43	191.5	57
6	10^4	3.5	192	480
7a	10^4	40.25	-	-
7b	10^4	87.25	1152	0
8	10^4	15.5	40	122
			62.5	57
			1140.5	0
9	10^3	24	46.5	126 (SSF1)
				54 (SSF2)
10	10^3	39.25	72	52
11	10^3	39.5	358.5	0
12	10^4	16.25	72	5
13	10^4	16	67.25	5
14	10^2	16	85	0 (SSF1)
				0 (SSF2)
15	10^3	18.75	n.d.	n.d.

* Elapsed time after start of MS2 seeding
n.d. - long-term sampling not done

The implication of long-term detachment of MS2 is that the true removal of phage (via inactivation and irreversible attachment) could be considerably less than the removals calculated in this study from samples collected while influent MS2 concentrations were at steady state. In order to quantify the magnitude of permanent MS2 removal by slow sand filtration due to inactivation and irreversible attachment, a mass balance would be required.

6.6 Concluding Remarks

This study showed that the pilot scale slow sand filters could reduce MS2 concentrations by 0.2 to 2.2 logs on average, depending on water temperature, hydraulic loading rate, sand depth, and schmutzdecke age. Based on these modest MS2 removals and the observations of long-term detachment from the SSFs in this pilot scale study, it is likely that viruses would pass through full scale SSFs and into the filter effluent, depending on the influent virus concentration.

At full scale, slow sand filtration (or multistage SSF) systems operating without pre- or post-disinfection or oxidation (in developing countries, for example) could be at risk of virus breakthrough leading to infection if the dose was high enough. Depending on the infectious doses of the viruses present in the filter effluent, slow sand filtration, even when preceded by roughing filtration or followed by multiple slow sand filters in series, may not be suitable as a stand-alone system to ensure virus free treated drinking water. Therefore, SSF or MSF systems would need to include pre- or post-disinfection or oxidation to ensure no infectious viruses reach the consumer.

To optimize virus removal by slow sand filtration, the filters should be operated at the lower range of literature-recommended HLRs and with sand depths in the upper range of that recommended in literature. This optimization is of particular importance during periods of cold water temperatures less than 10°C.

6.7 Summary of Findings

A total of 16 MS2 challenge tests were conducted between February and June 2006 at variable hydraulic loading rates (0.1 and 0.4 m/h), water temperatures (<10 vs. >20°C), sand depths (36-40 vs. 87-92 cm), and time since filter scraping. The following broader conclusions can be drawn from the results of the MS2 challenge tests:

- Average MS2 removals ranged from 0.2-2.2 logs in the SSFs and 0.1-0.2 logs in the RFs under all conditions tested.

- To maximize virus removal by SSF, the HLR should be in the lower range of that recommended in the literature (0.1-0.4 m/h) and in the upper range of the recommended bed depth (1.0-1.3 m), especially when treating cold water.
- Multiple SSFs in series can measurably improve virus removal.
- A SSF system would need to include other treatment technologies such as pre- or post-disinfection or oxidation in order to achieve regulatory virus removal targets.

Findings from challenge tests on the slow sand filters of pilot 1 (which had shallow bed depths of 36-40 cm) included the following (all statistical analyses at the 1% significance level):

- In cold water, average MS2 removals by the pilot 1 SSFs were significantly greater at an HLR of 0.1 m/h (1.5-2.2 logs) compared to 0.4 m/h (0.2-0.5 logs).
- In warm water, average MS2 removals by the pilot 1 SSFs were significantly greater at an HLR of 0.1 m/h (2.0-2.1 logs) compared to 0.4 m/h (0.9-1.3 logs).
- At 0.1 m/h, average MS2 removals were similar in warm water (2.0-2.1 logs) and cold water (1.5-2.2 logs), possibly because insufficient warm water acclimation time was provided.
- At 0.4 m/h, average MS2 removals were significantly higher in warm water (0.9-1.3 logs) compared to cold water (0.2-0.5 logs) because sufficient warm water acclimation time was provided for biological activity in the SSF to increase.
- It is possible that better MS2 removals would have been observed if experiments had been carried out later in the summer after longer acclimation times at warmer temperatures.
- Heat-induced inactivation of MS2 was deemed to be of little importance at the temperatures tested (2-24°C).
- Schmutzdecke scraping had only a minor and short-term negative effect on MS2 removals. Average MS2 removal was 2.2 logs in test 7a prior to schmutzdecke scraping, decreased to 1.6 logs one day after scraping (test 7b), then increased 1.9 logs on the second day after scraping. This demonstrated that the SSF had recovered relatively quickly.
- Long-term detachment of previously attached MS2 phage (10^1-10^2 PFU/mL) was observed for up to 260 hours after seeding was stopped, which indicates attachment was reversible.

Conclusions from the challenge tests on the pilot 2 SSF1 (bed depth was 87-92 cm, within the range recommended in the literature) and SSF2 (~45 cm sand depth) were as follows:

- Average MS2 removals in the SSF1 of pilot 2 (1.1-2.0 logs) were greater than removals in all other SSFs tested, which was attributed to the deeper sand bed in SSF1.
- The greater sand depth of SSF1 likely led to greater MS2 removals because of longer detention times for biological mechanisms to act (predation and inactivation), in addition to more contact opportunities for attachment to sand grains and biofilms.
- SSF1 average MS2 removals were greater in warm water (2.0 logs) than in cold water (1.1 logs).
- In cold water at 0.4 m/h, the average MS2 removal in SSF1 (1.1 logs) was greater than that of the pilot 1 SSFs (0.2-0.5 logs) and pilot 2 SSF2 (0.5 logs).
- In warm water at 0.4 m/h, the average MS2 removal by SSF1 (2.0 logs) was greater than the pilot 1 SSFs (0.9-1.3 logs) and pilot 2 SSF2 (0.6 logs).
- The second SSF in series contributed to overall MS2 removals. SSF2 average removal in warm water (0.56 logs) was greater than in cold water (0.46 logs).
- Combined SSF1 and SSF2 average removals were 1.6 and 2.6 logs in cold and warm water, respectively, thereby providing measurably higher MS2 removal compared to any single SSF tested.
- Long-term detachment of previously attached MS2 (10^1-10^2 PFU/mL) was observed for up to 46 hours after seeding was stopped.

Results from the roughing filter tests included the following:

- Roughing filters achieved minimal MS2 removals, averaging 0.1 logs (pilot 1 train 1 at 1.5 m/h) and 0.2 logs (pilot 2 at 0.95 m/h), which can be attributed to relatively large diameter (4-18 mm) filter media, cold water temperatures, high HLRs (0.95-1.5 m/h), and short detention times (20-30 minutes).

7.0 Conclusions and Recommendations

7.1 Conclusions

The conclusions drawn from the current study can be divided into three categories: virus removal; *Cryptosporidium* and *Giardia* removal; and turbidity removal and operational findings.

7.1.1 Virus Removal

Major conclusions:

1. Average removals of MS2 bacteriophage ranged from 0.2 to 2.2 logs in the slow sand filters and 0.1 to 0.2 logs in the roughing filters under all conditions tested.

2. Virus removal by slow sand filtration was strongly dependant on hydraulic loading rate, sand depth, and water temperature.

3. Virus removal increased with deeper sand depth and warmer water temperature, but decreased at higher hydraulic loading rates. Multiple SSFs in series can further improve virus removal.

4. To optimize virus removal, SSFs should be designed with bed depths as recommended in the literature (>100 cm) and be operated at the lower range of hydraulic loading rates recommended in literature (~0.1 m/h), especially in cold water conditions.

5. Attachment of MS2 was reversible, as demonstrated by the long-term detachment of previously attached phage for up to 260 hours after seeding stopped.

Minor conclusions:

- Schmutzdecke scraping had only a minor and short-term negative effect on MS2 removals.
- Average MS2 removals in the SSF1 of pilot 2 (1.1-2.0 logs) were greater than removals in all other SSFs tested, which was attributed to the deeper sand bed in SSF1.
- The second SSF in series in pilot 2 contributed to MS2 removals, demonstrating that the multi-barrier approach provided superior MS2 removal compared to any single SSF tested.
- SSF2 should be seeded independently of SSF1 in future challenge tests.

- Average MS2 removals were significantly greater at an HLR of 0.1 m/h (1.5-2.2 logs) compared to 0.4 m/h (0.2-0.5 logs) (for tests on the pilot 1 SSFs in cold water).

- Average MS2 removals were significantly greater in warm water (0.9-1.3 logs) compared to cold water (0.2-0.5 logs), likely due to increased biological activity in the filter (for tests at 0.4 m/h on the SSF of pilot 1 train 1).

- At 0.1 m/h (for the SSF of pilot 1 train 1), removals were similar in warm and cold water because insufficient warm water acclimation time had been provided.

- Average removals in the SSF1 of pilot 2 were better in warm water (2.0 logs) than in cold water (1.1 logs).

- Increased MS2 removals may have been observed if experiments had been carried out later in the summer after more acclimation time at warmer water temperatures.

7.1.2 *Cryptosporidium* and *Giardia* Removal

Major conclusions:

1. Removal of oocysts (2.6 - >4.4 logs) and cysts (>3.8 - >4.5) were good regardless of sand depth, hydraulic loading rate, and water temperature in the ranges tested.

2. Removals of oocysts and cysts were similar in the SSF of pilot 1 train 1 (~40 cm bed depth) and the SSF1 of pilot 2 (~100 cm bed depth), which demonstrated that bed depth was not an important factor. This suggested that oocysts and cysts were removed in the top of the SSF bed, likely by straining. Therefore, good oocyst and cyst removals can be achieved using shallower SSF bed depths and higher HLRs than recommended in the literature, especially for biologically mature filters.

3. Oocyst removals increased as the SSFs became more biologically mature, likely because of improved straining from greater biofilm coverage and solids accumulation in the sand bed.

4. Average removals demonstrated in this study were often greater than the removal credits given to slow sand filtration by regulatory agencies (3 logs oocyst removal – USEPA; 2 logs cyst removal – Ontario Ministry of Environment).

Minor conclusions:

- At cold water temperatures (~2°C), good removals of oocysts (3.9 logs) and cysts (>4.5 logs) were achieved in the biologically mature SSF of pilot 1 train 1.

- The good removals at various bed depths and at cold temperatures (when biological activity is low) suggest that physical (not biological) removal mechanisms predominate.

- Oocyst removals in the SSF of pilot 1 train 1 were somewhat higher at an HLR of 0.4 m/h compared to 0.8 m/h.

- Average *Cryptosporidium* removals ranged from 2.6 to >4.4 logs in the SSF of pilot 1 train 1 and ranged from 2.9 to >4.3 logs in the SSF1 of pilot 2.

- Average *Giardia* removals ranged from >3.8 to >4.5 logs in the SSF of pilot 1 train and averaged >3.7 logs in the SSF1 of pilot 2. Complete cyst removals (i.e. no recovery of cysts in the effluent samples) were frequently observed.

- The second slow sand filter in pilot 2 showed very low average removal of oocysts and cysts (~0 logs), which was likely because influent concentrations were too low for accurate removals to be measured. SSF2 should be seeded independently of SSF1 in future tests.

7.1.3 Turbidity Removal and Filter Runs

Major conclusions:

1. Effluent turbidity of all multistage SSF pilot systems were within the regulated effluent limits in Ontario for full-scale SSFs (below 1 NTU at least 95% of the time and never exceeded 3 NTU), despite measured raw water turbidity peaks over 300 NTU.

2. The roughing filter component of the multistage slow sand filtration process was responsible for the majority of the turbidity removal.

3. Most (50-80%) of SSF filter runs were short (1-3 weeks long), especially during periods of high raw water turbidity in the spring and fall.

4. Good turbidity removal was achieved despite SSFs having been operated at a high HLR of 0.4 m/h. To reduce excessive filter clogging and operator maintenance, the HLR should be decreased during long periods of elevated raw water turbidity (particularly in the spring and fall wet weather seasons).

Minor conclusions:

- Full-train turbidity removals averaged 95-97% for both pilot systems.
- For pilot 1, roughing filters contributed to ~75% of full-train turbidity removal, compared to ~25% for the slow sand filters. Ozone contactors provided negligible turbidity removal.
- For pilot 2, RFA contributed to 60% of full-train turbidity removal, compared to 87% for RFB (finer media than RFA), 37% for SSF1, and only 0.2% for SSF2.

7.1.4 Significance of Results for Public Health and Industry

1. Stand-alone MSF systems with modified designs that include shallow bed RFs and SSFs and operate at high HLRs (0.4 to 0.8 m/h) can achieve regulated *Cryptosporidium* and *Giardia* removal targets, provided that the system is biologically mature.
2. Stand-alone MSF systems without disinfection or oxidation would not be expected to achieve regulated virus removal/inactivation targets, even when designed according to recommendations in the literature. This finding is of particular importance for communities in some parts of developing countries where chemicals are not available for water treatment.
3. Virus removals can be optimized by providing deep SSF beds and operating at low HLRs.
4. Virus removal may be impaired in cold water, which could affect the viability of using SSF/MSF at northern climates if communities do not use disinfection or oxidation.
5. Even at pilot-scale, the MSF systems tested achieved effluent turbidities that met the regulations set out for full-scale SSF applications. At full-scale, the MSF configurations tested would be expected to achieve even better turbidity removals.
6. Roughing filters achieve the majority of turbidity removal in the MSF system and are therefore critical for protecting SSFs from excessive clogging, especially when treating raw water that is subject to high turbidity peaks.
7. Hydraulic loading rate should be decreased during long duration periods of high raw water turbidity to prevent SSF clogging, frequent SSF scraping, and the subsequent temporary impairment of filter performance.

7.2 Recommendations

1. Employ Virus Cocktail and Experimental Design in Further Virus Challenge Tests

- Pursue further virus challenge tests using a cocktail of bacteriophage (MS2, φX174 and PRD1) as surrogates for enteric viruses that cover a broad range of size, composition, surface charge, and attachment behaviours.

- Carry out a full experimental design to determine virus removal over a wide range of hydraulic loading rates, water temperatures, and sand depths. Perform regression analysis using result data in order to develop a mathematical model that can be used to predict virus removal by slow sand filtration systems tested.

- Virus challenge tests should be conducted at variable raw water turbidities to determine if turbidity influences virus removal.

- In order to determine if viruses are removed primarily by physical (attachment) or biological (predation) mechanisms, challenge tests could be conducted on biologically mature and immature (i.e. virgin) slow sand filters. Furthermore, slow sand filters could be seeded with filter-feeding protozoa and rotifers to determine if they contribute to virus removal via predation.

- The second slow sand filter in series (pilot 2) should be seeded with viruses and tested independently of SSF1.

2. Include Positively Charged Media in SSF Bed to Enhance Virus Removal

- Optimize virus removal by slow sand filtration by incorporating positively charged media in the slow sand filters to determine if the removal of negatively charged viruses increases.

- Research various configurations, such as incorporating a "sandwich" layer of positively charged media on top or within the slow sand filter bed. If a second slow sand filter in series is used, positively charged media could be used for all or part of the filter depth.

- Determine the effect of positively charged filter media on operational parameters such as head loss, filter run length, and turbidity removal.

- Determine if the positive charge decreases over time as adsorption sites become saturated.

- Positively charged media options include iron-oxide coated sand, zero-valent iron beads or filings, and naturally occurring zeolites (alumino-silicate minerals containing cations).
- The availability, cost and operational impacts of using positively charged media should be considered for both developed and developing country applications in keeping with the principles of slow sand filtration as a simple, cost-effective and sustainable technology.
- If iron is included in multistage slow sand filtration systems, the removal of arsenic could also be researched, which would beneficial for use in developing countries where arsenic in drinking water poses a significant health threat.

3. **Replicate *Cryptosporidium* and *Giardia* Challenge Tests**

- *Cryptosporidium* and *Giardia* challenge tests should be conducted on roughing filters to determine their contribution to oocyst and cyst removal. The results could be used to obtain additional removal credits from regulatory agencies.
- When testing a second slow sand filter in series it should be seeded with *Cryptosporidium* and *Giardia* and tested independently of the first.
- Investigating the removal of *Cryptosporidium* and *Giardia* by biologically mature and immature (i.e. virgin) slow sand filters would provide valuable insights on the effect of filter maturity on removal. Biological maturity had a major impact on ooycst removal in the current study.

4. **Develop Operational & Maintenance Protocols**

- Research should be carried out to optimize the operation and maintenance protocols for the treatment of challenging waters by pilot multistage slow sand filtration. The objective would be to maximize SSF filter run length and minimize SSF scraping frequency.
- Specifically, protocols should be developed that indicate how much hydraulic loading rates should be decreased under a range of turbidity (magnitude and duration) scenarios. For example, if raw water turbidity exceeds "X" NTU for "Y" days, then slow sand filter HLR should be decreased to "Z" m/h.

- A preventative maintenance protocol for roughing filter cleaning should also be developed based on turbidity conditions in order to optimize protection against SSF clogging.

- Multiple roughing filters in series could be tested to determine if they protect the slow sand filters from clogging during periods of high raw water turbidity. Alternative roughing filter designs (media type and depth, horizontal roughing filtration, etc.) could also be considered. Improved roughing filter performance would improve downstream SSF turbidity removal under a broader range of surface water qualities.

Appendix A

Chloride Tracer Test Results
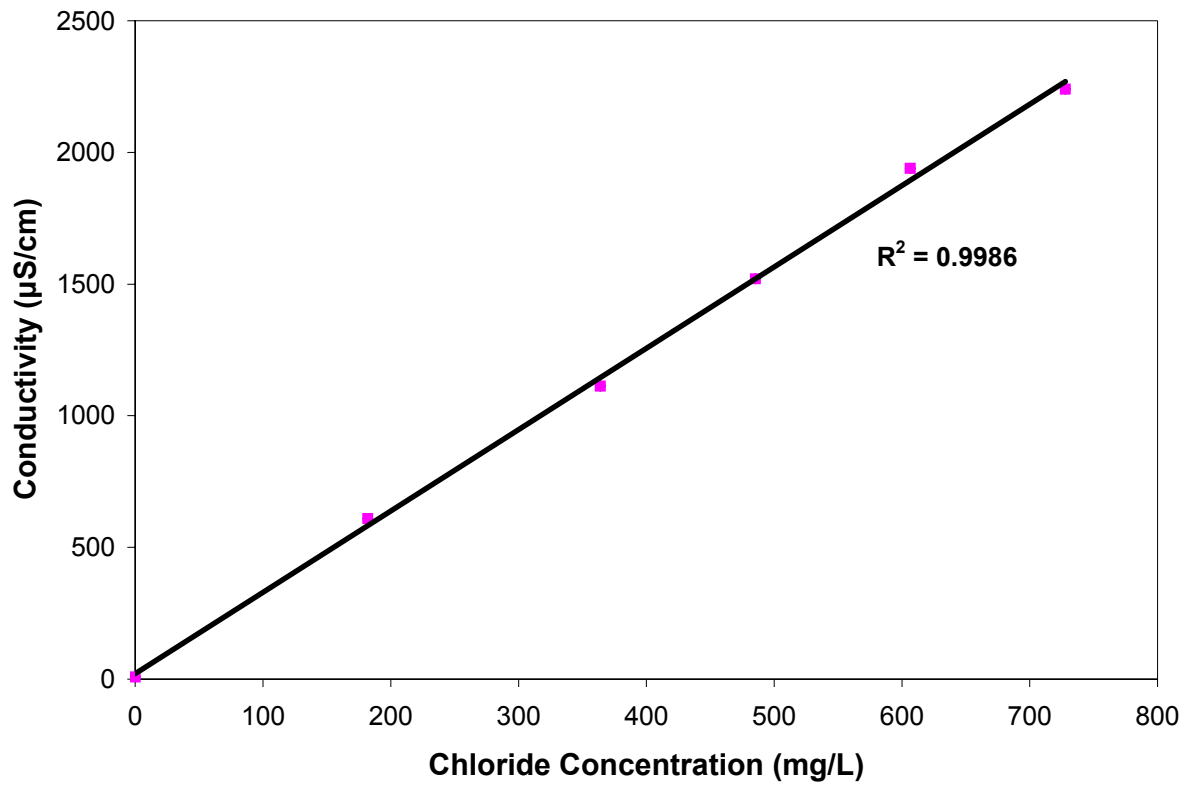
Figure A-1: Calibration curve
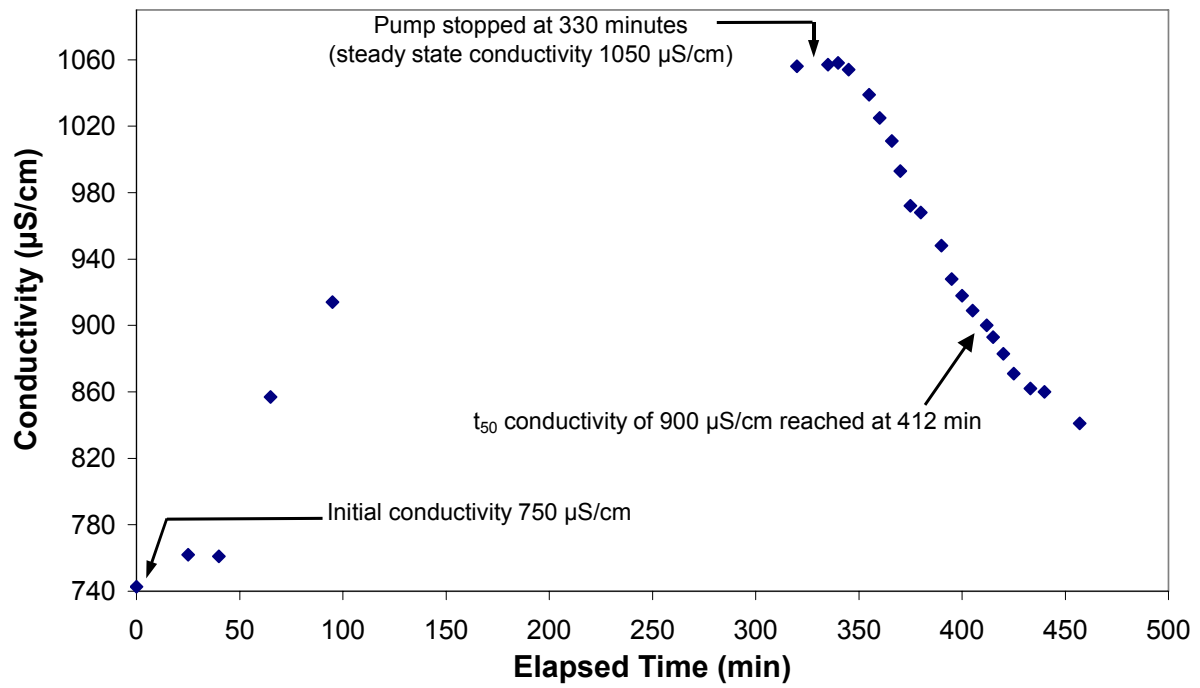
Figure A-2: Tracer test results for the SSF of pilot 1 train 1 at 0.4 m/h (0.5 L/min)
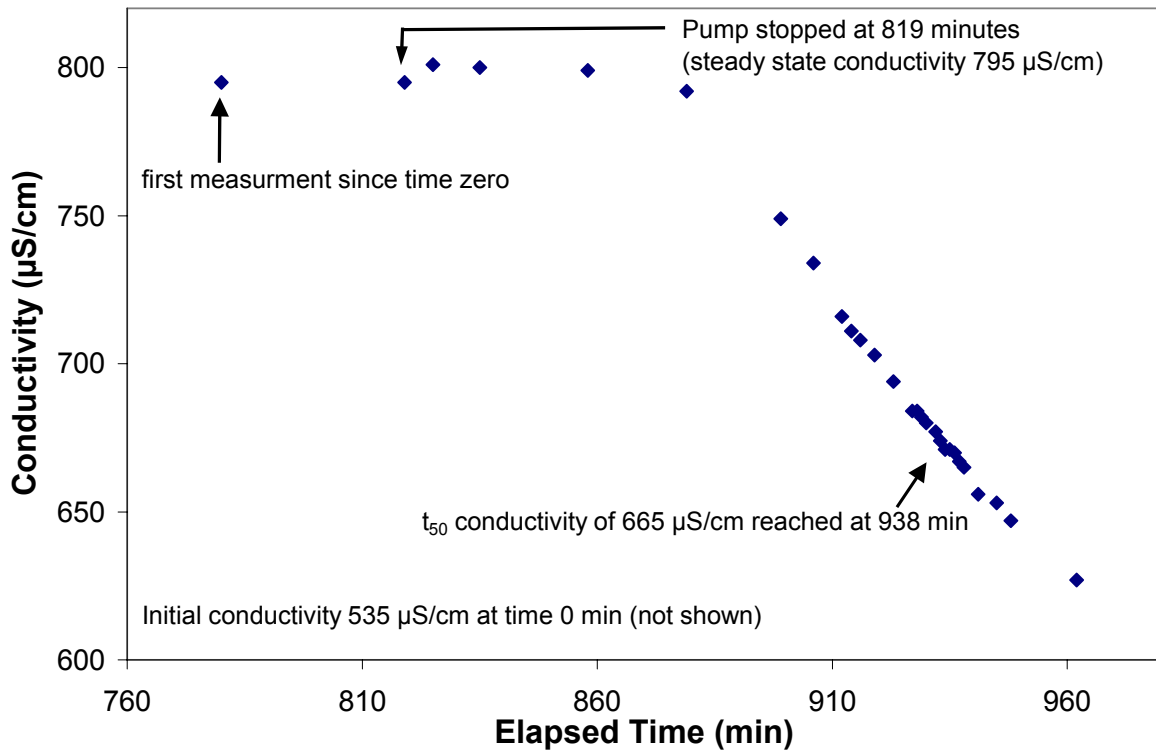
Figure A-3: Tracer test results for pilot 2 SSF1 at 0.4 m/h (0.5 L/min)
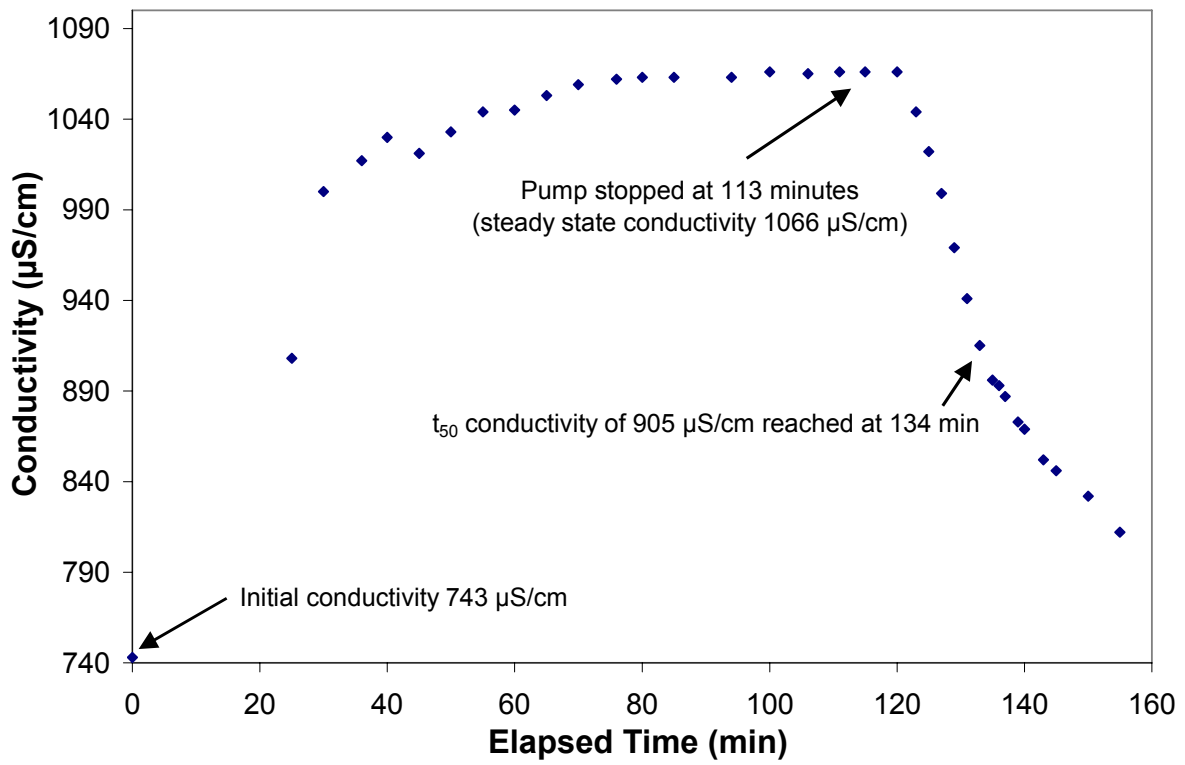
Figure A-4: Tracer test results for the RF of pilot 1 train 1 at 1.5 m/h (0.5 L/min)
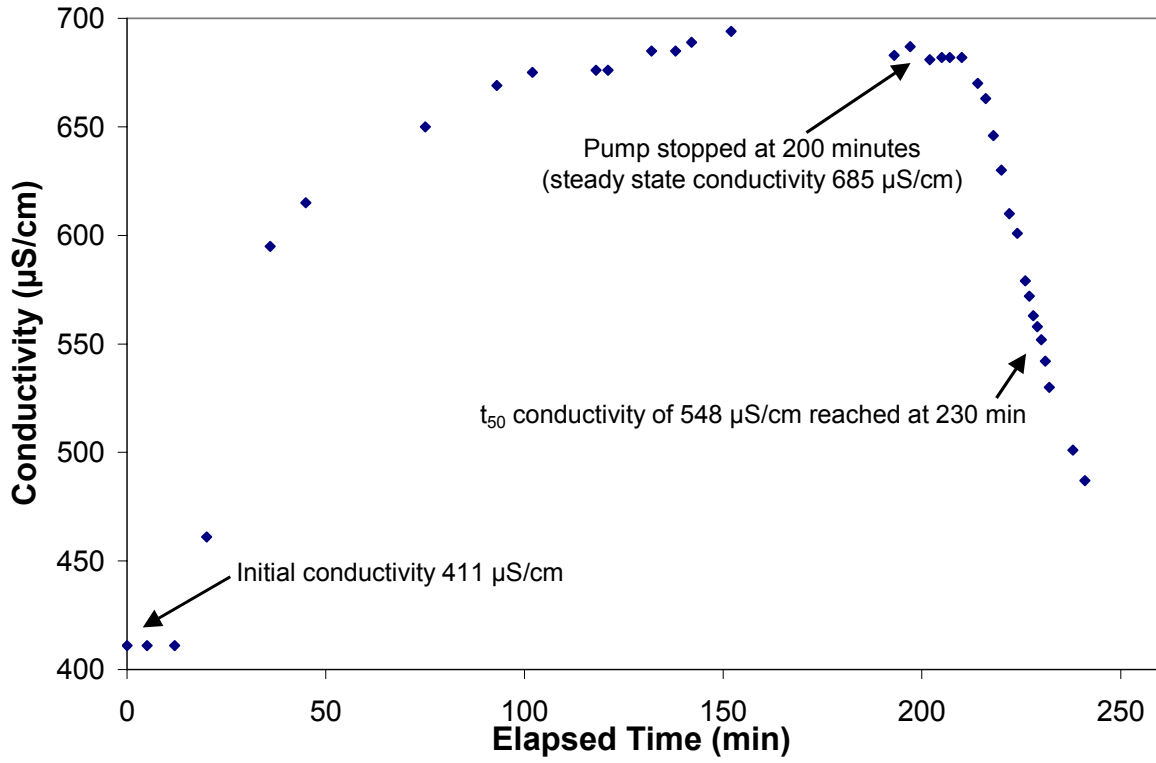
Figure A-5: Tracer test results for the RFA of pilot 2 at 0.95 m/h (0.5 L/min)

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Appendix B
Slow Sand Filter Run Lengths
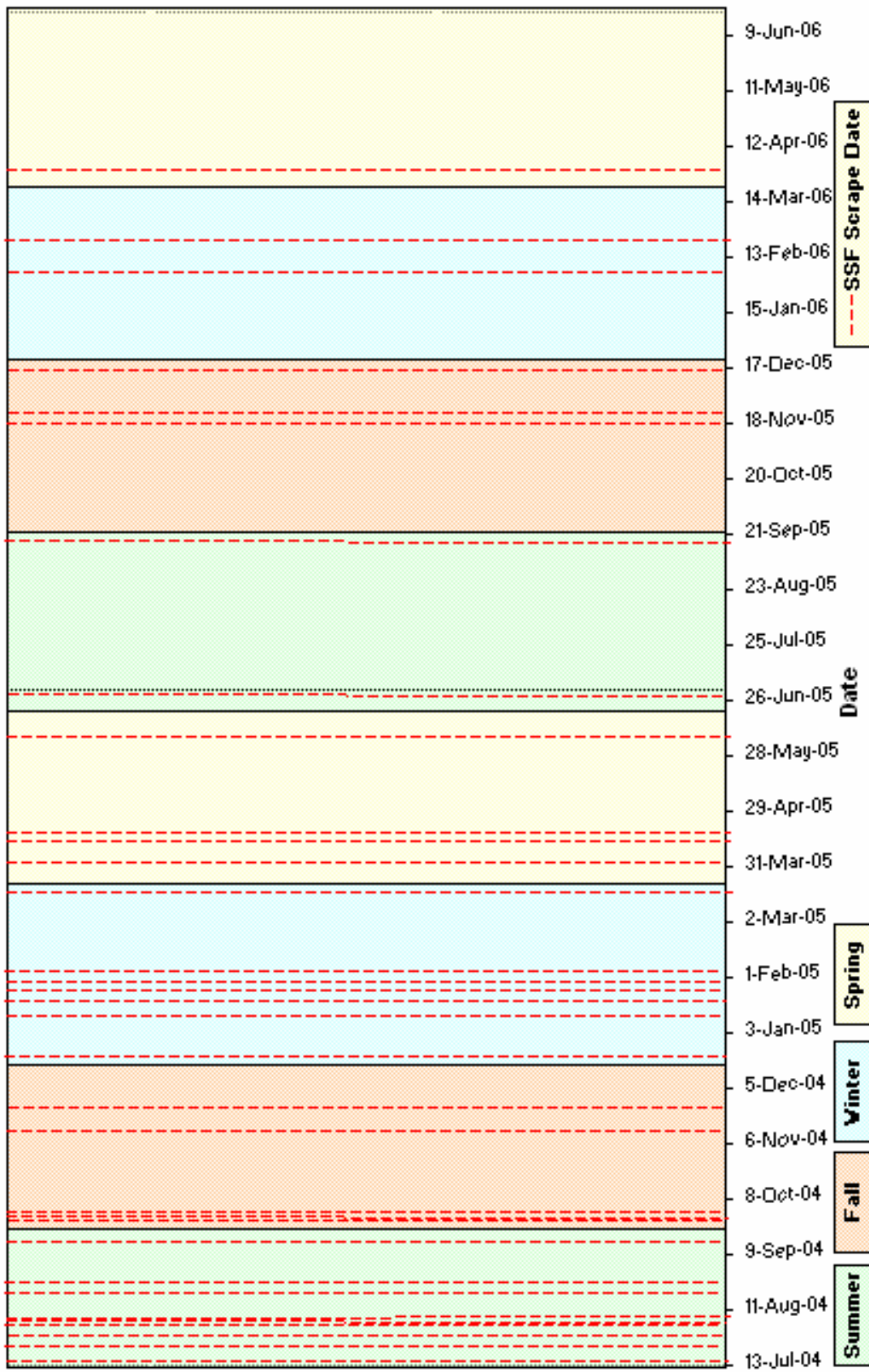
Figure B-6: Pilot 1 train 1 filter run contour map

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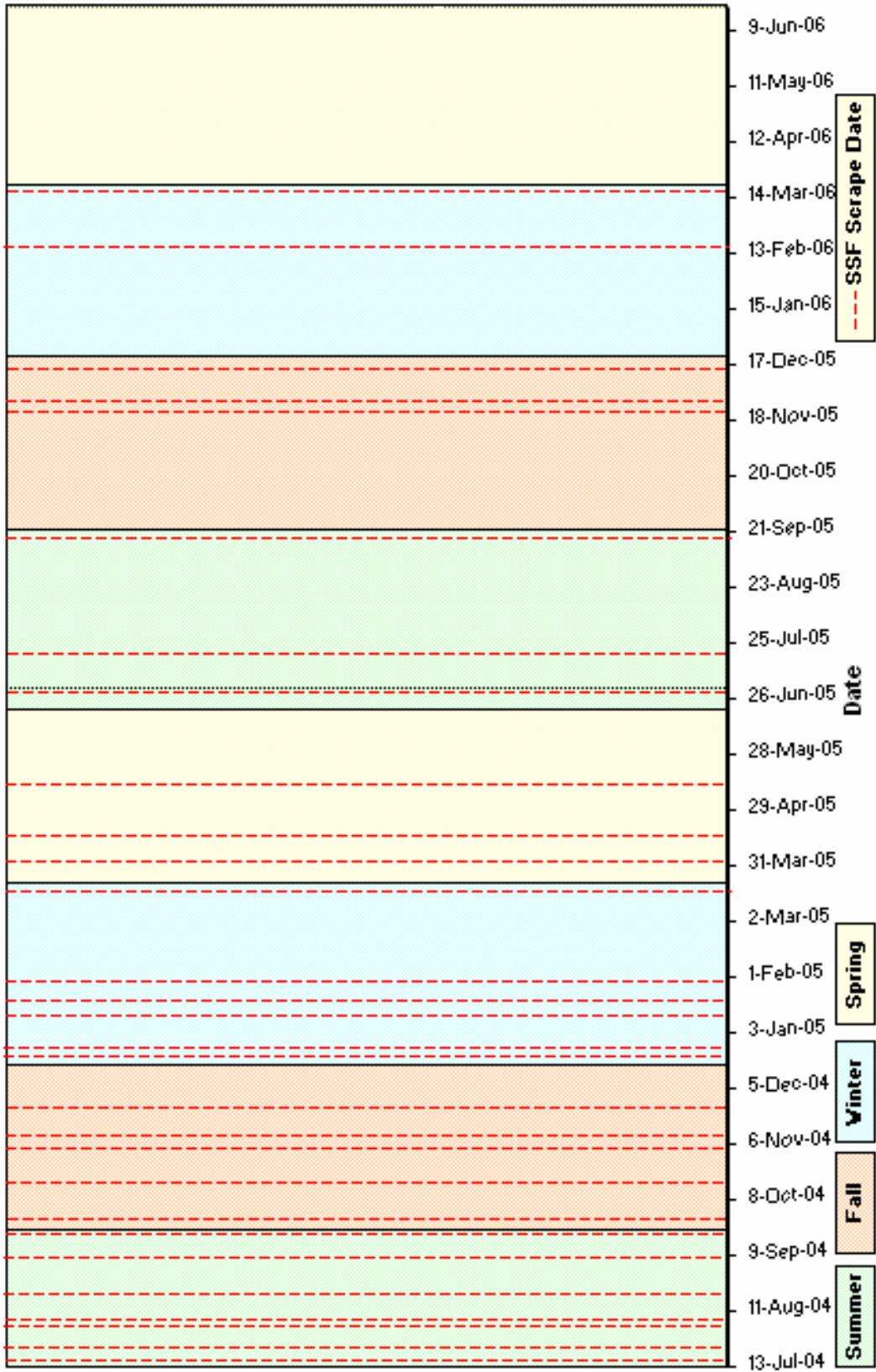
Figure B-7: Pilot 1 train 2 filter run contour map

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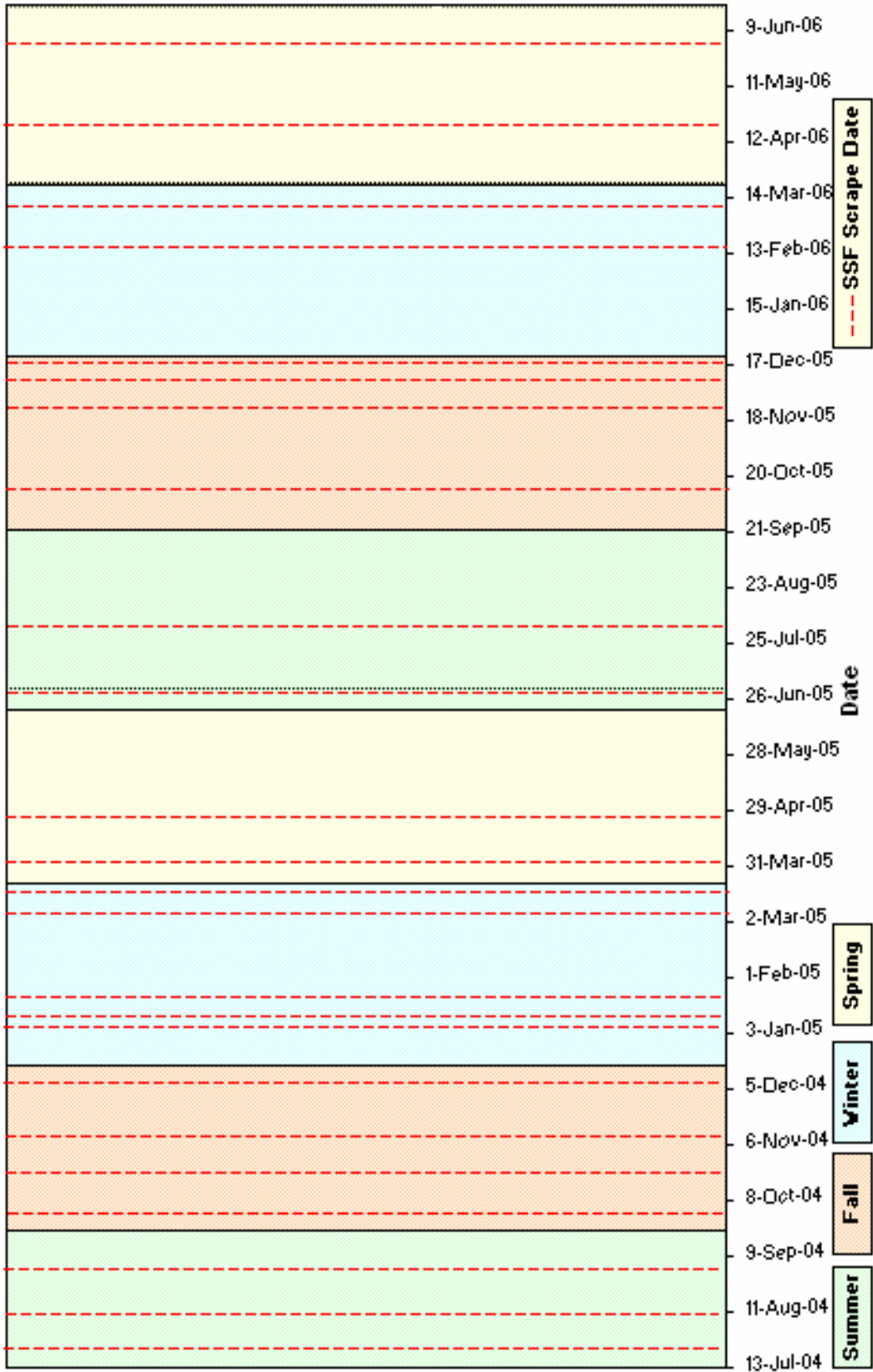
Figure B-8: Pilot 2 SSF1 filter run contour map

Table B-1: Pilot 1 train 1 SSF overflow and scraping dates

	Date		Filter Run	Distance from top SSF column to sand surface		Sand Removed	Sand Depth	
Overflow	Scrape P1/T1 SSF		(days)	Before	After	(cm)	(cm)	Notes
	05-Jan-04		-					
	09-Jan-04		4					Swirl clean (no scrape)
	18-Jan-04		9					Swirl clean (no scrape)
	30-Jan-04		12					
	14-Feb-04		15					
	16-Feb-04		2					
	21-Feb-04		5					upwash
	08-Mar-04		16					1 L sand removed
	15-Mar-04		7					
	21-Mar-04		6					
24-Mar-04	24-Mar-04		3					Scrape + upwash
	28-Apr-04		35					
	13-May-04		15					
	21-May-04		8					
	04-Jun-04		14					Scrape + upwash
	15-Jul-04		41		85.0		45.0	RESANDED
23-Jul-04	23-Jul-04		8	85.0	85.0	0.0	45.0	Horz flow (no scrape)
	29-Jul-04		6*	85.0	86.5	1.5	43.5	not o/f
03-Aug-04	03-Aug-04		5*	86.5	86.5	0.0	43.5	not o/f; Horz flow clean
06-Aug-04	06-Aug-04		3	86.5	86.0	-0.5	44.0	
	20-Aug-04		14*	86.0	87.5	1.5	42.5	not o/f, Crypto prep
	27-Aug-04		7*	87.5	88.6	1.1	41.4	not o/f, post Crypto scrape
15-Sep-04	15-Sep-04		19	88.6	90.0	1.4	40.0	
27-Sep-04	27-Sep-04		12	90.0	92.5	2.5	37.5	
29-Sep-04	29-Sep-04		2	92.5	93.4	0.9	36.6	0.8 m/h HLR
	01-Oct-04		2*	93.4	94.0	0.6	36.0	after crypto expt; not o/f
11-Nov-04	11-Nov-04		41	94.0	95.5	1.5	34.5	
21-Nov-04			10					
	25-Nov-04		-	95.5	81.0	-14.5	49.0	Resanded 14.5 cm
21-Dec-04	21-Dec-04		26	81.0	83.0	2.0	47.0	

* Filter run not ended because of terminal head loss

| Date | | Filter Run | Distance from top SSF column to sand surface | | Sand Removed | Sand Depth | |
Overflow	Scrape P1/T1 SSF	(days)	Before	After	(cm)	(cm)	Notes
08-Jan-05		18					
13-Jan-05	13-Jan-05	-	83.0	85.3	2.3	44.7	
16-Jan-05		3					
17-Jan-05		-					
	18-Jan-05	-	85.3	86.9	1.6	43.1	
	26-Jan-05	8*		85.9		44.1	not o/f; Upwash
	29-Jan-05	3*		86.4		43.6	not o/f; Upwash + scrape
	03-Feb-05	5*	86.4	88.0	1.6	42.0	not o/f; Post-Crypto test
10-Mar-05		35					
12-Mar-05		-					
16-Mar-05	16-Mar-05	-	88.0	90.0	2.0	40.0	
24-Mar-05		8					
28-Mar-05		-					
31-Mar-05		-					
02-Apr-05	02-Apr-05	-	90.9	93.4	2.5	36.6	
11-Apr-05		9					
13-Apr-05	13-Apr-05	-	92.6	95.0	2.3	35.0	
	15-Apr-05	2*					not o/f; upwash only
07-Jun-05	07-Jun-05	-	95.0	96.1	1.1	33.9	
28-Jun-05	28-Jun-05	21	96.0	97.5	1.5	32.5	
13-Sep-05		77					
16-Sep-05	16-Sep-05	-	97.5	85.8		44.3	scrape + resand
28-Oct-05		42					
01-Nov-05		-					
16-Nov-05		-					
	17-Nov-05	-	85.8	87.5	1.8	42.5	
21-Nov-05	21-Nov-05	4	88.1	89.0	0.9	41.0	
09-Dec-05		18					
14-Dec-05	14-Dec-05	-					Upwash only
	03-Feb-06	51*	87.4	89.6	2.2	40.4	not o/f
14-Feb-06		11					
15-Feb-06		-					
17-Feb-06		-					
20-Feb-06	20-Feb-06	-	90.1	92.0	1.95	38.0	
	29-Mar-06	37*	91.7	93.7	2.0	36.3	not o/f; MS2 test 7
	21-Jun-06	>84*					Decommissioned

* Filter run not ended because of terminal head loss

Table B-3: Pilot 1 train 2 SSF overflow and scraping dates

Date		Filter	Distance from top SSF column to sand surface		Sand	Sand	
	Scrape	Run			Removed	Depth	
Overflow	P1/T2 SSF	(days)	Before	After	(cm)	(cm)	Notes
	05-Jan-04	-					
	09-Jan-04	4					Swirl clean (no scrape)
	18-Jan-04	9					Swirl clean (no scrape)
	30-Jan-04	12					
	14-Feb-04	15					
16-Feb-04	16-Feb-04	2					
20-Feb-04	20-Feb-04	4					
	21-Feb-04	1					upwash
	08-Mar-04	16					1 L sand removed
24-Mar-04	24-Mar-04	16					Scrape + upwash
20-Apr-04		27					
21-Apr-04	21-Apr-04	-					upwash
28-Apr-04	28-Apr-04	7					
05-May-04	05-May-04	7					
07-May-04	07-May-04	2					upwash
13-May-04	13-May-04	6					
16-May-04	16-May-04	3					
21-May-04	21-May-04	5					
25-May-04		4					
	01-Jun-04	-					
	04-Jun-04	3					scrape + upwash
	15-Jul-04	41		84.0		46.0	RESANDED
	23-Jul-04	8*	84.0	86.0	2.0	44.0	not o/f; horz flow clean
	03-Aug-04	11*		86.0		44.0	not o/f; Horz flow clean
	06-Aug-04	3*	86.0	85.0	1.0	45.0	not o/f; scraped
	20-Aug-04	14*	85.0	87.2	2.2	42.8	not o/f
08-Sep-04	08-Sep-04	19	87.2	90.0	2.8	40.0	
20-Sep-04	20-Sep-04	12	90.0	92.0	2.0	38.0	
	27-Sep-04	7*	92.0	94.5	2.5	35.5	not o/f
18-Oct-04	18-Oct-04	21	94.5	96.0	1.5	34.0	
	03-Nov-04	16*	96.0	97.0	1.0	33.0	not o/f
11-Nov-04	11-Nov-04	8	97.0	98.0	1.0	32.0	
	25-Nov-04	14*	98.0	83.0	-15.0	47.0	RESANDED
21-Dec-04	21-Dec-04	26	83.0	85.0	2.0	45.0	
24-Dec-04	24-Dec-04	3	85.0	86.0	1.0	44.0	

* Filter run not ended because of terminal head loss

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Table B-4: Pilot 1 train 2 SSF overflow and scraping dates (continued)

Date Overflow	Scrape P1/T2 SSF	Filter Run (days)	Distance from top SSF column to sand surface Before	Distance from top SSF column to sand surface After	Sand Removed (cm)	Sand Depth (cm)	Notes
08-Jan-05		15					
13-Jan-05	13-Jan-05	-	86.0	87.1	1.1	42.9	
16-Jan-05		3					
17-Jan-05		-					
	18-Jan-05	-	86.8	89.2	2.4	40.8	
19-Jan-05		1					
26-Jan-05		-					
29-Jan-05	29-Jan-05	-	89.2	91.2	2.0	38.8	
10-Mar-05		40					
12-Mar-05		-					
16-Mar-05	16-Mar-05	-	91.2	92.6	1.4	37.4	
24-Mar-05		8					
28-Mar-05		-					
31-Mar-05		-					
02-Apr-05	02-Apr-05	-	92.6	94.8	2.1	35.3	
11-Apr-05		9					
13-Apr-05		-					
15-Apr-05	15-Apr-05	-	94.8	96.0	1.3	34.0	scrape + upwash
02-May-05		17					
09-May-05		-					
11-May-05	11-May-05	-	96.0	96.9	0.9	33.1	
	28-Jun-05	48*	96.5	97	0.5	33.0	not o/f
15-Jul-05		17					
19-Jul-05	19-Jul-05	-	98.7	100.4	1.7	29.7	
	16-Sep-05	59*	99.9	87.3	-12.6	42.8	RESAND
28-Oct-05		42					
04-Nov-04		-					
18-Nov-05		-					
21-Nov-05	21-Nov-05	-	87.3	89.2	1.9	40.8	
24-Nov-05		3					
28-Nov-05	28-Nov-05	-	89.0	91.2	2.2	38.8	
14-Dec-05	14-Dec-05	16				38.8	Upwash only
14-Feb-06		62					
15-Feb-06	15-Feb-06	-	91.2	92.5	1.3	37.5	
16-Mar-05	16-Mar-06	29	92.5	94.5	2.0	35.6	
	08-Apr-06	>23*				35.6	RF leak started

* Filter run not ended because of terminal head loss

Table B-5: Pilot 2 SSF1 overflow and scraping dates

| Date | | Filter | Sand | Sand | |
Overflow	Scrape P2 SSF1	Run (days)	Removed (cm)	Depth (cm)	Notes
	12-Mar-04	-			1 L sand scraped
	02-Apr-04	21			
	17-May-04	45		91.5	
23-Jul-04	23-Jul-04	67	3.5	88.0	
09-Aug-04	09-Aug-04	17	2.5	85.5	
	02-Sep-04	24*	1.5	84.0	not overflowing
01-Oct-04	01-Oct-04	29*	4.0	80.0	not o/f
22-Oct-04	22-Oct-04	21*	2.5	77.5	not o/f
08-Nov-04		17			
11-Nov-04	11-Nov-04	-	1.5	76.0	
08-Dec-04	08-Dec-04	27	3.0	73.0	
07-Jan-05	07-Jan-05	30	2.0	71.0	
13-Jan-05	13-Jan-05	6	3.0	68.0	
	21-Jan-05	8*	2.0	66.0	not o/f;likely Crypto prep
02-Mar-05		40			
07-Mar-05	07-Mar-05	-	1.9	63.6	
12-Mar-05		5			
16-Mar-05	16-Mar-05	-	3.2	60.4	
19-Mar-05		3			
24-Mar-05		-			
28-Mar-05		-			
31-Mar-05		-			
02-Apr-05	02-Apr-05	-	2.1	58.1	

* Filter run not ended because of terminal head loss

Table B-6: Pilot 2 SSF1 overflow and scraping dates (continued)

Date		Filter Run (days)	Sand Removed (cm)	Sand Depth (cm)	Notes
Overflow	Scrape P2 SSF1				
18-Apr-05		16			
22-Apr-05		-			
25-Apr-05	25-Apr-05	-	2.1	56.1	
07-Jun-05		43			
17-Jun-05		-			
22-Jun-05		-			
28-Jun-05	28-Jun-05	-	1.1	54.2	
	02-Aug-05	35*	34.6 add	100	RESANDING
14-Oct-05	14-Oct-05	73	2.4	96.4	
24-Nov-05	24-Nov-05	41	2.0	94.4	
01-Dec-05		7			
05-Dec-05		-			
09-Dec-05	09-Dec-05	-	1.5	93.0	
20-Dec-05	20-Dec-05	11	0.5	92.9	
	20-Dec-05		-3.2	96.1	upwashed
14-Feb-06	14-Feb-06	56	1.8	93.9	
06-Mar-06		20			
09-Mar-06	09-Mar-06	-	2.0	91.9	
08-Apr-06		30			
20-Apr-06	20-Apr-06	-	2.0	89.4	
01-Jun-06		42			
02-Jun-06	02-Jun-06	-	1.9	87.3	
	21-Jun-06	>19*			Decommissioned

* Filter run not ended because of terminal head loss

Appendix C

Water Temperature Measurements
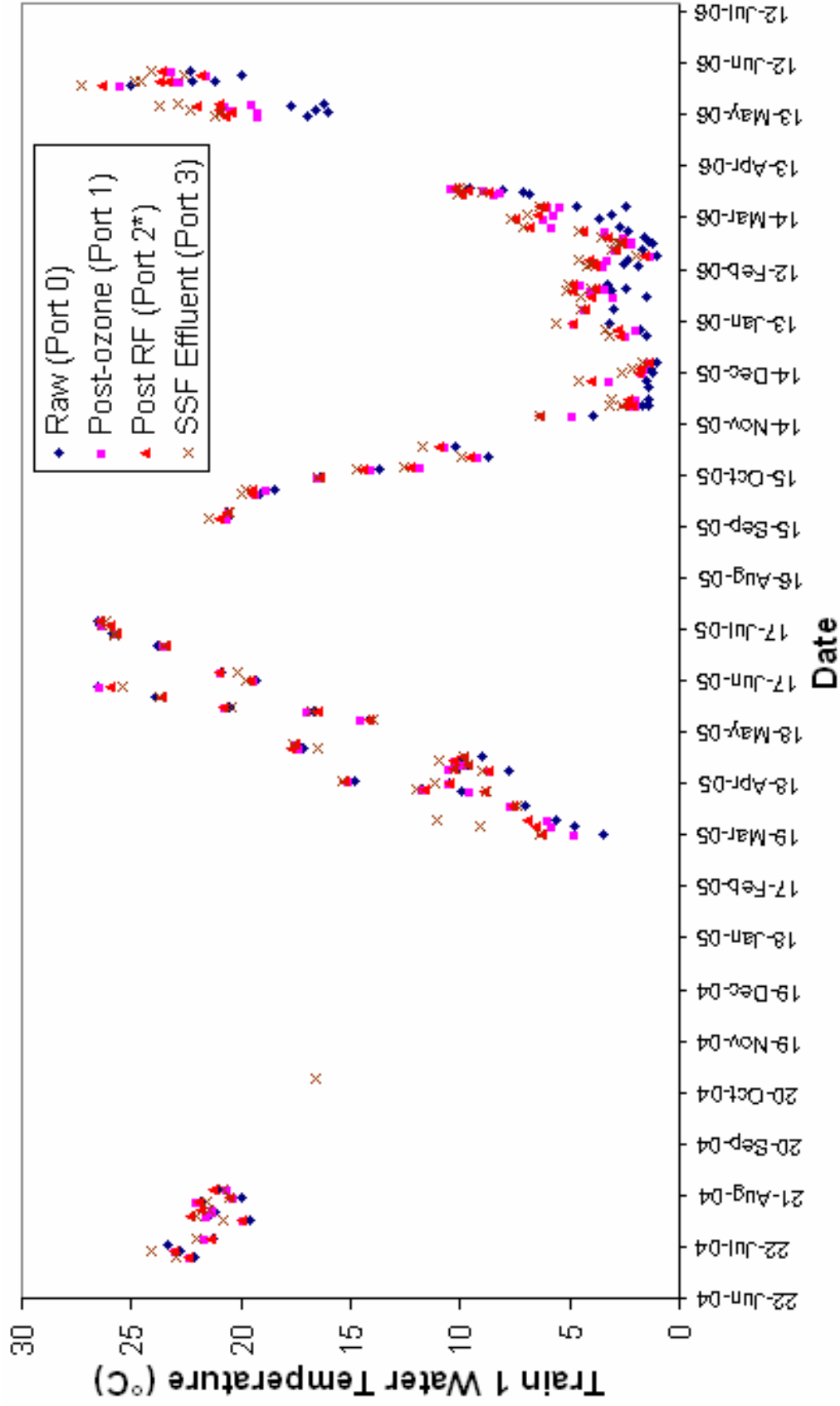
Figure C-9: Pilot 1 train 1 water temperature

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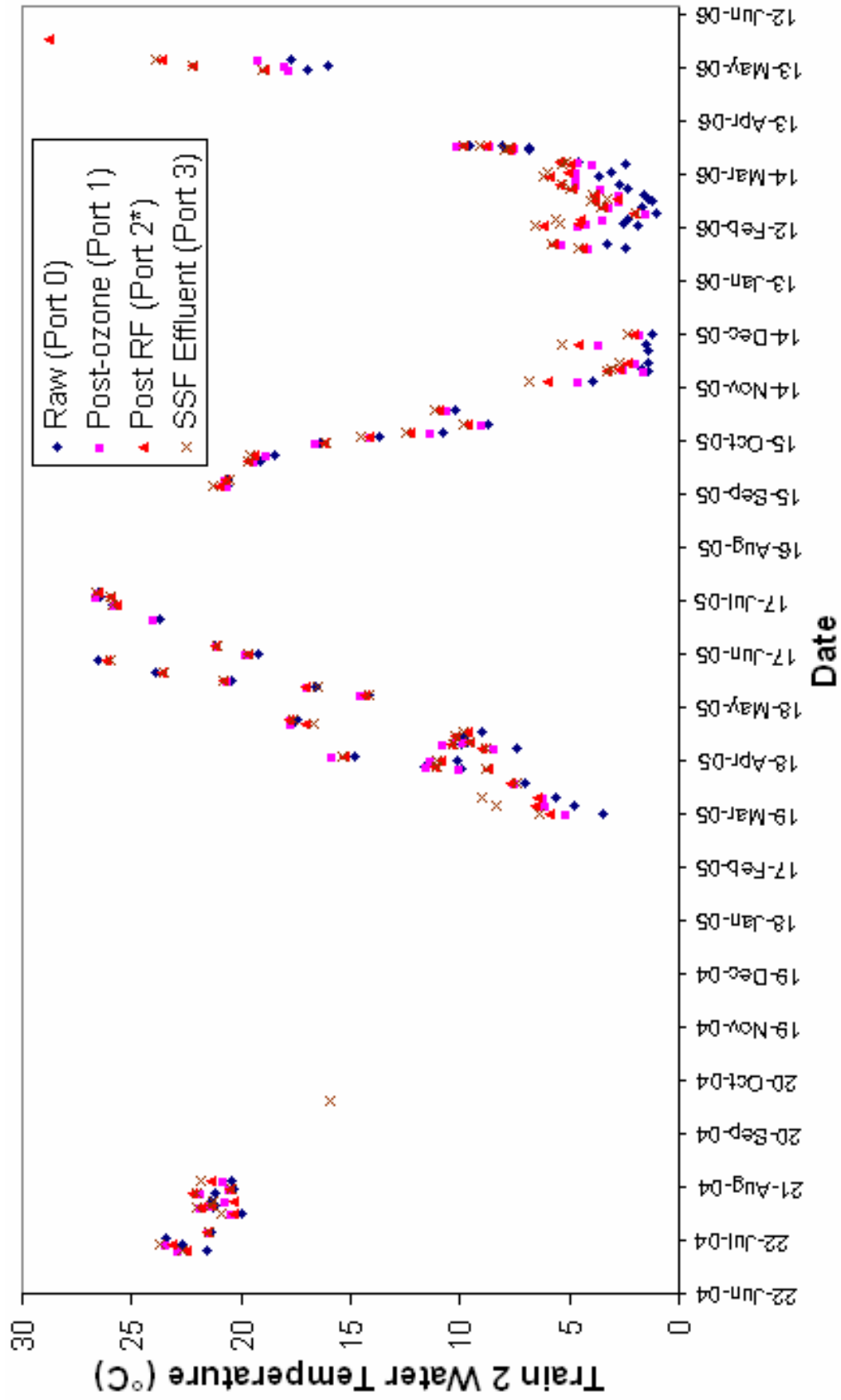
Figure C-10: Pilot 1 train 2 water temperature

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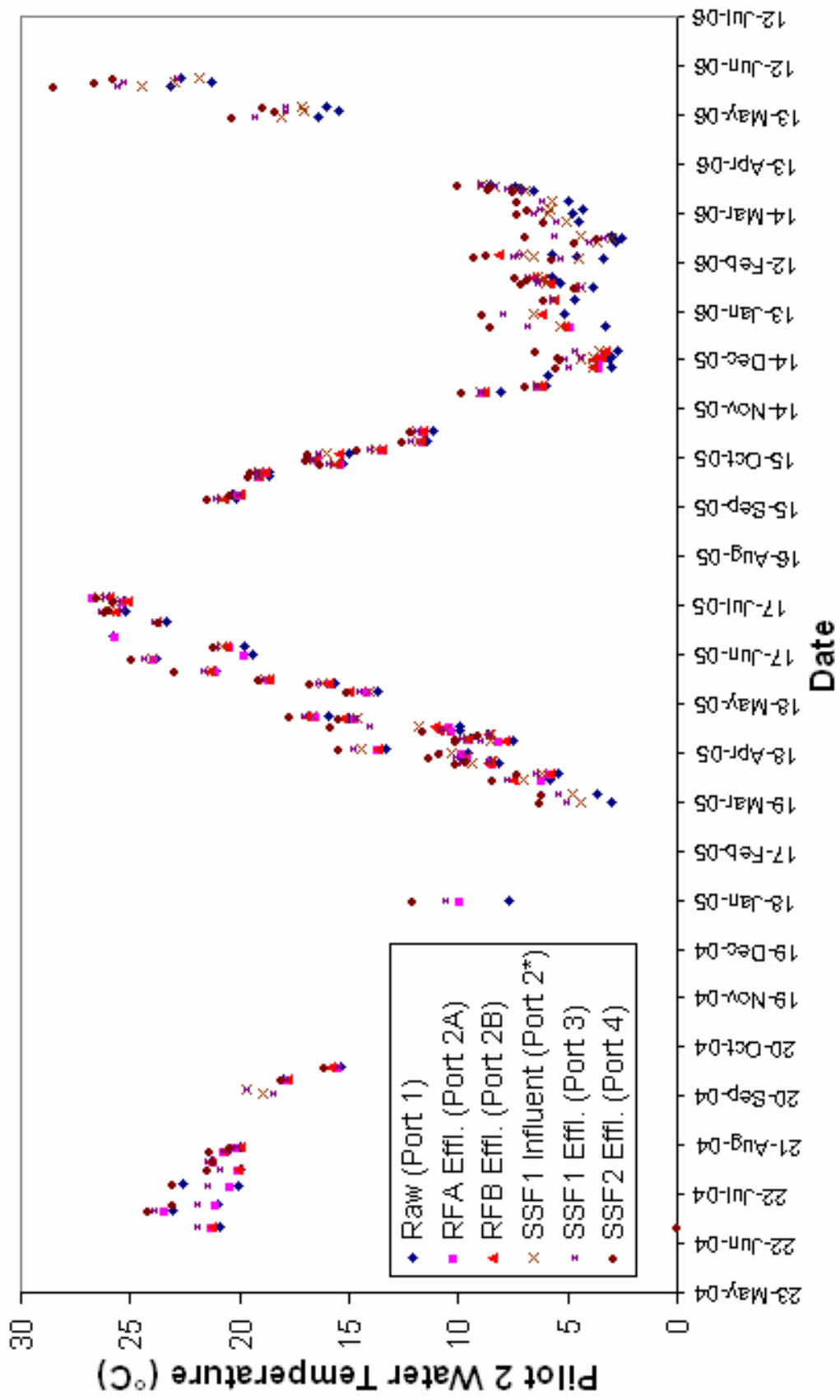
Figure C-11: Pilot 2 water temperature

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Appendix D

Water Quality Data for *Cryptosporidium* and *Giardia* Challenge Tests

Table D-7: Water Quality for *Cryptosporidium* Challenge Test 2

Time+ (hour)	Raw (Sampling Port 0) Turbidity* (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)	SSF Influent (Port 2*) Turbidity (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)	SSF Effluent (Port 3) Turbidity* (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)
1	5.46	6.46	17.5	2.54	6.37	18.8	0.16	3.98	19.5
2	5.34	6.58	17.6	2.44	6.98	18.5	0.15	4.20	18.8
3	6.05	6.48	17.5	2.56	6.29	19.1	0.15	3.75	19.7
4	6.08	6.30	17.6	2.68	6.30	18.7	0.15	3.73	19.2
5	8.08	7.92	17.1	2.86	6.45	19.2	0.15	3.53	20.3
6	8.90	7.77	17.7	3.22	6.23	18.5	0.16	3.76	19.3
7	8.52	7.75	17.9	3.05	6.47	18.9	0.15	4.06	19.2
8	9.24	7.39	18.3	2.97	6.29	19.1	0.14	4.29	19.9
9	10.0	8.21	18.3	3.15	6.47	19.1	0.16	4.51	19.1
Avg.	7.52	7.21	17.7	2.83	6.43	18.9	0.15	3.98	19.4
Sand Depth (cm)			40						
Water Depth (cm)			104.4						
Schmutzdecke Age++ (days)			10						

+ Elapsed time after start of seeding; * Handheld turbidity measurements

** Depth of water above SSF surface; ++ time since last filter cleaning (schmutzdecke scraping)

Note: Test 2 on SSF of Pilot 1 Train 1 at 0.4 m/h on 31-May-04

Table D-8: Water Quality for *Cryptosporidium* Challenge Test 3

Time+ (hour)	Raw (Sampling Port 0) Turbidity* (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)	SSF Influent (Port 2*) Turbidity (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)	SSF Effluent (Port 3) Turbidity* (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)
1.5	3.82	7.31	20.9	3.21	7.21	21.2	0.19	5.35	21.9
3	3.15	6.76	20.9	2.82	6.98	21.7	0.20	5.21	22.0
5	3.08	6.26	21.3	2.25	6.63	22.1	0.20	4.86	22.3
8	3.83	10.84	23.0	2.08	7.33	22.2	0.20	5.26	22.2
Avg.	3.47	7.79	21.5	2.59	7.04	21.8	0.20	5.17	22.1
Sand Depth (cm)			40						
Water Depth (cm)			101.5						
Schmutzdecke Age++ (days)			3						

+ Elapsed time after start of seeding; * Handheld turbidity measurements

** Depth of water above SSF surface; ++ time since last filter cleaning (schmutzdecke scraping)

Note: Test 3 on SSF of Pilot 1 Train 1 at 0.8 m/h on 7-June-04

Table D-9: Water Quality for *Cryptosporidium* and *Giardia* Challenge Test 4

Time+ (hour)	Raw (Sampling Port 0) Turbidity* (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)	SSF Influent (Port 2*) Turbidity (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)	SSF Effluent (Port 3) Turbidity* (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)
0	11.10/8.884	3.9	23.2	0.82	3.6	23.6	0.12/0.099	2.4	23.8
1	10.30/8.556	3.6	23.2	1.03	3.5	23.7	0.11/0.097	1.6	23.3
2	8.38/7.008	3.6	22.9	1.08	3.4	23.4	0.12/0.104	1.4	23.6
3	7.32/6.375	3.7	23.6	0.99	3.3	23.7	0.13/0.103	1.6	24.0
4	7.22/5.939	4.2	23.2	0.96	3.2	23.2	0.13/0.100	1.6	23.3
5	8.93/7.297	4.5	23.6	0.97	3.8	23.5	0.12/0.100	1.8	23.5
6	10.90/8.642	4.8	23.9	1.08	3.4	23.8	0.15/0.098	1.6	23.9
7	11.80/9.407	5.2	23.6	1.30	3.4	24.1	0.13/0.096	1.6	24.2
8	10.90/9.080	6.6	23.5	1.61	3.2	23.7	0.15/0.097	2	23.9
9	9.87/8.561	5.4	23.5	1.47	3.3	23.5	0.11/0.102	2.5	23.8
Avg.	9.67/7.975	4.6	23.4	1.13	3.4	23.6	0.13/0.100	1.8	23.7

Sand Depth (cm)	42.5
Water Depth (cm)	19.5
Schmutzdecke Age[++] (days)	6

[+] Elapsed time after start of seeding; * Handheld / online turbidity measurements

** Depth of water above SSF surface; ++ time since last filter cleaning (schmutzdecke scraping)

Note: Test 4 on SSF of Pilot 1 Train 1 at 0.4 m/h on 26-Aug-04

Table D-10: Water Quality for *Cryptosporidium* and *Giardia* Challenge Test 5

Time+ (hour)	Raw (Sampling Port 0)			SSF Influent (Port 2*)			SSF Effluent (Port 3)		
	Turbidity* (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)	Turbidity (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)	Turbidity* (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)
0	5.83/5.324	4.4	16.4	3.57	5.8	16.8	0.24/0.214	5.2	17.6
1	5.05/4.787	4.6	16.4	2.84	5.8	16.9			
1.5							0.28/0.247	5.2	17.0
3	3.46/3.420	4.7	17.1	2.56	5.7	17.6			
3.5							0.28/0.245	5.1	18.2
4	3.49/3.479	5.2	16.6	2.18	5.9	17.5			
4.5							0.27/0.241	5.0	17.2
5	3.48/3.338	5.6	17.4	2.55	6.0	17.6			
5.5							0.27/0.252	5.3	17.5
6	3.89/3.515	6.4	17.5	2.27	5.7	17.6			
6.5							0.28/0.258	5.2	17.3
8	3.22/3.023	5.8	17.5	3.14	5.8	18.0			
8.5							0.23	4.8	18.4
Avg.	4.06/3.841	5.2	17.0	2.7	5.8	17.4	0.26/0.241	5.1	17.6

Sand Depth (cm)	36.6
Water Depth (cm)	50.4
Schmutzdecke Age^{++} (days)	1

$^{+}$ Elapsed time after start of seeding; * Handheld / online turbidity measurements

** Depth of water above SSF surface; ++ time since last filter cleaning (schmutzdecke scraping)

Note: Test 5 on SSF of Pilot 1 Train 1 at 0.8 m/h on 30-Sept-04

Table D-11: Water Quality for *Cryptosporidium* and *Giardia* Challenge Test 6

Time+ (hour)	Raw (Sampling Port 0)			SSF Influent (Port 2*)			SSF Effluent (Port 3)		
	Turbidity* (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)	Turbidity (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)	Turbidity* (NTU)	Dissolved Oxygen (mg/L)	Temp. (°C)
0	2.44/2.148	10.81	1.4	2.06	12.04	2.5	0.21/0.177	9.21	2.7
1	2.49/2.102	10.87	1.8	1.87	10.59	3.2	0.23/0.184	8.91	3.4
2	2.36/2.121	11.14	3.2	1.88	11.50	3.7	0.24/0.188	8.94	3.9
3	2.34/2.101	10.45	3.3	1.95	10.42	3.9	0.24/0.197	8.75	4.4
4	2.32/2.086	10.89	2.5	1.80	10.30	4.1	0.26/0.206	9.18	3.8
5	2.39/2.088	10.95	2.2	1.88	11.78	3.4	0.25/0.212	8.92	4.1
6	2.34/2.071	10.63	2.0	1.76	11.61	3.4	0.27/0.216	9.11	3.8
7	2.29/2.085	10.87	3.3	1.79	10.67	3.6	0.26/0.221	9.22	4.7
8	2.24/2.076	12.13	2.9	1.76	10.99	4.3	0.27/0.211	8.71	4.8
9	2.39/2.064	11.98	3.3	1.84	10.02	3.5	0.26/0.201	8.50	3.7
Avg.	2.36/2.094	11.07	2.6	1.86	10.99	3.6	0.25/0.201	8.95	3.9

Sand Depth (cm)	43.6
Water Depth (cm)	36.4
Schmutzdecke Age++ (days)	4

+ Elapsed time after start of seeding; * Handheld / online turbidity measurements

** Depth of water above SSF surface; ++ time since last filter cleaning (schmutzdecke scraping)

Note: Test 6 on SSF of Pilot 1 Train 1 at 0.4 m/h on 2-Feb-05

Table D-12: Water Quality for *Cryptosporidium* Challenge Test 1

	Time[+] (hour)	Raw (Port 1)	SSF1 Influent (Port 2*)	SSF1 Effluent (Port 3)	SSF2 Effluent (Port 4)
Turbidity*	0.8	2.09	0.55	0.26	0.21
(NTU)	7.3	1.93	0.55	0.26	0.2
DO	0.8	11.62		7.67	8.45
(mg/L)	7.3	15.19	11.62	9.61	8.88
Temp	0.8	10		10.7	11
(°C)	7.3	9.9	10.8	11.1	12.6
pH	7.2	8.35			
Sand Depth (cm)	SSF1	100			
	SSF2	50			
Water Depth** (cm)	SSF1	50.5			
	SSF2	15.2			
Schmutzdecke Age[++] (days)	SSF1	26			
	SSF2	~120			

[+] Elapsed time after start of seeding; * Handheld turbidity measurements

** Depth of water above SSF surface; ++ time since last filter cleaning (schmutzdecke scraping)

Note: Test 1 on SSF1 and SSF2 of Pilot 2 at 0.4 m/h on 28-Apr-04

Table D-13: Water Quality for *Cryptosporidium* and *Giardia* Challenge Test 7

Time[+] (hour)	Turbidity* (NTU) Raw (Port 0)	SSF1 Influent (Port 2*)	SSF1 Effluent (Port 3)	SSF2 Effluent (Port 4)
0	4.80/3.268	1.62	0.13/0.128	0.12/0.119
1	2.68/2.771	0.95	0.13/0.130	0.16/0.118
2	2.75/2.668	0.92	0.18/0.136	0.53/0.120
3	2.70/2.668	0.89	0.13/0.129	0.12/0.129
4	2.56/2.727	0.92	0.15/0.126	0.31/0.121
5	2.61/2.802	0.87	0.12/0.125	0.13/0.120
6	3.77/4.237	0.87	0.12/0.127	0.13/0.121
9	2.46/2.744	1.02	0.13/0.127	0.16/0.121
Avg.	3.04/2.986	1.01	0.14/0.129	0.21/0.121
Sand Depth (cm)	100 (SSF1), 44 (SSF2)			
Water Depth (cm)	31.5 (SSF1), 28 (SSF2)			
Schmutzdecke Age[++] (days)	~7 (SSF1), ~100 (SSF2)			

[+] Elapsed time after start of seeding; * Handheld / online turbidity measurements

** Depth of water above SSF surface; ++ time since last filter cleaning (schmutzdecke scraping)

SSF1 influent water temperature was approx. 26°C on July 22-05

SSF1 influent dissolved oxygen conc. was approx. 4 mg/L on July 22-05

SSF1 resanded 7 days before test 7, but 35 day old schmutzdecke placed on top of new sand

Note: Test 7 on SSF1&2 of Pilot 2 at 0.4 m/h on 9-Aug-05

Appendix E

Cryptosporidium and *Giardia* Challenge Tests – Description and

Results of Individual Experiments

Cryptosporidium and *Giardia* Challenge Tests

Seven *Cryptosporidium* and *Giardia* challenge tests were conducted on the slow sand filters (SSFs) of pilots systems 1 and 2 between April 2004 and August 2005. The challenge tests aimed to quantify oocyst and cyst removal by slow sand filtration at different hydraulic loading rates, water temperatures, sand depths, and SSF biological maturities.

Cryptosporidium challenge tests 1 to 3 were carried out and documented by Cleary (2005). Test 1 was conducted on the relatively immature SSFs of pilot 2 after approximately 4 months of operation following commissioning at cold water temperatures. Tests 2 and 3 were conducted on the relatively immature SSF of pilot 1 train 1 after 5 months of operation.

The *Cryptosporidium* and *Giardia* challenge tests 4 and 5 were carried out by S. Ndiongue in warm water on the biologically mature SSF of pilot 1 train 1. Test 6 was performed in cold water by S. Ndiongue and J. DeLoyde on the SSF of pilot 1 train 1. Finally, test 7 was conducted by J. DeLoyde and K. Tabor on the mature SSFs of pilot 2 after more than 1.5 years of continuous operation.

All seven challenge tests were included in the following discussion to allow comparisons with a sufficient data set to increase the scope of observations and trends noted. Individual experiments are discussed below, which compliments the overall summary and analysis of results presented in Chapter 5 of this thesis.

Pilot 1 (Train 1) Challenge Tests

Challenge tests 2 to 6 were performed on the SSF of pilot 1 train 1. Although raw water was pre-ozonated (except during challenge test 6), no ozone residual was present in the SSF influent. The results and calculated removals are described below. Challenge tests 1 and 7 performed on pilot plant 2 are discussed later.

Challenge Test 2

Challenge test 2 was performed on May 31, 2004 and involved the seeding of formalin inactivated *C. parvum* oocysts into the SSF influent of pilot 1 train 1. The SSF had been in operation for approximately 7 months. The SSF was operated at an HLR of 0.4 m/h and the oocyst feedstock was injected continuously for six hours at 1 mL/min. Using a hemacytometer, the oocyst feedstock concentration averaged 4.6×10^8 oocysts/L based on five replicate counts. A detention time of one hour was used as the lag time between corresponding influent and effluent samples when calculating removals. Sampling times, oocyst counts and concentrations, and calculated removals are presented in Table E-14.

Table E-14: *Cryptosporidium* oocyst removals for challenge test 2

SSF Influent (Sample Port 2*)				SSF Effluent (Sample Port 3)				Oocyst % Removal
Sample Time (h)	Volume Processed (mL)*	Count** (# oocysts)	Influent Conc.$^{+}$ (oocysts/L)	Sample Time (h)$^{++}$	Volume Processed (mL)*	Count** (# oocysts)	Effluent Conc.$^{+}$ (oocysts/L)	
0	180	2	11	0	250	52	208	-
1	1	68	68000	2	250	22	88	99.87
3	2	706	353000	4	250	1	4	99.9989
4	1	140	140000	5	250	192	768	99.45
5	2	349	174500	6	200	96	480	99.72
6	1	83	83000	7	250	78	312	99.62
8	100	1376	13760	9	250	36	144	-

* Volume filtered through membrane

** Number of oocysts counted on entire membrane

$^{+}$ Concentration = (count/volume processed)x1000 mL

$^{++}$ Assumed 1 hour detention time through SSF

$^{+++}$ Arithmetic mean

Notes: Test 2 (31-May-04) on SSF of pilot 1 train 1 operated at 0.4 m/h; Samples processed June 2 to 21, 2004

Average^{+++} 99.73 (2.58 logs)

Standard Deviation 0.21

As shown in Table E-14, average oocyst removal was 99.73 ± 0.21 % (2.58 logs). The SSF influent had an approximate water temperature of 19°C, influent dissolved oxygen (DO) concentration of 6 mg/L, influent turbidity that ranged from 2 to 3 NTU, and effluent turbidity of 0.16 NTU (Table D-7, Appendix D). The depth of water above the SSF surface was 104 cm and the sand bed depth was approximately 40 cm. The SSF had been cleaned (i.e. had the top 2 cm of sand removed) 10 days before challenge test 2.

Challenge Test 3

Challenge test 3 was performed on June 7, 2004. Formalin inactivated *C. parvum* oocysts were seeded into the influent of the SSF of pilot 1 train 1, which had been in operation for approximately 7 months. The HLR was increased from 0.4 to 0.8 m/h the day before the test. The oocyst feedstock was injected continuously for six hours at 2 mL/min. Using a hemacytometer, the oocyst feedstock concentration averaged 4.4×10^8 oocysts/L based on five replicate counts. A detention time of 0.5 h was used as the lag time between corresponding influent and effluent samples. Sampling times, oocyst counts and concentrations, and calculated removals are presented in Table E-15.

Table E-15: *Cryptosporidium* oocyst removals for challenge test 3

SSF Influent (Sample Port 2*)				SSF Effluent (Sample Port 3)				Oocyst % Removal
Sample Time (h)	Volume Processed (mL)*	Count** (# oocysts)	Influent Conc.$^+$ (oocysts/L)	Sample Time (h)$^{++}$	Volume Processed (mL)*	Count** (# oocysts)	Effluent Conc.$^+$ (oocysts/L)	
0	100	1249	12490	0	200	6	30	-
1	1	37	37000	1.5	200	16	80	99.78
3	1	57	57000	3.5	250	92	368	99.35
4	1	103	103000	4.5	200	30	150	99.85
5	1	106	106000	5.5	200	73	365	99.66
6	1	74	74000	6.5	200	9	45	99.94
8	100	244	2440	8.5	200	14	70	-

* Volume filtered through membrane

** Number of oocysts counted on entire membrane

$^+$ Concentration = (count/volume processed)x1000 mL

$^{++}$ Assumed 0.5 hour detention time through SSF

$^{+++}$ Arithmetic mean

Notes: Test 3 (7-Jun-04) on SSF of pilot 1 train 1 operated at 0.8 m/h; Samples processed June 15 to 22, 2004

Average^{+++}   **99.72** (2.55 logs)

Standard Deviation   **0.23**

As shown in Table E-15, average oocyst removal was 99.72 ± 0.23 % (2.55 logs). Count results were based on single counts, except the count for the 3-hour influent sample count of 57 oocysts, which was an average of two replicate counts (47 and 67 oocysts) for the same membrane. The removal calculated using the 8-hour influent and 8.5-hour effluent samples was not included in the average because oocyst seeding had stopped after six hours and the influent concentration at the time of the 8-hour sample was very low and unrepresentative (Table E-15).

The SSF influent had an approximate water temperature of 22°C, influent DO concentration of 7 mg/L, influent turbidity that ranged from 2 to 3 NTU, and effluent turbidity of 0.2 NTU (Table D-8, Appendix D). The depth of water above the SSF surface was 101 cm and the sand bed depth was approximately 40 cm. The top of the SSF bed had been scraped for cleaning 3 days before challenge test 3.

Challenge Test 4

Challenge test 4 was performed on August 26, 2004. Formalin inactivated *C. parvum* oocysts and *G. muris* cysts were seeded into the influent of the pilot 1 train 1 SSF, which had been in operation for approximately 10 months. The SSF was operated at an HLR of 0.4 m/h and the oocyst and cyst feedstock was injected continuously for six hours at 1 mL/min. Using a hemacytometer, the feedstock concentration averaged 6.2×10^8 oocysts/L and 8.0×10^7 cysts/L based on five replicate counts. For the purpose of calculating removals, a detention time of one hour was used as the lag time between corresponding influent and effluent samples. Sampling times, oocyst count results, and calculated oocyst removals are presented in Table E-16.

Since no oocysts were recovered in the 2-hour and 4-hour effluent samples (i.e. complete removal was achieved) (Table E-16), removals were calculated based on an effluent concentration of 1 oocyst/L. Therefore, those removals were given a "greater than" sign to indicate that removals were at least (and possibly greater than) the value shown. Greater removals may have been observed if higher concentrations of oocysts had been seeded into the SSF influent so that some oocysts could be recovered in the effluent.

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Table E-16: *Cryptosporidium* oocyst removals for challenge test 4

SSF Influent (Sample Port 2*)				SSF Effluent (Sample Port 3)				Oocyst % Removal
Sample Time (h)	Volume Processed (mL)*	Count** (# oocysts)	Influent Conc.$^{+}$ (oocysts/L)	Sample Time (h)$^{++}$	Volume Processed (mL)*	Count** (# oocysts)	Effluent Conc.$^{+}$ (oocysts/L)	
0	100	0	0	0	200	0	0	-
1	2	64	32000	2	200	0	0	>99.997
3	2	328	164000	4	200	0	0	>99.9994
4	2	523	261500	5	200	1	5	99.998
5	2	241	120500	6	200	1	5	99.996
6	2	458	229000	7	200	5	25	99.989
8	100	252	2520	9	200	1	5	-

Average^{+++} >99.996 (>4.38 logs)

* Volume filtered through membrane
** Number of oocysts counted on entire membrane
$^{+}$ Concentration = (count/volume processed)x1000 mL
$^{++}$ Assumed 1 hour detention time through SSF
$^{+++}$ Arithmetic mean
Notes: Test 4 (26-Aug-04) on SSF of pilot 1 train 1 operated at 0.4 m/h; Samples processed Aug 31-Sept 1, 2004

In Table E-16, the average oocyst removal was at least 99.996% (at least 4.38 logs), but no standard deviation could be calculated because of the complete removal observed for some effluent samples. The oocyst removal corresponding to the 8-hour influent sample was not included in the average because the seeding had stopped after six hours. As such, the influent concentration at the time of the 8-hour influent sample was very low and unrepresentative (Table E-16). The removals calculated for *Giardia* cysts are presented in Table E-17.

Cyst removals averaged at least 99.98 % (at least 3.76 logs) (Table E-17). Similar to the effluent oocyst counts in Table E-16, complete removal of *Giardia* cysts was observed in some effluent samples (Table E-17). In such cases, removals were calculated based on an effluent concentration of 1 cyst/L and labeled with a "greater than" sign to indicate that removals were at least (and possibly greater than) the value shown. Greater removals may have been observed if higher cyst concentrations had been seeded into the SSF influent so that some cysts could be recovered in the effluent. The cyst removal corresponding to the 8-hour influent sample was not included in the average because the seeding had stopped after six hours. As such, the influent

concentration at the time of the 8-hour influent sample was very low and unrepresentative (Table E-17).

Table E-17: *Giardia* cyst removals for challenge test 4

SSF Influent (Sample Port 2*)				SSF Effluent (Sample Port 3)				*Giardia* Cyst % Removal
Sample Time (h)	Volume Processed (mL)*	Count** (# cysts)	Influent Conc.[+] (cysts/L)	Sample Time (h)[++]	Volume Processed (mL)*	Count** (# cysts)	Effluent Conc.[+] (cysts/L)	
0	100	0	0	0	200	0	0	-
1	2	10	5000	2	200	0	0	>99.98
3	2	31	15500	4	200	0	0	>99.994
4	2	56	28000	5	200	0	0	>99.996
5	2	26	13000	6	200	1	5	99.96
6	2	107	53500	7	200	2	10	99.98
8	100	25	250	9	200	0	0	-

* Volume filtered through membrane
** Number of cysts counted on entire membrane
[+] Concentration = (count/volume processed)x1000 mL
[++] Assumed 1 hour detention time through SSF
[+++] Arithmetic mean

Average[+++]   >99.98 (>3.76 logs)

Notes: Test 4 (26-Aug-04) on SSF of pilot 1 train 1 operated at 0.4 m/h; Samples processed Aug 31-Sept 1, 2004

The SSF influent had an average water temperature of 23.6°C, influent DO concentration of 3.4 mg/L, influent turbidity of 1.1 NTU, and effluent turbidity of 0.1 NTU (Table D-9, Appendix D). The depth of water above the SSF surface was 19.5 cm and the sand bed depth was 42.5 cm. The top of the SSF bed had been scraped for cleaning 6 days before challenge test 4.

Challenge Test 5

Challenge test 5 on September 30, 2004. Formalin inactivated *C. parvum* oocysts and *G. muris* cysts were seeded into the influent of the pilot 1 train 1 SSF, which had been in operation for approximately 11 months. The HLR was increased from 0.4 to 0.8 m/h three days before the test. The oocyst feedstock was injected continuously for six hours at 2 mL/min. Using a hemacytometer, the feedstock concentration averaged 5.6×10^8 oocysts/L and 1.0×10^8 cysts/L based on five replicate counts. A detention time of 0.5 hours was used as the lag time between corresponding influent and effluent samples when calculating removals. Sampling times, oocyst count results, and calculated oocyst removals are presented in Table E-18.

Table E-18: *Cryptosporidium* **oocyst removals for challenge test 5**

SSF Influent (Sample Port 2*)				SSF Effluent (Sample Port 3)				Oocyst % Removal
Sample Time (h)	Volume Processed (mL)*	Count** (# oocysts)	Influent Conc.$^+$ (oocysts/L)	Sample Time (h)$^{++}$	Volume Processed (mL)*	Count** (# oocysts)	Effluent Conc.$^+$ (oocysts/L)	
0	100	43	430	0	200	0	0	-
1	2	364	182000	1.5	200	8	40	99.98
3	2	604	302000	3.5	200	11	55	99.98
4	2	410	205000	4.5	200	9	45	99.98
5	2	534	267000	5.5	200	21	105	99.96
6	2	454	227000	6.5	200	58	290	99.87
8	100	713	7130	8.5	200	8	40	-

* Volume filtered through membrane

** Number of oocysts counted on entire membrane

$^+$ Concentration = (count/volume processed)x1000 mL

$^{++}$ Assumed 0.5 hour detention time through SSF

$^{+++}$ Arithmetic mean

Notes: Test 5 (30-Sep-04) on SSF of pilot 1 train 1 operated at 0.8 m/h; Samples processed Oct 5 and 6, 2004

Average^{+++}	**99.95**
	(3.34 logs)
Standard Deviation	**0.05**

As shown in Table E-18, average *Cryptosporidium* oocyst removal was 99.95 ± 0.05 % (3.34 logs). The oocyst removal corresponding to the 8-hour influent sample was not included in the average because the seeding had stopped after six hours. As such, the influent concentration at the time of the 8-hour influent sample was very low and unrepresentative (Table E-18). The removals calculated for *Giardia* cysts are presented in Table E-19.

Average *Giardia* cyst removal was at least 99.996% (>4.40 logs) (Table E-19). Complete cyst removal was observed for each effluent sample except the 4.5-hour effluent sample. In these cases, removals were calculated based on an effluent concentration of 1 cyst/L and labeled with a "greater than" sign. The cyst removal corresponding to the 8-hour influent sample was not included in the average because the seeding had stopped after six hours. As such, the influent concentration at the time of the 8-hour influent sample was very low and unrepresentative (Table E-19).

Table E-19: *Giardia* **cyst removals for challenge test 5**

SSF Influent (Sample Port 2*)				SSF Effluent (Sample Port 3)				*Giardia* Cyst % Removal
Sample Time (h)	Volume Processed (mL)*	Count** (# cysts)	Influent Conc.[+] (cysts/L)	Sample Time (h)[++]	Volume Processed (mL)*	Count** (# cysts)	Effluent Conc.[+] (cysts/L)	
0	100	0	0	0	200	0	0	-
1	2	64	32000	1.5	200	0	0	>99.997
3	2	140	70000	3.5	200	0	0	>99.9986
4	2	84	42000	4.5	200	1	5	99.988
5	2	118	59000	5.5	200	0	0	>99.998
6	2	122	61000	6.5	200	0	0	>99.998
8	100	34	340	8.5	200	0	0	-

Average[+++] >99.996 (>4.40 logs)

* Volume filtered through membrane
** Number of cysts counted on entire membrane
[+] Concentration = (count/volume processed)x1000 mL
[++] Assumed 0.5 hour detention time through SSF
[+++] Arithmetic mean
Notes: Test 5 (30-Sep-04) on SSF of pilot 1 train 1 operated at 0.8 m/h; Samples processed Oct 5 and 6, 2004

The SSF influent had an average water temperature of 17.4°C, influent DO concentration of 5.8 mg/L, influent turbidity of 2.7 NTU, and effluent turbidity of 0.26 NTU (Table D-10, Appendix D). The depth of water above the SSF surface was 50.4 cm and the sand bed depth was 36.6 cm. The top of the SSF bed had been scraped for cleaning 1 day before challenge test 5.

Challenge Test 6

Challenge test 6 was performed on February 2, 2005. Formalin inactivated *C. parvum* oocysts and *G. muris* cysts were seeded into the influent of the pilot 1 train 1 SSF, which had been in operation for approximately 15 months. The SSF was operated in cold water at a HLR of 0.4 m/h and the feedstock was injected continuously into the SSF influent for six hours at 1 mL/min. Using a hemacytometer, the feedstock concentration averaged 6.3×10^8 oocysts/L and 1.0×10^8 cysts/L based on five replicate counts. A detention time of one hour was used as the lag time between corresponding influent and effluent samples when calculating removals. Sampling times, oocyst count results, and calculated oocyst removals are presented in Table E-20.

Table E-20: *Cryptosporidium* **oocyst removals for challenge test 6**

SSF Influent (Sample Port 2*)				SSF Effluent (Sample Port 3)				Oocyst % Removal
Sample Time (h)	Volume Processed (mL)*	Count** (# oocysts)	Influent Conc.+ (oocysts/L)	Sample Time (h)++	Volume Processed (mL)*	Count** (# oocysts)	Effluent Conc.+ (oocysts/L)	
0	100	0	0	0	200	0	0	-
1	2	251	125500	2	200	8	40	99.97
3	2	507	253500	4	200	4	20	99.992
4	2	217	108500	5	200	2	10	99.991
5	2	589	294500	6	200	2	10	99.997
6	2	388	194000	7	200	3	15	99.992
8	50	1676	33520	9	200	0	0	-

* Volume filtered through membrane

** Number of oocysts counted on entire membrane

+ Concentration = (count/volume processed)x1000 mL

++ Assumed 1 hour detention time through SSF

+++ Arithmetic mean

Average+++	99.988 (3.92 logs)
Standard Deviation	0.01

Notes: Test 6 (2-Feb-05) on SSF of pilot 1 train 1 operated at 0.4 m/h; Samples processed Feb 7 and 8, 2005

Oocyst removals averaged 99.988 ± 0.01 % (3.92 logs) (Table E-20). Since seeding had stopped after six hours, the 8-hour influent oocyst concentration was unrepresentatively low and the removal was not included in the average. The removals calculated for *Giardia* cysts are presented in Table E-21.

Complete *Giardia* cyst removal was achieved for all effluent samples and no cysts were recovered in challenge test 6 (Table E-21). Calculated removals were based on an effluent concentration of 1 cyst/L. Average cyst removal was at least, and possibly greater than, 99.997% (4.48 logs). If it had been economically feasible to seed a higher concentration of cysts into the SSF influent, some cysts may have been recovered in the effluent and greater removals may have been observed. Since seeding stopped after six hours, the 8-hour influent count was low and the removal was not included in the average.

Table E-21: *Giardia* cyst removals for challenge test 6

SSF Influent (Sample Port 2*)				SSF Effluent (Sample Port 3)				*Giardia*
Sample Time (h)	Volume Processed (mL)*	Count** (# cysts)	Influent Conc.⁺ (cysts/L)	Sample Time (h)⁺⁺	Volume Processed (mL)*	Count** (# cysts)	Effluent Conc.⁺ (cysts/L)	Cyst % Removal
0	100	1	10	0	200	0	0	-
1	20	656	32800	2	200	0	0	>99.997
3	20	1346	67300	4	200	0	0	>99.9985
4	20	696	34800	5	200	0	0	>99.997
5	20	1365	68250	6	200	0	0	>99.9985
6	20	1583	79150	7	200	0	0	>99.9987
8	50	513	10260	9	200	0	0	-

* Volume filtered through membrane

** Number of cysts counted on entire membrane

⁺ Concentration = (count/volume processed)x1000 mL

⁺⁺ Assumed 1 hour detention time through SSF

⁺⁺⁺ Arithmetic mean

Average⁺⁺⁺ >99.997 (>4.48 logs)

Notes: Test 6 (2-Feb-05) on SSF of pilot 1 train 1 operated at 0.4 m/h; Samples processed Feb 7 and 17, 2005

Challenge test 6 was conducted in raw and SSF influent water with temperatures of 1.4 and 2.5°C, respectively, at the start of the test (Table D-11, Appendix D). The average SSF influent DO concentration was 11 mg/L, influent turbidity was 2 NTU, and effluent turbidity was 0.2 NTU. The depth of water above the SSF surface was 36.4 cm and the sand bed depth was 43.6 cm. The top of the SSF bed had been scraped for cleaning 4 days before challenge test 6.

Pilot 2 Challenge Tests

Challenge tests 1 and 7 were performed on the SSFs of pilot 2 and are described below.

Challenge Test 1

Challenge test 1 was performed on April 28, 2004 and involved the seeding of formalin inactivated *C. parvum* oocysts into the influent of SSF1 of pilot 2. Effluent samples were taken from SSF1 and SSF2 in order to quantify the removal in both filters. Since pilot 2 had been in operation for only 4 months at cold water temperatures, the SSFs were likely biologically immature at the time of challenge test 1.

The SSFs were operated at an HLR of 0.4 m/h and the oocyst feedstock was injected continuously into the influent of SSF1 for six hours at 1 mL/min. Using a hemacytometer, the oocyst feedstock concentration averaged 4.2×10^8 oocysts/L based on three replicate counts. For calculating removals, a two hour detention time was used as the lag time between corresponding SSF1 influent and effluent samples. In SSF2, a one hour detention time was assumed. Sampling times, oocyst counts and concentrations, and calculated oocyst removals for SSF1 are presented in Table E-22.

Table E-22: *Cryptosporidium* **oocyst removals by SSF1 for challenge test 1**

SSF1 Influent (Sample Port 2*)				SSF1 Effluent (Sample Port 3)				Oocyst % Removal
Sample Time (h)	Volume Processed (mL)*	Count** (# oocysts)	Influent Conc.+ (oocysts/L)	Sample Time (h)++	Volume Processed (mL)*	Count** (# oocysts)	Effluent Conc.+ (oocysts/L)	
0	200	34	170	0	400	0	0	-
				1	490	0	0	-
				2	560	0	0	-
1	2	231	115500	3	505	153	303	99.74
2	1	162	162000	4	505	77	152	99.91
3	2	348	174000					-
4	1	288	288000	6	485	77	159	99.94
6	1	292	292000	8	500	208	416	99.86
8	1	94	94000					-

* Volume filtered through membrane	
** Number of oocysts counted on entire membrane	**Average+++** **99.86**
+ Concentration = (count/volume processed)x1000 mL	**(2.86 logs)**
++ Assumed 2 hour and 1 hour detention times through SSF1 and SSF2, respectively	**Standard Deviation** **0.09**
+++ Arithmetic mean	

Notes: Test 1 (28-Apr-04) on SSF1 & 2 of pilot 2 operated at 0.4 m/h; Samples processed Apr 28 to May 20, 2004

As shown in Table E-22, average oocyst removal in SSF1 was 99.86 ± 0.09 % (2.86 logs). For quality control, some oocyst counts were averages based on replicate counts of the same membrane, including the 6-hour influent count (252 and 333 oocysts), the 6-hour effluent count (73 and 81 oocysts), and the 8-hour effluent count (260 and 156 oocysts). Table E-23 presents sampling times, oocyst counts and concentrations, and calculated removals for SSF2.

Table E-23: SSF2 and combined *Cryptosporidium* removals for challenge test 1

SSF1 Influent Sample Time (h)	SSF1 Effluent Sample Time (h)[++]	SSF2 Effluent (Sample Port 4)				SSF2 Oocyst % Removal	Combined Oocyst % Removal[‡]
		Sample Time (h)[++]	Volume Processed (mL)*	Count** (# oocysts)	Effluent Conc.[+] (oocysts/L)		
-	-	0	500	0	0	-	-
1	3	4	500	5	10	96.70	99.991
3	-	6	500	12	24	-	99.986
4	6	7	500	44	88	42.29	99.97
-	-	8	500	75	150	-	-

* Volume filtered through membrane	**Average**[+++]	69.49 (0.52 logs)	99.98 (3.75 logs)
** Number of oocysts counted on entire membrane		
[+] Concentration = (count/volume)x1000 mL	**Standard Deviation**	38.48	0.01

[++] Assumed 2 hour and 1 hour detention times through SSF1 and SSF2, respectively

[+++] Arithmetic mean

‡ Combined removals through SSF1 and SSF2

Notes: Test 1 (28-Apr-04) on SSF1 & 2 of pilot 2 at 0.4 m/h; Samples processed Apr 28 to May 20, 2004

In Table E-23, only two oocyst removals could be calculated for SSF2 based on a one hour detention time. The calculated removals in SSF2 were low because the incoming oocyst concentrations in the SSF1 effluent were low (note that SSF1 effluent serves as the SSF2 influent). The approximate average oocyst concentrations were 190,000 oocysts/L in the SSF1 influent, 170 oocysts/L in the SSF2 influent (i.e. SSF1 effluent), and 70 oocysts/L in the SSF2 effluent. To accurately quantify oocyst removal in SSF2, the filter's influent would need to be seeded with very high concentrations of oocysts. If this were done, removals could likely be in the range observed for the SSF of pilot 1 train 1 in challenge tests 2 to 6 since they had similar bed depths. Average oocyst removal in SSF2 was 69.5 ± 38.5 % (0.52 logs) (Table E-23).

Also shown in Table E-23 are combined removals calculated based on a combined three hour detention time through both SSFs (two hours through SSF1 plus one hour through SSF2). The combined oocyst removals averaged 99.98 ± 0.01 % (3.75 logs). However, it may be more meaningful to consider only the removals in SSF1 since SSF2 would need to be seeded with high oocyst concentrations in order to accurately determine its capacity to remove oocysts.

The SSF1 of pilot 2 had an influent water temperature of 10.8°C, DO concentration of 11.6 mg/L, and turbidity of 0.5 NTU (Table D-12, Appendix D). Effluent turbidities for SSF1 and SSF2 were 0.26 and 0.21 NTU, respectively. The sand bed depth of SSF1 was approximately 100 cm, which was about twice as deep as the approximately 45 cm deep sand bed of SSF2. The depth of water above SSF1 and SSF2 was 50 and 15 cm, respectively. The top of the SSF1 bed had been scraped for cleaning 26 days before challenge test 1. SSF2 had not been scraped since pilot 2 was commissioned in December 2003.

Challenge Test 7

Challenge test 7 was performed on August 9, 2005 and involved the seeding of formalin inactivated *C. parvum* oocysts and *G. muris* cysts into the influent of SSF1 of pilot 2. Effluent samples were taken from both SSF1 and SSF2 to quantify the additional removal provided by the second SSF in series. Pilot 2 was considered to be fully biologically mature during challenge test 7 because it had been in continuous operation for over 1.5 years.

The SSFs were operated at a HLR of 0.4 m/h and the oocyst and cyst feedstock was injected continuously into the influent of SSF1 for six hours at 1 mL/min. For calculating removals, a two hour detention time was used as the lag time between corresponding SSF1 influent and effluent samples. A one hour detention time was used for calculating SSF2 removals. *Cryptosporidium* oocyst counts and removals for SSF1 and SSF2 are presented in Table E-24 and Table E-25.

Oocyst removals in the biologically mature SSF1 averaged at least 99.994% (4.25 logs) (Table E-24). Since complete oocyst removal was observed in the 7-hour SSF1 effluent sample, a removal was calculated based on a concentration of 1 oocyst/L. If it had been economically feasible to seed a higher concentration of cysts into the SSF influent, some cysts may have been recovered in the effluent and greater removals may have been observed. For SSF2, average oocyst removal was low (>99.998%, >4.82 logs) (Table E-25).

Table E-24: *Cryptosporidium* oocyst removals in SSF1 for challenge test 7

SSF1 Influent (Sample Port 2*)				SSF1 Effluent (Sample Port 3)				SSF1 Oocyst % Removal
Sample Time (h)	Volume Processed (mL)*	Count** (# oocysts)	Influent Conc.$^+$ (oocysts/L)	Sample Time (h)$^{++}$	Volume Processed (mL)*	Count** (# oocysts)	Effluent Conc.$^+$ (oocysts/L)	
1	2	795	397500	3	200	7	35	99.991
2	2	933	466500	4	200	13	65	99.986
3	2	1545	772500	5	200	2	10	99.999
4	2	775	387500	6	200	3	15	99.996
5	2	1616	808000	7	200	0	0	>99.9998

Average^{+++} >99.994 (>4.25 logs)

* Volume filtered through membrane
** Number of oocysts counted on entire membrane
$^+$ Concentration = (count/volume processed)x1000 mL
$^{++}$ Assumed 2 hour and 1 hour detention times through SSF1 and SSF2, respectively
$^{+++}$ Arithmetic mean
Notes: Test 7 (9-Aug-05) on SSF1 & 2 of pilot 2 at 0.4 m/h; Samples processed Aug 10 to 17, 2005
Notes: Influent slides counted July 2006 except 4-hour sample counted 16-Aug-05; SSF1 effluent slides counted Aug 26 to Sept 9, 2005

Table E-25: SSF2 and combined *Cryptosporidium* removals for challenge test 7

SSF1 Influent Sample Time (h)	SSF1 Effluent Sample Time (h)$^+$	SSF2 Effluent (Sample Port 4)				SSF2 Oocyst % Removal	Combined Oocyst % Removal
		Sample Time (h)$^+$	Volume Processed (mL)	Count (# oocysts)	Effluent Conc. (oocysts/L)		
1	3	4	200	1	5	85.71	99.9987
2	4	5	200	1	5	92.31	99.9989
3	5	6	200	8	40	-300.0	99.9987
4	6	7	200	10	50	-233.3	99.996
5	7	8	200	3	15	>-1400	>99.9998
-	-	9	200	8	40	-	-

Average^{++} >-351.1 (>-0.65 logs) >99.998 (>4.82 logs)

* Calculated using SSF1 influent and SSF2 effluent counts
** Calculated using only SSF1 influent and effluent counts
$^+$ Assumed 2 hour and 1 hour detention times through SSF1 and SSF2, respectively
$^{++}$ Arithmetic mean
Notes: Test 7 (9-Aug-05) on SSF1 & 2 of pilot 2 at 0.4 m/h; Samples processed Aug 10 to 17, 2005
Notes: SSF2 effluent slides counted Aug 18 to 25, 2005

The poor removals in SSF2 were likely due to the low oocyst concentrations in the SSF1 effluent (which served as the SSF2 influent). For comparison, in test 7 the SSF1 influent concentration

averaged approximately 560,000 oocysts/L whereas the SSF1 effluent concentration averaged only 25 oocysts/L (Table E-24). The oocyst concentration of the SSF2 effluent averaged 26 oocysts/L. At such low oocyst concentrations for both the SSF2 influent and effluent, it was not possible to calculate large removals because all oocyst counts were in the range of the detection limit. In some cases (including the 6-, 7-, and 8-hour SSF2 effluent samples), counts were 3 to 7 oocysts greater than the SSF1 effluent, which led to the calculation of negative removals. When the positive and negative removals were taken together, the average SSF2 oocyst removal was approximately zero. However, to accurately quantify oocyst removal in SSF2, the filter would need to be seeded with very high concentrations of oocysts to achieve SSF2 influent oocyst concentrations in the same range as SSF1 influent concentrations. If seeded with such high oocyst concentrations, SSF2 could likely provide oocyst removals in the range observed for the SSF of pilot 1 train 1 in challenge tests 2 to 6 since they had similar bed depths.

The combined removals in Table E-25 were calculated based on a combined three hour detention time through both SSFs (two hours through SSF1 plus one hour through SSF2) in the case of the 1-hour and 2-hour SSF1 influent samples. "Combined" removals corresponding to the 3-, 4-, and 5-hour SSF1 influent samples were only for removals in SSF1 and did not include the negative removals calculated for SSF2. It may be more meaningful to consider only the removals in SSF1 since SSF2 needs to be seeded with high oocyst concentrations in order to accurately determine its capacity to remove oocysts.

Giardia cyst counts and removals for SSF1 and SSF2 are presented in Table E-26 and Table E-27. *Giardia* cyst removals in SSF1 averaged at least 99.987% (3.73 logs) (Table E-26). Since complete cyst removal was observed in the 5-hour SSF1 effluent sample, removal was calculated based on a concentration of 1 oocyst/L. For SSF2, cyst removals were small and negative on one occasion (Table E-27). This was attributed to the low cyst concentration in the SSF1 effluent, which served as the SSF2 influent. When the positive and negative removals were averaged together, the average SSF2 cyst removal was at least –520% (-0.79 logs). However, to accurately quantify cyst removal in SSF2, the filter would need to be seeded with very high

concentrations of cysts. If seeded with high cyst concentrations, SSF2 could likely provide cyst removals in the range observed for the SSF of pilot 1 train 1 in challenge tests 4 to 6.

Table E-26: *Giardia* cyst removals in SSF1 for challenge test 7

SSF1 Influent (Sample Port 2*)				SSF1 Effluent (Sample Port 3)				SSF1 Cyst % Removal
Sample Time (h)	Volume Processed (mL)*	Count** (# cysts)	Influent Conc.[+] (cysts/L)	Sample Time (h)[++]	Volume Processed (mL)*	Count** (# cysts)	Effluent Conc.[+] (cysts/L)	
1	2	113	56500	3	200	1	5	99.991
2	2	120	60000	4	200	4	20	99.97
3	2	212	106000	5	200	0	0	>99.9991
4	2	68	34000	6	200	2	10	99.97
5	2	197	98500	7	200	4	20	99.98

* Volume filtered through membrane

** Number of cysts counted on entire membrane

[+] Concentration = (count/volume processed)x1000 mL

[++] Assumed 2 hour and 1 hour detention times through SSF1 and SSF2, respectively

[+++] Arithmetic mean

Average[+++]   >99.98 (>3.73 logs)

Notes: Test 7 (9-Aug-05) on SSF1 & 2 of pilot 2 at 0.4 m/h; Samples processed Aug 10 to 17, 2005

Notes: Influent slides counted July 2006 except 4-hour sample counted 16-Aug-05; SSF1 effluent slides counted Aug 26 to Sept 9, 2005

Table E-27: SSF2 and combined *Giardia* removals for challenge test 7

SSF1 Influent Sample Time (h)	SSF1 Effluent Sample Time (h)[++]	SSF2 Effluent (Sample Port 4)				SSF2 Cyst % Removal	Combined Cyst % Removal[‡]
		Sample Time (h)[++]	Volume Processed (mL)	Count (# cysts)	Effluent Conc. (cysts/L)		
1	3	4	200	0	0	>80.0	>99.998
2	4	5	200	1	5	75.0	99.992
3	5	6	200	6	30	>-2900.0	>99.9991*
4	6	7	200	1	5	50.0	99.985
5	7	8	200	0	0	>95.0	>99.9990
-	-	9	200	2	10	-	-

[+] Calculated using SSF1 effluent counts

‡ Combined removals through SSF1 and SSF2

[++] Assumed 2 hour and 1 hour detention times through SSF1 and SSF2, respectively

[+++] Arithmetic mean

Average[+++]   >-520 (>-0.79 logs)   >99.995 (>4.27 logs)

Notes: Test 7 (9-Aug-05) on SSF1 & 2 of pilot 2 at 0.4 m/h; Samples processed Aug 10 to 17, 2005

Notes: SSF2 effluent slides counted Aug 18 to 25, 2005

The combined removals in Table E-27 were calculated based on a combined three hour detention time through both SSFs, except for the 3-hour SSF1 effluent sample where only the cyst removal in SSF1 is included because removal in SSF2 was negative. It may be more meaningful to consider only the removals in SSF1 since SSF2 needs to be seeded with a high concentration of *Giardia* cysts in order for its ability to remove cysts to be accurately determined.

The SSF1 of pilot 2 had an approximate influent water temperature of 26°C, DO concentration of 4 mg/L, and turbidity of 1 NTU (Table D-13, Appendix D). Effluent turbidities for SSF1 and SSF2 were 0.13 and 0.12 NTU, respectively. The sand bed depth of SSF1 was 100 cm, which was about twice as deep as the approximately 44 cm sand bed of SSF2. The depth of water above SSF1 and SSF2 was 31.5 and 28 cm, respectively. Although new sand had been added to SSF1 7 days before challenge test 7, the top 10 cm of the mature sand bed had been set aside prior to resanding and placed on top of the new sand. Prior to resanding, SSF1 had not been cleaned for 35 days. SSF2 had not been scraped for about 100 days prior to test 7.

Appendix F
Water Quality Data for MS2 Challenge Tests

Table F-28: Water quality data for MS2 tests 1 and 2

		Dates (Test 1)		Dates (Test 2)	
		14-Feb-06	**15-Feb-06**	**27-Feb-06**	**28-Feb-06**
Water Temperature (°C)					
Raw		1.9	2.5	1.2	1.4
Influent		3.8	3.5	2.6	2.6
Effluent		4.2	3.9	2.7	2.9
Turbidity (NTU)					
Raw	(Manual)	7.97	8.81	7.62	3.14
	(Online)	7.183	8.581	7.212	3.020
Influent		2.09	2.32	2.08	3.08
Effluent	(Manual)	0.19	0.22	0.23	0.23
	(Online)	0.180	0.221	0.220	0.220
Dissolved Oxygen (mg/L)					
Raw		10.17	9.69	10.4	10.4
Influent		8.50	8.55	8.7	8.7
Effluent		7.89	8.06	7.9	8.0
pH	Raw (Manual)	8.19	n.d.	8.14	8.24
	(Online)	8.28	8.33	8.36	8.41
Total Hardness (mg/L as $CaCO_3$)	Raw	268	n.d.	288	288
Conductivity (µs)	Raw	n.d	n.d.	756	817
Sand Depth (cm)		40.4	40.4	38.0	38.0
Water Depth Above Media (cm)		85.1	85.1	66.0	72.5
Schmutzdecke Age (days)*		11	12	7	8

n.d. – measurement not done

* Time since last scraping of slow sand filter surface

Table F-29: Water quality data for MS2 test 3

		Date
		6-Mar-06
Water Temperature (°C)		
Raw		2.3
Influent		3.4
Effluent		4.4
Turbidity (NTU)		
Raw Influent	(Manual)	4.07
	(Online)	3.792
Influent		n.d.
Effluent		1.0
Dissolved Oxygen (mg/L)		
Raw		9.9
Influent		9.7
Effluent		8.5
pH	Raw (Manual)	8.23
	(Online)	8.39
Total Hardness (mg/L as $CaCO_3$)	Raw	275
Conductivity (µs)	Raw	723
Media Depth (cm)*		58.5
Water Depth Above Media (cm)		11.5

n.d. – measurement not done

* Media depth including 30 cm gravel and 28.5 cm granular activated carbon (GAC)

Table F-30: Water quality data for MS2 tests 4 and 5

		Date (Test 4)	Dates (Test 5)	
		9-Mar-06	20-Mar-06	21-Mar-06
Water Temperature (°C)				
Raw		2.7	2.4	4.7
Influent		6.8	6.4	6.2
Effluent		7.1	6.4	6.4
Turbidity (NTU)				
Raw	(Manual)	38.9	17.3	14.2
	(Online)	33.71	17.34	13.36
Influent		0.41	2.34	2.34
Effluent	(Manual)	0.16	0.16	0.17
	(Online)	0.150	0.154	0.148
Dissolved Oxygen (mg/L)				
Raw		9.4	9.3	9.4
Influent		4.8	5.3	5.3
Effluent		2.3	3.5	3.5
pH	Raw (Manual)	7.98	7.94	7.96
	(Online)	8.30	n.d.	8.09
Total Hardness (mg/L as $CaCO_3$)	Raw	160	202	205
Conductivity (μs)	Raw	n.d.	504	546
Sand Depth (cm)		38.0	38.0	38.0
Water Depth Above Media (cm)		17.0	17.5	17.5
Schmutzdecke Age (days)*		17	28	29

n.d. – measurement not done
* Time since last scraping of slow sand filter surface

Table F-31: Water quality data for MS2 test 6

			Date
			21-Mar-06
Water Temperature (°C)			
	Raw/Influent		5.0
	Effluent		5.7
Turbidity (NTU)			
	Raw / Influent	(Manual)	14.3
		(Online)	14.22
	Effluent		8.05
Dissolved Oxygen (mg/L)			
	Raw / Influent		8.9
	Effluent		7.3
pH	Raw	(Manual)	7.97
		(Online)	8.09
Total Hardness (mg/L as $CaCO_3$)	Raw		205
Conductivity (μs)	Raw		533
Media Depth (cm)			120
Water Depth Above Media (cm)			15

Table F-32: Water quality data for MS2 tests 7a and 7b

			Dates (Test 7a)		Dates (Test 7b)	
			28-Mar-06	29-Mar-06	30-Mar-06	31-Mar-06
Water Temperature (°C)						
Raw			6.8	7.1	8.1	9.6
Influent			9.9	8.7	9.7	10.2
Effluent			10.1	9.0	9.9	10.0
Turbidity (NTU)						
Raw	(Manual)		4.07	3.75	4.31	5.74
	(Online)		3.979	3.687	4.135	5.093
Influent			0.79	0.76	n.d.	0.70
Effluent	(Manual)		0.14	0.13	0.16	0.13
	(Online)		0.146	0.135	0.150	0.135
Dissolved Oxygen (mg/L)						
Raw			8.3	9.3	7.6	8.1
Influent			3.3	4.2	4.2	3.6
Effluent			1.1	1.7	1.9	2.7
pH	Raw	(Manual)	8.05	8.04	8.19	8.17
		(Online)	8.30	8.31	8.30	8.30
Total Hardness (mg/L as $CaCO_3$)	Raw		255	256	250	222
Sand Depth (cm)			38.0	38.0	36.3	36.3
Water Depth Above Media (cm)			15.0	20.5	8.7	6.9
Schmutzdecke Age (days)*			35	36	1	2

n.d. – measurement not done

* Time since last scraping of slow sand filter surface

Table F-33: Water quality data for MS2 test 8

		Date
		29-Mar-06
Water Temperature (°C)		
Raw		6.8
Influent		7.7
Effluent		7.8
Turbidity (NTU)		
Raw	(Manual)	3.75
	(Online)	3.650
Influent		1.13
Effluent	(Manual)	0.17
	(Online)	0.155
Dissolved Oxygen (mg/L)		
Raw		9.3
Influent		6.3
Effluent		5.9
pH	Raw (Manual)	8.04
	(Online)	8.31
Total Hardness (mg/L as $CaCO_3$)	Raw	256
Sand Depth (cm)		35.5
Water Depth Above Media (cm)		60.5
Schmutzdecke Age (days)*		12

Table F-34: Water quality data for MS2 test 9

		Date
		30-Mar-06
Water Temperature (°C)		
Raw		7.4
Influent		8.3
Effluent		8.3 (SSF1) / 8.5 (SSF2)
Turbidity (NTU)		
Raw	(Manual)	2.72
	(Online)	3.301
Influent		1.54
Effluent	(Manual)	0.12 (SSF1) / 0.10 (SSF2)
	(Online)	0.115 (SSF1) / 0.088 (SSF2)
Dissolved Oxygen (mg/L)		
Raw		8.8
Influent		6.3
Effluent		4.9 (SSF1) / 5.1 (SSF2)
pH Raw	(Manual)	8.17
	(Online)	8.30
Total Hardness (mg/L as $CaCO_3$)	Raw	245
Conductivity (µs)	Raw	402
Sand Depth (cm)		92.0 (SSF1) / 44.0 (SSF2)
Water Depth Above Media (cm)		94.5 (SSF1) / 29.0 (SSF2)
Schmutzdecke Age (days)*		20 (SSF1) / 334 (SSF2)

* Time since last scraping of slow sand filter surface

Table F-35: Water quality data for MS2 tests 10 and 11

			Dates (Test 10)		Dates (Test 11)	
			15-May-06	**16-May-06**	**18-May-06**	**19-May-06**
Water Temperature (°C)						
	Raw		16.0	16.6	17.7	16.2
	Influent		20.4	21.0	22.0	21.0
	Effluent		20.9	22.3	23.7	22.9
Turbidity (NTU)						
	Raw	(Manual)	3.45	7.65	16.8	12.5
		(Online)	2.949	7.829	15.03	11.67
	Influent		0.38	0.49	0.38	0.93
	Effluent	(Manual)	0.10	0.10	0.12	0.10
		(Online)	0.071	0.096	0.072	0.068
Dissolved Oxygen (mg/L)						
	Raw		5.2	5.2	4.77	6.3
	Influent		2.3	2.0	1.29	2.0
	Effluent		0.9	1.0	0.74	0.64
pH	Raw	(Manual)	8.00	7.95	7.88	7.94
		(Online)	8.21	8.08	8.02	8.10
Total Hardness (mg/L as $CaCO_3$)	Raw		240	230	n.d.	n.d.
Sand Depth (cm)			36.3	36.3	36.3	36.3
Water Depth Above Media (cm)			8.2	7.4	7.7	6.7
Schmutzdecke Age (days)*			47	48	50	51

n.d. – measurement not done

* Time since last scraping of slow sand filter surface

Table F-36: Water quality data for MS2 tests 12, 13, and 15

			Test 12	Test 13	Test 15
			2-Jun-06	**5-Jun-06**	**8-Jun-06**
Water Temperature (°C)					
Raw			21.2	20.0	22.3
Influent			23.3	21.8	23.6
Effluent			24.6	22.6	24.1
Turbidity (NTU)					
Raw	(Manual)		79.5	30.7	25.2
	(Online)		68.45	27.03	21.48
Influent			16.3	2.51	1.91
Effluent	(Manual)		0.14	0.15	0.14
	(Online)		0.077	0.080	0.083
Dissolved Oxygen (mg/L)					
Raw			2.57	5.96	3.91
Influent			1.67	3.26	2.36
Effluent			0.43	2.15	1.14
pH	Raw	(Manual)	n.d.	n.d.	n.d.
		(Online)	7.80	8.26	8.03
Sand Depth (cm)			36.3	36.3	36.3
Water Depth Above Media (cm)			45.6	54.3	53.6
Schmutzdecke Age (days)*			64	67	70

n.d. – measurement not done

* Time since last scraping of slow sand filter surface

Table F-37: Water quality data for MS2 test 14

		Date
		5-Jun-06
Water Temperature (°C)		
Raw		22.7
Influent		21.8
Effluent		22.9 (SSF1) / 25.8 (SSF2)
Turbidity (NTU)		
Raw	(Manual)	17.1
	(Online)	n.d.
Influent		3.52
Effluent	(Manual)	0.15 (SSF1) / 0.14 (SSF2)
	(Online)	0.106 (SSF1) / 0.094 (SSF2)
Dissolved Oxygen (mg/L)		
Raw		5.00
Influent		3.52
Effluent		1.97 (SSF1) / 2.98 (SSF2)
pH	Raw (Manual)	n.d.
	(Online)	8.26
Sand Depth (cm)		87.4 (SSF1) / 44.0 (SSF2)
Water Depth Above Media (cm)		74.9 (SSF1) / 28.0 (SSF2)
Schmutzdecke Age (days)*		3 (SSF1) / 401 (SSF2)

n.d. – measurement not done
* Time since last scraping of slow sand filter surface

Appendix G

Plaque Count and Removal Data for MS2 Challenge Tests

Table G-38: Test 1 plaque counts and MS2 removals (SSF Pilot 1 Train 1, 0.4 m/h, Cold water)

Sample Date (2006)	Plating Date (2006)	SSF Influent (Sample Port 2*)								SSF Effluent (Sample Port 3)								MS2 % Removal+++
		Sample Time (h)	Dilution+	Plaque Count***					PFU/mL++	Sample Time (h)	Dilution+	Plaque Count**					PFU/mL++	
				1	2	3	Avg	SD*				1	2	3	Avg	SD*		
14-Feb	15-Feb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
15-Feb	21-Feb	1	3	27	28	33	29	3	2.9E+04	2	2	94	100	87	94	7	9.4E+03	68.1
	21-Feb	3	3	35	41	55	44	10	4.4E+04	4	3	21	18	23	21	3	2.1E+04	52.7
	17-Feb	3.5	3	95	95	87	92	5	9.2E+04	4.5	3	34	32	19	28	8	2.8E+04	69.3
	16-Feb	4	3	48	49	44	47	3	4.7E+04	5	3	28	31	16	25	8	2.5E+04	46.8
	21-Feb	4.5	3	44	40	39	41	3	4.1E+04	5.5	3	29	29	41	33	7	3.3E+04	19.5
	21-Feb	5	3	82	87	74	81	7	8.1E+04	6	3	28	24	33	28	5	2.8E+04	65.0
	16-Feb	5.5	3	105	95	84	95	11	9.5E+04	6.5	3	36	32	28	32	4	3.2E+04	66.2
	17-Feb	6	3	98	76	104	93	15	9.3E+04	7	3	30	33	34	32	2	3.2E+04	65.1
	16-Feb	22	4	22	30	20	24	5	2.4E+05	23	3	76	66	71	71	5	7.1E+04	70.4
	15-Feb	22.5	3	133	114	153	133	20	1.3E+05	23.5	3	49	46	55	50	5	5.0E+04	62.5
	17-Feb	23	3	121	111	129	120	9	1.2E+05	24	3	45	35	42	41	5	4.1E+04	66.2
	16-Feb	24	3	95	104	95	98	5	9.8E+04	25	3	40	32	39	37	4	3.7E+04	62.2
	16-Feb	25	3	99	110	100	103	6	1.0E+05	26	3	41	39	30	37	6	3.7E+04	64.4
	16-Feb	25.5	3	98	93	115	102	12	1.0E+05	26.5	3	62	56	70	63	7	6.3E+04	38.6
	17-Feb	26	3	104	101	119	108	10	1.1E+05	27	3	36	32	41	36	5	3.6E+04	66.4
20-Feb	21-Feb	144	0	1	1	2	1	0.58	1	144	0	12	20	27	20	8	20	-
	21-Feb									147	0	30	28		29	1	29	-
24-Feb	27-Feb	245.5	0	1	1	1	1	0	1	245.5	0	19	24	25	23	3	23	-
27-Feb	27-Feb	311								311	0	14	6	15	12	5	12	-

Average***	58.9	
Standard Deviation	14.1	(0.39 logs)
Number of Data Points	15	

* Standard deviation (SD) in logs for triplicate plate counts
** Each sample plated in triplicate
*** Arithmetic mean
+ 2 indicates a 10^2 dilution; 3 indicates a 10^3 dilution; 4 indicates a 10^4 dilution; 0 indicates no dilution
++ Sample MS2 concentration in plaque forming units (PFU) per mL
+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using a 1 hour detention time
Note: Test 1 conducted on the SSF of pilot 1 train 1 at 0.4 m/h in cold water

Table G-39: Test 2 plaque counts and MS2 removals (SSF Pilot 1 Train 1, 0.4 m/h, Cold water)

Sample Date (2006)	Plating Date (2006)	SSF Influent (Sample Port 2*)								SSF Effluent (Sample Port 3)								MS2 % Removal+++
		Sample Time (h)	Dilution+	Plaque Count***					PFU/mL++	Sample Time (h)	Dilution+	Plaque Count***					PFU/mL++	
				1	2	3	Avg	SD*				1	2	3	Avg	SD*		
27-Feb	27-Feb	0	0	1	0	-	0	0.7	0	0	0	14	6	15	12	5	12	-
	07-Mar	1	3	25	28	17	23	6	2.3E+04	2	3	51	43	28	40.7	12	4.1E+04	-
	27-Feb	1.5	3	105	130	61	99	35	9.9E+04	2.5	3	67	36	38	47	17	4.7E+04	52.4
		2	3	99	97	89	95	5	9.5E+04	3	3	55	45	55	52	6	5.2E+04	45.6
28-Feb		2.5	3	131	123	129	128	4	1.3E+05	3.5	3	50	52	67	56	9	5.6E+04	55.9
		3	3	120	121	114	118	4	1.2E+05	4	3	55	72	64	64	9	6.4E+04	46.2
		3.5	3	137	147	142	142	5	1.4E+05									-
		4	3	115	131	130	125	9	1.3E+05	5	3	51	63	57	57	6	5.7E+04	54.5
										5.5	3	53	67	80	67	14	6.7E+04	-
		5	3	151	156	178	162	14	1.6E+05	6	3	81	63	74	73	9	7.3E+04	55.1
		5.5	3	113	167	132	137	27	1.4E+05	6.5	3	77	88	69	78	10	7.8E+04	43.2
		6	3	156	153	157	155	2	1.6E+05	7	3	78	79	91	83	7	8.3E+04	46.8
28-Feb	07-Mar	24	3	89	95	64	83	16	8.3E+04	25	3	67	58	57	61	6	6.1E+04	26.6
		25	3	74	96	103	91	15	9.1E+04	26	3	68	74	74	72	3	7.2E+04	20.9
		25.5	3	94	95	64	84	18	8.4E+04	26.5	3	80	71	66	72	7	7.2E+04	14.2
		26	3	89	98	104	97	8	9.7E+04	27	3	74	69	68	70	3	7.0E+04	27.5
		26.5	3	126	117	149	131	17	1.3E+05	27.5	3	76	86	80	81	5	8.1E+04	38.3
		27	3	103	102	92	99	6	9.9E+04	28	3	70	59	82	70	12	7.0E+04	29.0
03-Mar	07-Mar	102.5	0	0	0	0	0	0	0	102.5	0	114	100	142	119	21	119	-
06-Mar	07-Mar									179	0	35	36	76	49	23	49	-

Average*** 39.7 (0.22 logs)
Standard Deviation 13.7
Number of Data Points 14

* Standard deviation (SD) in logs for triplicate plate counts
** Each sample plated in triplicate
*** Arithmetic mean
+ 3 indicates a 10^3 dilution; 0 indicates no dilution
++ Sample MS2 concentration in plaque forming units (PFU) per mL
+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using a 1 hour detention time
Note: Test 2 conducted on the SSF of pilot 1 train 1 at 0.4 m/h in cold water

Table G-40: Test 3 plaque counts and MS2 removals (RF Pilot 1 Train 1, 1.5 m/h, Cold water)

Sample Date (2006)	Plating Date (2006)	RF Influent (Sample Port 1) Sample Time (min)	Dilution+	Plaque Count*** 1	2	3	Avg	SD*	PFU/mL++	RF Effluent (Sample Port 2) Sample Time (min)	Dilution+	Plaque Count*** 1	2	3	Avg	SD*	PFU/mL++	MS2 % Removal+++
06-Mar	07-Mar	60	3	148	139	155	147	8	1.5E+05	0	0	0	0	0	0	0	0	-
		80	3	141	154	163	153	11	1.5E+05	80	3	124	99	107	110	13	1.1E+05	25.3
		100	3	205	182	179	189	14	1.9E+05	100	3	105	116	132	118	14	1.2E+05	22.9
		120	3	158	169	190	172	16	1.7E+05	120	3	115	128	114	119	8	1.2E+05	36.9
	08-Mar	140	3	127	113	138	126	13	1.3E+05	140	3	143	121	125	130	12	1.3E+05	24.8
	09-Mar	160	3	125	128	135	129	5	1.3E+05	160	3	94	110	73	92.3	19	9.2E+04	26.7
										180	3	87	86	93	88.7	4	8.9E+04	31.4

Average*** 28.0 (0.14 logs)

Standard Deviation 5.2

Number of Data Points 6

* Standard deviation (SD) in logs for triplicate plate counts

** Each sample plated in triplicate

*** Arithmetic mean

+ 3 indicates a 10^3 dilution; 0 indicates no dilution

++ Sample MS2 concentration in plaque forming units (PFU) per mL

+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using a 20 minute detention time

Note: Test 3 conducted on the RF of pilot 1 train 1 at 1.5 m/h in cold water

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Table G-41: Test 4 plaque counts and MS2 removals (SSF Pilot 1 Train 1, 0.1 m/h, Cold water)

Sample Date (2006)	Plating Date (2006)	SSF Influent (Sample Port 2*) Sample Time (h)	Dilution+	Plaque Count** 1	2	3	Avg	SD*	PFU/mL++	SSF Effluent (Sample Port 3) Sample Time (h)	Dilution+	Plaque Count** 1	2	3	Avg	SD*	PFU/mL++	MS2 % Removal+++
09-Mar	09-Mar[1]									0	0	53	63	52	56	6	56	-
		11	4	67	63	68	66	3	6.6E+05	12	2	43	45	50	46	4	4.6E+03	-
		11.5	4	65	67	59	64	4	6.4E+05	12.5	2	50	57	56	54	4	5.4E+03	-
		12	4	51	64	68	61	9	6.1E+05	13	2	50	48	45	48	3	4.8E+03	-
		12.5	4	67	72	68	69	3	6.9E+05	13.5	2	59	41	52	51	9	5.1E+03	-
		13	4	55	40	62	52	11	5.2E+05	14	2	39	47	43	43	4	4.3E+03	-
		13.5	4	63	59	96	73	20	7.3E+05	14.5	2	53	34	52	46	11	4.6E+03	-
		14	4	85	81	67	78	9	7.8E+05	15	2	48	36	48	44	7	4.4E+03	99.3
13-Mar	15-Mar	108	0	24	12	22	19	6	19	108	0	25	21	13	20	6	20	-
16-Mar	20-Mar	180	0	0	2	2	1	1	1	180	0	19	13	11	14	4	14	-
19-Mar	20-Mar	264	0	0	1	2	1	1	1	264	0	5	5	8	6	2	6	-

* Standard deviation (SD) in logs for triplicate plate counts

** Each sample plated in triplicate

+ 2 indicates a 10^2 dilution; 4 indicates a 10^4 dilution; 0 indicates no dilution

++ Sample MS2 concentration in plaque forming units (PFU) per mL

+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using 4 hour detention time; log removal = 2.18 logs

[1] Influent samples plated 09-Mar-06, effluent samples plated 10-Mar-06

Note: Test 4 conducted on the SSF of pilot 1 train 1 at 0.1 m/h in cold water

Table G-42: Test 5 plaque counts and MS2 removals (SSF Pilot 1 Train 1, 0.1 m/h, Cold water)

Sample Date (2006)	Plating Date (2006)	SSF Influent (Sample Port 2*)								SSF Effluent (Sample Port 3)								MS2 % Removal+++
		Sample Time (h)	Dilution+	Plaque Count** 1	2	3	Avg	SD*	PFU/mL++	Sample Time (h)	Dilution+	Plaque Count** 1	2	3	Avg	SD*	PFU/mL++	
19-Mar	20-Mar	0	0	0	0	2	1	1	1	0	0	5	5	8	6	2	6	-
20-Mar	22-Mar									11	3	23	23	21	22	1	2.2E+04	-
										12.5	3	21	12	18	17	5	1.7E+04	-
20-Mar	20-Mar	10.5	4	49	44	44	46	3	4.6E+05	14.5	2	207	196	182	195	13	2.0E+04	95.7
		11	4	57	59	61	59	2	5.9E+05	15	2	180	220	247	216	34	2.2E+04	96.3
		11.25	4	64	50	68	61	9	6.1E+05	15.25	2	211	190	175	192	18	1.9E+04	96.8
		11.5	4	50	49	71	57	12	5.7E+05	15.5	2	198	228	245	224	24	2.2E+04	96.1
		13.5	4	58	54	66	59	6	5.9E+05									
		15.5	4	70	62	44	59	13	5.9E+05									
21-Mar	22-Mar									38.5	3	29	17	32	26	8	2.6E+04	-
										41	3	30	18	26	25	6	2.5E+04	-
		38	4	109	122	111	114	7	1.1E+06	42	3	20	25	29	25	5	2.5E+04	97.8
		38.25	4	114	92	98	101	11	1.0E+06	42.25	3	32	37	31	33	3	3.3E+04	96.7
		38.5	4	120	117	139	125	12	1.3E+06	42.5	3	32	28	30	30	2	3.0E+04	97.6
		38.75	4	117	94	128	113	17	1.1E+06	42.75	3	30	30	35	32	3	3.2E+04	97.2
		39	4	122	138	117	126	11	1.3E+06	43	3	22	37	31	30	8	3.0E+04	97.6
		41	4	109	112	117	113	4	1.1E+06									
		43	4	114	119	100	111	10	1.1E+06									
27-Mar	28-Mar	191.5	0	41	30	27	33	7	33	191.5	0	64	64	44	57	12	57	-

Average*** 96.9 (1.51 logs)

Standard Deviation 0.7

Number of Data Points 9

* Standard deviation (SD) in logs for triplicate plate counts

** Each sample plated in triplicate

*** Arithmetic mean

+ 2 indicates a 10^2 dilution; 3 indicates a 10^3 dilution; 4 indicates a 10^4 dilution; 0 indicates no dilution

++ Sample MS2 concentration in plaque forming units (PFU) per mL

+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using 4 hour detention time

Note: Test 5 conducted on the SSF of pilot 1 train 1 at 0.1 m/h in cold water

Table G-43: Test 6 plaque counts and MS2 removals (RFA Pilot 2, 0.95 m/h, Cold water)

Sample Date (2006)	Plating Date (2006)	RFA Influent (Sample Port 1)								RFA Effluent (Sample Port 2A)								MS2 % Removal+++
		Sample Time (h)	Dilution+	Plaque Count**			Avg	SD*	PFU/mL++	Sample Time (h)	Dilution+	Plaque Count**			Avg	SD*	PFU/mL++	
				1	2	3						1	2	3				
21-Mar	22-Mar	2	3	91	100	97	96	5	9.6E+04	2.5	3	90	75	71	78.7	10	7.9E+04	18.1
		2.25	3	123	111	136	123	13	1.2E+05	2.75	3	86	47	68	67	20	6.7E+04	45.7
		2.5	3	121	127	145	131	12	1.3E+05	3	3	71	66	76	71	5	7.1E+04	45.8
		2.75	3	114	121	115	117	4	1.2E+05	3.25	3	56	85	91	77.3	19	7.7E+04	33.7
		3	3	132	115	102	116	15	1.2E+05	3.5	3	93	104	92	96.3	7	9.6E+04	17.2
29-Mar	07-Apr									192	1	40	56	-	48	11	480	-
15-May	16-May									1315	0	0	0	0	0	0	0	-

Average***   32.1   (0.17 logs)

Standard Deviation   14.1

Number of Data Points   5

* Standard deviation (SD) in logs for triplicate plate counts

** Each sample plated in triplicate

*** Arithmetic mean

+ 1 indicates a 10^1 dilution; 3 indicates a 10^3 dilution

++ Sample MS2 concentration in plaque forming units (PFU) per mL

+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using 30 minute detention time

Note: Test 6 conducted on RFA of pilot 2 at 0.95 m/h in cold water

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Table G-44: Test 7a plaque counts and MS2 removals (SSF Pilot 1 Train 1, 0.1 m/h, Cold water)

Sample Date (2006)	Plating Date (2006)	SSF Influent (Sample Port 2*)								SSF Effluent (Sample Port 3)								MS2 % Removal+++
		Sample Time (h)	Dilution+	Plaque Count***			Avg	SD*	PFU/mL++	Sample Time (h)	Dilution+	Plaque Count***			Avg	SD*	PFU/mL++	
				1	2	3						1	2	3				
27-Mar	28-Mar	0	0	41	30	27	33	7	33	0	0	64	64	44	57	12	57	-
28-Mar	28-Mar	11.5	4	179	172	164	172	8	1.7E+06	11.5	2	75	72	74	74	2	7.4E+03	-
		11.75	4	189	178	213	193	18	1.9E+06	14	2	121	105	94	107	14	1.1E+04	-
		12	4	155	185	169	170	15	1.7E+06	15.5	2	114	111	120	115	5	1.2E+04	99.3
		12.25	4	164	149	137	150	14	1.5E+06	15.75	2	102	109	124	112	11	1.1E+04	99.4
		14	4	147	135	154	145	10	1.5E+06	16	2	111	128	110	116	10	1.2E+04	99.3
		16.25	4	144	119	129	131	13	1.3E+06	16.25	2	132	129	114	125	10	1.3E+04	99.2
29-Mar	29-Mar	24	4	70	72	63	68	5	6.8E+05	24	2	171	135	156	154	18	1.5E+04	-
29-Mar	29-Mar	35.5	4	123	160	143	142	19	1.4E+06	35.5	2	65	66	53	61	7	6.1E+03	-
		35.75	4	144	103	117	121	21	1.2E+06	38	2	88	73	72	78	9	7.8E+03	-
		36	4	112	121	135	123	12	1.2E+06	39.5	2	75	82	91	83	8	8.3E+03	99.4
		36.25	4	104	90	90	95	8	9.5E+05	39.75	2	84	81	70	78	7	7.8E+03	99.4
		38	4	107	101	122	110	11	1.1E+06	40	2	68	68	77	71	5	7.1E+03	99.4
		40.25	4	79	89	99	89	10	8.9E+05	40.25	2	60	72	66	66	6	6.6E+03	99.3

Average*** 99.3 (2.18 logs)

Standard Deviation 0.1

Number of Data Points 8

* Standard deviation (SD) in logs for triplicate plate counts
** Each sample plated in triplicate
*** Arithmetic mean
+ 2 indicates a 10^2 dilution; 4 indicates a 10^4 dilution; 0 indicates no dilution
++ Sample MS2 concentration in plaque forming units (PFU) per mL
+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using 4 hour detention time

Note: Test 7 conducted on the SSF of pilot 1 train 1 at 0.1 m/h in cold water

211

Table G-45: Test 7b plaque counts and MS2 removals (SSF Pilot 1 Train 1, 0.1 m/h, Cold water)

Sample Date (2006)	Plating Date (2006)	SSF Influent (Sample Port 2*) Sample Time (h)	Dilution+	Plaque Count*** 1	2	3	Avg	SD*	PFU/mL++	SSF Effluent (Sample Port 3) Sample Time (h)	Dilution+	Plaque Count*** 1	2	3	Avg	SD*	PFU/mL++	MS2 % Removal+++
30-Mar	30-Mar	59.75	4	31	54	46	44	12	4.4E+05	59.75	2	83	101	119	101	18	1.0E+04	-
		60	4	45	55	67	56	11	5.6E+05	62	2	119	114	97	110	12	1.1E+04	-
		60.25	4	54	48	49	50	3	5.0E+05	63.75	2	163	141	118	141	23	1.4E+04	96.8
		60.5	4	78	68	54	67	12	6.7E+05	64	2	107	118	117	114	6	1.1E+04	98.0
		62	4	67	60	48	58	10	5.8E+05	64.25	2	124	99	88	104	18	1.0E+04	97.9
		64.5	4	71	80		76	6	7.6E+05	64.5	2	125	100	123	116	14	1.2E+04	98.3
31-Mar	03-Apr	82.5	4	78	102	99	93	13	9.3E+05	82.5	2	100	107	108	105	4	1.1E+04	-
		82.75	4	87	93	97	92	5	9.2E+05	86.5	2	123	112	85	107	20	1.1E+04	98.9
		83	4	88	79	93	87	7	8.7E+05	86.75	2	91	83	98	91	8	9.1E+03	99.0
		83.25	4	102	75	86	88	14	8.8E+05	87	2	115	121	113	116	4	1.2E+04	98.7
		87.25	4	34	38	34	35	2	3.5E+05	87.25	2	117	140	120	126	13	1.3E+04	98.6
14-May	15-May	1152	0	0	0	0	0	0	0	1152	0	0	0	0	0	0	0	-

Average*** 98.3 (1.76 logs)

Standard Deviation 0.7

Number of Data Points 8

* Standard deviation (SD) in logs for triplicate plate counts
** Each sample plated in triplicate
*** Arithmetic mean
+ 2 indicates a 10^2 dilution; 4 indicates a 10^4 dilution; 0 indicates no dilution
++ Sample MS2 concentration in plaque forming units (PFU) per mL
+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using 4 hour detention time
Note: Test 7 conducted on the SSF of pilot 1 train 1 at 0.1 m/h in cold water

212

Table G-46: Test 8 plaque counts and MS2 removals (SSF Pilot 1 Train 2, 0.4 m/h, Cold water)

Sample Date (2006)	Plating Date (2006)	SSF Influent (Sample Port 2*)								SSF Effluent (Sample Port 3)								MS2 % Removal+++
		Sample Time (h)	Dilution+	Plaque Count**			Avg	SD*	PFU/mL++	Sample Time (h)	Dilution+	Plaque Count**			Avg	SD*	PFU/mL++	
				1	2	3						1	2	3				
28-Mar	30-Mar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
		11.5	3	108	115	84	102	16	1.0E+05	12.5	3	41	33	36	37	4	3.7E+04	64.2
		12	3	109	110	129	116	11	1.2E+05	13	3	37	48	39	41	6	4.1E+04	64.4
		12.5	3	125	134	123	127	6	1.3E+05	13.5	3	42	41	39	41	2	4.1E+04	68.1
		13	3	137	140	141	139	2	1.4E+05	14	3	42	50	48	47	4	4.7E+04	66.5
		13.5	3	134	136	167	146	19	1.5E+05	14.5	3	48	43	50	47	4	4.7E+04	67.7
		14	3	134	125	129	129	5	1.3E+05	15	3	42	32	37	37	5	3.7E+04	71.4
29-Mar	30-Mar	14.5	3	136	131	127	131	5	1.3E+05	15.5	3	48	38	57	48	10	4.8E+04	63.7
30-Mar	30-Mar	40	0	1	4		3	2	3	40	0	131	108	127	122	12	122	-
31-Mar	31-Mar	62.5	0	0	0	0	0	0	0	62.5	0	51	73	47	57	14	57	-
15-May	15-May	1140.5	0	0	0	0	0	0	0	1140.5	0	0	0	0	0	0	0	-

Average*** 66.6 (0.48 logs)

Standard Deviation 2.8

Number of Data Points 7

* Standard deviation (SD) in logs for triplicate plate counts

** Each sample plated in triplicate

*** Arithmetic mean

+ 3 indicates a 10^3 dilution; 0 indicates no dilution

++ Sample MS2 concentration in plaque forming units (PFU) per mL

+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using 1 hour detention time

Note - Test 8 conducted on the SSF of pilot 1 train 2 at 0.4 m/h in cold water

Table G-47: Test 9 SSF1 plaque counts and MS2 removals (Pilot 2, 0.4 m/h, Cold water)

Sample Date (2006)	Plating Date (2006)	SSF1 Influent (Sample Port 2*)								SSF1 Effluent (Sample Port 3)								SSF1 MS2 % Removal++++
		Sample Time (h)	Dilution+	Plaque Count**					PFU/mL++	Sample Time (h)	Dilution+	Plaque Count**					PFU/mL++	
				1	2	3	Avg	SD*				1	2	3	Avg	SD*		
29-Mar	31-Mar									0	0	0	0	0	0	0	0	-
30-Mar	07-Apr	19.5	3	90	72	96	86	12	8.6E+04	20.5	2	49	48	58	52	6	5.2E+03	-
		20	3	84	73	68	75	8	7.5E+04	21	2	60	46	72	59	13	5.9E+03	-
		20.5	3	90	88	91	90	2	9.0E+04	21.5	2	64	49	63	59	8	5.9E+03	93.2
		21	3	70	69	74	71	3	7.1E+04	22	2	72	65	49	62	12	6.2E+03	91.7
		21.5	3	79	84	61	75	12	7.5E+04	22.5	2	57	67	81	68	12	6.8E+03	92.4
		22	3	80	89	75	81	7	8.1E+04	23	2	69	59	62	63	5	6.3E+03	91.1
		22.5	3	79	107	125	104	23	1.0E+05	23.5	2	72	65	74	70	5	7.0E+03	90.6
		23	3	78	70	71	73	4	7.3E+04	24	2	71	55	66	64	8	6.4E+03	92.1
31-Mar	07-Apr	46.5	1	31	25	43	33	9	330	46.5	0	121	126	131	126	5	126	-

Average*** **91.8** (1.09 logs)

Standard Deviation **0.9**

Number of Data Points **6**

* Standard deviation (SD) in logs for triplicate plate counts
** Each sample plated in triplicate
*** Arithmetic mean
+ 2 indicates a 10^2 dilution; 3 indicates a 10^3 dilution; 0 indicates no dilution
++ Sample MS2 concentration in plaque forming units (PFU) per mL
+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration)
++++ 2 hour detention time in SSF1 and 1 hour detention time in SSF2
+++++ 2 hour detention time in SSF1 and SSF2 at 0.4 m/h in cold water
Note: Test 9 conducted on pilot 2 SSF1 and SSF2 at 0.4 m/h in cold water

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Table G-48: Test 9 SSF2 plaque counts and MS2 removals (Pilot 2, 0.4 m/h, Cold water)

Sample Date[1] (2006)	SSF1 Effluent (Sample Port 3)								SSF2 Effluent (Sample Port 4)								SSF2[2] MS2 % Removal[+++]	Total[3] MS2 % Removal[+++]
	Sample Time (h)	Dilution[+]	Plaque Count** 1	2	3	Avg	SD*	PFU/mL[++]	Sample Time (h)	Dilution[+]	Plaque Count** 1	2	3	Avg	SD*	PFU/mL[++]		
29-Mar	0	0	0	0	0	0	0	0									-	-
30-Mar	20.5	2	49	48	58	52	6	5.2E+03	21	2	28	17	15	20	7	2.0E+03	-	-
	21	2	60	46	72	59	13	5.9E+03	21.5	2	21	13	26	20	7	2.0E+03	61.3	-
	21.5	2	64	49	63	59	8	5.9E+03	22	2	17	15	24	19	5	1.9E+03	68.5	97.7
	22	2	72	65	49	62	12	6.2E+03	22.5	2	24	19	17	20	4	2.0E+03	65.9	96.3
	22.5	2	57	67	81	68	12	6.8E+03	23	2	25	29	30	28	3	2.8E+03	54.8	98.0
	23	2	69	59	62	63	5	6.3E+03	23.5	2	12	22	20	18	5	1.8E+03	73.7	97.0
	23.5	2	72	65	74	70	5	7.0E+03	24	2	16	20	28	21	6	2.1E+03	66.3	-
	24	2	71	55	66	64	8	6.4E+03									-	-
31-Mar	46.5	0	121	126	131	126	5	126	46.5	0	56	39	68	54	15	54	-	-

Average***	65.1	97.2
	(0.46 logs)	(1.56 logs)
Standard Deviation	6.4	0.8
Number of Data Points	6	4

* Standard deviation (SD) in logs for triplicate plate counts
** Each sample plated in triplicate
*** Arithmetic mean
+ 2 indicates a 10^2 dilution; 3 indicates a 10^3 dilution; 0 indicates no dilution
++ Sample MS2 concentration in plaque forming units (PFU) per mL
+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration)
+++ 2 hour detention time in SSF1 and 1 hour detention time in SSF2
[1] Plating dates are 31-Mar for 29-Mar samples and 7-Apr for all other samples
[2] SSF1 effluents used at SSF2 influent concentrations
[3] Total log reductions are combination of SSF1 and SSF2 reductions
Note: Test 9 conducted on pilot 2 SSF1 and SSF2 at 0.4 m/h in cold water

Table G-49: Test 10 plaque counts and MS2 removals (SSF Pilot 1 Train 1, 0.1 m/h, Warm water)

Sample Date (2006)	Plating Date (2006)	SSF Influent (Sample Port 2*) Sample Time (h)	Dilution+	Plaque Count** 1	2	3	Avg	SD*	PFU/mL++	SSF Effluent (Sample Port 3) Sample Time (h)	Dilution+	Plaque Count** 1	2	3	Avg	SD*	PFU/mL++	MS2 % Removal+++
14-May	15-May	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
15-May	16-May	10.25	4	28	45	44	39	10	3.9E+05	10.25	2	43	51	39	44	6	4.4E+03	-
		10.5	4	49	52	57	53	4	5.3E+05	12.5	2	40	43	53	45	7	4.5E+03	-
		10.75	4	51	48	75	58	15	5.8E+05	14.25	2	25	41	44	37	10	3.7E+03	99.1
		11	4	52	53	54	53	1	5.3E+05	14.5	2	31	40	32	34	5	3.4E+03	99.3
		12.5	4	66	52	57	58	7	5.8E+05	14.75	2	33	34	22	30	7	3.0E+03	99.5
		15	4	51	57	50	53	4	5.3E+05	15	2	27	33	50	37	12	3.7E+03	99.3
16-May	17-May	34.5	4	53	43	57	51	7	5.1E+05	34.5	2	43	52	42	46	6	4.6E+03	-
		34.75	4	45	56	43	48	7	4.8E+05	37	2	37	39	32	36	4	3.6E+03	-
		35	4	52	57	33	47	13	4.7E+05	38.5	2	48	56	45	50	6	5.0E+03	99.0
		35.25	4	55	54	53	54	1	5.4E+05	38.75	2	36	45	31	37	7	3.7E+03	99.2
		37	4	45	56	64	55	10	5.5E+05	39	2	44	55	48	49	6	4.9E+03	99.0
		39.25	4	46	44	41	44	3	4.4E+05	39.25	2	44	54	44	47	6	4.7E+03	99.1
17-May	19-May	72	0	6	5	5	5	0.6	5	72	0	53	51	52	52	1	52	-

Average*** 99.2

Standard Deviation 0.2 (2.09 logs)

Number of Data Points 8

* Standard deviation (SD) in logs for triplicate plate counts

** Each sample plated in triplicate

*** Arithmetic mean

+ 2 indicates a 10^2 dilution; 4 indicates a 10^4 dilution; 0 indicates no dilution

++ Sample MS2 concentration in plaque forming units (PFU) per mL

+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using 4 hour detention time

Note: Test 10 conducted on the SSF of pilot 1 train 1 at 0.1 m/h in warm water

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Table G-50: Test 11 plaque counts and MS2 removals (SSF Pilot 1 Train 1, 0.1 m/h, Warm water)

Sample Date (2006)	Plating Date (2006)	SSF Influent (Sample Port 2*) Sample Time (h)	Dilution+	Plaque Count** 1	2	3	Avg	SD*	PFU/mL++	SSF Effluent (Sample Port 3) Sample Time (h)	Dilution+	Plaque Count** 1	2	3	Avg	SD*	PFU/mL++	MS2 % Removal+++
17-May	19-May	0	0	6	5	5	5	0.6	5	0	0	53	51	52	52	1	52	-
18-May	19-May	11.25	4	72	58	59	63	8	6.3E+05	11.25	2	39	32	39	37	4	3.7E+03	-
		11.5	4	51	52	62	55	6	5.5E+05	13.5	2	46	32	41	40	7	4.0E+03	-
		11.75	4	48	65	48	54	10	5.4E+05	15.25	2	36	35	39	37	2	3.7E+03	99.4
		12	4	42	50	49	47	4	4.7E+05	15.5	2	39	34	46	40	6	4.0E+03	99.3
		13.5	4	52	61	54	56	5	5.6E+05	15.75	2	36	42	37	38	3	3.8E+03	99.3
		16	4	56	52	55	54	2	5.4E+05	16	2	33	46	49	43	9	4.3E+03	99.1
19-May	19-May	34.75	4	59	53	71	61	9	6.1E+05	34.75	2	76	59	73	69	9	6.9E+03	-
		35	4	63	50	44	52	10	5.2E+05	38.75	2	57	64	82	68	13	6.8E+03	98.9
		35.25	4	58	58	50	55	5	5.5E+05	39	2	56	62	63	60	4	6.0E+03	98.8
		35.5	4	51	64	71	62	10	6.2E+05	39.25	2	72	58	74	68	9	6.8E+03	98.8
		39.25	4	53	54	53	53	0.6	5.3E+05	39.5	2	72	65	76	71	6	7.1E+03	98.9
01-Jun	02-Jun	358.5	0	0	0	1	0	0.6	0	358.5	0	0	0	0	0	0	0	-

Average*** 99.1

(2.02 logs)

Standard Deviation 0.2

Number of Data Points 8

* Standard deviation (SD) in logs for triplicate plate counts

** Each sample plated in triplicate

*** Arithmetic mean

+ 2 indicates a 10^2 dilution; 4 indicates a 10^4 dilution; 0 indicates no dilution

++ Sample MS2 concentration in plaque forming units (PFU) per mL

+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using 4 hour detention time

Note: Test 11 conducted on the SSF of pilot 1 train 1 at 0.1 m/h in warm water

Table G-51: Test 12 plaque counts and MS2 removals (SSF Pilot 1 Train 1, 0.4 m/h, Warm water)

Sample Date (2006)	Plating Date (2006)	SSF Influent (Sample Port 2*) Sample Time (h)	Dilution+	Plaque Count** 1	2	3	Avg	SD*	PFU/mL++	SSF Effluent (Sample Port 3) Sample Time (h)	Dilution+	Plaque Count** 1	2	3	Avg	SD*	PFU/mL++	MS2 % Removal+++
01-Jun	02-Jun	0	0	0	0	1	0	0.6	0	0	0	0	0	0	0	0	0	-
02-Jun	04-Jun	11.5	3	120	115	108	114	6	1.1E+05	12.5	2	210	183	179	191	17	1.9E+04	83.3
		12	3	123	102	122	116	12	1.2E+05	13	2	166	169	186	174	11	1.7E+04	85.0
		12.5	3	138	125	120	128	9	1.3E+05	13.5	2	148	147	169	155	12	1.5E+04	87.9
		13	3	136	115	117	123	12	1.2E+05	14	2	149	161	173	161	12	1.6E+04	86.9
		13.5	3	136	140	149	142	7	1.4E+05	14.5	2	168	158	161	162	5	1.6E+04	88.5
		14	3	132	107	129	123	14	1.2E+05	15	2	194	189	180	188	7	1.9E+04	84.7
		14.5	3	117	116	119	117	2	1.2E+05	15.5	2	173	176	186	178	7	1.8E+04	84.8
04-Jun	05-Jun	72	0	9	9	9	9	0	9	72	0	4	5	6	5	1	5	-

Average*** 85.9 (0.85 logs)

Standard Deviation 1.9

Number of Data Points 7

* Standard deviation (SD) in logs for triplicate plate counts

** Each sample plated in triplicate

*** Arithmetic mean

+ 2 indicates a 10^2 dilution; 3 indicates a 10^3 dilution; 0 indicates no dilution

++ Sample MS2 concentration in plaque forming units (PFU) per mL

+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using 1 hour detention time

Note: Test 12 conducted on SSF of pilot 1 train 1 at 0.4 m/h in warm water

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Table G-52: Test 13 plaque counts and MS2 removals (SSF Pilot 1 Train 1, 0.4 m/h, Warm water)

Sample Date (2006)	Plating Date (2006)	SSF Influent (Sample Port 2*)								SSF Effluent (Sample Port 3)								MS2 % Removal+++
		Sample Time (h)	Dilution+	Plaque Count**					PFU/mL++	Sample Time (h)	Dilution+	Plaque Count**					PFU/mL++	
				1	2	3	Avg	SD*				1	2	3	Avg	SD*		
04-Jun	05-Jun	0	0	9	9	9	9	0	9	0	0	4	5	6	5	1	5	-
05-Jun	05-Jun	12	3	127	94	88	103	21	1.0E+05	13	2	104	88	102	98	9	9.8E+03	90.5
		12.5	3	103	99	116	106	9	1.1E+05	13.5	2	97	101	87	95	7	9.5E+03	91.0
		13	3	105	81	101	96	13	9.6E+04	14	2	89	92	95	92	3	9.2E+03	90.4
		13.5	3	97	111	98	102	8	1.0E+05	14.5	2	96	86	109	97	12	9.7E+03	90.5
		14	3	108	114	93	105	11	1.1E+05	15	2	91	86	91	89	3	8.9E+03	91.5
		14.5	3	78	105	120	101	21	1.0E+05	15.5	2	98	107	129	111	16	1.1E+04	89.0
		15	3	110	100	115	108	8	1.1E+05	16	2	79	113	105	99	18	9.9E+03	90.9
08-Jun	08-Jun	67.25	0	0	-	-	0	-	0	67.25	0	1	14	1	5	8	5	-

Average*** 90.5 (1.02 logs)

Standard Deviation 0.8

Number of Data Points 7

* Standard deviation (SD) in logs for triplicate plate counts

** Each sample plated in triplicate

*** Arithmetic mean

+ 2 indicates a 10^2 dilution; 3 indicates a 10^3 dilution; 0 indicates no dilution

++ Sample MS2 concentration in plaque forming units (PFU) per mL

+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using 1 hour detention time

Note: Test 13 conducted on SSF of pilot 1 train 1 at 0.4 m/h in warm water

Table G-53: Test 14 SSF1 plaque counts and MS2 removals (Pilot 2, 0.4 m/h, Warm water)

Sample Date (2006)	Plating Date (2006)	SSF1 Influent (Sample Port 2*) Sample Time (h)	Dilution+	Plaque Count** 1	2	3	Avg	SD*	PFU/mL++	SSF1 Effluent (Sample Port 3) Sample Time (h)	Dilution+	Plaque Count** 1	2	3	Avg	SD*	PFU/mL++	SSF1 MS2 % Removal+++
04-Jun	06-Jun	0	0	0	0	-	0	0	0	0	0	0	0	-	0	0	0	-
05-Jun	06-Jun	11.5	3	77	65	45	62	16	6.2E+04	13.5	1	69	60	71	67	6	6.7E+02	98.9
		12	3	67	67	77	70	6	7.0E+04	14	1	77	69	90	79	11	7.9E+02	98.9
		12.25	3	75	66	76	72	6	7.2E+04	14.25	1	77	69	62	69	8	6.9E+02	99.0
		12.5	3	65	70	60	65	5	6.5E+04	14.5	1	67	73	57	66	8	6.6E+02	99.0
		12.75	3	80	60	61	67	11	6.7E+04	14.75	1	58	62	57	59	3	5.9E+02	99.1
		13	3	91	64	88	81	15	8.1E+04	15	1	66	69	66	67	2	6.7E+02	99.2
08-Jun	08-Jun	85	0	0	-	-	0	-	0	85	0	0	0	0	0	0	0	-

Average***	99.0
	(2.01 logs)
Standard Deviation	0.1
Number of Data Points	6

* Standard deviation (SD) in logs for triplicate plate counts
** Each sample plated in triplicate
*** Arithmetic mean
+ 3 indicates a 10^3 dilution; 1 indicates a 10^1 dilution; 0 indicates no dilution
++ Sample MS2 concentration in plaque forming units (PFU) per mL
+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration)
+++ 2 hour detention time in SSF1 and 1 hour detention time in SSF2
Note: Test 14 conducted on pilot 2 SSF1 and SSF2 at 0.4 m/h in warm water

Table G-54: Test 14 SSF2 plaque counts and MS2 removals (Pilot 2, 0.4 m/h, Warm water)

Sample Date[1] (2006)	SSF1 Effluent (Sample Port 3)								SSF2 Effluent (Sample Port 4)								SSF2[2] MS2 % Removal[+++]	Total[3] MS2 % Removal[+++]
	Sample Time (h)	Dilution[+]	Plaque Count**					PFU/mL[++]	Sample Time (h)	Dilution[+]	Plaque Count**					PFU/mL[++]		
			1	2	3	Avg	SD*				1	2	3	Avg	SD*			
04-Jun	0	0	0	0	-	0	0	0	0	0	0	0	-	0	0	0	-	-
05-Jun	13.5	1	69	60	71	67	6	6.7E+02	14.5	1	30	17	25	24	7	2.4E+02	64.0	99.6
	14	1	77	69	90	79	11	7.9E+02	15	1	14	17	29	20	8	2.0E+02	74.6	99.7
	14.25	1	77	69	62	69	8	6.9E+02	15.25	1	22	33	11	22	11	2.2E+02	68.3	99.7
	14.5	1	67	73	57	66	8	6.6E+02	15.5	1	16	19	12	16	4	1.6E+02	76.1	99.8
	14.75	1	58	62	57	59	3	5.9E+02	15.75	1	16	16	11	14	3	1.4E+02	75.7	99.8
	15	1	66	69	66	67	2	6.7E+02	16	1	11	23	17	17	6	1.7E+02	74.6	99.8
08-Jun	85	0	0	0	0	0	0	0	85	0	0	0	0	0	0	0	-	-
													Average***				72.2	99.7
																	(0.56 logs)	(2.56 logs)
													Standard Deviation				4.9	0.1
													Number of Data Points				6	6

* Standard deviation (SD) in logs for triplicate plate counts
** Each sample plated in triplicate
*** Arithmetic mean
+ 3 indicates a 10[3] dilution; 1 indicates a 10[1] dilution; 0 indicates no dilution
++ Sample MS2 concentration in plaque forming units (PFU) per mL
+++ Calculated as -log$_{10}$(Effluent concentration/Influent concentration)
+++ 2 hour detention time in SSF1 and 1 hour detention time in SSF2
[1] 04-Jun and 05-Jun samples plated on 6-Jun; 08-Jun samples plated on 08-Jun
[2] SSF1 effluents used at SSF2 influent concentrations
[3] Total log reductions are combination of SSF1 and SSF2 reductions
Note: Test 14 conducted on pilot 2 SSF1 and SSF2 at 0.4 m/h in warm water

Table G-55: Test 15 plaque counts and MS2 removals (SSF Pilot 1 Train 1, 0.4 m/h, Warm water)

Sample Date (2006)	Plating Date (2006)	SSF Influent (Sample Port 2*)								SSF Effluent (Sample Port 3)								MS2 % Removal[+++]
		Sample Time (h)	Dilution[+]	Plaque Count**					PFU/mL[++]	Sample Time (h)	Dilution[+]	Plaque Count**					PFU/mL[++]	
				1	2	3	Avg	SD*				1	2	3	Avg	SD*		
07-Jun	08-Jun	0	0	0	-	-	0	-	0	0	0	1	14	1	5	8	5	-
08-Jun	08-Jun	16.75	3	45	49	43	46	3	4.6E+04	17.75	2	44	33	30	36	7	3.6E+03	92.2
		17	3	67	59	68	65	5	6.5E+04	18	2	21	32	27	27	6	2.7E+03	95.9
		17.25	3	83	59	67	70	12	7.0E+04	18.25	2	37	35	37	36	1	3.6E+03	94.8
		17.5	3	73	56	71	67	9	6.7E+04	18.5	2	29	18	20	22	6	2.2E+03	96.7
		17.75	3	50	51	51	51	0.6	5.1E+04	18.75	2	41	25	38	35	9	3.5E+03	93.2

Average*** 94.5 (1.26 logs)

Standard Deviation 1.9

Number of Data Points 5

* Standard deviation (SD) in logs for triplicate plate counts

** Each sample plated in triplicate

*** Arithmetic mean

+ 2 indicates a 10^2 dilution; 3 indicates a 10^3 dilution; 0 indicates no dilution

++ Sample MS2 concentration in plaque forming units (PFU) per mL

+++ Calculated as $-\log_{10}$(Effluent concentration/Influent concentration) using 1 hour detention time

Note: Test 15 conducted on SSF of pilot 1 train 1 at 0.4 m/h in warm water

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Appendix H

MS2 Challenge Tests – Description and Results of Individual Experiments

Pilot 1 Slow Sand Filter MS2 Challenge Test Results

Most MS2 challenge tests were conducted on the SSFs of pilot 1. For the entire duration of MS2 testing, there was no pre-ozonation. In this section, the discussion of MS2 challenge test results has been organized according to temperature and HLR conditions. Challenge tests were conducted on the SSF of pilot 1 in cold water at a HLR of 0.4 m/h (tests 1 and 2 on train 1 and test 8 on train 2). Tests were also carried out on the train 1 SSF at a HLR of 0.1 m/h in cold water (tests 4, 5 and 7a/b) and in warm water (tests 10 and 11). Tests were then conducted on the train 1 SSF in warm water at a HLR of 0.4 m/h (tests 12, 13 and 15). Each challenge test is described in the subsections below.

Plaque count results and water quality data for all MS2 challenge tests are presented in Appendix G and Appendix F, respectively. Quality control procedures were followed each day samples were plated (Appendix I). Plaque counts for samples used to calculate removals were always between 20 and 200. Negative controls were plated before, during, and after the samples on each day and showed good *E. coli* lawns and no contamination by MS2 plaques. Positive controls using the QC stock yielded plaque counts in the expected range, even when plated along with 1 mL of raw water.

Cold Water Tests at 0.4 m/h

Influent water temperatures for the cold water tests ranged from 2 to 5°C for tests 1 and 2 and from 6 to 10°C for test 8 (Appendix F).

Test 1 – Cold Water, 0.4 m/h

MS2 challenge test 1 was conducted on the SSF of pilot 1 train 1 at 0.4 m/h in cold water on February 14 and 15, 2006 (Table 6-3). The average MS2 removal for test 1 was 0.4 logs based on 15 calculated removals (Table G-38 in Appendix G).

As noted in Section 3.1.5 of this thesis, a hydraulic detention time (HDT) of 60 minutes was used as the lag time between the collection of corresponding influent and effluent samples, even

though one chloride tracer test indicated the HDT was 82 minutes for the SSF of pilot 1 train 1. It is important to highlight that if a HDT of 90 minutes had been used instead, the average MS2 removal calculated from the data in Table G-38 would have still been approximately 0.4 logs. Therefore, the one hour HDT used for the pilot 1 train 1 SSF at 0.4 m/h was a reasonable estimate with no major impact on calculated removals.

Table G-38 shows the dates that samples were collected and plated. The target was to plate samples within 48 hours of collection. Test 1 samples were plated within 48 hours, except for the influent and effluent samples corresponding to influent sampling times of 1h, 3h, 4h, and 4.5h. These influent samples were initially plated within 48 hours, but a problem with the dilution series yielded zero plaques. Quality control results, including negative and positive controls, are included in Appendix I.

On the second day of test 1 (15-Feb) after more than 20 hours of seeding, influent concentrations were assumed to have reached a steady state of approximately 1×10^5 PFU/mL (Table G-38). On the first day (14-Feb), influent concentrations took 5 to 6 hours to reach approximately 1×10^5 PFU/mL, indicating that steady state was reached within the first 6 hours of seeding at a hydraulic loading rate of 0.4 m/h. While MS2 seeding was stopped after 27 hours, sampling continued for up to 311 hours. Long-term detachment of MS2 bacteriophage from the SSF was observed, although MS2 concentrations did decrease over time (Table G-38).

As shown in Table F-28 (Appendix F), water quality was similar for tests 1 and 2. Although raw water turbidity on February 28 was lower than those on the other test dates, influent and effluent turbidities were similar. The SSF of pilot 1 train 1 was scraped between test 1 and 2, as indicated by the sand depths and schmutzdecke ages in Table F-28.

Test 2 – Cold Water, 0.4 m/h

Challenge test 2, a replicate of test 1, was conducted on the SSF of pilot 1 train 1 at 0.4 m/h in cold water on February 27 and 28, 2006 (Table 6-3). The average MS2 removal in test 2 (0.2

logs) was lower than the test 1 average (0.4 logs) (Table G-39 in Appendix G). As in test 1, long-term detachment and release of MS2 from the slow sand filter was observed.

The average log removal calculated for test 2 was low due to the results from samples collected on Day 2, February 28, 2006 (Table G-39). The cause of this discrepancy was likely related to the fact that the February 28 samples were plated one week after collection due to problems with host *E. coli*, while the February 27 samples were plated within 48 hours of collection. The February 28 influent and effluent samples were originally plated on March 1, but all the plates that contained sample water or QC stock showed no plaques. On March 2, new host *E. coli* HFr was taken from the freezer stock and the February 28 samples were replated on March 7. Quality control results are presented in Appendix I.

On the first day of test 2 seeding, influent concentrations reached a steady state MS2 concentration of approximately 1.4×10^5 to 1.6×10^5 PFU/mL after 3.5 to 6 hours of seeding (Table G-39). However, as previously mentioned, influent concentrations were lower on the second day of the test.

Test 8 – Cold Water, 0.4 m/h

Test 8 was the first and only MS2 challenge test conducted on the SSF of pilot 1 train 2. The HLR was 0.4 m/h and a HDT of one hour was used as the lag time between corresponding influent and effluent samples. The average MS2 removal was 0.5 logs (Table G-46 in Appendix G). This was similar to the average MS2 removals for the 0.4 m/h tests on the SSF of pilot 1 train 1 (0.4 logs for test 1 and 0.2 logs for test 2). Long-term detachment of MS2 from the SSF was observed long after seeding had stopped (Table G-46).

Test 8 was considered to be a cold water experiment because it had taken less than a week for the raw water to warm up from less than 5°C to between 6 and 10°C (Table F-33, Appendix F). The sand depth was 35.5 cm, which was approximately the same as the sand depth of the SSF of pilot 1 train 1 in tests 1, 2, 4, 5, 7a, and 7b (Table 6-2). Quality control data are included in Appendix I.

Cold Water Tests at 0.1 m/h

MS2 challenge tests 4, 5, 7a, and 7b were carried out on the SSF of pilot 1 train 1 in cold water at a HLR of 0.1 m/h. Influent water temperatures ranged from 6 to 10°C for these tests (Appendix F).

Test 4 – Cold Water, 0.1 m/h

MS2 challenge test 4 was conducted in cold water on the SSF of pilot 1 train 1 at an HLR of 0.1 m/h. Since the HLR was decreased four-fold, it was reasonable to assume that the HDT would increase four-fold. Therefore, because a one hour HDT was used for an HLR of 0.4 m/h, a four hour HDT was used for an HLR of 0.1 m/h.

Although multiple samples were analyzed, an error in sampling protocol allowed for the calculation of only one log removal (2.2 logs) by comparing the 11-hour influent and 15-hour effluent sample (Table G-41, Appendix G).

In test 4, all influent and effluent samples collected on March 9 were plated on the same day (Table G-41). However, plaque counts for the effluent samples were below 20 when a 10^3 dilution was used. As such, effluent samples were replated on March 10 using a 10^2 dilution to achieve plaque counts in the range of 20 to 200. Similar to all previous SSF tests, long-term detachment of MS2 was observed in test 4 (Table G-41).

For the majority of 2004, 2005, and 2006, the SSF of pilot 1 train 1 was operated at 0.4 m/h. The HLR was decreased from 0.4 to 0.1 m/h on March 6 immediately following MS2 challenge test 3 (Table 6-3). Therefore, there was approximately 48 hours between the time when HLR was decreased to 0.1 m/h and when test 4 was started. It was assumed that the drop in nutrient loading associated with the drop in HLR would not impair the biological community in the SSF because it was assumed that the activity of the filter's biomass was limited to a greater extent by the cold water temperatures rather than nutrient loading.

Test 5 – Cold Water, 0.1 m/h

MS2 challenge test 5, a replicate of test 4, was conducted in cold water on the SSF of pilot 1 train 1 at 0.1 m/h. After the HLR was decreased from 0.4 to 0.1 m/h, 2 days of acclimation were given before starting test 4 and 13 days of acclimation were given before starting test 5. Despite the difference in acclimation times between test 4 and 5, the SSF effluent DO concentrations were similar (Table F-30, Appendix F). Since raw and SSF influent DO concentrations were also similar, this indicated that DO consumption and microbial activity in the SSF was similar in both tests.

The average test 5 MS2 removal was 1.5 logs (Table G-42 in Appendix G). For unknown reasons, this average removal was lower than the single removal of 2.18 logs calculated from test 4. As in test 4, an HDT of four hours was used. In test 5, other samples were analyzed but not used for log removal calculations (Table G-42). These samples were used for quality control to verify MS2 concentrations over the full sampling period. Long-term detachment of MS2 from the SSF was observed (Table G-42).

Tests 7a and 7b – Cold Water, 0.1 m/h

Test 7a and 7b were conducted on the SSF of pilot 1 train 1, operated at 0.1 m/h in cold water (Table 6-3). A four hour detention time was used as the lag time between corresponding influent and effluent samples. One objective of these tests was to determine the effect of schmutzdecke scraping on MS2 removals. Filter seeding during tests 7a and 7b lasted nearly four days (87 hours). In test 7a, the SSF was seeded for 40 hours before it was stopped for 30 minutes on March 29 while the schmutzdecke was scraped (Table 6-3). Seeding restarted following schmutzdecke scraping and continued for an additional 27 hours (test 7b).

The average MS2 removal was 2.2 in test 7a, which compared well with the average removals in test 4 (2.2 logs) and test 5 (1.5 logs). These tests were all conducted at an HLR of 0.1 m/h in cold water.

The scraping of the schmutzdecke layer between tests 7a and 7b resulted in a 0.3 to 0.5 log decrease in average MS2 removals. The average MS2 removal was measurably lower in test 7b (1.8 logs) compared to test 7a (2.2 logs) (Table G-44 and Table G-45 in Appendix G). The removal of the schmutzdecke layer likely caused the lower MS2 removals in test 7b due to the loss of biofilm, organic matter, and predators in that layer.

When the results from each day of test 7b were analyzed separately, it was apparent that the SSF quickly recovered its capacity to remove MS2 phage. On the first day of test 7b, the average MS2 removal (1.7 logs) was 0.5 logs lower than the test 7a average (2.2 logs). The average removal had already increased to 1.9 logs on the second day of test 7b. Therefore, it can be concluded that the removal of the schmutzdecke layer had no major long-term negative impact on MS2 removals. In practice, SSF effluent is typically diverted to waste following full-scale filter scraping until water quality parameters (such as turbidity and bacteria concentrations) return to acceptable ranges.

Tests 7a and 7b were considered to be cold water experiments because it had taken less than a week for the raw water to warm up from less than 5°C to between 6 and 10°C. Water quality data are listed in Table F-32 (Appendix F). Also of note is the fact that the schmutzdecke removal before test 7b reduced head loss to the point that water levels were below the influent sampling port and influent samples needed to be scooped out of the water column (Table F-32). Quality control data are included in Appendix I.

Warm Water Tests at 0.1 m/h

In May 2006, MS2 challenge tests were performed on the SSF of pilot 1 train 1 in warm water conditions at HLRs of 0.1 m/h (tests 10 and 11).

Test 10 – Warm Water, 0.1 m/h

On May 11, 2006, the air temperature inside the building that housed the pilot systems was increased to 35°C to raise the temperature of the water inside the filters. The objective was to

simulate summer conditions and promote biological growth in the filters. During test 10, raw water temperatures were between 16 and 17°C and SSF influent and effluent temperatures ranged from 20.4 to 23.7°C (Table F-35, Appendix F). Test 10 started on May 14, only three days after the air temperature in the building was raised.

Warm water test 10 was conducted on the SSF of pilot 1 train 1 using a four hour HDT (Table 6-3). By the time of test 10, the SSF of pilot 1 train 1 had been operated continuously at an HLR of 0.1 m/h for over two months. The average MS2 removal for test 10 was 2.1 logs (Table G-49 in Appendix G). This was very close to the average removals calculated for the cold water 0.1 m/h tests, including test 4 (2.2 logs), test 5 (1.5 logs), and test 7a (2.2 logs). This was unexpected and is attributed to a relatively short exposure to warmer water (~16°C on May 15, 2006) that appears not to have permitted biological activity in the SSF to increase sufficiently to enhance MS2 removal in the relatively short period of warm water conditions that preceded test 10.

As indicated by the schmutzdecke ages in Table F-35 (Appendix F), the SSF had not been scraped since March 29 (test 7), 47 days previously, because the low HLR provided minimal solids loading and hence minimal head loss. As was the case in test 7b, the head loss in the SSF was so low that the water level above the sand surface was below the SSF influent sampling port and influent samples were collected by scooping water from the water column above the SSF bed.

As shown in Table G-49 (Appendix G), some influent and effluent samples were not used to calculate log removals, but were instead used for quality control to verify concentrations over the full sampling period. MS2 was detected in samples collected after seeding had stopped, which indicated that MS2 were detaching from the SSF. Quality control data are shown in Appendix I.

Test 11 – Warm Water, 0.1 m/h

MS2 challenge test 11, a replicate of test 10, was conducted in warm water on the SSF of pilot 1 train 1 at 0.1 m/h. Seeding began on May 17, 2006, one day after test 10 seeding had stopped (Table 6-3). The average MS2 removal for test 11 was 2.0 logs (Table G-50 in Appendix G).

This average removal was similar to the average removals calculated for the other cold and warm water 0.1 m/h tests 4, 5, 7a, 7b, and 10. Water temperatures were similar to test 10 (Table F-35, Appendix F). The results of test 11 implied that the biological activity in the SSF had not, as previously discussed, increased noticeably in the warm water conditions (>20°C) compared to the cold water conditions (<10°C).

Warm Water Tests at 0.4 m/h

Warm water MS2 challenge tests were carried out on the SSF of pilot 1 train 1 at 0.4 m/h (tests 12, 13, and 15).

Test 12 – Warm Water, 0.4 m/h

MS2 challenge tests 12, 13, and 15 were replicates (Table 6-3). These tests were conducted on the SSF of pilot 1 train 1 operated at 0.4 m/h in warm water. Using a one hour HDT, the average MS2 removal for test 12 was 0.9 logs (Table G-51 in Appendix G). The average removal in test 12 was much greater than that calculated for cold water tests run at 0.4 m/h (0.4 logs for test 1 and 0.2 logs for test 2). This suggests that the warmer temperature and correspondingly higher biological activity in test 12 led to improved MS2 removals.

It is important to note that the raw water was warmer in tests 12, 13, and 15 (20 to 22°C) compared to tests 10 and 11 (16 to 17°C) (Appendix F), even though the SSF influent and effluent water temperatures were approximately the same (22 to 24°C) due to the warm indoor building temperature. Tests 12, 13, and 15 were conducted two to three weeks after tests 10 and 11, which allowed more warm water acclimation time for biological activity in the SSF to increase.

Test 13 – Warm Water, 0.4 m/h

MS2 challenge test 13 was conducted on the SSF of pilot 1 train 1 in warm water at a HLR of 0.4 m/h (Table 6-3). The average MS2 removal was 1.0 logs (Table G-52 in Appendix G). This was measurably greater than the average removal of 0.9 logs calculated in test 12 three days before. It

was likely that the persistent warm water conditions led to improved MS2 removals. The test 13 average removal was greater than those calculated for the cold water 0.4 m/h tests 1 and 2.

Test 15 – Warm Water, 0.4 m/h

MS2 challenge test 15 was conducted on the SSF of pilot 1 train 1 in warm water at an HLR of 0.4 m/h (Table 6-3). The average MS2 removal was 1.3 logs (Table G-52 in Appendix G). This was higher than the average removal of 1.0 logs calculated in test 13 three days earlier. The longer warm water acclimation time (compared to tests 10 to 13) and resulting increased biological activity likely caused the improved MS2 removals. The test 15 average removal was greater than those calculated for the cold water 0.4 m/h tests 1 and 2.

Pilot 2 Slow Sand Filter MS2 Challenge Tests

MS2 challenge tests 9 and 14 were conducted on the SSFs of pilot 2 at a HLR of 0.4 m/h in cold and warm water conditions, respectively.

Test 9 – Pilot 2, Cold Water

MS2 challenge test 9 was conducted on the SSFs of pilot 2 in cold water on March 30, 2006 (Table 6-3). MS2 phage were seeded into the effluent of roughing filter A (RFA). Both SSF1 and SSF2 were operated at a HLR of 0.4 m/h, although the flow rate was 0.5 L/min for SSF1 and 0.11 L/min for SSF2 because SSF2 was a smaller diameter column. Detention times of 2 hours and 1 hour were used for SSF1 and SSF2, respectively. To calculate removals for SSF2, the MS2 concentrations in the SSF1 effluent were used as SSF2 influent values.

The average MS2 removals in test 9 were 1.1 logs for SSF1 and 0.5 logs for SSF2 (Table G-47 and Table G-48 in Appendix G). When removals by SSF1 and SSF2 were combined to reflect the performance of two slow sand filters in series, the average combined removal was 1.6 logs.

The average MS2 removal by the 44 cm deep SSF2 was similar to the removals in other cold water 0.4 m/h tests on the 38 cm deep SSF of pilot 1 train 1 (0.4 logs in test 1 and 0.2 logs in test 2) and the 36 cm deep SSF of pilot 1 train 2 (0.5 logs in test 8).

However, the average MS2 log removal of 1.0 logs achieved by SSF1 at 0.4 m/h was approximately double that of the other 0.4 m/h cold water tests 1, 2, and 8. The sand depth of SSF1 (92 cm) was approximately double that of the other slow sand filters tested (36 to 38 cm). Therefore, the HDT in SSF1 would be approximately double that of the other SSFs for a given HLR. It was surmised that the greater MS2 removals in SSF1 were related to the increased HDT, which would provide greater time for biological removal mechanisms such as predation to reduce MS2 concentrations. Furthermore, the deeper sand bed of SSF1 would be expected to provide additional contact opportunities for attachment of MS2 to biofilms and sand grains. These results implied that sand depth (and corresponding increased detention time) was an important factor in MS2 removal, likely due to both biological and physical removal mechanisms.

It is worth noting that samples collected on March 31, 2006 were originally plated the same day but were replated on April 7 using a different dilution to obtain plaque counts in the acceptable range. Quality control results are presented in Appendix I. The pilot 2 SSF2 schmutzdecke had not been scraped in almost one year because solids loading and head loss were very low (Table F-34, Appendix F). The SSF2 effluent DO concentration was greater than that for SSF1 effluent because the water level in the SSF2 column was well below the inlet and water became re-oxygenated as it cascaded in (Table F-34, Appendix F).

Test 14 – Pilot 2, Warm Water

The results of MS2 challenge test 14 demonstrated that warm water conditions led to increased MS2 removals in the pilot 2 SSFs at a HLR of 0.4 m/h (Table 6-3). As shown in Table F-37 (Appendix F), water temperatures were above 20°C during test 14. The average MS2 removals were 2.0 logs in SSF1 and 0.6 logs in SSF2 (Table G-53 and Table G-54 in Appendix G). When SSF1 and SSF2 were combined, the average MS2 removal was 2.6 logs. The combination of the

deep-bed SSF1 followed by the shallow-bed SSF2 in series provided superior MS2 removals compared to any single SSF tested.

The average MS2 removal in SSF1 (2.0 logs) was approximatley double the average removal calculated in test 9 (1.1 logs) for SSF1 operated at the same HLR (0.4 m/h) in cold water. This implies that warmer water temperatures led to increased biological activity in SSF1, which in turn led to increased MS2 removals in test 14.

However, the average MS2 removal in SSF2 (0.6 logs) was only slightly greater than the average MS2 removal for SSF2 calculated in test 9 (0.5 logs) at the same HLR (0.4 m/h) in cold water. The removals in SSF2 had only marginally improved despite the warm temperatures likely because SSF2 received water pretreated by SSF1 and therefore had less biomass than SSF1.

Roughing Filter MS2 Challenge Test Results

MS2 challenge tests 3 and 6 were conducted in cold water (<5°C) on the RF of pilot 1 train 1 and on RFA of pilot 2, respectively. Average MS2 removals were 0.1 logs in test 3 (Table G-40) and 0.2 logs in test 6 (Table G-43) (Appendix G).

Despite the deeper gravel depth in the RFA of pilot 2 (120 cm, Table F-29) compared to the RF of pilot 1 train 1 (~60cm, Table F-31), the removals in tests 3 and 6 were similarly low. Although the roughing filters were biologically active, poor MS2 removals were likely due to the combined adverse effects of high HLR, cold water temperatures, and large grain sizes.

In these tests, only the roughing filters were seeded and sampled. The RF of pilot 1 train 1 had a shallow bed consisting of 30 cm of gravel below a 30 cm granular activated carbon cap (refer to Section 3.1.2 of this thesis). Conversely, RFA of pilot 2 was built according to recommendations in the literature and had a gravel bed depth of 120 cm (refer to Section 3.1.3). The flow rate was 0.5 L/min in both tests, which corresponded to SSF HLRs of 0.4 m/h. However, the roughing filter HLRs were different in test 3 (1.5 m/h) and test 6 (0.95 m/h). The

HDT was 20 minutes for test 3 and 30 minutes for test 6 (Section 3.1.5). Water temperature for both tests was below 5°C (Table F-29 and Table F-31 in Appendix F). In test 6, the high concentration of MS2 in the 192-hour effluent sample (480 PFU/mL) was unexpected and unexplained (Table G-43 in Appendix G). Quality control data are included in Appendix I.

Appendix I
Quality Control Data for MS2 Challenge Tests

Table I-56: Quality control data for MS2 challenge tests

Processing Date (2006)	Negative Control* Plaque Count**		Positive Control[+] QC Stock Dilution[++]	Plaque Count				PFU/mL	Notes
	Before	After		1	2	3	Avg		
27-Jan	0	0	5	32	23	34	29.7	3.0E+06	
			5	37			37	3.7E+06	with raw water
03-Feb	0	0	5	12	25	30	22.3	2.2E+06	
			5	29	28	17	24.7	2.5E+06	with raw water
08-Feb	n.d.	n.d.	5	45	41	29	38.3	3.8E+06	
15-Feb	0	0	5	38	29		33.5	3.4E+06	
			5	24	31		27.5	2.8E+06	with raw water
16-Feb	0	0	n.d.						
17-Feb	0	0	n.d.						
21-Feb	0	0	5	23	23	30	25.3	2.5E+06	
23-Feb	0	0	n.d.						
27-Feb	0	0	5	23	23	15	20.3	2.0E+06	
28-Feb	0	0	5	19	29		24	2.4E+06	
			5	30	36		33	3.3E+06	with 28-Feb raw
01-Mar			5	0	0	0	0	0.0E+00	*E.coli* problem
02-Mar	0	n.d.	5	30	13	20	21	2.1E+06	New Mar.2 *E.coli*
03-Mar			5	0	0	0	0	0.0E+00	Old *E. coli*
04-Mar			5	25	25	28	26	2.6E+06	New *E. coli*
			5	22	26		24	2.4E+06	New *E. coli*
05-Mar			5	0	0	0	0	0.0E+00	Old *E. coli*
			5	29	28	27	28	2.8E+06	New *E. coli*
06-Mar			5	44			44	4.4E+06	
07-Mar	0	0	5	25	28		26.5	2.7E+06	
			5	26	26		26	2.6E+06	with 6-Mar raw
09-Mar	0	0	5	39	30		34.5	3.5E+06	
			5	24			24	2.4E+06	with 9-Mar raw
10-Mar	0	0	5	31	27		29	2.9E+06	
15-Mar	0	0	5	19	17	38	24.7	2.5E+06	
20-Mar	0	0	5	22	18		20	2.0E+06	
			5	25			25	2.5E+06	with 20-Mar raw
22-Mar	0	0	5	24	21		22.5	2.3E+06	
			5	29			29	2.9E+06	with 21-Mar P1 raw
28-Mar	0	0	5	23	21		22	2.2E+06	
			5	26	25		25.5	2.6E+06	with 28-Mar P1 raw

* Negative control - agar with *E. coli*
** Negative controls plated before and after plating samples
+ Positive control - agar, *E. coli* and diluted quality control (QC) stock
++ 5 indicates a 10^5 dilution; 6 indicates a 10^6 dilution; 7 indicates a 10^7 dilution

Table I-57: Quality control data for MS2 challenge tests (continued)

Processing Date (2006)	Negative Control* Plaque Count**		Positive Control+ QC Stock Dilution++	Positive Control Plaque Count					
	Before	After		1	2	3	Avg	PFU/mL	Notes
29-Mar	0	0	5	24	25		24.5	2.5E+06	
			5	27			27	2.7E+06	with 29-Mar P1 raw
30-Mar	0	0	5	42	32		37	3.7E+06	
			5	23	22		22.5	2.3E+06	
			5	24			24	2.4E+06	with 30-Mar P1 raw
31-Mar	0	0	5	42	32		37	3.7E+06	
03-Apr	0	0	5	32			32	3.2E+06	
			5	28			28	2.8E+06	with 30-Mar P1 raw
07-Apr	0	0	5	16	17	15	16	1.6E+06	
15-May	0	0	5	13	12		12.5	1.3E+06	Old *E. coli*
			5	15	17		16	1.6E+06	New *E. coli*
16-May	0	0	5	18	12	19	16.3	1.6E+06	
17-May	0	0	5	18	21	15	18	1.8E+06	
			5	TNTC	TNTC	TNTC			QC-2
19-May	0	0	5	23	16	13	17.3	1.7E+06	
			6	181	169	162	171	1.7E+08	QC-2
			7	23	19		21	2.1E+08	QC-2
01-Jun	0	0	5	11	10	13	11.3	1.1E+06	
			7	14	12	17	14.3	1.4E+08	QC-2
02-Jun	0	0	5	21	13		17	1.7E+06	
			5	21			21	2.1E+06	with 2-Jun raw
			7	22	28		25	2.5E+08	QC-2
			7	28	25		26.5	2.7E+08	QC-2, with 2-Jun raw
04-Jun	0	0	5	11	13	19	14.3	1.4E+06	
05-Jun	0	0	5	183	177		180	1.8E+07	
06-Jun	0	0	5	9	14		11.5	1.2E+06	
			5	14			14	1.4E+06	with 2-Jun raw
			5	13			13	1.3E+06	with 5-Jun P1 raw
			5	13			13	1.3E+06	with 5-Jun P2 raw
			7	25	14		19.5	6.2E+20	QC-2
			7	19			19	1.9E+20	QC-2, with 2-Jun raw
			7	12			12	1.2E+13	QC-2, with 5-Jun P1 raw
			7	13			13	1.3E+14	QC-2, with 5-Jun P2 raw
08-Jun	0	0	7	15	7		11	1.1E+12	
			7	7			7	7.0E+07	QC-2, with 8-Jun P2 raw

* Negative control - agar with *E. coli*

** Negative controls plated before and after plating samples

+ Positive control - agar, *E. coli* and diluted quality control (QC) stock

++ 5 indicates a 10^5 dilution; 6 indicates a 10^6 dilution; 7 indicates a 10^7 dilution

Table I-58: Plaque count data for MS2 spikes

			MS2 Spike					
Preparation Date (2006)	Processing Date (2006)	Spike Dilution*	Plaque Count				PFU/mL**	Average (PFU/mL)
			1	2	3	Avg		
27-Jan	03-Feb	5	TNTC	TNTC	TNTC			
		6	79	81	87	82.3	8.2E+07	
		7	10	10				
	23-Feb-06	6	71	72	60	67.7	6.8E+07	
	22-Mar-06	6	95	81	83	86.3	8.6E+07	7.9E+07
23-Feb	23-Feb-06	5	TNTC	TNTC				
		6	79	67	86	77.3	7.7E+07	
		7	5	10				
	07-Mar-06	6	87	76	70	77.7	7.8E+07	
	22-Mar-06	6	78	84	91	84.3	8.4E+07	8.0E+07
15-Mar	22-Mar-06	6	100	92	115	102	1.0E+08	
27-Mar	30-Mar-06	5	TNTC	TNTC				
		6	89	86	73	82.7	8.3E+07	8.3E+07
28-Mar	30-Mar-06	5	TNTC	TNTC				
		6	92	72	97	87	8.7E+07	
	03-Apr-06	6	149	155	120	141	1.4E+08	1.1E+08
12-May	15-May-06	5	TNTC	TNTC				
		6	90	78	87	85	8.5E+07	
	16-May-06	6	90	75	86	83.7	8.4E+07	8.4E+07
17-May	17-May-06	6	116	99	100	105	1.1E+08	
	19-May-06	6	98	130	139	122	1.2E+08	1.1E+08
24-May	01-Jun-06	6	79	83	61	74.3	7.4E+07	
	06-Jun-06	6	59	77	53	63	6.3E+07	
	08-Jun-06	6	30	45	32	35.7	3.6E+07	5.8E+07
01-Jun	01-Jun-06	6	71	66	77	71.3	7.1E+07	
	06-Jun-06	6	55	63	51	56.3	5.6E+07	6.4E+07

* 5 indicates a 10^5 dilution; 6 indicates a 10^6 dilution; 7 indicates a 10^7 dilution
** MS2 concentration (PFU/mL) calculated by mulitiplying avg. plaque count by dilution
TNTC - too numerous to count

Bibliography

Aboytes, R., Di Giovanni, G.D., Abrams, F.A., Rheinecker, C., McElroy, W., Shaw, N., and LeChevallier, M.W. (2004). Detection of infectious *Cryptosporidium* in filtered drinking water, Journal American Water Works Association, 96(9), 88-98. [as cited by Amburgey *et al.* (2005)]

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

Amy, G., Carlson, K., Collins, M.R., Drewes, J., Gruenheid, S., and Jekel, M. (2006). Integrated comparison of biofiltration in engineered versus natural systems, In Recent Progress in Slow Sand and Alternative Biofiltration Processes (Edited by R. Gimbel, N.J.D. Graham, and M.R. Collins), IWA Publishing, London, UK, 3-11.

Arora, H., DiGiovanni, G.D., and LeChevallier, M.W. (2001). Spent filter backwash contaminants and treatment strategies, Journal American Water Works Association, 93(5), 100. [as cited by Amburgey *et al.* (2005)]

AWWA (1999). Waterbourne pathogens, AWWA Manual of Water Practices M48, American Water Works Association, Denver, CO. [as cited by Health Canada, 2004a]

AWWA (1991). Manual of Design for Slow Sand Filtration, American Water Works Research Foundation (Edited by David Hendricks), Denver, CO.

AWWA (1988). Roundtable: *Cryptosporidium*, Journal American Water Works Association, 80, 14-28. [as cited by Health Canada (2004b)]

Bales, R.C., Li, S., Maguire, K.M., Yahya, M.T., Gerba, C.P., Harvey, R.W. (1995). Virus and bacteria transport in a sandy aquifer, Cape Cod, MA, Ground Water, 33(4), 653-661.

Bales, R.C., Shimin, L. (1993). MS-2 and poliovirus transport in porous media: hydrophobic effects and chemical perturbations, Water Resources Research, 29(4), 957-963.

Bales, R.C., Hinkle, S.R., Kroeger, T.W., Stocking, K., Gerba, C.P. (1991). Bacteriophage adsorption during transport through porous media: chemical perturbations and reversibility, Environmental Science Technology, 25, 2088-2095.

Barrett, J.M., Bryck, J., Collins, M.R., Janonis, B.A., and Logsdon, G.S. (1991). Manual of Design for Slow Sand Filtration. AWWA Research Foundation, Denver, CO.

Bellamy, W.D., Silverman, G.P., Hendricks, D.W., and Logsdon, G.S. (1985a). Removing *Giardia* cysts with slow sand filtration, Journal American Water Works Association, 77(2), 52-60.

Bellamy, W.D., Hendricks, D.W., and Logsdon, G.S. (1985b). Slow sand filtration: influences of selected process variables, Journal American Water Works Association, 77(12), 62-66.

Bernardo, L.D. (1988). Upflow coarse-grained prefilter for slow sand filtration, In Slow Sand Filtration: Recent Developments in Water Treatment Technology (Edited by N.J.D. Graham), Ellis Horwood Ltd., Chichester, England.

Bradford, S.M., Bradford, A.W., Gerba, C.P. (1993). Virus transport through saturated soils, Wiener Mitteilungen, 12, 143-147. [as referenced by Schijven *et al*., 2000]

Bukhari, Z., Smith, H.V., Sykes, N., Humphreys, S.W., Paton, C.A., Girdwood, R.W.A., and Fricker, C.R. (1997). Occurrence of *Cryptosporidium* sp. oocysts and *Giardia* sp. cysts in sewage influents and sewage effluents from sewage treatment plants in England, Water Science and Technology, 35(11-12), 385-390. [as cited by Stott *et al*. (2001)]

Butkus, M.A., Bays, T.J., and Labare, M.P. (2003). Influence of surface characteristics on the stability of *Cryptosporidium parvum* oocysts, Applied and Environmental Microbiology, 69(7), 3819-3825.

Burge, W.D., Enkiri, N.K. (1978). Virus adsorption to five soils, Journal of Environmental Quality, 7(1), 73-76.

Byrd, T.L. and Walz, J.Y. (2005). Interaction force profiles between *Cryptosporidium parvum* oocysts and silica surfaces, Environmental Science and Technology, 39, 9574-9582.

Chauret, C., Springthorpe, S., and Sattar, S. (1999). Fate of *Cryptosporidium* oocysts, *Giardia* cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion, Canadian Journal of Microbiology, 45(3), 257-262. [as cited by Stott *et al*. (2003)]

Chauret, C., Nolan, K., Chen, P., Springthorpe, S., and Sattar, S. (1998). Aging of *Cryptosporidium parvum* oocysts in river water and their susceptibility to disinfection by chlorine and monochloramine, Canadian Journal of Microbiology, 44(12), 1154-1160.

Cleary, S. (2005). Sustainable drinking water treatment for small communities using multistage slow sand filtration. Master's Thesis, Department of Civil Engineering, University of Waterloo, Waterloo, Ontario, Canada.

Cliver, D.O. and Herrmann, J.E. (1972). Proteolytic and microbial inactivation of enteroviruses, <u>Water Research</u>, 6, 797-805.

Collins, M.R., Begin, A.N., Musich, J.P., and LeCraw, R.A. (2005). Pre-treatment enhancements to slow sand filtration – A case study North Haven, Maine, <u>Journal of the New England Water Works Association (NEWWA)</u>, 119(4), 204-212.

Collins, M.R., Cole, J.O., Westersund, C.M., and Paris, D.B. (1994). Evaluation of Roughing Filter Design Variables. AWWA Research Foundation, Denver, CO.

Considine, R.F., Dixon, D.R., and Drummond, C.J. (2002). Oocysts of *Cryptosporidium parvum* and model sand surfaces in aqueous solutions: an atomic force microscope (AFM) study, <u>Water Research</u>, 36, 3421-3428.

Craun, G.F., Hubbs, S.A., Frost, F., Calderon, R.L., and Via, S.H. (1998). Waterborne outbreaks of cryptosporidiosis, <u>Journal American Water Works Association</u>, 90(9), 81-91. [as cited by Amburgey *et al.* (2005)]

Current, W.L. and Garcia, L.S. (1991). Cryptosporidiosis, <u>Clinical Microbiology Reviews</u>, 4(3), 325-358.

Daniel, P.A. (1996). *Cryptosporidium*: A risk assessment, <u>Water Supply</u>, 14(3-4), 387. [as cited by Amburgey *et al.* (2005)]

DeBorde, D.C., Woessner, W.W., Kiley, Q.T., Ball, P.N. (1999). Rapid transport of viruses in a floodplain aquifer, <u>Water Research</u>, 33(10), 2229-2238.

DeBorde, D.C., Woessner, W.W., Lauerman, B., Ball, P.N. (1998). Virus occurrence and transport in a school septic system and unconfined aquifer, <u>Ground Water</u>, 36(5), 825-834.

Deng, M.Y. and Cliver, D.O. (1995). Persistence of inoculated Hepatitis A virus in mixed human and animal wastes, <u>Applied and Environmental Microbiology</u>, 61(1), 87-91.

Deng, M.Y. and Cliver, D.O. (1992). Inactivation of poliovirus type 1 in mixed human and swine wastes and by bacteria from swine manure, <u>Applied and Environmental Microbiology</u>, 58, 2016-2021.

Dizer, H., Grutzmacher, G., Bartel, H., Wiese, H.B., Szewzyk, R., and Lopez-Pila, J.M. (2004). Contribution of the colmation layer to the elimination of coliphage by slow sand filtration, <u>Water Science and Technology</u>, 50(2), 211-214.

Dorner, S.M., Anderson, W.B., Gaulin, T., Candon, H.L., Slawson, R.M., Payment, P., and Huck, P.M. (2007). Pathogen and indicator variability in a heavily impacted watershed, Journal of Water and Health, 5(2), 241-257.

Dorner, S.M., Huck, P.M., Slawson, R.M., Gaulin, T., and Anderson, W.B. (2004). Assessing Levels of Pathogenic Contamination in a Heavily Impacted River Used as a Drinking Water Source, Journal of Toxicology and Environmental Health, Part A, 67 (20-22), 1813-1823.

Dowd, S.E., Pillai, S.D., Wang, S., Corapcioglu, M.Y. (1998). Delineating the specific influence of virus isoelectric point and size on virus adsorption and transport through sandy soils, Applied and Environmental Microbiology, 64 (2), 405-410.

Dullemont, Y.J., Schijven, J.F., Hijnen, W.A.M., Colin, M., Magic-Knezev, A., and Oorthuizen, W.A. (2006). Removal of microorganisms by slow sand filtration, In Recent Progress in Slow Sand and Alternative Biofiltration Processes (Edited by R. Gimbel, N.J.D. Graham, and M.R. Collins), IWA Publishing, London, UK, 12-20.

Duncan, A. (1988). The ecology of slow sand filters, In Slow Sand Filtration: Recent Developments in Water Treatment Technology (Edited by N.J.D. Graham), Ellis Horwood Ltd., Chichester, England.

DuPont, H.L., Chappell, C.L., Sterling, C.R., Okhuysen, P.C., Rose, J.B., and Jakubowski, W. (1995). The infectivity of *Cryptosporidium parvum* in healthy volunteers, New England Journal of Medicine, 332, 855-859. [as cited by Health Canada (2004b) and Stott *et al*. (2001)]

Eighmy, T.T., Collins, M.R., Malley, J.P. Jr., Royce, J., Morgan, D. (1993). Biologically enhanced slow sand filtration for removal of natural organic matter, AWWA Research Foundation, Denver, CO.

Eighmy, T.T. and Collins, M.R. (1988). Modifications to the slow rate filtration process for improved trihalomethane precursor removal, In Slow Sand Filtration: Recent Developments in Water Treatment Technology (Edited by N.J.D. Graham), Ellis Horwood Ltd., Chichester, England.

Elliott, M.A., Stauber, C.E., Koksal, F., Liang, K.R., Huslage, D.K., DiGiano, F.A., Sobsey, M.D. (2006). The operation, flow conditions and microbial removals of an intermittently operated, household-scale slow sand filter, In Recent Progress in Slow Sand and Alternative Biofiltration Processes (Edited by R. Gimbel, N.J.D. Graham, and M.R. Collins), IWA Publishing, London, UK, 268-277.

Ellis, K.V. (1985). Slow Sand Filtration, Critical Reviews in Environmental Control, 15(4), 315-354.

Emelko, M.B. (2006). Personal communication, October 2006.

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

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

Farrah, S.R., Preston, D.R. (1993). Adsorption of viruses to sand modified by in situ precipitation of metallic salts, <u>Wiener Mitteilungen</u>, 12, 25-29. [as referenced by Schijven *et al*., 2000]

Fayer, R., Trout, J.M., Walsh, E., and Cole, R. (2000). Rotifers ingest oocysts of *Cryptosporidium parvum*, <u>J. Eukaryot. Microbiol.</u>, 47(2), 161-163.

Flemming, H.C. (1995). Sorption sites in biofilms, <u>Water Science and Technology</u>, 32(8), 27-33.

Fogel, D., Isaac-Renton, J., Guasparini, R., Moorehead, W., and Ongerth, J. (1993). Removing *Giardia* and *Cryptosporidium* by slow sand filtration, <u>Journal American Water Works Association</u>, 85(11), 77-84.

Fox, K.R., Miltner, R.J., Logsdon, G.S., Dicks, D.L., and Drolet, L.F. (1984). Pilot-plant studies of slow-rate filtration, <u>Journal American Water Works Association</u>, 76(12), 62-68.

Ford, T.E. (1999). Microbiological safety of drinking water: United States and global perspectives, <u>Environmental Health Perspectives</u>, 107(Suppl. 1), 191-206. [as cited by Amburgey *et al*. (2005)]

Funderburg, S.W., Moore, B.E., Sagik, B.E., Sorber, C.A. (1981). Viral transport through soil columns under conditions of saturated flow, <u>Water Research</u>, 15, 703-711. [as referenced by Schijven *et al*., 2000]

Galvis, G., Latorre, J., and Galvis, A. (2002). Multi-stage filtration technology. *In* Small Community Water Supplies, IRC Technical Paper Series No. 40, IRC International Water and Sanitation Centre, The Hague, The Netherlands.

Galvis, G., Latorre, J., and Visscher, J.T. (1998). Multi-stage filtration: An innovative water treatment technology, IRC Technical Paper Series No. 34E, IRC International Water and Sanitation Centre, The Hague, The Netherlands.

Galvis, G., Visscher, J.T., and Lloyd, B. (1992). Multi-stage surface water treatment for community water supply in Columbia, <u>Waterlines</u>, 10(3), 26-29.

Gerba, C.P. (1984). Applied and theoretical aspects of virus adsorption to surfaces, <u>Advances in Applied Microbiology</u>, 30, 133-168.

Goyal, S.M., and Gerba, C.P. (1979). Comparitive adsorption of human enteroviruses, simian rotavirus, and selected bacteriophage to soils, <u>Applied and Environmental Microbiology</u>, 38(2), 241-247.

Grabow, W.O.K (2001). Bacteriophage: Update on application as models for viruses in water, <u>Water SA</u>, 27(2), 251-268.

Graczyk, T.K., Evans, B.M., Shiff, C.J., Karreman, H.J., and Patz, J.A. (2000). Environmental and geographical factors contributing to watershed contamination with *Cryptosporidium parvum* oocysts, <u>Environmental Research</u>, 82(3), 263-271. [as cited by Stott *et al*. (2003)]

Hancock, C.M., Rose, J.B., and Callahan, M. (1998). Crypto and *Giardia* in US groundwater, <u>Journal American Water Works Association</u>, 90(3), 58-61. [as cited by Swertfeger *et al*. (1999)]

Harvey, R.W. and Ryan, J.N. (2004). Use of PRD1 bacteriophage in groundwater viral transport, inactivation, and attachment studies, <u>FEMS Microbiology Ecology</u>, 49, 3-16.

Health Canada (2004a). Guidelines for Canadian Drinking Water Quality: Supporting Documentation - Enteric Viruses. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario, Canada.

Health Canada (2004b). Guidelines for Canadian Drinking Water Quality: Supporting Documentation - Protozoa: *Giardia* and *Cryptosporidium*. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario, Canada.

Health Canada (2003). Guidelines for Canadian Drinking Water Quality: Supporting Documentation – Turbidity. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario, Canada.

Heller, L. and Brito, L.L.A. (2006). The retention of *Cryptosporidium* sp. oocysts at varying depths in slow sand filters: A pilot study, <u>Journal of Water Supply: Research and Technology - Aqua</u>, 55(3), 193-206.

Herbold-Paschke, K., Straub, U., Hahn, T., Teutsch, G., Botzenhart, K. (1991). Behaviour of pathogenic bacteria, phage and viruses in groundwater during transport and adsorption, <u>Water Science and Technology</u>, 24(2), 301-304.

Hijnen, W.A.M., Brouwer-Hanzens, A.J., Charles, K.J., and Medema, G.J. (2005). Transport of MS2 phage, *Escherichia coli*, *Clostridium perfringens*, *Cryptosporidium parvum*, and *Giardia intestinalis* in a gravel and sandy soil, Environmental Science and Technology, 39, 7860-7868.

Hijnen, W.A.M., Schijven, J.F., Bonne, P., Visser, A., and Medema, G.J. (2004). Elimination of viruses, bacteria and protozoan oocysts by slow sand filtration, Water Science and Technology, 50(1), 147-154.

Hoxie, N.J., Davis, J.P., Vergeront, J.M., Nashold, R.D., and Blair, K.A. (1997). Cryptosporidiosis-associated mortality following a massive waterborne outbreak in Milwaukee, Wisconsin, American Journal of Public Health, 87(12), 2032-2035. [as cited by Amburgey *et al*. (2005)]

Huisman, L. and Wood, W.E. (1974). Slow sand filtration, World Health Organization, Geneva, Switzerland.

Hunter, P., Syed, Q., and Naumova, E.N. (2001). Possible undetected outbreaks of cryptosporidiosis in areas of the north west of England supplied by an unfiltered surface water source, Commun. Dis. Public Health, 4, 136-138. [as cited by King *et al*., 2005]

Hurst, C.J., Gerba, C.P., and Cech, I. (1980). Effects of environmental variables and soil characteristics on virus survival in soil, Applied and Environmental Microbiology, 40(6), 1067-1079.

Jansons, J., Edmonds, L.W., Speight, B., and Bucens, M.R. (1989). Survival of viruses in groundwater, Water Research, 23(3), 301-306.

Jin, Y., Yates, M.V., Thompson, S.S., Jury, W.A. (1997). Sorption of viruses during flow through saturated sand columns, Environmental Science and Technology, 31, 548-555.

Keswick, B.H., Gerba, C.P. (1980). Viruses in groundwater, Environmental Science and Technology, 14(11), 1290-1297.

Kim, T.D. and Unno, H. (1996). The roles of microbes in the removal and inactivation of viruses in a biological wastewater treatment system, Water Science and Technology, 33(11), 243-250.

King, B.J., Keegan, A.R., Monis, P.T., and Saint, C.P. (2005). Environmental temperature controls *Cryptosporidium* oocyst metabolic rate and associated retention of infectivity, Applied and Environmental Microbiology, 71(7), 3848-3857.

Kinoshita, T., Bales, R.C., Maguire, K.M., Gerba, C.P. (1993). Effect of pH on bacteriophage transport through sandy soils, Journal of Contaminant Hydrology, 14, 55-70.

Kohne, R.W., Logsdon, G.S., and Logsdon, P.E. (2002). Slow Sand Filtration, In <u>Control of Microorganisms in Drinking Water</u>, American Society of Civil Engineers, Reston, VA, 113-126.

Kunzar, Z.A. and Elimelech, M. (2005). Role of surface proteins in the deposition kinetics of *Cryptosporidium parvum* oocysts, <u>Langmuir</u>, 21, 710-716.

Lance, J.C., Gerba, C.P. (1984). Virus movement in soil during saturated and unsaturated flow, <u>Applied and Environmental Microbiology</u>, 335-337.

Lance, J.C., Gerba, C.P., Wang, D.S. (1982). Comparative Movement of Different Enteroviruses in soil columns, <u>Journal of Environmental Quality</u>, 11(3), 347-351.

Lang, J.S., Giron, J.J., Hansen, A.T., Rhodes Trussell, R., and Hodges, W.E. (1993). Investigating filter performance as a function of the ratio of filter size to media size, <u>Journal American Water Works Association</u>, 85(10), 122-130.

LeChevallier, M.W., DiGiovanni, G.D., Clancy, J.L., Bukhari, Z., Bukhari, S., Hargy, T., Rosen, J.S., Sobrinho, J., and Frey, M.M. (2000). Source water assessment: variability of pathogen concentrations, <u>Proceedings, AWWA Water Quality Technology Conference (WQTC)</u>, Salt Lake City, Utah. Paper TU12-4.

LeChevallier, M.W. and Norton, W.D. (1995). *Giardia* and *Cryptosporidium* in raw and finished water, <u>Journal American Water Works Association</u>, 87(9), 54-68. [as cited by Swertfeger *et al*. (1999)]

LeChevallier, M.W., Norton, W.D., and Lee, R.G. (1991a). *Cryptosporidium* and *Giardia* spp. in filtered drinking-water supplies, <u>Applied and Environmental Microbiology</u>, 57, 2617-2621. [as cited by Byrd and Walz (2005) and Health Canada (2004b)]

LeChevallier, M.W., Norton, W.D., and Lee, R.G. (1991b). Occurrence of *Cryptosporidium* and *Giardia* spp. in surface-water supplies, <u>Applied and Environmental Microbiology</u>, 57, 2610-2616. [as cited by Byrd and Walz (2005) and Health Canada (2004b)]

Lloyd, B.J. (1996). The significance of protozoal predation and adsorption for the removal of bacteria by slow sand filtration, In <u>Slow Sand Filtration: Recent Developments in Water Treatment Technology</u> (Edited by N.J.D. Graham), Ellis Horwood Ltd., Chichester, England, 129-137.

Logan, A.J., Stevik, T.K., Siegrist, R.L., and Ronn, R.M. (2001). Transport and fate of *Cryptosporidium parvum* oocysts in intermittent sand filters, <u>Water Research</u>, 35(18), 4359-4369.

Loveland, J.P., Ryan, J.N., Amy, G.L., Harvey, R.W. (1996). The reversibility of virus attachment to mineral surfaces, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 107, 205-221.

Madigan, M.T., Martinko, J.M., and Parker, J. (2003). Brock Biology of Microorganisms – 10th Edition. Prentice Hall, Upper Saddle River, NJ.

Madore, M.S., Rose, J.B., Gerba, C.P., Arrowood, M.J., and Sterling, C.R. (1987) Occurrence of *Cryptosporidium* oocysts in sewage effluents and selected surface waters, Journal of Parasitology, 73(4), 702–705. [as cited by Stott *et al*. (2001)]

McConnell, L.K., Sims, R.C., Barnett, B.B. (1984). Reovirus removal and inactivation by slow-rate sand filtration, Applied and Environmental Microbiology, 48(4), 818-825.

Meinhardt, P.L., Casemore, D.P., and Miller, K.B. (1996). Epidemiologic aspect of human cryptosporidiosis and the role of waterborne transmission, Epidemiologic Reviews, 18(2), 118-136.

Melnick, J.L. and Gerba, C.P. (1980). The ecology of enteroviruses in natural waters, CRC Critical Reviews in Environmental Control, 10(1), 65-93.

Meschke, J.S., and Sobsey, M.D. (2003). Comparative removal of Norwalk virus, poliovirus type 1, F+ RNA coliphage MS2 and Escherichia coli in miniature soil columns, Water Science and Technology, 47(3), 85-90.

Meschke, J.S., and Sobsey, M.D. (1998). Comparative adsorption of Norwalk virus, poliovirus-1 and F+ RNA coliphage MS2 to soils suspended in treated wastewater, Water Science and Technology, 38(12), 187-189.

Ministry of Environment (2006). Procedure for Disinfection of Drinking Water in Ontario – Second Revision, June 2006. Ontario Ministry of the Environment, Publication 4448e01 (as adopted by reference by Ontario Regulation 170/03 under the Safe Drinking Water Act).

Mix (1974). Dev. Ind. Microbiol. 15, 136-142. [as cited by Gerba, 1984]

Montgomery (2005). Water Treatment Principles and Design – 2nd Edition. John Wiley and Sons, NJ.

Nasser, A.M., Glozman, R., and Nitzan, Y. (2002). Contribution of microbial activity to virus removal in saturated soil, Water Research, 36, 2589-2595.

Nasser, A.M. and Oman, S.D. (1999). Quantitative assessment of the inactivation of pathogenic and indicator viruses in natural water sources, Water Research, 33(7), 1748-1752.

O'Donoghue, P.J. (1995). *Cryptosporidium* and cryptosporidiosis in man and animals, <u>Int. J. Parasitol</u>, 25, 139-195. [as cited by Stott *et al*. (2001)]

Penrod, S.L., Olson, T.M., Grant, S.B. (1996). Deposition kinetics of two viruses in packed beds of quartz granular media, <u>Langnuir</u>, 12, 5576-5587.

Powelson, D.K., Gerba, C.P., Yahya, M.T. (1993). Virus transport and removal in wastewater during aquifer recharge, <u>Water Research</u>, 27(4), 583-590.

Poynter, S.F.B. and Slade, J.S. (1977). The removal of viruses by slow sand filtration, <u>Progress in Water Technology</u>, 9(1), 75-88.

Poynter, S.F.B. (1966). Studies in the access of enteroviruses to water supplies. Ph.D. thesis, University of London, UK.

Pyper, G.R. (1985). Slow sand filter and package treatment plant evaluation: operating costs and removal of bacteria, *Giardia*, and trihalomethanes, <u>USEPA/600/2-85/052</u>, U.S. Environmental Protection Agency, Cincinnati, Ohio. [as cited by Kohne (2002)]

Quanrud, D.M., Carroll, S.M., Gerba, C.P., and Arnold, R.G. (2003). Virus removal during simulated soil-aquifer treatment, <u>Water Research</u>, 37, 753-762.

Rachwal, A.J., Bauer, M.J., Chipps, M.J., Colbourne, J.S., and Foster, D.M. (1996). Comparisons between slow sand and high rate biofiltration, In <u>Slow Sand Filtration: Recent Developments in Water Treatment Technology</u> (Edited by N.J.D. Graham), Ellis Horwood Ltd., Chichester, England, 3-10.

Redman, J.A., Grant, S.B., Olson, T.M., Adkins, J.M., Jackson, J.L., Castillo, M.S., Yanko, W.A. (1999). Physiochemical mechanisms responsible for the filtration and mobilization of a filamentous bacteriophage in quartz sand, <u>Water Research</u>, 33(1), 43-52.

Redman, J.A., Grant, S.B., Olson, T.M. (1997). Filtration of recombinant Norwalk virus particles and bacteriophage MS2 in quartz sand: Importance of electrostatic interactions, <u>Environmental Science and Technology</u>, 31, 3378-3383.

Rose, J.B. (1988). Occurrence and significance of *Cryptosporidium* in water, <u>Journal American Water Works Association</u>, 80(2), 53-58. [as cited by Shaw *et al*. (2000)]

Ryan, J.N., Harvey, R.W., Metge, D., Elimelech, M., Navigato, T. Pieper, P. (2002). Field and laboratory investigations of inactivation of viruses (PRD1 and MS2) attached to iron oxide-coated quartz sand, <u>Environmental Science and Technology</u>, 36, 2403-2413.

Sanchez, L.D., Latorre, J., Galvis, G., and Visscher, J.T. (2006). Microbial conditions before and after cleaning in slow sand filters under tropical conditions, In Slow Sand Filtration: Recent Developments in Water Treatment Technology (Edited by N.J.D. Graham), Ellis Horwood Ltd., Chichester, England, 152-161.

Schijven, J.F., de Bruin, H.M., Hassanizadeh, S.M., Husman, A.M. (2003). Bacteriophage and clostridium spores as indicator organisms for removal of pathogens by passage through saturated dune sand, Water Research, 37, 2186-2194.

Schijven, J.F. and Simunek, J. (2002). Kinetic modeling of virus transport at the field scale, Journal of Contaminant Hydrology, 55, 113-135.

Schijven, J.F., Hassanizadeh, S.M., de Bruin, R. (2002). Two-site kinetic modeling of bacteriophage transport through columns of saturated dune sand, Journal of Contaminant Hydrology, 57, 259-279.

Schijven, J.F., Hassanizadeh, S.M., Dowd, S.E., Pillai, S.D. (2001). Modeling virus adsorption in batch and column experiments, Quantitative Microbiology, 2, 5-20.

Schijven, J.F., Hassanizadeh, S.M. (2000). Removal of viruses by soil passage: Overview of modeling, processes, and parameters, Critical Reviews in Environmental Science and Technology, 30(1), 49-127.

Schijven, J.F., Hoogenboezem, W., Hassanizadeh, S.M., Peters, J.H. (1999). Modeling removal of bacteriophage MS2 and PRD1 by dune recharge at Castricum, Netherlands, Water Resources Research, 35(4), 1101-1111.

Schuler, P.F., Ghosh, M.M., and Gopalan, P. (1991). Slow sand and diatomaceous earth filtration of cysts and other particulates, Water Research, 25(8), 995-1005.

Schuler, P.F., Ghosh, M.M., and Boutros, S.N. (1988). Comparing the removal of *Giardia* and *Cryptosporidium* using slow sand and diatomaceous earth filtration, Proceedings of the 1988 American Water Works Association Annual Conference, Orlando, FL.

Schulz, C.R. and Okun, D.A. (1984). Surface water treatment for communities in developing countries. John Wiley & Sons, New York, NY.

Slade, J.S. (1978). Enteroviruses in slow sand filtered water, J. Inst. Water Eng. Sci., 82, 530.

Smith, H.V. and Rose, J.B. (1998). Waterborne cryptosporidiosis: current status, Parasitology Today, 14(1), 14-22. [as cited by Stott *et al*. (2001)]

Smith, H.V., Parker, J.F.W., Bukhari, Z., Campbell, D.M., Benton, C., Booth, N., and McCreadie, A. (1993). Significance of small numbers of *Cryptosporidium* sp. oocysts in water, Lancet, 342(8866), 312-313. [as cited by Health Canada (2004b)]

Sobsey, M.D., Hall, R.M., Hazard, R.L. (1995). Comparative removals of hepatitis A virus, enteroviruses and coliphage MS2 in miniature soil columns, Water Science and Technology, 31(5-6), 203-209.

Sobsey, M.D., Dean, C.H., Knuckles, M.E., Wagner, R.A. (1980). Interactions and survival of enteric viruses in soil materials, Applied and Environmental Microbiology, 92-102.

Storey, M.V., and Ashbolt, N.J. (2003). Enteric virions and microbial biofilms – a secondary source of public health concern?, Water Science and Technology, 48(3), 97-104.

Storey, M.V., and Ashbolt, N.J. (2001). Persistence of two model enteric viruses (B40-8 and MS-2 bacteriophage) in water distribution pipe biofilms, Water Science and Technology, 43(12), 133-138.

Stott, R., May, E., Ramirez, E., and Warren, A. (2003). Predation of *Cryptosporidium* oocysts by protozoa and rotifers: implications for water quality and public health, Water Science and Technology, 47(3), 77-83.

Stott, R., May, E., Matsushita, E., and Warren, A. (2001). Protozoan predation as a mechanism for the removal of *Cryptosporidium* oocysts from wastewaters in constructed wetlands, Water Science and Technology, 44(11-12), 191-198.

Swertfeger, J., Metz, D.H., DeMarco, J., Braghetta, A., and Jacangelo, J.G. (1999). Effect of filter media on cyst and oocyst removal, Journal American Water Works Association, 91(9), 90-100.

Tillett, H.E., de Louvois, J., and Wall, P.G. (1998). Surveillance of outbreaks of water-borne infectious disease: categorizing levels of evidence, Epidemiol. Infect., 120, 37-42. [as cited by King *et al.,* 2005]

Timms, S., Slade, J.S., and Fricker, C.R. (1995). Removal of *Cryptosporidium* by slow sand filtration, Water Science and Technology, 31(5-6), 81-84.

Tufenkji, N. and Elimelech, M. (2005). Spatial distributions of *Cryptosporidium* oocysts in porous media: Evidence for dual mode deposition, Environmental Science and Technology, 39, 3620-3629.

Tufenkji, N., Miller, G.F., Ryan, J.N., Harvey, R.W., and Elimelech, M. (2004). Transport of *Cryptosporidium* oocysts in porous media: Role of straining and physiochemical filtration, Environmental Science and Technology, 38, 5932-5938.

United Nations (2005). World Urbanization Prospects: The 2005 Revision Population Database (http://esa.un.org/unup/).

United States Environmental Protection Agency (2006). The Long-term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) Implementation Guidance – Draft for Comment [EPA 816-R-06-006 June 2006], US EPA, Office of Water, 166pp.

United States Environmental Protection Agency (2005). Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA [EPA 815-R-05-002], US EPA, Office of Water, 76pp.

Urfer-Frund, D. (1998). Effects of oxidants on drinking water biofilters. Ph.D. Thesis, Department of Civil Engineering, University of Waterloo, Waterloo, Ontario, Canada.

Van Dyke, M.I., Morton, V., Anderson, W.B., Isaac-Renton, J.L., and Huck, P.M. (2006). The Occurrence of Selected Bacterial and Protozoan Pathogens in the Grand River Watershed, In: Proceedings of the 12th Canadian National Conference & 3rd Policy Forum on Drinking Water, Saint John, New Brunswick, April 1-4, 2006 (in press).

Van Loosdrecht, M.C.M., Eikelboom, D., Gjaltema, A., Mulder, A., Tijhuis, L., and Heijnen, J.J. (1995). Biofilm structures, Water Science and Technology, 32(8), 35-43.

Visscher, J.T. (1990). Slow sand filtration: Design, operation, and maintenance, Journal American Water Works Association, 82(6), 67-71.

Visscher, J.T., Paramasivam, R., Raman, A., Heijnen, H.A. (1987). Slow sand filtration for community water supply: Planning, design, construction, operation and maintenance. IRC International Water and Sanitation Centre, Technical paper series, No.24. The Hague, The Netherlands.

Wallis, P.M., Isaac-Renton, J.L., Erlandsen, S.L., and Olson, M.E. (1995). Risk assessment for waterborne giardiasis and cryptosporidiosis in Canada. Unpublished report to Health Canada. [as cited by Health Canada (2004b)]

Wang, D., Gerba, C.P., and Lance, J.C. (1981). Effect of soil permeability on virus removal through soil columns, Applied and Environmental Microbiology, 42(1), 83-88.

Ward, R.L, Knowlton, D.R., and Winston, P.E. (1986). Mechanism of inactivation of enteric viruses in fresh water, Applied and Environmental Microbiology, 52(3), 450-459.

Watling, M.T. (2004). Removal of *Cryptosporidium parvum* oocysts and potential surrogates in vertical and horizontal flow-through columns. Master's thesis, Department of Civil Engineering, University of Waterloo, Waterloo, Ontario, Canada.

Wegelin, M. (1996). Surface water treatment by roughing filters: A design, construction, and operational manual. Sandec Report No. 2/96, Swiss Centre for Development Cooperation in Technology and Management (SKAT), St. Gallen, Switzerland.

Wegelin, M. (1988). Roughing gravel filters for suspended solids removal, In Slow Sand Filtration: Recent Developments in Water Treatment Technology (Edited by N.J.D. Graham), Ellis Horwood Ltd., Chichester, England.

Wegelin, M. and Schertenleib, R. (1993). Roughing filters for water treatment. International Reference Centre for Waste Disposal (IRCWD) News No. 27, IRCWD, Dubendorf, Switzerland.

Wheeler, D., Bartram, J., and Lloyd, B.J. (1988). The removal of viruses by filtration through sand, In Slow Sand Filtration: Recent Developments in Water Treatment Technology (Edited by N.J.D. Graham), Ellis Horwood Ltd., Chichester, England, 207-229.

Windle-Taylor, E. (1969). The removal of viruses by slow sand filtration, Rep. Results Bact. Chem. Biol. Exam. Land. Waters, 44, 52, 1969-70.

World Health Organization (2006). Guidelines for Drinking Water Quality – First Addendum to Third Edition. World Health Organization, Geneva, Switzerland.

Yahya, M.T., Cluff, C.B., and Gerba, C.P. (1993a). Virus removal by slow sand filtration and nanofiltration, Water Science and Technology, 27(3), 445-448.

Yahya, M.T., Galsomies, L., Gerba, C.P., and Bales, R.C. (1993b). Survival of bacteriophage MS-2 and PRD-1 in ground water, Water Science and Technology, 27(3-4), 409-412.

Yates, M.V., Stetzenbach, L.D., Gerba, C.P., and Sinclair, N.A. (1990). The effect of indigenous bacteria on virus survival in ground water, Journal of Environmental Science and Health, A25(1), 81-100.

Yates, M.V. and Gerba, C.P. (1985). Factors controlling the survival of viruses in groundwater, Water Science and Technology, 17, 681-687.

Yates, M.V., Gerba, C.P., and Kelley, L.M. (1985). Virus persistence in groundwater, Applied and Environmental Microbiology, 49(4), 778-781.

Zuckerman, U., Gold, D., Shelef, G., and Armon, R. (1997). The presence of *Giardia* and *Cryptosporidium* in surface waters and effluents in Israel, <u>Water Science and Technology</u>, 35(11-12), 381-384. [as cited by Stott *et al*. (2003)]