Science of the Total Environment 1031 (2026) 181810

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

Physical and biological fate of protozoan (oo)cysts in slow sand filtration: A review

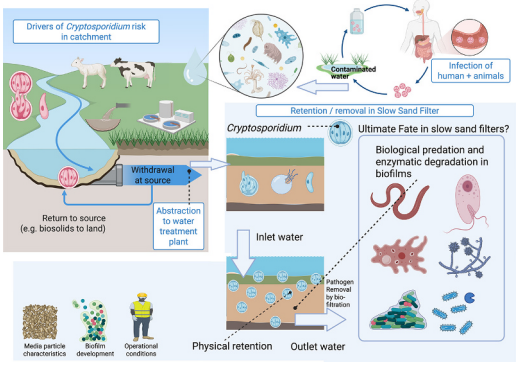
Sophie L. Bretagne [a], Michael Chipps [b], Jim Harris [a], Francis Hassard [a,*]

[a] Cranfield University, Cranfield, MK43 0AL, UK
[b] Thames Water Utilities Limited, Kempton Park AWTW, Hanworth, TW13 6XH, UK

HIGHLIGHTS

- Cryptosporidium LRVs in slow sand filtration span 0.09 to >7.2 log (median 2.64).
- Schmutzdecke maturation and stable operation improve oocyst retention.
- Turbidity, flow rate and temperature influence run length and removal variability.
- Ciliates, rotifers and nematodes ingest oocysts, but inactivation mechanism is uncertain.
- Viability/infectivity tests (e.g., PMA-PCR, ICC-qPCR) should be used to complement monitoring.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Cryptosporidium
Slow sand filtration
Protozoan oocysts
Biological predation
Schmutzdecke
Drinking water treatment

ABSTRACT

Cryptosporidium is a leading cause of waterborne disease globally, in part due to its chlorine-resistant oocysts, low infectious dose, and ability to cause widespread outbreaks. Multiple barriers are employed in drinking water treatment to reduce risks from *Cryptosporidium* and other pathogens. Slow sand filtration (SSF), a low-carbon drinking water treatment system, has widely varying log removal values (LRVs) (0.09 to >7.2-log; median 2.64-log across published studies). This has been attributed to filter maturity, sand grain size, filter depth, and hydraulic loading rates. Additional enhancement is linked to the stage of ripening of the biologically active layer (Schmutzdecke), which promotes the physical, chemical, and biological mechanisms responsible for oocyst retention. This review identifies key biological agents for oocyst degradation. Nematodes such as *Caenorhabditis elegans* ingest hundreds of oocysts per organism, while ciliates including *Paramecium* and *Euplotes* show high uptake rates. Rotifers (*Bdelloidea*, *Epiphanes*, *Euchlanis*) further internalize large numbers of oocysts. These organisms could contribute to degradation or sequestration of oocysts, reducing their presence in SSF. However, excreted oocysts can remain infective, so clarifying which fauna truly inactivate them versus those that merely immobilize them is needed. Pathogen load, temperature, and competition from other food sources also shape biological removal in SSF. Integrating physical straining and biological predation may help optimize SSF design and operation for *Cryptosporidium* control. Selecting or encouraging micro- and meiofauna with high ingestion or digestion capacities may further improve SSF performance, although the current evidence base remains limited

* Corresponding author.
E-mail address: francis.hassard@cranfield.ac.uk (F. Hassard).

https://doi.org/10.1016/j.scitotenv.2026.181810
Received 14 May 2025; Received in revised form 13 April 2026; Accepted 14 April 2026
Available online 17 April 2026
0048-9697/© 2026 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

and context-dependent. Future research should focus on elucidating the fate of excreted oocysts under realistic field conditions and identifying optimal operational parameters to maximize net *Cryptosporidium* removal.

1. Introduction

Cryptosporidium is a protozoan parasite that causes cryptosporidiosis, a gastrointestinal illness characterised by watery diarrhoea, abdominal pain, and fever. Its primary transmission occurs via the faecal-oral route, through ingestion of contaminated water (drinking or recreational), direct contact with infected animals (e.g., cattle, farm animals), or person-to-person spread in care settings (UK Health Security Agency, 2023). Globally, cryptosporidiosis outbreaks have been documented in the United States, Europe, and the UK; between 2009 and 2017, 444 outbreaks in the U.S. resulted in 7465 cases, primarily tied to swimming pools, infected livestock, and childcare environments (Ma et al., 2022). Worldwide, it is estimated that cryptosporidiosis substantially contributes to hospitalization rates, especially among young children (Shaposhnik et al., 2019). Large waterborne outbreaks have also been documented in Sweden and Italy: the 2010 outbreak in Östersund affected approximately 27,000 individuals (Widerström et al., 2014), the 2011 outbreak in Skellefteå affected approximately 18,500 individuals (Bjelkmar et al., 2017), and the 2019 outbreak in northeastern Italy involved 80 cases among 114 vacationers (Franceschelli et al., 2022). Smaller-scale incidents continue to be reported, highlighting *Cryptosporidium*'s continued threat to public health (Ma et al., 2022).

Cryptosporidium persists in multiple reservoirs including human, agricultural, and wildlife, making it difficult to contain. Sources of *Cryptosporidium* include wastewater treatment plants (WWTPs) (Madore et al., 1987; Robertson et al., 2000), while runoff from farmland or forests contaminate rivers, lakes, and reservoirs (Sturdee et al., 2007). Improved WWTP protocols, enhanced livestock infection management, and interception of agricultural runoff can lower environmental oocyst loads (Li et al., 2022). Such measures help protect immunocompromised populations, such as those with HIV/AIDS, who face a greater risk of severe illness (Hunter and Nichols, 2002).

Effective water treatment is vital for reducing cryptosporidiosis, but failures in operation, unplanned bypasses, or inadequate filtration can enable *Cryptosporidium* to persist into finished drinking water. The *Badenoch Report* outlines that insufficient process control and poorly maintained filtration systems were important in previous UK outbreaks, highlighting the necessity of more rigorous operational management for *Cryptosporidium*, including a multi-barrier defence, managing filtration, and processing wash waters to minimize risks of internal closed loop recirculation of oocysts (Badenoch, 1990). Although oocyst detection in final water is relatively rare, their presence can pose serious health risks to vulnerable groups (Shirley et al., 2012).

In regions like the UK, the Netherlands, the United States, and Canada, where surface water is commonly used as source water, slow sand filtration (SSF) is often employed as one component of the water treatment train. Depending on source-water quality, SSF may be preceded by pretreatment such as roughing filtration or coagulation/clarification to reduce suspended solids and protect the biologically active sand bed, whereas lower-turbidity supplies may require less extensive pretreatment (Abdiyev et al., 2023; Hashimoto et al., 2019). Ultraviolet (UV) disinfection can provide an additional safeguard by damaging *Cryptosporidium* DNA, therefore limiting infectivity without introducing chemical by-products (Rochelle et al., 2005).

Because SSF performance is strongly conditioned by influent particle and pathogen loading, source water quality (including turbidity dynamics during rainfall/runoff events) can influence both operational stability (e.g., headloss development, run length, and scraping frequency) and pathogen removal performance. Source water protection programmes, including catchment interventions to reduce faecal inputs, manage livestock access, improve wastewater discharges, and limit particulate mobilisation, act as an important upstream barrier that can reduce the stress placed on SSF and improve the reliability of downstream treatment (Sturdee et al., 2007; Li et al., 2022). Where raw water turbidity is high or highly variable, pretreatment such as roughing filtration or coagulation/clarification is often required to protect SSF and maintain finished-water quality (Hashimoto et al., 2019; Abdiyev et al., 2023).

SSFs rely on gradual water flow through sand, allowing extended contact with a biofilm layer (the Schmutzdecke) composed of microorganisms that remove pathogens via physical trapping, adsorption, and biodegradation (Abdiyev et al., 2023). Due to clogging of pores within the bed, headloss accumulates. Maintaining SSF performance involves periodic sand scraping or replacement, as well as regular monitoring, to preserve filtration efficiency (Cullen and Letterman, 1985). Although SSF's capacity to reduce *Cryptosporidium* can be significant, reported removal efficiencies vary widely, indicating a knowledge gap concerning the influence of biofilm ecology and operational conditions. By combining SSF with other barriers, water suppliers aim to mitigate *Cryptosporidium* risks. Biological reduction mechanisms within nature-based engineering systems (e.g., SSF) have received less research attention as explored in the following sections of this review.

1.1. Variability in protozoan removal using slow sand filtration

Log removal values (LRVs) are widely used to assess the performance of water treatment processes in eliminating microorganisms such as bacteria, viruses, and protozoa from water. The equation to calculate LRV is:

$$LRV = log_{10}\left(\frac{Concentration\ of\ X\ at\ inlet}{Concentration\ of\ X\ at\ outlet}\right) \quad (1)$$

Where X refers to the microorganism of interest, here *Cryptosporidium* or *Giardia*.

Although SSFs have proven effective for removing pathogenic bacteria and protozoa, published data reveal a wide variability in Cryptosporidium and Giardia (oo)cyst removal. The reported LRVs for Cryptosporidium and Giardia (oo)cysts in SSF systems range from 0.09 (Park et al., 2012) to >7.2 (Hijnen et al., 2005), with a median value of 2.64 (Table 1).

LRVs summarised here are drawn from clean-sand column studies, ripened column/pilot systems, and full-scale SSFs. These study types differ in ecological realism and in how well they represent Schmutzdecke maturity, food-web structure, hydraulics, and operational disturbances (e.g., scraping). Consequently, column studies are most informative for isolating physicochemical transport/retention mechanisms, whereas pilot and full-scale systems better represent net performance under operational conditions. Accordingly, Table 1 distinguishes study scale, maturity, and key test conditions to support interpretation rather than implying all LRVs are directly comparable.

Reported LRVs are also influenced by sampling strategy e.g., sample volumes, timing relative to challenges and maintenance, oocyst concentration and recovery efficiencies, and the analytical methods used to enumerate oocysts. In drinking-water practice, oocyst enumeration commonly relies on filtration followed by immunomagnetic separation (IMS) and immunofluorescence assay microscopy (IFA), as exemplified by USEPA Method 1623/1623.1 for *Cryptosporidium* and *Giardia* (USEPA, 2012); however, recovery efficiency and detection limits are method- and matrix-dependent and can propagate into LRV uncertainty. Some studies also employ flow cytometry, microscopy-only approaches,

Table 1
Summary of *Cryptosporidium* and *Giardia* removal in slow sand filtration (SSF). Columns detail the reference, target protozoan and initial challenge concentration, type of filter (e.g., lab-scale columns vs. pilot/full-scale SSF), sand characteristics (ripened vs. clean, grain size, depth), experimental duration, and resulting log removal values (LRVs). Where relevant, notable conditions such as sand preconditioning or inactivation methods (heat/formalin) are indicated.

Reference	Protozoa dosed	Challenge [org./L]	Type of filter	Nature of sand	Duration of experiment	Surface area of filter [m^2]	Sand depth [m]	Porosity	Grain size d_{50} [μm]	Sand uniformity coefficient	Hydraulic loading rate [m/h]	*Cryptospo–ridium* LRV	*Giardia* LRV
Bellamy et al. (1985b)	live *G. lamblia*	50–5075 cysts/L	Pilot-scale SSF	Matured filter	~ 510 days	0.07	1.42		340	1.46	0.04 0.12 0.4		4.05 4.22 3.72
Bellamy et al. (1985a)	Live *Giardia*		Sand columns	Matured in column (9 weeks)	11 months	0.07	0.97		340	1.46	0.47		>3.10 - >4 [a]
Schuler et al. (1991)	Live *Giardia muris* and formalin inactivated *C. parvum*	1200–3000 oocysts/L 220–2200 cysts/L	Sand columns	Matured in column (~1 month)	> 1 year > 1 year	0.064	0.9		270	1.63	0.4 0.3	>4 [a] >4 [a]	2.77 - >4 [a] >4 [a]
Fogel et al. (1993)	Live *Giardia* and *Cryptosporidium*		Full scale SSF	Matured in filters	21 months	258	1.05			3.3–3.8	0.19–0.4	0.14–0.49	0.96–1.4
Timms et al. (1995)	Heat inactivated *C. parvum*		Pilot scale SSF	Matured in filter (4–5 weeks)	3.5 to 6 h	1.13	0.5				0.3–0.4	3.52–5.52	
Brush et al. (1999)	Formalin-inactivated *C. parvum*		Sand columns	Clean sand	15 min	1.13×10^{-3}	0.11	0.33	850		1.0584	0.32	
Harter et al. (2000)	Live *C. parvum*		Sand Columns	Clean sand		2.04×10^{-3}	0.1	0.51 0.44 0.42	180 420 1400		0.3 0.3 0.3	2.28 0.7 0.13	
Logan et al. (2001)	Live *C. parvum*	65,000 oocysts/L	Sand columns	Matured in column (10 days)	61 days	0.018	0.6	0.46 0.48	310 1400	2.125 1.72	0.00167–0.00417 0.00167–0.00417	2.21–3.7 3.4–3.7	
Tufenkji et al. (2004)	Heat inactivated *C. parvum*		Sand Columns	Clean sand	approx. 30 min	7.85×10^{-5}	0.071	0.43				0.4 to 3	–
Bradford and Bettahar (2005)	Live *C. parvum*		Sand columns	Clean sand		1.66×10^{-3}	0.13	0.36 0.34 0.35	710 360 150	1.21 1.88 2.25	0.066 0.06 0.078	0.51 0.62 0.96	
Hijnen et al., 2005	Live *C. parvum* & *G. intestinalis*		Columns with sand soil		2 weeks	6.36×10^{-3}	0.5	0.32	180	1.6	0.02–0.03	3.3 and 3.9	6.2 and > 6.2 [a]
			Columns with gravel soil		2 weeks	6.36×10^{-3}	0.5	0.36	500	5.3	0.03–0.1	>6.7 [a] and > 7.2 [a]	>6.8 [a] and > 7.4 [a]
Abudalo et al. (2005)	Formalin-inactivated *C. parvum*		Sand columns	Clean sand		1.77×10^{-4}	0.1	0.38	920		0.03	0.12–1.48	
Hijnen et al. (2006) AWWA WQTC	Live *C. parvum*	10^6–10^7 oocysts/L	Sand columns	Mature full-scale SSF sand, no Schmutzdecke	30 h	6.36×10^{-3}	0.4		290 580		0.3 0.3	>5.3 [a] >6.5 [a]	
Hijnen et al. (2007)	UV inactivated *C. parvum*	4.5×10^9 oocysts/L	Pilot	Matured sand	250 days	2.56	1.5	0.41	280	–	0.3	4.7	
Abudalo et al. (2010)	Formalin-inactivated *C. parvum*	10^9 oocysts/L	Sand columns	Preconditioned with dissolved organic matter		1.77×10^{-4}	0.1	0.38	920	–	0.03	0.24–0.52	
Park et al. (2012)	Live *C. parvum*	10^6 oocysts/L	Sand columns	Clean sand	720 min	2.04×10^{-3}	0.2	0.33	425	–	0.296	0.09–4.85	

[a] No oocysts were observed in the effluent of the filter. LRV is determined using the input concentration and the detection limit (DL).

or PCR-based assays; each can introduce distinct recovery biases (e.g., losses during concentration) and endpoint differences (total vs viable/infective oocysts). In several studies, effluent oocysts were not detected and LRVs were inferred from method detection limits, meaning some values represent "≥LRV" rather than exact removal. Differences in oocyst preparation (live vs heat-, UV-, or formalin-inactivated), staining/labelling, and whether assays target total oocysts versus viable/infective oocysts can therefore contribute substantial variability when comparing studies.

Turbidity is a practical indicator linking source water quality to SSF operation and performance. Elevated or highly variable turbidity increases particle loading, accelerates headloss development, and can shorten filter runs, increasing scraping frequency and repeatedly resetting Schmutzdecke maturity; conversely, stable low turbidity supports longer runs and a more established biological community (Huisman and Wood, 1974; Barret et al., 1991; Visscher, 1990). Where raw water turbidity is high (e.g., during storm events), pretreatment such as roughing filtration or coagulation/clarification is often required to protect SSF and maintain finished-water quality (Hashimoto et al., 2019; Abdiyev et al., 2023).

Investigations by Bellamy et al. (1985a) and Bellamy et al. (1985b) showed an average 4.0-log removal of Giardia in SSF. Schuler et al. (1991) later confirmed removal exceeding 4.0-log for both *Cryptosporidium* and *Giardia* once filters had acclimatised and were biologically mature. In contrast, Fogel et al. (1993) noted lower removals (≤0.5 for *Cryptosporidium* oocysts to 1.4 log for *Giardia*) under prolonged cold conditions and when coarser sand was used, contributing to these suboptimal results. Low temperatures can reduce microbial activity in the Schmutzdecke, and larger sand grains decrease the surface area available for attachment (Maurya et al., 2020), reducing the removal efficiency.

Bench-scale experiments by Harter et al. (2000) and Brush et al. (1999) showed that Cryptosporidium removal declined with increasing sand grain size because larger pore spaces permit greater pathogen passage and reduce the straining effect (Bradford et al., 2003). However, Logan et al. (2001) achieved 2.2–3.7 log removal for fine sand and 3.4–3.7 log for coarse sand when operated at filtration rates as low as 0.0017–0.0042 m/h. This highlights the role of filtration velocity in pathogen contact time, a key factor for effective straining and biofilm-mediated removal: slower flow extends hydraulic retention, increasing the likelihood of oocysts colliding with sand grains or encountering predatory organisms in the biofilm. Bradford and Bettahar (2005) and Abudalo et al. (2005) investigated attachment, detachment, and straining mechanisms, with LRVs generally improving with decreasing filter media grain size. Tufenkji et al. (2004) and Park et al. (2012) both noticed enhanced deposition and attachment efficiencies with increasing ionic strength conditions. Hijnen et al. (2005, 2006, 2007) reported removals between 3.3 – >6.5 LRV in mature full-scale or pilot SSF.

Abudalo et al. (2010) demonstrated that higher concentrations of dissolved organic compounds reduce oocyst retention by blocking adsorption sites or competing with oocysts for attachment. Park et al. (2012) showed that modifying ionic conditions or sand polarity also influenced Cryptosporidium removal, since electrostatic charges can shift from repulsive to attractive and promote closer contact. These studies highlight the importance of water chemistry in modulating electrostatic interactions between oocysts and filter media. Specifically, both the net charge and ionic environment can alter attachment efficiency; for instance, oocyst surfaces often carry a negative charge that can be partly neutralised by cations, thereby promoting closer contact and increased deposition (Drozd and Schwartzbrod, 1996; Karaman et al., 1999; Ongerth and Pecoraro, 1996).

Multiple parameters shape SSF efficacy for oocyst retention. Key factors include sand grain size, filter depth, media uniformity, and hydraulic loading rates. In addition, filter maturity, initial conditioning, and experimental constraints can significantly alter reported LRVs. Some investigations also utilised large inoculum doses of oocysts, potentially influencing observed results. Several studies used inactivated (e.g., heat-, UV-, or formalin-treated) oocysts to enable safer handling and controlled transport/retention experiments; however, the inactivation method can alter oocyst surface properties and such studies do not provide direct information on infectivity (Logan et al., 2001; Park et al., 2012).

1.1.1. How do sand characteristics and ripening influence removal?

A key factor underlying the varied removal efficiencies is the interaction between sand properties and the gradual maturation of the filter bed, often referred to as ripening. For example, Bellamy et al. (1985b) showed that the "maturity" of a SSF refers to how well-established its microbial community is, particularly in the top Schmutzdecke layer but also in the lower strata. Bellamy et al. (1985a) also demonstrated that a mature SSF bed, with a fully formed Schmutzdecke, enhanced coliform removal by about 40%, rising from 60.1% removal in a SSF with very limited biological activity to 97.5 to 99.9% removal for a mature SSF. A similar trend is evident for other protozoa, where nine studies evaluated protozoan removal in ripened sand filters using column and pilot-scale systems (Table 1). Of these, six reported an LRV exceeding 3-log (Bellamy et al., 1985a, 1985b; Hijnen et al., 2005, 2006, 2007; Timms et al., 1995). In the remaining three (Fogel et al., 1993; Logan et al., 2001; Schuler et al., 1991), lower LRVs were linked to specific operational or environmental constraints such as extended cold periods or irregular flows (e.g., dry/wet cycles), likely inhibiting microbial metabolism and biofilm stability. Across all the studies where the SSF bed was mature, the average LRV reached 4.02 (±1.94-log), suggesting that microbial ripening and/or partial pore clogging can significantly increase the protozoan removal through enhanced biofilm-mediated mechanisms (e.g., predation, adsorption of oocysts on grains or biofilm surfaces prior to further degradation (Bellamy et al., 1985b; Fogel et al., 1993).

By contrast, in seven additional studies focused on physical and physicochemical processes, such as attachment, detachment, and straining, short bench-scale sand columns packed with clean sand were used. These experiments showed lower average LRVs of around 1-log (±1.34-log) in filter effluent, implying that maturity contributes roughly 3-log of extra oocyst removal beyond basic mechanical capture, since the established biofilm and partially clogged pores provide more contact opportunities between oocysts, media, and protozoan predators (Lefebvre et al., 2021). However, protozoan retention depends on multiple parameters, so ripening alone may not compensate for other suboptimal conditions (e.g., high hydraulic loading) or very high pathogen loads if the filter's contact time and headloss or microbial capacity (for degradation) is exceeded. For instance, shock loading from events such as firefighting or sudden snowmelt/runoff could elevate influent pathogen levels beyond what a mature biofilm can effectively handle. Such events commonly coincide with transient turbidity loading increases and changes in particle character, which can accelerate clogging and alter interception/attachment behaviour, further affecting net LRVs.

Sand grain size also plays an important role, as finer media typically captures more particles but clogs more readily, causing headloss development (i.e., elevated resistance to flow). Harter et al. (2000) and Bradford and Bettahar (2005) observed oocyst reductions declining by 0.45-log and 2.15-log respectively with larger grain sizes, likely because coarser pores permit easier passage of suspended particles. This aligns with the principle that removal efficiency is inversely proportional to grain diameter, evidenced by correlation coefficients of −0.82 (Harter et al., 2000) and − 0.91 (Bradford and Bettahar, 2005). Interestingly, Hijnen et al. (2005) and Logan et al. (2001) noted the opposite in ripened filters: *Cryptosporidium* LRVs rose by about 0.6-log (Logan et al., 2001) and 1.85-log (Hijnen et al., 2005) with larger grain size, suggesting that, once a robust microbial community develops (i.e., a thick, diverse biofilm below the Schmutzdecke), biological mechanisms like predation or expanded attachment sites on extracellular polymers may

compensate for less physical straining (Elemo et al., 2025). Biofilm-induced "pore bridging" can further hinder oocyst passage, akin to fouling phenomena in membrane filtration, by spanning gaps between larger grains and creating additional surfaces for attachment and biodegradation.

Uniformity similarly affects filtration because it governs the consistency of pore sizes throughout the bed. Although lower uniformity should, in theory, yield more variable pore spaces and thus enhance fine-particle capture (via localised straining), Bradford and Bettahar (2005) and Hijnen et al. (2005) reported reduced *Cryptosporidium* removal under smaller uniformity coefficients. Logan et al. (2001), however, saw increased protozoan removal in SSF with lower uniformity possibly because non-uniform grains provided diverse niches for biofilm formation, which can promote additional collision or adhesion events. Such inconsistent findings may arise because irregular grain shapes can create preferential flow paths in some setups, while in others, the biofilm matrix can bridge pore spaces to favour extra straining or predation. Hence, recommendations for SSF often advise a uniformity coefficient between 1.8 and 2.2 (Huisman and Wood, 1974) to balance pore-size distribution and a grain size of 150–400 μm (Ellis, 1985) to ensure adequate surface area for attachment without excessive clogging.

Fogel et al. (1993) illustrate how deviating from these guidelines can undermine SSF performance. Their full-scale system, constrained by limited sand-media availability, used coarser grains (> 600 μm) with a uniformity coefficient above recommended levels (≥3, rather than ≤2.2), yielding poorer removal of both *Cryptosporidium* oocysts and *Giardia* cysts (LRVs not exceeding 1.4). Moreover, how sand characteristics and microbial ripening interact may shift over time. A mature Schmutzdecke and deeper biofilm layers can mitigate certain physical limitations by supplying extensive surfaces for attachment and predation, which may explain contradictory patterns in grain size efficacy. The influence of bed depth on these processes is reviewed next.

1.1.2. Hydraulic loading, sand depth and other parameters

While sand characteristics and filter ripening strongly influence SSF performance, bed depth, hydraulic loading rates, and additional factors also influence consistent protozoan removal. To ensure effective filtration, a minimum depth of 0.7 m is recommended for SSF by Huisman and Wood (1974) under WHO guidelines, based on findings that shallower beds (<0.7 m) often provide insufficient residence time for filter efficiency. In practice, SSF bed depths typically range from 0.7 m to 1.3 m (Barret et al., 1991; Ellis, 1985; Huisman and Wood, 1974; Visscher, 1990), allowing more opportunities for suspended particles to collide with and adhere to sand grains, facilitating interception. Consequently, the probability of particle trapping and adsorption increases with filter depth.

Seven studies (Abudalo et al., 2005, 2010; Bradford and Bettahar, 2005; Brush et al., 1999; Harter et al., 2000; Park et al., 2012; Tufenkji et al., 2004) evaluated *Cryptosporidium* removal. Studies in short sand columns (0.1–0.2 m) often show notable *Cryptosporidium* breakthrough (LRVs <1), likely from limited contact time and minimal biofilm formation. While sand depth alone is not the sole factor in pathogen retention, deeper filters generally achieve higher LRVs, suggesting that greater media thickness extends the filtration path and adds layers for straining or biological processes. However, extrapolating from small-scale columns can be misleading: these setups are useful for isolating specific chemical or physical conditions but cannot replicate the diverse microbial communities and scaled flow dynamics of full-scale SSFs. Additionally, narrow columns can produce wall effects that distort flow around oocysts; thus, the column diameter should be at least 10 to 20 times the median grain size (≥10 cm) to minimize boundary artifacts.

Regarding hydraulic loading rates, Bellamy et al. (1985b) showed that slower filtration rates (e.g., 0.04–0.12 m/h) yielded greater (>0.3-log increase) *Giardia* removals. Schuler et al. (1991) reported a similar trend of increased protozoan retention even with modest changes in flow. Lower flow rates (e.g., <0.12 m/h) extend contact time between pathogens and collector grains, promoting collision, attachment, and potential predation by allowing microorganisms and suspended particles additional interaction time. At very low velocities, microscopic-scale sedimentation can also occur (Huisman and Wood, 1974), as gravitational settling exceeds convective transport. While reducing filtration velocity may improve protozoan attenuation, the trade-off is reduced water production, posing challenges for municipal-scale systems that need higher throughput.

Several other variables affect SSF efficacy. Temperature, for example, influences microbial activity within the bed; Bellamy et al. (1985a) observed a 10% decrease in removal efficiency under colder conditions, likely due to slower metabolic rates and reduced biofilm formation. SSFs that are directly exposed to seasonal fluctuations (i.e., outdoor/uncovered) can experience substantial changes in feed-water and bed temperatures, thereby affecting microbial predation rates and overall filtration kinetics. Likewise, the initial (oo)cyst dose is significant: studies challenging SSFs with substantially higher pathogen concentrations than those typically found in natural waters (Abudalo et al., 2010; Hijnen et al., 2006, 2007; Logan et al., 2001; Park et al., 2012; Schuler et al., 1991) risk exceeding the filter's capacity for retention or microbial predation, thus causing breakthrough. In some cases, longer run times or bed ripening reduced these effects (Hijnen et al., 2006, 2007; Schuler et al., 1991).

Finally, while LRV is a useful metric for assessing immediate pathogen retention, it has limitations. The LRV cannot exceed the initial pathogen influx, potentially underestimating a filter's capacity when inlet concentrations are low. Additionally, once the inlet concentration returns to zero, standard LRV calculations may not capture delayed or secondary release of pathogens, often termed post-challenge breakthrough. This can occur if retained oocysts detach or if abrupt flow shifts dislodge trapped particles (Helmi et al., 2008). A "delayed LRV" approach could address cumulative pathogen retention over time, providing a more holistic view of SSF performance.

1.2. Physicochemical retention mechanisms in SSF

Although slow sand filtration has been used in the UK for nearly 200 years, it remains largely viewed and operated as a "black box" owing to the interplay of biological, chemical, and physical mechanisms. While the fundamental physicochemical principles governing particle retention in clean-sand-only filters are well understood and can be modelled effectively, the additional biological activity in SSF adds complexity through microbial interactions and biofilm processes. A diverse community of micro- and meiofauna, along with feed-water-derived algae (Pfannes et al., 2015), can significantly enhance the removal of bacteria, viruses, and protozoa, including *Cryptosporidium* oocysts (Hijnen et al., 2007). However, predicting removal efficiencies from first principles alone is challenging because of these added factors.

Although most conventional treatment works in developed countries employ multiple steps (e.g., ozonation, coagulation, clarification) before SSF, the following discussion focuses on oocyst retention within SSF irrespective of pre-treatment. Historically, SSFs have been placed outdoors, subjected to weather extremes (including ultraviolet (UV) radiation). Smaller systems may be covered to limit algal growth and for use in harsher climates, but large, uncovered SSFs in the UK can occupy thousands of square meters (e.g., up to 7370 m^2 in London). Beyond straining and media adhesion, solar UV can damage Cryptosporidium oocysts in the supernatant above the sand (Connelly et al., 2007).

While UV-C (ultraviolet-C) inactivation of *Cryptosporidium parvum* oocysts is well documented (Rochelle et al., 2005), solar UV-A and UV-B effects in freshwater are less studied. Johnson et al. (1997) found that 3 days of solar exposure in marine water reduced oocyst viability by 90%, compared with 50% in phosphate-buffered saline (PBS) kept in the dark. Under solar water disinfection (SODIS) conditions, McGuigan et al. (2006) and Méndez-Hermida et al. (2005) reported declines in infectivity/excystation after 6–10 h of sunlight exposure, while Connelly

et al. (2007) reported >99.99% infectivity loss after 10 h of full-spectrum solar irradiation in PBS. Morris et al. (1995) noted that lower dissolved organic carbon (DOC) enhances UV penetration, implying that clearer supernatants may favour sunlight-mediated attenuation. King et al. (2008) also observed rapid reductions in infectivity during the first hour of sunlight exposure. However, Busse et al. (2019) modelled approximately 6-log inactivation specifically for the top 20 cm of an outdoor swimming pool at 320 nm after one hour, so this result should not be generalised to deeper or more optically complex waters. Collectively, these studies suggest that sunlight may contribute to oocyst attenuation in uncovered systems, but its effect is highly context dependent and unlikely to provide a consistent primary barrier in municipal SSF. By contrast, engineered low-pressure UV systems can deliver validated doses; Morita et al. (2002) reported that approximately 10–12 mJ/cm^2 produced around 2-log (99%) inactivation of *Cryptosporidium* oocysts under controlled conditions.

Physical straining remains a factor in *Cryptosporidium* oocyst retention. Tufenkji et al. (2004) found oocyst capture exceeded Derjaguin–Landau–Verwey–Overbeek (DLVO) theory predictions, indicating **a** mechanical removal mechanism that is less dependent on electrostatic interactions. Hijnen et al. (2005) similarly documented oocyst retention above predictions from the Tufenkji and Elimelech (2004) model. These data emphasize particle-size straining, particularly when oocysts approach the critical size ratio relative to sand grains (Bradford et al., 2003; Sakthivadivel, 1967). Tufenkji et al. (2004) further demonstrated that irregular grain shapes produce small interstices capable of trapping oocyst-sized particles, **as** media geometry influences filtration efficacy.

Beyond straining, physicochemical adhesion also immobilizes suspended particles. Yao et al. (1971) integrated colloidal theory (Smoluchowski, 1917) and air filtration theory (Friedlander, 1958; Spielman and Goren, 1970) to account for gravity, interception, and Brownian diffusion. Tufenkji and Elimelech (2004) refined the Yao et al. (1971) numerical method by incorporating particle size ratio, convective/diffusive transport, approach velocity, and van der Waals forces. This model offers more accurate predictions of how effectively a filter can capture particles of varying sizes under different flow conditions than the earlier, more generalised theory of Yao et al. (1971). When Cryptosporidium oocysts are treated as inert, oocyst-sized particles with the same electrostatic characteristics, these models generate realistic deposition estimates in clean sand, indicating that such approaches capture most aspects of filtration performance in controlled conditions (Tufenkji and Elimelech, 2004).

In fully ripened SSFs, however, it is difficult to separate purely physical from simultaneous biological mechanisms. Over time, biofilm development on sand grains can alter grain shape, pore geometry, and surface polarity (Abudalo et al., 2010; Lefebvre et al., 2021). Such changes may reduce oocyst attachment if electrostatic factors are modified or pore channels narrow, implying that some filtration parameters evolve as the filter matures (e.g., headloss, interstitial flow paths) (Dai and Hozalski, 2002)**.** Consequently, purely physical models may under- or over-estimate oocyst retention in operational SSFs, where microbial processes also strongly promote pathogen removal. Thus**,** advancing our understanding of protozoan degradation under these bioactive conditions, especially in SSF, requires additional exploration.

1.3. Biological removal mechanisms: the ingestion and subsequent fate of oocysts by higher organisms

Slow sand filters and other aquatic environments harbour diverse communities of higher trophic organisms, including protozoa and metazoan meiofauna (e.g., ciliates, rotifers, nematodes) (Goddard, 1980; Ibrahim, 1989). These communities benefit from the relatively stable hydrodynamic conditions and continuous supply of organic detritus in SSF beds and, in doing so, may contribute to the removal of *Cryptosporidium* oocysts (Fayer et al., 2000; Trout et al., 2002). However, several questions persist: are these organisms degrading oocysts, and is *Cryptosporidium* a preferred, indifferent, or suboptimal food source compared to other particulates?

Cryptosporidium oocysts are often trapped in filtration media but limited direct in situ data make it unclear whether they remain immobilised, undergo secondary transport, or are consumed (Siqueira-Castro et al., 2016; Gómez-Couso et al., 2007; Scheid and Schwarzenberger, 2011). Some researchers propose that oocysts serve as an additional nutrient source for higher trophic organisms (King et al., 2007; Stott et al., 2001)**,** thereby enhancing oocyst clearance in drinking water, wastewater, and natural systems (Stott et al., 2003; Trout et al., 2002). In nutrient-poor drinking water systems, predation can be a key mechanism of oocyst removal because microfauna must scavenge limited organic compounds. By contrast, in eutrophic environments (e.g., many wastewater treatment systems), abundant organic matter could reduce direct oocyst ingestion, yet overall microbial activity (e.g., extracellular enzymes) may still incidentally degrade oocysts (Hassard et al., 2018).

Studies in reservoir sediments show that benthic and planktonic communities can reduce Cryptosporidium levels by physically trapping and biologically ingesting oocysts (King and Monis, 2007). Slower flow rates allow oocysts to accumulate, making these sediments natural arenas for diverse organisms to prey on or degrade them, offering insights into removal processes. Similarly, research on river sediments, activated sludge, and wetlands reveals a broad distribution of oocyst-ingesting species (Siqueira-Castro et al., 2016; Stott et al., 2001), suggesting that leveraging natural predation could bolster *Cryptosporidium* removal in engineered systems (Stott et al., 2001). For example, backwash from rapid gravity filters often returns to wastewater streams or the start of drinking water treatment flowsheets, potentially reintroducing oocysts (Fig. 1). Therefore, SSF and other nature-based technologies may help close the loop and curb further spread by acting as a sink for *Cryptosporidium*. Additionally, laboratory trials tested the feasibility of introducing monocultures (e.g., *Paramecium* spp.) into biological filtration systems, measuring survival, ingestion rates, and potential oocyst degradation (Fayer et al., 2000; Stott et al., 2001). Bioaugmentation could therefore offer a strategy to enhance *Cryptosporidium* removal when existing microbial communities are insufficient.

Experimental approaches typically began by isolating test organisms from environmental samples and briefly starving them to standardize feeding, after which they are exposed to fluorescently labelled oocysts (Table 2). Epifluorescence microscopy is then used to measure ingestion and excretion, revealing how many oocysts each organism consumed (Fayer et al., 2000; Stott et al., 2001, 2003). Many species can rapidly ingest multiple oocysts, but it is still uncertain how well these lab-based findings carry over to more complex, nutrient-rich environments. Further studies are needed to determine if such biological removal can be consistently leveraged in operational water treatment or for cleanup of waste streams from these assets (e.g., reservoir sediments).

1.3.1. Ingestion of Cryptosporidium parvum oocysts

Building on the broader discussion of *Cryptosporidium* predation in aquatic systems, this section focuses on the specific evidence for oocyst ingestion by organisms and the factors that influence this process**.** Research has shown substantia**l** variability in the ingestion of *Cryptosporidium* oocysts by aquatic fauna, demonstrating that broad taxonomic classifications alone do not reliably predict which species will ingest oocysts (Table 2). Instead, feeding behaviour seems linked to morphological, ecological, and possibly chemical cues**.** For instance, King et al. (2007) reported “uncountable” oocyst ingestion by Bdelloid rotifers under high oocyst density andminimal alternative prey**.** Whereas *Lepadella*, another rotifer genus, ingested only two oocysts. Several ciliate taxa exhibited no uptake (Siqueira-Castro et al., 2016), suggesting that even closely related groups can differ in feeding responses, as a consequence of differences in feeding apparatus, mouth-part size, or prey-recognition cues.

A range of biological attributes, including organism size, feeding mode (filter feeders vs. bacterivores), and motility influences whether

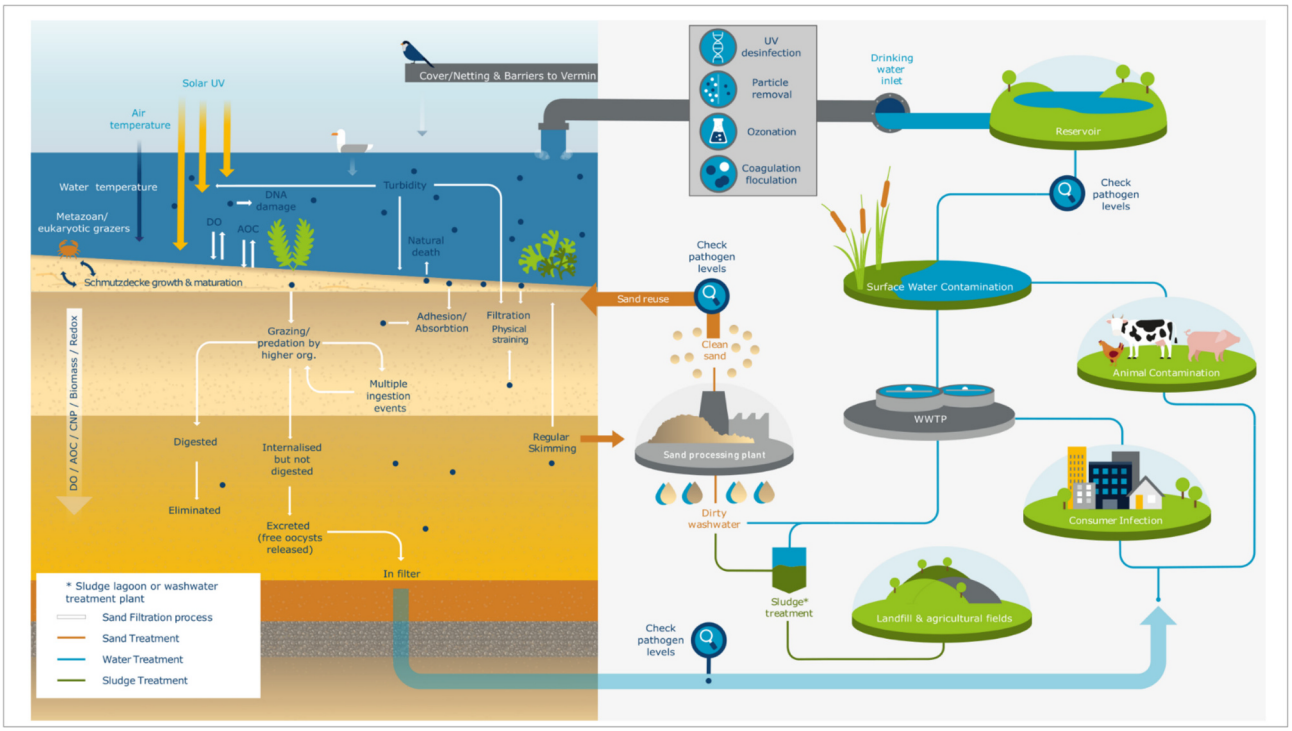


Fig. 1. Schematic illustrating (left) the key processes in slow sand filtration, biological predation, physical straining, and solar UV exposure, and (right) the broader water cycle, including runoff pathways, municipal treatment steps (e.g., coagulation, UV disinfection), and contamination checks. The left half shows a cross-section of the slow sand filter bed, highlighting *Cryptosporidium* oocyst capture, microbial interactions, and routine maintenance; the right half depicts how treated water and residual wastes cycle back into the environment, emphasizing potential sources and interventions for pathogen control.

and how oocysts are ingested. Larger organisms, such as the nematode ***C. elegans*** (~1 mm), can ingest up to 500 oocysts per individual (Huamanchay et al., 2004), whereas smaller rotifers (0.2–0.5 mm) often ingest fewer (10%) compared to *C. elegans*, due to smaller gut capacity. Even within the same genus, behaviours can vary markedly: *Vorticella infusionum* ingested oocysts in 8 of 10 test specimens, whereas ***Vorticella convallaria***, despite a similar suspension-feeding mechanism, did not ingest any (Siqueira-Castro et al., 2016). Potential explanations include slight variations in ciliary structure, chemical sensing, or particle size preferences. Similarly, some *Spirostomum* species readily ingested oocysts, while closely related taxa did not (Siqueira-Castro et al., 2016), suggesting feeding behaviour can be highly species-specific and reflective of unique ecological niches or trophic strategies. These species-level differences have clear relevance for both in situ studies and engineered nature-based treatment systems. Identifying which organisms thrive under certain conditions and their feeding preferences can guide biofiltration design, operation, and optimization (e.g., SSF, constructed wetlands) to maximize pathogen removal.

Although specific mechanisms of oocyst ingestion vary widely, differences in oral structures and feeding currents help explain why some taxa more readily capture larger particles like *Cryptosporidium* oocysts. For instance, certain ciliates possess a specialised buccal cavity or ciliated peristome that channels suspended particles toward the cell's oral groove (Kudo, 1954), whereas others rely on a simpler suspension-feeding approach (Pauli et al., 2005). Some rotifers likewise use ciliated coronae to generate water currents, but their relatively small mouthparts and rapid feeding motions may reduce contact with larger particles such as (oo)cysts (Trout et al., 2002). Amoebae, in contrast, typically employ phagocytosis by enveloping food items, yet may reject oocysts if they lack necessary surface receptors or chemical cues indicating nutritional value (Thorp and Covich, 2001). Morphological and behavioural differences can thus help explain why ingestion rates vary, even among closely related species (Siqueira-Castro et al., 2016; Fayer et al., 2000).

Such patterns of selective ingestion extend beyond ciliates and rotifers. Certain amoebae merely encountered oocysts without ingesting them, indicating a rejection mechanism based on surface recognition or poor nutritional signals (Siqueira-Castro et al., 2016; King et al., 2007). Scheid and Schwarzenberger (2011) likewise observed amoebae moving around oocysts with no uptake. Additionally, some rotifers briefly ingested oocysts then expelled them via the corona, a behaviour noted in 3 of the 6 genera studied (Fayer et al., 2000). This rapid expulsion may imply that oocysts are not consistently favoured and may be avoided.

Alternative food sources also affect uptake. Stott et al. (2003) reported that *Paramecium* exposed to high oocyst densities (1800–3000 oocysts/organism) consumed an average of 20 per organism, whereas 91% of *Paramecium* exposed to lower densities (9 oocysts/organism) showed no ingestion. One rationale is that when oocysts are abundant, they are less avoidable, heightening incidental or opportunistic ingestion; at lower densities, *Paramecium* may favour other particles with superior nutritional returns relative to the energy cost of ingesting oocysts.

From an ecological standpoint, these findings underscore how certain microorganisms can naturally remove Cryptosporidium from water, sediment, or biofilms. Even among related taxa, variations in ingestion rates indicate that feeding apparatus morphology, chemical cues, and competing prey significantly affect oocyst uptake. Further lab and field studies are critical for shaping effective strategies to curb Cryptosporidium contamination. In SSF contexts, pinpointing which species thrive in low-nutrient conditions and exhibit elevated oocyst ingestion can guide filter design, operations, and maintenance, creating new opportunities for both natural and engineered interventions to mitigate cryptosporidiosis risks and meet regulatory requirements.

1.3.2. *Ingestion rates*

Ingestion rates of *Cryptosporidium* oocysts by aquatic organisms vary widely, from 0.003 oocysts per organism per minute in *Acanthamoeba* (Gómez-Couso et al., 2007) to 5.1 oocysts per organism per minute in

Table 2
Summary of *Cryptosporidium* oocyst ingestion by various aquatic taxa (rotifers, amoebae, ciliates, nematodes, etc.). Listed are the phylum, genus, and species tested, the presence or absence of ingestion, oocyst exposure levels, ingestion rates, contact times, and key references. Some entries denote missing data (N/A) or annotate the test environment (e.g., wetland rotifers, activated sludge isolates).

Phylum	Genus	Species	Ingestion observed	Specific exposure [oocysts/org.]	Specific ingestion [oocysts/org.]	Contact time [min]	Specific ingestion rate [oocysts/org./min]	References
Rotifera	*Philodina*	species B	+	1000–2000	5–15 (25)			Fayer et al. (2000)
	Monostyla	Unidentified	+	1000–2000	<5–15 [a]			
	Epiphanes	*brachionus*	+	1000–2000	<5–15 [a]			
	Euchlanis	*triquetra*	+	1000–2000	up to 25			
	Brachionus	*quadridentatus*	+	1000–2000	up to 25			
	Asplanchna	Unidentified	+	1000–2000	<5–15 [a]			
	Lepadella	Unidentified	+	19,300 [b]	At least 2	90	0.022 [f]	King et al. (2007)
	Dicranophorus	Unidentified	+	19,300 [b]	No data	90	0.01 [f]	
	Bdelloid	3 Unidentified species	+	19,300 [b]	Uncountable	90	0.01 [f]	
	Unidentified (wetland rotifer)	Unidentified	+	67	1.6 (7)	60	0.027 [f]	Stott et al. (2003)
Amoebozoa	*Acanthamoeba*	group II; Petz	+	200,000 [b]	1 (3)			Scheid and Schwarzenberger (2011)
		group II; aqua iso	–	N/A	N/A	N/A	N/A	
		commandoni PL 30/40	–	N/A	N/A	N/A	N/A	
		Unidentified	+	2,000,000 [b]	2 (6)	720	0.003	Gómez-Couso et al. (2007)
		culbertsoni	+	1	1.8 (3)	120	0.015 [f]	Stott et al. (2003)
	Vannella	Dent G1	–	N/A	N/A	N/A	N/A	Scheid and Schwarzenberger (2011)
	Saccamoeba	*limax* SL 1	–	N/A	N/A	N/A	N/A	
		stagnicola aqss	–	N/A	N/A	N/A	N/A	
Amoebozoa (cont'd)	*Hartmannella*	sp.	–	N/A	N/A	N/A	N/A	Scheid and Schwarzenberger (2011)
	Thecamoeba	*quadrilineata* d. c.h.1.	+	200,000 [b]	8 (15)			
	Thecamoeba	unidentified	+	19,300	No data	240		King et al. (2007)
	Mayorella	unidentified	+	19,300	approx. 13	240	0.05	
	Microchlamys	unidentified	–	19,300	N/A			
	Willaertia	unidentified	+	19,300	No data	240	0.004	
	Cochliopodium	unidentified	+	19,300	No data	240	0.004	
Ciliophora	*Stylonychia*	*mytilus*	+	1000	11(50)	Overall	1	Stott et al. (2001)
						5	2.9	
						60	0.2	
			+	10–30		Overall	0–0.03 (0.2)	
					0.2	10	0.02 (0.2)	
					0.6	20–40	0.03	
					0.2	40–60	0.01	
			+	67	0.3 (3)	60	0.005	Stott et al. (2003)
					0.6 / 0–2	180	0.003	
	Paramecium	unidentified	+	19,300 [b]	at least 13	90	0.14 [f]	King et al. (2007)
		caudatum	+	1000	35 (90)	overall	2.8	Stott et al. (2001)
						5	5.1	
Ciliophora (cont'd)	*Paramecium*	*caudatum*				30	1.6	Stott et al. (2001)
			+	10–30		overall	0–0.03 (0.2)	
					0.33	30	0.01	
					1.22	60	0.02	
			+	67	1.9 (9)	60	0.03 [f]	Stott et al. (2003)
					0.5/ 0–2	180	0.003 [f]	
			+	9	0.5/ 1–11 (11)	60	0.01 (0.2)	
			+	18–30	0.5 / 1–11(11)	60	0.05 (0.4)	
			+	1800–3000	20 / 3–45	60	1.11 (2.3)	
	Euplotes	*patella*	+	1000	1(10)	overall	0.07 (0.2)	Stott et al. (2001)
						5	0.2	
						60	>0.1	
		aediculatus	+	7	in 4/10 specimen [c]	6 to 40	0.025 to 0.17 [e]	Siqueira-Castro et al. (2016)
		unidentified	+	19,300 [b]	at least 15	90	0.16	King et al. (2007)
	Unidentified (Wetland ciliate)	unidentified	+	1000	3 (20)	overall	0.2	Stott et al. (2001)
						5	0.4	
						60	>0.1	
	Stentor	*coeruleus*	+	7	in 4/10 specimen [c]	35	0.03 [f]	Siqueira-Castro et al. (2016)
	Blepharisma	*sinuosum*	–	7	N/A	N/A	N/A	
Ciliophora (cont'd)	*Blepharisma*	unidentified	+	19,300 [b]	Unknown [g]	90	0.01	King et al. (2007)
	Oxytricha	unidentified	+	19,300 [b]	>100 [d]	90	1.11	
		granulifera	+	7	in 3/10 specimen [c]	45	0.02 [f]	Siqueira-Castro et al. (2016)

(*continued on next page*)

Table 2 (*continued*)

Phylum	Genus	Species	Ingestion observed	Specific exposure [oocysts/org.]	Specific ingestion [oocysts/org.]	Contact time [min]	Specific ingestion rate [oocysts/org./min]	References
	Spirostomum	minus-complex	+	7	in 7/10 specimen [c]	30	0.03 [f]	
		teres	–	7	N/A	N/A	N/A	
	Sterkiella	sp.	–	7	N/A	N/A	N/A	
		cavicola	–	7	N/A	N/A	N/A	
	Vorticella	infusionum	+	7	in 8/10 specimen [c]	30	0.03 [f]	
		convallaria	–	7	N/A	N/A	N/A	
	Aspidisca	*cicada*	–	7	N/A	N/A	N/A	
	Epistylis	*plicatilis*	–	7	N/A	N/A	N/A	
	Gastronauta	*aloisi*	–	7	N/A	N/A	N/A	
	Loxodes	*striatus*	–	7	N/A	N/A	N/A	
	Metopus	*laminarius*	–	7	N/A	N/A	N/A	
	Holosticha	unidentified	+	19,300 [b]	No data	90	0.01	King et al. (2007)
	unidentified (wetland ciliate)	unidentified	+	67	0.2 (3)	60	0.003	Stott et al. (2003)
Platyhelminth	*Rhabdocoel*	unidentified	+	19,300 [b]	Uncountable [d]	90	0.01	King et al. (2007)
Gastrotricha	*Chaetonotus*	unidentified	+	19,300 [b]	at least 4	90	0.04 [f]	King et al. (2007)
Nematode	*Caenorhabditis*	*elegans*	+	unknown	0–500	60	8.3	Huamanchay et al. (2004)
Percolozoa	*Tetramitus*	*pararusselli Rhodos*	–	N/A	N/A	N/A	N/A	Scheid and Schwarzenberger (2011)
	Naegleria	*gruberi* aq 2	–	N/A	N/A	N/A	N/A	

[a] Oocysts momentarily ingested then expelled.
[b] Not specific.
[c] Number of organisms positive for *Cryptosporidium*.
[d] Too many oocysts present in organism to count.
[e] Assumption of a minimum of 1 oocyst ingested.
[f] Calculated based on available data.
[g] Oocysts present but impossible to count due to autofluorescence of organism.

Paramecium (Stott et al., 2001). Nematodes can ingest even more, up to 8.3 oocysts per organism per minute under high oocyst density and limited alternative prey. These rates derive from direct observation or extrapolation, and factors like species, pathogen load, incubation period, and nutrient availability all influence feeding frequency.

In contrast to the rapid ingestion seen in some nematodes, amoebae typically show slower rates due to their phagocytic mode, which encapsulates oocysts in vesicles. This encapsulation may prolong oocyst survival in SSF or aid further breakdown if amoebae are later consumed by higher-level predators. Whether oocysts within amoebic vesicles remain infective post-excretion is still uncertain (Lefebvre et al., 2023; Scheid and Schwarzenberger, 2011).

To capture the full extent of amoebal uptake, King et al. (2007) and Stott et al. (2003) extended incubation periods, while Gómez-Couso et al. (2007) employed contact times of up to 12 h. Although SSF contact times are often shorter (e.g., hours vs. days), prolonged exposure in upper filter layers or within biofilms might mirror these extended scenarios, allowing ongoing amoebal interaction with oocysts especially as trapped particles could remain in a SSF for weeks.

In other taxa, both exposure duration and oocyst density shape ingestion dynamics. Higher pathogen loads can stimulate more frequent feeding encounters, whereas longer contact times may cause saturation or fatigue. Stott et al. (2001) observed initial high rates (2.9 and 5.1 oocysts per minute) in *Stylonychia* and *Paramecium* at elevated oocyst densities, dropping to 0.2 and 1.6 after one hour, potentially from feeding capacity saturation. No such decline occurred at lower doses (10–30 oocysts/organism), closer to natural concentrations.

Even so, prolonging contact time generally yielded higher total ingestion, reflecting a cumulative effect. Temperature and other filtration steps also influence these rates: colder conditions suppress metabolic activity, while warmer conditions promote robust microbial and meiofaunal communities and predation (Weisse, 2024). Coagulants can further affect ingestion by changing oocyst properties or particle aggregation. These factors help explain why SSFs exhibit variable pathogen removal efficiencies, highlighting the importance of both biological and operational parameters, including temperature, loading rates, and pretreatment, when assessing SSF performance.

1.3.3. Excretion, digestion, and viability of oocysts

Excretion or egestion of *Cryptosporidium* oocysts may explain declining ingestion rates over time: once internalised, some organisms expel oocysts quickly rather than invest energy in digestion, freeing capacity to ingest more nutritious particles. Fayer et al. (2000) observed up to eight oocysts released within rotifer faecal pellets (e.g., Epiphanes and Euchlanis) after 15 min, while Stott et al. (2001) documented excretion at 145 min. King et al. (2007) similarly noted near-complete oocyst expulsion by rotifers around 90 min post ingestion, suggesting an adaptive strategy to avoid harbouring non-beneficial particles. Amoebae gradually expel oocysts prior to encystation (Gómez-Couso et al., 2007; Scheid and Schwarzenberger, 2011) and ciliates egest them within 60 min with no net accumulation (Stott et al., 2001, 2003). In an SSF context, rapid turnover means predators may not retain oocysts long enough for inactivation, posing a potential public health concern if excreted oocysts remain infective (Bretagne et al., 2026).

A key reason ingestion may not translate to inactivation is that *Cryptosporidium* oocysts are adapted to withstand harsh physicochemical conditions and enzymatic catalysis. The multi-layered oocyst wall limits permeability and can resist digestive processes, so gut passage through aquatic predators may result in short-term sequestration (e.g., within faecal pellets) rather than structural failure. Predator-specific gut residence time, mechanical processing, and enzyme complements, along with temperature and alternative prey availability, are therefore likely determinants of whether ingestion causes meaningful damage across species and environmental conditions.

Despite these excretory observations, little is known about the ultimate fate of expelled oocysts. Gómez-Couso et al. (2007) and Scheid and Schwarzenberger (2011) report minimal morphological change, whereas Fayer et al. (2000) and King et al. (2007) note reduced

fluorescence signals in expelled oocysts, implying partial enzymatic or chemical modification. Intriguingly, Huamanchay et al. (2004) discovered empty oocysts excreted by certain nematodes, raising questions about whether the contents were digested or shed. Overall, the thick glycoprotein wall likely resists enzymatic breakdown, although species-specific differences in enzyme systems could influence oocyst alteration and warrant further investigation.

The ability of organisms like rotifers to fully degrade oocysts is doubtful. Fayer et al. (2000) were among the first to question whether rotifers produce the enzymes needed to penetrate and digest the oocyst's acidic glycoprotein layer (Tufenkji et al., 2006), which is specifically adapted to protect the parasite in harsh environments. Consequently, the hypothesis that repeated ingestion cycles might incrementally weaken oocyst walls, reducing infectivity, remains largely untested, requiring advanced methods (e.g., SEM, cell-culture infectivity, cryo-EM) to detect subtle damage. One way to test this hypothesis is to use sequential passaging experiments (repeated cycles through the same predator, or through multiple predator taxa) and then compare structural integrity and infectivity before and after each passage. A practical methodological approach is to combine high-resolution imaging (e.g., SEM/TEM or imaging flow cytometry) for wall disruption, permeability or fluorescence changes as supportive indicators, and viability/infectivity endpoints (e.g., propidium monoazide quantitative polymerase chain reaction (PMA-qPCR) for viable oocysts and integrated cell culture qPCR (ICC-qPCR) or cell-culture assays for infectivity) to confirm whether cumulative damage translates to reducing the public health risks from this pathogen. If such cumulative damage does occur, these biological interactions could profoundly affect Cryptosporidium control in natural waters, wastewater, and SSF systems, as repeated passage through different predators might enhance inactivation.

1.3.4. Strategies for optimizing biological water treatment systems to enhance Cryptosporidium removal

Designing biological water treatment systems to maximize *Cryptosporidium* removal requires understanding the variable ingestion rates and ecological needs of different aquatic organisms. One approach is to selectively introduce species with high ingestion rates, such as *Paramecium* or certain nematodes so the system is biologically primed for effective oocyst capture (Pauli et al., 2005). However, it is equally important to consider whether these species excrete viable oocysts under specific conditions, as re-liberation could negate net removal gains (Fayer et al., 2000; King et al., 2007; Stott et al., 2001).

Bioaugmentation or selective encouragement of high-ingestion taxa also carries ecological and operational risks. Ecologically, favouring one functional group may shift community structure, reduce resilience, or promote unintended trophic interactions (including establishment of undesired taxa). Operationally, increased biomass production and grazing activity could accelerate headloss development, shorten run length, increase scraping frequency, and contribute to effluent biological instability if sloughing occurs (Bretagne et al., 2025). Any bioaugmentation strategy should therefore be trialled cautiously and coupled to monitoring that links predator abundance and community composition to both oocyst counts and oocyst viability/infectivity, alongside routine operational indicators such as turbidity/particle counts, headloss, dissolved oxygen, and run time between maintenance interventions (Elemo et al., 2024).

Incorporating a diverse predator community, encompassing ciliates, rotifers, amoebae, and nematodes, creates conditions of ecological redundancy and enhances overall system resilience (Brookes et al., 2005). If one species becomes less efficient due to environmental shifts or pathogen adaptation, others can maintain robust *Cryptosporidium* removal. Ongoing monitoring of predator abundance and oocyst concentrations can inform timely operational adjustments, keeping the system both adaptive and effective. Moreover, aligning biofiltration design and operation with emerging research is seen as a priority for SSF systems (Hassard et al., 2022). As new findings clarify which organisms exhibit stable ingestion rates and which conditions promote oocyst degradation, filters can be retrofitted or reconfigured to harness these insights. Adopting a holistic approach not only improves *Cryptosporidium* removal for current demands but also provides a flexible platform for integrating future technological or biological advances, ultimately bolstering water quality and public health protection.

1.3.5. Limitations of translating ingestion/digestion experiments to real aquatic matrices

Although numerous laboratory studies demonstrate that certain aquatic organisms can ingest significant numbers of *Cryptosporidium parvum* oocysts, these controlled experiments often maximize uptake by limiting alternative prey, raising questions about how such behaviours translate under field conditions (Fayer et al., 2000; Trout et al., 2002). In natural or engineered systems, organisms typically have access to more assimilable food particles, potentially reducing the motivation to consume oocysts. Moreover, some species exhibit avoidance strategies, while others opportunistically ingest oocysts, yet the overall impact on oocyst removal in full-scale water or wastewater treatment remains uncertain.

Most ingestion studies quantify uptake under controlled conditions, but net *Cryptosporidium* risk reduction in full-scale SSF depends on predator density and distribution in the Schmutzdecke, ecological interactions, hydraulics, and critically, whether ingestion causes inactivation rather than rapid egestion. Laboratory studies may therefore over- or under-estimate net removal due to starvation or limited alternative prey, unrealistically high oocyst-to-predator ratios, simplified biofilm architecture and grazing networks, temperature differences, and hydrodynamics that do not represent field shear and residence time. A practical translation approach is to treat lab ingestion rates as an upper bound and scale expected net removal by (i) predator abundance, (ii) contact/residence time, and (iii) the fraction of ingested oocysts demonstrably inactivated versus egested viable.

Compounding these uncertainties is the fate of expelled oocysts, which can be released in boluses 20–30 times larger than a single oocyst (Fayer et al., 2000). While this may enhance sedimentation in filters (e.g., slow sand or rapid gravity systems), such aggregates could also shield oocysts from disinfection. Additionally, many protozoa that ingest *Cryptosporidium* serve as vectors for other human pathogens. Amoebae can harbour viable microbes post-encystation (Winiecka-Krusnell and Linder, 2001), and Scheid and Schwarzenberger (2011) suggest that *Cryptosporidium* oocysts might likewise remain infective, similar to *Toxoplasma gondii* oocysts, following amoebal ingestion and exocytosis.

Other aquatic organisms also accumulate and retain infective oocysts: Fayer et al. (1997) and Graczyk et al. (1998) showed that shellfish such as estuarine oysters and freshwater clams can harbour *Cryptosporidium* oocysts, indicating a potential role in environmental retention and transport. Méndez-Hermida et al. (2006, 2007) similarly showed that the aquatic arthropod *Artemia* can carry over 30 viable oocysts, which may be passed on to predatory fish. Hence, lab-based ingestion data may overestimate net removal, given complex species interactions, diverse feeding options, and potential for oocyst transport or protection in real-world environments. Addressing these gaps is important for accurately predicting and ultimately optimizing *Cryptosporidium* control in water treatment processes.

1.3.6. Novel methodologies to assess oocyst ingestion and viability

1.3.6.1. Detection endpoints: presence, viability and infectivity. It is useful to distinguish between (i) total oocyst detection (presence/absence or enumeration regardless of viability), (ii) viability (oocysts that remain intact and potentially capable of infection), and (iii) infectivity (oocysts that can invade and reproduce in a host). These endpoints are not equivalent, and the choice of method can influence both perceived removal and the interpretation of predator-mediated effects.

Studying *Cryptosporidium* removal by biological means demands methods to track oocyst ingestion, digestion, and viability. Emerging techniques are improving the ability to quantify these processes. For instance, researchers use fluorescently labelled oocysts (stained with FITC-conjugated antibodies) to feed protozoan predators and then examine gut contents by epifluorescence microscopy (Fayer et al., 2000; Stott et al., 2001). This allows direct enumeration of oocysts ingested per predator and observation of any morphological changes (e.g. degraded oocyst walls) post-ingestion (Stott et al., 2001). In one approach, paired fluorescence and DIC (differential interference contrast) microscopy images were used to confirm oocysts inside rotifers and to capture excreted oocyst clusters (Fayer et al., 2000). Beyond microscopy, flow cytometry has become a powerful tool. Researchers have noted that when oocysts are partially digested by predators, the specific antibody binding (FITC fluorescence intensity) can diminish, presumably due to epitope degradation (King et al., 2005). This change in signal can serve as a marker to distinguish ingested/damaged oocysts from intact ones. Proposed methods include flow cytometric sorting of oocysts based on fluorescence intensity to isolate those that have passed through predators, for downstream viability testing (King et al., 2005). Viability and infectivity assays themselves have seen significant innovation. Traditional dye permeability tests (using dyes like DAPI/PI) provided a rough live/dead count, but newer molecular assays give more definitive results. One breakthrough is the use of propidium monoazide PCR (PMA-PCR), which combines a DNA-intercalating dye with PCR to estimate the fraction of oocysts with intact versus compromised walls. PMA preferentially penetrates oocysts with compromised walls and blocks amplification of their DNA, while intact oocysts are more likely to remain amplifiable. This makes PMA-PCR a useful viability proxy, but not a direct measure of infectivity. The *Cryptosporidium* PMA-PCR method was shown to distinguish live from dead oocysts in mixed samples and to reduce false PCR signals from non-viable oocysts (Brescia et al., 2009). Researchers demonstrated that PMA-PCR can quantify viable oocysts in environmental waters and even genotype them, an important advance for risk assessment (Brescia et al., 2009). Another cutting-edge approach integrates cell culture with quantitative PCR (ICC-qPCR). Here, filtered water samples are applied to a susceptible cell monolayer (e.g. HCT-8 cells); after an incubation, any infective oocysts will invade and reproduce. The culture is then assayed by PCR for *Cryptosporidium* DNA, indicating the presence of viable (infective) oocysts. This combined cell culture TaqMan-PCR technique has been used to measure oocyst inactivation rates under various environmental conditions (King et al., 2005). It combines the gold-standard infectivity endpoint with the sensitivity of molecular detection. Additionally, microfluidic lab-on-chip devices and imaging flow cytometry are emerging for high-throughput analysis. For example, one on-chip system (CryptoDetect CARD) integrates immunomagnetic separation of oocysts and a microfluidic sensor, aiming to concentrate and detect oocysts from water in a single automated platform (Nemati et al., 2023).

While still in development, such tools could be deployed to assess how effectively biological processes are ingesting and inactivating *Cryptosporidium*. These techniques are helping to unveil the fate of oocysts after predation (are they merely sequestered or truly eliminated?) and to quantify true risk reduction beyond simple counting. Advanced analytical methodologies are shedding light on subtler aspects of *Cryptosporidium* removal (digestion status, residual infectivity), helping to link microbial community activity with public health protection benefits.

Given that predator ingestion may result in sequestration without inactivation, pairing enumeration with viability or infectivity endpoints is particularly valuable when evaluating biological removal mechanisms. For research, process optimisation, and validation trials, viability- and infectivity-informed methods (e.g., PMA-qPCR or ICC-qPCR) can better resolve whether observed reductions reflect true risk reduction. For routine regulatory compliance, presence/absence approaches remain conservative and operationally established; however, targeted use of viability/infectivity assays can complement compliance monitoring by supporting investigative assessments, troubleshooting, and evidence-based optimisation of multi-barrier treatment.

1.4. Future work

Future investigations should address the gap between *controlled laboratory findings and the complexities* of full-scale SSF systems. Although certain micro- and meiofauna (e.g., rotifers, ciliates, nematodes) demonstrate the ability to ingest *Cryptosporidium* oocysts, the *efficacy* of these interactions under real-world conditions, where *competing food sources*, variable flow regimes, and fluctuating environmental parameters (e.g., temperature, dissolved organic carbon, seasonal changes) are present, remains uncertain. It is also important to determine how SSF design features (e.g., *sand grain size*, filter depth, hydraulic loading) shape *predator* community development and activity over time, particularly *once the filter is fully ripened (due to ecological changes)*.

Moreover, the *viability and infectivity* of excreted oocysts require closer examination. *Partial digestion* does not necessarily result in oocyst inactivation, and certain organisms can expel intact oocysts, thus reducing net removal efficiency. Whether *repeated ingestion cycles* by the same species or higher trophic-level organisms further weaken the oocyst wall and diminish infectivity is an additional avenue of exploration.

The *intentional addition* of high-ingestion species (e.g., *Paramecium* or *Oxytricha*) into SSF could potentially enhance pathogen removal; however, *any such bioaugmentation* must be assessed for *ecological impact* and long-term stability. Integrating *computational models* (e.g., fluid dynamics, ecosystem simulations) with empirical data may yield *predictive tools* for designing, operating, and scaling bioaugmented SSF. Researchers are beginning to view biological communities as customisable treatment agents. Through bioaugmentation or optimised design, an engineered filter could host a dense population of oocyst predators and competitors, augmenting the physical filtration barrier. Key challenges remain, such as ensuring introduced species survive and do not cause issues (e.g. filter clogging or unintended regrowth of pathogens). Concurrently, high-volume detection methods and rapid sensors for *Cryptosporidium*, ideally capable of both presence/absence and viability assessments, could enable near–real-time monitoring of filter performance, facilitating timely interventions in case of sudden oocyst breakthrough or shifts in microbial community structure. Such technologies may significantly reduce operational response times, improve overall system resilience, and enhance water safety.

If high-ingestion species are intentionally introduced or selectively encouraged, long-term monitoring should address both performance and unintended impacts. Operational indicators could include influent/effluent turbidity (or particle counts), headloss development, run time between maintenance events, and effluent biological stability, while biological monitoring could track key grazer taxa (e.g., microscopy-based counts or molecular community profiling where feasible). Critically, monitoring programmes should link biological community structure to both total oocyst concentrations and viability/infectivity measures to confirm that any apparent gains represent sustained risk reduction rather than temporary sequestration.

Additionally, lower-impact maintenance practices such as underwater skimming of the Schmutzdecke rather than draining or mechanically scraping the entire filter could help preserve the established microbial community and biofilm integrity. By minimizing disruption, these gentler procedures may sustain high predator densities, maintain beneficial interstitial structures, and prolong the overall pathogen-removal efficiency of SSF. Finally, because *regulatory standards* typically hinge on *Cryptosporidium* presence/absence rather than oocyst viability, further work should assess *how biologically enhanced filtration* meets compliance benchmarks, and whether *viability-based assays* might more accurately reflect the benefits of these natural removal processes.

1.5. Conclusion

This review demonstrates that slow sand filtration is a multifaceted process capable of significantly reducing *Cryptosporidium* oocysts through a combination of physical, chemical, and biological mechanisms. While physical straining and adsorption are long-established contributors to pathogen removal, the maturing of the sand filter bed and the formation of a Schmutzdecke enhance overall efficacy by supporting a complex biological community. Diverse micro- and meiofauna, including nematodes, rotifers, and ciliates, can ingest *Cryptosporidium* oocysts, potentially contributing to greater reductions in oocyst numbers. Nonetheless, considerable variability in LRVs exists, ranging from 0.09-log to >7.2-log (with some values inferred from method detection limits when oocysts are not detected in effluent), highlighting the critical influence of parameters such as sand grain size, filter depth, hydraulic loading rates, and operational maturity. A key insight from the literature is that ingestion of oocysts by higher organisms does not necessarily equate to permanent removal. The possibility of excretion with retained viability introduces uncertainty regarding the net effect of biological predation. Furthermore, environmental conditions, including temperature fluctuations and the availability of alternative food particles, can modulate both ingestion rates and overall filtration performance. High experimental doses of (oo)cysts, or the use of inactivated pathogens, may also alter observed LRVs, highlighting the need for standardised challenge protocols that better represent real-world source water conditions.

CRediT authorship contribution statement

Sophie L. Bretagne: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Michael Chipps:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Jim Harris:** Writing – review & editing, Supervision. **Francis Hassard:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors acknowledge the financial support of the Engineering and Physical Sciences Research Council (EPSRC), through PhD award to Sophie Bretagne (EP/T518104/1), and support from Thames Water.

Data availability

Data associated with this manuscript are available at: doi: https://doi.org/10.6084/m9.figshare.28881467

References

Abdiyev, K., Azat, S., Kuldeyev, E., Ybyraiymkul, D., Kabdrakhmanova, S., Berndtsson, R., Khalkhabai, B., Kabdrakhmanova, A., Sultakhan, S., 2023. Review of slow sand filtration for raw water treatment with potential application in less-developed countries. Water 15 (11), 2007. https://doi.org/10.3390/W15112007.

Abudalo, R.A., Bogatsu, Y.G., Ryan, J.N., Harvey, R.W., Metge, D.W., Elimelech, M., 2005. Effect of ferric oxyhydroxide grain coatings on the transport of bacteriophage PRD1 and Cryptosporidium parvum oocysts in saturated porous media. Environ. Sci. Technol. 39 (17), 6412–6419. https://doi.org/10.1021/es050159h.

Abudalo, R.A., Ryan, J.N., Harvey, R.W., Metge, D.W., Landkamer, L., 2010. Influence of organic matter on the transport of Cryptosporidium parvum oocysts in a ferric oxyhydroxide-coated quartz sand saturated porous medium. Water Res. 44 (4), 1104–1113. https://doi.org/10.1016/J.WATRES.2009.09.039.

Badenoch, John, 1990. Cryptosporidium in Water Supplies: Report of the Group of Experts. HMSO.

Barret, J.M., Bryck, J., Collins, M.R., Janonis, B.A., Logsdon, G.S., 1991. Manual of Design for Slow Sand Filtration. AWWA Research Foundation and American Water Works Association, USA.

Bellamy, W.D., Hendricks, D.W., Logsdon, G.S., 1985a. Slow sand filtration: influences of selected process variables. J. Am. Water Works Assoc. 77 (12), 62–66. https://doi.org/10.1002/j.1551-8833.1985.tb05659.x.

Bellamy, W.D., Silverman, G.P., Hendricks, D.W., Logsdon, G.S., 1985b. Removing giardia cysts with slow sand filtration. J. Am. Water Works Assoc. 77 (2), 52–60. https://doi.org/10.1002/J.1551-8833.1985.TB05492.X.

Bjelkmar, P., Hansen, A., Schönning, C., Bergström, J., Löfdahl, M., Lebbad, M., Wallensten, A., Allestam, G., Stenmark, S., Lindh, J., 2017. Early outbreak detection by linking health advice line calls to water distribution areas retrospectively demonstrated in a large waterborne outbreak of cryptosporidiosis in Sweden. BMC Public Health 17, 328. https://doi.org/10.1186/s12889-017-4233-8.

Bradford, S.A., Bettahar, M., 2005. Straining, attachment, and detachment of cryptosporidium oocysts in saturated porous media. J. Environ. Qual. 34 (2), 469–478. https://doi.org/10.2134/JEQ2005.0469.

Bradford, S.A., Simunek, J., Bettahar, M., Van Genuchten, M.T., Yates, S.R., 2003. Modeling colloid attachment, straining, and exclusion in saturated porous media. Environ. Sci. Technol. 37 (10), 2242–2250. https://doi.org/10.1021/es025899u.

Brescia, C.C., Griffin, S.M., Ware, M.W., Varughese, E.A., Egorov, A.I., Villegas, E.N., 2009. Cryptosporidium propidium Monoazide-PCR, a molecular biology-based technique for genotyping of viable cryptosporidium oocysts. Appl. Environ. Microbiol. 75 (21), 6856. https://doi.org/10.1128/AEM.00540-09.

Bretagne, S.L., Chipps, M., Doggett, I., Bradwell, M.C., Hardiman, H., Harris, J., Hassard, F., 2025. Underwater skimming improves retention and degradation of Cryptosporidium oocysts in slow sand filters. Clean. Eng. Technol. 101134.

Bretagne, S.L., Chipps, M., Doggett, I., Bradwell, M.C., Hardiman, H., Harris, J., Hassard, F., 2026. Evaluating Cryptosporidium oocyst removal in SSF: impact of underwater skimming and dry skimming. J. Environ. Manag. 397, 128062.

Brookes, J.D., Aldridge, K., Wallace, T., Linden, L., Ganf, G.G., 2005. Multiple interception pathways for resource utilisation and increased ecosystem resilience. Hydrobiologia 552 (1), 135–146. https://doi.org/10.1007/S10750-005-1511-8/METRICS.

Brush, C.F., Ghiorse, W.C., Anguish, L.J., Parlange, J., Grimes, H.G., 1999. Transport of Cryptosporidium parvum oocysts through saturated columns. J. Environ. Qual. 28 (3), 809–815. https://doi.org/10.2134/JEQ1999.00472425002800030011X.

Busse, M.M., Becker, M., Applegate, B.M., Camp, J.W., Blatchley, E.R., 2019. Responses of Salmonella typhimurium LT2, Vibrio harveyi, and Cryptosporidium parvum to UVB and UVA radiation. Chem. Eng. J. 371, 647–656. https://doi.org/10.1016/J.CEJ.2019.04.105.

Connelly, S.J., Wolyniak, E.A., Williamson, C.E., Jellison, K.L., 2007. Artificial UV-B and solar radiation reduce in vitro infectivity of the human pathogen Cryptosporidium parvum. Environ. Sci. Technol. 41 (20), 7101–7106. https://doi.org/10.1021/ES071324R.

Cullen, T.R., Letterman, R.D., 1985. The Effect of Slow Sand Filter Maintenance on Water Quality. J. Am. Water Works Assoc. 77 (12), 48–55. https://doi.org/10.1002/j.1551-8833.1985.tb05657.x.

Dai, X., Hozalski, R.M., 2002. Effect of NOM and biofilm on the removal of Cryptosporidium parvum oocysts in rapid filters. Water Res. 36 (14), 3523–3532. https://doi.org/10.1016/S0043-1354(02)00045-3.

Drozd, C., Schwartzbrod, J., 1996. Hydrophobic and electrostatic cell surface properties of Cryptosporidium parvum. Appl. Environ. Microbiol. 62 (4), 1227–1232. https://journals.asm.org/journal/aem.

Elemo, T., Chipps, M., Graham, N., Turner, A., Jefferson, B., Hassard, F., 2024. Predicting the impact of underwater skimming on dissolved oxygen consumption in slow sand filters for potable water treatment. Sci. Total Environ. 954, 176730.

Elemo, T., Chipps, M., Graham, N., Turner, A., Bretagne, S., Jefferson, B., Hassard, F., 2025. Evaluating the impact of underwater skimming on slow sand filter performance and operation. Water Res. 276, 123234.

Ellis, K.V., 1985. Slow sand filtration. CRC Crit. Rev. Environ. Control. 15, 255–315.

Fayer, R., Farley, C.A., Lewis, E.J., Trout, J.M., Graczyk, T.K., 1997. Potential role of the Eastern oyster, Crassostrea virginica, in the epidemiology of Cryptosporidium parvum. Appl. Environ. Microbiol. 63 (5), 2086–2088. https://doi.org/10.1128/AEM.63.5.2086-2088.1997.

Fayer, R., Trout, J.M., Walsh, E., Cole, R., 2000. Rotifers Ingest Oocysts of Cryptosporidium parvum. J. Eukaryot. Microbiol. 47 (2), 161–163. https://doi.org/10.1111/j.1550-7408.2000.tb00026.x.

Fogel, D., Isaac-Renton, J., Guasparini, R., Moorehead, W., Ongerth, J., 1993. Removing Giardia and Cryptosporidium by slow sand filtration. J. Am. Water Works Assoc. 85 (11), 77–84. https://doi.org/10.1002/j.1551-8833.1993.tb06105.x.

Franceschelli, A., Bonadonna, L., Cacciò, S.M., Sannella, A.R., Cintori, C., Gargiulo, R., Coccia, A.M., Paradiso, R., Iaconelli, M., Briancesco, R., Tripodi, A., 2022. An outbreak of cryptosporidiosis associated with drinking water in north-eastern Italy, August 2019: microbiological and environmental investigations. Eurosurveillance 27 (35), 2200038. https://doi.org/10.2807/1560-7917.ES.2022.27.35.2200038.

Friedlander, S.K., 1958. Theory of aerosol filtration. Ind. Eng. Chem. 50 (8), 1161–1164. https://doi.org/10.1021/ie50584a036.

Goddard, M.R., 1980. The Ecology of Protozoan Populations of Slow Sand Filters, with reference to Ciliates. Royal Holloway, University of London.

Gómez-Couso, H., Paniagua-Crespo, E., Ares-Mazás, E., 2007. Acanthamoeba as a temporal vehicle of Cryptosporidium. Parasitol. Res. 100 (5), 1151–1154. https://doi.org/10.1007/s00436-006-0377-7.

Graczyk, T.K., Fayer, R., Cranfield, M.R., Conn, D.B., 1998. Recovery of waterborne Cryptosporidium parvum oocysts by freshwater benthic clams (Corbicula fluminea).

Appl. Environ. Microbiol. 64 (2), 427–430. https://doi.org/10.1128/AEM.64.2.427-430.1998.
Harter, T., Wagner, S., Atwill, E.R., 2000. Colloid transport and filtration of Cryptosporidium parvum in sandy soils and aquifer sediments. Environ. Sci. Technol. 34 (1), 62–70. https://doi.org/10.1021/es990132w.
Hashimoto, Y., Takashima, H., Jayamohan, S., 2019. Application of roughing filter to pre-treat 1,000 NTU raw water for slow sand filter. Water Pract. Technol. 14 (2), 355–364. https://doi.org/10.2166/WPT.2019.021.
Hassard, F., Biddle, J., Harnett, R., Stephenson, T., 2018. Microbial extracellular enzyme activity affects performance in a full-scale modified activated sludge process. Sci. Total Environ. 625, 1527–1534. https://doi.org/10.1016/J.SCITOTENV.2018.01.073.
Hassard, F., Elemo, T., Chipps, M., Turner, A., Jefferson, B., Graham, N., 2022. Underwater remote skimming of slow sand filters for sustainable water production. ACS ES T Water 2 (9), 1471–1474. https://doi.org/10.1021/ACSESTWATER.2C00313.
Helmi, K., Skraber, S., Gantzer, C., Willame, R., Hoffmann, L., Cauchie, H.M., 2008. Interactions of Cryptosporidium parvum, Giardia lamblia, vaccinal poliovirus type 1, and bacteriophages φX174 and MS2 with a drinking water biofilm and a wastewater biofilm. Appl. Environ. Microbiol. 74 (7), 2079–2088. https://doi.org/10.1128/AEM.02495-07.
Hijnen, W.A.M., Brouwer-Hanzens, A.J., Charles, K.J., Medema, G.J., 2005. Transport of MS2 phage, *Escherichia coli, Clostridium perfringens*, *Cryptosporidium parvum*, and *Giardia intestinalis* in a gravel and a sandy soil. Environ. Sci. Technol. 39 (20), 7860–7868. https://doi.org/10.1021/es050427b.
Hijnen, W.A.M., Dullemont, Y.J., Bosklopper, K.Th.G.J., Schijven, J.F., Medema, G., 2006. Assessment of the Capacity of Slow sand Filtration to Eliminate Cryptosporidium oocysts.
Hijnen, W.A.M., Dullemont, Y.J., Schijven, J.F., Hanzens-Brouwer, A.J., Rosielle, M., Medema, G.J., 2007. Removal and fate of Cryptosporidium parvum, Clostridium perfringens and small-sized centric diatoms (Stephanodiscus hantzschii) in slow sand filters. Water Res. 41 (10), 2151–2162. https://doi.org/10.1016/j.watres.2007.01.056.
Huamanchay, O., Genzlinger, L., Iglesias, M., Ortega, Y.R., 2004. Ingestion of Cryptosporidium oocysts by Caenorhabditis elegans. J. Parasitol. 90 (5), 1176–1178. https://doi.org/10.1645/GE-253R.
Huisman, L., Wood, W.E., 1974. Slow sand Filtration. World Health Organization. https://iris.who.int/handle/10665/38974.
Hunter, P.R., Nichols, G., 2002. Epidemiology and clinical features of cryptosporidium infection in immunocompromised patients. Clin. Microbiol. Rev. 15 (1), 145. https://doi.org/10.1128/CMR.15.1.145-154.2002.
Ibrahim, M.G., 1989. Ecological Studies on the Ciliates and Bacterial Populations of Slow Sand Filters. Royal Holloway, University of London & Bedford New College.
Johnson, D.C., Enriquez, C.E., Pepper, I.L., Davis, T.L., Gerba, C.P., Rose, J.B., 1997. Survival of giardia, cryptosporidium, poliovirus and salmonella in marine waters. Water Sci. Technol. 35 (11−12), 261–268. https://doi.org/10.2166/WST.1997.0744.
Karaman, M.E., Pashley, R.M., Bustamante, H., Shanker, S.R., 1999. Microelectrophoresis of Cryptosporidium parvum oocysts in aqueous solutions of inorganic and surfactant cations. Colloids Surf. A Physicochem. Eng. Asp. 146 (1–3), 217–225. https://doi.org/10.1016/S0927-7757(98)00796-1.
King, B.J., Monis, P.T., 2007. Critical processes affecting Cryptosporidium oocyst survival in the environment. Parasitology 134 (Pt 3), 309–323. https://doi.org/10.1017/S0031182006001491.
King, B.J., Keegan, A.R., Monis, P.T., Saint, C.P., 2005. Environmental temperature controls Cryptosporidium oocyst metabolic rate and associated retention of infectivity. Appl. Environ. Microbiol. 71 (7), 3848–3857. https://doi.org/10.1128/AEM.71.7.3848-3857.2005.
King, B.J., Monis, P.T., Keegan, A.R., Harvey, K., Saint, C.P., 2007. Investigation of the Survival of Cryptosporidium in Environmental Waters. Research Report No. 47. Cooperative Research Centre for Water Quality and Treatment, Salisbury, South Australia (ISBN 9781876616731).
King, B.J., Hoefel, D., Daminato, D.P., Fanok, S., Monis, P.T., 2008. Solar UV reduces Cryptosporidium parvum oocyst infectivity in environmental waters. J. Appl. Microbiol. 104 (5), 1311–1323. https://doi.org/10.1111/J.1365-2672.2007.03658.X.
Kudo, R.R., 1954. Protozoology. In: Protozoology, 4th ed. C. C. Thomas. https://doi.org/10.5962/bhl.title.7431.
Lefebvre, M., Razakandrainibe, R., Villena, I., Favennec, L., Costa, D., 2021. Cryptosporidium-biofilm interactions: a review. Appl. Environ. Microbiol. 87 (3), e02483-20. https://doi.org/10.1128/AEM.02483-20.
Lefebvre, M., Razakandrainibe, R., Schapman, D., François, A., Genty, D., Galas, L., Villena, I., Favennec, L., Costa, D., 2023. Interactions between free-living amoebae and Cryptosporidium parvum: an experimental study. Parasite 30, 31. https://doi.org/10.1051/PARASITE/2023033.
Li, Y., Wang, M., Chen, X., Cui, S., Hofstra, N., Kroeze, C., Ma, L., Xu, W., Zhang, Q., Zhang, F., Strokal, M., 2022. Multi-pollutant assessment of river pollution from livestock production worldwide. Water Res. 209, 117906. https://doi.org/10.1016/J.WATRES.2021.117906.
Logan, A.J., Stevik, T.K., Siegrist, R.L., Rønn, R.M., 2001. Transport and fate of Cryptosporidium parvum oocysts in intermittent sand filters. Water Res. 35 (18), 4359–4369. https://doi.org/10.1016/S0043-1354(01)00181-6.
Ma, J.Y., Li, M.Y., Qi, Z.Z., Fu, M., Sun, T.F., Elsheikha, H.M., Cong, W., 2022. Waterborne protozoan outbreaks: An update on the global, regional, and national prevalence from 2017 to 2020 and sources of contamination. Sci. Total Environ. 806, 150562. https://doi.org/10.1016/J.SCITOTENV.2021.150562.
Madore, M.S., Rose, J.B., Gerba, C.P., Arrowood, M.J., Sterling, C.R., 1987. Occurrence of Cryptosporidium oocysts in sewage effluents and selected surface waters. J. Parasitol. 73 (4), 702–705. https://doi.org/10.2307/3282398.
Maurya, A., Singh, M.K., Kumar, S., 2020. Biofiltration technique for removal of waterborne pathogens. In: Waterborne Pathogens: Detection and Treatment, pp. 123–141. https://doi.org/10.1016/B978-0-12-818783-8.00007-4.
McGuigan, K.G., Méndez-Hermida, F., Castro-Hermida, J.A., Ares-Mazás, E., Kehoe, S.C., Boyle, M., Sichel, C., Fernández-Ibáñez, P., Meyer, B.P., Ramalingham, S., Meyer, E. A., 2006. Batch solar disinfection inactivates oocysts of Cryptosporidium parvum and cysts of Giardia muris in drinking water. J. Appl. Microbiol. 101 (2), 453–463. https://doi.org/10.1111/J.1365-2672.2006.02935.X.
Méndez-Hermida, F., Castro-Hermida, J.A., Ares-Mazás, E., Kehoe, S.C., McGuigan, K.G., 2005. Effect of batch-process solar disinfection on survival of Cryptosporidium parvum oocysts in drinking water. Appl. Environ. Microbiol. 71 (3), 1653–1654. https://doi.org/10.1128/AEM.71.3.1653-1654.2005.
Méndez-Hermida, F., Gomez-Couso, H., Ares-Mazas, E., 2006. Artemia is capable of spreading oocysts of Cryptosporidium and the cysts of Giardia. J. Eukaryot. Microbiol. 53 (6), 432–434. https://doi.org/10.1111/j.1550-7408.2006.00126.x.
Méndez-Hermida, F., Gómez-Couso, H., Ares-Mazás, E., 2007. Possible involvement of Artemia as live diet in the transmission of cryptosporidiosis in cultured fish. Parasitol. Res. 101, 823–827. https://doi.org/10.1007/s00436-007-0543-6.
Morita, S., Namikoshi, A., Hirata, T., Oguma, K., Katayama, H., Ohgaki, S., Motoyama, N., Fujiwara, M., 2002. Efficacy of UV Irradiation in Inactivating Cryptosporidium parvum Oocysts. Appl. Environ. Microbiol. 68 (11), 5387. https://doi.org/10.1128/AEM.68.11.5387-5393.2002.
Morris, D.P., Zagarese, H., Williamson, C.E., Balseiro, E.G., Hargreaves, B.R., Modenutti, B., Moeller, R., Queimalinos, C., 1995. The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. Limnol. Oceanogr. 40 (8), 1381–1391. https://doi.org/10.4319/LO.1995.40.8.1381.
Nemati, S., Shalileh, F., Mirjalali, H., Omidfar, K., 2023. Toward waterborne protozoa detection using sensing technologies. Front. Microbiol. 14, 1118164. https://doi.org/10.3389/fmicb.2023.1118164.
Ongerth, J.E., Pecoraro, J.P., 1996. Electrophoretic mobility of Cryptosporidium oocysts and Giardia cysts. J. Environ. Eng. 122 (3), 228–231. https://doi.org/10.1061/(ASCE)0733-9372(1996)122:3(228).
Park, Y., Atwill, E.R., Hou, L., Packman, A.I., Harter, T., 2012. Deposition of Cryptosporidium parvum Oocysts in porous media: a synthesis of attachment efficiencies measured under varying environmental conditions. Environ. Sci. Technol. 46 (17), 9491–9500. https://doi.org/10.1021/es300564w.
Pauli, W., Jax, K., Berger, S., 2005. Protozoa in wastewater treatment: function and importance. In: Biodegradation and Persistance, pp. 203–252. https://doi.org/10.1007/10508767_3.
Pfannes, K.R., Langenbach, K.M.W., Pilloni, G., Stührmann, T., Euringer, K., Lueders, T., Neu, T.R., Müller, J.A., Kästner, M., Meckenstock, R.U., 2015. Selective elimination of bacterial faecal indicators in the Schmutzdecke of slow sand filtration columns. Appl. Microbiol. Biotechnol. 99 (23), 10323–10332. https://doi.org/10.1007/s00253-015-6882-9.
Robertson, L.J., Paton, C.A., Campbell, A.T., Smith, P.G., Jackson, M.H., Gilmour, R.A., Black, S.E., Stevenson, D.A., Smith, H.V., 2000. *Giardia* cysts and *Cryptosporidium* oocysts at sewage treatment works in Scotland, UK. Water Res. 34 (8), 2310–2322. https://doi.org/10.1016/S0043-1354(99)00408-X.
Rochelle, P.A., Upton, S.J., Montelone, B.A., Woods, K., 2005. The response of Cryptosporidium parvum to UV light. Trends Parasitol. 21 (2), 81–87. https://doi.org/10.1016/j.pt.2004.11.009.
Sakthivadivel, R., 1967. Theory and mechanism of filtration of non-colloidal fines through a porous medium. https://search.proquest.com/openview/f08bf9e9f60006a4d232d5e88de7bdf2/1.pdf?pq-origsite=gscholar&cbl=18750&diss=y.
Scheid, P.L., Schwarzenberger, R., 2011. Free-living amoebae as vectors of cryptosporidia. Parasitol. Res. 109, 499–504. https://doi.org/10.1007/s00436-011-2287-6.
Schuler, P.F., Ghosh, M.M., Gopalan, P., 1991. Slow sand and diatomaceous earth filtration of cysts and other particulates. Water Res. 25 (8), 995–1005.
Shaposhnik, E.G., Abozaid, S., Grossman, T., Marva, E., On, A., Azrad, M., Peretz, A., 2019. The prevalence of cryptosporidium among children hospitalized because of gastrointestinal symptoms and the efficiency of diagnostic methods for Cryptosporidium. Am. J. Trop. Med. Hyg. 101 (1), 160. https://doi.org/10.4269/AJTMH.19-0057.
Shirley, D.A.T., Moonah, S.N., Kotloff, K.L., 2012. Burden of disease from Cryptosporidiosis. Curr. Opin. Infect. Dis. 25 (5), 555. https://doi.org/10.1097/QCO.0B013E328357E569.
Siqueira-Castro, I.C.V., Greinert-Goulart, J.A., Bonatti, T.R., Yamashiro, S., Franco, R.M. B., 2016. First report of predation of Giardia sp. cysts by ciliated protozoa and confirmation of predation of Cryptosporidium spp. oocysts by ciliate species. Environ. Sci. Pollut. Res. 23 (11), 11357–11362. https://doi.org/10.1007/s11356-016-6689-y.
Smoluchowski, M.v., 1917. Versuch einer Mathematischen Theorie der Koagulationskinetik Kolloider Lösungen. Z. Phys. Chem. 92 (1), 129–168. https://doi.org/10.1515/ZPCH-1918-9209.
Spielman, L.A., Goren, S.L., 1970. Capture of small particles by london forces from low-speed liquid flows. Environ. Sci. Technol. 4 (2), 135–140. https://doi.org/10.1021/ES60037A007.
Stott, R., May, E., Matsushita, E., Warren, A., 2001. Protozoan predation as a mechanism for the removal of cryptosporidium oocysts from wastewaters in constructed wetlands. Water Sci. Technol. 44 (11–12), 191–198. https://doi.org/10.2166/WST.2001.0828.

Stott, R., May, E., Ramirez, E., Warren, A., 2003. Predation of Cryptosporidium oocysts by protozoa and rotifers: implications for water quality and public health. Water Sci. Technol. 47 (3), 77–83. https://doi.org/10.2166/WST.2003.0166.

Sturdee, A., Foster, I., Bodley-Tickell, A.T., Archer, A., 2007. Water quality and Cryptosporidium distribution in an upland water supply catchment, Cumbria, UK. Hydrol. Process. 21 (7), 873–885. https://doi.org/10.1002/HYP.6278.

Thorp, J.H., Covich, A.P., 2001. Ecology and classification of North American freshwater invertebrates. Academic Press.

Timms, S., Slade, J.S., Fricker, C.R., 1995. Removal of Cryptosporidium by slow sand filtration. Water Sci. Technol. 31 (5–6), 81–84. https://doi.org/10.1016/0273-1223(95)00245-I.

Trout, J.M., Walsh, E.J., Fayer, R., 2002, October 1. Rotifers ingest giardia cysts. J. Parasitol. 88 (5), 1038–1040. https://doi.org/10.1645/0022-3395(2002)088[1038:RIGC]2.0.CO;2.

Tufenkji, N., Elimelech, M., 2004. Correlation equation for predicting single-collector efficiency in physicochemical filtration in saturated porous media. Environ. Sci. Technol. 38 (2), 529–536. https://doi.org/10.1021/ES034049R.

Tufenkji, N., Miller, G.F., Ryan, J.N., Harvey, R.W., Elimelech, M., 2004. Transport of cryptosporidium oocysts in porous media: role of straining and physicochemical filtration. Environ. Sci. Technol. 38 (22), 5932–5938. https://doi.org/10.1021/ES049789U.

Tufenkji, N., Dixon, D.R., Considine, R., Drummond, C.J., 2006. Multi-scale Cryptosporidium/sand interactions in water treatment. Water Res. 40 (18), 3315–3331. https://doi.org/10.1016/J.WATRES.2006.07.036.

U.S. Environmental Protection Agency (USEPA), 2012. Method 1623.1: Cryptosporidium and Giardia in Water by Filtration/IMS/FA. Office of Water, Washington, DC.

UK Health Security Agency, 2023, November 1. Cryptosporidium: Public Advice. https://www.gov.uk/guidance/cryptosporidium-public-advice.

Visscher, J.T., 1990. Slow sand filtration: design, operation, and maintenance. J. Am. Water Works Assoc. 82 (6), 67–71. https://doi.org/10.1002/j.1551-8833.1990.tb06979.x.

Weisse, T., 2024. Thermal response of freshwater ciliates: can they survive at elevated lake temperatures? Freshw. Biol. 69 (9), 1245–1260. https://doi.org/10.1111/FWB.14302.

Widerström, M., Schönning, C., Lilja, M., Lebbad, M., Ljung, T., Allestam, G., Ferm, M., Björkholm, B., Hansen, A., Hiltula, J., Långmark, J., Löfdahl, M., Omberg, M., Reuterwall, C., Samuelsson, E., Widgren, K., Wallensten, A., Lindh, J., 2014. Large outbreak of Cryptosporidium hominis infection transmitted through the public water supply, Sweden - Volume 20, Number 4—April 2014 - emerging infectious diseases journal - CDC. Emerg. Infect. Dis. 20 (4), 581–589. https://doi.org/10.3201/EID2004.121415.

Winiecka-Krusnell, J., Linder, E., 2001. Bacterial infections of free-living amoebae. Res. Microbiol. 152 (7), 613–619. https://doi.org/10.1016/S0923-2508(01)01240-2.

Yao, K.-M., Habibian, M.T., O'Melia, C.R., 1971. Water and Waste Water Filtration: Concepts and Applications. Environ. Sci. Technol. 5 (11), 1105–1112. https://doi.org/10.1021/es60058a005.

https://dspace.org/

Physical and biological fate of protozoan (oo)cysts in slow sand filtration: a review

Bretagne, Sophie L.; Chipps, Michael; Harris, Jim A.; Hassard, Francis

2026-05-20
Elsevier
https://doi.org/10.1016/j.scitotenv.2026.181810
Bretagne SL, Chipps M, Harris J, Hassard F. (2026) Physical and biological fate of protozoan (oo)cysts in slow sand filtration: a review. Science of The Total Environment, Volume 1031, May 2026, Article number 181810

Downloaded from a DSpace repository. https://dspace.org/