Fluorescent microspheres as surrogates to assess oocyst removal efficacy from a modified slow sand biofiltration water treatment system

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ABSTRACT

There has been a resurgence of interest, technological advancement, and implementation of biologically active slow sand filtration technologies for small-scale potable water treatment in North America. Modelling the fate and transport of pathogenic microorganisms is vital to assess technological safety and for licensing, permitting and regulatory validation. The efficacy of a modified slow sand (MSS) filter treatment technology to produce drinking water and remove *Cryptosporidium* oocyst-sized particles was tested using a rural raw water source seeded with 4–4.9 μm (mean 4.5 μm) microsphere surrogates. The turbidity, temperature, pH and total organic carbon content of raw water were 1.46 ± 0.010 nephelometric turbidity unit (NTU), 3.40 ± 0.30 °C, 8.05 ± 0.16 and 22.7 ± 0.64 mg/L, respectively, and those of the MSS filter effluent with biologically active carbon filter influent were 0.47 ± 0.0 NTU, 3.36 ± 0.42 °C, 7.90 ± 0.27 and 22.6 ± 0.65 mg/L. The decimal elimination capacity of the MSS and biologically active carbon filters for the microspheres were at least 2 and 1 log at 95% confidence, respectively. These systems are capable of removing oocyst-sized particles under extreme conditions, providing safe, effective and economical treatment solutions for small-scale municipal drinking water.

Key words | elimination capacity, fluorescent microspheres, modified slow sand filtration, oocyst surrogates, potable water treatment, rural raw water

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INTRODUCTION

Significant challenges exist for the design and implementation of small-scale potable water treatment systems in rural and remote communities across Canada and the globe. Solutions are often not cost effective, appropriately scaled or operationally manageable within community structures. The rates of non-compliance to potable water standards tend to be inversely correlated to population served (Cleary 2005). Higher rates of non-compliance are associated with smaller population centres having inadequate potable water treatment systems and typically poorer quality water sources than those of urban centres. Furthermore, urban communities have access to resources that promote and protect potable water development, treatment and security for the stable production of reliable water quality and quantity (WHO 2011). With economies of scale, larger communities can also develop more sophisticated water treatment infrastructure and recruit and retain more highly skilled operators (Cleary 2005).

The slow sand filtration (SSF) process is the oldest, least expensive and most reliable water treatment process used to produce potable water worldwide (Fogel *et al.* 1993; Gafvert *et al.* 2002; Pokhrel *et al.* 2005). SSF is commonly used to remove *Cryptosporidium parvum* and *Giardia* oocysts, and is currently used in potable water treatment in London, UK, and other cities in Europe (Schuler *et al.* 1991). In the Netherlands, SSFs are used as the last stage

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of a multi-barrier treatment system (Hijnen *et al.* 2007). However, since conventional SSFs can be labour intensive and occupy a relatively large footprint, it has not always been the technology of choice for smaller communities.

A labour-intensive system is not deemed suitable for many small communities based on budgetary and other restrictions related to certified operation. While it can be argued that small communities tend to have adequate space available for water treatment plants (WTPs) requiring a larger footprint, this is not always the case. For budgetary reasons, communities often choose to upgrade within buildings that house simple chlorine or chemical plants, thus limiting the space available. Furthermore, heating can be costly in extremely cold Canadian winter climates. Thus, many small communities on tight budgets may not readily choose modified slow sand (MSS) WTPs.

During the operation of SSFs, a thin layer of detritus and biological material called the *schmutzdecke* (dirty skin) forms on the sand bed (Bellamy *et al.* 1985a; Weber-Shirk & Dick 1997b). Although it is acknowledged that the *schmutzdecke* aids in purification through physical, chemical and biological means, the associated mechanisms are poorly defined. Beyond this layer, there is also biological growth within the sand bed and the gravel support layer. Biological activity is considered to assist in particle removal by providing an enhanced physical screening mechanism within the biofilm (Haarhoff & Cleasby 1991). Furthermore, production of exopolysaccharide (a sugar polymer) by the microbial biofilm can mitigate the transport of particles through the lower layers of the filtration system and improve adsorption to sand grains (Mauclaire *et al.* 2004). Finally, bacterial contaminants, those potentially harmful to humans, are thought to be removed through predation and grazing by more complex organisms (Bellamy *et al.* 1985b; Lloyd 1996; Weber-Shirk & Dick 1997a; Bichai *et al.* 2010).

The physicochemical removal (screening and attachment) of microbiological particles, such as *C. parvum* and *Giardia* oocysts, is thought to be statistically significant for particles with diameters (d) in the range 0.75–10 μm (Weber-Shirk & Dick 1997a), with no measureable difference in particle ($d > 2$ μm) removal when biological activity was arrested. Weber-Shirk & Dick (1999) demonstrated that bacterivory is primarily the purview of flagellates that graze on bacteria smaller than 2 μm. More recently, predation by zooplankton has been shown to affect the transport and fate of oocysts; however, internalized oocysts account for less than 1% of total oocysts (Bichai *et al.* 2010).

The use of multistage filtration (MSF) treatment with SSF commonly begins with some level of pre-treatment (often ozonation and roughing filtration) to improve overall system performance, particularly for treating substantially polluted water sources. Ochieng *et al.* (2004) demonstrated that MSF can remove both suspended solids and turbidity similar to or better than conventional WTPs, and that MSF systems greatly reduce bacteria requiring less disinfection. An MSF with a horizontal flow roughing filter was capable of removing 99 and 98% of *Escherichia coli* and total coliforms (Ochieng *et al.* 2004), respectively, consistent with treated water from two commissioned rural Canadian MSS filter WTPs shown to be free of microorganisms (Gottinger *et al.* 2011). The water treatment community remains sceptical regarding the potential of biological filters to release their associated microorganisms. Sketchell *et al.* (1997) showed that activated carbon fines release some microorganisms by biomass sloughing and transport, but subsequent research identified these organisms as common non-pathogenic species not posing health risks (Sketchell *et al.* 1997; Bouwer & Crowe 1988; Camper & Jones 2000; Amburgey *et al.* 2005). Further barriers to the uptake of SSF and modified SSF treatments often relate to the unfamiliarity of consulting engineers and regulatory agencies with the technology.

This research was undertaken, in part, to demonstrate within a formal Environmental Technology Verification (ETV) process that MSS filter treatment technologies can constitute reasonable, safe and effective options for rural and remote communities. The Mainstream Water Solutions Inc. filtration processes operate similarly to conventional SSF, allowing growth of biological activity within the media bed. The system consists of an MSS filter and a biological activated carbon (BAC) filter. Modified maintenance procedures for the MSS filter involved hydraulic filter cleaning rather than scraping and re-sanding, as for conventional SSF. The biological activity combined with physicochemical removal and/or uptake processes are designed to sufficiently purify ground and/or surface water to meet or exceed drinking water standards.

To test this expectation, a fate and transport monitoring research project was initiated for the MSS and BAC filtration processes to remove microsphere analogues simulating *C. parvum* oocyst sizes ($d = 4$–$4.9\ \mu m$; mean $d = 4.5\ \mu m$). A rural raw water source (Craik, Saskatchewan) was seeded with these microspheres, and the filtration units operated under a variety of conditions to test filter ruggedness and robustness to remove oocyst-sized particles. The *C. parvum* oocysts have a d between 3.5 and 6 μm, and experiments conducted on the laboratory scale have shown that microspheres of this size closely match oocyst behaviour in terms of breakthrough velocity and filtration efficiency (Amburgey 2002). The microspheres exhibit similar peak arrival (breakthrough) time and attenuation within solids. However, it is important to note that: (1) oocyst structure is much more complex than that of microspheres; and (2) microsphere transport in some tests may be quite different from that of oocysts, thus resulting in either over- or under-predicted transport in both laboratory- and field-scale investigations. Therefore, care should be exercised when microspheres are employed as analogues for the filtration and removal of oocysts (Dai & Hozalski 2003; Mohanram *et al.* 2011). Here, we evaluate the MSS and BAC filter components of an SSF system for its ability to effectively and consistently remove the protozoan pathogen sized particles from water.

METHODS AND MATERIALS

The verification tests were designed to operate each of the MSS and BAC filters independently to assess their ability to remove particles in the size range of *Cryptosporidium* oocysts, and to recover particle removal efficiency after maintenance activities (backwash). Experiments were conducted on complex surface water (high organics, total dissolved solids and colour) at low temperature (<5 °C) without pre-treatment.

Four seeding and simultaneous filtration events were used to determine the performance of MSS and BAC filters under routine operation at near terminal headloss (NTH), and their recovery time required to return to turbidity <1 NTU (nephelometric turbidity unit) and efficient particle removal after backwash.

Pilot WTP design and operation

The system (Figure S1, available online at http://www.iwaponline.com/jws/062/104.pdf) included pilot-scale MSS and BAC filters along with a treated water storage tank and a microsphere injection system composed of a flowmeter, a Carter Manostat cassette pump (Fisher Scientific, Edmonton, AB) and a static mixer. A detailed description of the system components is provided in Table 1. Verification testing was conducted in a small rural community of approximately 400 people whose surface water source is 1.5 km from the WTP. A sodium chloride (Acros Organics) tracer test was used to determine the true hydraulic residence time (HRT) of both the MSS and BAC filters and the time required for the filters to reach steady state.

Table 1 | Pilot MSS and BAC filter specifications

Unit process	Specifications
Modified biological slow sand (MSS) filter	• 107 cm (42′) $d \times 213$ cm (84′) h tank • Piping: 3.2 cm (1.25′) d Schedule 80 PVC with true union valves • Media: pea gravel [3.0–3.5 mm, d_{10}; uniformity coefficient, $d_{60}/d_{10} < 1.7$] (base); filtration sand [0.3–0.4 mm, d_{10}; uniformity coefficient, $d_{60}/d_{10} < 1.6$] (bed) • Filter surface area: 0.89 m^2 (9.62 ft^2) • Total bed depth: 107 cm (30.5 cm base material; 76.5 cm sand bed) • Volume of filter material: 0.95 m^3 • Bed volume = 270.64 L (55 min) • HRT = 3.5 h
Biological activated carbon (BAC) filter	• 66 cm (26′) $d \times 165$ cm (65′) h tank • Piping: 3.2 cm (1.25′) d Schedule 80 PVC with true union valves • Media: pea gravel [3.0–3.5 mm, d_{10}; uniformity coefficient, $d_{60}/d_{10} < 1.7$] (base); filtration sand [0.3–0.4 mm, d_{10}; uniformity coefficient, $d_{60}/d_{10} < 1.6$]; activated carbon [0.7–0.9 mm, d_{10}; uniformity coefficient, $d_{60}/d_{10} < 2.1$] • Filter surface area: 0.34 m^2 (3.69 ft^2) • Total bed depth: 89 cm (30.5 cm base material; 30 cm sand bed; 28.5 cm biological carbon bed) • Volume of filter material: 0.31 m^3 • Bed volume = 121.6 L (25 min) • HRT = 1.25 h
Product water storage	• 66 cm (26′) $d \times 165$ cm (65′) h tank

PVC, polyvinyl chloride; h, height, d, diameter; d_{60}/d_{10}, uniformity coefficient.

During the testing period, the water flow rate was maintained between 5 and 5.2 litres per minute (LPM), corresponding to a hydraulic loading rate of between 0.34–0.35 and 0.88–0.92 m/h for MSS and BAC filters, respectively. The hydraulic loading rate of MSS filters is similar to that of the full-scale SSF employed in WTPs. The activated carbon (Hydrodarco 4000, Norit Americas, Inc.) used in the BAC filter was biologically ripened carbon obtained from the municipal WTP BAC filter, ensuring that it was biologically active and did not simply function as granular activated carbon. Both filters were installed and operating 4 months prior to the commencement of testing to ensure they were biologically active. This time frame is consistent with the ripening period experienced for a similar full-scale municipal system operating with that water source.

Filters were backwashed manually using collapse pulsing (sub-fluidization) by applying a light air scour and clean wash water upward through the filter under-drain. The water flow rates for the MSS and BAC filters were 179 and 468 LPM/m^2 at 193 kPa, respectively, while the airflow rates were 225 and 588 LPM/m^2 at 276 kPa. The municipal plant (product water and air compressor) was used for backwashing filters when significant headloss (15 cm) developed. The approximation of NTH was determined on the basis of three factors: (1) visual observations of the operating head through site glasses installed on filters, (2) review of backwash frequency and clogging of pilot equipment since its operation and (3) review of municipal WTP backwash intervals treating the same raw water source but with ozone and roughing filter pre-treatment. The simplified backwash procedures included: (1) filter isolation, (2) partial filter head drain, (3) reverse filter flow, (4) dirty water drainage from filter surface, (5) filter drainage to reduce turbidity (<1 NTU) and (6) return to service.

Performance verification testing included four filtration trials with continuous seeding (Table 2) to determine the performance of the MSS and BAC filters when operating at NTH, and immediately after backwashing.

Table 2 | Description of filtration experiments during verification testing

	MSS NTH	MSS backwash (MSS-BW)	BAC NTH	BAC backwash (BAC-BW)
Duration (h)	12	11	8	21
Water volume treated (L)	3,679	3,373	2,453	6,439
Bed volumes treated (L)	13.6	12.5	20.2	53.0
Microspheres				
Colour	Nile red	Nile red	Yellow	Yellow
Influent suspension (n/L)[a]	50,000	50,000	20,000	20,000
Number of sampling intervals (after steady state)	11	12	10	10

[a]Number spheres per litre, n/L.

Evaluation of feed and finished water, and operational conditions

Influent and effluent water quality parameters were monitored throughout the filtration experiments to provide the most comprehensive indication of filter performance. SSF turbidity, a key parameter, is regulated at <1 NTU in 95% of measurements (SK Environment 2002). Onsite turbidity readings were measured (HACH 2100P portable turbidimeter), along with pH and temperature (HACH SensION 256 multimeter, with platinum pH probe). Filter flow rate was determined by installing a flowmeter (Blue-White micro digital with 6.35 mm fitting; 30–7,000 mL/min) on the MSS filter influent and verified manually using bucket and stopwatch measurements of filter effluent flow.

Microsphere preparation and seeding

Yellow (FCM-4052-2; 1.13×10^8/mL) and Nile red (FCM-4056-2; 1.6×10^8/mL) fluorescent carboxylate-coated polystyrene microspheres (4–4.9 μm; Spherotech; Lake Forest, IL) were used as substitutes for the *Cryptosporidium* oocysts. Stock microsphere suspensions (10^8/L) were mixed using BAC filter effluent water and stored in foil-wrapped beakers to prevent photobleaching and continuously stirred to prevent aggregation. The Carter Manostat cassette pump continuously applied suspension to the influent line of the selected filter and was mixed post-injection statically (clear PVC pipe with 1 mm glass beads). Based on evaluation of feedstock concentration, injection rate and flow rates in comparison to filter influent enumerations (which were directly on target, n/L), the static mixer performed as intended without filtering. There was a separate injection point in front of the BAC filter to inject suspension for separate trials.

For the MSS filter challenge, influent water was seeded with microsphere suspensions (52,000–57,000 n/L), corresponding to that required for a 3 log decimal elimination capacity (DEC) (NSF/ANSI Standard 53). For these trials, microsphere suspensions (24,000–26,000 n/L) suitable to determine a 2 log DEC were supplied to the BAC filter. A constant flow rate was maintained throughout the experiments to ensure a uniform microsphere concentration. Microspheres were applied to filters until the final effluent sample was collected.

Sample collection, microsphere enumeration and analysis

All effluent samples (1 L) were collected in triplicate every consecutive hour, starting at hour 1, which corresponded to the void volume (minimum exit time for microspheres and less than the time required for steady state). Influent water samples were also collected every hour after seeding to assess the proper injection volume to deliver a precise amount of microspheres. The results of these samples, taken directly at the influent of the filter, were used to calculate log reductions for the filters. Microsphere number in the feed stock was verified by enumerating serial dilutions (10^{-3}) of microsphere suspensions. Samples for microsphere enumeration were vigorously shaken, filtered (3.0 μm Nucleopore Track Etch polycarbonate membrane) on a filter holder (Millipore) with a vacuum pump and then transferred to a slide for manual enumeration by fluorescence microscopy (Motic A30) with corresponding green (excitation wavelength (λ_{ex}) = 500 nm; emission wavelength (λ_{em}) = 525 nm) or red (λ_{ex} = 540 nm; λ_{em} = 605 nm) optical filter cubes. Triplicate samples were enumerated and the arithmetic mean calculated to arrive at one discrete data point representing operation at a specific time during a seeding event. Standard microsphere suspensions (1,000 n/L) were prepared in a volumetric flask and used for comparison.

Data analysis

The DEC (log removal) was calculated using the following relationship:

$$\mathrm{DEC} = \log_{10}\{[C_{\mathrm{Inf}}]/[C_{\mathrm{Eff}}]\} \quad (1)$$

where C_{Inf} and C_{Eff} are the number of microspheres counts in 1 L of influent and effluent water, respectively. All data are reported as mean ± standard deviation with confidence intervals calculated at 95% as follows:

$$X \pm t_{n-1},\ 1 - a/(2(\mathrm{SD}/\sqrt{n})) \quad (2)$$

where X is the mean, t is the distribution value with $n - 1$ degrees of freedom, n is the number of measurements, a is the significance level of 95% and SD is the standard deviation.

RESULTS AND DISCUSSION

Supporting documentation and data are available within the ETV report (Physical Removal of *Giardia* and *Cryptosporidium*-sized Particles from Drinking Water; http://www.etvcanada.ca/English/PMVP.aspx) that was the basis for this study.

Influent water quality

The source water for this study was chosen to provide a challenging water matrix for *Cryptosporidium* removal. Water quality data are presented in Table S1 (available online at http://www.iwaponline.com/jws/062/104.pdf), indicating that the source water was high in organic material, metal ions and conductivity, moderate in alkalinity and low in pathogens. The water temperature and pH during the testing period were 3.4 ± 0.3 °C and 8.05 ± 0.01, respectively. The turbidity of the influent water averaged 1.46 NTU.

Microsphere removal by MSS and BAC filters

Hydraulic retention time analysis of the MSS and the BAC filters using an NaCl tracer test confirmed that steady state was reached in 4.33 and 1.33 h, respectively (data not shown), and samples collected after this time period were used for enumeration. Based on preferred flow-path structure and size exclusion effects (Mohanram *et al.* 2011), microsphere flow velocity through the filters was expected to be much faster, reaching steady state long before the NaCl tracer. Thus, the only data considered were those collected once steady state was reached.

Effluent water quality data are summarized in Table S2 (available online at http://www.iwaponline.com/jws/062/104.pdf). Microsphere enumeration in stock suspensions and influent feed (Table 3) indicate that microsphere seeding exceeded target values (50,000 and 20,000 n/L). The mean log removal of the MSS filter for duplicate experiments, when operation was approaching terminal headloss (0.34 m/h hydraulic loading rate), was 2.22 ± 0.08. The MSS filter effluent turbidity was 0.46 ± 0.02 NTU.

Similarly, following backwash and a return to turbidity below 1 NTU, the average log removal of the MSS filter was 2.19 ± 0.08 (Table 3). Turbidity was reduced to 1 NTU at an interval coinciding with steady state (4.33 h). The mean log removal for the BAC filter operating at 0.89 m/h NTH was 2.19 ± 0.09 (Table 3) and turbidity in the BAC filter effluent was 0.52 ± 0.01. The BAC filter was aggressively backwashed, therefore, turbidity did not return to below 1 NTU until 3.5 h. The filtration experiment continued for 95 h; however, the BAC filter did not achieve log reductions above 2 as experienced in pre-backwash. Filter performance remained stable with DEC above 1 log and turbidity below 1 NTU. The mean log removal for the BAC filter post-backwash was 1.37 ± 0.08.

Removal of *Cryptosporidium* surrogates

The results of this study clearly demonstrate the capacity of MSS and BAC filters to successfully remove *C. parvum*-sized microsphere surrogates from raw water. During prolonged loading of the MSS filter at steady state, it was able to consistently remove particles with a minimum DEC of 2 log.

Slow sand filters are economical, simple to operate and suitable for small-scale municipal drinking water facilities (Logsdon *et al.* 2002). Slow sand filters have demonstrated the potential to remove most human toxins (Bourne *et al.* 2006) and pathogens (Grutzmacher *et al.* 2002; Weber-Shirk 2002; Dizer *et al.* 2004; Rooklidge *et al.* 2005; Stauber *et al.* 2006; Hijnen *et al.* 2007; Elliott *et al.* 2008; Jenkins *et al.* 2011).

Although traditional slow sand filters have been studied for DEC, there are limited data available on the removal capacity for oocyst-sized particles for more modern MSS filters. A similar study of an MSS filter reported a removal capacity of 100 and 99.98% for *Giardia* and *Cryptosporidium* oocysts, respectively (Palmateer *et al.* 1999).

The BAC filter was less capable of resuming pre-backwash particle removal efficiency after an aggressive filter cleaning; however, it effectively reduced the particle number by at least 1 log under these conditions. It is typical for BAC filters to be employed as either second or third stage treatment (after MSS filters), and in this instance, the BAC filter was operating in harsh conditions and fed raw water with no pre-treatment.

Effect of water quality on microsphere removal

The present study demonstrates that the combination of MSS and BAC filters have the capacity to remove oocyst-sized particles from a very low temperature (3–4 °C), high

Table 3 | Summary of influent and effluent microsphere numbers, log removal and filter effluent turbidity for MSS and BAC filters at near terminal headloss (NTH) and after backwash (BW)

	Influent particles (n/L) [ave. ± SD)	Effluent particles (n/L) [ave. ± SD]	Percent removal [ave. ± SD]	Log reduction [ave. ± SD]	Turbidity [ave. ± SD]
MSS-NTH	57,600 ± 4,800	363 ± 55	99.37 ± 0.09	2.22 ± 0.08	0.46 ± 0.02
95% confidence interval	52,562; 62,639	326; 400	99.30; 99.44	2.16; 2.27	0.45; 0.047
MSS-BW	52,200 ± 2,200	346 ± 60	99.34 ± 0.11	2.19 ± 0.08	N/A[a]
95% confidence interval	48,700; 55,700	307; 384	99.27; 99.41	2.13; 2.24	N/A[a]
BAC-NTH	26,600 ± 5,600	174 ± 39	99.35 ± 0.15	2.19 ± 0.09	0.52 ± 0.01
95% confidence interval	19,649; 33,551	146; 202	99.24; 99.46	2.12; 2.26	0.49; 0.55
BAC-BW	24,200 ± 4,000	1,048 ± 181	95.67 ± 0.75	1.37 ± 0.08	N/A[a]
95% confidence interval	19,234; 29,166	918.30; 1,177.24	95.14; 96.21	1.31; 1.43	N/A[a]

[a]Backwash influenced turbidity variability, precluding mean value calculations.

carbon content water source without any pre-treatment. Previous studies demonstrate the impact of different variables including media grain size, hydraulic loading rate, influent water composition and temperature on particle removal capacity. Logan *et al.* (2001) studied the transport and behaviour of *Cryptosporidium* in intermittent sand filters and concluded that larger filter grain sizes and high hydraulic loading rates had a detrimental effect on *Cryptosporidium* removals, but that there was no difference in oocyst removal efficiency when spiked into clean or waste water. The studies by Logan *et al.* confirmed the earlier findings of Chauret *et al.* (1995), which showed no correlation between pH, turbidity and effluent oocyst concentration.

The present study appears to support these earlier studies, indicating that the removal of *Cryptosporidium* and *Giardia* surrogates was not affected by the influent water turbidity, pH, organic content or high ionic content. However, since influent turbidity, pH, organic and ionic content were relatively stable throughout the pilot testing, it is not possible to definitively state that significant changes in influent quality may not have resulted in changes to the removal of the *Cryptosporidium* and *Giardia* surrogates. The relationship between turbidity, filter backwash and log reduction for both the MSS and BAC filters is presented in Figures S2 and S3 (available online at http://www.iwaponline.com/jws/062/104.pdf), showing that the MSS filter resumed pre-backwash DEC despite somewhat elevated effluent turbidity. Taken together, these results demonstrate the relevance of <1 NTU turbidity standards for the production of safe water by SSFs.

On the other hand, the BAC filter did not resume pre-backwash log reduction efficiency, despite an effluent filter turbidity of <1 NTU. While normal filter cleaning procedures were followed for this smaller filter, it was backwashed at higher air and water pressure than recommended, simply as a function of equipment limitations. Thus, it is difficult to ascertain whether the log removal pattern observed after cleaning is characteristic of this multimedia filter or a function of this unusually aggressive cleaning. Nevertheless, such results highlight the danger of relying on turbidity standards as surrogate indicators for safe drinking water from multimedia filters.

The current study further demonstrates that microsphere removal was not affected by the low influent water temperature (<5 °C) used throughout this study, contrary to a study conducted by Fogel *et al.* (1993) showing that SSF at lower temperatures had reduced oocyst removal efficiency. The results of the latter study by Fogel *et al.* could be explained by reduced performance as a result of decreased biological activity at colder temperatures. However, the satisfactory performance observed in the current study supports the conclusions of Weber-Shirk & Dick (1997a, 1999), and indicates that the majority of oocyst removal is a function of physical screening rather than biological processes, where internalized oocysts appear at a frequency 100-fold lower than free oocysts (Bichai *et al.* 2010).

Dissolved organic matter has also been shown to have a detrimental effect on oocyst removal. Dai & Hozalski (2003) showed natural organic matter (NOM) significantly decreased oocyst removal by biological rapid filters, proposing that it increased the negative surface charge on the oocysts, enhancing electrostatic repulsive forces between the media and the oocysts. The present study shows the efficient removal of oocyst surrogates, even at a very high organic content (~22 mg/L), which again may reflect the difference between the ability of oocysts and abiotic surrogates to adsorb to NOM. If anything, the microsphere surrogates likely underestimate oocyst removal, as oocysts of *C. parvum* adhere when in intimate contact with model sand surfaces, a process possibly mediated by surface proteins (Considine *et al.* 2002).

Since microsphere surrogates are likely not subject to predation as are pathogenic oocysts (Bichai *et al.* 2010), their fate and transport may slightly differ. However, uptake and retention through other biological and physical removal processes are anticipated to be within reasonable agreement with results obtained from the microsphere surrogate pilot.

CONCLUSIONS

The efficacy testing of the MSS and BAC filtration system to remove *C. parvum* oocyst-sized particles using 4–4.9 μm microsphere surrogates revealed reductions of greater than

2 and 1 log, respectively. This study demonstrates that MSS and BAC filters can remove oocyst-sized particles in extreme conditions such as low temperature and high organic content. Combining MSS and BAC filtration gives rise to systems equal in potential to conventional slow sand filters to provide safe, effective and economical treatment solutions for small-scale municipal drinking water systems.

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