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Underwater skimming improves retention and degradation of *Cryptosporidium* oocysts in slow sand filters

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ABSTRACT

Cryptosporidium oocysts are resilient protozoan pathogens that resist conventional disinfection, posing significant challenges to drinking water quality. Filtration processes like slow sand filters (SSFs) effectively remove these oocysts, but limited data exist on their fate in SSFs, particularly following maintenance practices such as skimming. This study examined the spatial and temporal distribution of inactivated *Cryptosporidium parvum* oocysts in pilot-scale SSF operated under two skimming regimes: dry skimming and underwater skimming. The underwater skim approach offers benefits in terms of production volume gains and reduced downtime, but pathogen removal has not been comprehensively assessed using this approach. Across two 4-day dosing periods, oocyst breakthrough was lower under UWS (UWS: 7.6 % vs DS: 47.0 % of filtrate samples were positive for oocysts). In addition, core samples were collected at six time points to track oocyst retention and vertical migration. In both underwater skim and dry skim slow sand filters, most oocysts were captured in the top 100 mm of the filter, gradually moving downward over time. Notably, underwater skim filters retained more oocysts in the upper layers than dry skim filters, resulting in lower breakthrough frequency. Although skimming did remove some oocysts in both regimes, the majority were rendered undetectable *in situ* through processes such as predation, enzymatic digestion, and natural decay—evidenced by the increasing proportion of oocyst-like bodies and their near-complete absence from the filtrate. Thus, underwater skim is a viable alternative to dry skim for *Cryptosporidium* removal, sustaining filter performance by trapping oocysts in the upper layers and maintaining similar rates of oocyst degradation. These insights support improved SSF maintenance strategies that enhance pathogen removal.

Nomenclature

Variable/ Parameter	Definition	Value [Units]
η_T	Overall single-collector contact efficiency	[-]
η_D	Single-collector contact efficiency from diffusion	[-]
η_I	Single-collector contact efficiency from interception	[-]
η_G	Single-collector contact efficiency from gravity	[-]
N_R	Aspect ratio between the filter media and the particle	[-]
N_{Pe}	Peclet number	[-]
N_G	Ratio of the particle velocity and the fluid approach velocity	[-]
N_{vdW}	Ratio of the van der Waals interaction energy and the thermal energy of the fluid	[-]
D_∞	Bulk diffusion coefficient in an infinite medium	[m^2/s]
A_s	Happel's porosity-dependent parameter	[-]
T	Temperature, absolute	[K]
α	Attachment efficiency	[-]
L_{layer}	Depth of specific layer of filter	[m]
$N_{IN,i}$	Input number of oocysts for layer i, determined by first order decay regression	[oocysts]

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(*continued*)

Variable/ Parameter	Definition	Value [Units]
$N_{OUT,i}$	Output number of oocysts for layer i	[oocysts]
$R_{model,i}$	Retention of oocysts in layer i	[oocysts]
U	Fluid approach velocity, here reservoir stored water	8.33×10^{-5} [m/s]
d_c	Spherical collector spherical grain diameter: sand	3.64×10^{-4} [m]
	Spherical collector grain diameter: GAC	7×10^{-4} [m]
d_p	Spherical particle diameter	4.8×10^{-6} [m]
L	Total depth of filter media	0.82 [m]
ρ_p	Density of particle, here *Cryptosporidium* oocysts	1050 [kg/m^3]
ρ_w	Density of fluid, here reservoir stored water	1000 [kg/m^3]
ε	Estimated porosity of the SSF	0.364
H	Hamaker constant	6.2×10^{-21} [J]
g	Earth's gravity constant	9.81 [m/s^2]
k	Boltzmann constant	1.3805×10^{-23} [J/K]

1. Introduction

Cryptosporidium is a gastrointestinal protozoan parasite prevalent in surface waters and presents a significant public health concern due to its resistance to conventional disinfection processes such as chlorination (Fayer and Xiao, 2007; Karanis et al., 1998). The robust outer wall of *Cryptosporidium* oocysts allows them to survive chemical treatments and persist in the environment for extended periods (Collins et al., 1994). Consequently, physical filtration methods or alternative disinfectants (e.g., ozone and UV) are often required to effectively remove oocysts and ensure safe drinking water supplies (Weber-Shirk and Dick, 1997; Burke et al., 2025).

Among the available filtration methods, slow sand filters (SSFs) have proven both effective and sustainable for removing a range of microbial pathogens, including *Cryptosporidium* oocysts (Collins et al., 1992; Weber-Shirk and Dick, 1997). SSFs operate with minimal energy and chemical inputs, relying on physical straining, adsorption, and biological mechanisms. Critical to their success is the Schmutzdecke – a biologically active layer composed of particulates, organic matter, algae, and a rich microbial community (Haig et al., 2011). However, the entire SSF bed contributes to the removal of bacteria, viruses, and protozoan parasites (Bellamy et al., 1985b; Elliott et al., 2008; Fogel et al., 1993; Schuler et al., 1991).

The Schmutzdecke operates under aerobic conditions, and dissolved oxygen (DO) can modulate biofilm activity and community interactions that influence particle and pathogen fate in slow sand filters (Elemo et al., 2024; Kato et al., 2004; Steele and Odumeru, 2004). Although SSFs effectively reduce *Cryptosporidium* oocysts from the filtrate, the fate of retained oocysts and the relative contributions of physical, chemical, and biological processes remain poorly quantified. *Cryptosporidium* oocysts can remain viable for long periods in aquatic environments (King et al., 2005), but some authors, like Tufenkji et al. (2004), suggest that favourable cell surface properties enhance migration within granular media (including SSFs) and that aspect warrants further investigation to inform long-term safety and operational protocols.

A primary operational challenge in SSFs is the gradual build-up of particles and biofilm within the Schmutzdecke, leading to increased headloss and eventual filter clogging (Collins et al., 1992; Logsdon et al., 2002). Regular maintenance via sand 'skimming' removes the top layer, which helps restore flow rates but may disrupt the microbial community and temporarily reduce treatment performance (Campos et al., 2002; Collins et al., 1992; Maiyo et al., 2023). The conventional dry skimming (DS) approach involves draining the SSF before sand removal, resulting in operational downtime (3–5 days) and disturbances to the microbial assemblages (Campos et al., 2002; Hassard et al., 2022). This downtime necessitates lengthy re-ripening periods which can vary from 6 h (Cullen and Letterman, 1985) to 100 days (Pyper, 1985) to re-establish optimal filtration conditions, usually determined by microbial indicator and turbidity removal. The ripening period is dependent on multiple aspects such as the nutrient content in the inlet raw water, hydraulic conditions, and overall SSF design.

Underwater skimming (UWS) occurs while the filter remains submerged (Buzunis, 1995; Campos et al., 2002) and has already been successfully field-tested in France (Back, 2006). This method reduces downtime and can preserve the biological integrity of the SSF by maintaining aquatic habitats (Miao et al., 2023). Nevertheless, concerns persist regarding potential reductions in filtrate quality, altered headloss development patterns, and increased particle penetration to deeper layers of the bed (Campos et al., 2002; Hassard et al., 2017). Determining how UWS impacts pathogen retention rates and biological degradation of *Cryptosporidium* oocysts is vital to further deployment of this approach.

Previous research on *Cryptosporidium* fate in SSFs has focused primarily on short-term retention (Timms et al., 1995; Couso-Pérez et al., 2023) and distribution within the filter bed (Heller and de Brito, 2006; Hijnen et al., 2007). Timms et al. (1995) found that oocysts were retained mainly in the top 25 mm of sand immediately after dosing, while Heller and de Brito (2006) and Hijnen et al. (2007) observed deeper penetration over time. Hijnen et al. (2007) also emphasized the effectiveness of SSFs in *Cryptosporidium* removal but noted a dearth of data on the long-term fate of retained oocysts. In addition, *Cryptosporidium* log removal values vary greatly, ranging from 0.09 (Park et al., 2012) to $>$ 7.2 (Hijnen et al., 2005) depending on the study conditions and parameters such as sand maturity, the depth of the sand layer (Bellamy et al., 1985a), the hydraulic loading (Bellamy et al., 1985b; Hijnen et al., 2005), the sand grain size (Harter et al., 2000; Logan et al., 2001). Moreover, whether skimming dry or underwater removes a significant fraction of these oocysts or if they degrade *in situ* is largely unknown (Siqueira-Castro et al., 2016; Fayer et al., 2000; Scheid and Schwarzenberger, 2011). Data on removal of other pathogens such as *Escherichia coli* suggests higher organisms (e.g. metazoans) were responsible for the biodegradation of *E. coli* in pilot-scale SSF (Haig et al., 2015). There is limited information on the interplay between oocyst retention and migration, and *in situ* degradation processes are important for optimizing SSF operation. The testing of novel skimming approaches (e.g., UWS) is also a key component of this work.

Accordingly, this study addressed these knowledge gaps by systematically investigating the temporal and spatial distribution of *Cryptosporidium* oocysts within pilot-scale SSFs subject to either DS or UWS regimes. The influences of each skimming method on oocyst retention and migration were explored, and evidence of degradation over time was provided. The findings will help refine SSF maintenance practices, enhancing pathogen removal and safeguarding drinking water quality.

2. Materials and methods

2.1. Pilot filtration setup

Pilot experiments were undertaken at the Thames Water Research, Development and Innovation Centre, located in Kempton Park Advanced Water Treatment Works (AWTW), Hanworth, UK. A dosing experiment was conducted over an eight-week period, from October to December 2023, using a pilot-scale system of ten SSFs that had been operating continuously since March 2021, with details reported previously (Elemo et al., 2025). Media characteristics and operational conditions of the pilot-scale SSFs were identical to full-scale SSFs, but bed area and supernatant water depth had to be adapted due to the scale. The filter media was supported by a metal plate containing 24 filter nozzles, each with 36 slots of 0.2 mm width (Fig. 1). The filtrate from each SSF exited through the base nozzles and was collected in clean 20-litre vessels that overflowed to waste, enabling continuous sampling without interrupting filtration. The upper sand layers were re-sanded biannually to maintain optimal performance, with the most recent

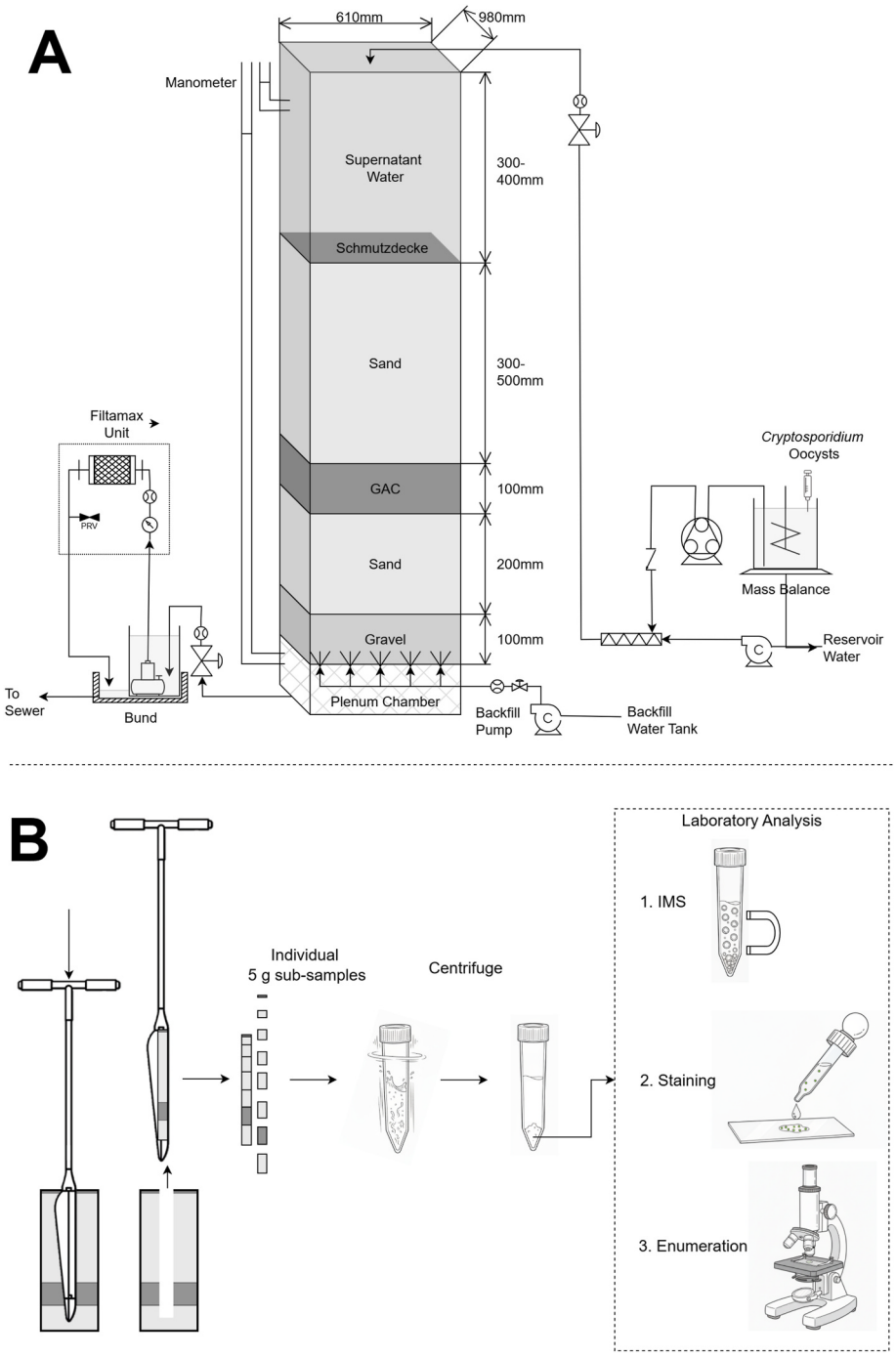


Fig. 1. (A) Diagram of the pilot-scale slow sand filtration system in its setup for the dosing experiment, (B) Flowchart of the sampling and sectioning of the SSF core, the sandwashing step to detach the particulate matter from the sand grains, followed by the IMS isolation, staining and enumeration of oocysts.

re-sanding in early August 2023, prior to the start of this study.

2.2. SSF monitoring and skimming procedures

To maintain optimal SSF operation, skimming was performed by removing the top 40 mm of sand when the headloss reached ~1300 mm or after the seasonal maximum runtime (40 days in summer, 70 days in winter) for Thames Water in London. Two skimming techniques were employed in this experiment. In the DS method, the filter was completely drained before skimming and then slowly refilled to prevent disturbing the remaining media. In contrast, the UWS technique involved skimming while the filter remained submerged, removing the same amount of sand through a suction device without draining. Two UWS conditions were tested: UWS 0 %, where flow was completely interrupted for 6 h during skimming, and UWS 33 %, where the outlet flow rate was reduced by two-thirds (to a Hydraulic Loading Rate (HLR) of 0.1 m/h) without interrupting the flow to reflect the conditions of the operational HLR of day 1 post skim of a traditional DS SSF. To replicate full-scale SSFs operational practices for the pilot-scale, UWS 0 % beds had flow interrupted for 6 h during skimming. DS beds were drained and exposed to air for 28 h as draining and refilling in these systems takes longer. UWS took less than one day, whereas DS spanned over two days. After skimming, flow through all filters was gradually increased by one-third (0.1 m/h) every 24 h until reaching the full flow rate of 0.3 m/h

over three days (two days for UWS 33 %, as it started at one-third full flow). Each skimming regime was implemented on the pilot-scale SSF for a year before the *Cryptosporidium* dosing experiment to allow development and acclimation of microbial ecosystems specific to each technique.

2.3. Dosing parameters

To ensure operator safety and prevent environmental contamination, inactivated, γ-irradiated *Cryptosporidium parvum* oocysts (Iowa isolate; TCS Biosciences UK; 10^9 oocysts) were used for dosing. The oocysts were inactivated using a γ-irradiation dose of 50,000 Gy, which ensured complete elimination of infectivity while preserving wall integrity and antigenicity; morphology was indistinguishable from live material on microscopy (validated through microscopy by UKAS accredited laboratory). Prior to use, the oocysts were stored at 4 °C for less than 1 year. Oocysts were continuously dosed at a target concentration of 3000 oocysts/L over two periods, each lasting three and four consecutive weekdays respectively. Each day's dosing lasted approximately 8 h , resulting in a daily theoretical dose of 3.89×10^6 oocysts and a total theorethical dose of 3.11×10^7 oocysts for each SSF. Alternative surrogates, such as *Bacillus subtilis* or *Clostridium perfringens* spores, were deemed unsuitable due to differences in surface properties, size, and morphology, which influence their interactions with sand surfaces and biofilm, thereby affecting their behaviour in SSFs (Tufenkji et al., 2006). Because irradiated oocysts lack metabolic activity, some biofilm–oocyst interactions relevant to live organisms may differ; thus any observed decay reflects loss of detectability, rather than loss of infectivity. However, identical dosing and processing across UWS and DS mean relative comparisons of retention and breakthrough are unaffected and comparable between experiments.

The dosing solution was prepared by adding 125 mL of a working solution to 28.3 L of inlet reservoir water. The working solution consisted of 1.25 mL of vortexed oocyst stock solution (equivalent to 3.11×10^8 oocysts) suspended in 1 L of phosphate-buffered saline (PBS) containing 0.05 % Tween 20 (Sigma Aldrich, UK) to aid in cell dispersal. The dosing solution was fed into the SSF inlet flow (1616.5 L/h), at a flow rate of 3.52 L/h, thoroughly mixed into the bulk reservoir inlet water via a static mixer to ensure homogeneity, and then distributed equally to individual SSFs (at 162 L/h). The addition of Tween 20 to the working solution and constant mechanical stirring of the dosing solution limited aggregation of oocysts in solution and ensured a homogenous distribution of the oocysts in the dosing tank, with further mixing achieved by a static mixer in the bulk fluid flow prior to the inlet of the SSF beds. The dispersal of oocysts was not standardised during sampling and analysis to avoid biasing the results.

During dosing events, the filters were operated in "non-overflow" mode to ensure equal distribution of oocysts to all beds. The inlet flow rate was matched to the outlet flow rate to prevent overflow and avoid potential uneven distribution of oocysts between adjacent beds. Outside of dosing periods, the SSFs were switched back to overflow mode.

Several quality control measures ensured accuracy and consistency in the dosing process. Equipment, such as dosing pumps and flow meters, was calibrated prior to dosing and regularly checked during dosing to maintain consistent dosing rates**.** The dosing solution was continuously stirred with an automated paddle stirrer to prevent oocyst settling, ensuring consistent oocyst concentration in the feed water. Daily influent water samples were analysed using immunomagnetic separation (IMS) to verify oocyst concentrations. The oocyst recovery and enumeration and statistical data analysis are provided in supplementary material (Table A-1).

2.4. Sampling and analysis

Over the 56-day experiment, comprising an 11-day dosing phase, a 25-day post-dosing observation, a 2-day skimming event, and an 18-day post-skim observation, four of the ten SSF beds were designated as "sacrificial" filters for core sampling to assess oocyst distribution within the filter media. This design allowed for the assessment of both temporal and spatial variations in oocyst distribution. These four beds represented two of the three skimming regimes (UWS 0 % and DS), with two replicates for each regime. The remaining six filters were used for filtrate sampling and were left undisturbed to preserve their integrity and accurately reflect oocyst retention as expected in full-scale SSFs. Core samples were collected at six specific time points: before dosing (Control, August 2023), after the first dosing event (Day 5), after the second dosing event (Day 12), one week post-dosing (Day 19), pre-skim (Days 37 and 38), and post-skim (Day 50). These time points were strategically chosen to capture dynamic changes in oocyst distribution due to dosing and subsequent skimming interventions including potential temporal changes in the depth distribution.

2.4.1. Core sampling approach

Core sampling assessed the spatial distribution of oocysts within the filter media. A Russian peat corer (Eijkelkamp Soil & Water, Netherlands) was used to extract undisturbed vertical profiles of wet sand, thereby preserving the filter layer stratification (Fig. A-2, in Supplementary Information). Prior to sampling, the SSFs were briefly drained to facilitate access without disrupting the media structure. Coring commenced within minutes of drain-down and the surface mat remained intact with no observable sloughing. Additionally, these sampled SSF were not being monitored for oocyst breakthrough due to disruption in filter integrity and potentially of the pore structure during the coring activity.

Six cores were collected from each sacrificial filter – one per sampling event – to capture spatial variability across the filter bed. Each core was immediately sectioned *in situ* into predefined depth intervals corresponding to the filter media layers: Layer 1 (Schmutzdecke): 0–25 mm; Layer 2: 25–50 mm; Layer 3: 50–100 mm; Layer 4: 100–200 mm; Layer 5: 200–300 mm; Layer 6: 300–500 mm; GAC Layer: 500–600 mm; Underlying sand layer: below 600 mm. These discrete intervals enabled detailed profiling of oocyst distribution, with a particular focus on the upper layers where biological activity and particle retention are expected to be the most pronounced. Sectioning was performed aseptically to prevent cross-contamination, and each sample was placed into a sterile, labelled bag and frozen onsite at −20 °C within 2 h of collection to preserve oocyst integrity prior to analysis. Post-coring, the holes were refilled with clean media (sand and GAC where appropriate) and the filter beds were backfilled immediately with filtrate to minimise the filters' exposure to the air. This destructive sampling technique affected an area of 0.00125 m^2 for each core, which corresponds to approximately 0.2 % of the bed surface.

2.4.2. Oocyst recovery and enumeration

To recover and enumerate *Cryptosporidium* oocysts from sand samples, a Standing Committee of Analysts, (2014) Blue Book 234 (part 14) method was used. The protocol (section C) was adapted to meet the specific sample requirements: a 5 g subsample of homogenized sand was placed into a sterile 50 mL polypropylene centrifuge tube containing 25 mL of 0.1 % (w/v) Tween 20 solution, a non-ionic surfactant that facilitates the detachment of the oocyst biofilm and particulates from sand grains. The tubes were gently agitated for 5 min by hand to dislodge oocysts. After allowing the sand to settle for 1 min, the supernatant was carefully decanted into a clean centrifuge tube and centrifuged at $1100 \times g$ for 15 min to concentrate oocysts into a pellet. This elution process was then repeated with an additional 20 mL of fresh elution buffer, and the resulting supernatants were combined and centrifuged as before. The resulting pellets were combined and resuspended in 7.5 mL of sterile distilled water for subsequent IMS. For samples expected to contain high oocyst concentrations (upper layers), the resuspended pellet was diluted tenfold, whereas samples from lower layers were processed without dilution.

IMS was performed using a concentration and extraction kit (Dynabeads™ *Cryptosporidium* IMS, Thermo Fisher Scientific, UK). Magnetic beads coated with monoclonal antibodies specific to *Cryptosporidium* oocyst surface antigens were then added to the samples to selectively bind and separate the oocysts from the surrounding particulate matter. The isolated oocysts were stained with fluorescein isothiocyanate (FITC)-labelled monoclonal antibodies and counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Enumeration was performed by epifluorescence microscopy at 400 × magnification. Oocysts were identified based on their morphology and fluorescence properties, and the number of oocysts and oocyst-like bodies (OLBs), defined as empty oocysts lacking any trophozoites (Fig. 2) were recorded. The detection limit of the IMS method was approximately 1 oocyst per concentrated sample, which is consistent with detection limits reported in other environmental studies (Bukhari et al., 1998; Sturbaum et al., 2002). To evaluate the efficiency of the oocyst extraction and enumeration protocols, recovery experiments were conducted using sand samples spiked with known quantities of inactivated oocysts at concentrations of 5, 10, 50, 100, 1,000, and 10,000 oocysts per 5 g of sand. These spiked samples were processed alongside experimental samples. Recovery efficiencies were calculated by comparing the number of oocysts detected in the processed spiked samples to the known number added, providing data on the effectiveness of the methods across a range of concentrations (Table A-1 in Supplementary Information). Recovery efficiencies varied with oocyst concentration. Samples containing 5 oocysts per 5 g of sand had ~20 % recovery rates, with high variability likely due to the sparse distribution of oocysts. Recovery improved with higher oocyst concentrations (10, 50, or 100 oocysts) but remained below 50 %. Only samples with more than 1000 oocysts achieved recovery rates exceeding 50 %, with an overall efficiency of 40.3 % for samples containing 5 to 1000 oocysts. Data were not corrected for recovery efficiency; thus, reported values represent uncorrected data.

2.4.3. Data analysis and calculations

The oocyst counts obtained from the 5 g sand subsamples were extrapolated to estimate the oocyst abundance within each filter layer. The mass of sand in each layer was calculated using the filter surface area (0.54 m^2), the thickness of the layer, and the bulk density of moist sand (1682 kg/m^3). The oocyst concentration (oocysts per gram of sand) was scaled up to the equivalent oocyst count in the total mass of sand in the corresponding layer. The amount of sand processed for oocyst determination corresponded to 0.02–0.08 % of the mass of sand in 25 mm–100 mm layers respectively. Statistical analyses were undertaken to determine differences in oocyst concentrations between filters, sampling times, and the SSF cleaning methodologies. Data were checked for normality using the Shapiro-Wilk test ($W(20) = 0.906$, $p = 0.054$) and the homogeneity of variance ($W(3, 16) = 0.408$, $p = 0.7494$) and met the assumptions of parametric tests. Analysis of variance (two-factor ANOVA with replication) tests were employed with a significance level of $p < 0.05$.

2.4.4. High volume filtrate sampling

The filtrate of the remaining six pilot-scale SSFs in the 10-cell installation (beds where media and filter integrity were not disturbed) was monitored for oocyst breakthrough over the duration of the experiment. A UK water industry-standard cumulative sampling technique was employed for continuous filtrate monitoring using IDEXX Filta-Max® cartridges in duplicate on both UWS and DS SSFs. Filta-Max cartridges were replaced at 24-h intervals for one SSF per skimming condition and at 96-h intervals for the replicate filter during weekdays; during weekends, cartridges collected filtrate over a 72-h period. To prevent clogging, flow rates to the cartridges for the 72-h and 96-h samples were reduced from 1 L/min (approximately 37 % of filtrate outflow) to 0.5 L/min (approximately 18.5 % of filtrate outflow). Oocyst isolation, staining, and enumeration via microscopy were performed according to the Standing Committee of Analysts (Part 14, 2014) Blue Book 234 method, section A. The overall recovery efficiency of the method, evaluated daily by spiking separate Filta-Max cartridges with 100 oocysts, averaged at 50.01 ± 8.19 % (n = 109). Each spiked filter was operated on the rig for 24 h, during which approximately 1000 L of

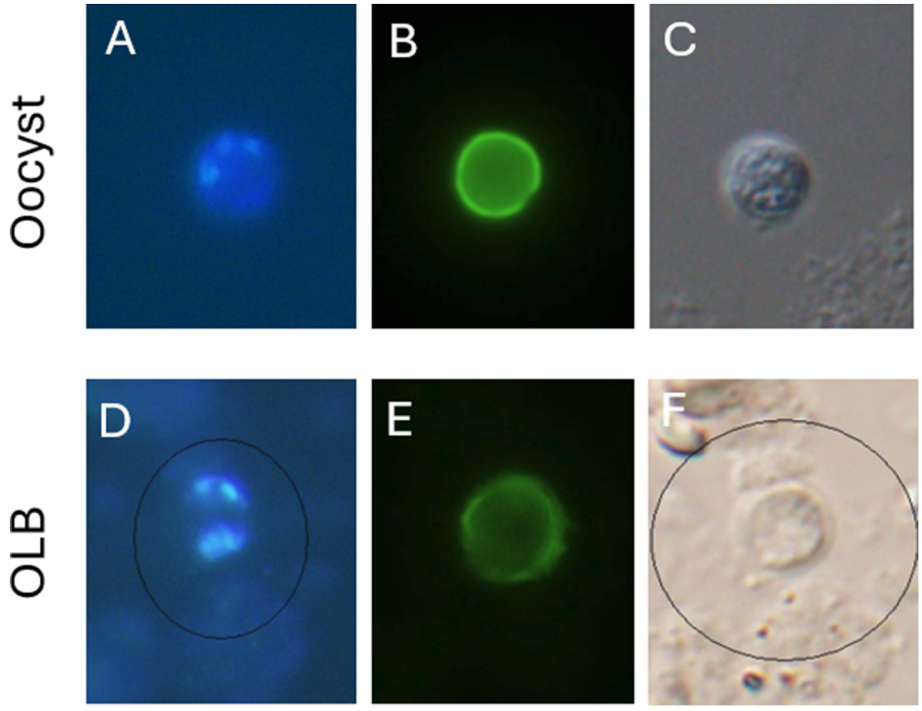


Fig. 2. Photographs of a *Cryptosporidium* oocyst and an oocyst-like body (OLB) captured using oil microscopy at 100× magnification: (A) Oocyst with DAPI stain, (B) Oocyst with FITC stain, (C) Oocyst under Differential Interference Contrast (DIC), (D) Environmental OLB with DAPI stain, (E) Environmental OLB with FITC stain, (F) Environmental OLB under DIC. OLBs are defined as damaged oocysts with a compromised oocyst wall, as show in (F).

water was filtered.

The concentration of oocysts per high volume sample was calculated as follows:

$$C_{OUT} = \frac{N_{OUT}}{V_{Filta-Max}}$$

Where C_{OUT} [oocysts/L] is the concentration of oocysts in the filtrate, N_{OUT} the number of oocysts captured in the high volume Filta-Max sample and $V_{Filta-Max}$ [L] the volume of water that has gone through the Filta-Max cartridge.

2.5. Overall single collector efficiency model calculation

The retention of *Cryptosporidium* oocysts in each layer of the SSF was modelled using the single collector contact efficiency equation with the numerical resolution proposed by Tufenkji and Elimelech (2004) which was derived in part from the work of Yao et al. (1971). The individual collector efficiencies were calculated using the correlations provided by Tufenkji and Elimelech (2004), with constants derived from the physicochemical properties of the oocysts (size and density), the filter media (porosity, and grain size), and the SSF operating conditions (HLR).

The total collector efficiency (η_T), in a clean sand filter is expressed as the sum of three transport mechanisms: diffusion (η_D), interception (η_I) and gravity sedimentation (η_G):

$$\eta_T = \eta_D + \eta_I + \eta_G \tag{1}$$

expressed as:

$$\eta_D = 2.4 A_s^{1/3} N_R^{-0.081} N_{Pe}^{-0.715} N_{vdW}^{0.052} \tag{2}$$

$$\eta_I = 0.55 A_s N_R^{1.55} N_{vdW}^{0.125} N_{Pe}^{-0.125} \tag{3}$$

$$\eta_G = 0.22 N_R^{-0.24} N_G^{1.11} N_{vdW}^{0.053} \tag{4}$$

Where A_s is Happel's porosity-dependent parameter defined as follows.

$$A_s = \frac{2(1-\gamma^5)}{2-3\gamma+3\gamma^5+2\gamma^6} \tag{5}$$

$$\gamma = (1-\varepsilon)^{1/3} \tag{6}$$

N_R is the aspect ratio between the collector (filter media) and the particle size of interest.

$$N_R = \frac{d_p}{d_c} \tag{7}$$

Where d_p is the particle diameter [m] and d_c the collector grain diameter [m]. N_{Pe} is the Peclet number that characterises the ratio of convective to diffusive transport.

$$N_{Pe} = \frac{Ud_c}{D_\infty} = \frac{3\pi\mu d_p U d_c}{kT} \tag{8}$$

With the viscosity $\mu(T)$ [kg/s/m], U the approach velocity set to 0.3 [m/h] and the absolute temperature T [K] of the fluid and k the Boltzmann constant 1.3805×10^{-23} [kg m^2/s^2/K]. The viscosity of the approach fluid is temperature dependent and obtained using the following equation.

$$\mu(T) = A \cdot 10^{\left(\frac{B}{T-C}\right)} \tag{9}$$

Where A, B, C are empirical constants set to $A = 2.41 \times 10^{-5}$ [Pa•s], $B = 247.8$ [K] and $C = 140$ [K].

N_G is defined as the ratio of the particle's gravitational settling velocity to the fluid's approach velocity and calculated as:

$$N_G = \frac{d_p^2(\rho_p - \rho_w)g}{18\mu U} \tag{10}$$

Where ρ_p is the density of the target particle 1050 [kg/m^3], here *Cryptosporidium* oocysts, ρ_w is the density of the fluid (set to 1000 [kg/m^3]), and g earth's gravitational acceleration (9.81 [m/s^2]).

N_{vdW} is the ratio of the van der Waals interaction energy and the thermal energy of the fluid.

$$N_{vdW} = \frac{H}{kT} \tag{11}$$

For this model, the porosity ε was assumed to be constant with time and skim regime and was set to a fixed value of 0.36 (Abudalo et al., 2005; Tufenkji and Elimelech, 2004).

The retention capacity of a granular porous medium also depends on the interaction between suspended particles and collector grains. Due to repulsive colloidal interactions, not all particles that collide with the sand grains remain attached. Here, α represents the empirical attachment efficiency, which defines the fraction of particles that adhere to the collector grains upon collision.

For this model, empirical data from the number of oocysts recovered from the 24-h high-volume filtrate samples of the corresponding duplicate bed were used to determine the attachment efficiency α.

$$\alpha = -\frac{2}{3}\frac{d_c}{(1-\varepsilon)L\eta_T} ln\left(\frac{C}{C_0}\right) = -\frac{2}{3}\frac{d_c}{(1-\varepsilon)L\eta_T} ln\left(\frac{N_{OUT,dosing}}{N_{IN,dosing}}\right) \tag{12}$$

Where L is the depth of the entire filter (here 0.82 [m]), N_{IN} is the cumulative amount of oocysts added over the dosing period, and N_{OUT} is the product of the number of oocysts recovered in the high volume Filta-Max samples and the volume correction coefficient c_v.

$$N_{OUT,dosing} = N_{FM} \cdot c_v \tag{13}$$

The empirical particle diameter for the filter media is calculated using the specific d_{10} and d_{60} values

$$d_c \approx d_{50} \approx \sqrt{d_{10} d_{60}} \tag{14}$$

The attachment coefficient α can be established for each skim condition, UWS 0 % and DS, using empirical data from the duplicate beds during the dosing period. Then, by rewriting equation (12), the number of oocysts exiting the filter can be modelled as:

$$N_{OUT} = N_{IN}\, e^{-\frac{3}{2}\alpha\frac{(1-\varepsilon)L_{layer}\eta_T}{d_c}} \tag{15}$$

Where L_{layer} is the thickness of each layer, which varies according to the segmentation of the cores. By considering each layer to be a shallow filter, where the output from layer i ($N_{OUT,i}$) becomes the input for the subsequent layer $N_{IN,i+1}$, the number of oocysts exiting layer can be modelled. The retention capacity is determined by subtracting the $N_{OUT,i}$ from $N_{IN,i}$.

$$R_{model,i} = N_{IN,i} - N_{OUT,i} \tag{16}$$

The decrease of number of oocysts present in the sand cores with time was estimated using a first order reaction (Alum et al., 2014). The averaged total number of oocysts detected in the post-dosing, pre-skim core samples collected on Days 12, 19, and 37 were used to calculate the instantaneous oocyst presence in the sand layers at a time (t) and enabled the adjustment of the initial input value N_{IN} to be time dependent.

$$N_{IN} = N_0 \cdot e^{-kt} \tag{17}$$

Where N_0 is the estimated number of oocysts at time $t = 0$, and k is the decay rate constant for each DS and UWS beds. The Day 5 samples were excluded from this analysis because the full oocyst load had not yet been administered at that point, and the post-skim samples were omitted due

to the potential physical removal of oocysts during skimming, which could bias the results.

The normalised root mean squared error (NRMSE) was used to evaluate the model by comparing the estimated retention with the core data. The error was calculated using the difference between observed y_i and estimated $\hat{y}_i$ values for layer *i*.

$$\mathrm{RMSE} = \frac{1}{n}\sqrt{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2} \tag{18}$$

As the error is highly dependent on the number of oocysts present in each layer and to ensure comparison of error values was possible, the RMSE was normalised by the highest observed value.

$$\mathrm{NRMSE} = \frac{\mathrm{RMSE}}{max\left(y_{obs,i\to n}\right)} \tag{19}$$

As filtration efficiency is porosity dependent, a sensitivity analysis was performed by varying the porosity factor from 0 to 1 for all of the terms that are a function of porosity, while keeping the attachment coefficient fixed since it was determined using oocyst breakthrough data specific to the filtering conditions of the pilot-scale SSF used in this study.

2.6. Feed water characteristics and filter operation

The average (± standard deviation) feed water quality parameters included total organic carbon (TOC) concentration of 3.18 ± 0.07 mg/L, turbidity of 0.6 ± 0.6 NTU, and pH of 8.1 ± 0.2 (Table A-3 in Supplementary Information). The filters operated under air temperatures ranging from −4 to 17 °C and water temperatures of 9–17 °C. Initially backfilled with filtrate, the SSFs were started at a hydraulic loading rate of 0.1 m/h, which was increased by 0.1 m/h daily until reaching an operational rate of 0.3 m/h. No pre-treatment was applied to the raw water due to its low turbidity (<1 NTU) and the need to accelerate filter headloss development to simulate full-scale conditions in this pilot plant. Headloss was measured by the difference in water levels between the supernatant surface and outlet using piezometric tubes attached to the plenum of each SSF, with a maximum operating headloss of 1300 mm. Measurements were recorded daily to monitor headloss progression, assess filter performance, and schedule maintenance activities (e.g., skimming). The values were temperature normalised to account for dynamic viscosity (Elemo et al., 2025).

3. Results and discussion

3.1. Accumulation of oocysts in the filter media

The *Cryptosporidium* oocysts were enumerated from core samples to ascertain what portion of the oocysts were captured within the SSF media during and after dosing with ~9.3 million inactivated oocysts per SSF bed (measured dose). The total number of retained oocysts peaked in the Day 12 cores (Fig. 4), reaching a maximum of ~4.3 million oocysts in DS-SSF 10, corresponding to 50.24 % of the total oocysts dosed to this SSF. These cores were collected less than 24 h after the final dosing day, which explains the significant increase in oocyst counts, with the addition of 1,957,628 oocysts in UWS SSF 1, 3,301,623 oocysts in UWS SSF 6, 1,840,540 oocysts in DS SSF 5 and 2,888,506 oocysts in DS SSF 10, compared to the Day 5 core samples since only 23 % of the total oocysts had been dosed to the SSF.

On days 19 and 37, the total number of oocysts detected decreased. The UWS beds (SSF 1 and SSF 6) had a rapid reduction in oocyst numbers of 46 % (1,283,110 oocysts) and 57 % (2,336,997 oocysts), respectively, from Day 12 to Day 19. This was followed by a slower reduction (31 %, 547,801 oocysts) for UWS SSF 6 during the remainder of the observation phase (Table A-2 in Supplementary Information). In contrast, the DS beds (SSF 5 and SSF 10) showed less substantial reductions of 20 % (483,772 oocysts) and 34 % (1,454,415 oocysts) one week post-dosing, with reduction rates ranging from 9 % (165,629 oocysts) to 59 % (1,683,851 oocysts) between Day 19 and the pre-skim core on Day 37 (Table A-2 in Supplementary Information).

The Day 50 cores, collected 12 days after the skimming event, contained the lowest oocyst counts, 335,560 oocysts in UWS SSF 1, 658,731 oocysts in UWS SSF 6, 695,522 oocysts in DS SSF 5, 163,919 oocysts in DS SSF 10. Cores from UWS SSF 1 and DS SSF 10 revealed significant reductions in oocyst numbers from the counts recorded on Day 37, with decreases of 80 % (1,325,875 oocysts) and 86 % (1,032,137 oocysts), respectively. UWS SSF 6 and DS SSF 5 showed reductions of 45 % (538,217 oocysts) and 60 % (1,025,221 oocysts), respectively. However, despite these reductions, further analysis suggested that the skimming regime had a limited effect on oocyst removal compared to other processes that may be taking place in the SSFs.

Notably, the UWS SSF 6 and DS SSF 10 Day 12 cores exhibited significantly higher oocyst counts, roughly double those of the other two filters - with 4.33 million and 4.08 million oocysts detected, respectively. In contrast, UWS SSF 1 and DS SSF 5 peaked at 2.82 million and 2.37 million oocysts, respectively. This phenomenon does not imply differences in the retention capacity of the filters but is more likely due to increased activity of primary producers, such as algae, induced by uncontrolled environmental factors such as greater sunlight exposure for UWS SSF 1 and DS SSF 5 than the other filters located on the opposite side of the installation. Increased photosynthetic activity may lead to higher production of organic matter, which could have contributed to oocyst recovery (Andersson et al., 2024).

In terms of the total number of oocysts detected in the core samples, the skimming regime appears to have little discernible influence on oocyst retention. Statistical analyses using Two-Factor ANOVA with replication ($F(4,10) = 0.29$, $p > 0.05$) indicated no significant difference in retention capacity between the DS and UWS SSFs. These findings indicate that the inherent retention capacity of the entire filter bed is the primary factor in oocyst capture - since only a shallow 40 mm layer is skimmed infrequently, a substantial amount of oocysts remain retained in the layers below the Schmutzdecke.

3.2. Spatial distribution of oocysts within the depth column

Developing an understanding of the distribution of oocysts with depth is critical for evaluating the effectiveness of SSFs in pathogen removal and for assessing whether novel maintenance practices such as UWS impact removal performance.

In both UWS SSFs, a substantial portion of the total oocysts was detected in the Schmutzdecke (Fig. A-1 in Supplementary Information). Specifically, 51 % of the total oocysts in UWS SSF 6 on Day 12 (2,076,584 oocysts) and 65 % in UWS SSF 1 (1,844,598 oocysts) were located within the first 25 mm of the filter media (Table 1). This is likely due to the Schmutzdecke's dense biofilm and organic matter, which facilitate both physical straining and biological interactions that readily capture oocysts (Bellamy et al., 1985b; Schijven et al., 2013). In contrast, the DS filters exhibited deeper penetration of oocysts less than 24 h post-dosing, with only 38 % (906,936 oocysts) in DS SSF 5 and 33 % (1,433,183 oocysts) in DS SSF 10 retained in the Schmutzdecke. Such

Table 1
Percentage of total number of oocysts retained in the Schmutzdecke (0–25 mm) and layers 2 & 3 (25–100) mm of pilot-scale SSF.

	Schmutzdecke (Layer 1)			Layers 2 & 3		
	Day 12	Day 19	Day 37	Day 12	Day 19	Day 37
UWS SSF 1	65.4	46.4	30.5	27.4	29.0	52.1
UWS SSF 6	50.9	37.1	34.2	27.8	51.5	56.8
DS SSF 5	38.3	14.5	28.9	51.7	66.3	46.8
DS SSF 10	33.1	41.5	53.5	34.7	40.7	41.9

deeper oocyst penetration may result from the draining and refilling cycles in DS, which can disturb the top layer and transiently reduce microbial density or biofilm cohesion (Türetgen et al., 2007). A higher proportion of oocysts was detected in layers 2 and 3 (25–100 mm depth), accounting for 52 % (1,224,439 oocysts) in DS SSF 5 and 35 % (1,503, 266 oocysts) in DS SSF 10, compared to 27 % (773,070 oocysts) in UWS SSF 1 and 28 % (1,133,812 oocysts) in UWS SSF 6. This suggests that the UWS method may enhance retention of oocysts in the upper layers shortly after dosing, potentially due to less disturbance of the filter bed structure, preservation of the microbial community, and accelerated ripening (Elemo et al., 2025; Türetgen et al., 2007). All these factors help maintain a stable and biologically active surface layer that traps oocysts before they migrate deeper (Searcy et al., 2006; Wolyniak et al., 2009). While disturbance of the Schmutzdecke during DS is a plausible contributor to deeper oocyst penetration, alternative mechanisms include (i) hydraulic-rate effects, where increased loading or transients can drive deeper advective transport, and (ii) preferential flow paths formed by media heterogeneity and biofilm structuring. Both mechanisms are well-documented to enhance particle/oocyst migration in granular media (Bak et al., 2019; Bottero et al., 2013; Emelko, 2003; Kurz et al., 2022; Thullner and Baveye, 2008). These filters operated at identical HLR setpoints with no systematic between-regime differences in recorded flows, but these pathways are suggested as complementary explanations.

A general downward migration of oocysts was observed in three of the four SSF beds (UWS SSF 1, DS SSF 5, and UWS SSF 6). This gradual percolation and redistribution can occur under constant flow, with dynamic biofilm attachment and detachment processes occurring, as well as biological processes such as microbial grazing also contributing to oocyst movement (Gómez-Couso et al., 2007; Scheid and Schwarzenberger, 2011). Thus, over time, the proportion of oocysts in the Schmutzdecke decreased, accompanied by a consistent increase in the number of oocysts in the layers beneath (Table 1 and Fig. A-1 in Supplementary Information). For example, in UWS SSF 6, the proportion in the Schmutzdecke declined from 51 % (2,076,584 oocysts) on Day 12 to 34 % (409,151 oocysts) on Day 37, while the proportion in layers 2 and 3 (75 mm cumulatively) increased from 28 % (1,133,812 oocysts) to 42 % (680,040 oocysts) over the same period. In contrast, SSF 10 exhibited little change (less than an 8 % difference) in the Schmutzdecke during the post-dosing period. This deviation may be attributed to macro- and microscopic structural differences inherent between filters or environmental factors (Attiani et al., 2025), such as variations in flow patterns (Helmi et al., 2008) or microbial activity (Searcy et al., 2006; Wolyniak et al., 2009, 2010).

In layer 4 (100–200 mm depth), oocyst counts varied slightly with time and between filters, ranging from 1 % (37,053 oocysts) to 19 % (287,771 oocysts) of the total oocysts detected in the core samples. Layer 5 (200–300 mm) exhibited further reduced oocyst counts, with a maximum of only 3.5 % (54,589 oocysts). This indicates a cumulative filtering effect in the overlying layers, including physical straining and microbial interactions, which limits deeper oocyst penetration (Trikannad et al., 2023). No oocysts were observed in the GAC samples.

Table 2
Oocyst breakthrough in filtrate of adjacent SSF operated consistent with planned skim regime.

	24 h		96 h	
	UWS SSF 2	DS SSF 4	UWS SSF 7	DS SSF 9
Max. oocysts per sample	1	3	2	1
Frequency of positive detections [%]	7.89	47.37	13.33	46.67
Number of samples	38	38	15	15
Subsample for high volume sampling [%][a]	37.52	37.41	19.45	17.66

The GAC layer, generally situated below 450–500 mm of filter media, showed an absence of oocysts - a finding corroborated by minimal detections in layer 6 (immediately above the GAC layer). This indicates that the GAC layer does not play a significant role in oocyst retention in these SSFs, likely due to its increased porosity and the resulting lower straining efficiency (Sakthivadivel, 1967). Hijnen et al. (2010) observed a 1.3 to 2.7-log removal of *Cryptosporidium* oocysts and *Giardia* cysts in a 1 m depth GAC filter, operated at a 5 m/h filtration rate, contrasting with the null detection of oocysts in the GAC layer in this study. However, the authors attribute most of the retention to attachment, with very limited straining. The shorter contact time for oocyst capture due to the size of the GAC layer (0.1 m) of this study will affect the attachment efficiency (Bellamy et al., 1985a), thus the retention capacity.

Detections of oocysts in the sand below the GAC layer were noted during the early stages of the trial. Specifically, positive detections occurred on Days 5 and 12 for the DS filters (accounting for 7 % and 18 % of the total oocysts, respectively) and on Days 12 and 19 for the UWS filters (with detections of 2 %, 5 %, and 18 %). While these detections indicate that some oocysts penetrate deeper into the filter bed, movement may occur via advective transport or minor preferential flow paths (Diehl et al., 2025). The lack of a direct correlation with filtrate samples positive for oocysts suggests that oocyst presence in the bottom layers is not a conclusive indicator of breakthrough into the treated water. Deeper oocysts may still be trapped by residual biofilm or fine particulates, preventing them from exiting with the filtrate (Trikannad et al., 2023). Despite detections below the GAC layer in 30 % of the UWS cores, oocysts were more frequently detected in the filtrate of DS filters (~47 % oocyst-positive samples) than in UWS filters (8–13 % oocyst-positive samples; Table 2). The wet/dry cycling could thin or disrupt the existing biofilm and potentially kill and/or dislodge key microbial populations. Because biofilm provides both a physical barrier (through exopolymeric substances (Searcy et al., 2006)) and a biological barrier (via predation, enzymatic degradation, etc. (Siqueira-Castro et al., 2016; Nasser et al., 2007)) to oocyst transport, any reduction in biofilm thickness or integrity can reduce the filter's capacity to trap oocysts. Additionally, draining and refilling may create minor gaps or heterogeneities in the media (Türetgen et al., 2007), which can lead to preferential flow paths or "short-circuiting," where a portion of the flow bypasses the usual tortuous route through the media (Dai and Hozalski, 2002). Both effects, biofilm disruption and more direct flow pathways, can increase the risk of deeper oocyst penetration and ultimately allow more oocysts to exit with the filtrate.

These findings are consistent with other studies on the spatial distribution of *Cryptosporidium* oocysts in SSF media. For example, Timms et al. (1995) reported that oocysts were present only in the top 25 mm of sand. This was attributed to the biologically active nature of the surface layer, which effectively filtered out oocysts (Searcy et al., 2006). Heller and de Brito (2006) found significant numbers in the top layer (up to 150 mm) but also in lower layers, suggesting gradual migration. Such observations highlight the potential for downward movement through pore spaces after detachment from upper strata in the SSF or via biologically driven transport over time (Gómez-Couso et al., 2007; Wolyniak et al., 2009). Hijnen et al. (2007) detected oocysts as deep as approximately 500 mm after 184 days of operation, with an exponential decrease in concentration with depth and further declines between 184 and 253 days, indicative of degradation or continued downward migration. In that study, multiple physical and biological mechanisms worked together to reduce oocyst numbers in deeper strata over extended periods (Bradford and Bettahar, 2005; Fayer et al., 2000; Stott et al., 2001, 2003; Tufenkji et al., 2004).

Understanding the spatial distribution of oocysts within the filter media has important implications for SSF operation and maintenance. Knowing where most oocysts accumulate and how they move helps operators tailor skimming intervals and manage filter depth more effectively (Heller and de Brito, 2006). The retention of a significant proportion of oocysts in the Schmutzdecke and upper layers

demonstrates that these zones form a primary barrier against pathogen passage, with high microbial activity and dense biofilm contributing to efficient oocyst capture (Chen et al., 2021; Hijnen et al., 2006). This suggests that more regular skimming could effectively remove a substantial portion of retained oocysts, as it physically discards the top layer containing the highest oocyst loads, thereby reducing the risk of downward migration and subsequent breakthrough. By removing the surface layer, filters can be re-ripened with fresh sand that quickly re-develops its active microbial community, further limiting oocyst penetration (Bai et al., 2024). The observed downward migration over time further highlights the need for regular monitoring and timely filter depth management. While UWS appears to retain more oocysts in the upper layers shortly after dosing, the long-term differences between UWS and DS in terms of oocyst distribution are less pronounced, indicating that both skimming regimes are effective, though their short-term impacts differ (Elemo et al., 2025). In addition, while dry coring may slightly perturb the top millimetres, the protocol was identical for UWS and DS and conclusions are supported by independent filtrate measurements.

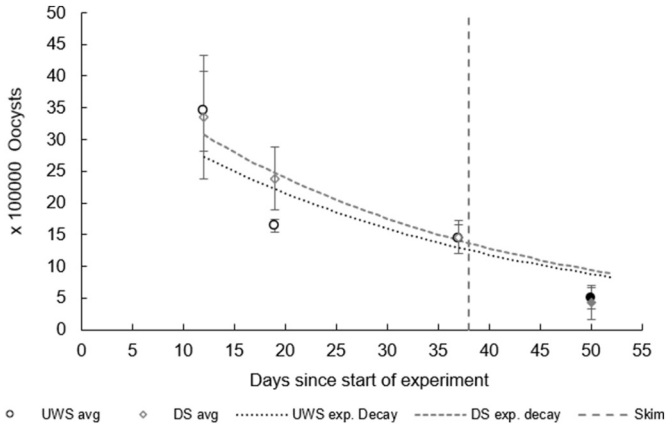


Fig. 3. Averaged total number of oocysts detected in post-dosing and pre-skim cores Days 12, 19 and 37 (with standard error bars) and first order decay regression curves to model the decrease in oocysts with time. $R^2_{UWS} = 0.58$ and $R^2_{DS} = 0.65$.

3.3. Decrease of oocysts in SSF post-dosing

A decrease in the total number of detected *Cryptosporidium* oocysts was observed over time following the dosing period. The number of unaccounted-for oocysts, those that were dosed but are not detected in the core samples, increased from an initial range of 5×10^6 to 7×10^6 oocysts on Day 12, corresponding to approximately 54 % (5,004,860 oocysts) to 75 % (6,518,163 oocysts) of the total oocysts dosed, to 82 % (7,618,440 oocysts) – 87 % (8,143,127 oocysts) pre-skim and up to 98 % (9,175,264 oocysts) post-skim (Table 3). This progression indicates a steady decline in oocysts measurable in the filter media, regardless of the skimming regime, and highlights that many oocysts become undetectable over time due to *in situ* processes. Results of the two ANOVA with replication indicated a significant difference in retained oocysts with time regardless of skim regime ($F(4,10) = 12.69$, $p < 0.05$).

The exponential regression (eq. (17)) yielded daily decay rate constants of $k_{UWS} = 0.0298\ d^{-1}$ for UWS beds and $k_{DS} = 0.0311\ d^{-1}$ for the DS filters. Although the DS filters exhibited a slightly higher daily reduction rate, the difference between the two rates is within the margin of error of the averaging. This close similarity suggests that both filters, operated concurrently, share comparable biological and physical conditions that govern oocyst decay, indicating minimal impact from the type of skimming on the natural rate of oocyst reduction (Alum et al., 2014; Kato et al., 2004). To assess the effect of the skimming event on oocyst removal, the expected number of oocysts that would be "lost" between the pre-skim core (Day 37) and the post-skim core (Day 50) was extrapolated using the established exponential regression rates. For the UWS filters, an additional 4.16×10^5 oocysts would be expected to be lost over the 13-day period following the Day 37 core, while for the DS filters, the expected loss was 8.14×10^5 oocysts.

Comparing these predicted values to the observed data from the Day 50 cores (represented by the solid markers in Fig. 3), the predicted oocyst counts for Day 50 were found to be higher than the standard deviation of the duplicate data points for both UWS and DS filters. This indicates that the actual oocyst counts after skimming deviated by 3.82×10^5 oocysts for UWS and 5.12×10^5 oocysts for DS beds from what would be expected based on the natural decline observed prior to skimming.

Calculating the proportion of oocyst removal attributable to the skimming event revealed that, only 4 % of the total oocyst dose removed in the UWS filters and 5.5 % in the DS filters could be attributed to skimming. These findings suggest that skimming does not seem to be the primary nor the most efficient mechanism for the removal of *Cryptosporidium* oocysts retained in SSF media. Instead, a range of *in situ* processes contributes to the ongoing reduction of detectable oocysts, indicating that normal biological and physical activities within the filter media are the dominant drivers of decline in retained oocysts (Trikannad et al., 2023).

This finding aligns with observations from other studies that have reported a decay rate coefficient as low as 0.014 d^{-1} to 0.02 d^{-1} for SSF (Hijnen et al., 2007) and 0.048 d^{-1} for the natural attenuation of oocysts on various surfaces when incubated at 12 °C (Alum et al., 2014) and on average 0.028 d^{-1} within a soil matrix (Kato et al., 2004). Possible mechanisms for this natural decline include predation by microbial communities (Siqueira-Castro et al., 2016; Fayer et al., 2000; Scheid and Schwarzenberger, 2011), enzymatic degradation (Nasser et al., 2007), and physical processes such as adsorption and reversible attachment to filter media particles (Bradford and Bettahar, 2005).

If skimming does not significantly enhance the removal of retained oocysts, its main purpose shifts to managing headloss and sustaining consistent hydraulic performance, rather than actively removing pathogens. By controlling headloss, operators help ensure that filtration rates and residence times remain optimal, creating stable conditions that support the microbial communities responsible for oocyst attenuation (Luo et al., 2017). Additionally, preserving these essential microbial populations is more feasible with less disruptive skimming practices, such as UWS, which can also offer increased production volume through reduced downtime without compromising oocyst retention efficiency (Türetgen et al., 2007). Recognising that biological processes, such as predation and enzymatic breakdown, are key drivers of oocyst attenuation underscores the importance of stable filter conditions - adequate

Table 3
Percentage of unaccounted-for oocysts, with percentage of oocyst-like bodies (OLBs) detected in brackets.

Filter	Day 5	Day 12	Day 19	Day 37	Day 50
UWS SSF 1	60.69 (4.93)	69.79 (N/A)	83.53 (5.55)	82.21 (3.53)	96.41 (3.92)
UWS SSF 6	64.48 (7.89)	56.29 (N/A)	81.32 (5.73)	87.18 (7.66)	92.95 (0.00)
DS SSF 5	75.89 (14.69)	74.62 (N/A)	79.80 (4.66)	81.58 (2.51)	92.55 (4.06)
DS SSF 10	34.18 (18.68)	53.59 (N/A)	69.16 (0)	87.19 (5.85)	98.24 (3.02)

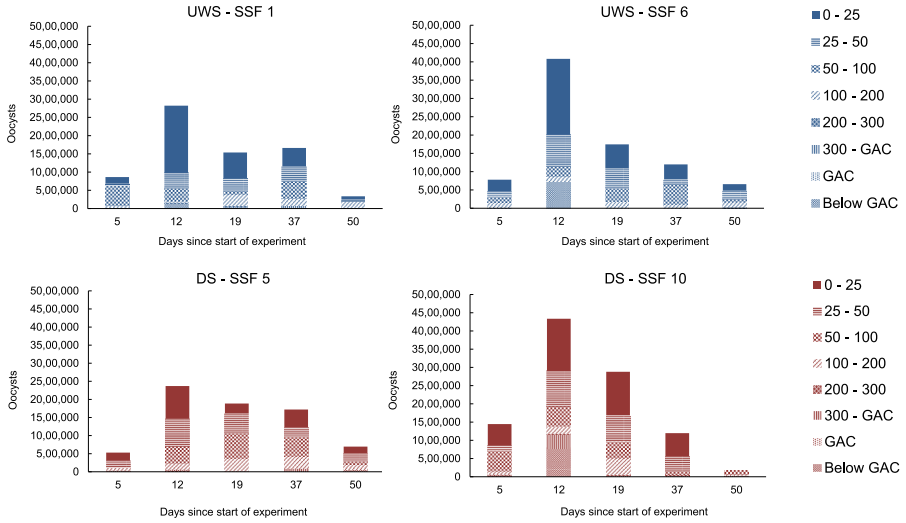


Fig. 4. *Cryptosporidium* oocysts retained in each layer for all cores for underwater and dry skimmed duplicate slow sand filters.

DO (Elemo et al., 2024), favourable temperatures (Wolyniak et al., 2010), and consistent flow rates (Helmi et al., 2008) - for sustaining these beneficial microbial populations.

In addition to counting intact oocysts, OLBs which are empty oocyst shells were also enumerated (Fig. 5). OLBs appeared in nearly every core sample during the dosing period, including as early as Day 5, when 5–19 % of oocysts exhibited compromised walls (Table 3). Control cores taken before the trials contained no OLBs, and none were found in inlet water samples, indicating that OLB formation occurred *in situ*. Although OLB abundance increased in later cores (Fig. 5), it did not consistently correlate with a decline in intact oocysts, and the proportion of OLBs remained between 2.5 % and 7.6 % after Day 19 (data for Day 12 were not recorded). Because oocyst isolation relies on antibody-coated magnetic particles binding to intact walls, any damage may lead to underestimation of OLBs. Still, their presence confirms that oocysts underwent physical alterations in the SSF, providing further evidence of biological or chemical degradation processes.

3.4. Oocyst mass balance

A daily decrease of approximately 50,000 oocysts per filter was observed post dosing, but the fate of oocysts remains unclear. Oocyst breakthrough into the filtrate, while positive for oocysts 47 % of the time for DS and only 8–14 % for UWS (Table 2), was minimal and insufficient to account for the observed decrease in core samples. With no other exit pathways identified, it is likely that the inactivated *Cryptosporidium* oocysts underwent *in situ* processes rendering them undetectable. One probable mechanism is degradation within the filter bed. The presence of OLBs indicates that some oocysts experienced damage sufficient to rupture their resistant walls while retained in the filter. OLBs were most abundant in the top three layers (first 100 mm), where the majority of retained oocysts were found, supporting the hypothesis of active *in situ* processing. Natural decay seems an unlikely predominant factor, given the experiment's duration and the known longevity of oocysts under similar conditions. Oocysts can remain viable for up to 12 weeks in raw reservoir water at 15 °C (King et al., 2005), comparable to the experimental conditions, and up to 7 months in cleaner water (Jenkins et al., 2002) or 120 days in soil (Kato et al., 2004).

Biological activity within the SSF beds likely contributes to oocyst degradation. Studies have shown that higher organisms such as rotifers, paramecia, and certain ciliates can rapidly ingest significant numbers of *Cryptosporidium* oocysts (Siqueira-Castro et al., 2016; Fayer et al., 2000; King et al., 2007; Stott et al., 2003). The fate of internalized oocysts may include:

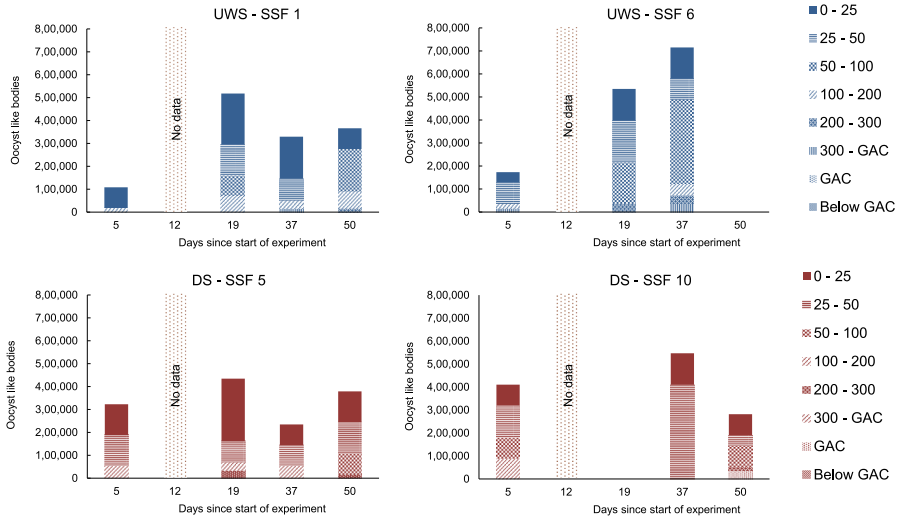


Fig. 5. Oocysts-like bodies (OLB) retained in each layer for all cores for underwater and dry skimmed duplicate slow sand filters.

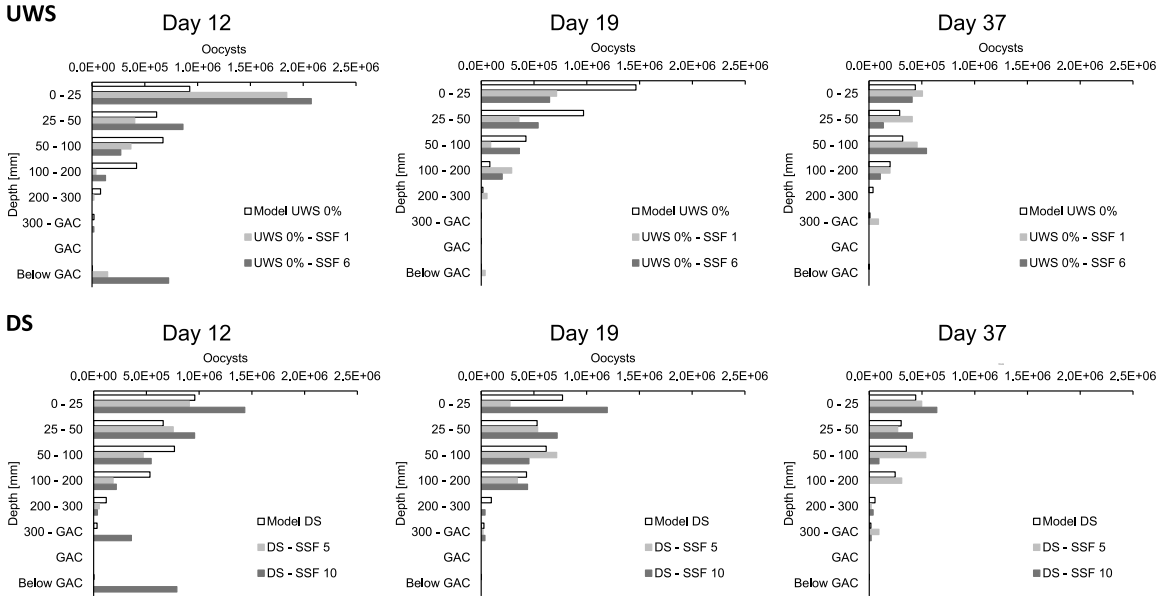


Fig. 6. Predicted and measured oocyst retention per layer in cores on Day 12, 19 and 37 for underwater and dry skimmed duplicate filters.

- Excretion without digestion: oocysts are internalized but not digested, then excreted back into the filter media, potentially clumped in larger pellets (Gómez-Couso et al., 2007; Scheid and Schwarzenberger, 2011).
- Complete digestion: oocysts are fully digested, leading to permanent degradation (Huamanchay et al., 2004).
- Partial digestion and re-internalization: oocysts are partially digested, excreted back into the media, and may be re-internalized multiple times until the oocyst wall is compromised (Fayer et al., 2000; King et al., 2007).

The presence of such organisms in UK SSFs has been confirmed by Goddard (1980) and Ibrahim (1989). While only one ciliate species known to graze on *Cryptosporidium* (*Oxytricha granulifera*) was identified in a full-scale SSF core (unpublished data), other oocyst-ingesting organisms are likely present. Additionally, diurnal migration patterns of these organisms within sediment layers are well documented (Kuczyńska-Kippen, 2001);(Loose and Dawidowicz, 1994) , potentially affecting oocyst distribution by acting as vectors for oocyst transport within the filter media. Another possible mechanism is the action of bacterial extracellular enzymes within the biofilm-coated filter media. Nasser et al. (2007) reported a 1-log reduction in oocyst viability after exposure to such enzymes, which can digest components of the oocyst wall, leading to compromised wall integrity and potential loss of detectability. This could reduce the efficiency of IMS during oocyst isolation, thereby contributing to the decrease in detectable oocysts. Together, these biological processes provide circumstantial evidence of *in situ* degradation of *Cryptosporidium* oocysts within the SSF media.

3.5. Can oocyst retention be predicted in SSF?

Single-collector contact efficiency (η_T) values for the sand layers were consistently low, averaging 2.74×10^{-3} under both DS and UWS assumptions (see nomenclature). Because η_T depends mainly on temperature, assuming constant porosity, it showed negligible variation across regimes and over time ($\sigma = 0.0002$). Although grain size also factors into collector efficiency, the GAC layer ($\eta_{T\,GAC} = 3 \times 10^{-3}$) exhibited nearly the same value as sand, implying that stable porosity largely negates particle-size effects.

Attachment efficiencies (α) were derived from filtrate data using a fixed porosity of 0.36. Both DS and UWS α values exceeded unity ($\alpha_{UWS} = 2.22$ and $\alpha_{DS} = 2.01$) (Table 4), suggesting that additional physical processes (e.g., straining) complement basic gravity, diffusion, and interception (Hijnen et al., 2005). Model inputs were corrected for oocyst decay at Day 12, 19, and 37. In general, the model predicted higher oocyst breakthrough than actually occurred. On Day 12, it projected 25 oocysts for UWS and 87 for DS, whereas measured breakthroughs were 1 and 14, respectively—yet it still captured the empirically higher DS breakthrough (2.82×10^{-3} % vs. 9.33×10^{-4} % for UWS). The model underestimated retention in the Schmutzdecke, especially for UWS on Day 12 (45–50 % of observed), and overestimated it in deeper layers by up to 238 %. However, discrepancies lessened at Days 19 and 37 (Fig. 6 and Fig. A-3 in Supplementary Information). No GAC retention was modelled, and the stepwise approach further underestimated retention below the GAC, causing errors of up to 7.9×10^5 oocysts for DS.

Table 4
Calculated input parameters to model the retention of oocysts in clean SSF.

	Underwater skim	Dry skim
Averaged efficiency η_T for sand layers	2.74×10^{-3}	
Averaged efficiency η_T for GAC layer	3×10^{-3}	
Averaged α for sand layers	2.22	2.01
Averaged α for GAC layer	0	
N_{IN} input value on Day 12 [oocysts]	2.73×10^6	3.07×10^6
N_{IN} input value on Day 19 [oocysts]	2.22×10^6	2.47×10^6
N_{IN} input value on Day 37 [oocysts]	1.30×10^6	1.41×10^6

These mismatches were anticipated. The pilot-scale SSF is a mature system with robust biofilm growth, while the single-collector model assumes only physical retention mechanisms (sedimentation, diffusion, interception) and treats porosity as constant. Factors such as predation, biofilm formation, microbial grazing and time-dependent changes in pore structure are omitted. Single-collector model retentions must therefore be treated as a lower-bound baseline and deviations from the pilot-scale retention values should be interpreted as evidence of additional biological/structural processes.

A sensitivity analysis (Figure A-4 in Supplementary Information) varying porosity from 0.1 to 1 did not appreciably improve model accuracy (normalised mean squared errors ranged from 0.14 to 0.49), likely because biological processes, not just porosity, shape actual retention. As a result, model predictions did not differ substantially

between DS and UWS, reflecting the greater influence of processes beyond the scope of the single-collector approach. Additionally, dynamic porosity and changing collector grain size by depth would be a good proxy for biofilm development as a function of time and filter depth. A fixed porosity of 0.36 was used here (Elemo et al., 2024; Tufenkji and Elimelech, 2004), because the detailed measurements needed to implement the model suggested by Diehl et al. (2025)—which incorporates time- and depth-dependent biofilm growth—were not collected when the cores were sampled. Overall, the estimated oocyst retention did not align more accurately with one skim regime over the other, since overestimation in the lower layers balanced underestimation in the top layer under UWS. As previously noted, oocysts tend to penetrate deeper in DS beds, whereas they remain more concentrated near the surface in UWS beds. This distribution explains the smaller over- and underestimations immediately after dosing (Day 12).

4. Perspectives of UWS for *cryptosporidium* removal

A key advantage of UWS, when viewed through the lens of *Cryptosporidium* and other pathogen removal, lies in how it affects the post-skimming ripening period. In conventional DS, draining the filter disrupts the surface biofilm (Türetgen et al., 2007), where much of the biological degradation of oocysts and other pathogens takes place (Chen et al., 2021; Schijven et al., 2013)—and often prolongs the time needed for that community to re-establish full removal capacity (Elemo et al., 2025). Because UWS keeps the filter bed submerged, it preserves much of the biofilm's integrity and shortens ripening, which helps sustain consistent oocyst capture and degradation immediately following maintenance.

Additionally, the headloss patterns characteristic of UWS are often more stable during a filter run, as the top layer is disturbed less abruptly (Elemo et al., 2025). This stability helps maintain the hydraulic conditions that favour *Cryptosporidium* entrapment and biological interactions (e.g., protozoan or bacterial grazing) (Helmi et al., 2008; Luo et al., 2017). When DS leads to a sharper switch from “slow” to “fast” headloss, there is a risk of uneven flow or channelling, which can facilitate oocyst detachment and penetration deeper into the bed (Helmi et al., 2008). Under UWS, the gradual transition and smoother hydraulic profile reduce the likelihood of oocyst breakthrough or short-circuiting.

Moreover, repeated skimming under UWS helps preserve deeper microbial communities that continue to degrade or intercept oocysts that do migrate beyond the top layers (Türetgen et al., 2007). Although the Schmutzdecke is widely recognized as crucial for pathogen removal (Chen et al., 2021), healthy biofilms in lower layers also contribute significantly to overall treatment (Trikannad et al., 2023), especially once the surface sand has been disturbed or replaced. By minimally disturbing the filter with each skim, UWS promotes resilient subsurface biofilms capable of maintaining steady pathogen attenuation over repeated cleaning cycles. Consequently, UWS not only stabilizes the top layer crucial for initial oocyst capture but also safeguards the deeper layers necessary for sustained *Cryptosporidium* retention and degradation.

5. Conclusions

These findings confirm that filters managed using both UWS and DS can reliably retain and degrade *Cryptosporidium* oocysts in SSFs. For both skimming regimes, more than 80 % of the dosed oocysts became undetectable within 40 days, suggesting that biological processes, particularly predation and enzymatic digestion, influence overall oocyst attenuation. Although most oocysts initially accumulated in the top 25 mm (the Schmutzdecke), gradual penetration into deeper layers was observed in either skimming regime. UWS appeared to slow initial oocyst migration more effectively than DS, although the difference between the two regimes became less pronounced as time progressed. Overall, the skimming regime did not influence the retention of oocysts significantly. However, oocyst detection in the filtrate was 3.6 times more frequent for DS than for UWS SSFs.

Skimming itself accounted for only a small fraction of oocyst removal, indicating that these *in situ* decay processes play a far more important role. This pattern was further supported by modelling results, which showed that simple physical-filtration parameters (e.g., single-collector efficiency) could not fully explain observed retention. Rather, biofilm development, grazing by protozoa and metazoans, as well as straining and interception likely interact in complex, time-dependent ways to reduce oocyst numbers.

Under these conditions, the primary function of skimming, whether performed underwater or dry, is to manage headloss and maintain steady flow rather than to directly remove pathogens. In this regard, UWS offers practical advantages by minimizing filter downtime and preserving the integrity of the Schmutzdecke community. Maintaining a stable hydraulic regime and a sufficient DO concentration for filter organisms appears critical for sustaining high removal efficiency. From a utility perspective, adopting UWS can therefore achieve comparable or better oocyst retention while enabling greater production volumes and reduced filter offline time. Overall, these results highlight the importance of maximizing and maintaining robust biological activity in slow sand filters to ensure consistent protection against *Cryptosporidium* and similar resilient pathogens.

CRediT authorship contribution statement

Sophie L. Bretagne: Writing – original draft, Methodology, Investigation, Formal analysis. **Michael Chipps:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Ian Doggett:** Writing – review & editing, Methodology. **Michael C.J. Bradwell:** Methodology, Formal analysis, Data curation. **Heide Hardiman:** Methodology, Investigation, Data curation. **Jim Harris:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Francis Hassard:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clet.2025.101134.

Data availability

Data supporting this study are openly available from Figshare at 10.6084/m9.figshare.28882868.

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Underwater skimming improves retention and degradation of Cryptosporidium oocysts in slow sand filters

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