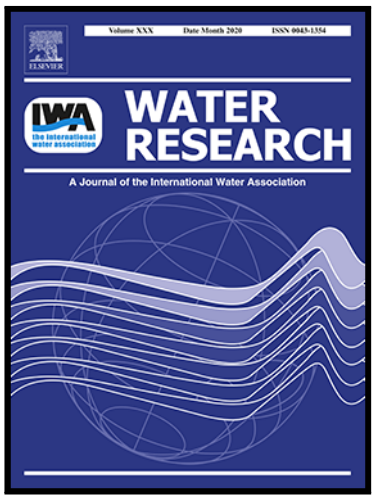

Influence of slow sand filter cleaning process type on filter media biomass: backwashing versus scraping

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Highlights:

- For the first time, a biomass approach was used to investigate backwashed SSF.
- Biomass accumulated differently in scraped and backwashed slow sand filters.
- 16S rRNA sequencing indicated variation of the bacterial community between filters.
- Biomass was better preserved in different trophic levels after backwash.
- The filtrate was of good quality in BFS and ScSF despite their biomass differences.

Influence of slow sand filter cleaning process type on filter media biomass: backwashing versus scraping

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Abstract

Biomass was assessed as a new approach for evaluating backwashed slow sand filters (BSF). Slow sand filtration (SSF) is a simple technology for water treatment, where biological mechanisms play a key role in filtration efficiency. Backwashed slow sand filters were previously recommended for small-scale filters (~1 m² of filtration area) as an alternative to conventional filters that are usually cleaned by scraping (ScSF). Biomass was never evaluated in BSF, which is a gap in the knowledge of this technology, considering the importance of its biological mechanisms. Therefore, for the first time, two filters operating under the same conditions were used to compare the influence of backwashing on biomass; one filter was cleaned by backwashing and the other by scraping. Biomass along the filter media depth (40 cm) was assessed by different techniques and compared in terms of cellular biomass (by chloroform fumigation), volatile solids, bacterial community (by 16S rRNA gene sequencing), and observations by scanning electron and fluorescence microscopy. Filters were also monitored and compared regarding filtered water quality and headloss; their differences were related to the different cleaning processes. Overall, filtered water quality was acceptable for slow sand filter standards (turbidity < 1 µT and total coliform removal > 1 log). However, headloss developed faster on scraped filters, and biomass was different between the two filters. Backwashing did not significantly disturb biomass while scraping changed its surface sand layers. Cell biomass was more abundant and spread across the filtration depth, related to lower headloss, turbidity, and cyanobacterial breakthrough. These results agreed with the water quality and microscopy observations. The bacterial community was also less stratified in the backwashed filter media. These results expand the knowledge of backwashing use in slow sand filters, demonstrating that this process

preserves more biomass than scraping. In addition, biomass preservation can lead to bacterial selectivity and faster filter ripening. Considering the importance of biomass preservation on slow sand filtration and its biological filtration mechanisms, the results presented in this paper are promising. The novel insight that BSF can preserve biomass after backwashing may contribute to increasing its application in small communities.

Keywords: slow sand filtration; biomass; *schmutzdecke*; 16S rRNA gene sequencing; microbial community profile; water treatment.

Abbreviations and Symbols

Bio	Cell biomass
BVK	*Live/Dead® BacLight Invitrogen*™ cell viability kit
BFW	BSF filter effluent
BSF	Backwashed slow sand filter
C	Final concentration
C_0	Initial concentration
d_{10}	Effective diameter
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DOC	Dissolved organic carbon
FM	Filter media
HMDS	Hexamethyldisilazane
LP	*Lagoa do Peri* Lake
OTU	Operational taxonomic unit

PCoA Principal coordinate analysis

RFW Roughing filter effluent

ScFW Scrapped filter effluent

ScSF Scrapped slow sand filter

SEM Scanning electronic microscopy

SSF Slow sand filtration

SUVA Specific Ultraviolet Absorbance

TOC Total organic carbon

UC Uniformity coefficient

URF Upflow roughing filter

VS Total volatile solids

WHO World Health Organization

WTP Water treatment plant

1 INTRODUCTION

Slow sand filtration (SSF) is likely one of the oldest techniques used for water treatment in public water assessment. (Erba et al., 2014; Huisman and Wood, 1974). Nevertheless, it is a technology still used worldwide owing to the high quality filtered water produced (Graham and Collins, 2014). Interactions between the filter's biological community and the physicochemical separation process result in high SSF effluent quality (Gimbel et al., 2006; Nakamoto et al., 2014).

These interactions tend to improve with biomass accumulation and are

responsible for the removal of turbidity and most of the biological pathogens such as bacteria, viruses, and protozoa cysts (Bellamy et al., 1985a; Hijnen et al., 2004; Huisman and Wood, 1974; D. R. McNair et al., 1987; Pizzolatti et al., 2014). This SSF biomass development is related to filtration efficiency and filter operation, especially on the sand surface; however, it is still considered a "black box" for SSF technology (Campos et al., 2002; Graham and Collins, 2014).

In terms of operational impacts, biomass accumulation is related to increasing headloss. At a certain accumulation point, the filter becomes clogged and the *schmutzdecke* layer must be removed by scraping to recover the hydraulic loading (Campos et al., 2002). Furthermore, biomass complexity makes filtration mechanisms difficult to understand and predict, depending on variations in SSF design, operation, and raw water characteristics (Bellamy et al., 1985a; Campos et al., 2002; Huisman and Wood, 1974).

Previous studies have suggested backwashing for SSF cleaning as an alternative to scraping (de Souza et al., 2017, 2016; Michelan et al., 2011; Pizzolatti et al., 2014). The application of backwashing is particularly recommended for medium- and small-scale filters (<1 m^2) that can be easily applied in small and isolated communities or small agroindustry (FUNASA, 2019; Michelan et al., 2011; Pizzolatti et al., 2014). This is because the backwashing operation is simple and lasts only a few minutes, while scraping is laborious and time-consuming.

The upfront economic investment for a backwashed slow sand filter (BSF) is higher than that for a scraped slow sand filter (ScSF), especially because of the valves and backwashing water reservoir. However, less sand can be used because progressive scraping and final re-sanding are not necessary, minimizing costs and sand loss (de

Souza et al., 2016, 2018; FUNASA, 2019; Michelan et al., 2011).

In addition to BSF filtered water quality and operation, there is no specific research regarding the effect of SSF filter media (FM) fluidisation on biomass development, an important feature of SSF mechanisms. Studies on biomass in backwashed biofilters have diverged on the influence of backwash on biomass, evidence on their complexity and dependency on filtration operational aspects, and filtration media. Previous BSF studies have reported differences in headloss behaviour compared to ScSF and, in some cases, lower effluent quality. They have suggested that biomass could influence this (de Souza et al., 2016; Michelan et al., 2011; Pizzolatti et al., 2014, 2010). However, biomass aspects have not been assessed in other BSF studies.

This paper discusses the influence of backwashing on BSF biomass by comparing a BSF to an ScSF with similar characteristics. Biomass evaluation was based on biomass quantification and distribution through filter media depth, bacterial community by *high-throughput* 16S rRNA sequencing, and biomass distribution (solids and bacteria) on sand grain surfaces by microscope images. As a result, biomass was evaluated in different aspects to provide more information about BSF.

2 METHODOLOGY

2.1 FILTRATION SYSTEM AND RUNS

The filtration system used in this study was composed of two parallel SSFs, an ScSF and a BSF (Figure 1A). These filters, used and described in other studies, follow the design recommendations from these studies and SSF literature (FUNASA, 2019;

Huisman and Wood, 1974; Pizzolatti et al., 2010, 2014). Prior to the study, the filters were in operation for tests; therefore, the system was mature and stable. In addition, an upflow roughing filter (URF) was used for phytoplankton excess removal prior to SSF.

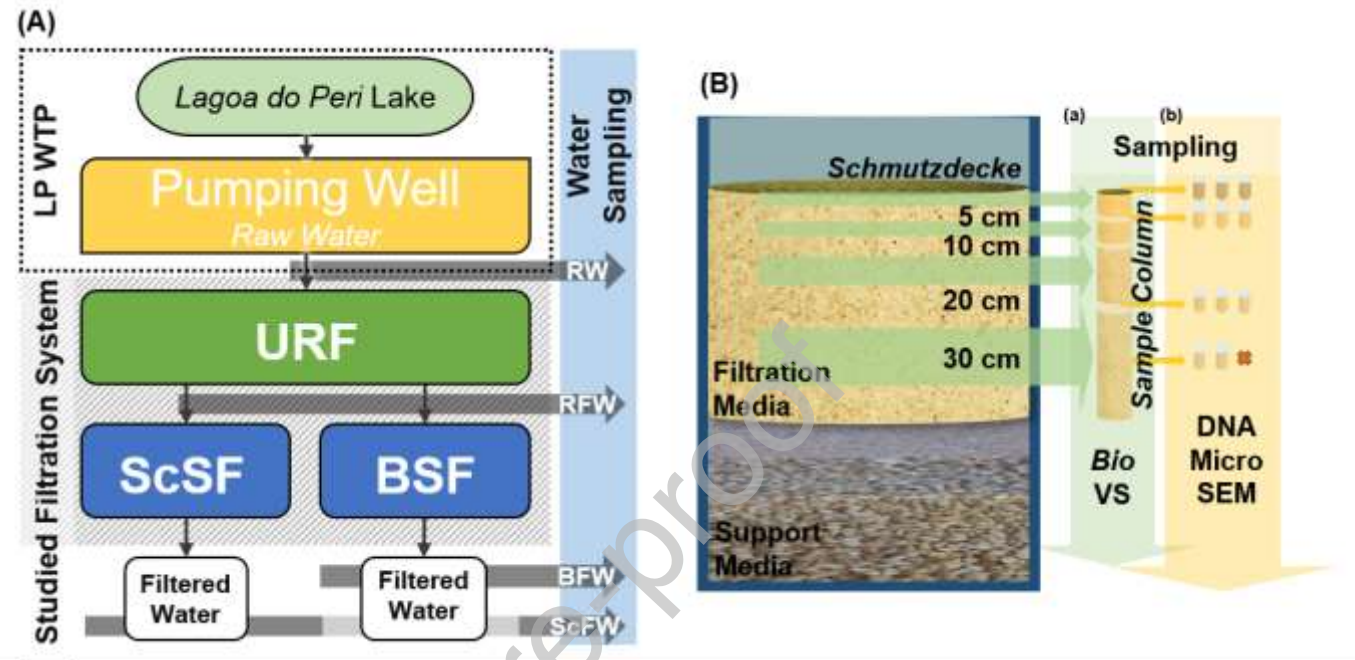

Figure 1 – Filtration system water sampling points (A) and *schmutzdecke* and filtration media sampling points and separation strategy (B). Labels: (LP WTP) *Lagoa do Peri*'s water treatment plant; (RW) Raw water; (URF) Upflow rough filter; (ScSF) Slow sand filter with scraping and external cleaning; (ScSW) Water sampled from ScSF; (BSF) Backwashed slow sand filter; (BFW) Water sampled from BSF; (*Bio*) Cell biomass samples; (VS) volatile solids samples; (DNA) samples for DNA extraction, (Micro) optical microscopy samples; and (SEM) scanning electron microscopy samples.

Table 1 describes the main characteristics of both ScSF and BSF. A schematic representation of each is shown in the *Supplementary Material* (Figures S1 and S2).

Table 1 – Main design characteristics and operational aspects of ScSF and BSF.

Filtration rate	4 m/d
Filtration area	0.64 m²
Maximum headloss	100 cm
Filtration run	15 d
Support layer characteristics	Gravel: • L = 10 cm d = 6.65 – 12.7 mm • L = 7.5 cm d = 3.18 a 6.65 mm • L = 7.5 cm d = 2 – 3.18 mm
Filter media characteristics	Sand: L = 40 cm d_{10} = 0.30 mm UC = 1.6

Note: (L) Layer depth; (d) diameter; (d_{10}) effective diameter; and (UC) uniformity coefficient.

A sand medium with a low uniformity coefficient (UC = 1.6) was used as the filter media to minimise size stratification after backwashing (FUNASA, 2019). Uniform media (<1.8) are recommended for BSF to avoid excessive size stratification that could lead to high initial headloss, especially for a low effective diameter (d_{10} = 0.30 mm) (de Souza et al., 2016; Pizzolatti et al., 2014).

At the end of the 15 days of filtration, *schmutzdecke* and the top 5–6 cm of the sand from ScSF were scraped and washed manually with fresh raw water. The BSF was cleaned by backwashing for 4 min with total bed fluidisation and 40% average expansion. Both SSFs operated at a filtration rate of 4 m/d and were not covered (Pizzolatti et al., 2014, 2010).

2.2 WATER SAMPLING AND QUALITY ANALYSIS

Water quality parameters, such as turbidity and coliforms, were monitored. Filter influent and effluent water were sampled and analysed for comparison and filtration

process evaluation (Figure 1A). All analysed water quality parameters are listed in Table S1, along with the equipment used and sampling frequency. Turbidity was analysed using a *HACH 2100P* Turbidimeter, and total coliforms and *Escherichia coli* using the *Collilert Quanti-tray®* system.

Sampling was always performed at least 24 h after cleaning to allow system maturation and effluent turbidity stabilisation, as has been previously reported in other studies (de Souza et al., 2016; Pizzolatti et al., 2014). The methodologies for the water sample preparation and analysis are described in the *Supplementary Material*.

2.3 FILTER MEDIA SAMPLING AND ANALYSIS

Sand across the entire depth of the filtration column was sampled as a "sample column" (Figure 1B, a). For better sample representativity, three distinct sample columns (Ø 20 mm) were taken from three different locations on the filter surface area. Each column sample was portioned according to its depth (depth-portioned samples): *schmutzdecke* plus 0–5 cm depth sand layer, as well as 5–10, 10–20, and 20–40 cm sand layers (Figure 1B, a). Then, the depth-portioned samples from the different sand columns were combined (sample pool) according to depth (> 80 g of sand).

Before the sampling pool, 1-g samples were taken from the sample columns at depths of 0 (*schmutzdecke*), 5, 20, and 30 cm, and subsequently combined into 3-g samples for each depth. These were used for microscopy observation and DNA extraction (Figure 1B, b). The *schmutzdecke* in the filters was thin and mixed with the top millimetres of sand; therefore, it was impossible to separate it from the sand. This top mixture (1-g of sand + *schmutzdecke*) was used for DNA extraction and microscopy as a representation of the *schmutzdecke*.

Biomass was measured indirectly as cell biomass (*Bio*) using the chloroform fumigation method (Campos et al., 2002), with volatile solids (VS) per sand dry weight after 30 min at 550 °C burning (Manav Demir et al., 2018). *Bio* was calculated based on the total organic carbon (TOC) extracted from the sand samples before and after chloroform fumigation (Campos et al., 2002), as described in the *Supplementary Material*.

Sand samples were observed using scanning electron microscopy (SEM) and brightfield and fluorescence optical microscopy. For fluorescence microscopy, a *Live/Dead BacLight Invitrogen*™ stains kit (BVK) was used to assess bacterial distribution and viability on freshly sampled sand. Glutaraldehyde preservation, ethanol dehydration, hexamethyldisilazane (HMDS) final dehydration, and golden coating were used prior to SEM observations (See *Supplementary Material*). *ImageJ* (Schindelin et al., 2012) and *Leica Application Suite*/LAS 3.3 software were used for image processing.

2.4 HIGH-THROUGHPUT DNA SEQUENCING

Sand samples had genomic DNA extracted from the pellets of the filter media using *DNeasy PowerSoil* (©QIAGEM, Hilden, Germany) according to the manufacturer's protocol. For representativity, the DNA was extracted using a sample pool from two different sampling times, one at the middle and another at the end of the study. The extracted products were sent to the company *Neoprospecta Microbiome Technologies,* Inc. (Florianópolis, Brazil) for high-throughput 16S rRNA sequencing analysis using the *MiSeq* platform (Miseq™, Illumina Inc., USA). All 16S rRNA reads were analysed by sequencing the V3-V4 region on the extracted DNA using the universal primers 341F 5´-CCTACGGGRSGCAGCAG-3´ (Wang and Qian, 2009) and 806R 5´-

GGACTACHVGGGTWTCTAAT-3´ (Caporaso et al., 2011). De-multiplexed *.fastq* files were imported and analysed using *QIIME2*™, version 2 (2019.4) (Bolyen et al., 2019), following the *MiSeq* standard operating procedure with some modifications on VirtualBox. For quality control, sequences were filtered, denoised, merged, and chimeras were removed using DADA2 (Callahan et al., 2016). Sequences were classified using the *Greengenes* database 13_8 (99% OTUs full-length sequences) (DeSantis et al., 2006) and features related to mitochondria or chloroplasts were removed.

The count table and metadata from the *QIIME2*™ taxonomic annotation were imported as *.csv* and a complete workflow was developed for data exploration, statistical analyses, and graphics.

2.5 STATISTICAL ANALYSIS

Medians and means were compared using *Kruskal-Wallis* and *ANOVA* with the *Tukey* comparison method. The *Spearman* coefficient was used to compute data correlations using *Minitab®* 18. Removals were calculated in percentage terms (%) using the subtraction of final (C) from initial (C_0) concentrations divided by C_0, while log removal was calculated as $Log_{10}(C_0/C)$. The removals were presented followed by *p*-values from the *Tukey* comparison between C and C_0 means.

The data from high-throughput sequencing were normalized and rarefaction analysis was employed to evaluate the sample coverage. Subsequently, alpha diversity (observed richness and *Shannon*) and beta diversity (Principal Coordinates Analysis - PCoA) based on the Bray-Curtis distance metric were applied to evaluate the patterns of similarities between the samples, and how they clustered according to their metadata information. Finally, *QIIME2*™ was also used to compare bacterial communities in ScSF

and BSF by pairwise *PERMANOVA* analysis.

3 RESULTS AND DISCUSSION

3.1 FILTRATION EFFICIENCY

The effluent water quality of both filters was classified as acceptable (<1 NTU) according to the WHO recommendation for SSF (WHO, 2017). ScSF and BSF significantly removed most of the monitored water quality parameters and, in the case of turbidity, its removal by the ScSF was different from that in the BSF (Table S2). For instance, turbidity decreased from 3.0 NTU to 0.64 in ScSF and 0.83 NTU in BSF, representing 79% (p=0.000) and 73% (p=0.000) removal, respectively. Mean values did not differ between the filters (p=0.962). However, due to the increasing filtration efficiency for suspended solids removal during filtration running, median values were lower (ScSF=0.47 NTU and BSF=0.70 NTU) and differed between the two filters (p=0.000). This result indicates that, despite the suitable water quality, there are some differences in filtration mechanisms in both filters.

Meanwhile, total coliforms were successfully removed by ScSF (1.5 log, p=0.003) and BSF (1.3 log, p=0.029) but with no statistical difference between the two filters. This removal is in line with the values previously reported for SSF (1–3 log) (Amy et al., 2006).

While turbidity is removed by physical filtration mechanisms, *schmutzdecke* plays a key role in water purification e.g. coliform removal (Huisman and Wood, 1974; Weber-Shirk and Dick, 1997a, 1997b). Distinct removal in turbidity may indicate differences in these mechanisms among the two filters, as reported by previous studies

and may be related to the different biomass on those filters (de Souza et al., 2016; Pizzolatti et al., 2014).

3.2 BIOMASS QUANTIFICATION AND HEADLOSS

Biomass decreased with column depth in both filters (Figure 2 and Table 2). Before cleaning, the ScSF surface biomass contents were 114.1 µg-*Bio*/g-sand and 5.3 mg-VS/ g-sand on average, which agree with other studies using similar methodology (Campos et al., 2002; Manav Demir et al., 2018). These studies also reported decreasing biomass with depth, as observed in the ScSF (Figure 2 and Table 2). However, Campos et al. (2002) reported that biomass reduction with depth was not as evident in covered ScSF with less *schmutzdecke* formation.

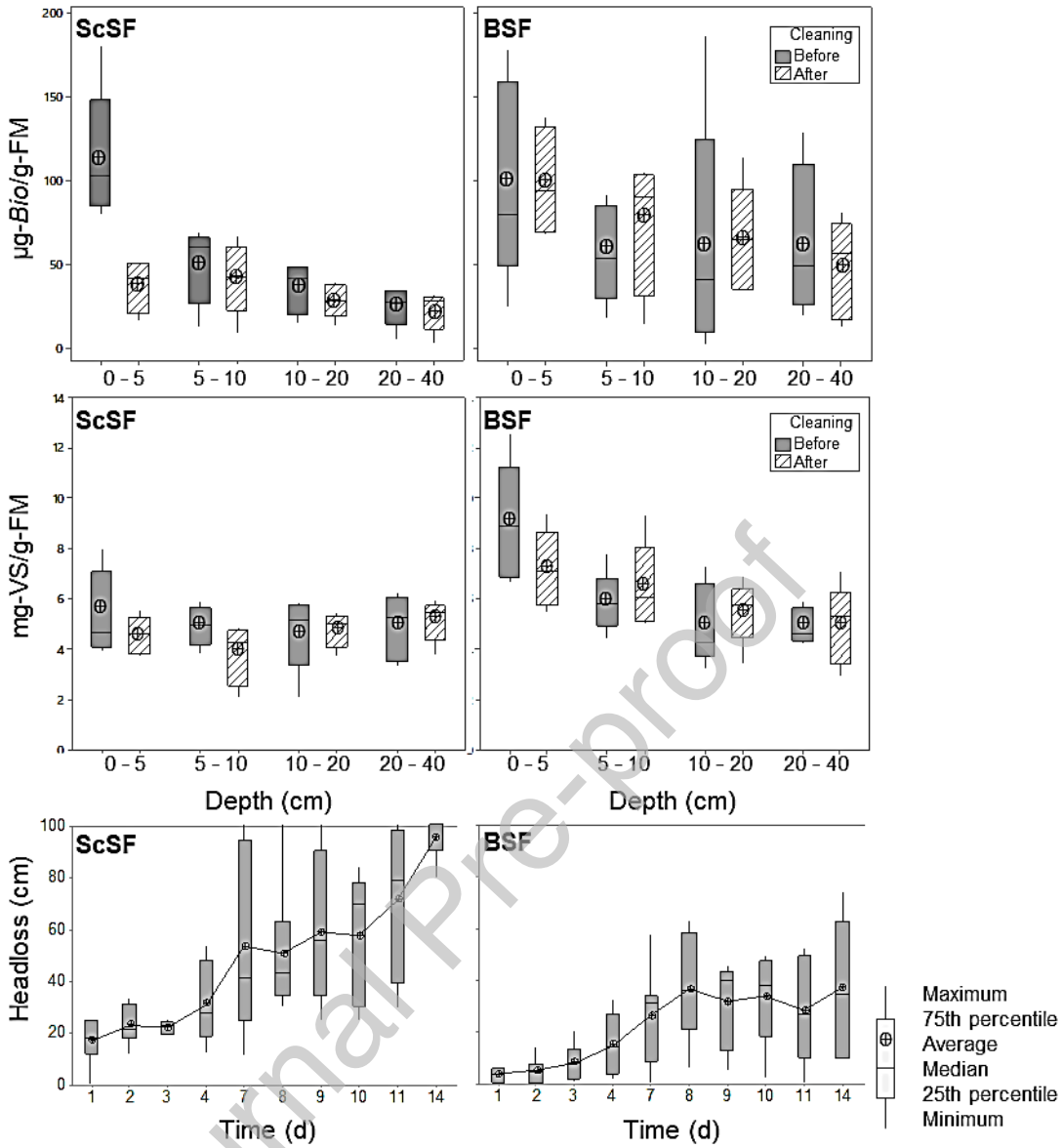

Figure 2 – Headloss development and biomass on scraped (ScSF) and backwashed slow sand filter (BSF). Biomass is presented as cell biomass (*Bio*) and total volatile solids (VS) along the filtration depth before, and after cleaning.

Table 2 - Average cell biomass (*Bio*) and total volatile solids (VS) on scraped (ScSF) and backwashed slow sand filter (BSF) along the filtration depth, before (BC) and after (AC) cleaning.

		ScSF		BSF	
Biomass	**Depth (cm)**	**BC**	**AC**	**BC**	**AC**
Bio **(µg/g-sand)**	**0–5**	114.1*#	38.0	99.7	99.0
	5–10	49.7	42.0	57.3	75.5
	10–20	37.0	28.6	62.0	65.7
	20–40	25.2	22.7	62.3	48.5
VS (mg/g-sand)	**0–5**	5.4	4.6	9.0*	7.2
	5–10	4.9	3.9	5.9	6.5
	10–20	4.7	4.8	5.0	5.5
	20–40	4.9	5.2	4.9	5,0

Note: * Statistically different from the deeper layer; # Statistically different after cleaning.

Biomass decreased with depth on BSF with less significant variation before and after backwashing. The biomass on the surface was 99.7 µg-*Bio*/g-sand and 9.0 mg-VS/g-sand before cleaning and 99.0 µg-*Bio*/g-sand ($p = 0.983$) and 7.2 mg-VS/g-sand ($p = 0.185$) after backwashing (Table 2). Biomass was also more distributed along the filtration column depth on BSF (45.5–99.7 µg-*Bio*/g-sand and 4.9–9.0 mg-VS/g-sand) than on ScSF (22.7–114.1 µg-*Bio*/g-sand and 3.9–5.4 mg-VS/g-sand) and with more *Bio* and VS on deeper layers (Figure 2 and Table 2).

However, surface scraping reduced the biomass on the top layer from 114.1 µg-*Bio*/g-sand and 5.4 mg-VS/g-sand to 38.0 µg-*Bio*/g-sand ($p = 0.009$) and 4.6 mg-VS/g-sand ($p = 0.332$), respectively. Biomass values in the clean sand were similar to the deeper layers that were not scraped (22.7–49.7 µg-*Bio*/g-sand and 3.9–5.2 mg-VS/g-sand). This means that the *schmutzdecke* formed on the top surface was successfully removed by scraping.

The BSF headloss increased from 3.5 cm after 24 h of operation to 37 cm on average after 15 days (Figure 2). Meanwhile, the ScSF headloss increased from 17 cm

(24 h) to 97 cm (15 d) on average. In this case, distinct biomass profiles were reflected in different headloss behaviours but there were almost no significant variations in filtered water quality between ScSF and BSF (Table S2). *Schmutzdecke* maturation affects filter effluent quality, especially for microorganism removal (Coliforms removal > 2 Log); it may take weeks to form (Bellamy et al., 1985a, 1985b). Nevertheless, biomass on the surface layer significantly increased on ScSF within 15 days (Figure 2 and Table 2). In addition, the lower disturbance on the top layer biomass of BSF was not as evident as it was on ScSF.

Higher biomass concentration on the sand surface is favourable because many materials are trapped by sieving (Weber-Shirk and Dick, 1997a, 1997b). In addition, substrates and oxygen are more available for different organisms, forming a complex food chain on SSF (Bellamy et al., 1985b; Huisman and Wood, 1974; Nakamoto, 2014). After scraping the ScSF, the headloss decreased because the biomass was removed. On BSF, biomass distribution with depth indicates deeper filtration and consequently a significant occurrence of biological mechanisms in the deeper layers, making headloss development slower than on ScSF, where the surface became clogged with time. This could be explained by the higher particle penetration due to the increased porosity and grain mixture caused by backwashing. De Souza et al. (2016) observed that higher impurity breakthrough in BSF was influenced by the filter media grain size, finding higher porosity in BSF than in ScSF due to the removal of fine grains by backwashing. Consequently, the water quality deteriorated. Marnoto (2008) reported that hydraulic conductivity recovered to initial running levels after cleaning, even with *schmutzdecke* preservation. In this study, it was observed that these organic materials were not the most influential in the headloss development of the BSF as they clogged the ScSF

surface.

3.3 ATTACHMENT OBSERVATION

In SEM micrographs, attached material observations were evident by changes in the texture of sand samples from different filter medium depths (Figure 4 and 4) and by comparison to the new sand before use (Figure S4). This indicates the attachment of suspended material and biofilm on both filter media.

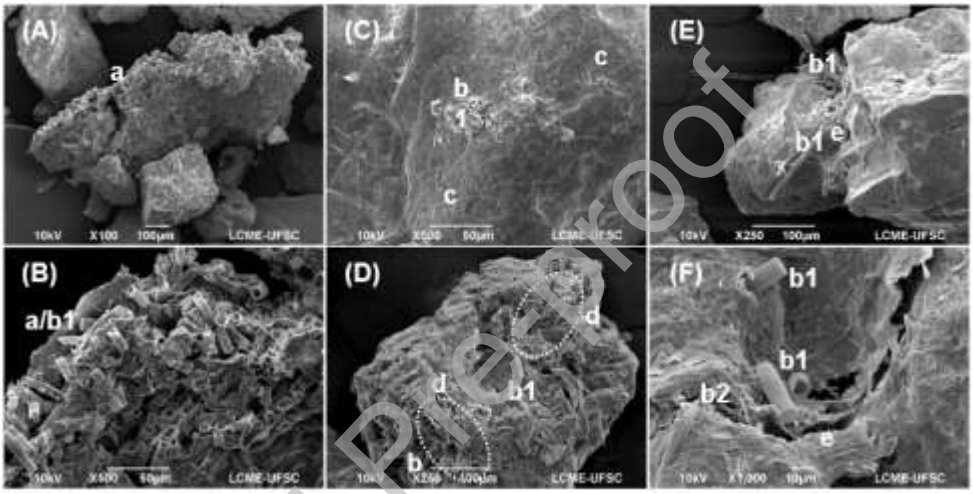

Figure 3 – SEM micrographs of BSF top layer sand before and after cleaning. (A) *Schmutzdecke* and sand, X100; (B) diatoms forming a cohesive *schmutzdecke*, X500; (C) sand grain surface covered by filamentous Cyanobacteria in *schmutzdecke*, X250; (D) sand grains covered by biomass, X250; (E) sand after cleaning, X250; and (F) sand after cleaning, X1000. (a) *Schmutzdecke* biomass agglomerate; (b) diatoms, (1) *Aulacoseira ambigua* and (2) *Navicula* sp.; (c) filamentous cyanobacteria; (d) filamentous cyanobacteria agglomerate; and (e) biomass maintaining grains cohesion.

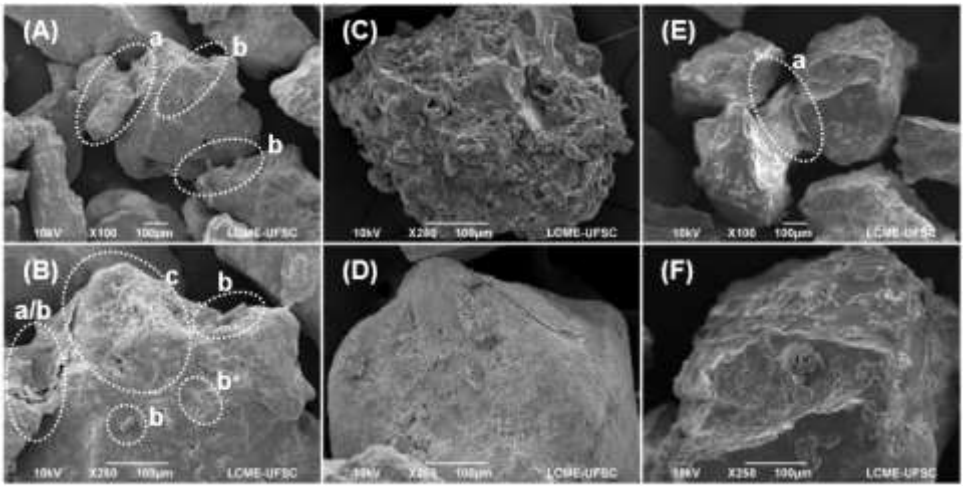

Figure 4 – SEM micrographs showing ScSF sand grains before and after cleaning. (A) Sand grains mixed with *schmutzdecke* at X100 (before scrapping); (B) sand grains uniformly covered by biomass in *schmutzdecke*, X250 (before scraping); (C) sand grains with cavities covered by biomass, X250 (5 cm depth); (D) sand grains with uniform discreet biomass cover, X250 (30 cm depth); (E) sand grains after manual external cleaning, X100; and (F) sand grains after manual external cleaning, X250. (a) Biomass and grains cohesion; (b) diatoms; and (c) filamentous cyanobacteria agglomerate.

Fluorescent microscopy observations using BVK showed potentially viable bacteria (green) within the *schmutzdecke* and on the sand grain surface (Figure 5). Bacteria with membrane damage appeared as red (or red-yellowish) and might not be viable. Pfannes et al. (2015) also used fluorescent microscopy for bacterial viability and extracellular polymeric substance observation. They reported isolated and small bacterium aggregates in SSF *schmutzdecke,* while bacteria in the deeper filtration layers were isolated or in the biofilm.

In this study, the distribution of extracellular polymeric substances was not specifically assessed. However, it was possible to see bacteria distributed on the grain surface, rather than small aggregates, indicating biofilm formation with predominantly viable bacteria (Figure 5) (Pfannes et al., 2015).

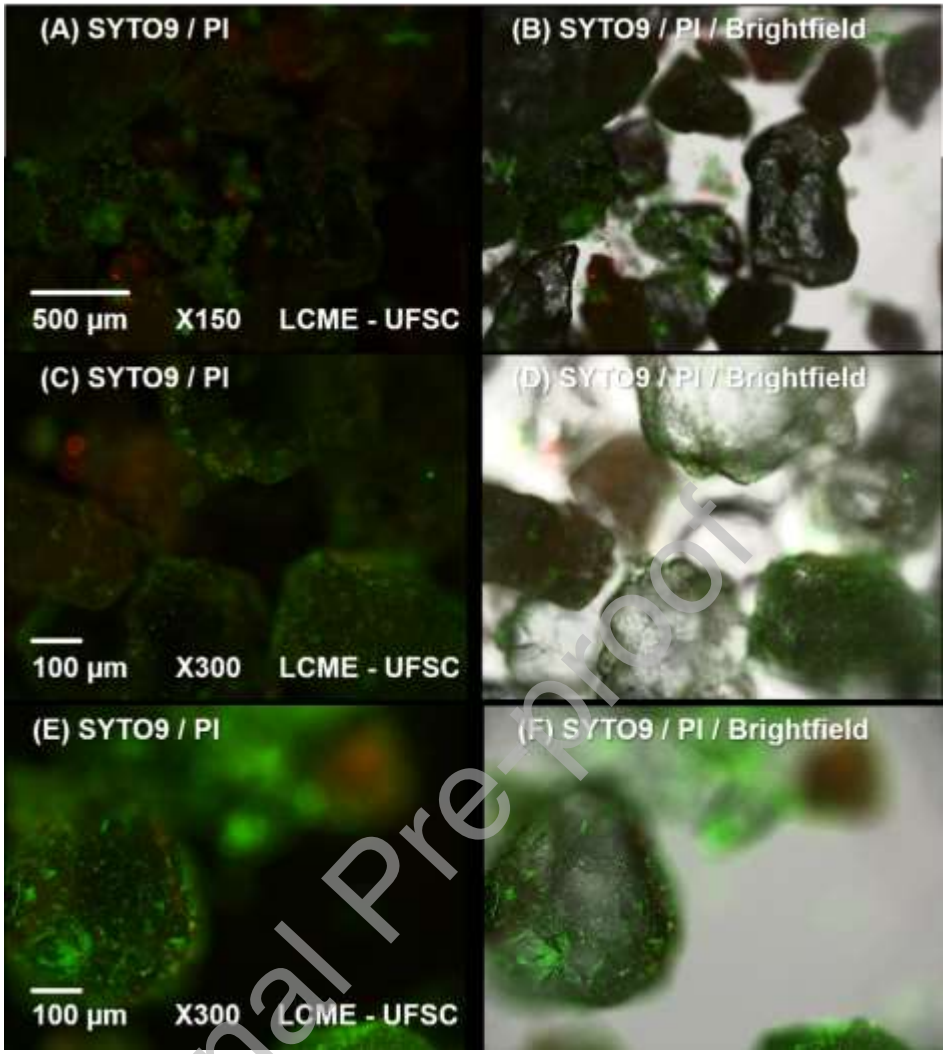

Figure 5 – Fluorescent microscopy micrographs showing *schmutzdecke* and the top sand layers before cleaning of ScSF (A;B) and BSF (E;F) and after cleaning of ScSF (C;D). Fluorescence microscopy shows the viable bacteria stained green by SYTO9 and unviable bacteria stained yellow-red by SYTO9 and Propidium Iodide (PI). Brightfield microscopy (B; D; and F) also give an idea of the surface of the sand grains.

Microscope images similar to biomass results show that cleaning was not sufficient to remove all of the attached material (Figure 3, Figure 4 E and F), especially on BSF (Figure 4 E and F). Furthermore, by fluorescent microscopy, it was possible to see viable bacteria attached to the sand before and after cleaning, especially in the aggregates on sand cavities. Viable bacteria were observed, even after scraping, which

removed biomass significantly (Figure 5). This is evidence that although biomass was mostly removed, bacteria were still attached to the sand grains immediately after cleaning. This suggests that immediate resanding, if necessary, could be a suitable option for SSF maturation (Barret et al., 1991; Huisman and Wood, 1974).

3.4 16S rRNA SEQUENCING OF THE DYNAMICS OF MICROBIAL COMMUNITY

For a more specific characterization of bacterial biomass, the microbial community dynamics of the sand samples were investigated by 16S rRNA sequencing as well as the identification of the main genera found in the microbiota and their relative abundance. This study aimed to investigate the influence of backwashing on SSF at the bacterial community structure level.

A total of 790k sequences were retrieved from 16 samples of high-throughput 16S rRNA Illumina MiSeq™ sequencing. After quality control by *QIIME2*™ and the removal of chimera and low-quality reads (Phred<24), 555k high-quality sequences remained for further analysis (Table S4).

3.4.1 Bacterial Community Identification and Relative Abundance

At the phylum level, the most abundant bacteria were Proteobacteria (42%–80%), Acidobacteria (3%–22%), Verrucomicrobia (5%–16%), Chloroflexi (3%–15%), Bacteroidetes (4%–12%), Actinobacteria (2%-6%), Nitrospirae (0%–6%), Chlorobi (1%–6%), and Cyanobacteria (0%–2%) (Figure S6).

These phylum relative abundances were similar to those found in other studies, with Proteobacteria, Nitrospirae, Planctomycetes, Actinobacteria, Bacteroidetes, and Chloroflexi being the most common (D'Alessio et al., 2015; Haig et al., 2015; Hwang et

al., 2014; Lautenschlager et al., 2014; Liao et al., 2015; Oh et al., 2018). Proteobacteria are usually predominant in SSF due to the availability and variability of this phylum metabolism in the environment. Its presence is related to the degradation of diverse organic compounds on biofilters (D'Alessio et al., 2015; Haig et al., 2015; Lautenschlager et al., 2014; Liao et al., 2015).

Other organic matter degradation-associated bacteria phyla were Verrucomicrobia, Chloroflexi, Bacteroidetes, and Actinobacteria (Sangwan et al., 2004; Servin et al., 2008; Speirs et al., 2019; Thomas et al., 2011). Chloroflexi is usually present in the sand bed rather than the *schmutzdecke* (D'Alessio et al., 2015; Haig et al., 2015). However, these phyla did not change after scraping, as previously reported by Haig et al. (2015). Bacteroidetes have been reported in other studies with decreasing abundance in *schmutzdecke* with time (Haig et al., 2014; Zhao et al., 2019). On the other hand, Nitrospirae bacteria are indicative of the nitrification process on SSF and more common in deeper layers (Lautenschlager et al., 2014).

The relative abundances of identified bacterial genera are shown in Figure 6. In decreasing order, the most abundant identified genera were *Geobacter* (1%–23%), *Nitrospira* (1%–9%), *Anaeromyxobacter* (0%–8%), *Hyphomicrobium* (1%–10%), *Candidatus* Solibacter (0%–9%), *Rhodoplanes* (1%–6%), *Mycobacterium* (0%–6%), and *Chthoniobacter* (0%–6%).

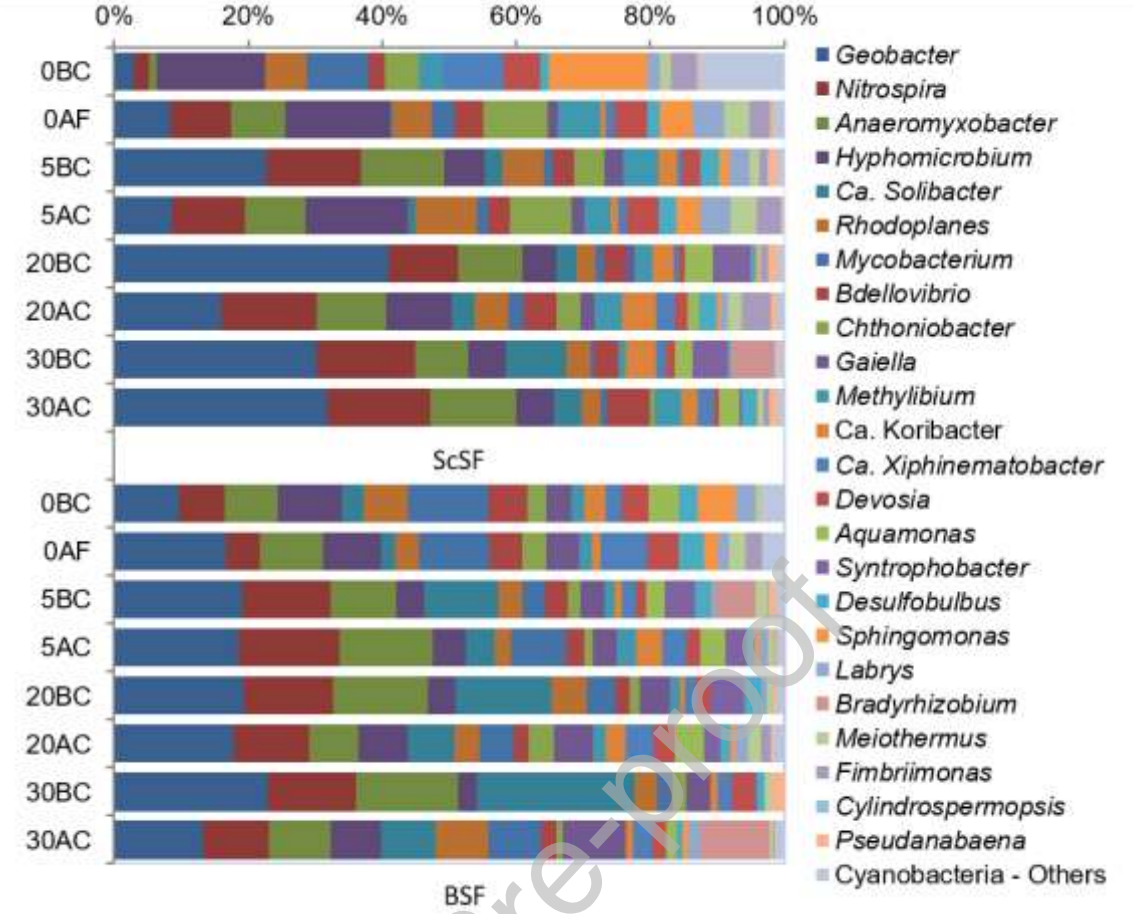

Figure 6 – Relative abundance at the genus level found in the datasets from ScSF and BSF, through filter depth and before (BC) and after cleaning (AC). Numbers indicate depth (0, 5, 20, and 30 cm)

Geobacter (Proteobacteria) was the most abundant genus. Bacteria from this genus are anaerobic and use Fe (III) or Mn (IV) as electron acceptors for organic carbon degradation (Childers et al., 2002). The abundance of *Geobacter* had an inverse correlation with depth, which indicates that this degradation process may occur when oxygen is present at lower concentrations. Fe and Mn were not quantified in this study. However, SSF is capable of removing these ions via physical mechanisms (e.g. sieving) after oxidation and precipitation (Demir, 2016; Manav Demir et al., 2018; Michelan et

al., 2011), when the presence of associated bacteria is expected (Tekerlekopoulou et al., 2013).

Nitrospira (Nitrospirae phylum) is the second most common genus and is known for its role in the complete nitrification process (Daims et al., 2015; Palomo et al., 2016). This genus was also found to be dominant in other SSF studies and is associated with the nitrogen cycle (Oh et al., 2018; Wang et al., 2014). Other nitrogen cycle-related genera (*Anaeromyxobacter, Hyphomicrobium, Rhodoplanes*, and *Candidatus* Solibacter) and phylum (Verrucomicrobia and Planctomycetes) have also been identified (Gupta et al., 2012; Hiraishi and Ueda, 1994; Pearce et al., 2012; Sanford et al., 2002; Urakami et al., 1995; Van Teeseling et al., 2015; Wang et al., 2019) and reported in other drinking water studies (Demir, 2016; Kaarela et al., 2015; Lautenschlager et al., 2014; Liao et al., 2013; Oh et al., 2018; Vandenabeele et al., 1995; Wang et al., 2018). The presence of nitrogen-cycle organisms confirms the complexity of bacterial activities on SSF, which have already been reported to be capable of complete nitrification (Aslan and Cakici, 2007; Nakhla and Farooq, 2003).

3.4.2 Bacterial Community Spatial Distribution and Alterations due to Cleaning Processes

Overall, as relative abundances indicated, samples closer to the surface were more influenced by the cleaning processes (Figure 6). The relative bacterial abundance also changed due to the different cleaning processes and sand depths. *Spearman* correlations between relative abundance and depth were significant ($p<0.05$) in ScSF for the most abundant genera, excluding *Anaeromyxobacter*, and most of the phylum, such as Proteobacteria, Acidobacteria, and Nitrospirae. These correlations were less obvious

in BSF (Table S5) and are probably related to bacterial characteristics and their attachment strength to grains (Haig et al., 2015; Lautenschlager et al., 2014; Oh et al., 2018). In addition, they indicate the complexity and different roles bacteria may have as SSF ultimately relies on biological treatment, which is affected by factors such as food availability, nutrients, and filter operation (Haig et al., 2015; Lautenschlager et al., 2014; Oh et al., 2018).

Principal coordinate analysis (PCoA) based on the *Bray-Curtis* distance metric showed differences between the BSF and ScSF groups (Figure 7, a and b). Pairwise PERMANOVA also highlighted statistical differences between the overall bacterial community diversity ($p = 0.001$) in both filters.

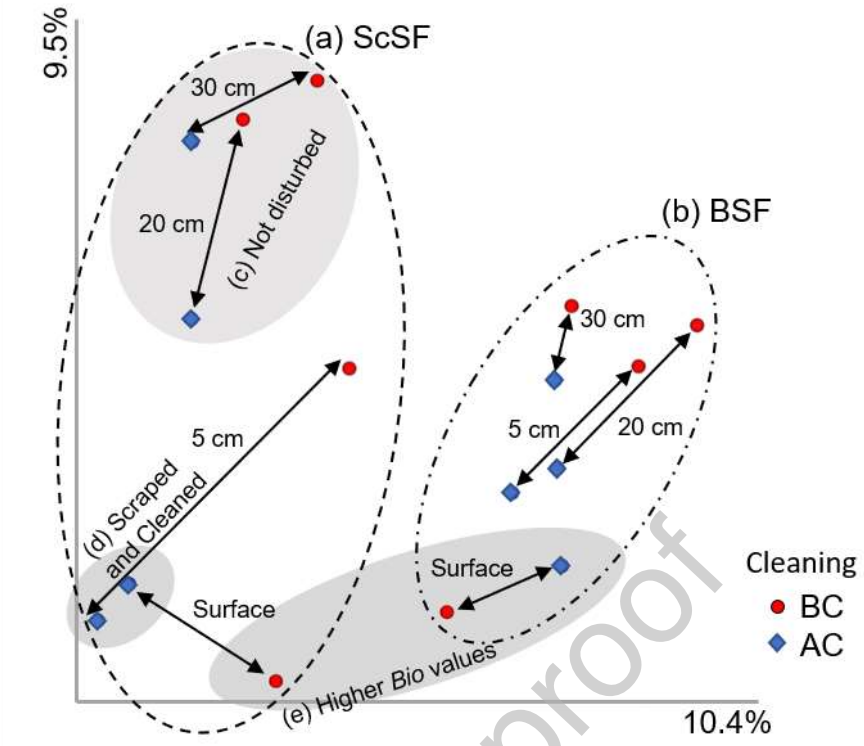

Figure 7 – Principal coordinate analysis (PCoA) plot using *Bray-Curtis* distances for ScSF and BSF samples from different sand depths, before (BC) and after (AC) cleaning process; (a) ScSF sample group, (b) BSF sample group, (c) samples not disturbed by any cleaning process, (d) samples cleaned after scraping, and (e) samples at the top layer presenting higher biomass as *Bio*.

BSF sample coordinates appear closer to each other (Figure 7b), showing less variation in bacterial community diversity. It is also remarkable that the sample coordinates from the top sand layers were also close, indicating similarity with the *schmutzdecke* samples (Figure 7e). Conversely, on ScSF, the top surface and 5 cm samples were similar after cleaning, probably because both sampling points were influenced by scraping (~6 cm deep) (Figure 7d). Meanwhile, the undisturbed sample coordinates appear close (Figure 7c). The ScSF sample coordinates (Figure 7a) were more dispersed on the PCoA graphic, indicating depth stratification of the bacterial community related to the filtration process and higher biomass values on the top layers

(Figure 7e).

These results agree well with the biomass distribution in the sand layers (Figure 2), confirming the differences in filtration mechanisms between ScSF and BSF. Other studies also reported differences between bacterial communities in the raw water (not assessed in this study) and deeper sand layers (D'Alessio et al., 2015; Lautenschlager et al., 2014; Oh et al., 2018; Pfannes et al., 2015).

Based on the alpha diversity indices (*Shannon* and *Evenness*) and number of OTUs, the BSF samples were considered more uniform than ScSF and were more diverse on the top layers (Table S4). Dalahmeh et al. (2014) also reported similar results. They argued that the low genetic diversity in *schmutzdecke* bacteria was due to the high food chain complexity on the sand surface, while competition and predation by other organisms decreased with depth. Food is also less available at the lower layers, making the bacterial community more homogenous. However, in BSF, these indexes became more uniform due to the sand fluidisation, indicating backwashing mixture and bacterial selectivity (Table S4).

3.5 OTHER MICROORGANISMS COMPOSING BIOMASS

Overall, as reported in other studies, *schmutzdecke* was the most diverse layer, forming a complex food chain with microcrustaceans, midge larvae, nematodes, rotifers, algae, and bacteria (Hurley and Wottom, 2006; Joubert, 2008; Law et al., 2001; D. McNair et al., 1987; Nakamoto, 2014; Ranjan and Prem, 2018). This diversity was mainly observed in the BSF but not in the ScSF. Algae were also visible under fluorescence (Figure 8) but their viability cannot be related to the BVK due to chlorophyll-a natural fluorescence (in red) (Reavie et al., 2010). *A. ambigua*, other

diatoms, and filamentous cyanobacteria were the most common microorganisms present (Figure 3, 3, and 8). The appearance of these microorganisms is not surprising as they are common in the *Lagoa do Peri* water and have been previously reported as filter clogging phytoplankton (de Souza et al., 2017; Saavedra del Aguila and Di Bernardo, 2003; Saupe and Mosimann, 2003).

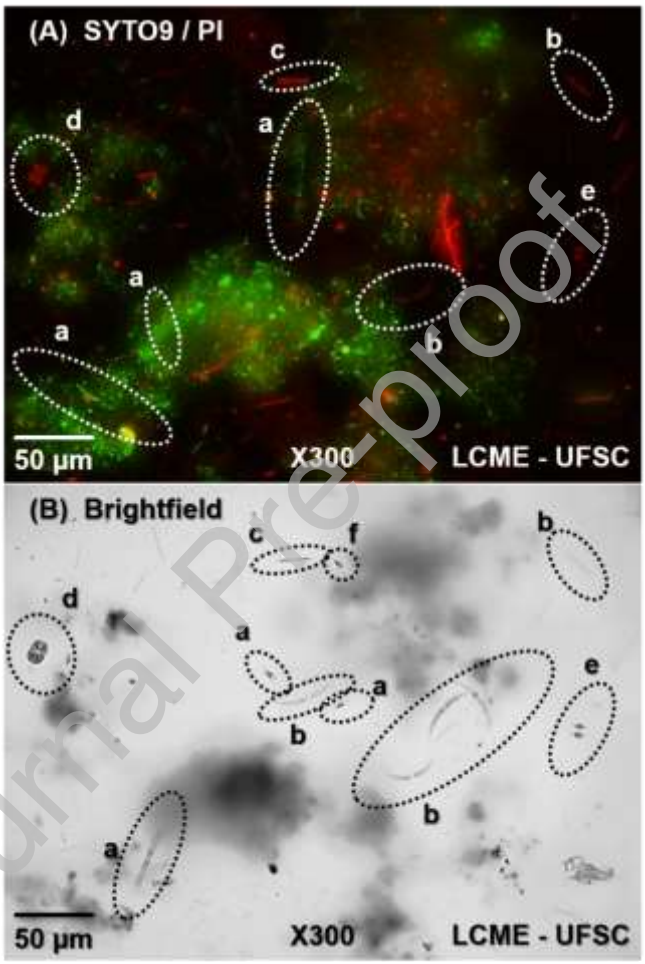

Figure 8 – Miscellaneous materials composing *schmutzdecke* with microalgae, especially diatoms, cyanobacteria, bacteria and protozoa: (a) *Aulacoseira sp.*; (b) *Closterium sp.*; (c) *Fragilaria sp.*; (d) *Cosmarium sp.*; (e) *Staurastrum sp.*; and (f) Rotifer.

Cyanobacteria are highlighted in this study because of their abundance in the raw water, especially the genera *Cylindrospermopsis* and *Pseudanabaena*. They are related to filter clogging, are usually removed by SSF, and explain filtration runs of 15 days on

average (de Souza et al., 2017; Mondardo, 2009; Pereira et al., 2012; Pizzolatti et al., 2014; Schöntag et al., 2015). Both the *Cylindrospermopsis* (0.00%–0.18%) and *Pseudanabaena* (0.00%–2.10%) genera were identified in both ScSF and BSF, being more abundant in the *schmutzdecke* (0.88%–12.67%), and were visible under the microscope (Figure 3 and 4). Their presence on the ScSF surface can also be related to faster headloss development. Moreover, their presence along the depth (Figure 6) may explain their breakthrough to effluent water (Table S2). The more significant chlorophyll-*a* removal is probably due to diatom trapping at the surface because of their size (Figure 8 and Table S2).

The presence of these different organisms is an example of *schmutzdecke* complexity (Nakamoto, 2014). Its diversity preservation by BSF, as observed by microscopy, is a promising result of this cleaning method. Lower disturbances of *schmutzdecke* are recommended by some SSF researchers for a better preservation of biological filtration mechanisms (Hurley and Wottom, 2006; Iwase et al., 2006; Nakamoto, 2014, 2011).

These results show that regular scraping also preserves bacteria. However, other organisms were not observed (e.g. diatoms, nematodes, or microcrustaceans). Biomass decreased significantly after scraping (Figure 2). Such differences were not observed on BSF, demonstrating that backwashing preserves the biomass diversity in the filter bed, as speculated by other studies (de Souza et al., 2016; Marnoto et al., 2008). However, when scraping was used, biomass decreased. This suggests that scraping disturbs the microbial community in ScSF, explaining the need for a filter maturation period (Barret et al., 1991; Huisman and Wood, 1974).

3.6 BIOMASS CONSIDERATIONS REGARDING BACKWASHING

In this study, backwashing alone was not sufficient to significantly remove biomass from the sand surface. This may be explained by the backwashing hydrodynamics. The lower d_{10} results in lower fluid/media tension, as water velocity is low (Cleasby et al., 1977; Fitzpatrick, 1998; Valencia and Cleasby, 1979). This probably results in less *Bio* detachment from sand grains, although there is evidence of variation in the backwash water turbidity in other studies (de Souza et al., 2016; Pizzolatti et al., 2014). In backwashing, friction forces between grains are dominant at the beginning of the bed expansion. After bed fluidisation, the major forces acting on the sand grains are the drag tension between the media and fluid, pulling the attached material out of the filter. (Fitzpatrick, 1998; Valencia and Cleasby, 1979).

On average, a fluidized bed with 40% expansion is adopted for backwashing, based on recommendations for rapid filters, although other values might be suggested for rapid filters (Cleasby et al., 1977; Crittenden et al., 2012). However, expansion varies throughout the backwashing duration. At the beginning of backwashing, dirt is usually removed due to initial friction forces, higher velocities, and porosity augmentation liberating interstitial trapped material (de Souza et al., 2016; Fitzpatrick, 1993; Pizzolatti et al., 2014). Nevertheless, because of the smaller grain size used in the BSF, backwashing flow rates are lower than those in rapid sand filters, leading to smaller drag tension between water and sand grains after complete fluidisation (de Souza et al., 2016; Fitzpatrick, 1998; Valencia and Cleasby, 1979).

The lower tension may explain the differences in *Bio* and VS attached to the sand media before and after backwashing. *Bio* and VS have different compositions (Figure 2). While *Bio* represents cell biomass that can be strongly attached to the sand surface by

exopolymer substances, VS represents any organic material attached to the filter media that might be easily removed when the sand bed is expanding. The nature of the trapped material (organic and non-organic) and their separate ways of attachment on the grain surface (e.g. attachment mechanisms, position on the sand grain surface, size, and shape) may have led to different detachment modes and, consequently, initial headloss recovery. This *Bio* preservation may also be confirmed by the low variability of the bacterial community and microscopy observations (Figure 3, 4, and 5).

3.7 BIOMASS CONSIDERATIONS REGARDING BSF OPERATION

The results regarding biomass in BSF may be considered preliminary, due to the short period of the study and its pioneering quality. However, these results are promising for introducing backwashing in small- and medium-scale slow sand filters.

Considering the importance of biological degradation of certain compounds in SSF, biomass preservation can be considered an advantage of BSF (Summers, 2014). Biofilm preservation on the filter could maintain the microbial community despite consecutive cleaning, reducing the ripening period. Although this requires further investigation, it has been previously reported that the ripening period could be eliminated due to biomass preservation in biosand filters (Ikhlef and Basu, 2017). A concern about this is that the maintenance of biomass could result in an outbreak of persistent pathogens if they are present in the sand bed or *schmutzdecke* (Hwang et al., 2014; Karon et al., 2011).

Despite possible advantages and concerns, initial headloss was recovered after cleaning (Figure 2), indicating that it was mostly due to the interstitial or non-organic materials that were removed during backwashing. Furthermore, headloss was lower in

BSF than in ScSF at the end of the operation (Figure 2), which allows a longer operational time and higher productivity (de Souza et al., 2016).

Characterization of bacterial communities by 16S rRNA gene sequencing is also promising, as Figure 6, 7, and S6 show that there is bacterial community stratification in ScSF with depth (less evident on BSF), especially at the genera level. Additionally, it shows that scraping changes the bacterial community in sand, which may have a higher impact on ScSF ripening than on BSF. Few studies have used 16S rRNA sequencing for bacterial community characterization on SSF. This technique is promising because bacterial degradation pathways could be better understood in SSF in the future (Haig et al., 2011). In the case of BSF, specific conditions such as the maintenance of bacterial community may be an indication of bacterial selectivity after consecutive backwashing, which could also lead to a faster ripening, favouring the removal of target contaminants (Flemming et al., 2016; Ikhlef and Basu, 2017). Other studies have reported the importance of bacterial degradation on SSF for the removal of target contaminants, such as organic compounds and nutrients, and the selectivity of specific bacteria due to these contaminants over operational time or treatment processes (Aslan and Cakici, 2007; D'Alessio et al., 2015; Li et al., 2018, 2017; Liu et al., 2019; Miltner et al., 1995; Summers, 2014; Zearley and Summers, 2012).

These results are representative of the complexity of SSF biological mechanisms. Bacterial activity should be further studied in future research. Long-term studies could investigate the possible backwashing role in selecting specific and better-attached bacteria for biofilm preservation and reduce the filter maturation period in BSF.

4 CONCLUSIONS

The following are the main conclusions of this study:

- Biomass was developed in the sand bed differently depending on the filter depth and cleaning process. The top sand layers and *schmutzdecke* developed more biomass in terms of *Bio* (99.7–114.1 μg-*Bio*/g-sand) and VS (5.43–9.04 mg-VS/g-sand). In addition, biomass stratification was more evident in the deeper ScSF layers than in the BSF, resulting in faster ScSF clogging.
- Microscopy observations confirmed the biomass quantification results, showing biomass diversity preservation on BSF. The different techniques, namely, SEM and fluorescence microscopy, highlighted different aspects of the filter media biomass and overall attached material. SEM analyses showed the material attached to the sand grain surfaces, while fluorescence microscopy showed that viable bacteria were spread across the *schmutzdecke* and sand media even after backwashing and scraping.
- *High-throughput* 16S rRNA sequencing complements the indirect biomass quantification and is a useful tool for bacterial community structure characterization. In this study, bacterial communities changed significantly due to the cleaning process, indicating microbial selectivity of the fluidisation process.
- Proteobacteria was the predominantly identified phylum (42%–80%). Meanwhile, *Geobacter* (1%–23%) and *Nitrospira* (1%–9%) were the most prevalently identified genera, being associated with the iron and nitrogen cycles. Other significantly identified genera were associated with organic matter

degradation, demonstrating the complexity of SSF bacterial activity across the filter depth.

- Differences in biomass across the filter depth helped to understand the differences between ScSF and BSF water qualities. Both filters had acceptable efficiencies, according to WHO recommendations for drinking water standards, especially turbidity (<1.0 NTU) and total coliforms (>1 log).
- Overall, scraping and backwashing affected both slow sand filters, resulting in distinct biomass accumulation and bacterial communities. Both filters, ScSF and BSF, could improve water quality, but the BSF was simpler to operate. Therefore, BSF is recommended for small and community-scale filters as an alternative to conventional SSF to produce good effluent quality through less laborious cleaning processes.
- Further studies on biological pathways are recommended to better understand the SSF bacterial purification mechanisms and possible selectivity. In addition, the speed up of the BSF maturation period, especially for the removal of target contaminants such as iron, nitrogen, and other biodegradable compounds, could be investigated.

5 SUPPLEMENTARY MATERIAL

Supplementary Material presents additional methodology and data to support the authors' statements (Table S and Figure S). The SEM original size micrographs were also included for better observation.

All SEM micrographs taken from the SSF samples used for this work are available

at http://dx.doi.org/10.17632/b26d6fbg2t.1 .

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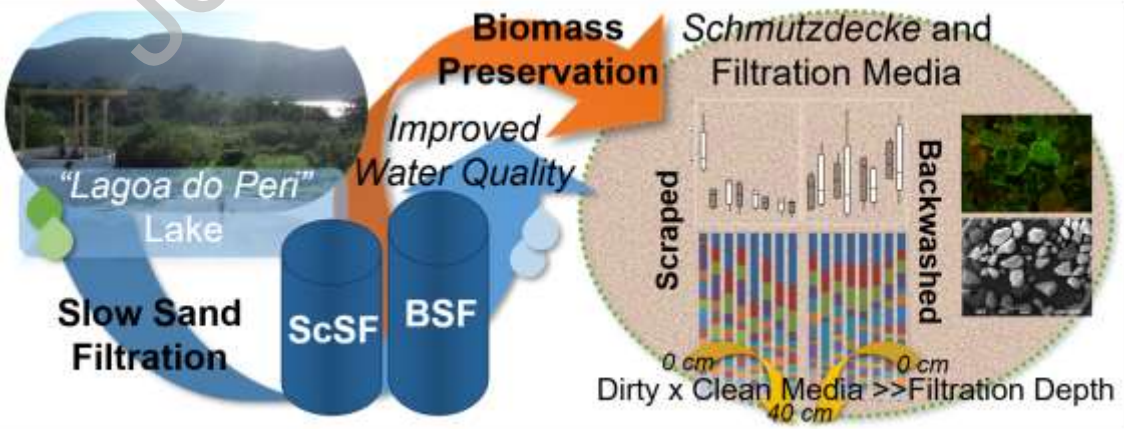
Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Graphical abstract



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