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Cryptosporidium Oocysts Removal by Upflow Direct Filtration: Pilot Scale Assessment

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Abstract: Studies on the removal of *Cryptosporidium* oocysts by direct filtration suggested that high removal efficiencies (>3.0 log) can be achieved, but the vast majority of the studies focused on the assessment of downflow direct filtration. However, in comparison with downflow direct filtration, filters in upflow direct filtration systems use lower filtration rates, deeper stratified bed, and water flows from coarse to fine sand grain, which may improve the removal of oocysts. In this context, we evaluated the removal of *Cryptosporidium* oocysts using upflow direct filtration, on a pilot scale, to treat Paranoá Lake water (Brazil) seeded with *Cryptosporidium* oocysts. The experiments were conducted with raw water with low turbidity (3.2–5.7 NTU) and induced higher turbidity (28–30 NTU). Non-parametric statistical analysis was used to verify correlations between the filtered water values and removal of oocysts, particles, and turbidity. In general, no correlation was observed between the parameters analyzed, nor between their removals. The exception was the correlation between residual values of *Cryptosporidium* oocysts and particles of 2 to 7 μ m during ripening, an aspect that needs further evaluation. Under stable operation, average removal of *Cryptosporidium* oocysts by upflow direct filtration. Under stable operation, removal of oocysts was around 1 log lower.

Keywords: Cryptosporidium oocysts; particle counts; turbidity; upflow direct filtration

1. Introduction

The absence and deficiency of water and sanitation infrastructure and services in different regions of Brazil represents a continuous public health risk. This problem has intensified due to the increase in population in the country's urban areas, industrialization, and accelerated land occupation, leading to a deterioration of water resources and increasing risk of waterborne diseases, including those caused by intestinal protozoa that are resistant to chlorination, which is the disinfection process commonly used in Brazil.

Cryptosporidium transmission occurs by untreated surface water, improper water treatment or water contaminated in distribution systems. Previous studies have demonstrated the occurrence of *Cryptosporidium* oocysts in several regions of Brazil and in different environments, such as groundwater, surface water, drinking water systems, both in untreated raw water (influent) and treated finished water, as well as wastewater [1–7]. In this context, concerns regarding the risk associated with *Cryptosporidium* spp. have led the Ministry of Health to enforce, through ordinance 2914/2011 (reedited as Annex XX of Ordinance 5/2017 [8]), regulation for filtered water produced by conventional or direct filtration, where turbidity must be less than or equal to 0.5 NTU in at least 95% of the samples. Moreover, turbidity values must be less than or equal to 0.3 NTU in filtered water when

the concentration of *Cryptosporidium* spp. oocysts in the source water is equal to or greater than 3.0 oocysts/L.

Cryptosporidium oocysts are resistant to environmental stress and to inactivation by chlorine [9]. Due to the small oocyst size, filtration is considered an essential barrier in water treatment plants for reducing risks to human health associated with this pathogen. Therefore, despite the development of disinfection technologies capable of achieving reasonable levels of *Cryptosporidium* oocysts inactivation, traditional physicochemical technologies remain important tools for achieving desirable removal levels of this pathogen.

In Brazil, although conventional treatment is still the most widely used method, the use of direct filtration systems has increased due to the lower implementation costs of these technologies. According to Di Bernardo et al. [10], in the beginning of the 21st century over 350 water treatment plants in Brazil used upflow direct filtration, with capacities of 5 to 2000 L/s, and the use of the direct filtration process has already exceeded conventional treatment in various states of the country. The 2008 national survey on water and sanitation [11] revealed that, daily, around 4 million cubic meters of water are treated using non-conventional techniques and over 13 million cubic meters of the water distributed to the population is only disinfected, mostly with chlorine. So, direct filtration may play an important role in producing safe water in Brazil.

Studies on the removal of *Cryptosporidium* oocysts by direct filtration have suggested that, with efficient coagulation and stable operation, high removal efficiencies (>3.0 log) can be obtained [12–18]. Most of the research on the removal of *Cryptosporidium* oocysts by direct filtration has assessed downflow direct filtration.

In this context, how upflow direct filtration systems behave with the occurrence of *Cryptosporidium* oocysts in raw water must be assessed. According the Brazilian Society of Technical Standards (ABNT) [19], if it is not possible to conduct pilot plant filtration experiments, upflow filters must be designed to operate with filtration rates up to 5 m/h, using deep stratified bed (≥ 2 m) with water flowing from coarse- to fine-sand grain. These design characteristics may positively influence on the removal of *Cryptosporidium* oocysts.

In this study, we evaluated the removal of *Cryptosporidium* oocysts using the upflow direct filtration process (coagulation + filtration), by performing experiments on a pilot scale. The evaluation considered the removal efficiency during the beginning of the filtration run (ripening) as well as during stable filtration. Non-parametric statistical techniques were used to verify correlation between the filtered water turbidity, particle counts and *Cryptosporidium* oocysts and their removal.

2. Materials and Methods

Pilot-scale investigations were performed with source water collected from Lake Paranoá (Brasilia-Federal District, Brazil). Paranoá lake water, in the point of withdrawal, is typically 20 to 27 °C, turbidity < 10 NTU, true color < 15 HU, alkalinity < 30 mg CaCO₃/L, pH from 6.0 to 7.3 and dissolved organic carbon < 1.5 mg/L—characteristics that are compatible with the upflow direct filtration [10]. Paranoá Lake water was seeded with inactivated *Cryptosporidium parvum* oocysts supplied by the School of Medicine at Triângulo Mineiro (Minas Gerais State, Brazil) to create the raw water (RW) to be used in filtration experiments. Concentration of *Cryptosporidium* oocysts in the seeded raw water ranged from 10^2 to 10^3 oocysts/L. The high initial concentration of *Cryptosporidium* oocysts in the raw water is a strategy used in various studies [12,13,20] to ensure detection in the filtered water and allow evaluation of the removal efficiency.

Before the filtration experiments, jar tests, adapted for direct filtration according to a routine described elsewhere [10], were carried out using alum as the coagulant. With Paranoá Lake water (turbidity = 2.7 to 4.8 NTU), alum doses from 0 to 14 mg of $Al_2(SO_4)_3/L$ were tested. However, when Paranoá Lake water was spiked with kaolinite to induce higher turbidity (approximately 30 NTU), the doses tested ranged from 12 to 22 mg $Al_2(SO_4)_3/L$. The coagulation pH ranged from 5.0 to 7.5. The data obtained in the jar tests were used to plot turbidity removal isovalue lines, with pH

coagulation on the X axis, coagulant dose on Y axis and turbidity removal (after filtration) on the Z axis. The coagulation conditions (pH and optimal dose) were selected based on higher removal regions in these graphics.

2.1. Pilot Plant Apparatus

The upflow direct filtration experiments were conducted in a pilot plant. The upflow pilot filter had a total height of 4.0 m with sand medium depth of 1.86 m over a 0.85 m support layer. The sand medium consisted of four sub-layers with the granulometric characteristics proposed by Sens et al. [21] (Figure 1).



Figure 1. Schematics of pilot-scale upflow direct filtration plant (not in scale).

The filter was operated at constant filtration rate of 5 m/h. Flow rate was measured volumetrically and pressure losses were monitored using piezometers. Twelve filtration experiments were performed: nine with low turbidity raw water and three with induced higher turbidity raw water. After each experiment, the filtration medium was washed with pumped tap water during 20 min at 30% medium expansion (upflow velocity around 85 m/h).

Considering the objective of the study, evaluation of the filter during ripening and stable operation, the filtration experiments were stopped after 6 to 7 h of filter operation. Thus, within the stable operation period.

2.2. Sampling and Analysis

During filtration experiments pH (pHmeter Orion 210, Thermo Fisher Scientific, Waltham, MA, USA), turbidity (HACH 2100 AN, Loveland, CO, USA), total coliforms and *Escherichia coli* (Colilert), as well as *Cryptosporidium* oocysts, were monitored in both raw and filtered water. Coagulated water samples were also analyzed to check the coagulation pH. During filter operation, pH, and turbidity were monitored every 15 min during the first hour and every 30 min in the following hours. Samples for coliform and *Cryptosporidium* oocysts analysis (using the USEPA method 1623 [22]) were collected at the beginning of filtration run (ripening period) and after around 6 h of operation (stable period). For quantification of oocysts, a 1 L composite sample of the water from the raw water reservoir was

collected during the experiment; the samples of filtered water were collected during ripening period and stable operation taking about 30 min, with a sample volume of 30 L. The first sample of filtered water was collected after the remaining wash water in the filter was displaced by the water being treated. The time necessary for displacement of the wash water, 23 min, was determined experimentally using of a tracer.

In eight experiments, particle size distribution, both in raw and filtered water, were monitored using an on-line particle counter (Hach, model 2200 PCX, Loveland, CO, USA). The particle counter was set to size particles into 21 bins, varying from 2 to 70 μ m.

2.3. Statistical Analysis

Data on particle counts, turbidity, and *Cryptosporidium* oocysts levels in raw and filtered water were recorded during the filtration experiments and treated to obtain their descriptive statistics, mainly the average and standard deviation. Non-parametric statistical techniques were applied to determine the existence of statistically significant correlations between the parameters and the removals in log units. Spearman's non-parametric correlation test was applied with variation between -1 and 1 using Version 9.0 of the Statistica StatSoft software. Non-parametric statistics represent a set of techniques most appropriate for use in studies where the distribution of a population and its parameters are not well known. Box-whisker plots of log removals were also produced.

3. Results and Discussion

During the period when the upflow direct filtration experiments were conducted, Paranoá Lake water turbidity, total coliforms and *E. coli* varied from 2.7 to 4.8 NTU, from 1.46×10^3 to 3.85×10^4 MPN/100 mL and from not detected to 1.83×10^3 MPN/100 mL, respectively. pH values ranged from 6.0 to 6.9. Paranoá Lake water has low turbidity values, even in the rainy season, and its turbidity is largely associated with the presence of algae.

3.1. Turbidity and Cryptosporidium Oocysts

Cryptosporidium oocysts data from upflow filtration experiments are shown in Figure 2. Table 1 summarizes the average of turbidity, turbidity removal and *Cryptosporidium* oocysts removal obtained during filtration experiments. As can be seen in Table 1, experiments E1 to E9 were conducted with low turbidity raw water (3.2 to 5.7 NTU) and experiments E10 to E12 were performed with higher turbidity (kaolinite spiked) water to evaluate filtration efficiency under turbidity level near the threshold recommended by Di Bernardo et al. [10] for the use of upflow direct filtration.



Figure 2. *Cryptosporidium* oocysts in raw and filtered water (RW—Raw Water; FWR—filtered water during ripening; FWS—filtered water during stable operation).

Experiment Number	Turbidity							Oocysts	
	Observations (N)	RW (NTU)	Residual-FW (NTU)		Removal (log)		- RW (ooc./L)	Kemoval (log)	
		Mean	Mean	SD	FWR	FWS		FWR	FWS
E1	15	4.8	0.27	0.14	1.20	1.24	565	2.98	3.70
E2	17	5.7	0.28	0.14	1.15	1.31	674	2.96	3.10
E3	17	5.4	0.32	0.06	1.06	1.22	292	2.62	>4.20
E4	17	4.4	0.31	0.11	1.25	1.15	902	2.60	3.80
E5	17	5.0	0.27	0.03	1.16	1.26	522	3.19	3.90
E6	17	3.5	0.25	0.04	1.05	1.14	76	2.68	>4.20
E7	16	5.4	0.21	0.03	1.15	1.40	1122	3.37	4.20
E8	14	4.4	0.18	0.06	1.10	1.42	752	(*)	3.00
E9	15	3.2	0.16	0.01	1.18	1.30	1864	(*)	2.80
E10	18	28	0.32	0.03	1.81	1.93	1796	3.18	3.80
E11	17	30	0.43	0.30	1.07	1.85	(**)	(**)	(**)
E12	17	30	0.22	0.04	2.09	2.13	1246	3.24	3.60

Table 1. Turbidity and *Cryptosporidium* oocysts values and log removals in the upflow direct filtration experiments.

RW—Raw Water; FW—filtered water; FWR—filtered water during ripening; FWS—filtered water during stable operation; SD—Standard Deviation; (*) no sample collected for *Cryptosporidium* oocysts detection; (**) filtration experiment without seeded *Cryptosporidium* oocysts.

Figure 2 shows that in the experiments with data of *Cryptosporidium* oocysts in both filtered water during ripening period and during stable operation (E1 to E7, E10, and E12), the quantification of oocysts in filtered water during stable operation (FWS) was slightly lower. Mann-Whitney's nonparametric test indicated that such differences were statistically significant at a 95% confidence (p value = 0.0009), indicating the filter's vulnerability in the early stages of filtration run. Filters vulnerability during ripening period was also observed by other researchers who evaluated the efficiency filtration on *Cryptosporidium* oocysts removal [13,23–25].

The Cryptosporidium oocysts removal data presented in Table 1 point out the difference in the performance of the filter during ripening period and during stable operation. Although the large range of removal in both ripening and stable operation, log removal under these operational conditions was statistically different at a 95% confidence level (p value < 0.0001, Mann-Whitney).

For experiments E1 to E7, carried out with low turbidity water, the average *Cryptosporidium* oocysts removal was 2.91 log during ripening and was >3.87 under stable operation. Therefore, the decrease in oocysts removal was near 1 log. This value is higher than those reported by Huck et al. [23] and Emelko [13], but it is in the range of 0.5 to 1 log reported by these authors based on other works [24,26].

According to O'Melia and Aly [27], "particles retained in a packed-bed filter during the initial stages of a run can act as collectors for other suspended particles applied to the bed at late time". Those collectors provide additional sites for further removal, and due to coagulation, they have surface charges that favor attachment forces, and higher retention during stable operation is expected. So, the higher passage of *Cryptosporidium* oocysts observed during ripening in this work is thought to be the result of the poor retention of the coagulated particles (including oocysts) on the surface of the cleaned grains in the early stage of operation, and was not influenced by the effect of the particles in wash water remnants due to the wash procedure carried out between the filtration experiments (20 min at 30% medium expansion) and the interval before collecting the first sample.

During stable operation, removal of *Cryptosporidium* oocysts was in the range of 2.80 to >4.20 log, which is consistent with USEPA credit of 2.5 log for direct filtration [28] as well as the values reported in various studies summarized by Emelko et al. [26], and others after that [15–18].

As can be seen in Table 1 and Figure 3, the removal of turbidity was lower during the ripening period than during stable operation, similar to *Cryptosporidium* oocysts removal. Despite this, no statistical correlation between removal of turbidity and removal of *Cryptosporidium* oocysts was observed, or

between residual turbidity and residual of *Cryptosporidium* oocysts. The lack of correlation is in agreement with other studies [20,24,25,29]. In this work (Figure 3), turbidity removal was consistently lower than *Cryptosporidium* oocysts removal in both ripening and stable operation of the upflow filter, suggesting that turbidity removal may be a conservative indicator for *Cryptosporidium* oocysts removal, as previously reported in the literature [30,31].



Figure 3. Comparative log removal of turbidity and of *Cryptosporidium* oocyst during ripening (FWR) and during stable operation (FWS).

Comparing data obtained in this study with previous results of the same group [32], that evaluated downflow direct filtration (bed depth—1.1 m; sand; effective size—1 mm; uniformity coefficient—1.15; filtration rate—8.75 m/h) and similar raw water (Paranoá Lake water seeded with *C. parvum* inactivated oocysts), the upflow direct filtration provided an average *Cryptosporidium* oocysts removal of more than 1.5 log higher. Both filters were operated at optimal coagulation conditions and similar flow rate.

The better result obtained with the upflow direct filtration was expected due to the differences in the design and operation of the filters. Considering the filtration rates (upflow, 5 m/h; downflow, 8.75 m/h) and the necessary surface filter area (cross section) for each pilot filter when treating the same flow rate, in m³/h, the deeper upflow filter provided more attachment sites (total surface area of grains more than twice of the downflow) which, together with the lower filtration rate, provided more retention of particles, including *Cryptosporidium* oocysts. The beneficial effect of a deeper bed (more attachment sites) was reported by Liu et al. [33] and agrees with filtration theory.

Another advantage of the upflow filter that became clear in the comparison with the downflow filter was the head loss development. Whereas the downflow filter head loss reached 1.8 m before 5 h of operation, the upflow filter head loss in the same period was always less than 0.3 m, regardless of the influent turbidity. Considering the filtration rates and respectively pilot filters surface areas, in 5 h of operation both filters, downflow and upflow, produced around 300 L of filtered water.

This comparison suggests that, considering the differences in design, upflow direct filtration may be a more robust option to downflow direct filtration for treating low turbidity water with *Cryptosporidium* oocysts.

3.2. Particle Counts and Cryptosporidium Oocysts

Due to the difficulty associated with the direct detection of *Cryptosporidium* oocysts, besides the turbidity measurements, particle counting is a parameter that has been investigated in several studies as surrogate measure for oocysts removal in water treatment systems [14,15,20,24,34,35]. Generally, previous research reported discrepancies regarding the existence of correlations between particle counts and *Cryptosporidium* oocysts removals.

Le Chevallier and Norton [34] combined data from four water supply systems, representing three watersheds with different turbidity levels (low, moderate, and high turbidity) of raw water, treated at conventional water treatment plants. The authors found a good correlation (r = 0.83, p < 0.05)

between *Cryptosporidium* oocysts removal and the number of particles larger than 5 μ m. Nieminski and Ongerth [20] suggested that the best correlation with *Cryptosporidium* oocysts occurs for particles with sizes between 4 and 7 μ m, a range also analyzed by Ribas et al. [35].

Therefore, in the current study the particle size distribution in raw water (RW) and filtered water (FW) were monitored in eight of the filtration experiments (E5–E12) using an online particle counter connected to both raw and filtered water lines of the pilot filter. Particle counts were monitored in the range of 2 to 70 μ m sizes. To compare the results of this study with those obtained in previous studies, for each experiment, particle sizes distribution data for the water samples were grouped in three ranges: 2–7, 8–15, and 20–70 μ m.

The results of average particle count monitoring of filtration experiments, both for low turbidity and higher turbidity of raw water, are shown in Figure 4. Table S1, presents the particle count monitoring and removal throughout the experiments E5 to E12.



■≥2 to 7 µm ■8 to 15 µm ■ 20 to 70 µm

Figure 4. Average particle count: (**a**,**c**) raw water and filtered water, respectively, in experiments with low turbidity raw water; (**b**,**d**) raw water and filtered water, respectively, in experiments with higher turbidity raw water. Error bars represent the standard deviation from average particle count.

In experiments conducted with low turbidity water (E5 to E9), total particle counts in influent varied from 1.45×10^3 /mL to 3.32×10^3 /mL; in experiments with induced higher turbidity raw water (E10 to E12) total particle counts were slightly higher (3.77×10^3 /mL– 3.99×10^3 /mL). Considering the quantification of *Cryptosporidium* oocysts (Table 1), the contribution of oocysts to particle counts in the range 2–7 µm was estimated to be between 0.02% and 0.10%. Thus, the oocysts concentrations likely did not influence the behavior of particle counts in this size range in the raw water.

The difference in the total particle counts in raw water was mainly due to the increase in the number of particles in the ranges 8–15 and 20–70 μ m. This behavior is associated with the particle size distribution of the kaolinite suspension added to the water, whose granulometric analysis revealed that nearly 70% of kaolinite particles were greater than 7 μ m [36].

Concerning ripening period, a reduction in the total particle counts (TPC) in filtered water was observed. For the experiments with low turbidity water, the particle counts decreased by one to two orders of magnitude relative to the TPC of raw water, corresponding to an average removal of 1.13 log

units. In the experiments with high-turbidity raw water, TPC reduction was smaller, 0.56 log units on average. In general, the higher particle removal was obtained for ranges 8–15 and 20–70 μ m sizes.

In the experiments using higher turbidity of raw water the particle removal in the size range of $2-7 \mu m$ was on average 0.9 log lower than in the experiments with low turbidity of the raw water. This behaviour may be due to the improvement of the kaolinite particles transport to the filter material surface by means of the interception and sedimentation mechanisms in the experiments conducted with higher turbidity raw water, as a function of the increase of the particle-to-collector size ratio, the particle sizes and its specific gravity. Other factor that may have favored the removal of kaolinite is associated to its structure, described by Gitis et al. [37] to be arranged in layers positively charged on their edges. Therefore, it is expected that, after coagulation at pH ~6.5, destabilized kaolinite particles preserve their positive charges. Under these conditions, the electrostatic kaolinite-sand interactions may have enhanced the adhesion to the clean and negatively charged filter material surface. Once deposited, kaolinite most likely covered the collector surface preventing other particles, such as those of 2 to 7 μm from Paranoá lake water, adhering to sand grains. The unfavorable interaction with kaolinite, may also have hindered the adhesion of these particles to kaolinite previously deposited onto collector surface.

Despite the reasonable removal, a significant number of particles in the range 2–7 μ m remained in the filter effluent throughout the filtering run, indicating the filter's vulnerability to this specific particle size range during the initial filtration period, including the filter ripening. Amirtharajah [38] highlighted that the combination of transport mechanisms through granular media filters yields a minimum net transport efficiency for particles around 1 μ m in size, as the efficiency of each transport mechanisms on particles removal is highly dependent of the particle size and is drastically reduced for particles that are approximately 1 μ m.

Figure 4 shows a gradual decrease in the particle counts until the second hour of filter run, due to the repulsing forces between influent particles and the negatively charged sand surface. This period was followed by an improvement in the quality of the effluent. At the end of the run, the average log TPC removal in the experiments with low turbidity raw water was slightly higher (1.2 log) than in the experiments with higher turbidity raw water (1.0 log), mainly due to the increase in number of particles of 2 to 7 μ m in the filtered water. When compared with the first five experiments (E5–E9), the average removal of particles with sizes 2 to 7 μ m for the last group of experiments (E10–E12) declined almost two-fold, suggesting that, similar to the initial filtering period, kaolinite particles removal may have improved as a result of the combination of the higher transport mechanisms efficiency and better kaolinite–sand interaction that preferentially attached to the sand surface in comparison with small particles formerly present in low turbidity raw water.

Figure 5 shows the removal of *Cryptosporidium* oocysts and particle counts for the three assessed size ranges. The values shown for both parameters correspond to the measurements recorded for the raw and filtered water at stable operation in each filtration experiment.



Figure 5. Removal of Cryptosporidium oocysts and particle counts in pilot-scale filtration experiments.

In all experiments shown in Figure 5 the oocysts removals were considerably higher than particle removals. In experiments E5 to E12, on average, oocysts removal (3.6 log) was at least 2.1 higher than particles log removals for the size ranges 2–7, 8–15, and 20–70 μ m (0.92, 1.42, and 1.49 log, respectively). It is also observed that the relationship between both parameters does not seem to be proportional. These observations are consistent with those reported by Edzwald et al. [39] and Huck et al. [24] in pilot conventional water treatment systems with dual-media filters operated under optimized coagulation conditions. According to Edzwald et al. [39], this behavior is most likely due to the differences among the analytical methods used to quantify both parameters, which are influenced by the small portion of oocysts in the raw water as well as the wider variety of particles characteristics such as size, shape and color. Based on the results of both studies, the authors have suggested that, under stable or optimal conditions, particle counts should be considered a conservative indicator of *Cryptosporidium* oocysts removal along with turbidity removal, as low levels of these parameters can be associated with good operational conditions for removal *Cryptosporidium* oocysts.

Remarkable differences were not observed among average oocysts removals obtained in the experiments conducted with low turbidity and higher turbidity raw water (Table 1). According to earlier studies, suspended clay particles can impact the transport and attachment of different biocolloids, including *Cryptosporidium* oocysts, in granular media. Gitis et al. [37] assessed the dynamics of *Cryptosporidium* oocysts and kaolinite particles $(0.78 \pm 0.32 \,\mu\text{m})$ adhesion to a quartz sand surface in experiments conducted in batch reactor, and verified that after coagulation with alum for complete destabilization of suspension, oocysts adhered more effectively onto sand grains in the presence of kaolinite in the filter influent than in its absence. As a result, *Cryptosporidium* oocysts increased from 1 log in the experiment performed with no addition of coagulant to 3.2 log in the experiment with the addition of 20 mg/L of alum. According to the authors *Cryptosporidium* oocysts adsorbed preferentially on kaolinite particles previously deposited onto the sand grains, as the edges positively charges of kaolinite most likely functioned as electrostatic adhesives of oocysts onto the sand surface. In the conditions tested in this study, despite the kaolinite addition to the raw water in experiments E10 to E12, the kaolinite concentration might not have been high enough to influence the oocysts–sand grains interactions.

The differences in the oocyst removals observed in both studies may also be associated to the differences in the operational mode used each the experimental system. While this study was carried out on a upflow filter operating in continuous mode, the study of Gitis et al. [37] was conducted on reactors operating in batch mode. Under batch condition the hydrodynamic forces, responsible for the dynamic equilibrium between the attachment and detachment processes were suppressed, likely leading to the higher oocysts removal.

Except in experiment E9, in the remaining experiments using raw water with low turbidity (E5 to E8), the particles removal for the three evaluated sizes ranges were very close. In contrast, in experiments E10 to E12, conducted with higher turbidity raw water, particles removals in the ranges 8-15 and $20-70 \mu m$ improved, whereas the particles removal in the range $2-7 \mu m$ decreased compared to experiments with low turbidity raw water. As previously mentioned, the structural features of kaolinite may have positively influenced the kaolinite–sand interaction producing a better retention of kaolinite than small–sized particles originally present in the raw water (from Paranoá Lake).

3.3. Correlations Analysis between Cryptosporidium Oocysts and Particle Counts

Spearman's non-parametric correlation tests were applied to assess possible correlations between *Cryptosporidium* oocysts and particle counts in filtered water, as well as the correlations between removals (log) of both parameters for ripening and stable operation periods. For the correlation analysis, all the filtered water data obtained from experiments E5 to E12, in which *Cryptosporidium* oocysts were seeded in the filter influent and the particle counts were monitored throughout the filtration run, were considered (except experiment E11).

The particle size ranges included in the correlation tests were 2–7, 4–6, 8–15, and 20–70 μ m, which were selected based on studies performed by Nieminski and Ongerth [20], Morse et al. [40], and Yu et al. [15].

Regarding the data obtained throughout the filtration experiments, Spearman's non-parametric tests did not show significant correlations between the levels of the parameters or between their removals (data not shown). However, when the data from ripening and stable operation periods were analysed independently, barely for ripening period the Spearman's tests showed moderately strong correlation (r = 0.70), significant at 90% level, between *Cryptosporidium* oocyst levels and particle counts for ranges 2–7 and 4–6 µm, as illustrated in Table 2.

Oocvsts	Particle Size Range (µm)									
	2–7	4–6	8–15	20–70						
Correlation Coefficient	0.70 *	0.70 *	0.18	0.46						
Log Removal										
Oocysts	Particle Size Range (µm)									
	2–7	4–6	8–15	20–70						
Correlation Coefficient	-0.1	0.30	0.30	0.00						

Table 2. Spearman's correlation coefficients between *Cryptosporidium* oocysts, particle counts, and their removals (log) at the ripening period.

* Significant at 90% confidence.

Cryptosporidium oocysts are reported to have sizes varying from 3 to 7 μ m [24,41]. This size range of *Cryptosporidium* oocysts is considered in the Method 1623 established by the USEPA [22], aimed to recover, identify, and count *Cryptosporidium* oocysts and *Giardia* cysts. Particle counts that showed a statistically significant strong correlation with *Cryptosporidium* oocysts levels in filtered effluent at the ripening period comprise *Cryptosporidium* oocysts-sized particles (Table 2). Considering source water used as raw water in this study was low to moderate in turbidity and particles, these findings suggest a possible application of particle counts monitoring in the ranges 2–7 and 4–6 μ m in the effluent of upflow filters as a complement of monitoring turbidity levels during the ripening of upflow filters, considered as critical due to the higher probability of *Cryptosporidium* oocysts passage.

The results of correlations tests observed herein approach to those of Yu et al. [15] that found strong correlations (Pearson) among the levels of *Cryptosporidium* oocysts and particle counts (r = 0.732 and r = 0.691, respectively) for sizes ranges 4–7 µm and 7–14 µm in the effluent from pilot single medium and dual-media rapid downflow filters treating waters with low turbidity and particle levels. However, differently to the current study, such correlations were observed throughout the operation filters run.

Despite the strong correlation observed between both parameters, similarly to other studies already mentioned, the authors have proposed that monitoring the particle counts should not be used as an enough indicator of *Cryptosporidium* levels or its removal.

As mentioned previously, Huck et al. [24] also evaluated the relationship between removals of *Cryptosporidium* oocyst and particle counts (2–150 μ m) in three pilot scale conventional water treatment systems with double layer filters, treating raw water with low turbidity and particle counts levels (average of 5000 particles/mL). The study included the influence of the filter stages (ripening, stable or optimal operation and breakthrough) besides the operational effects such as suboptimal coagulation on the relationship between both parameters.

When statistical correlation test was applied to the data obtained in all coagulation conditions from two pilot-plants, the correlations coefficients varied from moderate to strong, suggesting that the sensitivity of particle counts may be site specific.

Similar to the current study, no proportional relationship among removals of both parameters were found when the three pilot systems were operated at stable or optimal operation conditions, indicating that particle counts should not be considered as a quantitative indicator of *Cryptosporidium* removal capability when filters are operating well under stable operating conditions.

During the ripening period, although no statistical correlation tests were applied, the reduction in particles removal in relation to oocyst removal was higher than in the stable operation was observed. Sub-optimal coagulation conditions also resulted in a similar considerable average reduction both in oocyst and particle removals. Regarding the breakthrough, despite the filtration run in two pilot systems were stopped before reaching the late breakthrough, deterioration in *Cryptosporidium* oocysts removal was verified. Although the decrease was more significant in one of the systems, about of 3.5 log, these evidences indicate that, as in ripening, the breakthrough deserves special attention in relation to light increases in particle counts. Based on these evidences, Huck et al. [24] proposed particle counts as a useful tool for monitoring possible deterioration of filter capability for *Cryptosporidium* oocysts removal.

Considering a source water with low to moderate turbidity and particle counts as the raw water used in this study, the results of correlations tests corroborate with the literature, that has not consistently recommended the use of particle counts as a quantitative indicator for *Cryptosporidium* oocysts removal in water treatment systems operating well at stable or optimal operational conditions. However, it is possible using particle counts in the ranges 2–7 and 4–6 µm for monitoring the filtered water produced by the upflow direct filter during the ripening period, as particle counts in these size ranges seems to be sensitive to variations in the concentration of *Cryptosporidium* oocysts in filtered water during this vulnerable period to the *Cryptosporidium* oocyst passage. Based on this evidence, further studies need to be performed under other operational conditions different to those evaluated herein, such as filtration rate, composition of the filter bed and raw water quality, to confirm the relationship between particle counts and *Cryptosporidium* oocyst levels, not only considering the ripening and stable periods but also the breakthrough which is crucial for defining the end of filter run.

4. Summary

In Brazil, millions of people still receive water with only disinfection or no treatment. In this scenario, direct filtration systems, due to the lower implementation costs, play an important role in producing safe water.

Upflow direct filtration, consisting of coagulation and filtration, without the need of flocculation, became a popular option to treat water with low to moderate turbidity. In Brazilian design practice, filters used in upflow direct filtration systems are deeper, stratified, and operate with lower filtration rates compared with the ones used in downflow direct filtration.

This article assesses the removal of *Cryptosporidium* oocysts treating a water with low and low-moderate turbidity. The study, conducted in pilot scale, evaluated two periods within a filter cycle, ripening and stable operation, as well as correlations between filtered water values and removal of oocysts, turbidity and particle counts.

Under stable operation and optimal coagulation conditions, the average removal of *Cryptosporidium* oocysts was >3.87 log, ranging from 2.80 to >4.20 log. During the ripening period, removal of *Cryptosporidium* oocysts was around 1 log lower than during stable operation and this difference was statistically significant.

Removal of turbidity and particles (including those in the range of 2 to 7 μ m) were lower than the removal of *Cryptosporidium* oocysts, suggesting that the removal of turbidity and particles may be a conservative indicator of oocysts removal.

For the specific raw water (Paranoá Lake seeded with inactivated *Cryptosporidium* oocysts), and treating similar flow rate, a comparison of data obtained in this study with previous results of downflow direct filtration suggested that, considering the differences in design, upflow direct filtration

may be a more robust option regarding *Cryptosporidium* oocysts removal and head loss development. Further evaluation, in a broad range of conditions, is necessary to confirm these preliminary findings.

No correlation between the removal of *Cryptosporidium* oocysts, turbidity removal, and particle counts removal was observed, regardless of the filtration run stage. However, during ripening, a moderately strong correlation between residual values of *Cryptosporidium* oocysts and particles in the range of 2 to 7 μ m was obtained, suggesting the need for further evaluation of this behavior, particularly in the vulnerable periods within filter cycle—ripening and breakthrough.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/5/1328/s1, Table S1. Particle counts monitoring throughout the experiments E5 to E12.

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