Removal of *Cryptosporidium* by wastewater treatment processes: a review

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ABSTRACT

Cryptosporidium is a protozoan parasite that infects humans and various animal species. The environmental stability and the low infectious dose of *Cryptosporidium* facilitate its transmission by water and food. Discharge of untreated wastewater may result in waterborne or foodborne *Cryptosporidium* outbreaks, therefore a suitable treatment may prevent its dissemination. Most studies on the prevalence of *Cryptosporidium* oocysts in wastewater have reported a concentration range between 10 and 200 oocysts/L and a prevalence of 6 to 100%. Activated sludge has been found to be ineffective for the removal of *Cryptosporidium* oocysts. Stabilization ponds and constructed wetlands are efficient for the reduction of *Cryptosporidium* from wastewater, especially when the retention time is longer than 20 days at suitable sunlight and temperature. High rate filtration and chlorine disinfection are inefficient for the reduction of *Cryptosporidium* from effluents, whereas ultrafiltration and UV irradiation were found to be very efficient for the reduction of *Cryptosporidium* oocysts. Adequate tertiary treatment may result in high quality effluent with low risk of *Cryptosporidium* for unrestricted irrigation and other non-potable applications.

Key words | *Cryptosporidium*, effluents, inactivation, reduction, treatment, wastewater

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INTRODUCTION

Cryptosporidium is a protozoan parasite that infects humans and various animal species. The occurrence of outbreaks of cryptosporidiosis have increased awareness concerning the public health significance of *Cryptosporidium*. The average diameter of *Cryptosporidium* oocysts is 4–6 μm. The oocyst of *Cryptosporidium* is protected by an outer wall that allows it to persist in the environment for long periods of time, which facilitates its environmental transmission through contaminated water and food. Human infections are mostly caused either by *C. hominis* or *C. parvum*, the cattle genotype. Other species of *Cryptosporidium*, such as *C. meleagridis*, *C. felis*, and *C. canis*, may occasionally infect humans (Gatei *et al.* 2002; Plutzer & Karanis 2007; Xiao 2010). These genotypes may infect immunocompromised individuals. The concentration and prevalence of *Cryptosporidium* oocysts in sewage is influenced by the infection and excretion rates in the population served, and

by the contribution of infected domestic animals to the *Cryptosporidium* load in domestic sewage. However, rainfall may result in the dilution of oocysts in wastewater. Routes of *Cryptosporidium* transmission include person-to-person, contact with infected animals, recreational water, especially in swimming pools, drinking water and contaminated food (Long *et al.* 2002; Rose *et al.* 2002; Pönka *et al.* 2009; Baldursson & Karanis 2011). Two factors may be responsible for the transmission of *Cryptosporidium* through the water route: the environmental stability of oocysts and the low infectious dose of the parasite.

Most of the reported waterborne outbreaks of cryptosporidiosis have been in developed countries such as North America, the United Kingdom (UK), Europe, Australia, New Zealand and Japan (Baldursson & Karanis 2011). The waterborne outbreaks were caused by the consumption of contaminated drinking water from surface sources or

ground sources and swimming pools (Yoder & Beach 2007; Yoder *et al.* 2008; Baldursson & Karanis 2011). *Cryptosporidium* oocysts can enter drinking water sources through sewage contamination, failure of drinking water treatment plants or leakage into the distribution system. As the oocysts of *Cryptosporidium* are very resistant to chlorine disinfection, in some waterborne outbreaks the water quality complied with the fecal coliform guideline (Craun *et al.* 1998). The cryptosporidiosis outbreak with the highest number of cases occurred in Milwaukee, Wisconsin, USA in 1993, and involved about 400,000 infected individuals; this outbreak was the result of runoff of wastes due to heavy rain and failure of the drinking water plant to handle heavy contamination (MacKenzie *et al.* 1994).

The main purpose of advanced wastewater treatment is to reduce the levels of pathogens in order to prevent their environmental transmission. The wastewater treatment applied is governed by the planned application of the effluents and the public health risks involved. Wastewater treatment consists of physical, biological, and chemical processes for the removal of suspended solids, organic materials, and pathogenic microorganisms. The nature of pathogenic microorganisms determines their removal/reduction efficiency by the wastewater treatment process. Advanced wastewater treatment is applied, in particular, in arid regions, where treated effluents are used for irrigation and other non-potable applications. Efficient wastewater treatment is important to prevent the dissemination of pathogenic protozoan parasites by irrigated crops and water bodies that receive the treated wastewater effluents.

PREVALENCE OF *CRYPTOSPORIDIUM* IN WASTEWATER

Detection of oocysts in stools is taken as a sign of infection. Shepherd *et al.* (1988) reported that the median time of *Cryptosporidium* oocysts shedding from onset of symptoms to the last positive stool specimen was 8 days (range from 2 to 35 days). Infections with *Cryptosporidium* are associated strongly with the age of patients; *Cryptosporidium* DNA was detected in 21.8% stool samples of children aged <5 years submitted for examination (Ten Hove *et al.* 2007). Infected persons shed high concentrations of *Cryptosporidium*

oocysts, which can reach up to 10^{5-7} oocysts per gram of feces (Chappell *et al.* 1999). Similar levels of *Cryptosporidium* oocysts have been reported for infected calves, where 10^{4-7} oocysts per gram of feces have been detected (Xiao & Herd 1994).

Numerous studies have been conducted around the world to evaluate the prevalence and levels of *Cryptosporidium* oocysts in raw wastewater. Prior to 1998, most studies applied the sucrose flotation method to purify oocysts of *Cryptosporidium* from raw wastewater before staining and/or microscopic examination (US Environmental Protection Agency 1996; Bukhari *et al.* 1998). To improve the recovery efficiency of oocysts from wastewater samples or concentrated water samples, the immune magnetic separation (IMS) method was developed for affinity purification and concentration of oocysts (Bukhari *et al.* 1998; USEPA method 1622). Detection and enumeration methods have been developed from conventional detection techniques, such as acid-fast Ziehl–Neelsen staining and microscopic examination with a light microscope, or staining with anti-*Cryptosporidium* oocysts fluorescein isothiocyanate (FITC) labeled monoclonal antibodies (immune fluorescence (IF)) and examination by epi-fluorescent microscope (USEPA method 1623). Microscopic examination of concentrated wastewater or surface water samples may be tedious, time-consuming and require very experienced analysts to accurately identify *Cryptosporidium* oocysts. In parallel, researchers have attempted to develop alternative methods with greater specificity and sensitivity than the existing methods (Morgan *et al.* 1998; Guy *et al.* 2003; Zhou *et al.* 2003; Feng *et al.* 2009). Studies have demonstrated that the polymerase chain reaction (PCR) and more recently real time PCR have proved to be more sensitive than the indirect fluorescent antibody (IFA) method for the detection of *Cryptosporidium* oocysts in raw wastewaters (Guy *et al.* 2003; Zhou *et al.* 2003; Feng *et al.* 2009).

Most reviewed studies have not reported on the detection efficiency of *Cryptosporidium* oocysts in raw wastewater. The reported detection efficiency of *Cryptosporidium* oocysts from raw wastewater ranged from 5.5 ± 1.3% to as high as 85% (Gennaccaro *et al.* 2003; Robertson *et al.* 2006). It is interesting to note that large variations were reported in the recovery efficiency of oocysts from raw wastewater, which ranged from the lowest detection

sensitivity values reported for studies using USEPA method 1623, while less reliable methods such as flotation reported the highest (85%) recovery efficiency (Robertson *et al.* 2000). McCuin & Clancy (2005) evaluated the recovery efficiency of *Cryptosporidium* oocysts from samples of 250, 500, and 1,000 mL of raw wastewater by IMS and demonstrated a negative influence of the sample volume on the recovery efficiency. The mean recovery efficiency values from 250 mL, 500 mL, and 1,000 mL of raw wastewater were 33%, 31.8%, and 24.3%, respectively. Since the recoveries achieved from wastewater effluents with sodium chloride or sucrose density flotation were low and very variable, those researchers suggested that the IMS technology is suitable for the efficient recovery of *Cryptosporidium* oocysts from raw wastewater (McCuin & Clancy 2005).

Table 1 presents the results of 25 studies on the prevalence and concentration of *Cryptosporidium* oocysts from 13 countries from five continents. The prevalence of *Cryptosporidium* oocysts in raw wastewater ranged from 0 (where samples contained oocysts below the detection level) to 100% (where *Cryptosporidium* oocysts were detected in all samples analyzed). Differences in the prevalence of *Cryptosporidium* oocysts were observed in various studies even in the same country. For example, in Brazil three studies were reviewed, in which *Cryptosporidium* oocyst prevalence was reported in 6.4, 58.3, and 100% of the samples (Santos *et al.* 2004; Cantusio Neto *et al.* 2006; Hachich *et al.* 2013). In the USA, six studies were reviewed and the reported prevalence ranged from 27.9 to 78% (Mayer & Palmer 1996; Gennaccaro *et al.* 2003; Zhou *et al.* 2003; Clancy *et al.* 2004; Harwood *et al.* 2005).

The concentration of *Cryptosporidium* oocysts detected in raw wastewater ranged from 0 (below the detection limit) to 60,000 oocysts/L. The highest values for *Cryptosporidium* in raw wastewater were reported from Brazil (Cantusio Neto *et al.* 2006). The dominant values reported

Table 1 | Prevalence and concentration of *Cryptosporidium* oocysts in raw wastewater

Country	Samples #	*Cryptosporidium* oocysts/L (range)	% positive	Recovery efficiency	Reference
Brazil	8	1–560[a]	100	NR[b]	Santos *et al.* (2004)
	53	0–60,000[a]	6.4	46.2	Cantusio Neto *et al.* (2006)
	24	<2.5–2,700[a]	58.3	NR	Hachich *et al.* (2013)
China	90	NR	70	NR	Feng *et al.* (2009)
	22	240 ± 88[a]	100	NR	Fu *et al.* (2010)
Germany	206	(0–1,745)[a]	31.1		Gallas-Lindemann *et al.* (2013)
	51	50–1,280[a]	100	NR	Ajonina *et al.* (2012)
Italy	3	4.5 ± 0.8[a]	100	NR	Carraro *et al.* (2000)
Japan	73	8–50[a]	10	14	Suwa & Suzuki (2001)
Kenya	11	12.5–72.97[a]	18.2	NR	Grimason *et al.* (1993)
Malaysia	24	1–80[a]	50	NR	Lim *et al.* (2007)
Norway	32	100–1,100 (200)[a]	80	NR	Robertson *et al.* (2006)
Spain	10	45.7 ± 7.5[a]	NR	44.1 ± 10.8	Reinoso *et al.* (2008)
	46	103–139[a]	100	NR	Montemayor *et al.* (2005)
	48	8–970[a]	100	NR	Castro-Hermida *et al.* (2008)
S. Africa	56	0–150 (31.7)[a]	60.7	NR	Dungeni & Momba (2010)
Sweden	19	5 (3–220)[a]	26.3	15 ± 4.6	Ottoson *et al.* (2006)
UK	33	111 ± 127[a]	NR	85 ± 18	Robertson *et al.* (2000)
USA	179	NR[c]	27.9	NR	Zhou *et al.* (2003)
	95	<2–24[a]	29.5	NR	Clancy *et al.* (2004)
	18	<5.5–263[a]	78	5.5 ± 1.3	Gennaccaro *et al.* (2003)
	30	3–21[a]	74	NR	Harwood *et al.* (2005)

[a]detection method immune fluorescence (IF).
[b]not reported.
[c]PCR.

for the concentration of *Cryptosporidium* in raw wastewater were from 10 to 200 oocysts/L (Table 1). Dungeni & Momba (2010) studied the concentration and prevalence of *Cryptosporidium* oocysts in four wastewater treatment plants in South Africa. A concentration of up to 150 oocysts/L was detected in the influent of the wastewater treatment plant with up to 79% prevalence level.

Several factors may influence the prevalence and concentration of *Cryptosporidium* in wastewater. The incidence of cryptosporidiosis in the community may influence the levels of *Cryptosporidium* oocysts in wastewater. In the USA, the reported cases of cryptosporidiosis for the years 2009 and 2010 were from a high level of 17.4 in 100,000 in Wisconsin in 2010 to a low level of 0.6 in 100,000 in 2009 in Mississippi. The high incidence was reported in states that experience cryptosporidiosis outbreaks, or have more efficient reporting systems (Yoder *et al.* 2012). In Europe, cryptosporidiosis is notifiable through the European Basic Surveillance Network. In 2005, 7,960 cryptosporidiosis cases were reported from 16 countries, which makes a crude incidence rate of 1.9 cases per 100,000. A clear peak of cryptosporidiosis was recorded in the autumn of 2005. The highest incidence was reported in Ireland with a rate of 13.7 followed by the UK with an incidence rate of 9.3 cases per 100,000. No cases of cryptosporidiosis were reported for eight out of the 16 European countries included in the study (Semenza & Nichols 2007). The incidence rate of *Cryptosporidium* may be influenced by the socio-economic and hygienic status of the community. Garvey & McKeown (2009) reported higher incidence rates of *Cryptosporidium* in rural than in urban settings.

The seasonal distribution of *Cryptosporidium* in wastewater has been examined in various parts of the world (Table 2). Ajonina *et al.* (2012) showed that oocysts of *Cryptosporidium* are predominant during autumn and winter in Germany. In Ireland, Cheng *et al.* (2012) reported high abundance of *Cryptosporidium* in sewage, especially in tourist towns; sudden season-dependent traveler influx may be a potential source of pathogens. *Cryptosporidium* was detected in over 93% of wastewater samples in summer, autumn, and winter; however, the detection frequency in spring was 69%. In Spain, the highest numbers of oocysts were detected in spring and summer in influent and effluent

Table 2 │ Seasonality of *Cryptosporidium* prevalence in wastewater

Country	Season	Reference
Germany	Autumn and winter	Ajonina *et al.* (2012)
Ireland	Summer, autumn, and winter	Cheng *et al.* (2012)
Spain	Spring and summer	Castro-Hermida *et al.* (2008)
	Spring and autumn	Montemayor *et al.* (2005)
Italy	Spring	Caccio *et al.* (2003)

wastewater samples (Castro-Hermida *et al.* 2008), while Montemayor *et al.* (2005) demonstrated the occurrence of two peaks in oocyst concentration, one in early spring and another in autumn. Robertson *et al.* (2006) reported no pattern of seasonality in the occurrence of *Cryptosporidium* in Norway. A study conducted in Italy has shown that the oocysts of *Cryptosporidium* were detected during the spring in raw wastewater (Caccio *et al.* 2003).

REMOVAL OF *CRYPTOSPORIDIUM* BY SECONDARY WASTEWATER TREATMENT

Various groups of pathogenic microorganisms (viruses, bacteria, protozoan parasites, and worms) may be present in wastewater effluents. Therefore, to prevent the environmental transmission of pathogens, treatment is applied to reduce or inactivate these pathogens in effluent before discharge. The wastewater treatment applied is governed by the planned application of the effluents and the public health risks involved. Wastewater treatment consists of physical (sedimentation and filtration), biological (activated sludge, trickling filters, and stabilization ponds), and chemical processes (chlorine, ozone, and chlorine dioxide disinfection). Multi-barrier treatment (mostly consisting of sedimentation, biological treatment, filtration, and disinfection) is applied to wastewater when reclaimed wastewater effluents are utilized for unrestricted irrigation of food crops or for groundwater recharge.

A prevalence of 12% was demonstrated for *Cryptosporidium* oocysts in activated sludge-treated effluent at a concentration range of 0.05 to 1.6 oocysts/L in Japan (Suwa & Suzuki 2001) (Table 3). A study on the prevalence

Table 3 | Prevalence of *Cryptosporidium* in secondary activated sludge-treated effluents

Country	Wastewater treatment	Prevalence (%)	Concentration oocysts/L	Reference
Italy	Activated sludge	NR[a]	0.21 ± 0.06	Carraro *et al.* (2000)
Japan	Activated sludge	12	0.05–1.6	Suwa & Suzuki (2001)
S. Africa	Activated sludge	50–79	1–150	Dungeni & Momba (2010)
Spain	Activated sludge	NR	2–390	Castro-Hermida *et al.* (2008)
Malaysia	Extended aeration	25%	20–40	Lim *et al.* (2007)
USA	Activated sludge	58.5	<0.1–40.8	Clancy *et al.* (2004)
	Activated sludge	84%		Harwood *et al.* (2005)
	Activated sludge	83	<12.8–345	Gennaccaro *et al.* (2003)
Spain	Activated sludge	100	1.8–5.6	Montemayor *et al.* (2005)
Ireland	Activated sludge	97%	10–32	Graczyk *et al.* (2009)
	Oxidation ditch	NR	4 ± 2	Cheng *et al.* (2009)
	Activated sludge	NR	8 ± 3.6	Cheng *et al.* (2009)
England	Activated sludge	15.4–46.6	10–69	Bukhari *et al.* (1997)
Sweden	Activated sludge	5.2		Ottoson *et al.* (2006)

[a]Not reported.

and removal efficiency of *Cryptosporidium* oocysts by activated sludge in four wastewater treatment plants in South Africa has shown a concentration of up to 40 oocysts/L in effluent samples and a prevalence of up to 86% of oocysts in effluent samples. The researchers reported no significant differences in the number of oocysts isolated from the effluent samples ($P \geq 0.05$) of larger and smaller plants (Dungeni & Momba 2010).

In Spain, a concentration range of 2 to 390 oocysts/L of *Cryptosporidium* oocysts was reported in activated sludge effluent (Table 3). Even though the concentration of oocysts in the final effluent throughout the year did not differ significantly among the wastewater treatment plants (WWTP), there is a possibility that oocysts were stably removed in WWTP. It was also found that the concentration of oocysts per liter was higher in the treated than in the untreated wastewater (Castro-Hermida *et al.* 2008). On the other hand, in the USA, 55 out of 94 (58.5%) activated sludge-treated effluent samples were found positive for *Cryptosporidium* at a concentration range of <0.1 to 40.8 oocysts/L. It was reported that in many cases the concentration of *Cryptosporidium* oocysts in effluents was found to be higher than that detected in the influent samples. Four reasons were mentioned by the authors as possibly contributing to this observation: (1) the low levels of *Cryptosporidium* detected in the influent make it difficult

to show significant $\log_{10}$ differences; (2) the improvement of recovery of oocysts in cleaner water matrices than that obtained in raw wastewater; (3) the low removal efficiency of *Cryptosporidium* in secondary and tertiary treatments; and (4) aggregated oocysts with organic material may be dispersed through the treatment processes, resulting in greater numbers in the effluent. Robertson *et al.* (2006) reported a removal efficiency of 0 to 50% for *Cryptosporidium* by activated sludge treatment and a concentration range of 230 to 3,300 oocysts/L. The authors also reported that in some of the effluent samples, the numbers of parasites detected was greater than those detected in the influent samples (Robertson *et al.* 2006). They explained the results as an artifact of higher method recovery efficiencies with the cleaner effluent samples or a reflection of temporal fluctuations in parasite concentrations, the lack of pairing of the samples, the uneven distribution of parasites in the sample matrices, or a combination of these factors.

In a study conducted by Lim *et al.* (2007) in Malaysia, the researchers reported that a secondary treatment process in which extended aeration was employed resulted in 73% reduction of the oocysts; however, a treatment process that encompassed an aerated lagoon did not reduce the concentration of *Cryptosporidium* oocysts significantly. Treated wastewater effluents contained *Cryptosporidium* oocysts a a concentration of 20 to 40 oocysts/L in three out of 12

(25%) effluent samples examined (Lim *et al.* 2007). The results are in agreement with previously reported results on the inefficient removal of *Cryptosporidium* by secondary wastewater treatment. Harwood *et al.* (2005) reported that following biological treatment, the frequency of detection of *Cryptosporidium* increased from 75% in the influent samples to 84%. The increase was explained to be due to the more sensitive detection in secondary effluent (21 to 94 oocysts/100 L) (Harwood *et al.* 2005). Gennaccaro *et al.* (2003) studied the removal efficiency of *Cryptosporidium* oocysts from wastewater by activated sludge treatment. The removal efficiency was estimated by IFA and cell-culture infectivity. A prevalence of 83% was recorded in secondary effluent at a concentration range of <12.8 to 345 oocysts/100 L, and the arithmetic mean was 112 ± 153 oocysts/100 L. The arithmetic mean of infectious oocysts in secondary effluent was 37 ± 28 oocysts/100 L. The results indicate that activated sludge treatment is not efficient for the removal of *Cryptosporidium* from wastewater (Gennaccaro *et al.* 2003).

Costán-Longares *et al.* (2008) studied the comparative removal of indicator and pathogenic microorganisms by wastewater treatment processes. Their findings have shown that the reductions in the numbers of viable *Cryptosporidium* oocysts were significantly lower than those of all other parameters and only approached by the reduction of sulphite reducing clostridia. The prevalence of *Cryptosporidium* in secondary effluent was 94% and the mean values of viable *Cryptosporidium* oocysts were $1.9 \log_{10}$ oocysts/100 L (Costán-Longares *et al.* 2008).

The removal efficiency of *Cryptosporidium* by sewage treatment processes depends upon their concentration in the influent and the processes applied (Cheng *et al.* 2009). The removal by typical sewage treatments is critical, and never guarantees a complete abatement of these protozoa. Primary and secondary sedimentation allows a variable removal due to the difference between the sedimentation velocities of oocysts with respect to the process (Enriquez *et al.* 1995; Withmore & Robertson 1995). Medema *et al.* (1998) pointed out that the ability of sedimentation to settle cysts and oocysts is related to their attachment to particles. The removal efficiency of the activated sludge process for *Cryptosporidium* is related to the clarification process: the flocculated material in the activated sludge was believed to contribute to the removal, acting as a 'settling blanket' (Enriquez *et al.* 1995; Withmore & Robertson 1995).

CRYPTOSPORIDIUM REMOVAL FROM WASTEWATER BY NATURAL TREATMENT METHODS

Biological treatment of domestic wastewater by natural systems can be accomplished by either constructed wetland (CWL) or waste stabilization ponds (WSPs). CWL and WSP systems are applied to treat wastewater of small communities and in regions were land is not limited. Two types of CWLs, subsurface flow (SSF) and free-surface flow (FSF), were evaluated for their efficiency in removing *Cryptosporidium* from domestic wastewater. The mechanisms responsible for the reduction of *Cryptosporidium* may be influenced by the configuration of the CWL, biological factors such as predation, microbial activity and chemical factors such as oxidation reactions, adsorption and exposure to toxins excreted from plants and bacteria. Additional mechanisms for pathogen reduction in CWLs include sedimentation, natural die-off, inactivation or death related to temperature, inactivation or death related to unfavorable water chemistry, biofilm interaction, mechanical filtration, exposure to biocides and UV radiation. Pathogen removal in CWL has also been shown to correlate with hydraulic retention time (Kadlec & Knight 1996). Table 4 presents results of *Cryptosporidium* oocysts reduction by extensive treatment methods.

A study was conducted to compare the removal efficiency of *Cryptosporidium* by SSF and FSF wetlands. The concentration of *Cryptosporidium* in the influent samples varied from 10 to 118 oocysts/L. A removal of 97.5% was recorded for *Cryptosporidium* in SSF wetlands, whereas no reduction was observed in FSF wetlands where greater concentration of *Cryptosporidium* was observed in the effluent as compared to the influent (Table 4). The concentration of *Cryptosporidium* was 10 to 32 oocysts/L in the influent samples compared to 22 to 78 oocysts/L in the effluent samples (Graczyk *et al.* 2009).

Reinoso *et al.* (2008) studied the comparative removal of protozoan parasites and indicator microorganisms in facultative pond (FP), surface flow (SF) wetland, and SSF

Table 4 | Removal of *Cryptosporidium* from wastewater by natural treatment processes

Country	Wastewater treatment	Removal %/log10	Reference
Ireland	Wetlands (SSF)	97.5%	Graczyk *et al.* (2009)
Kenya	Stabilization pond	100%	Grimason *et al.* (1993)
Spain	Oxidation pond	97%	Araki *et al.* (2001)
	Waste stabilization pond	40%	Reinoso & Bécares (2008)
	Anaerobic pond	4.6	Reinoso *et al.* (2011)
	Facultative pond	5.2	
	maturation	3.7	
	Facultative pond	2.81	Reinoso *et al.* (2008)
	Surface flow wetland (SFW)	3.21	
	Subsurface flow wetland (SSFW)	2.13	
USA	Duckweed pond	89%	Falabi *et al.* (2002)
	Subsurface flow wetland (SSFW)	64.2%	Thurston *et al.* (2001)

wetland, in Spain. The concentration of *Cryptosporidium* oocysts in the effluent samples was 45.7 oocysts/L and a prevalence of 7.55% was recorded. Removal efficiency values of 2.81, 3.21, and 2.13 $\log_{10}$ were recorded for *Cryptosporidium* oocysts in FB, SF, and SSF, respectively. No significant difference was observed between the removal of *Cryptosporidium* during the summer and winter months (Reinoso *et al.* 2008). It is worth noting that the removal rates were not determined by infectivity of *Cryptosporidium*. Thurston *et al.* (2001) studied the removal efficiency of oocysts by SSF-constructed wetland from secondary effluent. The concentration of the *Cryptosporidium* oocysts ranged from 0.9 to 108.9/100 L in the influent and <1.0 to 49.9/100 L in the effluent. The reduction ranged from 31.7 to 98.3% and the average removal was 64.2% (Thurston *et al.* 2001). Sedimentation is thought to be one of the major mechanisms of microbial reduction from wetlands used for wastewater treatment. This observation was supported by Karim *et al.* (2004), who reported concentrations of one to three orders of magnitude greater in the sediment compared to the water column for *Giardia* cysts and *Cryptosporidium* oocysts (Karim *et al.* 2004).

Cryptosporidium oocysts decreased by 89% using duckweed ponds, with an average number of 1.58 oocysts/L in the influent and 1.17 oocysts/L in the effluent. The duckweed pond was more effective in reducing the number of protozoan parasites (*Giardia* 98%; *Cryptosporidium* 89%) than indicator bacteria (total coliform 61%, fecal coliform 62%) or coliphages (Falabi *et al.* 2002). Therefore, the researchers concluded that the removal of protozoan parasites in the pond appeared to be related to their size.

WSPs are large, man-made water bodies in which wastewater is treated by natural processes. There are three types of ponds: (1) anaerobic, (2) facultative, and (3) aerobic (maturation), each with different treatment and design characteristics. Anaerobic ponds are built to a depth of 2 to 5 m and have a relatively short retention time of 1 to 7 days. Facultative ponds are constructed to a depth of 1 to 2.5 m and have a retention time between 5 and 30 days. Aerobic ponds are usually between 0.5 and 1.5 m deep with a retention time of 15 to 20 days. Ideally, several aerobic ponds can be built in series to provide a high level of pathogen removal. Reduction of pathogenic parasites in WSPs results from numerous processes, such as sedimentation, sunlight, predation, natural die-off, hydraulic residence time, and environmental variables, such as temperature, pH, dissolved oxygen, water potential, and ammonia.

Table 4 also presents results on the reduction of *Cryptosporidium* oocysts by WSPs. Reinoso *et al.* (2011) studied the mechanisms for the removal of parasites from waste stabilization ponds in northern Spain. They compared the removal efficiency of parasites and indicator microorganisms by anaerobic, facultative, and maturation ponds. *Cryptosporidium* oocysts were reduced by an average of 96%. The anaerobic ponds showed significantly higher surface removal rates (4.6, 5.2, and 3.7 log oocysts removed m^{-2} day^{-1}, respectively) than facultative and maturation ponds. Sunlight and water physicochemical conditions were the main factors influencing the removal of *C. parvum* oocysts both in the anaerobic and maturation ponds, whereas other factors like predation or natural mortality were more important in the facultative ponds. The results showed that sedimentation, the most commonly proposed mechanism for oocyst removal, was found to be negligible, ranging from zero in the maturation pond to 5.56% in the facultative pond. Reinoso & Bécares (2008) studied factors affecting the reduction of *C. parvum* oocysts in a waste stabilization pond. They evaluated the effects of sunlight, pond depth,

and type of treatment pond on oocyst viability by an assay based on the exclusion or inclusion of two vital dyes, 4′,6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI). Sunlight exposure was the most influential factor for oocyst inactivation. A 40% reduction was observed after 4 days of exposure to sunlight conditions compared with dark conditions. Inactivation rates suggested that the facultative ponds were the most aggressive environment for oocyst inactivation, followed by the maturation ponds and the anaerobic ponds (Reinoso & Bécares 2008).

Araki *et al.* (2001) studied the effect of high-rate algal ponds on the infectivity of *C. parvum*. A reduction of 97% in the infectivity of *Cryptosporidium* oocysts in neonatal mice was recorded within 6 days of exposure. The researchers suggested that pH, ammonia, and/or sunlight were major factors for the inactivation of oocysts in wastewater.

Gómez-Couso *et al.* (2009) studied the effect of water turbidity on the inactivation efficacy of *Cryptosporidium* by the solar water disinfection (SODIS) method. Polyethylene terephthalate (PET) bottles containing the contaminated turbid waters were exposed to full sunlight for 4, 8, and 12 h. SODIS significantly reduced the potential viability of *C. parvum* oocysts to increase the percentage of oocysts that took up the dye PI. The opposite correlation was demonstrated between the water turbidity level and the loss of viability of the oocysts (Gómez-Couso *et al.* 2010). These results are in agreement with data reported by Nasser *et al.* (2007), who demonstrated that sunlight enhances the reduction of infectivity of *C. parvum* and at the end of the 7-day study period a 3 log reduction in infectivity was measured in the sunlight- and seawater-exposed oocysts.

A study conducted on 11 WTPs in Kenya demonstrated that oocyst levels in the influent wastewater samples ranged from 12.5 to 72.97 oocysts/L, whereas no *Cryptosporidium* oocysts were detected in any of the final effluents of the studied pond systems (Grimason *et al.* 1993).

REDUCTION OF *CRYPTOSPORIDIUM* BY TERTIARY TREATMENT

Tertiary or advanced wastewater treatment is employed for the removal of contaminants that are not efficiently removed by secondary treatment and are harmful to the environment and public health. In many countries, wastewater is treated for reuse purposes to increase the water budget either for unrestricted crop irrigation, stream restoration, or other non-potable applications. To produce high quality effluent in intensive WTPs, a common treatment train consists of chemical coagulation of the secondary clarified effluent, followed by sedimentation, rapid sand filtration, and final disinfection (Rose 2007). Membrane ultrafiltration has also been used for tertiary treatment of secondary effluents and has proved to be efficient in the removal of *Cryptosporidium* oocysts (Lonigro *et al.* 2006). Disinfection normally involves the injection of a chlorine solution at the head end of a chlorine contact basin. Ozone and UV irradiation can also be used for disinfection. This level of treatment is assumed to produce an effluent free from detectable pathogens.

The prevalence of *Cryptosporidium* oocysts in tertiary effluents was reported by Harwood *et al.* (2005). The studied wastewater treatment train consisted of activated sludge, filtration and, in some cases, flocculation and chlorination. The concentration of *Cryptosporidium* oocysts did not decrease substantially in comparison to their prevalence in untreated influent and was recorded at 70% with a concentration range of 0.01 to 1 oocysts/100 L (Harwood *et al.* 2005) (Table 5). One explanation for the poor removal was an artifact related to the greater sensitivity of the detection method as the quality of the effluents is improved through treatment stages. The researchers reported that infectious oocysts were present in 20% of the reclaimed effluent samples (Harwood *et al.* 2005). Flocculation and sand filtration produced tertiary effluent with a concentration of *Cryptosporidium* consistently below 0.4 oocysts/L and a removal efficiency of 1.69 log (Fu *et al.* 2010). The results from a study conducted in Spain have shown that all samples of tertiary treated effluent (sand filtration, UV irradiation, and chlorination) were found positive for oocysts of *Cryptosporidium* with a mean value of 0.4 oocysts/L (Montemayor *et al.* 2005). Removal efficiencies of viable oocysts by sand filtration, UV, and chlorination were found to be 97.9% (Montemayor *et al.* 2005) (Table 5).

Membrane technologies have significant potential to produce high quality effluents and ultrafiltration can achieve high removal of protozoan cysts by physical sieving. Lonigro *et al.* (2006) evaluated the removal efficiency of

Table 5 | Prevalence of *Cryptosporidium* in tertiary-treated effluents

Country	Concentration oocysts/L	Prevalence (%)	Wastewater treatment	Reference
USA	0.01–1	70%[a]	Filtration, flocculation, and chlorination	Harwood *et al.* (2005)
	<1.8–178	67	Filtration and disinfection	Gennaccaro *et al.* (2003)
	<0.008–0.226	18.8	Filtration and disinfection	Clancy *et al.* (2004)
Spain	0.14–0.22	75	Sand filtration + UV	Montemayor *et al.* (2005)
Ireland	4 ± 2		Biofilm-coated filter	Cheng *et al.* (2009)
	<0.4	41	Flocculation and sand filtration	
	<0.2[b]	0	Membrane filtration	
Italy	0	0	Ultrafiltration	Lonigro *et al.* (2006)
China	0–0.4	41	Flocculation and sand filtration	Fu *et al.* (2010)
	0	0	Ultrafiltration	

[a]20% of the detected oocysts were infectious.
[b]Below detection limit.

Cryptosporidium oocysts from secondary effluents by membrane ultrafiltration and demonstrated high removal of parasites from the effluents, with all collected permeate samples testing negative for *Cryptosporidium* oocysts. These results were supported by Fu *et al.* (2010), who demonstrated that the levels of *Cryptosporidium* oocysts in all the ultrafiltration-treated effluents were below the detection limit and up to 1.84 $\log_{10}$ oocyst reduction was demonstrated. Similar results were recorded for the removal efficiency of *Cryptosporidium* oocysts by microfiltration and ultrafiltration, which resulted in a reduction of >7 $\log_{10}$ (Hirata & Hashimoto 1998).

The results presented suggest that membrane ultrafiltration provides high log removal of *Cryptosporidium* oocysts, indicating its usefulness for removal of pathogenic protozoan *Cryptosporidium* oocysts from wastewater.

Disinfection is considered the last barrier to prevent the environmental transmission of *Cryptosporidium* oocysts by wastewater effluents. The concentration X time (CT) product of free chlorine per 1 log reduction in infectivity of *Cryptosporidium* was estimated to be in the range of 800 to 900 mg min/L (Hirata *et al.* 2001); while a CT product of 2,700 mg min/L was needed for 3 log reduction of animal infectivity of *Cryptosporidium* oocysts (Hirata *et al.* 2001). The resistance of *Cryptosporidium* to free chlorine and monochloramine was also reported by Rennecker *et al.* (2000), who reported that a CT of about 2,000 mg min/L and 12,000 mg min/L were required to reach inactivation of 99.9% of *C. parvum* by chlorine and monochloramine, respectively. Similar results on the resistance of *Cryptosporidium* to free chlorine were reported earlier by Korich *et al.* (1990). Alternative disinfection methods were evaluated in order to replace chlorine as the sole disinfectant and to efficiently inactivate oocysts present in tertiary effluent. Ozone was proved to be efficient for the inactivation of *Cryptosporidium*, where greater than 90% inactivation, as measured by infectivity, was achieved by treating *Cryptosporidium* oocysts with 1 ppm of ozone (1 mg/L) for 5 min (Rennecker *et al.* 2000). The CT products of ozone per 1 log reduction in infectivity were 3 mg min/L. However, a 99% inactivation of *C. parvum* oocysts was achieved with an ozone CT of 5 mg × min/L at 20 °C. When used as primary disinfectant, ozone resulted in a strong synergy and the inactivation rates of *C. parvum* by free chlorine and monochloramine were 1.1–2.8 and 2.4–9.2 times faster, respectively, than the corresponding primary inactivation rate (Rennecker *et al.* 2000).

In the late 1990s, UV irradiation was found efficient for inactivating *Cryptosporidium* oocysts (Carpenter *et al.* 1999). After that, numerous studies demonstrated the effectiveness of low doses of UV irradiation to inactivate *Cryptosporidium* oocysts in various water matrixes. For example, Morita *et al.* (2002) showed that a UV dose of 1.0 mWs/cm^2 at 20 °C was needed to produce a 2-$\log_{10}$ reduction in infectivity (99% inactivation). No recovery in infectivity following treatment was observed after exposure to fluorescent-light irradiation or storage in darkness. The results indicate that the effects of UV irradiation on *C. parvum* oocysts as determined by

animal infectivity can conclusively be considered irreversible (Morita *et al.* 2002). These results are in agreement with numerous studies conducted on the inactivation of *Cryptosporidium* oocysts in water and wastewater. Using either animal infectivity or cell culture, *Cryptosporidium* was reported as sensitive to low UV radiation doses (Clancy *et al.* 2004).

CONCLUSIONS

This review paper presents results of studies concerning the prevalence of *Cryptosporidium* in wastewater and its removal by wastewater treatment processes. *Cryptosporidium* oocysts have been detected in wastewater at an average concentration of 10 to 200 oocysts/L. Activated sludge is inefficient for the removal of *Cryptosporidium* oocysts from wastewater and similar results were observed for high-rate sand filtration. UV radiation is the most effective disinfection process for the inactivation of *Cryptosporidium*. WSPs with a retention time longer than 20 days and SSF wetlands resulted in high removal of *Cryptosporidium* oocysts from wastewater. Application of efficient wastewater treatment processes may prevent the environmental transmission of *Cryptosporidium*.

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