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Review

Drinking water treatment processes for removal of *Cryptosporidium* and *Giardia*

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Abstract

Major waterborne cryptosporidiosis and giardiasis outbreaks associated with contaminated drinking water have been linked to evidence of suboptimal treatment. *Cryptosporidium parvum* oocysts are particularly more resistant than *Giardia lamblia* cysts to removal and inactivation by conventional water treatment (coagulation, sedimentation, filtration and chlorine disinfection); therefore, extensive research has been focused on the optimization of treatment processes and application of new technologies to reduce concentrations of viable/infectious oocysts to a level that prevents disease. The majority of the data on the performance of treatment processes to remove cysts and oocysts from drinking water have been obtained from pilot-tests, with a few studies performed in full-scale conventional water treatment plants. These studies have demonstrated that protozoan cyst removal throughout all stages of the conventional treatment is largely influenced by the effectiveness of coagulation pretreatment, which along with clarification constitutes the first treatment barrier against protozoan breakthrough. Physical removal of waterborne *Cryptosporidium* oocysts and *Giardia* cysts is ultimately achieved by properly functioning conventional filters, providing that effective pretreatment of the water is applied. Disinfection by chemical or physical methods is finally required to inactivate/remove the infectious life stages of these organisms. The effectiveness of conventional (chlorination) and alternative (chlorine dioxide, ozonation and ultra violet [UV] irradiation) disinfection procedures for inactivation of *Cryptosporidium* has been the focus of much research due to the recalcitrant nature of waterborne oocysts to disinfectants. This paper provides technical information on conventional and alternative drinking water treatment technologies for removal and inactivation of the protozoan parasites *Cryptosporidium* and *Giardia*.

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Keywords: *Cryptosporidium parvum*; *Giardia lamblia*; Drinking water treatment; Removal; Inactivation

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1. Introduction

1.1. Drinking water supply and regulatory activity in the USA

Protecting drinking water supplies against the parasitic protozoa *Cryptosporidium parvum* and *Giardia lamblia* is a major concern for water utilities worldwide. The guiding principle for providing safe water is the multiple-barrier concept that involves source water protection (surface and groundwater sources), optimization of the water treatment plant process and a properly maintained distribution system. [Tables 1 and 2](#) describe the treatment processes and filtration schemes in current use at large and medium-size water utilities across the USA.

In the United States, the US Environmental Protection Agency (USEPA) establishes national drinking water regulations under the Safe Drinking Water Act (SDWA), which was originally enacted in 1974 and further reauthorized in 1986 and 1996. USEPA's drinking water regulations have been developed, implemented, and revised under this law. [Roberson \(2003\)](#) and [Pontius \(2002, 2003\)](#) provide excellent reviews on the evolution, complexity, and current status of drinking water regulatory activity in the USA.

The Surface Water Treatment Rule (SWTR), promulgated in 1989, constituted one of the first regulations that used treatment technology to control *Giardia* in water by requiring 3-log cyst removal or inactivation ([USEPA, 1989](#)). The 3-log (99.9%) removal is accomplished by properly operated treatment plants, which achieve 2-log removal by conventional treatment and then requiring the disinfection process to achieve the remaining

Table 1
Water treatment processes used at large and medium-size water utilities in the USA^a

Process	Number of facilities	Percent ^b
Filtration	170	84.6
Clearwell or finished water reservoir	166	82.6
Flocculation	142	70.6
Fluoridation	140	69.7
Corrosion control	135	67.2
Sedimentation	131	65.2
Mixing basin or rapid mix	123	61.2
Disinfection contact basin	78	38.8
Preoxidation	55	27.4
Upflow solids clarifier	49	24.4
Softening	37	18.4
Raw water storage or presedimentation	36	17.9
Aeration or stripping	33	16.4
Other ^c	25	12.4

^a Survey conducted by the American Water Works Association (AWWA) Water Quality Division's System Committee.

^b Total more than 100% because some facilities had more than one process.

^c Recarbonation, activated carbon, powdered activated carbon, permanganate.

removal (Edzwald and Kelley, 1998). As with *Cryptosporidium*, the removal requirements for *Giardia* will also depend on the cyst concentration in the source water.

The USEPA promulgated the "Interim Enhanced Surface Water Treatment Rule" (IESWTR) on 16 December 1998, as a mean to control *Cryptosporidium* in drinking water. Within the regulation, compliance is defined by performance requirements for water treatment plants and by monitoring indices (e.g. turbidity, performance of individual filters) that aim to optimize the filtration process and in some cases the disinfection process. The regulation is still undergoing some revisions (USEPA, 1998, 2002). The key provisions in the IESWTR establish a Maximum Contaminant Level Goal (MCLG) of zero for

Table 2
Water filtration practices used at large and medium-size water utilities in the USA^a

Filtration practice	Number of facilities	Percent ^b
Dual media, rapid	93	54.7
Rapid sand, conventional	40	23.5
Granular carbon cap on any of the above filters	30	17.6
Trimedia, rapid	24	14.1
Do not filter	15	8.8
Granular carbon without any other filtration	5	2.9
Diatomaceous earth	1	<1
Slow sand	0	0
Other ^c	3	1.7

^a Survey conducted by the American Water Works Association (AWWA) Water Quality Division's System Committee.

^b Total more than 100% because some facilities had more than one type of filtration.

^c Mixed media; deep bed, high rate; membrane.

Cryptosporidium and require a 2-log₁₀ (99%) *Cryptosporidium* removal when using filtration only. According to this rule, public drinking water sources with levels of *Cryptosporidium* >0.075 oocysts/L (7.5 oocysts/100 L) will be required to upgrade to 3-log (99.9%) removal or inactivation; levels >1 oocyst/L (100 oocysts/100 L) will elicit a requirement to provide 4-log (99.99%) removal or inactivation; and >3 oocysts/L (300 oocysts/100 L) will elicit a requirement to provide 4.5-log (99.995%) removal or inactivation of *Cryptosporidium*. The IESWTR applies to public water systems that use surface water or ground water under the direct influence of surface water and serve 10,000 or more people. A long-term ESWTR to extend the IESWTR to systems serving 10,000 or fewer people, known as the Long-term 1 ESWTR (LT1ESWTR) was proposed 10 April 2000 (USEPA, 2000). Key provisions of the final LT1ESWTR reflect those of the IESWTR.

The “Information Collection Rule” is another regulation implemented by the USEPA, which ran from July 1997 to December 1998 (Messner and Wolpert, 2000) to address source water quality and the need for treatment for both *Cryptosporidium* and *Giardia*. This has fed into the rule development for “Long-term 2 Enhanced Surface Water Treatment Rule” (LT2ESWTR; and <http://www.epa.gov/safewater/lt2/st2eswtr.html> for further information). The purposes of the proposed LT2ESWTR are to improve control of microbial pathogens, including specifically *Cryptosporidium*, in drinking water (USEPA, 2003). Under the LT2ESWTR, water plants using conventional treatment will require monitoring for *Cryptosporidium*, *E. coli* and turbidity for a period of 24 months. The results of the 2-year monitoring will be used for determining the level of treatment requirements for *Cryptosporidium*.

2. Principles of conventional water treatment processes

Conventional water treatment includes a series of processes (coagulation, flocculation, clarification through sedimentation, filtration and disinfection) that when applied to raw water sources contribute to the reduction of microorganisms of public health concern (Geldreich, 1996). While these processes have been evaluated for turbidity and *Giardia* removal (cyst size: 8 µm × 12 µm), it is only relatively recently that investigations into removal of the smaller *Cryptosporidium* (oocyst size: 4.5 µm × 5.0 µm) have been published.

2.1. Coagulation–flocculation

Coagulation is a primary processing step used to hasten the agglomeration of fine particles in turbidity. This process is followed by flocculation and combined constitute a solid–liquid separation process in water treatment for destabilizing dissolved and colloidal impurities and producing large floc aggregates that can be removed from the water in the subsequent clarification/filtration processes (Gao et al., 2002). Aluminum-based salts, iron-based salts (ferric chloride) or organic polymers are the most common water treatment coagulant chemicals. Precipitate enmeshment is considered the optimal mechanism of coagulation for removal of protozoan cysts in water treatment systems (Jakubowski, 1990;

Jakubowski and Craun, 2002; Butkus et al., 2003). Studies have demonstrated that *Cryptosporidium* removal throughout all stages of the conventional treatment process is largely influenced by the effectiveness of coagulation pretreatment (Dugan et al., 2001).

Enhanced coagulation and enhanced precipitative softening are two treatment techniques included within the Stage 1 of the Disinfectants/Disinfection By-product Rule [D/DBPR] (USEPA, 1998). Briefly, this rule applies to community water systems that treat their water with a chemical disinfectant for either primary or residual treatment as a mean to reduce the levels of disinfectants and disinfection by-products (DBPs) in drinking water supplies. The goal of the treatment techniques is to provide additional removal of DBP precursors (i.e., natural organic matter (NOM)) for US water systems using surface waters or groundwater under the direct influence of surface waters. Enhanced coagulation is defined as the process of obtaining improved removal of DBP precursors through modified conventional treatment that includes reduction of pH to levels of 5–6 and the use of higher doses of coagulants (States et al., 2002). Pilot-scale trials were conducted by States et al. (2002) to study the enhanced coagulation approach on *C. parvum* removal using different coagulants (ferric chloride, polyaluminum chloride and alum). The mean log unit *Cryptosporidium* removal attributable to this treatment approach was 5.8-log units and no impairment on oocyst removal was observed due to pH reduction. Lime softening is a process that uses chemical precipitation with lime and other chemicals to promote the removal of hardness and particle matter (Cornwell et al., 2003). Logsdon (1994) reported oocyst removals ranging from 2.5 to 3.5 logs from 13 full-scale lime-softening plants. Bell (2000) reported a 2-log reduction of *Cryptosporidium* and *Giardia* during precipitative lime softening in bench-scale jar tests.

2.2. Clarification

Clarification is the first treatment barrier against protozoan passage during conventional water treatment (Edzwald and Kelley, 1998). This process is accomplished through sedimentation, which allows large floc-particle masses to settle prior to filtration (Jakubowski and Craun, 2002). Dissolved air flotation (DAF), which is a clarification process alternative to sedimentation, allows removal of fragile floc particles found in water treatment via adherence to air bubbles (Braghetta et al., 1997; Edzwald et al., 2000; French et al., 2000). Although the SWTR and the IESWTR do not address DAF plants for *Giardia* and *Cryptosporidium* removals, bench-scale and pilot-plant studies have demonstrated that DAF is much more effective than sedimentation for removal of protozoan cysts (Plummer et al., 1995; Edzwald and Kelley, 1998; Edzwald et al., 2000).

2.3. Filtration

Physical removal of turbidity and microorganisms from water is ultimately accomplished by filtration. Filters within a conventional water treatment process are considered as the last barrier to the release of particles and protozoan cysts into the distribution system (Cornwell et al., 2003). During filtration, water passes through a pore structure made up of a variety of bed materials that can be composed of the following: (i) a bed of sand (sand filtration) or (ii) a layer of diatomaceous earth (diatomaceous earth

filtration), or (iii) a combination of coarse anthracite coal overlying finer sand (dual- and tri-media filtration) (Trojan and Hansen, 1989). The removal of particles in suspension occurs by straining through the pores in the filter bed, by adsorption of the particles to the filter grains, by sedimentation of particles while in the media pores, by coagulation while traveling through the pores, and by biological mechanisms such as slow sand filtration (Trojan and Hansen, 1989). The latter is accomplished by the filtering action of the *schmutzdecke*. The *schmutzdecke* is the top layer (a few centimeters in depth) of sand and particulate materials (fine soil particles, plant debris, algae, free-living or non-pathogenic protozoa) that have been removed from the water as it percolates downward through the sand filter bed (Fox and Reasoner, 1999).

2.4. Disinfection

Disinfection is the process by which an organism's viability/infectivity is destroyed with a specific percentage of the population dying over some time frame defined as a rate. Water disinfection is accomplished with chemical or physical disinfectants and the most common of these is chlorine (added to water as a gas or solid) and the specific disinfection referred to as chlorination. While it was known that *Giardia* was much more resistant than bacteria to such disinfection it was possible to kill the cysts given a high enough concentration of the disinfectant and contact time (Korich et al., 1990; Finch et al., 1994). However, *Cryptosporidium* is one of the most resistant organisms in water and no inactivation was observed even after 18 h of contact time with chlorine at very high levels, and no inactivation was seen with chloramines (Korich et al., 1990; Gyürék et al., 1997). Thus the primary target for effective disinfection for protozoa has been on *Cryptosporidium*.

Alternative disinfectants including chlorine dioxide, ozone and ultra violet (UV) irradiation are now the focus of much research. Chlorine dioxide can inactivate oocysts (about 90%); however UV light and ozone have received much more attention (Peeters et al., 1989). Ozone and UV have many similar advantages and disadvantages. UV technology was first utilized in water treatment in Ft. Benton, MT, USA in the early 1970s (Wolfe, 1990). Ultraviolet light has several advantages: (i) it is a physical process that does not rely on the use of chemical additions; (ii) it has been shown to be highly effective in the inactivation of protozoa, while viruses remain the most resistant; (iii) it requires relatively short contact times; and (iv) no UV disinfection by-products have been currently identified. The disadvantages are: (i) differences in output amongst various types of UV lamps, reactor design and scale-up issues; (ii) inability to measure the lamp dose in practice; (iii) interference by turbidity; and (iv) no lasting residual disinfection effect.

It is now fairly well established that vital dyes such as 4,6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) and even in vitro excystation cannot be relied upon to accurately estimate *Cryptosporidium* oocyst infectivity post-UV treatment, and that some measure of infectivity was required using animals or cell culture. Vital dye inclusion/exclusion has been used as a measure of the integrity of an oocyst's outer wall as well as its inner cytoplasmic and nuclear membranes (Smith et al., 1991; Campbell et al., 1992; Jenkins et al., 1997). Excystation measures the enzymatic capabilities of the oocyst to open up upon exposure to trypsin at 37 °C (Neumann et al., 2000). UV has been shown to affect

Table 3

Removal of *Cryptosporidium* oocysts and *Giardia* cysts at pilot- and full-scale conventional water treatment plants

Scale	Sources of parasites	Total log removal		Reference
		<i>Cryptosporidium</i>	<i>Giardia</i>	
Pilot plant	Spiked: 10 ³ /L	–	1.75–4.0	DeWalle et al. (1984)
Pilot plant	Spiked: 10 ³ /L	–	>5	Bellamy et al. (1985)
Pilot plant			>2 to >3	Jakubowski (1990)
Full-scale plants	Environmental	2.2–2.4	2.0–2.5	LeChevallier et al. (1991)
Full-scale plants	Environmental	1.4–1.8	1.5	Kelley et al. (1995)
Full-scale plants	Spiked: 10 ⁷ (oo)cysts	1.9–2.8	2.8–3.7	Nieminski and Ongerth (1995)
Pilot plant	Spiked: 10 ⁴ (oo)cysts/mL	1.9–4.0	2.2–3.9	Nieminski and Ongerth (1995)
Full-scale plant	Environmental	1.5	1.5–1.7	States et al. (1997)

the DNA such that, while the membranes and enzymes seem to be intact, the organism is no longer capable of reproducing (Huffman et al., 2000; Morita et al., 2002). Huffman et al. (2000) reported that vital dyes overestimated *Cryptosporidium* infectivity, while predictions using the Focus Detection Method-Most Probable Number (FDM-MPN) cell culture method were comparable to the use of animal infectivity. Morita et al. (2002) reported excystation also overpredicted infectivity by demonstrating resistance up to 100 times greater UV doses using excystation compared to animal infectivity.

Relatively low doses of UV (1–9 mJ/cm²) have been shown to inactivate 2–4 log₁₀ (99–99.9%) of *C. parvum* oocysts and *G. lamblia* cysts (Craik et al., 2000, 2001; Linden et al., 2002). Studies by Shin et al. (2001), Oguma et al. (2001) and Belosevic et al. (2001) have shown that while *C. parvum* oocyst have the capability to repair UV-induced pyrimidine dimers in their DNA the oocysts were not capable of recovering their infectious nature post-UV exposure. Oguma et al. and Belosevic et al. performed their studies using animal

Table 4

Water disinfection studies with ozone and UV used for inactivation of *Giardia* cysts and *Cryptosporidium* oocysts

Disinfectant	Protozoan	Contact time concentration	Water condition	Log ₁₀	Reference
UV	<i>C. parvum</i>	50 mJ/cm ²	Treated filtered surface water pilot study	3.9	Bukhari et al. (1999)
UV	<i>C. parvum</i>	1 mJ/cm ²	Buffered saline	1.5	Zimmer et al. (2003)
UV	<i>C. parvum</i>	3 mJ/cm ²	Buffered saline	>3.2	Zimmer et al. (2003)
UV	<i>G. muris</i>	5 mJ/cm ²	Mili Q water	2	Craik et al. (2000)
UV	<i>G. lamblia</i>	1 mJ/cm ²	Buffered saline	>4	Linden et al. (2002)
Ozone	<i>C. parvum</i>	4 mg/L, 10 min	1 °C tap water	2	Corona-Vaszuez et al. (2002)
Ozone	<i>C. parvum</i>	2 mg/L, 1 min	20 °C tap water	2	Corona-Vaszuez et al. (2002)
Ozone	<i>G. muris</i> (50,000 cysts)	0.3 mg/L, 0.25 min	15 °C demand free buffer water	2	Haas and Kaymak (2003)
Ozone	<i>G. muris</i> (100 cysts)	0.3 mg/L, 3 min	15 °C demand free buffer water	2	Haas and Kaymak (2003)

infectivity assays while Shin et al. utilized both cell culture as well as animal infectivity analysis with comparable results between cell culture and animal infectivity assay. The inactivation of *Cryptosporidium* oocysts and *Giardia* cysts with UV is quite similar (Table 4). Linden et al. (2002) evaluated the kinetics and extent of inactivation of *G. lamblia* cysts infectivity by various doses of UV irradiation. The results of this investigation demonstrated that UV disinfection at practical doses (1 mJ/cm²) inactivated >4 log₁₀ *G. lamblia* cysts in water. Key results show that temperature does not affect the inactivation and have confirmed the inability for the parasites to reactivate post-UV exposure whether in the dark or light. Pilot studies with actual treated water and turbidities below 1 NTU could be designed to adequately control protozoa.

Ozone was first used as a disinfectant of drinking water in France almost 100 years ago. There are over 2000 drinking water treatment plants in the world using ozone and over 40 plants that use ozone have been built in the USA in the last two decades (Tate, 1991). Advantages of ozone disinfection are: (i) it is a highly effective disinfectant for all groups of microorganisms, particularly viruses and bacteria; (ii) it produces very few disinfection by-products; and (iii) ozone generators can treat high volumes of water. The disadvantages of ozone are: (i) it can produce bromate as disinfection by product if the water has bromide in it; (ii) there is no lasting residual effect; and (iii) reduced efficacy in cold water.

In temperate climates ozone may be appropriately used for disinfection and fairly low doses (0.3–1 mg/L) for very short contact times (1 min) are capable of inactivating up to 99% of cysts and oocysts (Table 4). However, studies on the inactivation kinetics for ozone disinfection of *C. parvum* and *G. muris* have demonstrated several phenomena: (i) inactivation is characterized by a lag phase followed by pseudo-first order kinetics (Rennecker et al., 1999); (ii) disinfection is temperature dependent with dramatic increases in concentration and contact time need to achieve inactivation with increasing temperatures; and (iii) initial concentration of *Giardia* cysts influenced the inactivation. The study by Haas and Kaymak (2003) is quite interesting because it suggests that in real world waters with low concentrations of organisms the inactivation rate is less than observed in seeded studies where the level of microorganisms is artificially elevated for the purpose of observing several orders of magnitude inactivation. These researchers put forth two hypotheses: first, that large concentration of organisms generates other disinfecting substances during the inactivation process, thus leading to a ripple effect; and second, that quorum sensing whereby sensitivity in the population to the disinfectant is influenced. Further studies on the biology of the protozoa and mechanisms of their destruction will be of significant interest.

3. Removal of *Cryptosporidium* and *Giardia* using conventional and advanced water treatment

The effectiveness of conventional and advanced water treatment techniques on removal of protozoan cysts has been evaluated through bench, pilot and/or full-scale water treatment systems (Jakubowski, 1990; Tanner and Ongerth, 1990; Nieminski and Ongerth, 1995; Kelley et al., 1995; Ongerth and Hutton, 1997; Swertfeger et al., 1999; Edzwald et al., 2000; States et al., 2000; Shaw et al., 2000; Huck et al., 2002). Bench-scale and

pilot-plant studies provide reliable information on protozoan cysts removal and process efficiency that can be used in a treatment technology-based approach to control protozoan parasites in water (Edzwald and Kelley, 1998; Edzwald et al., 2000). Removal of protozoa through the treatment process is expressed either as percent removal (i.e., 99%) or in terms of the logarithmic reductions (base 10). Log reductions are currently calculated as the difference between the \log_{10} of the influent concentration and the \log_{10} of the filtrate concentrate. Log removals that incorporate non-detects (no protozoan cysts detected in filtrate) are prefixed with the > symbol (Dugan et al., 2001; Jakubowski and Craun, 2002).

Cryptosporidium oocysts, like *Giardia* cysts, are organisms that can be physically removed from water supplies by conventional particle separation processes including chemical coagulation–flocculation, clarification (sedimentation), and granular media filtration (Bellamy et al., 1993; Dai and Hozalski, 2003). Efficient protozoan cyst removal can be achieved by properly functioning conventional filters when the water is effectively treated through coagulation, flocculation and settling prior to filtration (Jakubowski, 1990; Swertfeger et al., 1999; Shaw et al., 2000; Huck et al., 2002; Jakubowski and Craun, 2002; Emelko, 2003). Data on *Cryptosporidium* and *Giardia* removals through pilot- and full-scale conventional treatment plants are summarized in Table 3.

LeChevallier et al. (1991) examined 66 conventional water systems in the USA and found that most of the utilities achieved 2–2.5 \log_{10} cyst and oocyst removal by clarification and filtration as recommended by the SWTR. The investigation revealed that compliance with criteria outlined by the SWTR did not ensure that filtered water was free of waterborne parasites, therefore LeChevallier et al. indicated that high disinfection levels or more efficient disinfection procedures were ultimately required in order to protect against passage of the waterborne protozoa *Cryptosporidium* and *Giardia*. Water treatment plants using granular activated carbon (GAC) and rapid sand filters were more likely to have effluent samples positive for cysts and oocysts than those plants using dual- or mixed-media filters (LeChevallier et al., 1991).

Plummer et al. (1995) investigated the effectiveness of the clarification process (sedimentation versus dissolved air flotation) for the removal of *Cryptosporidium* oocysts under a variety of conditions. The results of this investigation demonstrated that oocyst removals were highest at pH of 5.0 when coagulant doses higher than those currently applied for turbidity removal were used. Oocyst reductions by sedimentation were below 1-log removal while those achieved by DAF were one or two orders of magnitude higher. Studies have shown that clarification through sedimentation provide *Cryptosporidium* removals of only 0.5–1 log (States et al., 1995).

Nieminski and Ongerth (1995) conducted a 2-year evaluation of *Giardia* and *Cryptosporidium* at a full-scale treatment plant and a pilot plant operating under conventional treatment and direct filtration regimes (without clarification). Consistent removal rates of protozoan cysts were achieved when the treatment plant produced water of consistently low turbidity (0.1–0.2 nephelometric turbidity units (NTU)). Removal of protozoan in seeding experiments conducted in the pilot plant averaged 3.40 log for *Giardia* and 2.9 log for *Cryptosporidium* while removals obtained for full-scale seeding experiments were of the order of 0.5 log less than in the corresponding pilot-tests. This study indicated that removal of cyst-size organisms and removal of turbidity could be used as indicators of the effectiveness of removal of cysts and oocysts.

States et al. (1997) investigated the efficiency of parasite removal in a full-scale conventional treatment plant and observed *Giardia* removal of 1.54 log and *Cryptosporidium* removal of 1.49 log. Small numbers of these protozoan cysts were found in finished water even in the absence of treatment problems; recycling of backwash water was considered a potential source of contamination to the treatment plant intake. The results of more recent studies have suggested that a conventional treatment plant can successfully treat spent filter backwash water (SFBW) for *Cryptosporidium* when such water is recycled continuously or intermittently without treatment before recycle (Cornwell et al., 2003).

Edzwald et al. (2000) evaluated removals of *Giardia* and *Cryptosporidium* by clarification (DAF and lamella sedimentation) combined with dual media filtration under challenge conditions of high cyst and oocyst levels. DAF and filtration together achieved average >5-log removals, which were comparable to those achieved by sedimentation and filtration. DAF clarification was superior to lamella sedimentation; the latter also provided a more effective barrier ahead of filtration. According to this investigation, particle counting was an excellent tool for monitoring process performance, however absolute numbers were not meaningful indicators of cysts or oocysts removal performance.

Shaw et al. (2000) demonstrated that the application of electropositive coatings with Fe Al (hydr) oxide to granular filtration media provided a 2.9-fold improvement in filter coefficient for removal of *C. parvum*. The increased removal was attributed to the change in zeta potential (from electronegative to electropositive) resulting from the coating, which decreased electrostatic repulsion between the sand and the electronegative *Cryptosporidium* oocysts. The study revealed that coated sand substantially increased the reliability of rapid and slow sand filtration systems and prevented breakthrough of *Cryptosporidium* oocysts during periods of suboptimal chemical conditioning.

Dugan et al. (2001) carried out pilot-scale tests to examine the impact of the filter media, filter-loading rates, and coagulant type on removal of *Cryptosporidium* seeded at high concentrations. Filtration removals of *Cryptosporidium* were not significantly different for the different filter media designs; however such removals were dramatically affected by suboptimal coagulation conditions (average 1.5 log). Optimal and enhanced coagulation conditions provided improved removal of *Cryptosporidium* oocysts and turbidity; turbidity was considered the most conservative indicator of total oocyst removal.

Diatomaceous earth filtration has been shown to be more effective than other conventional or granular media filtration in reducing concentrations of *Cryptosporidium* oocysts and *Giardia* cysts (Schuler and Ghosh, 1990; Ongerth and Hutton, 1997; Ongerth and Hutton, 2001). Up to 6-log *Cryptosporidium* removal can be expected under conditions practical in full-scale water treatment and several possibilities for application in municipal water treatment have been suggested (Ongerth and Hutton, 1997; Ongerth and Hutton, 2001).

4. Recent advances in membrane technology

Pressure-driven membrane processes (microfiltration [MF], ultrafiltration [UF], nanofiltration [NF], reverse osmosis [RO]) are playing an important role in drinking water production in the US and in Europe. These processes are being employed in water

treatment for multiple purposes including control of disinfection by-products (DBPs), pathogen removal, clarification, and removal of inorganic and synthetic organic chemicals (Jacangelo et al., 1997; Van der Bruggen et al., 2003). Low-pressure MF and UF, has received a great deal of attention as an alternative to conventional treatment and the removal of protozoan cysts has been well documented for selected membranes as it is described below (Jacangelo et al., 1995).

Potential mechanisms of action of low pressure membranes include: (i) sieving or size exclusion, (ii) adsorption to the membrane surface or internal structure, (iii) attachment to particles in the feedwater and subsequent removal by the membrane, (iv) removal by the cake layer formed at the membrane surface, (v) removal by non-hydraulically reversible membrane foulants, (vi) the characteristics of the membrane (i.e., charge). Mechanisms of removal depend on the microorganism and the chemistry of the solution being filtered (Jacangelo et al., 1995). MF membranes have the largest pores, ranging from 0.1 to 10 μm , and the highest permeability so that a sufficient water flux is obtained at a low pressure. MF is an efficient process to remove particles that may cause problems in further treatment steps. Applications of MF membranes in water treatment include clarification, pretreatment and particle and microbial removal (Jacangelo et al., 1997; Van der Bruggen et al., 2003). UF membranes have smaller pore sizes (0.002–0.1 μm), therefore the permeability is considerably lower than in MF and higher pressures are needed. Current applications of UF membranes in water treatment include particle and microbial removal. Physical sieving is considered as the major mechanism of removal of protozoan cysts. The pore sizes for MF and UF used in water treatment processes range from 0.01 to 0.5 μm , which is at least one order of magnitude lower than the size of protozoan cysts (4–15 μm) (Jacangelo et al., 1997).

It has been generally accepted that MF and UF can provide complete removal of all protozoan cysts of concern as long as the associated system components are intact and operating correctly. Recent investigations have demonstrated that different MF and UF membranes provide log removals of *C. parvum* oocysts and *G. muris* cysts ranging from >4 log to 6 log (Jacangelo et al., 1995, 1997).

5. Monitoring water systems

Monitoring has taken place for *Cryptosporidium* and *Giardia* throughout the world. The usefulness of monitoring has been acknowledged based on the recalcitrant nature of the protozoan cysts and the fact that the current indicator systems for water quality do not reflect the safety of the water in relation to the protozoa (Rose et al., 2002). Monitoring has been used for risk assessment purposes to determine the necessary treatment and risk to the population, to evaluate both pilot and full-scale water treatment system's reliability and efficacy, to examine sources in a watershed particularly wildlife and domestic animals, to determine the impact of rainfall, and to assist with epidemiological and waterborne outbreak investigations.

Laws governing water in both the USA and United Kingdom have utilized monitoring in the development of rules for protection of drinking water and public health. In the U.K., regulations have been developed for continuous monitoring post-filtration (using foam

filtration, immunomagnetic separation [IMS], and the immunofluorescence assay [IFA] for 1000 L with a standard of 1 oocyst/10L (100/1000 L) (Lloyd and Drury, 2002). The rationale is based on assessing and monitoring the efficacy of filtration systems. Given the costs of outbreaks the cost of monitoring was considered reasonable. As mentioned at the beginning of this chapter, laws and regulations for drinking water in the USA are governed by the “Safe Drinking Water Act”. Although it is not currently required by federal regulations voluntary routine monitoring for *Cryptosporidium* and *Giardia* have been implemented by some water utilities, typically once per month or once per quarter.

Detection of waterborne *Cryptosporidium* and *Giardia* require specialized equipment and procedural skills in order to provide reliable data that can be used for compliance monitoring and for determining microbial disease risks associated with drinking water (Rose et al., 2002). The USEPA validated and approved Method 1623 for simultaneous detection of waterborne *Cryptosporidium* and *Giardia*. USEPA method 1623 requires filtration, immunomagnetic separation of the cysts and oocysts, and an immunofluorescence assay for determination of protozoan concentrations, with confirmation through vital dye staining (4,6-diamidino-phenylindole (DAPI)) and differential interference contrast (DIC) microscopy (USEPA, 2003). Alternate procedures are allowed, provided that required quality control tests are performed and all quality control acceptance criteria in these methods are met. While method 1623 is now available for monitoring *Cryptosporidium* and *Giardia* in water perhaps the most powerful methods include molecular assays such as the polymerase chain reaction (PCR) alone or combined with cell culture infectivity assays (cell culture-PCR, CC-PCR). These methods have been used for genotyping and determining viability/infectivity and genotypes of waterborne *Giardia* cysts and *Cryptosporidium* oocysts, respectively (Xiao et al., 1999; Di Giovanni et al., 1999; Slifko et al., 1999; Xiao et al., 2001; Quintero-Betancourt et al., 2002, 2003; Guy et al., 2003). Such tools should be used in studies to further understand the transmission of these pathogens.

6. Concluding remarks

Cryptosporidium and *Giardia* remain two of the most important waterborne pathogens and while great advances have been made in water treatment, a better understanding of the mechanisms by which these parasites can be adequately controlled via new and innovative treatment, which can serve both developing and industrialized nations, is needed. This can only be accomplished via integrated studies, which examine the sources, concentrations, survival and transport of waterborne parasites, the impact of environmental factors, and finally the ability of treatment systems to reliably reduce the risk of protozoan waterborne disease.

The application of new methods such as USEPA method 1623 in combination with molecular and tissue culture methods has improved our ability to detect low levels of waterborne protozoa contamination. For instance, preliminary investigations have demonstrated the presence of low levels of the human pathogen *Cryptosporidium hominis* in finished effluents of conventional drinking water facilities (unpublished data). To meet the challenges of the stringiest drinking water regulations established to improve control of

Cryptosporidium, additional physical and chemical water treatments is required. As mentioned before, pressure-driven membrane processes such as microfiltration and ultrafiltration are playing an important role in drinking water production in the USA and in Europe. The use of UV light for drinking water disinfection is not common in the USA; however this alternative disinfection practice has been demonstrated to inactivate protozoa effectively, and is also amenable to the upgrading of conventional treatment plants. The multi-barrier approach for drinking water treatment in which a combination of various disinfectants and filtration technologies are applied for removal and inactivation of different microbial pathogens will guarantee a lower risk of microbial contamination.

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