Microcystis aeruginosa and microcystin-LR removal by household slow sand filters operating in continuous and intermittent flows

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A household slow sand filter (HSSF) is a widely used water treatment technology recognized as one of the most effective and sustainable in reducing waterborne diseases. However, there is a lack of knowledge concerning its behaviour in the presence of cyanobacteria and cyanotoxins. In this context, the study aimed to evaluate HSSF ability to remove *Microcystis aeruginosa* cells (stain BB005) and microcystin-LR from water, among other parameters, when operated under continuous (C-HSSF) and intermittent (I-HSSF) flows. C-HSSF was operated at a constant filtration rate (1.22 m^3 m^{-2} day^{-1}), while I-HSSF was operated at a variable filtration rate (starting at 2.95 m^3 m^{-2} day^{-1} and finishing at zero). Each filter produced 60 L day^{-1}. The influence of the pause period was also tested in the I-HSSF. The water from the study was prepared by inoculating *M. aeruginosa* culture in water from a well to a final cell density of ± $1x10^5$ cells mL^{-1}. *M. aeruginosa* removal rates were 2.39 ± 0.34 log and 2.01 ± 0.43 log by C-HSSF and I-HSSF, respectively. Microcystin-LR concentration in studied water was 5.55 µg L^{-1}, and both filters produced filtered water with microcystin concentrations below 1.0 µg L^{-1}, the maximum value recommended by the World Health Organization (WHO), for most of the samples. Turbidity and apparent colour were also within WHO guidelines. Filters operating with different flow regimes and distinct residence times did not statistically influence treatment efficiencies. Both filters showed promising results in the *M. aeruginosa* and microcystin-LR removals from water; nevertheless, more research is needed to understand the mechanisms involved in the reduction of both cyanobacteria and cyanotoxin through household slow sand filtration.

Keywords: biosand filter; developing countries; water treatment; drinking water; cyanobacteria; cyanotoxin.

1. Introduction

According to WHO and United Nations Children's Fund (UNICEF), 844 million people lack basic drinking water services, living without access to improved drinking water sources within 30 minutes' round trip (WHO and UNICEF, 2017). The ingestion of inappropriately treated or untreated water raises particular concerns when the main water source is a eutrophic river, lake or reservoir. In events of eutrophication, the environmental conditions can sustain a high-density proliferation of cyanobacteria, called "bloom" (Chorus and Bartram, 1999). According to Chen *et al.* (2011), 75% of cyanobacteria blooms are toxic. Cyanotoxins can present a variety of effects on human health ranging from allergic reactions, fever, headaches and diarrhoea to more severe cases of liver failure, respiratory arrest and, in rare cases, death (USEPA, 2014). Produced by several species of cyanobacteria and frequently found in strains of *Microcystis* sp. and *Anabaena* sp., microcystin is the most common class of cyanotoxin in eutrophic water bodies, presenting more than 80 variants (Rastogi et al, 2014; USEPA 2014). Due to their toxicity and wide distribution, microcystins were included as mandatory parameters for drinking water quality control in several countries. WHO guidelines for drinking water quality recommend a maximum concentration of 1.0 µg L^{-1} for total microcystin-LR (WHO, 2017).

Household, or point-of-use (POU), water treatments can help supply the demand for safe water in isolated communities with no reliable water source (WHO, 2012). In addition to making water safe for consumption, it is important that POU technologies meet other requirements such as operational simplicity and low-cost. Among a number of alternatives, the household slow sand filter (HSSF) is presented as one of the most effective (Sobsey et al., 2008). HSSF technology has shown promising results in the removal of turbidity, organic matter, chemical compounds and microorganisms such as total coliforms, *Escherichia coli*, viruses and protozoa cysts and oocysts (Elliott et al., 2015, 2011; Jenkins et al., 2011; Palmateer et al., 1999; Wang et al., 2016, 2014). Field studies also related HSSF implementation to the reduction of diarrhoea cases and an increase in quality of life (Cawst, 2012; Divelbiss et al., 2013; Liang et al., 2010). Additionally, HSSF presents the ability to be operated in both continuous and intermittent flows (Young-Rojanschi and Madramootoo, 2014).

Although microcystin removal through laboratory biological sand filters and conventional slow sand filters have been reported (Chorus et al., 2003; Eleuterio and Batista, 2010; Grützmacher et al., 2005, 2002, Ho et al., 2007, 2006; Somdee et al., 2014), there is insufficient understanding about HSSF behaviour when fed with water containing cyanobacteria and cyanotoxins. In this respect, a field study investigated HSSF efficiency to remove microcystin and lipopolysaccharide endotoxin from natural waters in Maputo, Mozambique (Bojcevska and Jergil, 2003). The authors reported microcystin removal efficiency ranging from -20% to 100%. However, due to low concentrations of microcystin in raw water, with the majority of the samples presenting concentrations below 1.0 µg L^{-1}, they were unable to reach a conclusion regarding HSSF efficiency to remove microcystin and more studies to confirm the real HSSF potential were recommended.

Considering the lack of research on cyanobacteria and cyanotoxin removal in HSSF, this paper aims to investigate the potential of HSSFs made of polyvinyl chloride (PVC) operating in intermittent and continuous flow regimes in the removal of *M. aeruginosa* cells and microcystin-LR.

2. Materials and Methods

2.1. Household slow sand filters

Two full-scale HSSFs were constructed in opaque PVC, using the prototype CAWST (2012) as a base model. One HSSF was designed to operate intermittently (I-HSSF) and the other to operate continuously (C-HSSF). Both filters were constructed with a 250 mm inside diameter (cross sectional area = 0.049 m²). HSSF designs are presented in Figure 1.

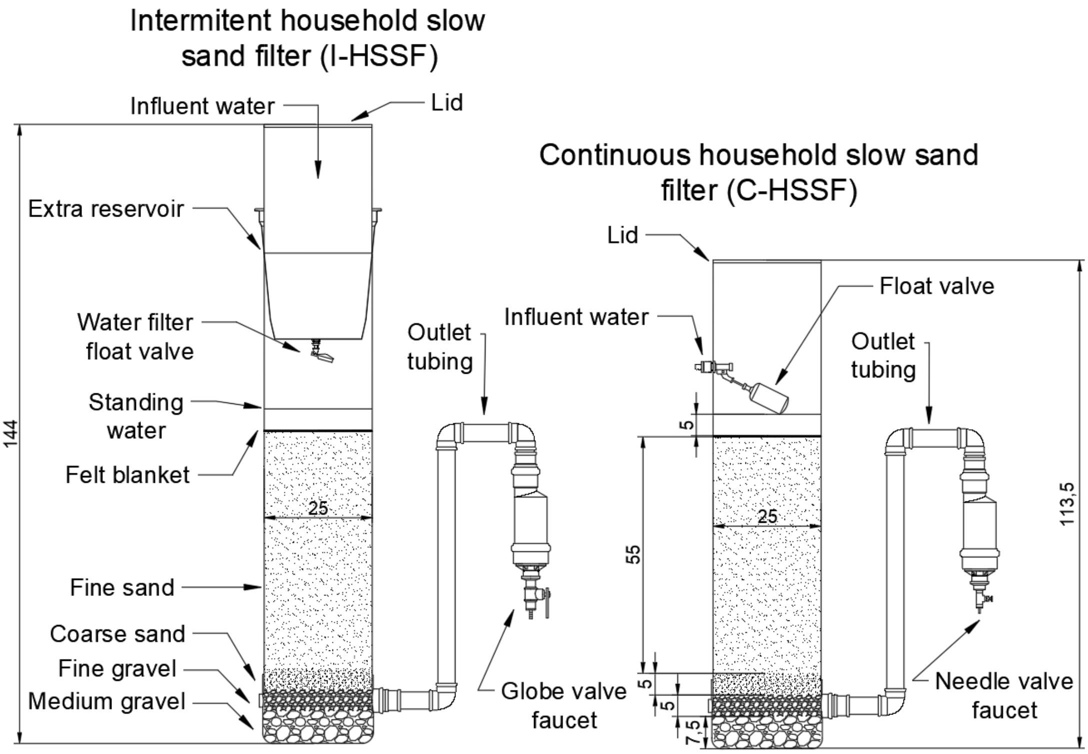

Figure 1 - Cross-section of full-scale HSSFs (units in centimetres).

The filter media was a 55 cm fine sand layer with an effective size (D_{10}) of 0.153 mm, uniformity coefficient (UC) of 1.68 and porosity of 45%. The fine sand layer weight was, approximately, 39 Kg. Support materials consisted of a 5 cm layer of coarse sand, 5 cm layer of fine gravel and 7.5 cm layer of medium gravel. Materials were washed, sun-dried and sieved using commercial sieves.

A non-woven synthetic fabric blanket, or felt blanket (specific gravity: ± 0.2 g cm^{-3}, composition: 100% polyester and thickness: 2 mm), was positioned at the filter media top, in order to facilitate HSSF operation and maintenance. All material used in construction and filling of the HSSFs were bought at local stores in São Carlos City (SP, Brazil). Precautions were taken to ensure the use of simple, easy-to-find tools and materials throughout the HSSF construction and operation processes.

2.2. Household slow sand filter operation

HSSFs were operated for 63 continuous days (from November 22, 2016, to January 23, 2017). The studied water was prepared daily at 8 am by diluting ± 1.5 L of *M. aeruginosa* culture into 133.5 L of well water. No pH correction was made. The preparation provided 135 L of studied water (Table 1).

Table 1 - Studied water characteristics.

Parameter	Mean value	Standard deviation	Coefficient of variation (%)
Temperature (K)	295.4	1.7	0.6
pH	6.7	0.3	4.5
M. aeruginosa (cell mL^{-1})	1.1×10^5	0.2×10^5	18.7
Microcystin-LR (µg L^{-1})	5.6	2.7	47.9
Turbidity (NTU)	2.4	0.7	30.1
Apparent colour (uH)	8.2	2.4	29.2
TOC (mg L^{-1})	1.5	0.6	39.2
Total coliforms (CFU $100mL^{-1}$)	5.6	6.2	112.4
Partial alkalinity ($mgCaCO_3$ L^{-1})	16.8	2.1	12.3
Total alkalinity ($mgCaCO_3$ L^{-1})	26.1	2.0	7.7
Conductivity (S m^{-1})	0.48	0.05	9.7

HSSF filtration rates were determined based on the daily water volume needed for activities in a simple residence, estimated to be approximately 60 L day^{-1}. The C-HSSF design maintained a steady hydraulic head and its filtration rate was kept constant at 1.22 m^3 m^{-2} day^{-1} (0.04 L min^{-1}). The C-HSSF filtration rate was controlled by a needle valve faucet installed in the outlet pipe. On the other hand, the I-HSSF hydraulic head was variable and, consequently, the filtration rate as well. I-HSSF maximum filtration rate was 2.95 m^3 m^{-2} day^{-1} (0.06 L min^{-1}) right after feeding and it decreased to zero.

For continuous operation, 75 L of the studied water was pumped to an elevated 150 L water tank, placed next to the filters. A submersible water pump HM-5063 (*Jeneca Electromechanical Co., Ltd.*, Hong Kong) was used to maintain a homogeneous mixture inside the water tank. A hose connected to the elevated water tank outlet tube conducted the water to the filter. Studied water entered C-HSSF through a side perforation above the filter media top, equipped with a float valve. The continuous feeding system was designed to maintain a steady hydraulic head and to equalize the water supply in the C-HSSF, avoiding oscillations that could influence the treatment. This adaptation resulted in a constant 10 cm hydraulic head. The remaining 60 L inside the elevated water tank was used for I-HSSF feeding. There was an extra reservoir coupled to the top of the I-HSSF constituted by a 13.6 L plastic container with a bottom hole equipped with a plastic float valve. A PVC section with 30 cm length and 250 mm diameter was attached to the container in order to increase its working volume. Studied water was poured into the container and the float valve controlled it entering into I-HSSF. This device was used as a diffuser surrogate with the advantage of being able to control the maximum hydraulic head within the filter. It can be observed that the float did not reduce the volume that could be added to the filters, it only limited the maximum water level above the standing water, and consequently, the maximum filtration rate.

Based mainly on a daily household routine I-HSSF feeding was done four times a day (8 am, 12 pm, 4 pm and 8 pm). The adopted I-HSSF water charge intervals resulted in two distinct retention times (4 h between successive feedings) and 12 h (between the last feeding and the first feeding of the next day). Charge volume was 15 L, the sum of standing water volume, and filter media and support layer pore volumes - named filter volume. Each feeding was done transferring the water from the elevated water tank into the I-HSSF using a plastic container.

2.3. Tracer tests

The C-HSSF and I-HSSF flow characterizations were carried out using 100 mg L^{-1} sodium chloride (NaCl) solution as a tracer, which was poured into the filters considering the particularities of each design. The variation of electric conductivity in the filtered water was detected in real time using a conductivity probe (*Vernier Software & Technology*, USA) positioned at the end of the outlet pipe. Probe calibration allowed correlating conductivity variation with tracer concentration. The data was collected by the software Logger Lite (*Vernier Software & Technology*, EUA) and processed by the software Excel 2016 (*Microsoft*, EUA) and Origin 8.6 (*OriginLab*, EUA). Tracer tests were performed in triplicate.

In I-HSSF tracer tests, the first feeding was made with NaCl solution and the subsequent feedings with well water. The filtration rate declined to zero when the hydraulic head reached the minimum level, at which time a new charging was performed. The volume of each filter charging was the filter volume, 15 L. The procedure resulted in concentration *versus* filter volume (C *vs* v) curves with a positive step followed by a negative step (*i.e.*, increase in concentration followed by a decrease in concentration). I-HSSF tracer test results were analysed according to those proposed by Elliott *et al.* (2008) and Bradley (2011). Hydraulic retention time (HRT) was obtained and Morrill Dispersion Index (MDI) was calculated in accordance with Tchobanoglous *et al.* (2003). MDI is the ratio between the time in which 10% and 90% of the tracer exits the filter, respectively. The index ranges from 1.0 (for ideal plug-flow rectors) to 22 (for ideal complete-mix reactors).

In C-HSSF tracer tests, the step input was applied for tracer injection, as recommended by Levenspiel (2000). The filtration rate was the same used during the filter operation, 1.22 m^3 m^{-2} day^{-1}. The HRT was determined and concentration *versus* time (C *vs* t) curves were plotted. The flow pattern was adjusted into three hydrodynamic mathematical models reported in Levenspiel (2000): Dispersion models (low dispersion and high dispersion) and N-continuous stirred tank reactors model (N-CSTRs). Adjusted mathematical models estimated whether the C-HSSF flow pattern resembled a plug-flow reactor or N ideal stirred tanks in series. MDI was also calculated. Between each test, C-HSSF was cleaned with well water until the NaCl solution from the previous test was completely removed.

2.4.Cyanobacterial culture

M. aeruginosa cultivation was carried out in an experimental facility. The inoculum (strain BB005) was supplied by the Laboratory of Phycology from the Federal University of São Carlos (UFSCar – Brazil). Cyanobacteria were grown in a batch culture system at WC medium, according to Guillard and Lorenzen (1972), in 2 L Erlenmeyer flasks aerated by aquarium pumps. Before being injected into the culture, the air passed through a 0.2 μm porosity polytetrafluoroethylene (PTFE) filter (*Sartorius Stedim*, USA). Luminous intensity, provided by cool white tubular fluorescent lamps, was approximately 60 μmol m^{-2} s^{-1}. A 12 h photoperiod was applied and the average temperature was 25 ± 2 °C. To maintain an aseptic environment, germicidal lamps were turned on for 15 min prior and after the doors in the experimental facility were opened. The subculture was executed inside a laminar flow cabinet; the new medium and all the materials were autoclaved and received a UV radiation bath before the procedure. The experimental facility provided conditions that allowed maintenance of the needed *M. aeruginosa* suspension volume during the HSSF operational period.

2.5. Sample collection and analysis

Filtered water samples and studied water samples were collected in 350 mL amber glass bottles. The C-HSSF sample collection was performed once a day, while the I-HSSF sample collection was made a few minutes after each water charge. I-HSSF samples were divided by its residence time. Samples collected at 12 pm, 4 pm and 8 pm (relative to 8 am, 12 pm and 4 pm water charges, respectively), with 4-hour feeding intervals were collected as a composed sample (I-HSSF(4h)). Samples collected at 8 am (relative to 8 pm water charge from the day before), with 12-hour feeding intervals were labelled as I-HSSF (12h). All the samples were stored in a cold chamber and analysed the day after collection. For microcystin-LR analyses, sample aliquots were frozen in Eppendorf tubes with locking caps. The microcystin-LR determination method used was an Enzyme-Linked Immunosorbent Assay (ELISA) – Microcystin Plate Kit (Cat # 20-0068 - *Beacon*, EUA). Based on studied water microcystin-LR concentrations from previous analyses, all the samples were diluted in deionized water at a 1:10 ratio in order to maintain microcystin-LR concentration within the method's limits of quantification (0.1 $\mu g\ L^{-1}$ – 2.0 $\mu g\ L^{-1}$).

Water quality parameters analysed were apparent colour (DM-COR Colorimeter – *Digimed*, Brazil), turbidity (2100N Turbidimeter – *Hach Company*, USA) microcystin-LR concentration, absorbance (λ 254 nm) (DR5000 spectrophotometer – *Hach Company*, USA), total coliforms (9222 Membrane filter technique for members of the coliform group - APHA, 2012), *Escherichia coli* (9222 Membrane filter technique for members of the coliform group - APHA, 2012), total organic carbon (TOC) (TOC-L – *Shimadzu*, Japan) and *M. aeruginosa* cell density using Fuchs-Rosenthal counting chamber and BX51 microscope (*Olympus*, Japan). Moreover, monitoring analyses were performed to assure the filters' operational stability. Monitoring analyses were pH (DM20 pH meter – *Digimed*, Brazil), temperature, partial and total alkalinity and conductivity (DM32 conductivity meter – *Digimed*, Brazil). Studied water was also analysed for the aforementioned parameters and I-HSSF and C-HSSF efficiency were determined, as well as their ripening times. By the end of the two-month operation, *schmutzdecke* samples were collected from the felt blanket and from the filter media's first millimetres. The BX51 microscope (*Olympus*, Japan) was used to capture images from *schmutzdecke* microorganisms for visual identification. Furthermore, scanning electron microscope (SEM) (ZEISS LEO 440 - *Cambridge*, England) was used to capture felt blanket photomicrographs before and after operation time in order to visualize potential fibre alterations caused by the development of microorganisms. Finally, acute ecotoxicological assays were performed with *Chironomus xanthus* larvae. The aim was to investigate acute toxicity by residual microcystin-LR in filtered water or by contact with the filter materials. In the assay, six (6) *C. xanthus* IV instar larvae were exposed to 250 mL of studied water or filtered water for 96-hours, after which the number of viable larvae was determined. Tests were carried out in triplicate in two operational moments: at 14 days of operation and at 63 days of operation. 1 L samples were taken from each filter for ecotoxicological assays and no composed sample was used.

The Kruskal-Wallis test was used to compare data from the filtered water samples among each other and with studied water (95% confidence interval). When statistical analyses indicated that the mean values were significantly different, the Mann-Whitney test was used to determine which sample was significantly different from another (95% confidence interval).

3. Results and Discussion

3.1. Tracer tests

I-HSSF tracer tests results are shown in Figure 2a. The increase in effluent tracer concentration, ranging from 0 mg L^{-1} to the maximum concentration of 83 mg L^{-1}, occurred on 1 filter volume. The same happened when the concentration declined (between the second and third filter volumes). This behaviour is expected from a plug flow reactor (Bradley et al., 2011).

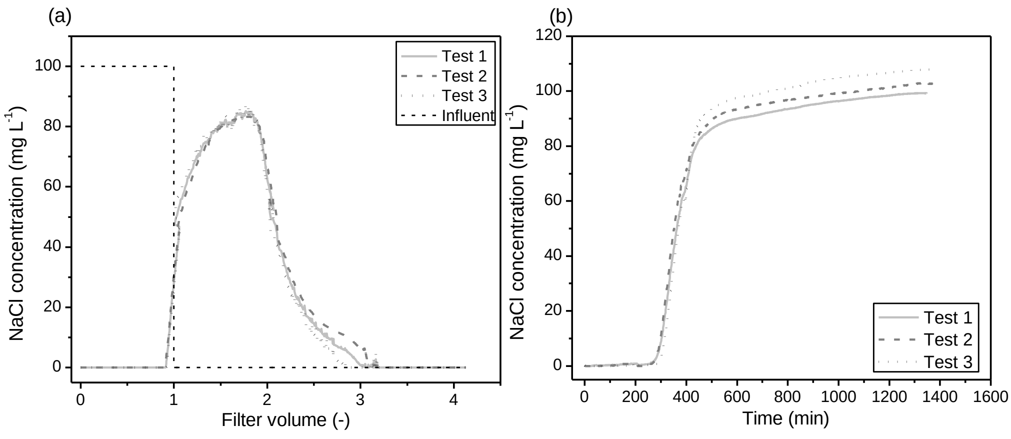

Figure 2 – (a) Results of tracer tests conducted with the intermittent household slow sand filter (I-HSSF). (b) Results of tracer tests conducted with the continuous household slow sand filter (C-HSSF).

Figure 2a also shows that the I-HSSF effluent tracer concentration (83 mg L^{-1}) did not reach the influent tracer concentration (100 mg L^{-1}). The approximately 27% difference can be attributed to the filter's hydraulic head, which may have diluted the tracer solution. I-HSSF HRT was approximately 239 min (3 hours and 59 min). Feeding intervals of 4 h between successive feedings were close to I-HSSF HRT, which resulted in practically no pause period. According to CAWST (2012), the HSSF pause period should not be lower than 1 h or higher than 48 h. However, CAWST's HSSF has a maximum filtration rate of 9.6 m^3 m^{-2} day^{-1} and an HRT of about 1.0 h, while the present PVC design operates at a maximum filtration rate of 2.95 m^3 m^{-2} day^{-1} and almost 4.0 h HRT. It was expected that the lower filtration rate compensated the absence of a pause period. Furthermore, a daily genuine household routine was given more attention than the pause period in the decision of feeding intervals. For the overnight feeding interval, the pause period was 8 h.

I-HSSF MDI was 1.88 ± 0,03, slightly higher than some reports in the literature, such as Elliot et al. (2008) who observed an MDI of 1.3 and Bradley (2011) who obtained an MDI of 1.4 in commercial plastic HSSFs (*HydrAid*, USA). However, the result obtained was similar to that observed by Young-Rojanschi and Madramootoo (2015), who found an MDI value of 1.8. In addition, the obtained MDI characterizes the I-HSSF flow as an effective plug-flow reactor, according to the USEPA classification (USEPA, 1986).

The C-HSSF experimental HRT was 417 min (6 h and 57 min), used to determine the sample collection time. Tracer tests C *vs* t curves are presented in Figure 2b. The N-CSTR model better-adjusted the C-HSSF flow pattern with an r^2 of 0.69 and N=6. The higher the N value is, the closer to the plug-flow the reactor is (Levenspiel, 1999). C-HSSF MDI was 2.69 ± 0.08, which means that a continuous flow filter could not be considered an effective plug-flow reactor, according to the USEPA classification (USEPA, 1986). C-HSSF's low N-value and high MDI may not be representative of the flow through filter media itself. Using a needle faucet to control the flow might have caused water to accumulate on the larger diameter section in the outlet tube leading to the formation of a mixing zone. Considering that in I-HSSF the needle faucet does not control its flow, there was no water accumulation on the larger diameter section in the outlet tube (*i.e.*, no mixing zone formation), which could explain the MDI differences between I-HSSF and C-HSSF.

3.2. Household slow sand filter operation

Both filters´ maturation (ripening) were observed to happen slowly. According to CASWT (2012), the HSSF maturation time lasts about 30 days, during which time the efficiency of the filter increases and filtration rate declines as *schmutzdecke* develops. Although the filtration rate decline was observed, neither of the HSSFs needed maintenance of the felt blanket or filter media top layer in 63 days of continuous operation. Reduced presence of microorganisms and nutrients in studied water resulted in a slow *schmutzdecke* development (Elliott et al., 2008). Microcystin-LR presence may also lead to the delay in microbiological development due to their ability to alter microbiological communities (Giaramida et al., 2013) and retard the growth of some bacteria (Miguéns and Valério, 2015). No evident increases in treatment efficiencies were observed throughout the operation for most of the parameters, including *M. aeruginosa* (for all filtered samples) and microcystin-LR (for I-HSSF samples). This behaviour was different from that reported in the literature for conventional slow sand filters and HSSFs, as an increase in treatment efficiencies over time was described (Cawst, 2012; Grützmacher et al., 2002; Ho et al., 2007). Water quality parameters in filtered water and filter removal rates are presented in Table 2.

According to the Kruskal-Wallis test, there was no significant difference between I-HSSF(4h), I-HSSF(12h) and C-HSSF results for *M. aeruginosa* removal (p = 0,2006). Therefore, operational conditions, such as intermittent and continuous operation, different filtration rates and HRT, did not influence the removal of *M. aeruginosa* cells. Studied water *M. aeruginosa* cell density was $1.06\times10^5 \pm 1.98\times10^4$ cells mL^{-1} (Table 2). Cell density variations in filtered waters over time are shown in Figure 3.

Table 2 - Water quality parameters in filtered water and removal rates from intermittent flow and continuous flow HSSFs

Parameter	C-HSSF		I-HSSF(4h)		I-HSSF(12h)	
	Value	Reduction	Value	Reduction	Value	Reduction
Temperature (°C)						
Mean	24.4	N/A	24.7	N/A	21.9	N/A
Standard deviation	2.10	N/A	1.70	N/A	1.61	N/A
pH						
Mean	7.31	N/A	6.94	N/A	7.00	N/A
Standard deviation	0.14	N/A	0.19	N/A	0.24	N/A
M. aeruginosa cell (cell mL^{-1})						
Mean	4.34E+02	2.40 log	9.90E+02	2.03 log	1.09E+03	1.99 log
Standard deviation	7.40E+02	0.34 log	1.34E+03	0.40 log	2.73E+02	0.47 log
Microcystin-LR (µg L^{-1})						
Mean	1.09	75.30%	1.12	74.35%	1.00	76.41%
Standard deviation	0.44	0.24 %	0.32	0.23 %	0	0.24 %
Turbidity (NTU)						
Mean	0.37	84.39 %	0.44	81.41 %	0.38	83.78 %
Standard deviation	0.16	8.85 %	0.26	17.03 %	0.23	13.60 %
Apparent colour (uH)						
Mean	0.19	97.73 %	0.44	94.73 %	0.23	97.17 %
Standard deviation	0.35	5.62 %	0.64	8.81 %	0.42	0.35 %
TOC (mg L^{-1})						
Mean	1.31	11.46 %	2.24	-51.57 %	1.44	2.55 %
Standard deviation	0.74	72.14 %	1.04	191.23 %	0.66	100.75 %
Absorbance (λ = 254 nm)						
Mean	2.01E-02	65.27 %	2.35E-02	59.48 %	1.85E-02	68.10 %
Standard deviation	2.9E-03	6.98 %	7.2E-03	18.19 %	5.9E-03	12.08 %
Total coliforms (CFU 100mL^{-1})						
Mean	3.33	-0.041 log	6.11	- 0.107 log	7.11	0.222 log
Standard deviation	2.40	0.491 log	3.95	0.546 log	3.76	0.624 log

Notes: Intermittent household slow sand filter with 4 h feeding interval (I-HSSF(4h)); intermittent household slow sand filter with 12 h feeding interval (I-HSSF(12h)) and continuous household slow sand filter (C-HSSF); not applicable (N/A).

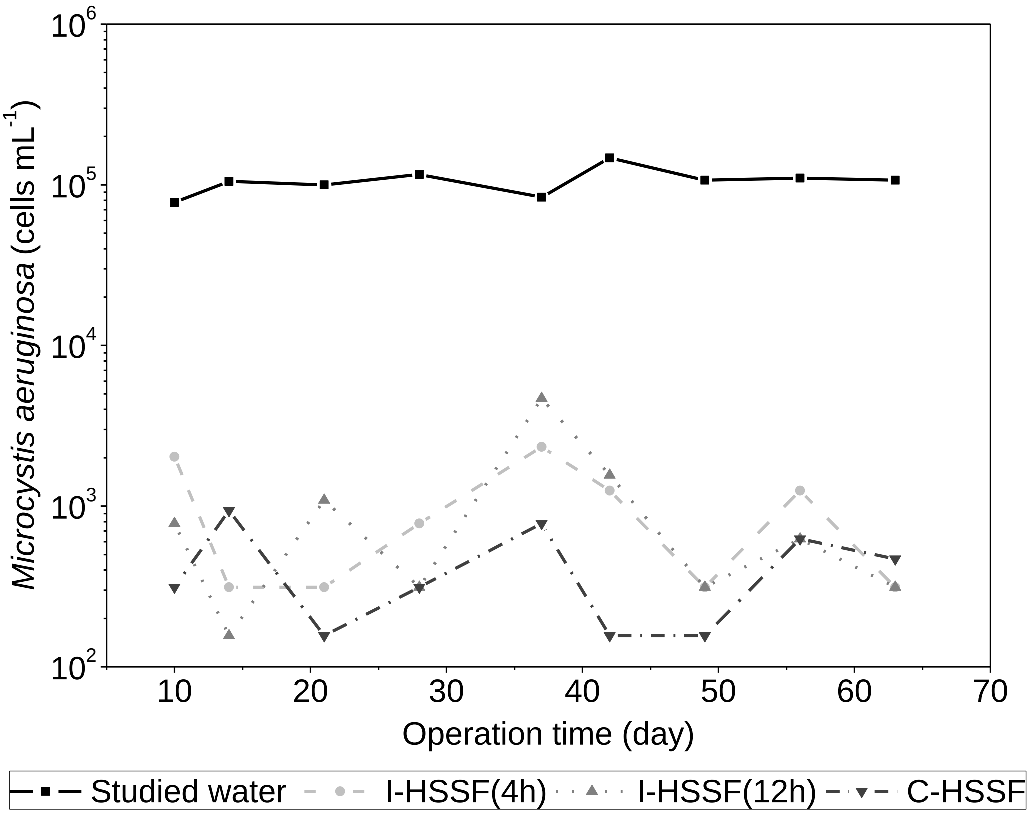

Figure 3- *M. aeruginosa* cell density in studied water and filtered water from intermittent HSSF with 4 h feeding interval (I-HSSF(4h)), intermittent HSSF with 12 h feeding interval (I-HSSF(12h)) and continuous HSSF (C-HSSF) over time.

The absence of statistical differences between the filters and I-HSSF HRTs could show evidence of the crucial role of grain size in *M. aeruginosa* removal. Grain size is considered to be critical in HSSF design, and is able to increase efficiency regardless of conditions such as the hydraulic head, influent turbidity and maintenance (Chan et al., 2015; Jenkins et al., 2011). Another critical condition in HSSF design reported by Jenkins et al (2011), the HRT, did not show to be as important in *M. aeruginosa* removal. The obtained results are similar to those observed by Bojcevska and Jergil (2003). The authors observed 99.33% cyanobacteria biomass removal using an HSSF feed with water from a lagoon containing large colonies of *Microcystis botrys* and *Microcystis novacekii*. Further information on filter media characteristics (e.g. D10 and UC) and pause periods were not provided in the report. *Microcystis* spp. behaviour of forming colonies in natural waters is well known (Holt et al., 1994) and it was observed in some episodes through *schmutzdecke* microscopy analysis (Figure 4). The presence of large colonies in influent water could increase the filter removal potential. Due to larger sizes, colonies may be more susceptible to physical trapping. However, laboratory cultivation with controlled conditions resulted in *M. aeruginosa* culture mostly in isolated cells, which combined with their small size, might have facilitated cyanobacteria passage through the filter media.

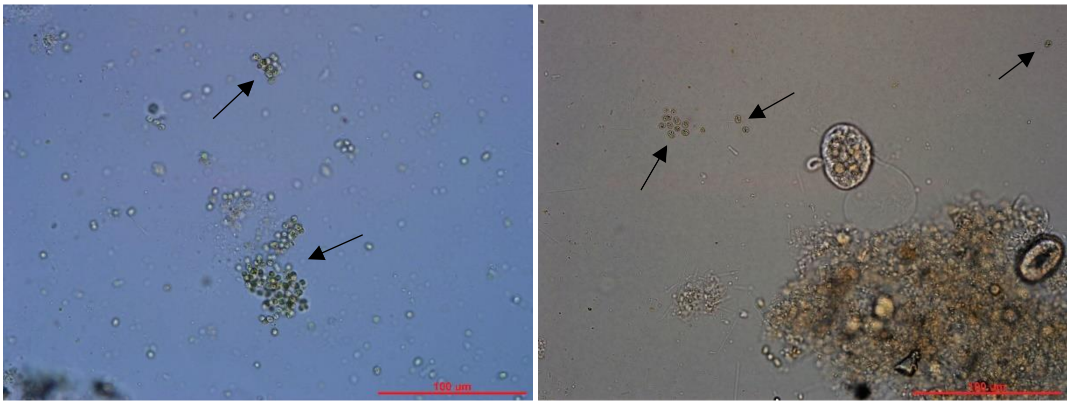

Figure 4 - *M. aeruginosa* presence as colonies and as single cells in *schmutzdecke*.

Studied water and filtered samples' microcystin-LR concentrations are shown in Figure 5.

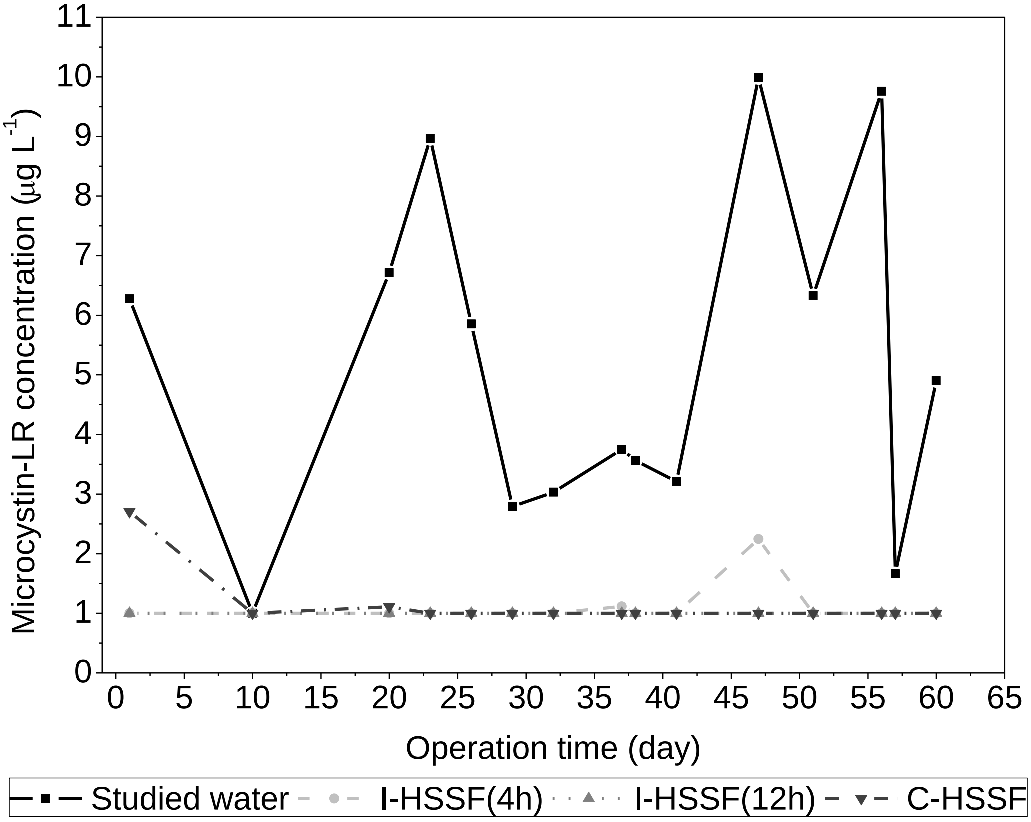

Figure 5- Microcystin-LR concentrations in studied water and filtered water from intermittent HSSF with 4 h feeding interval (I-HSSF(4h)), intermittent HSSF with 12-hour feeding interval (I-HSSF(12h)) and continuous HSSF (C-HSSF) over time.

Mean cyanotoxin concentration in studied water was 5.187 ± 2.83 µg L^{-1} (Table 2), ranging from ≤ 1.00 µg L^{-1} to 9.99 µg L^{-1}. Almost all the filtered water samples presented microcystin-LR concentrations below the microcystin kit limit of quantification (0.1 µg L^{-1}). Average removal efficiencies were at least 75.30% to I-HSSF(4h), 76.41% to I-HSSF(12h) and 74.35% to C-HSSF. Microcystin-LR removal could be attributed mainly to cell retention and biodegradation, as proposed in the available literature for conventional slow sand filters (Chorus et al., 2003; Eleuterio and

Batista, 2010; Grützmacher et al., 2005, 2002, Ho et al., 2007, 2006; Somde et al., 2014).

Bojcevska and Jergil (2003) observed a great influence from inlet microcystin concentrations and water characteristics in HSSF removal efficiencies. The authors reported removal rates ranging from -20% to 100% using concrete HSSF feed with raw water. Laboratory controlled conditions provided a more constant result in the present research, with removal rates ranging from 39.91% to 89.75% for I-HSSF(4h) and from 39.91% to 89.99% for I-HSSF(12h) and C-HSSF. Microcystin-LR concentrations are presented in Table 3. The influence of the influent microcystin-LR concentrations on the removal rates was not as evident, as seen by Bojcevska and Jergil (2003).

Table 3 – Microcystin-LR concentration and removal rates from intermittent HSSF and continuous HSSF.

Day of operation	Microcystin-LR concentration (µg L^{-1})				Removal rates (%)		
	Studied water	I-HSSF(4h)	I-HSSF(12h)	C-HSSF	I-HSSF(4h)	I-HSSF(12h)	C-HSSF
1	6.28	≤ 1.0	≤ 1.0	2.70	84.1	84.1	56.9
10	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0	N/A	N/A	N/A
20	6.71	≤ 1.0	≤ 1.0	1.11	85.1	85.1	83.5
23	8.97	≤ 1.0	≤ 1.0	≤ 1.0	88.8	88.8	88.8
26	5.85	≤ 1.0	≤ 1.0	≤ 1.0	82.9	82.9	82.9
29	2.79	≤ 1.0	≤ 1.0	≤ 1.0	64.2	64.2	64.2
32	3.03	≤ 1.0	≤ 1.0	≤ 1.0	67.0	67.0	67.0
37	3.75	1.11	≤ 1.0	≤ 1.0	70.3	73.3	73.3
38	3.56	≤ 1.0	≤ 1.0	≤ 1.0	71.9	71.9	71.9
41	3.21	≤ 1.0	≤ 1.0	≤ 1.0	68.8	68.8	68.8
47	9.99	2.25	≤ 1.0	≤ 1.0	77.5	90.0	90,0
51	6.33	≤ 1.0	≤ 1.0	≤ 1.0	84.2	84.2	84.2
56	9.76	≤ 1.0	≤ 1.0	≤ 1.0	89.7	89.7	89.7
57	1.66	≤ 1.0	≤ 1.0	≤ 1.0	39.9	39.9	39.9
60	4.90	≤ 1.0	≤ 1.0	≤ 1.0	79.6	79.6	79.6

Notes: Intermittent household slow sand filter with 4 h feeding interval (I-HSSF(4h)); intermittent household slow sand filter with 12 h feeding interval (I-HSSF(12h)) and continuous household slow sand filter (C-HSSF); not applicable (N/A).

For all filtered samples, cyanotoxin concentrations higher than 1.0 µg L^{-1} occurred only in four episodes. Two of those episodes happened for C-HSSF, on the first day of operation (2.70 µg L^{-1}) and on the 20^{th} day (1.11 µg L^{-1}). That might show that C-HSSF *schmutzdecke* development required at least 20 days to biodegrade the influent microcystin-LR to a concentration below 1.0 µg L^{-1}. The same behaviour was not observed in samples from I-HSSF. I-HSSF presented residual microcystin-LR concentrations ≤ 1.00 µg L^{-1} since day one for the majority of both pause period samples (Figure 5). I-HSSF(4h) presented microcystin-LR concentration higher than 1.0 μg L^{-1} on the 37th and 47th days of operation (1.12 μg L^{-1} and 2.67 μg L^{-1}, respectively). Results show that I-HSSF(12h) presented the most stable behaviour with residual microcystin-LR in filtered waters ≤ 1.0 μg L^{-1} throughout the whole operation. Moreover, greater stability can be observed in C-HSSF results when compared to I-HSSF(4h). While C-HSSF higher microcystin-LR concentration episodes happened at the beginning of the operation, which was expected since *schmutzdecke* was not well developed, I-HSSF(4h) higher concentration episodes happened days later. The reduced time between feedings, which resulted in no pause periods, could be the reason for I-HSSF(4h) instability in the aforementioned case.

Studies with laboratory biological sand filters found that the time required for the biolayer to develop on a sterile sand and efficiently remove microcystin-LR could vary from 7 months (Wang et al., 2007) to only 4 days (Ho et al., 2007, 2006), in similar conditions such as sand columns, microcystin analogues and water source . The authors from the aforementioned studies inoculated microcystin in water from a treatment plant with low organic matter concentration, likewise the studied water from the present study. The wide range of time between the studies are attributed to grain size and extracellular polymeric substances in the sand that could have been facilitated bacteria attachment (Wang et al., 2007). Therefore, even with a slow *schmutzdecke* development, exposure to the cyanotoxin since day one could have led to the rapid formation of an acclimated microbiological community able to degrade microcystin-LR, both in C-HSSF and in I-HSSF. In the first days of operation, however, the biodegradation processes were not well established, since *schmutzdecke* was yet at its earlier stages of development. Hence, biological activity cannot have been responsible for cyanotoxin removal at the beginning of operation.

Although no correlation between cell density and microcystin-LR concentration was found, the main hypothesis is that cell retention compensates the absence of biodegradation at the beginning of the experiment in the I-HSSF case. According to WHO (1999), more than 90% of the microcystins in healthy cyanobacterial populations, *i.e.* cyanobacterial culture which is not in stages of ageing or population decline, are intracellular. Hence, cell retention is one of the main removal process in slow sand filters when fed with water containing health cyanobacteria (Chorus et al., 2003; Grützmacher et al., 2002). Considering that studied water was composed of well water inoculated with *M. aeruginosa* culture, it is probable that the majority of influent microcystin-LR was intracellular, therefore, able to be removed by cell retention. A less likely hypothesis is that extracellular microcystin-LR and the sand´s first contact resulted in a sorption process, removing the cyanotoxin from water. Even though physical processes were considered negligible when compared to microcystin biodegradation (Grützmacher et al., 2005, 2002; Ho et al., 2007; Somdee et al., 2014), this explanation for microcystin-LR removal through clean sand was proposed (Grützmacher et al. 2005). Adsorption on the HSSF body might also have happened as a result of PVC capacity to adsorb microcystin (Codd and Bell, 1996).

The accumulation of toxic cyanobacteria cells inside the filter media, due to cell retention, can be a concern in the long term. Cyanobacteria can release intracellular compounds and increase cyanotoxin presence in filtered water. During operational time, no increase in the microcystin-LR concentration was observed in the filtered water, which is strong evidence of the biodegradation processes in the filters. Biodegradation was proposed by Jones and Orr (1994) and may be the key for the treatment. According to the authors, microcystin biodegradation was bi-phasic, indicating the action of at least two different bacteria strains. He et al., (2016) indicated that bacteria capable of degrading microcystin were described in the literature and currently belong to two phyla: Proteobacteria and Actinobacteria (e.g. *Sphingomonas*, *Sphingomonas*, *Sphingopoxyis*, *Methylobacillus, Arthrobacter*, *Brevibacterium* and *Rhodococous*). Moreover, microbiological communities with previous contact with microcystin can efficiently and quickly degrade this toxin (Christoffersen et al., 2002). Although there are few studies focusing on the HSSF efficiency to remove cyanobacteria and cyanotoxins, it is expected that field studies might present promising results, similar to those obtained in our laboratory study. However, further testing is needed to assess the safety of filtered water over time as a function of the use of natural sources.

As already mentioned, biological activity is one of the main responsible aspects for the degradation of microcystin in slow sand filtration and in HSSF (Bojcevska and Jergil, 2003; Bourne et al., 2006; Chorus et al., 2003; Eleuterio and Batista, 2010; Grützmacher et al., 2005, 2002, Ho et al., 2007, 2006; Somdee et al., 2014). In field studies, Chorus et. al. (2003) evaluated the removal of microcystins during the sediment passage in bank filtration and slow sand filters. Although the authors expressed concerns about the biodegradation, which should be studied, they considered sand and soil filtration as promising treatments for microcystin removal. Furthermore, the main removal mechanisms proposed were the retention of intracellular microcystin through physical filtration (cell retention) and biodegradation of the released extracellular microcystin (Chorus et. al. 2003). Additionally, technologies not related to sand/soil passage also highlighted the crucial role of biodegradation in the treatment of microcystin in water. Wang et al., (2007) proposed a dual removal mechanism in granular activated carbon (GAC) filters in two phases: phase I (adsorption) and phase II (adsorption and biodegradation), in which biodegradation is the major removal mechanism in phase II. GAC columns were inoculated with biofilm from a conventional

GAC filter and the biodegradation indicated to be dependent on the initial concentration of bacteria inoculated that degraded microcystin and temperature. Gravity driven membrane (GDM) filters, which are low cost technologies as the HSSFs, also showed potential to remove microcystin by biodegradation (Kohler et al., 2014; Silva et al., 2018). Although the mechanisms of degradation were not the objective of our study, it is believed that biodegradation might be the main removal process that took place in our HSSFs, besides cell retention.

It should be noted that low organic matter concentration might influence cyanotoxin removal by biodegradation, considering that other carbon sources would compete with microcystin-LR as substrate, thus biodegradation could be significantly reduced (Eleuterio and Batista, 2010). Eleuterio and Batista (2010) affirmed that the microcystin-LR removal by drinking water biofilters depends on cyanotoxin concentration and organic matter availability. In our study, TOC removals (Table 2) were 11.46% for C-HSSF and 2.55% for I-HSSF(12h). On the other hand, I-HSSF(4h) presented a 51.57% increase in organic matter when compared to the studied water. An increase in organic matter concentrations might have been a result of *M. aeruginosa* lysis inside the filters, which generated solubilisation of intercellular composts, other than microcystin, that were not completely biodegraded, which are present in filtered water. However, according to the Kruskal-Wallis test, there was no significant difference between TOC in filtered samples and studied water ($p = 0.1213$).

HSSF turbidity removal rates were 81.41 ± 17.03 % to I-HSSF(4h), 83.78 ± 13.60 to I-HSSF(12h), and 84.39 ± 8.85 % to C-HSSF (Table 2). Average removal rates were not as high as reported in the literature, which could be attributed to low turbidity in the studied water (1.05 to 4.36 NTU, which eliminated the need to use a pre-treatment and justified the long operational time without needing maintenance). Despite low removal rates, I-HSSF(4h) presented water turbidity below 1.0 NTU in 95.24% of the samples. Filtered water turbidity below 1.0 NTU in 95% of the samples is associated with a 1-2 log reduction of viruses and 2.5-3 log reduction of *Cryptosporidium* (WHO, 2017). For I-HSSF(12h) and C-HSSF, 98.14% and 100% of the samples presented turbidity below 1.0 NTU, respectively. Figure 6 presents turbidity of studied water and filtered water over time. Filtered water presented some instability on the first 20 days, a period in which results over 1.0 NTU were registered. After that, residual turbidity was more stable for both filters, with eventual peaks. Although there were no evident turbidity decreases in filtered water over time, there was a slight increase in removal efficiencies, which was more evident for I-HSSF. On the first 20 days, in addition to higher instability, lower removal rates were observed when compared to the rest of the operation time for both filters. Removal efficiencies were not significantly different between filter samplings according to the Kruskal-Wallis test ($p = 0.2753$).

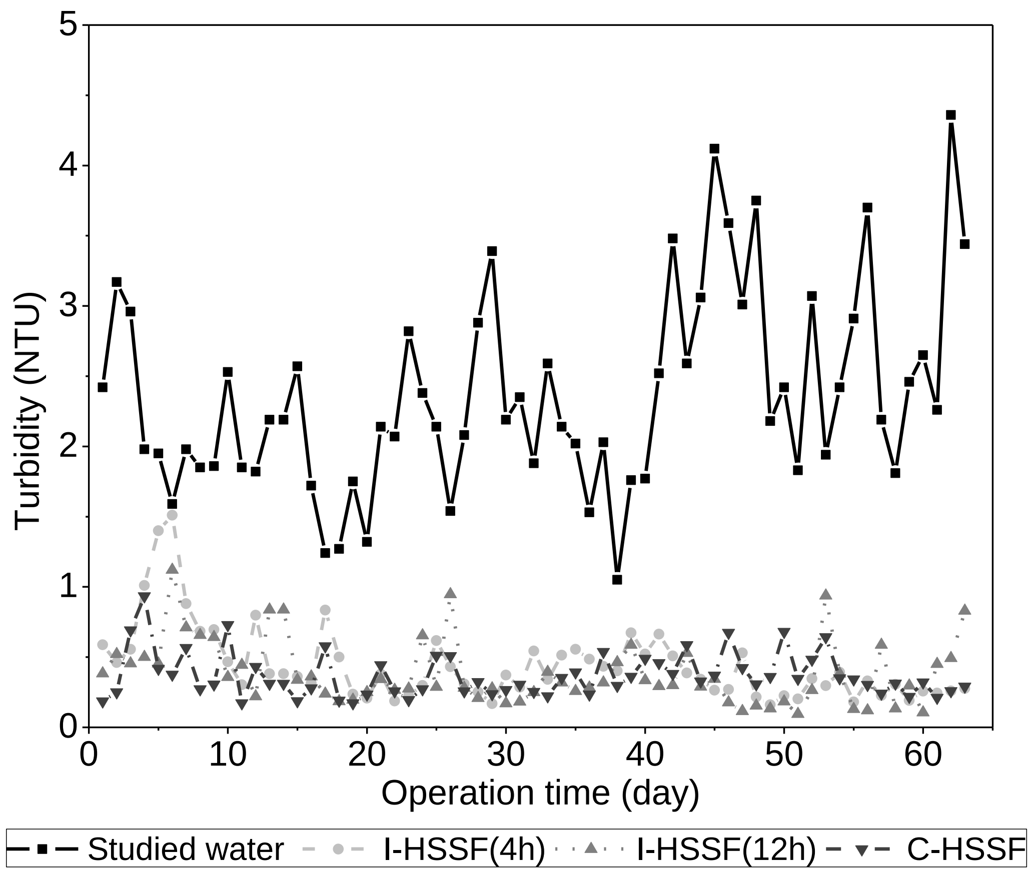

Figure 6 – Turbidity values in studied water and filtered water from intermittent HSSF with 4 h feeding interval (I-HSSF(4h)), intermittent HSSF with 12 h feeding interval (I-HSSF(12h)) and continuous HSSF (C-HSSF) over time.

The studied water´s mean apparent colour was already below the recommended WHO limit values (15 uH), and therefore all the samples presented an apparent colour within the WHO recommendations for human consumption. Removal rates were 91.57 ± 12.23 % to I-HSSF(4h), 95.48 ± 8.15 % to I-HSSF(12h) and 96.37 ± 6.80% for C-HSSF (Table 2). There was a significant difference between the apparent colour in filtered water ($p = 0.03414$) according to the Kruskal-Wallis test. The Mann-Whitney test showed that C-HSSF and I-HSSF(4h) presented significantly different residual apparent colour ($p = 0.01642$). I-HSSF(12h) results were not significantly different from the other samplings. *M. aeruginosa* was mainly responsible for attributing colour to studied water. Therefore, cell retention within HSSF was mainly responsible for apparent colour removal rates. During *M. aeruginosa* growth, a strong correlation was found between the apparent colour and cell density. However, the application of the physical-chemical parameter as an *M. aeruginosa* indirect measure could be subject to interference. Additionally, complete apparent colour removal was observed for 49.21% of I-HSSF(4h) samples, 65.08% of IHSSF(12h) samples and 68.25% of C-HSSF samples.

Regarding the microbiological risk, a low total coliform presence was detected both in the studied water and in the filtered water. *E. coli* was not detected in any sample. It was observed that total coliforms increase in filtered waters from I-HSSF, 0.041 log to I-HSSF(4h) and 0.107 log to I-HSSF(12h); and C-HSSF presented total coliform removal of 0.222 log (Table 3). HSSF coliform removal largely depends on *schmutzdecke* development (Elliott et al., 2008; Jenkins et al., 2011). Slow maturation may be responsible for the low removal rate to C-HSSF and also increases observed in the I-HSSF results. Even when there was an absence of total coliforms in the studied water, filtered samples showed the presence of the microorganism in both filters (Figure 7).

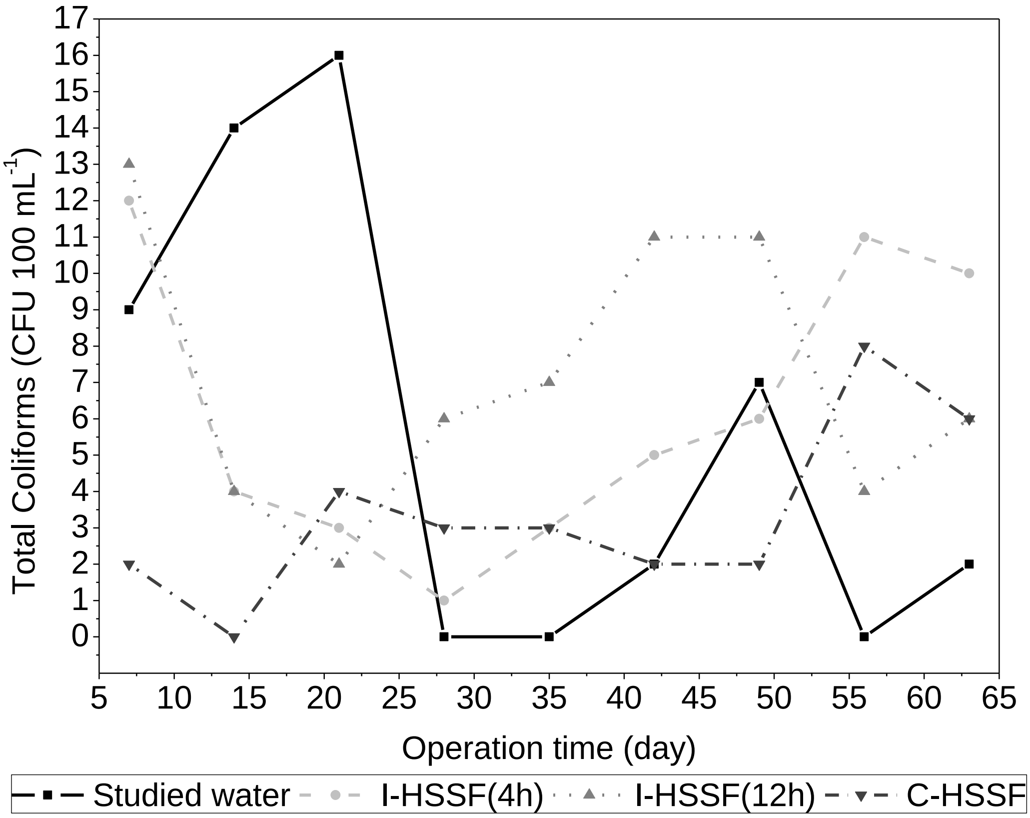

Figure 7 - Total coliforms in studied water and filtered water from intermittent HSSF with 4 h feeding interval (I-HSSF(4h)), intermittent HSSF with 12 h feeding interval (I-HSSF(12h)) and continuous HSSF (C-HSSF) over time.

This result could be related to coliform accumulation within the filter media. Without a biolayer developed enough, the accumulation was followed by an eventual breakthrough. Hence, a longer operation could improve total coliform removal (Elliott et al., 2015; Faria Maciel and Sabogal-Paz, 2018). The relationship between the filtration rate and coliform breakthrough is reported in the literature. Higher filtration rates are related to greater presence of faecal coliforms in HSSF filtered water (Elliott et al., 2008). I-HSSF samples were collected right after feeding when the filtration rate was at its highest (2.95 m^3 m^{-2} day^{-1}). The C-HSSF filtration rate remained constant, 1.22 m^3 m^{-2} day^{-1}, less than half of the I-HSSF filtration rate, which explains the different results between the filters.

Schmutzdecke microscopy analyses showed an apparent predominance of microorganisms morphologically similar to *M. aeruginosa* for both I-HSSF and C-HSSF (Figure 4). In addition to *M. aeruginosa*, microorganisms were identified morphologically similar to *Vorticella* sp, Rotifers, Nematodes and algae, among others, for both I-HSSF and C-HSSF. Although I-HSSF presented a greater diversity of microorganisms, the C-HSSF biolayer was more developed. *Vorticella* sp and Nematodes presented greater apparent predominance after *M. aeruginosa*. The presence of *Vorticella* sp and Rotifers are of special interest as they were reported as predators of the protozoa *Giardia* and *Cryptosporidium* (Bichai et al., 2014; Siqueira-Castro et al., 2016). Microorganism development was not confined to the felt blanket surface which was also present, to a less extent, in the filter media surface. Maintenance would not be limited to the felt blanket washing; the filter media top layer would also need maintenance. However, the felt blanket evidently reduced the biolayer formation in the sand surface, which can considerably simplify filter cleaning in events of clogging.

Figure 8 shows SEM micrographs of the felt blanket prior to and after using it in both filters. Fibre overlay is evident after 63 days of operation. The adhered material is expected to be composed of extracellular polymeric substances (Lewandowski and Boltz, 2011) and trapped materials. C-HSSF presented greater fibre coverage than I-HSSF, which can be related to C-HSSF´s more robust *schmutzdecke*. I-HSSF higher filtration rate, thus higher shear forces, could have led to greater biofilm detachment when compared to C-HSSF.

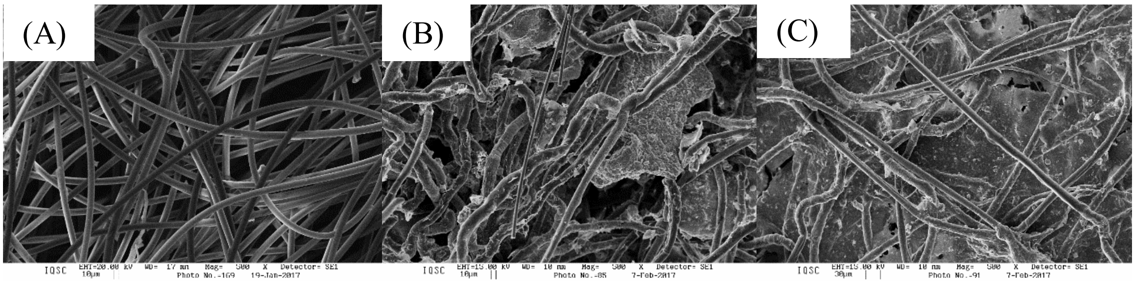

Figure 8 - Felt blanket SEM photomicrographs after 63 days of operation. (A) Clean felt blanket 500x; (B) Intermittent HSSF felt blanket 500x after 63 days of operation; (C) Continuous HSSF felt blanket 500x after 63 days of operation.

Results from acute ecotoxicological essays using *C. xanthus* larvae showed that both filters presented mortality rates of 5.6 ± 0.58 % in the first test (14 days of operation). This corresponds to the death of one of the 18 larvae used. In the first assay, the control test also showed a mortality rate of 5.6 ± 0.58 %; however, it was the result of one larva developing into the pupa stage and dying probably because of the lack of space to complete its life cycle. In the second test (63 days of operation), I-HSSF presented a mortality rate of 5.6 ± 0.58 % while C-HSSF presented 0% mortality. Despite the satisfactory results, further studies within this scope are required for more robust determination as to the safety of the filtered water. Chronic effect tests, for example, would be a great contribution since the adverse effects of long-term exposure to microcystin are commonly reported (Rastogi et al, 2014).

4. Conclusions

- Full-scale HSSF presented promising results in the removal of *M. aeruginosa* cells and microcystin-LR, with the majority of filtered water samples presenting residual microcystin-LR concentrations below 1.0 µg L^{-1}.

- Despite the slow development of *schmutzdecke*, both filters produced water with microcystin-LR below 1.0 µg L^{-1} in the first days of operation.
- Cell retention within filter media and biodegradation were the most likely mechanisms acting on microcystin-LR removal.
- Operational differences related to continuous and intermittent operation did not influence filter efficiencies, neither did distinct pause periods or filtration rates.
- Further research is recommended to better understand HSSF behaviour when fed with water containing *M. aeruginosa* cells and microcystin-LR, especially with natural surface waters, in a long-term operation and after events such as maintenance and flow stoppages.
- Mechanisms involved in the removal of both cyanobacteria and cyanotoxin (intra and extracellular) by HSSF (*i.e.* contributions of sand sorption and cellular retention) still need specific studies.

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Statement

The 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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