

Ripening of household slow sand filter by adding fish food

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Abstract

Vulnerable communities can improve their life quality using point-of-use water treatment technologies. Among these technologies, household slow sand filters (HSSF), which are filters adapted to domestic operations, stand out as one of the most effective and sustainable alternatives. However, some technical issues are not fully understood, such as the ripening process, which may take a long time to happen. In this context, this research evaluated the performance of a HSSF, in real scale and operated in continuous flow when a source of nutrients (fish food) was added to influent water, as a potential ripening agent. Physicochemical and microbiological parameters were evaluated to estimate the filter efficiency. According to the results, the HSSF reached a partial ripeness level in a short time with target parameter reduction in filtered water. Nevertheless, the instability observed in the filtered water quality reveals the significant health risks associated to human consumption when the HSSF is not yet ripened.

Keywords Biological maturation; biosand filter; drinking water; slow sand filtration; tracer tests; treatment

INTRODUCTION

Despite the progress made in water and sanitation services over the last decades, deficiencies related to equality and accessibility persist affecting populations all over the world. For instance, approximately 840 million people still lack access to basic drinking water services (WHO & UNICEF 2017). Ingestion of water contaminated by faecal matter cause almost 4 billion cases of diarrhoeal disease per year, from which 1.8 million are fatal (UNEP 2016). Further global data show the high level of exposition to inappropriate water sources and highlight the vulnerability of low- and middle-income countries to waterborne disease (WHO 2016).

While reliable, safe, and piped water is not accessible to every household, temporary actions, such as household water treatment and safe storage (HWTS) are needed to reduce waterborne diseases (WHO 2012). In this context, biosand filters (BSFs) or household slow sand filters (HSSF) stand out as one of the most promising alternatives due to their affordability, simplicity, and efficiency (Sobsey *et al.* 2008; Sabogal-Paz *et al.* 2020). The benefits derived from using these filters can be seen in studies in real-world implementations, where diarrhoea and other gastroenteritis symptoms have been drastically reduced (Stauber *et al.* 2012; Sisson *et al.* 2013).

Some questions about this technology remain unclear, such as those related to the biological processes responsible for water purification (Haig *et al.* 2015) and those linked to the maturing process (Palmateer *et al.* 1999). Ripening of sand beds is a critical factor influencing particle and microorganism removals in slow sand filters (SSF). A filter is 'mature' when coliform removal reaches its optimum level (Barrett *et al.* 1991). The development of a biologically active layer on the top of the filter media (*schmutzdecke*) is considered as one of the main processes responsible for parasites and particle removal. The low filtered water quality obtained before filter ripening and development of this biological layer, which may take about 30 days or more (Elliott *et al.* 2008; CAWST 2012), can increase health risks and may affect the user's acceptance of the technology as a result of waiting a long time for drinking water.

Studies have been conducted aiming to optimise the HSSF efficiency through design and operational modifications (Young-Rojanschi & Madramootoo 2014; Young-Rojanschi & Madramootoo 2015; Maciel & Sabogal-Paz 2018). Regarding operations, some works have confirmed that although HSSFs were developed for intermittent operations, a significantly better performance may be reached in a continuous operation, with higher reductions of microbial indicators (e.g. *Escherichia coli* and bacteriophage MS2) and turbidity (Young-Rojanschi & Madramootoo 2014; Maciel & Sabogal-Paz 2018).

A few works have focused on identifying substances that contribute to SSF ripening and its enhancement (none related to HSSFs specifically). There is evidence that constituents in raw water play a key role in enhancing the performance of SSFs (Weber-Shirk & Dick 1997; Jellison *et al.* 2000; Weber-Shirk 2002; Weber-Shirk & Chan 2007; Arora 2017). For instance, the biological ripening of SSFs and the *schmutzdecke* development are associated with the concentration of bacteria, biodegradable dissolved organic carbon and nutrients in the influent water (Weber-Shirk & Dick 1997; Weber-Shirk & Chan 2007; Arora 2017).

This suggests that a simple, low-cost method, which introduces a small amount of essential nutrients, can speed up the maturing process and consequently improve HSSF performance in its first days of operation (Arora 2017). In this context, exploratory research for a ripening agent focused on products that potential users (inhabitants of rural areas) could find and buy locally, and fish food emerged as a possible solution. This study evaluated the effect of adding fish food to the influent in order to rapidly mature an HSSF, constructed on a full

scale and operated in continuous flow mode. Fish food was selected mainly because it is cheap, accessible to rural communities and has a reasonable amount of organic matter, and macro and micro-nutrients, which may collaborate to the biological ripening process.

METHODS

Filter design

A HSSF was built using PVC pipes and fittings, which can be found in plumbing suppliers (Figure 1). The sand layer was 55 cm in thickness (effective size = 0.23 mm, uniformity coefficient = 2.0, and porosity = 36%). The support layer required a thickness of 18 cm comprising coarse sand (size range 1-2 mm) and gravel (size range 6-12 mm) with porosity of 37%. In addition, a non-woven synthetic fabric (thickness 2.8 mm, 25 μm fibres, 100% polyester, and 0.20 g/cm^3) was installed on the top sand layer.

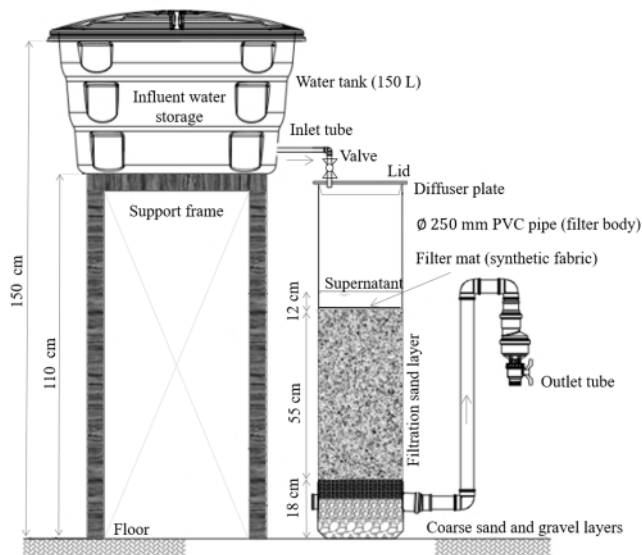


Figure 1. HSSF researched in continuous flow.

Filter operation

The HSSF was operated continuously with decreasing filtration rates caused by the water head variation in the water feed tank. The filtration rate was checked once a day and it was controlled by using a ball valve in the influent water tube. The maximum and minimum filtration rates were 0.08 $\text{m}^3/\text{m}^2\text{h}$ and 0.04 $\text{m}^3/\text{m}^2\text{h}$. This adopted range was based on the typical values (0.10 to 0.40 $\text{m}^3/\text{m}^2\text{h}$) used in conventional SSFs and considering Huisman and Wood's suggestions (Huisman & Wood 1974) of using reduced filtration rates during the filter ripening period. The average daily filtered volume was 70 L. The supernatant (standing water zone) was kept between 10 and 12 cm thickness as a result of the filter design, operation and hydraulic losses. The total water volume inside the HSSF during its operation was estimated in 20.34 L, including the outlet tube (1.37 L), gravel layer pores (3.25 L), sand layer pores (9.83 L) and supernatant (5.89 L).

Residence distribution time (RDT)

Three tracer tests were carried out to evaluate the water flow regime in the filter media (two at the beginning of the filter operation and one at the end). The tracer input followed the step injection method. In each tracer test, 300 L of sodium chloride solution (200 mg/L) was prepared, which continuously fed the filter until the outlet concentration was constant. An electrical conductivity probe was used to measure salt concentrations every minute. The tracer tests were performed using filter rates between 0.08 m³/m² h and 0.06 m³/m² h.

Ripening agent dosages

Fish food (Pirá Alevino 55, Guabi®), containing 55% protein level for the juvenile fish stage, was used as a potential ripening agent in this exploratory study, which aimed to evaluate the benefits and risks associated with this product (the fish food composition is shown in the Supplementary Material). The first step was to define the dosage range to be tested in the HSSF performance evaluation. Preliminary tests were conducted to verify the fish food effects in the water quality. Nitrate, nitrite and pH were measured for each dosage according to APHA *et al.* (2012). Based on the results and drinking-water quality guidelines (WHO 2017), the dosages to be used were defined.

HSSF performance evaluation – first phase

The first phase was developed to evaluate the fish food dosage, based on the evaluation of physico-chemical parameters in the filtered water and on the microbiological analysis of the non-woven fabric used on the top sand layer. The studied water was a mixture of 400 L of well water (turbidity ≤ 0.5 NTU) and kaolinite solution, presenting a final turbidity of approximately 25 NTU. The kaolinite solution was obtained from the supernatant formed, 24 h after mixing, in six beakers in which 43 g of kaolinite were mixed in 2 L of water during 120 min at a velocity gradient of 200 s⁻¹. A new studied water solution was prepared each week and the HSSF operation was conducted over one week for each dosage, with a water tank recharge every 24 h.

At each operation day, a known fish food mass was added in the filter supernatant water. Water samplings were taken daily from the influent, supernatant and filtered water in 200 mL plastic bottles. The intervals between samplings varied between 20 to 28 h. The parameters analysed were turbidity, apparent colour, electrical conductivity, pH, and nitrate according to APHA *et al.* (2012). After each weekly operation, the non-woven synthetic fabric was removed and examined by an optical microscope (Olympus BX-60) aiming to identify any microbial communities.

HSSF performance evaluation – second phase

The second phase lasted twelve days (purposefully a short period to evaluate maturation) and the fish food dosage used was the best obtained in the first phase. HSSF influent water was a mixture of 400 L of well water and 20 L of domestic wastewater filtered using cotton cloth. Domestic wastewater was added to simulate extremely contaminated water sources, which is a possible scenario in rural areas without a proper sewage system; therefore the ripening agent was tested in the most critical situation.

A new influent water solution was prepared each week. The HSSF operation and fish food additions were carried out as in the first phase. Samples were analysed daily, collected from both the supernatant and filtered water in 400 mL plastic bottles. The sampling interval varied between 23 to 25 h. The physico-chemical parameters analysed were the same as those presented for the first phase operation. Additionally, total coliforms and *Escherichia coli* were monitored as indicators of the ripening process and the Colilert method (IDEXX Quanti-Tray, Westbrook, ME) was used to determine their concentration.

At the end of the second operation phase, the non-woven synthetic fabric and the two samples (approximately 50 g each) of the filter media sand, collected at depths less than 5 cm, were analysed using an optical microscope, previously described.

Filter cleaning procedures

At the end of each week, the synthetic fabric used on the top of the sand layer was replaced, and the HSSF was cleaned and disinfected. To do this, the supernatant was reduced from 12 cm to 2 cm high. Subsequently, 50 mL of a sodium hypochlorite solution (concentration of 10%) was left in contact with the sand layer, slightly decompressed, during 24 h. Afterwards, the HSSF operated over three consecutive days using well water as the influent, at a maximum filter rate of 0.08 m³/m² h, aiming to completely remove the sodium hypochlorite solution from the filter.

RESULTS AND DISCUSSION

Tracer tests

RDT functions, experimentally determined, are presented in Figure 2, allowing direct comparison between the different tests carried out.

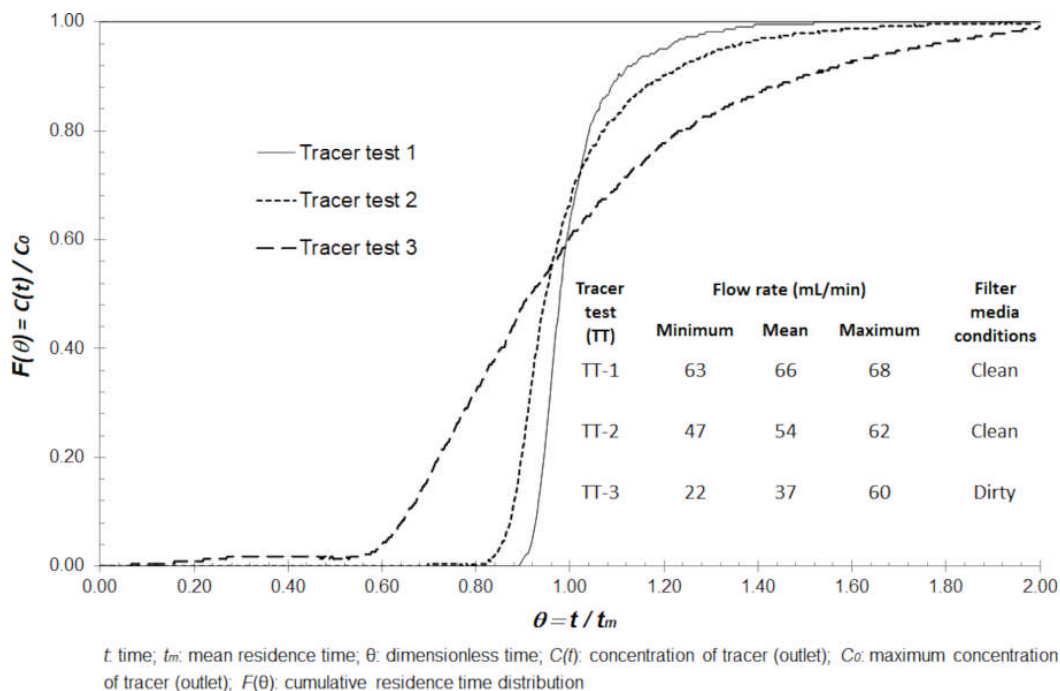


Figure 2. Experimental cumulative residence time distributions (RDT) resulting from the tracer tests.

The mean residence time (t_m) determined for each test was $t_{m1} = 5.47$ h; $t_{m2} = 6.71$ h and $t_{m3} = 8.03$ h. The effective unit volumes (EV) and the Morrill dispersion index (MDI) were calculated for the three tests, leading to the following results: $EV_1 = 21.6$ L, $EV_2 = 21.8$ L and $EV_3 = 17.8$ L and $MDI_1 = 1.18$, $MDI_2 = 1.37$, $MDI_3 = 2.29$, respectively. The tests carried out with the clean filter media (beginning of the filter operation) indicated a behaviour closer to an ideal plug flow reactor (MDI closer to 1), when compared to the test conducted at the end of the filter operation ('old filter media'). This finding should be considered to optimise the filter operation according to expected changes in residence time (Young-Rojanschi & Madramootoo 2015).

Fish food dosage definition

Tests were carried out with the different fish food concentration mixtures. Organic matter decomposition and the nitrification process associated with higher fish food concentrations generated reductions in the pH value (from 7.3 to 5.7 as the concentration increased from 0.1 to 10 g/L). Nitrite concentration was up to 20 $\mu\text{g/L}$, which is coherent with the high instability of this ion in water (Murphy *et al.* 2010). Considering the accumulation of fish food on the non-woven filter fabric and based on the possibility of the influent water to contain a high concentration of nitrate, the daily fish food dosages of 0.10, 0.25, and 0.50 g/day were defined to be tested in the first phase of the HSSF operation.

Performance evaluation – first phase

Table 1 presents the monitoring water quality during a period of five days for each daily fish food dosage.

Table 1. Water quality parameters obtained over five days of operation for each daily fish food dosage (temperature of water samples was 26.0 ± 2.6 °C). The paired sample t-test was used to determine p-values.

Dosage (g/day)	Parameter	Influent water (R)	Supernatant water (S)	Filtered water (F)	Statistically significant difference (SS) when p-value < $\alpha = 0.05$		
					R compared to S	S compared to F	
0.10	Turbidity (NTU)	8.2±3.2	8.5±2.5	3.0±0.8	p=0.626	p=0.000	SS
	Apparent colour (mg Pt-Co/L)	9.4±4.5	9.8±3.9	3.0±1.8	p=0.456	p=0.000	SS
	Electrical conductivity ($\mu\text{S/cm}$)	51.9±1.2	50.8±0.8	51.2±1.0	p=0.024	SS	p=0.755
	Nitrate ($\mu\text{g/L}$)	125.8±16.1	135.6±25.1	128±66	p=0.209		p=0.472
	pH	7.1±0.4	6.9±0.6	7.6±0.1	p=0.503		p=0.068
0.25	Turbidity (NTU)	11.2±1.0	11.4±1.3	2.7±0.5	p=0.089	p=0.000	SS
	Apparent colour (mg Pt-Co/L)	20.2±7.6	20.4±6.4	4.2±1.4	p=0.689	p=0.000	SS
	Electrical conductivity ($\mu\text{S/cm}$)	49.2±2.0	50.4±2.3	52.8±1.0	p=0.141		p=0.152
	Nitrate ($\mu\text{g/L}$)	101.3±56.1	80±56	163±82	p=0.647		p=0.114
	pH	7.0±0.2	7.2±0.3	7.7±0.1	p=0.005	SS	p=0.001
0.50	Turbidity (NTU)	16.1±4.6	15.2±5.0	4.7±2.3	p=0.083	p=0.000	SS
	Apparent colour (mg Pt-Co/L)	24.9±2.5	23.4±3.5	5.8±3.2	p=0.026	SS	p=0.000
	Electrical conductivity ($\mu\text{S/cm}$)	50.0±1.0	48.0±1.9	49.7±1.9	p=0.052		p=0.090
	Nitrate ($\mu\text{g/L}$)	302±44	282±62	136±71	p=0.173		p=0.000
	pH	7.0±0.2	7.4±0.3	7.7±0.2	p=0.034	SS	p=0.049

Turbidity and apparent colour were reduced, however there was an increase in the pH and the electrical conductivity remained stable. Nitrate concentrations did not increase after fish food was added. Since most of the observed data approximately followed a normal distribution (Anderson-Darling normality tests verified this condition), a paired t-test (Minitab® Statistical Software) was used to determine whether there were statistically significant differences between the observed water quality parameters when comparing influent, supernatant and filtered water. When comparing the dosages, it can be observed that 0.25 g/day resulted in a slightly better performance with respect to turbidity reductions; therefore, this value was assumed in the second phase.

Concerning the microbial community evaluation, the microscopic examinations of the synthetic fabric indicated a predominance of suspended bacterial cells and ciliates, amoebae and nematodes. These findings suggest that some microbial diversity was created in the filter as a result of adding fish food since the influent water was a simple mixture of well water with kaolinite, in which a significant presence of microorganisms is not expected. These results indicate the possible development of conditions for microbiological removal, including the predator-prey relationship between bacteria and protozoa, as reported by Lloyd (1973) and Haig *et al.* (2015).

Although the fish food additions may have contributed to a faster microbial community development, the instability of the effluent water quality was a persistent problem. This means that the mechanisms (biological and/or physico-chemical) were not sufficient to reduce the influent water turbidity over the short operation periods.

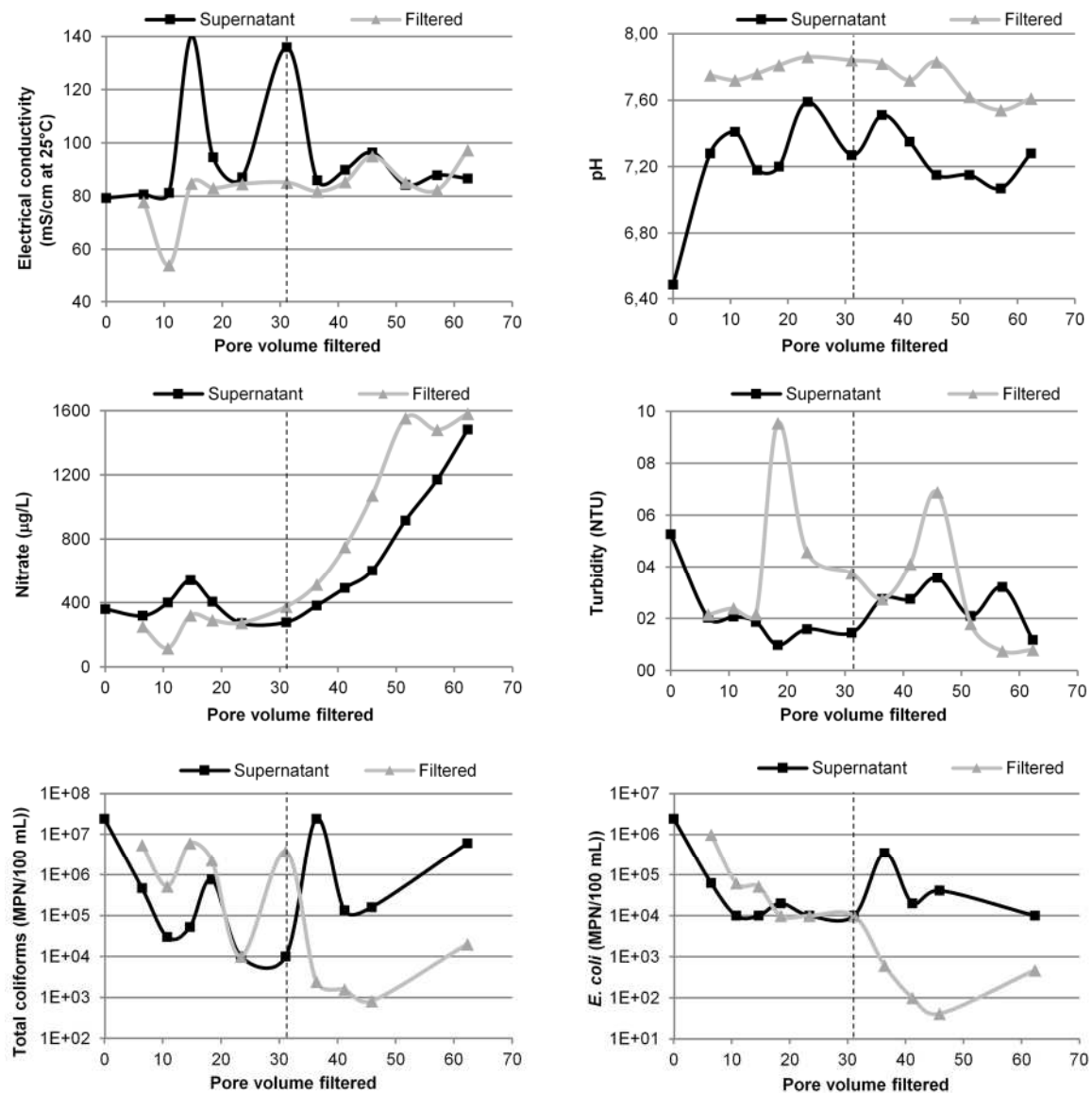
Performance evaluation – second phase

The filter operated continuously for twelve days in the second phase and 0.25 g of fish food was added daily. Figure 3 shows the water quality variation with the pore volume filtered. Electrical conductivity was relatively stable in the filtered water and a few peaks happened in the supernatant water, which may be a result of the organic matter decomposition.

The pH consistently increased similarly to the findings of Young-Rojanschi & Madramootoo (2015). To verify the existence of compounds such as calcium carbonate in the sand, which could explain the pH increase, solubility tests in hydrochloric acid of two sand samples (100 g each, collected at depths less than 10 cm from the top of the sand layer) were conducted at the end of the filter operation. The average solubility was 0.4%, which may corroborate to the hypothesis of leaching from the filter media. Since detailed investigation about this question was not conducted, there is still a need for future studies to better understand this process where pH increase takes place, commonly reported in studies on HSSF (Young-Rojanschi & Madramootoo 2015; Murphy *et al.* 2010).

Nitrate concentrations increased throughout the operation, both in the supernatant water and in the filtered water. This was expected due to the nitrification process, which tends to occur in the water under aerobic conditions (Murphy *et al.* 2010), and due to the short mean residence time (8 h), which does not allow for the occurrence of the denitrification process before the water leaves the filter.

An unexpected pattern was observed with respect to turbidity as shown in Figure 3. During the first days, the filtered water turbidity increased. A possible explanation is that solids adhered to the filter media surface were somehow released to the water during the first hours of operation.



$$\text{Pore volume filtered} = [\text{cumulative filtered volume}] / [\text{pore volume}], [\text{pore volume}] = 13.1 \text{ L}$$

Figure 3. Filter performance adding 0.25 g/day of fish food using highly contaminated influent water during the second phase (water temperature = 24.5 ± 0.5 °C) for twelve sequential days of operation. Vertical dashed lines indicate when the influent water was renewed.

Despite the high removal efficiency obtained at the end of the filter operation, total coliforms and *E. coli* in the filtered water also presented an unstable pattern. The observed peaks for these parameters in the supernatant water (cumulative filtered volume = 475 L, 8th day) happened due to renewing the studied water (influent), prepared with a new volume of domestic wastewater. From this point, it was possible to observe over the following two days that the variation in the effluent water (higher organic and microbiological loads) did not affect the filtered water quality, demonstrating some filter maturity level.

During the first days, the filtered water quality was worse than the influent water in terms of the microbiological parameters. This occurred due to an unexpected fact, which was the presence of total coliforms and *E. coli* in the fish food used. When the fish food was selected in this exploratory study as a potential ripening agent, a possible microbiological contamination was not expected since the manufacturer does not provide any

warning in this sense. The possibility of contamination was only questioned when the filter operation results showed anomalies. Then, it was confirmed in specific tests. A mixture prepared with 0.0042 g of fish food in 100 mL of distilled water (concentration similar to the daily fish food dosage in the supernatant, 0.25 g/5.89 L) presented more than 2.4×10^4 MPN/100 mL of total coliforms and 10 MPN/100 mL of *E. coli*. Hence, the use of this product or similar ones must be evaluated according to the inherent microbiological risk. Although the fish food selection represented a drawback in view of its human health risk, this study brings out the importance and some of the challenges of finding potential ripening agents in HSSFs. Clearly, our results open up possibilities for the water treatment of fish farming and encourage research for other ripening agents for rural communities.

On the best performance day (cumulative filtered volume = 540 L, 9th day), the removals of total coliforms and *E. coli* were 3.7 log and 3.1 log, respectively, which are higher than the values obtained by Stauber *et al.* (2006) for intermittently operated filters. Young-Rojanschi & Madramootoo (2014), who operated the filters continuously, obtained *E. coli* reductions of 3.71 ± 0.59 log in samples collected in the second month of operation. Nevertheless, the high removal efficiencies were not maintained over the following days, showing evidence of the instability of the HSSF behaviour during its ripening period. Regarding the statistical analysis, the Mann-Whitney nonparametric test was used, since most of the observed data in the second phase did not present a normal distribution, and only the parameter pH presented statistically significant results ($p = 0.000 < \alpha = 0.05$) when comparing the supernatant to the filtered water.

The microscopic examinations of the synthetic fabric and of the sand samples indicated an apparently more diverse microbial community than that observed in the previous phase, likely due to the use of domestic wastewater in the studied water (influent) (Table 2). Moreover, amounts of suspended bacterial cells, ciliates, flagellates, rotifers, nematodes and worm eggs observed in the filter fabric were higher than the amounts observed in the sand samples (further details can be found in the Supplementary Material). The results of water quality analysis and microbial colonisation, along with other studies (Weber-Shirk & Dick 1997; Wang *et al.* 2014), may support further understanding of the biological mechanisms responsible for removing particles and pathogens in HSSFs.

Table 2. Comparison of observed amounts of microorganisms found in the non-woven fabrics and sand samples.

Phase	Sample	Observed microorganisms	Quantification
First phase	Nonwoven fabric	Suspended bacteria	++++
		Rhizopoda (Amoebae)	++
		Free-swimming ciliates (like- <i>Tetrahymena</i>)	++++
		Nematode worms	+
Second phase	Nonwoven fabric	Suspended bacteria	++++
		Flagellates	++
		Free-swimming ciliates (like- <i>Tetrahymena</i>)	+++
		Nematode worms	+++
		Worm eggs	+++
		Rotifers	+++
	Sand (1)	Suspended bacteria	+++
		Flagellates	++
		Free-swimming ciliates	+++
		Attached ciliates (like- <i>Vorticella</i>)	+++
		Worm eggs	+++
		Rotifers	++
Sand (2)	Suspended bacteria	++	
	Flagellates	++	
	Rhizopoda -	+	

(Naked Amoebae and Testate Amoebae)	
Nematode worms	++
Worm eggs	+

Note: (+): a few; (++): some; (+++): many; (++++): predominant.

Ripening process

Water quality improvement in terms of turbidity or microbiological parameters indicated partial HSSF ripening. However, the incapacity of reaching stability in a short period indicates a challenge to be addressed in future studies. Since the filter operation was conducted in periods shorter than usual (one to two weeks rather than one or more months) to meet the objectives of our study, the lack of stability indicates an incomplete development of the biological layer. Although water treatment technologies based on slow sand filtration have been widely used for many years, difficulties related to the ripening process acceleration persist, motivated, among other aspects, by the limited understanding of the biological interactions responsible for the water purification (Weber-Shirk & Chan 2007).

Although fish food was found not to be adequate as a ripening agent, as it may contain *E. coli* and indicate a human risk, this study revealed some interesting characteristics of the filter performance over its first days of operation when an intentional and controlled worsening of the influent was provoked. The quantification of similar approaches, suggested by Weber-Shirk & Dick (1997) and Arora (2017) in the slow sand filtration literature, has not been explored in recent HSSF research.

Another important point is that intermittent and continuous operations lead to specific characteristics of oxygen distribution, nutrients supply, and microorganism colonisation. Then, different processes are expected to be associated with the filter ripening, as well as the selection of a suitable ripening agent. Hence, replicating similar experiments with other ripening agents (e.g. a source of nutrients, but without potential pathogens) seems to be a reasonable strategy to be carried out in future studies.

CONCLUSIONS

Adding fish food to the studied water contributed to a fast development of the microbial community on the sand layer and synthetic fabric, suggesting that the addition of nutrients, as a potential ripening agent, can be considered in future investigations. However, it should be noted that the microbiological quality of any selected product should be evaluated before being used in real-scale tests.

HSSF performance evaluation during the first operation days is essential because instabilities in the filtered water quality were observed, showing evidence of possible microbiological risk associated with the filtered water consumption when the filter is not completely ripened.

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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.

SUPPLEMENTARY MATERIALS

Images from the microscopic examinations of the non-woven fabric installed on the top sand layer and sand samples are presented as supplementary material. A table including constituents of the fish food used in the study is also provided.

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Supplementary Material

Figure 1 and Figure 2 present some details and the microscopic examinations regarding the first and second operational phases, respectively. Table 1 presents the constituents of the fish food used in the study.

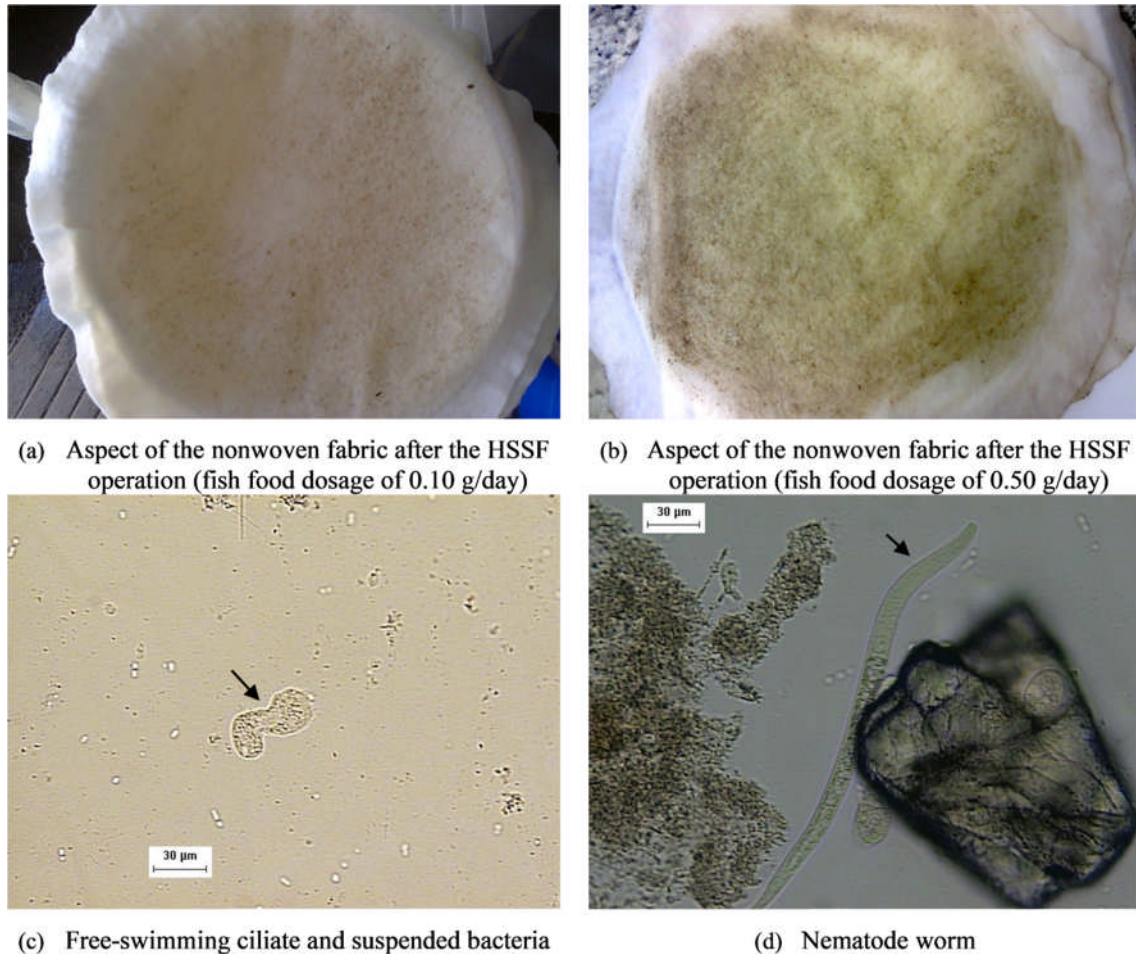
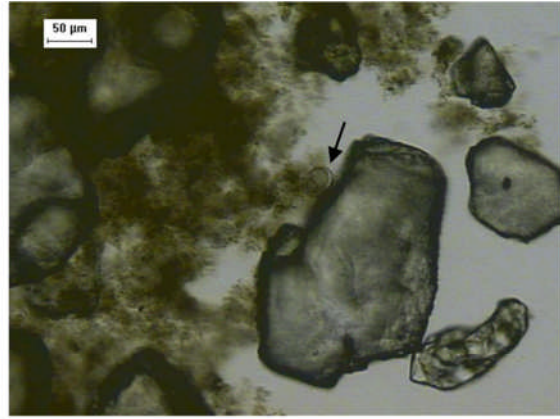


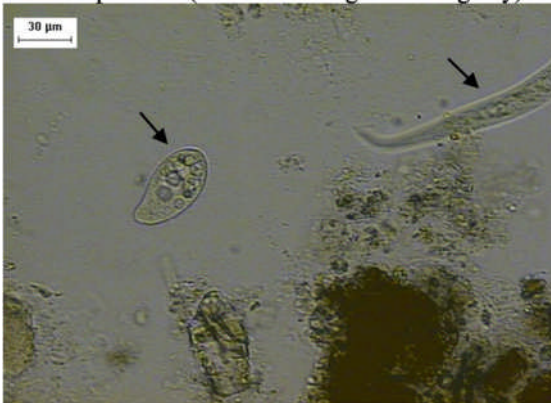
Figure 1 - Synthetic nonwoven fabrics installed on the top sand layer of the household slow sand filter (HSSF) during the first operation phase and some corresponding microscopic examinations



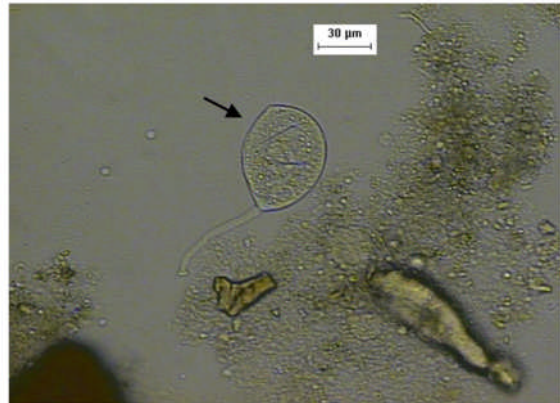
(a) Aspect of the nonwoven fabric after the HSSF operation (fish food dosage of 0.25 g/day)



(b) Grain of sand and worm egg



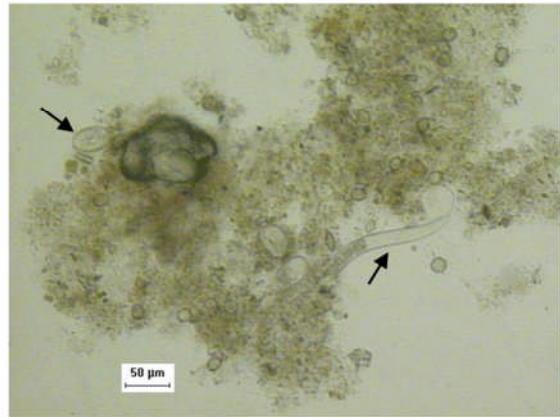
(c) Free-swimming ciliate and nematode worm



(d) Attached ciliate (like-*Vorticella*)

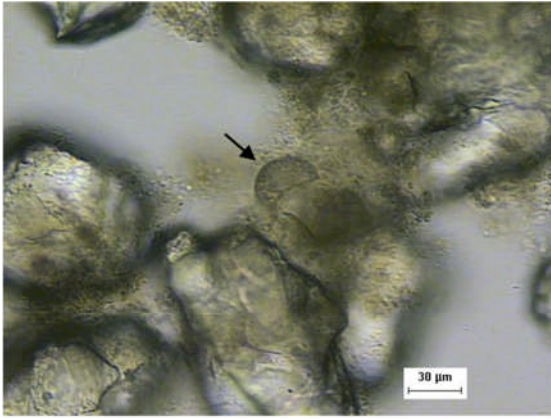


(e) Rotifer

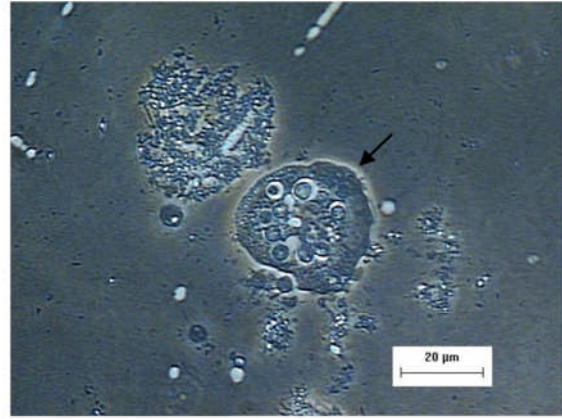


(f) Nematode worm and worm eggs

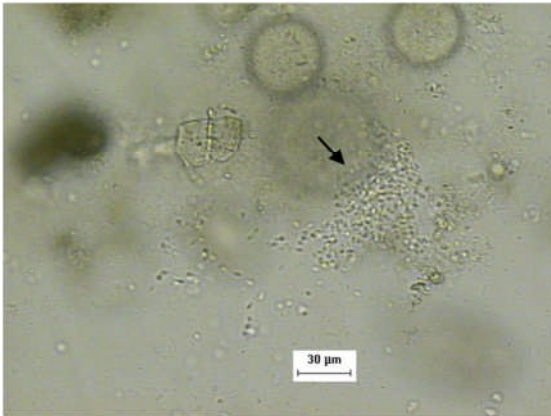
Figure 2 - Microscopic examinations corresponding to the second operation phase (fish food dosage = 0.25 g/d)



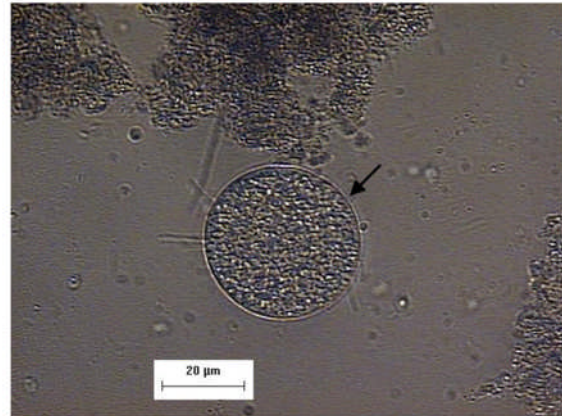
(g) Testate amoebae



(h) Naked amoebae



(i) Flagellates



(j) Protozoan cyst

Figure 2 (continued) - Microscopic examinations corresponding to the second operation phase (fish food dosage = 0.25 g/d)

Table 1. Constituents of the fish food used in the study (Pirá Alevino 55 Guabi ®).

Content	Concentration
Folate (min.)	10 mg/kg
Biotin (min.)	10 mg/kg
Calcium (max.)	38 g/kg
Calcium (min.)	42 g/kg
Copper (min.)	25 mg/kg
Choline (min.)	2,500 mg/kg
Ethereal extract (min.)	70 g/kg
Iron (min.)	150 mg/kg
Phosphorus (min.)	15 mg/kg
Mannan-oligosaccharides (min.)	60 mg/kg
Manganese (min.)	75 mg/kg
Fibrous matter (max.)	28 g/kg
Mineral matter (max.)	170 g/kg
Niacin (min.)	350 mg/kg
Protein (min.)	550 g/kg
Selenium (min.)	1.0 mg/kg
Moisture (max.)	100 g/kg
Vitamin A (min.)	35,000 IU/kg
Vitamin B1 (min.)	25 mg/kg
Vitamin B12 (min.)	100 mcg/kg
Vitamin B2 (min.)	35 mg/kg
Vitamin B6 (min.)	40 mg/kg
Vitamin B6 (min.)	40 mg/kg
Vitamin C (min.)	800 mg/kg
Vitamin D3 (min.)	2,000 IU/kg
Vitamin E (min.)	120 IU/kg
Vitamin E (min.)	120 IU/kg
Zinc (min.)	140 mg/kg

Notes: data informed by the fabricant; IU: International Units. **min.:** minimum; **max.:** maximum