Detection of *Cryptosporidium parvum* oocysts in artificially contaminated filter backwash water and ozone treatment on a pilot scale

Allan Pretti Ogura • Lyda Patricia Sabogal-Paz

A.P. Ogura

Department of Hydraulics and Sanitation, São Carlos School of Engineering, University of São Paulo, Avenida Trabalhador São-Carlense 400. Zip code: 13566-590. São Carlos, São Paulo. Brazil

e-mail: allan.ogura@usp.br

ORCiD: 0000-0001-8549-5820

L.P. Sabogal-Paz (corresponding author)

Department of Hydraulics and Sanitation, São Carlos School of Engineering, University of São Paulo. Avenida Trabalhador São-carlense 400. Zip code: 13566-590. São Carlos, São Paulo. Brazil. Phone: +55 16 3373 9548. Fax: +55 16 3373 9550.

e-mail: lysaboga@sc.usp.br

ORCiD: 0000-0003-2753-3248

Abstract

Waterborne diseases are a relevant concern for public health systems since commonly applied treatment techniques may not remove all water contaminants. *Cryptosporidium* spp. oocysts are an issue in water treatment plants due to their reduced size and resistance to the disinfection process (e.g., chlorination). Moreover, oocysts retained on the filter media might recirculate amongst the filter backwash water (FBW). This study aimed to detect *Cryptosporidium parvum* oocysts artificially inoculated on the FBW and evaluate the ozone treatment performance. A synthetic FBW underwent three concentration methods followed by immunomagnetic separation: calcium carbonate flocculation, direct centrifugation, and direct centrifugation with the 7X ICN dispersion solution. The latter method was selected as it presented less interference on oocyst viability (37.2 % reduction) and higher recovery (22.1 %) on preliminary assays. The recovery for the commercial suspensions was 15.4 ± 3.3 %, although the analytical quality performed with EasySeed® suspension obtained a recovery of 2.8 ± 0.8%. These limitations and methodologies for protozoan detection are challenging due to low recoveries, especially in complex matrices. Finally, FBW ozonation was performed on a pilot scale, and the propidium iodide dye indicated oocyst viability decreased after treatment. Oocyst inactivation was 2.83 log and 3.44 log for dosages of 7.5 mg O_3 L^{-1} for 10 min (i.e., 75 mg min L^{-1}) and 10 mg O_3 L^{-1} for 5 min (i.e., 50 mg min L^{-1}), respectively. Disinfection is a crucial pathway for addressing outbreak scenarios, and ozone treatment should be further studied.

Keywords

Disinfection; immunomagnectic separation; protozoa; propidium iodide; waterborne disease

Introduction

Waterborne diseases are relevant for adequate water resources management, although epidemiological data may be scarce in developing countries (Efstratiou, Ongerth, and Karanis 2017a; Neto et al. 2010). The main challenges are analysis expenses, methods complexity, and inefficient water treatment and monitoring (Efstratiou, Ongerth, and Karanis 2017a; Ramírez-Castillo et al. 2015), which could jeopardize public health in outbreak scenarios (Li and Wu 2019). From 2011 to 2016, *Cryptosporidium* spp. caused 63% of worldwide protozoan parasite outbreaks, while *Giardia* spp. corresponded to 37% of them (Efstratiou, Ongerth, and Karanis 2017a). Oocysts are infectious forms that cause cryptosporidiosis, commonly associated with diarrhea and dehydration (Current and Navin 1986; D. B. Huang and White 2006). The waterborne *Cryptosporidium* spp. transmission route is the fecal-oral pathway, infecting humans by ingesting contaminated water from only 1 to 10 oocysts (Dillingham, Lima, and Guerrant 2002). Besides, *Cryptosporidium* spp. oocysts have higher environmental resistance than *Giardia* spp. cysts (Fayer, Morgan, and Upton 2000; Keegan et al. 2008; Olson et al. 1999).

Flocculation, filtration, and direct centrifugation (DC) are standard concentration methods to detect oocysts in water samples (Efstratiou, Ongerth, and Karanis 2017b). Considering the United States Environmental Protection Agency (USEPA) guidelines, these methods are usually followed by the immunomagnetic separation (IMS) purification step (USEPA 2012). However, there are currently no standard protocols for complex matrices, including water treatment residues (Sammarro Silva and Sabogal-Paz 2020; Ogura and Sabogal-Paz 2021). The calcium carbonate flocculation (FCC) method is recommended for high turbidity water (Andreoli and Sabogal-Paz 2017; Feng et al. 2011; Giglio and Sabogal-Paz 2018; Vesey et al. 1993), although pH variations may interfere with oocyst viability (Franco et al. 2012). The DC method concentrates the sample volume in a pellet using a high-speed centrifuge. After

the DC, Boni de Oliveira (2012) applied the detergent dispersion solution ICN 7X at 1.0% and obtained cleaner reading wells even for soil samples, achieving 37.6% recovery for 500 inoculated oocysts.

Water treatment is crucial for public health as it aims to eliminate waterborne pathogenic microorganisms, ensuring safe drinking water (Li and Wu 2019). In water treatment plants (WTP), the settling and filtration stages remove most oocysts, although some can pass through the filter medium due to their small size (from 4.5 to 5.0 µm) and compressibility (Betancourt and Rose 2004; Franco 2007). Besides, the filter backwash water (FBW) recirculation could reinsert oocysts in the treatment process (Betancourt and Rose 2004; Karanis, Schoenen, and Seitz 1996), reinforcing the need for proper disinfection. Furthermore, disinfection is also required for FBW from swimming pools since *Cryptosporidium* spp. oocysts have been reported in these matrices (Greinert et al. 2004; Murphy et al. 2018).

Although chlorine is the most widely used disinfectant (Collivignarelli et al. 2017), it is not as effective against oocysts as ozone (Korich et al. 1990) and ultraviolet (UV) irradiation (Adeyemo et al. 2019; Nasser 2016). Moreover, chlorination undesired disinfection by-products (e.g., trihalomethanes) concern WTP management, especially in water with natural organic matter (Sorlini et al. 2015; Kim et al. 2003). In general, *Cryptosporidium* spp. oocysts are more resistant to common disinfection processes than *Giardia* spp. cysts (Korich et al. 1990). For instance, ozone provided higher *Cryptosporidium parvum* inactivation rates than chlorine and dioxide chlorine in the Brazilian public water supply (Pereira et al. 2008). Also, oocyst inactivation might be impaired in high turbidity water (Ran et al. 2010). Korich et al. (1990) obtained more than 90% oocyst inactivation by treating water with 1 mg O_3 L^{-1} for 5 min; *C. parvum* oocysts were 30 times more resistant than *Giardia* spp. cysts. Ran et al. (2010) observed higher inactivation efficiencies with a temperature increase from 5 to 25ºC,

and longer contact time caused damage to the oocysts' cellular structure. In this scenario, the cell wall disruption facilitates the incorporation of viability indicator dyes (e.g., propidium iodide – PI) (Campbell, Robertson, and Smith 1992).

To the best of our knowledge, there is a gap concerning oocyst detection in FBW from high turbidity water and evaluating the ozone treatment. Previous studies showed the relevance of investigating protozoa for each evaluated matrix as variabilities provide different results and limitations (Franco et al. 2012; Sammarro Silva and Sabogal-Paz 2020). Although Method 1623.1 (USEPA 2012) establishes the protocol for oocyst detection in drinking water, the recoveries for WTP residues might not meet this standard (Ogura and Sabogal-Paz 2021). In addition, considering ozone potential to inactivate oocysts (Ran et al. 2010), this disinfection technique could address the risks of recirculating FBW. In this scenario, this research aimed to detect *C. parvum* oocysts artificially inoculated into the FBW and to evaluate the ozone's treatment performance. In this regard, an artificial FBW sample was generated in jar testing, and the performances of three oocyst concentration methods were compared: FCC, DC, and DC + ICN 7X, followed by IMS. Then, the FBW passed through ozonation on a pilot scale, and the PI dye indicated viability to estimate disinfection efficiency.

Methods

This study consisted of three stages: synthetic FBW production using jar test assays; oocyst's concentration and detection methods comparison; and FBW treatment with ozone.

Filter backwash water production

First, high turbidity (110 NTU) artificial water was prepared with 0.16 g L^{-1} kaolinite (*Sigma-Aldrich®/Fluka* 60609) in non-contaminated groundwater. This procedure was chosen to minimize interference from freshwater samples (e.g., pH changes, metals, organic matter, and

other microorganisms), as stated by previous studies (Ogura and Sabogal-Paz 2021; Giglio and Sabogal-Paz 2018). The jar test treatability assays (Figure 1) were performed with 25 mg L^{-1} of PACl (specific weight of 1.362 g L^{-1} and 16.68% content of Al_2O_3). Coagulant dosage, coagulation pH, mixing conditions, and decantation time were optimized from Giglio and Sabogal-Paz (2018), considering turbidity and color removal on filtered water. The 2 L jar tests operated automatically (fast mixing of 1000 s^{-1} for 10 s and slow mixing of 25 s^{-1} for 30 min), and the decantation rate was 1.5 cm min^{-1}. Further treatment details, including drinking-water treatment sludge generation, were presented in Ogura and Sabogal-Paz (2021). After decantation, the clarified water was removed until the 80 mL mark. This water was redirected to the attached laboratory filters (ALF), composed of previously washed and dried sand (grain size between 0.30 and 0.59 mm, with an effective size of 0.42 mm). The ALF consisted of an acrylic tube with 19 mm in internal diameter and 40 cm high on a metallic support. One ALF was installed for each jar test, totaling six units. A silicone tube connected the ALF with a register, used to control filtered water flow (100 m^3 m^{-2} day^{-1} filtration rate). After the filtration step, each sand filter media was poured into a beaker and washed with 300 mL of distilled water. The supernatant water from this procedure was considered the filter backwash water (FBW), whose samples consisted of multiple jar test assays. Physical, chemical, and microbiological parameters were analyzed following APHA (2012) procedures.

Concentration and detection methods

The *C. parvum* oocysts commercial suspension was obtained from Waterborne® (New Orleans, USA). After homogenization, 5 µL of suspension was applied to three reading wells, which dried overnight. The procedures for microscope slide preparation followed the

recommendations from the Merifluor® kit (Meridian Bioscience, Inc.) and DAPI solution (Sigma-Aldrich®, F6057). The PI (Sigma-Aldrich®, P4170) solution was used for estimating viability since non-stained oocysts were considered potentially viable.

Three methods were evaluated for concentrating oocysts from 1.0 L FBW into a 5.0 mL sample: FCC, DC, and DC + ICN 7X. Each method was followed by IMS (USEPA, 2012), considering two acid dissociations and the instructions from the Dynabeads® kit. The volume of oocyst suspension (20 μL) was added directly into 1.0 L of FBW samples to minimize losses throughout the treatment processes. The estimated initial concentration was ± 2000 oocysts L^{-1}, and the sample was homogenized with a magnetic stirrer for 20 min.

The FCC protocol was adapted from Giglio and Sabogal-Paz (2018). In the FBW, 10 mL of calcium chloride ($CaCl_2$, 1M) and 10 mL of sodium bicarbonate ($NaHCO_3$, 1 M) were added. After 10 min of agitation, 1.5 mL of sodium hydroxide (NaOH, 5 M) was added to increase the pH to 10. After 10 min mixing, the beaker covered with a watch glass rested overnight. The next day, the supernatant was removed, and 20 mL of 10% sulfamic acid (H_3NSO_3) was added into the remaining 100 mL, while the sample stirred for 10 min. The sample was transferred to three 50 mL graduated centrifuge tubes. The beaker was washed with 30 mL Tween 80 (0.1%), and this content was distributed to the tubes. After centrifugation (20 min at 1500xg), the supernatant was discarded from each tube. The remaining 1.0 mL pellets were transferred to a single tube, and the pH was corrected to 7.0 using 4.2 mL of PBS (phosphate-buffered saline solution – from *Sigma-Aldrich*®). The tube was centrifuged (10 min at 1500xg), then the supernatant was removed up to the 5 mL mark.

The DC method divided the sample into 20 graduated 50 mL Falcon® tubes, centrifugated at 1500xg for 20min. The remaining 0.5 mL pellet was transferred to a single tube with the content of three washes (0.5 mL of Tween 80, 0.1%). Another centrifugation was carried out,

and the final pellet was 5 mL. The DC + ICN7X method followed the previous procedures for DC, and it was adapted from Boni de Oliveira (2012). The 5 mL pellet was transferred to a flat-bed tube (FBT) with 3.0 mL of 1.0% MP BIO® ICN detergent dispersion solution 7X. The FBT was homogenized in a rotatory mixer (20 rpm for 1.0 h). Then, its content was transferred to a Falcon® tube, which was centrifugated (1500xg for 20min). In the end, the supernatant was removed, and a 5 mL pellet remained.

As the chosen method for this research, the preliminary analytical quality assay (n = 3) evaluated the protocol for CD + ICN 7X followed by IMS with the *C. parvum* commercial suspension, with an initial concentration of ± 2000 oocysts L^{-1}. Besides, the EasySeed® (BTF Bio – Australia) suspension (99 ± 1.6 oocysts of *C. parvum*) validated the CD + ICN 7X and IMS method. The results were compared to the USEPA Method 1623.1 for water as guidance (USEPA 2012).

Ozone treatment

Figure 2 shows the pilot-scale ozonation unit. The acrylic column was 2.2 m long and 4 mm thick with a total capacity of 3.6 L and supported by rubberized clamps. At the top of the column, the 3.0 L contaminated FBW sample entered through a plastic funnel. The upper stainless-steel ball register prevented the gas loss, while a silicone hose directed the off-gas to the flask with 400 mL of 2% potassium iodide (KI) solution. At the bottom, a porous stone dispersed ozone bubbles formed by the generator. The treated sample was collected through a register.

The two studied conditions for ozone treatment were: a dosage of 7.5 mg O_3 L^{-1} for 10 min and 10 mg O_3 L^{-1} for 5 min. For each replicate (n = 3), the ozone disinfection treated FBW with an initial concentration of 5 000 oocysts L^{-1}. Approximately 20 s after turning off the

ozone generator, the gas passed through the entire column, and the silicone hose was disconnected. The treated sample (50 mL) was collected and evaluated on the DR 2800 spectrophotometer with the AccuVac® glass ampoule, according to the Indigo HACK® Method 8311 (APHA 2012). The iodometric method calibrated the ozone generator by the gas collection flask. Finally, the oocyst inactivation was estimated by Equation 1 (where I: Inactivation log of oocysts; N: average number of non-stained oocysts at the end of the treatment process; No: average number of non-stained oocysts inoculated in the sample). The log reduction expressed the relative number of inactivated oocysts, whereas every log unit corresponded to a 10-reduction factor. To evaluate statistical differences of the treatments, the Shapiro-Wilk normality test was performed, and the data were submitted to the analysis of variance (ANOVA) and Student's t-test, with 95% confidence (p-value < 0.05).

$$I = -\log(\frac{N}{No}) \qquad \text{(Equation 1)}$$

Results and discussion

Water treatment and filter backwash water production

Groundwater had total alkalinity of 15.3 mg $CaCO_3$ L^{-1}, conductivity of 33.2 μS cm^{-1}, turbidity of 0.2 NTU and apparent color of 0.0 HU, pH 6.78, and 24.3ºC. The concentration of 0.16 g L^{-1} of kaolinite achieved an average turbidity of 112 NTU, 114 HU apparent color, 3.3 HU true color, 8.8 mg $CaCO_3$ L^{-1}, total alkalinity, and 53.2 μS cm^{-1} conductivity. Table 1 presents the characterization of the study water, clarified water, filtered water and FBW. The turbidity in filtered water (0.32 NTU) characterized an estimated removal efficiency higher than 99%. However, 1 CFU of total coliforms was found in the filtered water, which not complied with Brazilian Potability Standards (Brazil 2011). The FBW turbidity (14.5 NTU) might influence concentration methods as solid particles may adhere to oocysts (Dai et al.

2004; Franco et al. 2012). Metal concentrations in the FBW (0.55 mg Al L^{-1} and 0.374 mg Fe L^{-1}) are higher than those from study water (0.32 mg Al L^{-1} and 0.232 mg Fe L^{-1}), probably due to coagulant residues, which could interfere with IMS procedures (USEPA 2012).

Concentration and detection methods

Regarding preliminary assays for comparing concentration methods, the recoveries were 17.6%, 15.6%, and 22.1% for the CCF, DC, and DC + ICN 7X, respectively. The CCF presented an inferior recovery on the first (5.8%) dissociation compared to the second (11.8 %), while the DC + ICN 7X had a recovery of 13.9% and 8.1% on the first and second dissociations, respectively. However, these efficiency rates did not meet the standard from Method 1623.1 (USEPA 2012), which requires values higher than 32% for water. The viability reduction was 60.1%, 72.2% and 37.2% for CCF, DC and DC + ICN 7X methods, respectively. These results indicated that the DC + ICN 7X method had the highest recovery rate and the lowest viability interference. By raising the pH to 10, the CCF method might cause damage to oocyst cell walls, making them more susceptible to PI dye incorporation (Giglio and Sabogal-Paz 2018). In addition, Campbell, Robertson, and Smith (1992) indicated that CCF reduces the viability of *C. parvum* oocysts up to 30% through pH changes. Therefore, the DC + ICN 7X protocol was selected for oocysts in the BFW samples.

The results for the analytical quality assays were shown in Table 2. The preliminary analytic control of the DC + ICN 7X method considered the average number of (oo) cysts in the suspensions, and the number of protozoa inoculated in the FBW was 2085 oocysts L^{-1}. Oocyst recoveries were higher for the second acid dissociations (13.9%). The average recovery for oocysts was 13.5 ± 7.6%, while the average viability of oocysts was 23.6 ± 2.5%. In this case, after applying the DC + ICN 7X method, the percentage of non-stained oocysts with PI was higher than the initial value. It might indicate that non-stained oocysts

are more easily attached to the magnetic beads from IMS, compared to stained oocysts. The Analytical Quality Control with EasySeed® was performed with 99.0 ± 1.6 oocysts L^{-1}. The highest average recoveries were obtained for the first acid dissociations for *C. parvum* oocysts (1.8 ± 1.5%) compared to the recovery of the second dissociation for oocysts (1.5 ± 1.7%). Considering both acid dissociations, recoveries were 3.3 ± 2.0% for oocysts. For *C. parvum*, these results were less than those required, which is 32% according to the USEPA Method 1623.1 (USEPA 2012).

Other studies have tested detection protocols for FBW with oocysts. The recovery results of this research were similar to the results of Silva and Sabogal-Paz (2020) for DC + ICN 7X in FBW produced in treatability assays with flotation. These authors achieved 19.9 ± 16.3% recovery of *C. parvum* oocysts using commercial suspensions and 2.3 ± 1.4 % using EasySeed®. The drinking-water treatment sludge from Ogura and Sabogal-Paz (2021) presented an average recovery of 13.5 ± 26.4 % *Cryptosporidium* spp. oocysts using commercial suspensions, and 3.3 ± 59.7 with EasySeed®. This study highlighted the aluminium and iron concentrations (14960 mg L^{-1} and 1128 mg L^{-1}, respectively) as interferences to oocyst adhesion to IMS's magnetic microspheres.

Regarding other concentration methods, Sammarro Silva and Sabogal-Paz (2020) performed membrane filtration and IMS for recovering *Cryptosporidium* spp. oocysts from FBW (6.7 NTU) and obtained similar results of 2.0 ± 1.9 % efficiency with EasySeed®. Nonetheless, Ladeia et al. (2018) did not find *Cryptosporidium* spp. on spent FBW from WTP by the FCC method, which could be due to lower recoveries or losses throughout treatment processes (Maciel and Sabogal-Paz 2016). Di Giovanni et al. (1999) detected *C. parvum* oocysts from 5.8% of the analyzed FBW samples, and the recovery was 9.1% for the IMS protocol compared to 5.8% for the Percoll-sucrose flotation method. Greinert et al. (2004) combined

CCF and DC and had 2.7 ± 1.2 % efficiency on *Cryptosporidium* spp. oocyst recovery, similar to this study even though they used swimming pools BFW.

Ozone treatment

The ozone mass balance is presented in Table 3. For 7.5 mg O_3 L^{-1}, the average percentage of ozone transferred was 80.2%, and the percentage was 78.5% for 10.0 mg O_3 L^{-1}. The ozone mass transfer can be an issue regarding disinfection efficiency and costs (Graça et al. 2020). The first suspension (36.8 ± 15.4%) showed more than twice the percentage of non-stained parasites in the second (14.8 ± 8.8%), which could influence disinfection results. The first ozone treatment (7.5 mg O_3 L^{-1} at 10 min) corresponded to a concentration-time (CT) of 75 mg min L^{-1}. This assay used 535 µL of *C. parvum* suspension in 3.0 L of FBW, and the estimated initial number of non-stained oocysts was 5489. At the end of the process, 8 non-stained oocysts were found, which corresponded to 2.83 log inactivation. The second treatment (10.0 mg O_3 L^{-1} at 5 min) consisted of 50 mg min L^{-1} CT. The expected quantity of non-stained oocysts was 5489 in 625 µL of *C. parvum* suspension. After the ozonation stage, only 1 non-stained oocyst was found, corresponding to 3.44 log inactivation. The results are presented in Table 3.

The microscopy images after FBW ozonation for both treatments were presented in Figures 3 and 4. In the DIC images, oocysts were still attached to microspheres, which indicated a limitation on the dissociation stage. The application of PI dye could be a limitation to this study since it can overestimate treatment efficiency by staining still infectious oocysts on dry slides (Petersen and Enemark 2018). However, this procedure is more feasible than the animal infectivity (Koehler et al. 2014), and presented a correlation with excystation methods (Campbell, Robertson, and Smith 1992).

Based on the estimated oocyst suspensions' initial viability (from 14.8 to 36.8 %), the CT value interfered with oocyst disinfection, and the dosage provided more significant interference compared to time ($p < 0.05$). The second treatment had better conditions due to greater oocyst inactivation (3.44 log) and lower CT values (50 mg min L^{-1}), reflecting lower operational costs. However, the low recovery of the detection protocol (DC + ICN 7X) might interfere with the estimated inactivation since few non-stained microorganisms were a limitation in this study. Korich et al. (1990) obtained inactivation of 2 logs of *C. parvum* with tenfold smaller CT (5 mg min L^{-1}). Considering 10^3 oocysts to induce mouse contamination, Peeters et al. (1989) obtained no infectivity of 1 x 10^7 oocysts L^{-1} with 6.66 mg min L^{-1}. After treating FBW from flotation treatment with CT 50 and 75 mg min L^{-1}, Silva and Sabogal-Paz (2020) did not find any oocysts after ozonation, and they associated this absence with total removal or disintegration.

On the other hand, some of the ozone treatment limitations should be considered in designing WTP disinfection units, including its high costs (Dore et al. 2013), mass transfer efficiency (Graça et al. 2020), and ecotoxicity to aquatic organisms (Coler and Asbury 1980; Jaylet, Gauthier, and Lévi 1991; Leynen et al. 1998). Ozone demand, decay rates in water, and CT analysis are solid aspects for evaluating feasibility (Schulz et al. 2005). Besides, drinking water ozonation generates disinfection byproducts that should be monitored, including assimilable organic carbon, aldehydes, carboxylic acids, ketones, and bromate (W. J. Huang, Fang, and Wang 2005; Richardson et al. 1999; Wert et al. 2007).

In 2020, the SARS-CoV-2 outbreak reinforced the concern about water and wastewater disinfection (Arslan, Xu, and Gamal El-Din 2020; Bhatt, Arora, and Prajapati 2020), especially considering its environmental contamination and potential waterborne transmission (Carraturo et al. 2020; La Rosa et al. 2020). In this scenario, ozone was presented as an alternative for dealing with SARS-CoV-2 spread due to its strong oxidation properties

(Tizaoui 2020; Martins et al. 2021; Morrison et al. 2021). Furthermore, since fungi and protozoa showed more resistance to ozonation than viruses and bacteria (Wen et al. 2020), optimizing oocyst disinfection in aquatic matrices might be a way to address virus outbreaks.

Therefore, studying and developing protozoan disinfection techniques could contribute to water resources management, especially considering negligible matrices (e.g., WTP residues). High turbidity matrices are challenging in terms of detection and disinfection (Giglio and Sabogal-Paz 2018; Ogura and Sabogal-Paz 2021). Considering FBW, proper disinfection techniques should be sought to overcome the risks inherent to recirculation. Ozone treatment showed potential as a strong oxidant for disinfection purposes, although its limitations should be studied and discussed for other matrices, including natural samples. In this context, pilot-scale experiments could address some limitations in laboratory-controlled conditions. Moreover, *in situ* studies on actual WTP could investigate the ozone treatment performance on a large scale and provide operational and engineering aspects on this topic.

Conclusions

Disinfection of aquatic matrices is fundamental for dealing with outbreak scenarios of pathogenic microorganisms. The three studied concentration methods on the preliminary assays presented low recovery rates (up to 22.1%). Furthermore, the CD + ICN 7X + IMS method had 15.4 ± 3.3 % and 2.8 ± 0.8 % with the commercial and EasySeed® suspensions, respectively, which did not comply with the standards recommended by the USEPA Method 1623.1 (≤ 32%). Besides, *C. parvum* suspension's low initial viability (up to 36.8 %) was a limitation to this study. Even higher ozonation CT (75 mg min L^{-1}) did not inactivate all oocysts in the FBW, reinforcing this protozoan resistance. Despite this, the PI viability assessment could overestimate disinfection efficiency as the oocyst could be stained but

remain infectious. Future research should investigate alternatives for protozoan purification as IMS procedures are expensive for monitoring water resources, especially in developing countries. Finally, this methodology should be applied for natural water samples to evaluate if the variabilities (e.g., pH changes and organic matter) interfere with the oocysts' recovery and inactivation.

Acknowledgements

The authors are grateful to the São Paulo Research Foundation (FAPESP) (Process 12/50522-0) and the Global Challenges Research Fund (GCRF) UK Research and Innovation (SAFEWATER; EPSRC Grant Reference EP/P032427/1) for the research support. The authors are also thankful to the National Council for Scientific and Technological Development (CNPq-Brazil) for the master's scholarship awarded to A.P.O.

Statement

Authors hereby declare previous originality check, no conflict of interest and open access to the repository of data used in this paper for scientific purposes.

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FIGURES

Detection of *Cryptosporidium parvum* oocysts in artificially contaminated filter backwash water and ozone treatment on a pilot scale

Allan Pretti Ogura • Lyda Patricia Sabogal-Paz

A.P. Ogura

Department of Hydraulics and Sanitation, São Carlos School of Engineering, University of São Paulo, Avenida Trabalhador São-Carlense 400. Zip code: 13566-590. São Carlos, São Paulo. Brazil

e-mail: allan.ogura@usp.br

ORCiD: 0000-0001-8549-5820

L.P. Sabogal-Paz (corresponding author)

Department of Hydraulics and Sanitation, São Carlos School of Engineering, University of São Paulo. Avenida Trabalhador São-carlense 400. Zip code: 13566-590. São Carlos, São Paulo. Brazil. Phone: +55 16 3373 9548. Fax: +55 16 3373 9550.

e-mail: lysaboga@sc.usp.br

ORCiD: 0000-0003-2753-3248

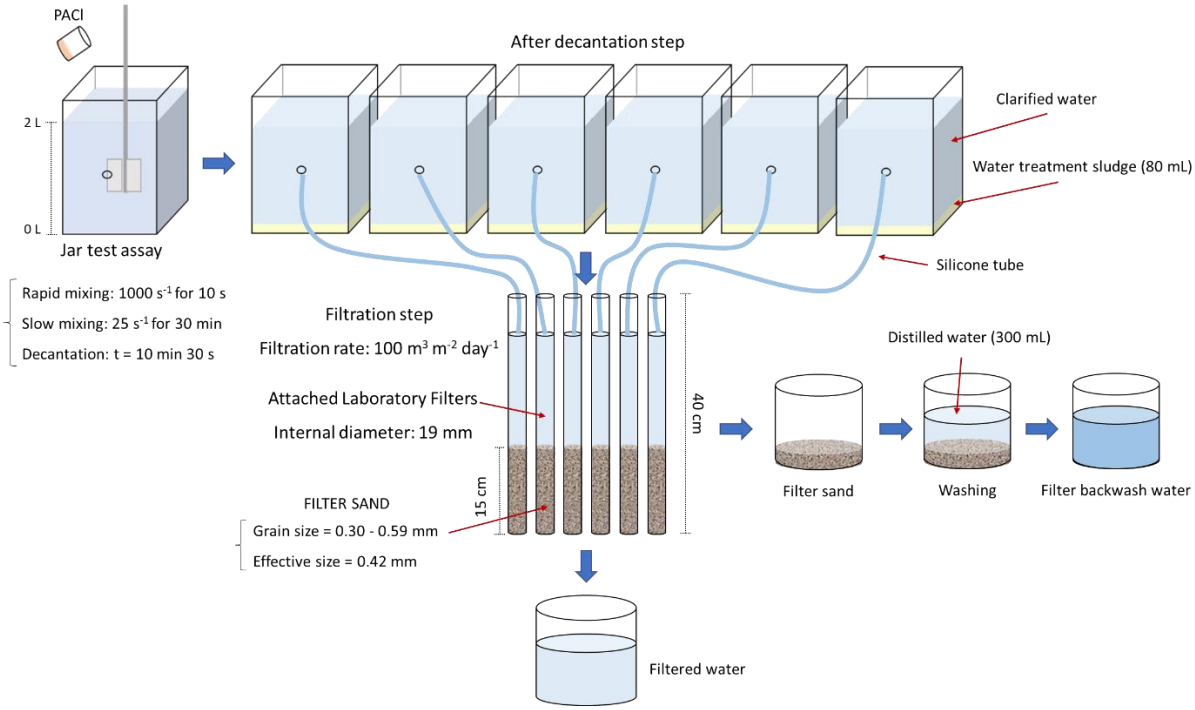

Figure 1 – Representative scheme of the jar test treatability assays, attached laboratory filters, and filter backwash water generation.

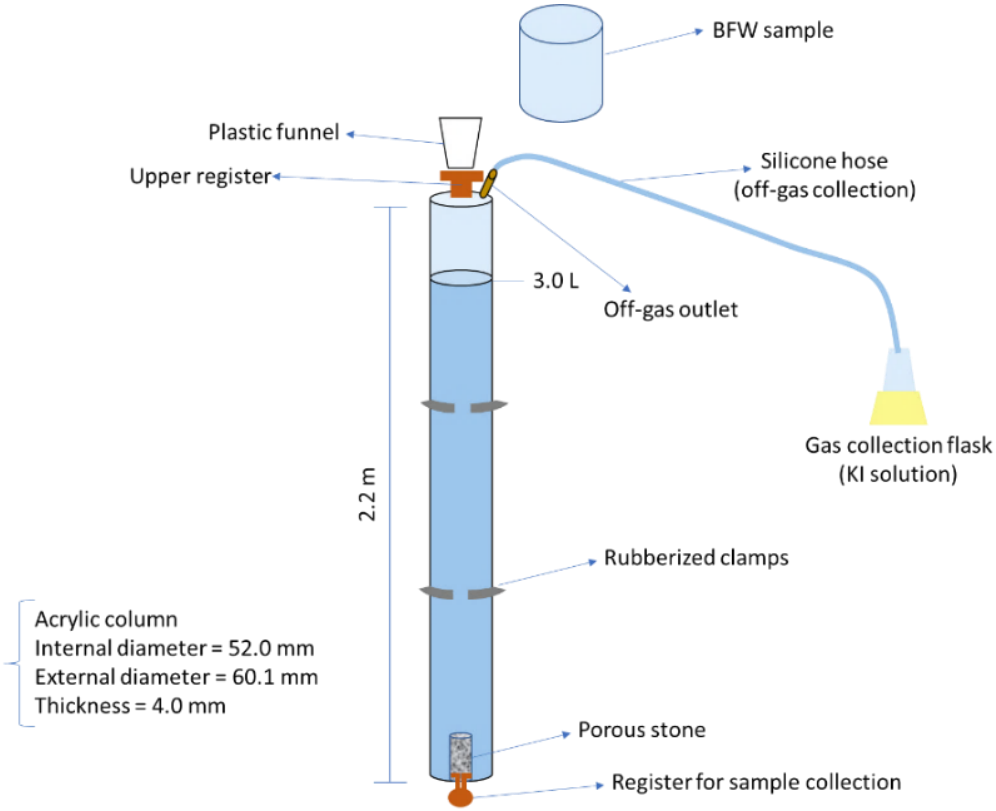

Figure 2 - Representative scheme of the ozonation column used for FBW disinfection experiments.

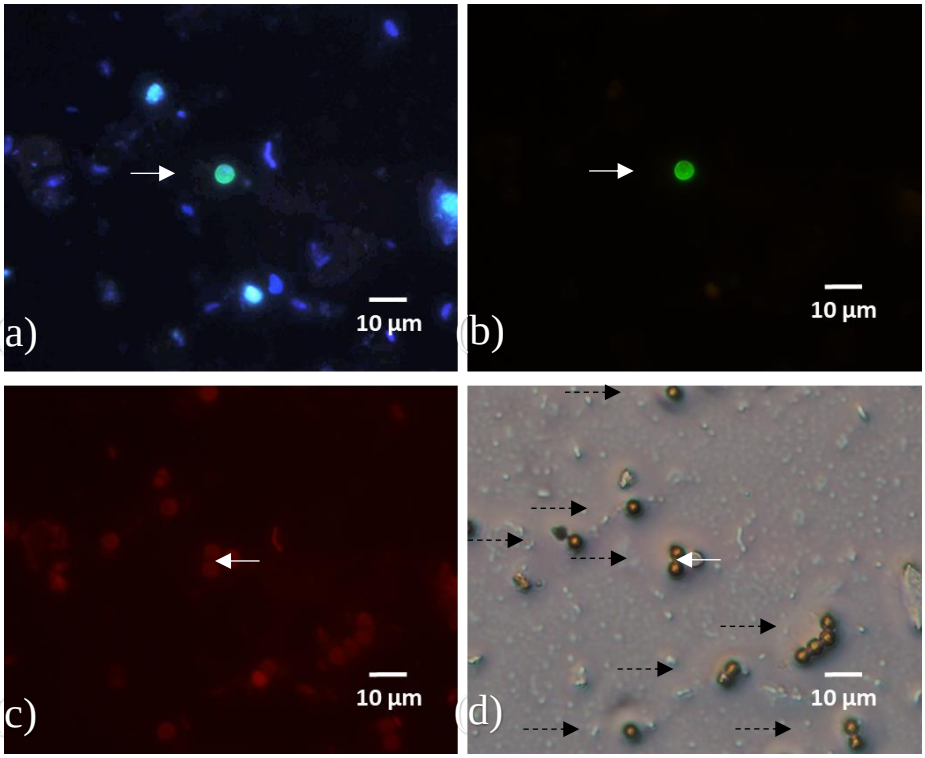

Figure 3 - Stained *C. parvum* cyst after ozone treatment (7.5 mg O_3 L^{-1} at 10 min for FBW). Oocyst indicated with arrows and microspheres with dashed arrow. Images (400X): (a) DAPI = 4 ', 6-diamino-2-phenyl-indole; (b) FITC = Fluorescein isothiocyanate; (c) PI = propidium iodide; (d) DIC = differential interferential contrast

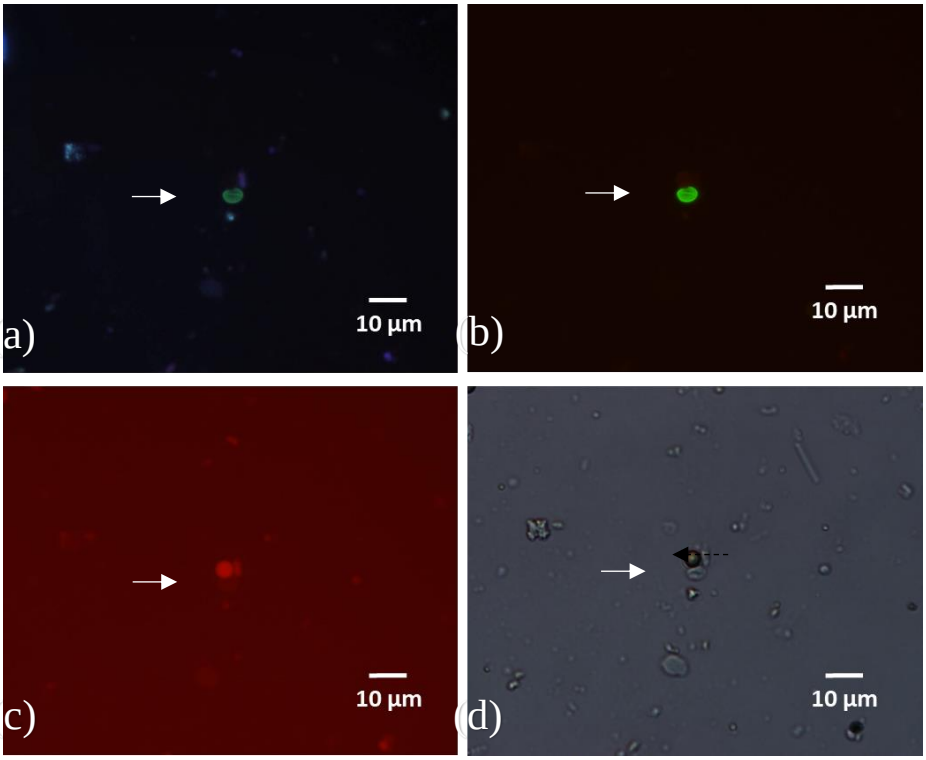

Figure 4 – Non-stained *C. parvum* cyst after ozone treatment (10 mg O_3 L^{-1} at 5 min for FBW). Oocyst indicated with arrows and microsphere with dashed arrow. Images (400X): (a) DAPI = 4 ', 6-diamino-2-phenyl-indole; (b) FITC = Fluorescein isothiocyanate; (c) PI = propidium iodide; (d) DIC = differential interferential contrast

TABLES

Detection of *Cryptosporidium parvum* oocysts in artificially contaminated filter backwash water and ozone treatment on a pilot scale

Allan Pretti Ogura • Lyda Patricia Sabogal-Paz

A.P. Ogura

Department of Hydraulics and Sanitation, São Carlos School of Engineering, University of São Paulo, Avenida Trabalhador São-Carlense 400. Zip code: 13566-590. São Carlos, São Paulo. Brazil

e-mail: allan.ogura@usp.br

ORCiD: 0000-0001-8549-5820

L.P. Sabogal-Paz (corresponding author)

Department of Hydraulics and Sanitation, São Carlos School of Engineering, University of São Paulo. Avenida Trabalhador São-carlense 400. Zip code: 13566-590. São Carlos, São Paulo. Brazil. Phone: +55 16 3373 9548. Fax: +55 16 3373 9550.

e-mail: lysaboga@sc.usp.br

ORCiD: 0000-0003-2753-3248

Table 1 – Aquatic matrix characterization from the jar testing assays.

Water quality characterization	Study water	Clarified water [1]	Filtered water [1]	FBW [1]
Turbidity (NTU)	112	3.45	0.32	14.5
Apparent colour (HU)	114	1	0.5	9.1
True colour (uH)	3.3	0.6	0.0	1.2
pH	7.15	6.18	7.14	6.10
Zeta potential (mV)	-24.03	-0.367	-0.288	-0.056
Conductivity (µS cm^{-1})	53.2	49.2	60.5	52.1
Absorbance (λ 254 nm)	0.07	0.02	0.01	0.01
Alkalinity (mg $CaCO_3$ L^{-1})	8.8	10.9	19.3	12.3
Total solids (mg L^{-1})	NM	NM	NM	113
Total volatile solids (mg L^{-1})	NM	NM	NM	2
Total fixed solids (mg L^{-1})	NM	NM	NM	111
Sedimentable solids (mL L^{-1})	0.1	0.0	0.0	0.05
TOC (mg L^{-1})	1.63	0.42	1.6	0.94
COD (mg L^{-1})	< DL	< DL	< DL	< DL
Total aluminium (mg Al L^{-1})	0.32	< DL	< DL	0.55
Total iron (mg Fe L^{-1})	0.232	< DL	< DL	0.374
Total manganese (mg Mn L^{-1})	< DL	< DL	< DL	< DL
Total coliforms (CFU/100mL)	16	4	1	6
Escherichia coli (CFU/100mL)	0	0	0	0

Notes:

NM: not measured; < DL: less than detection limit; FBW = filter backwash water.

[1]: multiple treatability tests were performed for obtaining a compound sample.

Table 2 - Analytical quality assays with suspensions of *C. parvum* detected by the CD + ICN 7X method followed by IMS.

Sample	Average recovery (%)	Coefficient of variation (%)
Commercial suspensions [1] (3 samples)	15.36 ± 3.33	1.70
EasySeed® suspension [2] (4 samples)	2.8 ± 0.80	30.2
Method 1623.1 USEPA (2012)	32-100	≤ 37

Notes: [1] Inoculated oocysts in the FBW: 2085 oocysts L^{-1}.

[2] Inoculated oocysts in the FBW: 99.0 ± 1.6 oocysts L^{-1}.

FBW = filter backwash water.

Table 3 - Ozone mass balance analyses of the FBW ozonation tests (n = 3), recoveries for DC + ICN 7X + IMS protocol, and percentage of non-stained parasites with PI after treatments.

Ozonation parameter	7.5 mg O_3 L^{-1}, 10 min, 25ºC	10.0 mg O_3 L^{-1}, 5 min, 25ºC
Off-gas ozone (mg O_3 L^{-1})	0.62 ± 0.31	1.07 ± 0.71
Residual dissolved ozone (mg O_3 L^{-1})	0.87 ± 0.35	1.09 ± 0.16
Consumed ozone (mg O_3 L^{-1})	6.00 ± 0.65	7.85 ± 0.56
Transferred ozone (%)	80.2 ± 8.7	78.5 ± 5.6

Ozone treatment	Oocyst recovery (%)	Variance (%)	Non-stained oocysts (%)	Variance (%)	Inactivation Efficiency
7.5 mg O_3 L^{-1} FBW, 10 min, 25ºC [1]	2.15 ± 0.76	35.12	2.17 ± 0.59	27.22	2.83-log
10.0 mg O_3 L^{-1} FBW, 5 min, 25ºC [2]	0.82 ± 0.23	28.69	0.70 ± 0.65	92.52	3.44-log

Notes: [1]: Viability estimated according to inoculated protozoa: 4993 oocysts L^{-1}.

[2]: Viability estimated according to inoculated protozoa: 5000 oocysts L^{-1}.

PI = propidium iodide; FBW = filter backwash water.