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 backwashed slow sand filters evaluation. Slow sand filtration (SSF) is a simple technology for water treatment and biological mechanisms have a key role on filtration efficiency of those filters. Backwashed slow sand filters (BSF) 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 and which is a gap in the knowledge of this technology, considering its biological mechanisms importance. For the first time, two filters operating at the same conditions were used to compare the influence of backwashing on biomass. One filter was cleaned by backwashing and the other by scraping, so they were compared at the same conditions. 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 observation by scanning electron and fluorescent microscopy. Filters were also monitored and compared regarding filtered water quality and headloss and their differences were related to the different cleaning process. Overall, filtered water quality was acceptable for slow sand filter standards (turbidity < 1 uT and total coliforms 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 it on surface sand layers. Cell biomass more present and was spread across the filtration depth and was related to lower headloss and turbidity

38   and cyanobacteria breakthrough. These results were also in agreement with water quality

39   and microscopy observations. Bacterial community was also less stratified in the

40   backwashed filter media depth. These results go beyond the knowledge of the

41   backwashing use in slow sand filters, demonstrating that this process preserve more

42   biomass than scraping. In addition, biomass preservation can lead to bacteria selectivity

43   and a faster filter ripening. Considering the importance of biomass preservation on slow

44   sand filtration and its biological filtration mechanisms, the results presented in this paper

45   are promising. The novel insight that BSF has the capacity to preserve biomass after

46   backwashing may contribute to increase its application in small communities.

47   **Keywords:** slow sand filtration; biomass; *schmutzdecke*; 16S rRNA gene sequencing;

48   microbial community profile; water treatment.

49

50   **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

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1 INTRODUCTION

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

These interactions tend to improve with biomass accumulation, and it is 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, and it is still considered a "black box" for the SSF technology (Campos et al., 2002; Graham and Collins, 2014).

In terms of operational impacts, biomass accumulation is related to headloss increasing. At a certain point of accumulation the filter get clogged, then the so called *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, and it depends on variations in SSF design, operation and raw water (Bellamy et al., 1985a; Campos et al., 2002; Huisman and Wood, 1974).

Previous studies 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

79   filters (<1m²) that can be easily applied in small and isolated communities or in small

80   agroindustry (FUNASA, 2019; Michelan et al., 2011; Pizzolatti et al., 2014). This is because

81   backwashing operation is simple and lasts only a few minutes, while scraping is laborious

82   and time-consuming.

83   The upfront economic investment for a Backwashed Slow Sand Filter (BSF) is higher

84   than for Scraped Slow Sand Filter (ScSF), especially due to the valves and backwashing

85   water reservoir. However, less sand can be used because progressive scraping and final

86   re-sanding are not necessary, also minimizing costs and sand loss (de Souza et al., 2016,

87   2018; FUNASA, 2019; Michelan et al., 2011).

88   Besides BSF filtered water quality and operation, there is no specific research

89   regarding the effect of SSF filter media (FM) fluidization on biomass development, an

90   important feature of SSF mechanisms. Studies about biomass in backwashed biofilters

91   diverge about backwash influence on biomass, an evidence about the complexity and

92   dependency on filtration operational aspects and filtration media. Previous BSF studies

93   reported differences on headloss behaviour compared to ScSF and, in some cases, lower

94   effluent quality. They suggested that biomass could have an influence on this (de Souza

95   et al., 2016; Michelan et al., 2011; Pizzolatti et al., 2014, 2010). However, biomass aspects

96   were never assessed in other BSF studies.

97   This paper discusses the influence of backwashing on BSF biomass by comparing a

98   BSF to a ScSF with similar characteristics. Biomass evaluation was based on biomass

99   quantification and distribution through filter media depth, bacterial community by *high-*

100   *throughput* 16S rRNA sequencing, and biomass distribution (solids and bacteria) on sand

101   grain surface by microscope images. Doing so, biomass was evaluated in different aspects

102   in order to provide more information about BSF.

2   METHODOLOGY

104   2.1   FILTRATION SYSTEM AND RUNS

105   The filtration system used in this study was composed of two parallel SSFs, a ScSF

106   and a BSF (Figure 1A). These filters were used and described in other studies and follow

107   the design recommendations from other studies and SSF literature (FUNASA, 2019;

108   Huisman and Wood, 1974; Pizzolatti et al., 2010, 2014). Prior to the study, the filters were

109   in operation for tests, so the system was mature and stable. Also, an upflow roughing

110   filter (URF) was used for phytoplankton excess removal prior to SSF.
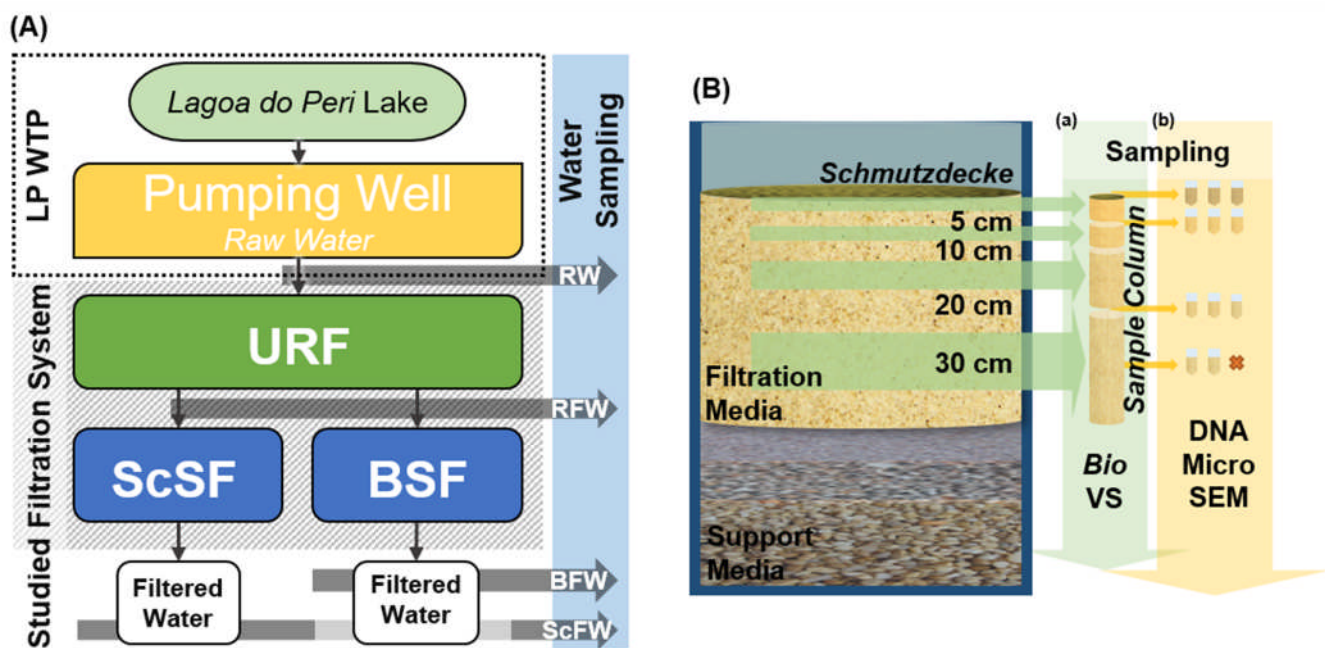
111

112   Figure 1 – Filtration system water sampling points (A) and *Schmutzdecke* and filtration media sampling

113   points and separation strategy (B). Labels: (LP WTP) *Lagoa do Peri*'s water treatment plant; (RW) Raw

114   water; (URF) Upflow rough filter; (ScSF) Slow sand filter with scraping and external cleaning; (ScSW)

115   Water sampled from ScSF; (BSF) Backwashed slow sand filter; (BFW) Water sampled from BSF; (*Bio*) Cell

116   biomass samples; (VS) volatile solids samples; (DNA) samples for DNA extraction, (Micro) optical

117   microscopy samples; and (SEM) scanning electron microscopy samples.

118   Table 1 describes the main characteristics of both ScSF and BSF, and a schematic

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119   representation of each one of them is shown in the *Supplementary Material* (Figures S1

120   and S2).

121   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

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

123

124   A sand media with low uniformity coefficient (UC = 1.6) was used as filter media to

125   minimize size stratification after backwashing (FUNASA, 2019). Uniform media (<1.8) are

126   recommended for BSF to avoid excessive size stratification that could lead to high initial

127   headloss, especially for a low effective diameter (d_{10} = 0.30 mm) (de Souza et al., 2016;

128   Pizzolatti et al., 2014).

129   At the end of the 15 days of filtration, *schmutzdecke* and the top 5-6 cm of sand

130   from ScSF were scraped and washed manually with fresh raw water. The BSF was cleaned

131   by backwashing for 4 min with total bed fluidization and 40% average expansion. Both

132   SSFs operated at 4 m/d filtration rate and were not covered (Pizzolatti et al., 2014, 2010).

133   2.2   WATER SAMPLING AND QUALITY ANALYSIS

134   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 water quality parameters analysed are listed in Table

S1, along with equipment used and sampling frequency. Turbidity was analysed in a *HACH*

2100P Turbidimeter, and Total Coliforms and *Escherichia coli* by *Collilert Quanti-tray®*

system.

Sampling was always performed at least 24h after cleaning to allow system

maturation and effluent turbidity stabilization, as it was 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 whole depth of the filtration column was sampled as a "sample

column" (Figure 1B, a). For a 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; $5-10$ cm; $10-20$ cm; 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 cm (*schmutzdecke*), 5 cm, 20 cm, and 30 cm, and were subsequently

combined into 3 g samples of each depth. These were used for microscopy observation

and DNA extraction (Figure 1B, b). *Schmutzdecke* in the filters were thin and mixed with

the top millimetres of sand, so it was impossible to separate it from sand. This top mixture

(1 g of sand + *schmutzdecke*) was used for DNA extraction and microscopy as a

9

158   representation of the *schmutzdecke*.

159   Biomass was measured indirectly as cell biomass (*Bio*) using the chloroform

160   fumigation method (Campos et al., 2002), and as volatile solids (VS) per sand dry weight

161   after 30 min at 550°C burning (Manav Demir et al., 2018). *Bio* was calculated based on

162   the total organic carbon (TOC) extracted from the sand samples before and after

163   chloroform fumigation (Campos et al., 2002), as described in the *Supplementary Material*.

164   Sand samples were observed using scanning electron microscopy (SEM) and

165   brightfield and fluorescence optical microscopy. For fluorescence microscopy, a

166   *Live/Dead® BacLight Invitrogen*™ stains kit (BVK) was used to assess bacteria distribution

167   and viability on freshly sampled sand. Glutaraldehyde preservation, ethanol dehydration,

168   hexamethyldisilazane (HMDS) final dehydration, and golden coating were used prior to

169   SEM observations (See *Supplementary Material*). *ImageJ* (Schindelin et al., 2012) and

170   *Leica Application Suite*/LAS 3.3 software was used for image processing.

171   2.4   HIGH-THROUGHPUT DNA SEQUENCING

172   Sand samples had genomic DNA extracted from pellets of filter media using *DNeasy*

173   *PowerSoil* (©QIAGEM, Hilden, Germany) according to the manufacturer's protocol. For

174   representativity, the DNA was extracted using a sample pool from two different sampling

175   times, one at the middle and another at the end of the study. The extracted products

176   were sent to the company *Neoprospecta Microbiome Technologies,* Inc. (Florianópolis,

177   Brazil) for high-throughput 16S rRNA sequencing analysis using the *MiSeq* platform

178   (Miseq™, Illumina Inc., USA). All 16S rRNA reads were analysed by sequencing the V3-V4

179   region   on   the   extracted   DNA   using   the   universal   primers   341F   5´-

180   CCTACGGGRSGCAGCAG-3´   (Wang   and   Qian,   2009)   and   806R   5´-

181   GGACTACHVGGGTWTCTAAT-3′ (Caporaso et al., 2011). De-multiplexed *.fastq* files were

182   imported and analysed using *QIIME2™*, version 2 (2019.4) (Bolyen et al., 2019), following

183   the *MiSeq* standard operating procedure with some modifications on a VirtualBox. For

184   quality control, sequences were filtered, denoised, merged, and chimeras were removed

185   using DADA2 (Callahan et al., 2016). Sequences were classified using the *Greengenes*

186   database 13_8 (99% OTUs full-length sequences) (DeSantis et al., 2006), and features

187   related to mitochondria or chloroplast were removed.

188   The count table and metadata from the *QIIME2™* taxonomic annotation were

189   imported as *.csv*, and a complete workflow was developed for the data exploration,

190   statistical analyses, and graphics.

191   2.5   STATISTICAL ANALYSIS

192   Medians and means were respectively compared using *Kruskal-Wallis* and *ANOVA*

193   with the *Tuckey* comparison method. The *Spearman* coefficient was used to compute

194   data correlations using *Minitab®* 18. Removals were calculated in percentage terms (%)

195   using the subtraction of final (C) from initial (C_0) concentrations divided by C_0, while Log

196   removal was calculated as $Log_{10}(C_0/C)$. The removals are presented followed by *p*-values

197   from the *Tuckey* comparison between C and C_0 means.

198   The data from high-throughput sequencing were normalized, and the rarefaction

199   analysis was employed to evaluate the sample coverages. Afterwards, alpha diversity

200   (observed richness and *Shannon*) and beta diversity (Principal Coordinates Analysis -

201   PCoA) based on the Bray–Curtis distance metric were applied to evaluate the patterns of

202   similarities between the samples, and how they cluster according to their metadata

203   information. Finally, *QIIME2™* was also used to compare bacterial communities in ScSF

204   and BSF by pairwise *PERMANOVA* analysis.

205   **3   RESULTS AND DISCUSSION**

206   3.1   FILTRATION EFFICIENCY

207   The effluent water quality of both filters was classified as good (<1NTU) according

208   to the WHO recommendation for SSF (WHO, 2017). ScSF and BSF significantly removed

209   most of the monitored water quality parameters, and in the case of turbidity, its removal

210   by the ScSF was different from the one in the BSF (Table S2). For instance, turbidity

211   decreased from 3.0 NTU to 0.64 in ScSF and 0.83 NTU in BSF, respectively, which

212   represent 79% (p=0.000) and 73% (p=0.000) removal. Mean values did not differ between

213   the filters (p=0.962). However, due to the increasing filtration efficiency on suspended

214   solids removal during filtration running, median values were lower (ScSF=0.47 NTU and

215   BSF=0.70 NTU) and differed between the two filters (p=0.000). This result indicates that,

216   despite the good water quality, there are some differences on filtration mechanisms in

217   both filters.

218   Meanwhile, total coliforms were successfully removed by ScSF (1.5 Log, p=0.003)

219   and BSF (1.3 Log, p=0.029), but with no statistical difference between the two filters. This

220   removal is in line with the values previously reported for SSF (1-3 Log) (Amy et al., 2006).

221   While turbidity is removed by physical filtration mechanisms, it is well known that

222   *schmutzdecke* plays a key role on water purification e.g. coliforms removal (Huisman and

223   Wood, 1974; Weber-Shirk and Dick, 1997a, 1997b). Distinct removal in turbidity may

224   indicate differences in these mechanisms among the two filters and it was reported by

225   previous studies and may be also related to the different biomass on those filters (de

226   Souza et al., 2016; Pizzolatti et al., 2014).

227   3.2   BIOMASS QUANTIFICATION AND HEADLOSS

228   Biomass decreased with column depth in both filters (Figure 2 and Table 2). Before

229   cleaning, the ScSF surface biomass contents were 114.1 µg-*Bio*/g-sand and 5.3 mg-VS/ g-

230   sand on average, which agree with other studies using similar methodology (Campos et

231   al., 2002; Manav Demir et al., 2018). These studies also reported decreasing biomass with

232   depth as observed in the ScSF (Figure 2 and Table 2). However, Campos et al. (2002)

233   reported that biomass reduction with depth was not so evident in covered ScSF with less

234   *schmutzdecke* formation.

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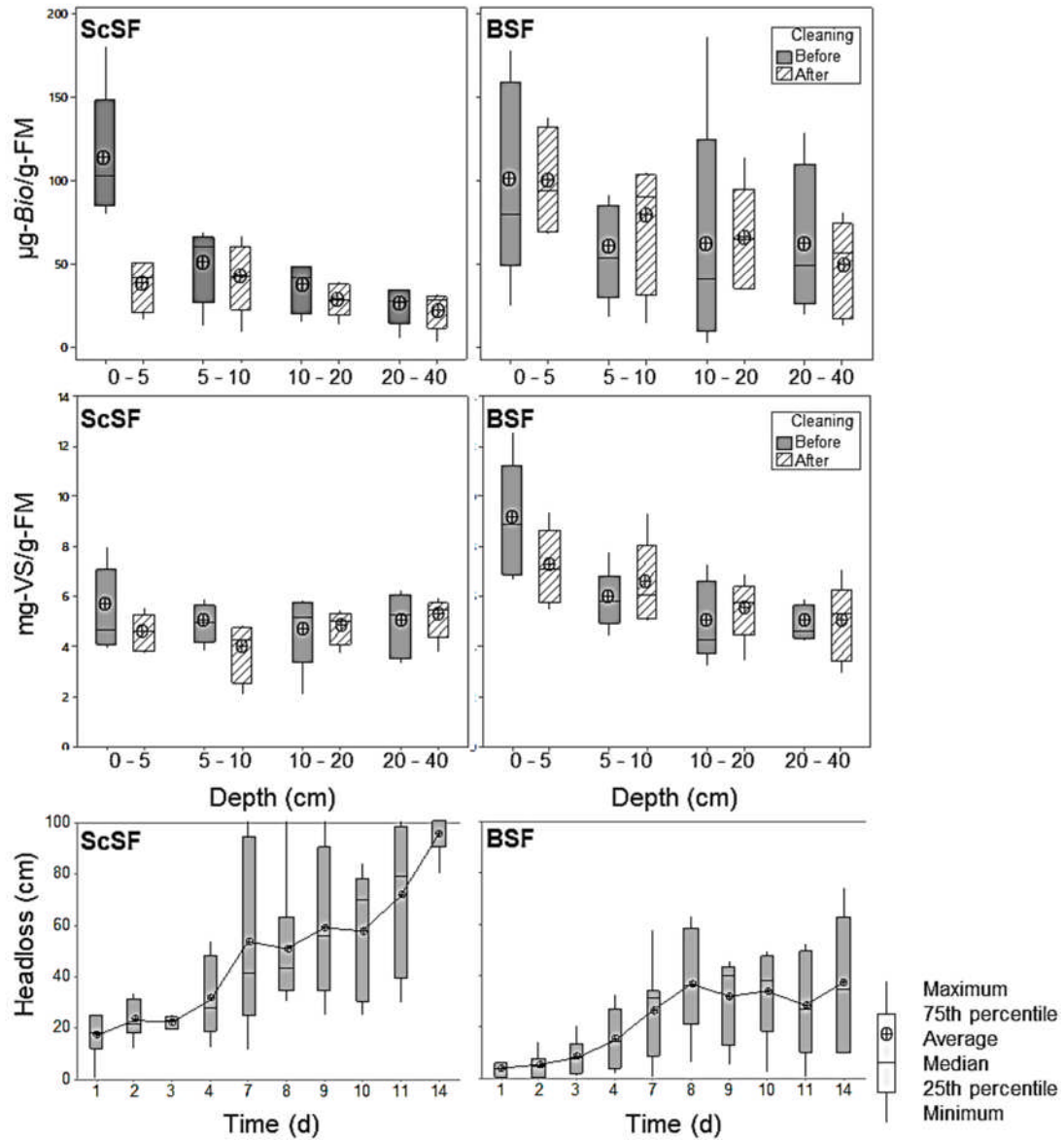
236

237   Figure 2 – Headloss development and biomass on Scraped (ScSF) and Backwashed Slow Sand Filter (BSF).

238   Biomass is presented as Cell biomass (*Bio*) and Total Volatile Solids (VS) along the filtration depth, before

239   and after cleaning.

240

241   Table 2 - Average Cell biomass (*Bio*) and Total Volatile Solids (VS) on Scraped (ScSF) and Backwashed

242   Slow Sand Filter (BSF) along the filtration depth, before (BC) and after (AC) cleaning.

		ScSF		BSF	
Biomass	**Depth (cm)**	**BC**	**AC**	**BC**	**AC**
Bio	**0 - 5**	114.1*#	38.0	99.7	99.0
(µg/g-sand)	**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	**0 - 5**	5.4	4.6	9.0*	7.2
(mg/g-sand)	**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

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

244

245   Biomass decreased with depth on BSF with less significant variation before and

246   after backwashing. Biomass on the surface was 99.7 µg-*Bio*/g-sand and 9.0 mg-VS/g-sand

247   before cleaning, and 99.0 µg-*Bio*/g-sand (p = 0.983) and 7.2 mg-VS/g-sand (p = 0.185)

248   after backwashing (Table 2). Biomass was also more distributed along the filtration

249   column depth on BSF (45.5-99.7 µg-*Bio*/g-sand and 4.9-9.0 mg-VS/g-sand) compared

250   with ScSF (22.7-114.1 µg-*Bio*/g-sand and 3.9-5.4 mg-VS/g-sand), and with more *Bio* and

251   VS on deeper layers (Figure 2 and Table 2).

252   On the other hand, surface scraping reduced the biomass on the top layer from

253   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-

254   VS/g-sand (p = 0.332), respectively. Biomass values in the clean sand were similar to the

255   deeper layers that were not scraped (22.7-49.7 µg-*Bio*/g-sand and 3.9-5.2 mg-VS/g-

256   sand). This means that *schmutzdecke* formed on the top surface was successfully

257   removed by scraping.

258   BSF headloss increased from 3.5 cm after 24 hours operation to 37 cm on average

259   after 15 days (Figure 2). Meanwhile, ScSF headloss increased from 17 cm (24 h) to 97 cm

260   (15 d) on average. In this case, distinct biomass profiles were reflected in different

261   headloss behaviour, but there were almost no significant variations in filtered water

262   quality between ScSF and BSF (Table S2). *Schmutzdecke* maturation affects filter effluent

263   quality, especially for microorganism removal (Coliforms removal > 2 Log) and it may take

264   weeks to form (Bellamy et al., 1985a, 1985b). Nevertheless, biomass on the surface layer

265   significantly increased on ScSF within 15 days (Figure 2 and Table 2). Also, the lower

266   disturbance on the top layer biomass of BSF was not as evident as it was on ScSF.

267   Higher biomass concentration on the sand surface is favourable since many

268   materials are trapped by sieving (Weber-Shirk and Dick, 1997a, 1997b). Also, substrates

269   and oxygen are more available for different organisms, forming a complex food chain on

270   SSF (Bellamy et al., 1985b; Huisman and Wood, 1974; Nakamoto, 2014). After scraping

271   the ScSF, the headloss decreased because biomass was removed. On BSF, biomass

272   distribution with depth indicates deeper filtration and consequently a significant

273   occurrence of biological mechanisms in the deeper layers, making headloss development

274   slower than on ScSF, where the surface became clogged with time. This could be

275   explained by higher particle penetration due to increased porosity and grain mixture

276   caused by backwashing. De Souza et al. (2016) observed that higher impurity

277   breakthrough in BSF was influenced by the filter media grain size, and they found higher

278   porosity in BSF than in ScSF due to the removal of fine grains by backwashing.

279   Consequently, the water quality was deteriorated. Marnoto (2008) reported that

280   hydraulic conductivity was recovered to initial running levels after cleaning even with

281   *schmutzdecke* preservation. In this study, it was observed that these organic materials

282   were not the most influential in the headloss development of the BSF as they clogged the

283   ScSF surface.

284   3.3   ATTACHMENT OBSERVATION

285   In SEM micrographs, attached material observation was evident by a change in the

286   texture of sand samples from different filter medium depths (Figure 4 and 4), and by

287   comparison to the new sand before use (Figure S4). This indicates the attachment of

288   suspended material and biofilm on both filter media.
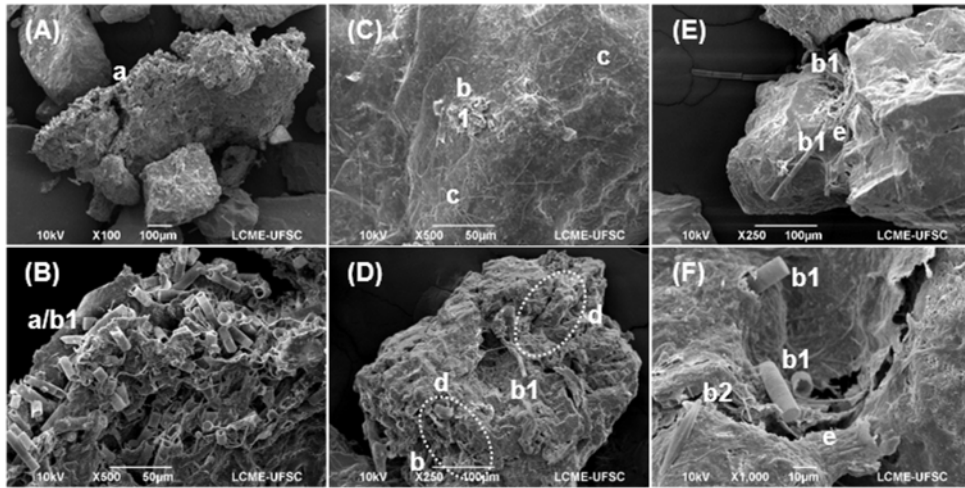
289

290   Figure 3 – SEM micrographs of BSF top layer sand before and after cleaning. (A) *Schmutzdecke* and sand,

291   X100; (B) Diatoms forming a cohesive *schmutzdecke*, X500; (C) Sand grain surface covered by filamentous

292   Cyanobacteria in *schmutzdecke*, X250; (D) Sand grains covered by biomass, X250; (E) Sand after cleaning,

293   X250; (F) Sand after cleaning, X1000; (a) *Schmutzdecke* biomass agglomerate; (b) diatoms, (1)

294   *Aulacoseira ambigua* and (2) *Navicula* sp.; (c) filamentous cyanobacteria; (d) filamentous cyanobacteria

295   agglomerate; and (e) biomass maintaining grains cohesion.

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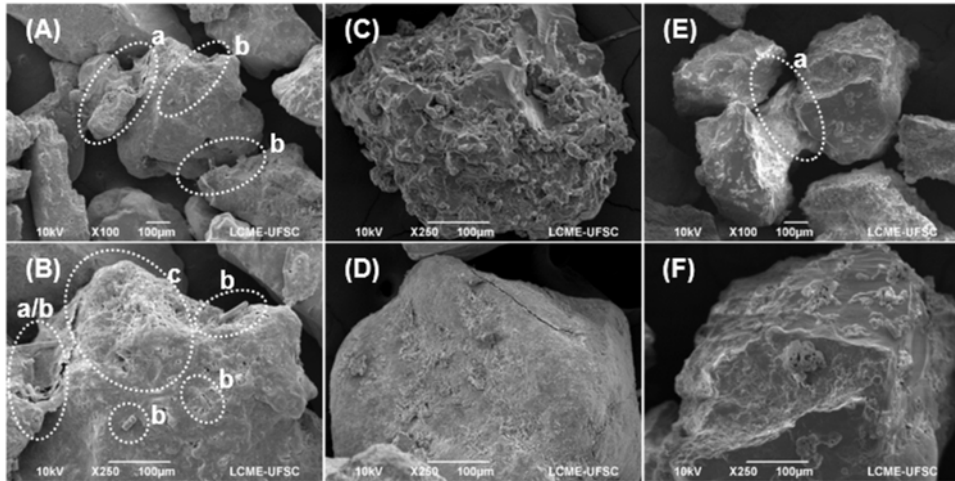
Figure 4 – SEM micrographs showing ScSF sand grains before and after cleaning. (A) sand grains mixed with *schmutzdecke* at X100 (before scrapping); (B) Sand grains uniformly covered by biomass in *schmutzdecke*, X250 (before scraping); (C) Sand grains with cavities covered by biomass, X250 (5 cm depth);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

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

In this study, extracellular polymeric substances distribution 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 (**Error! Reference source not found.**) (Pfannes et al., 2015).
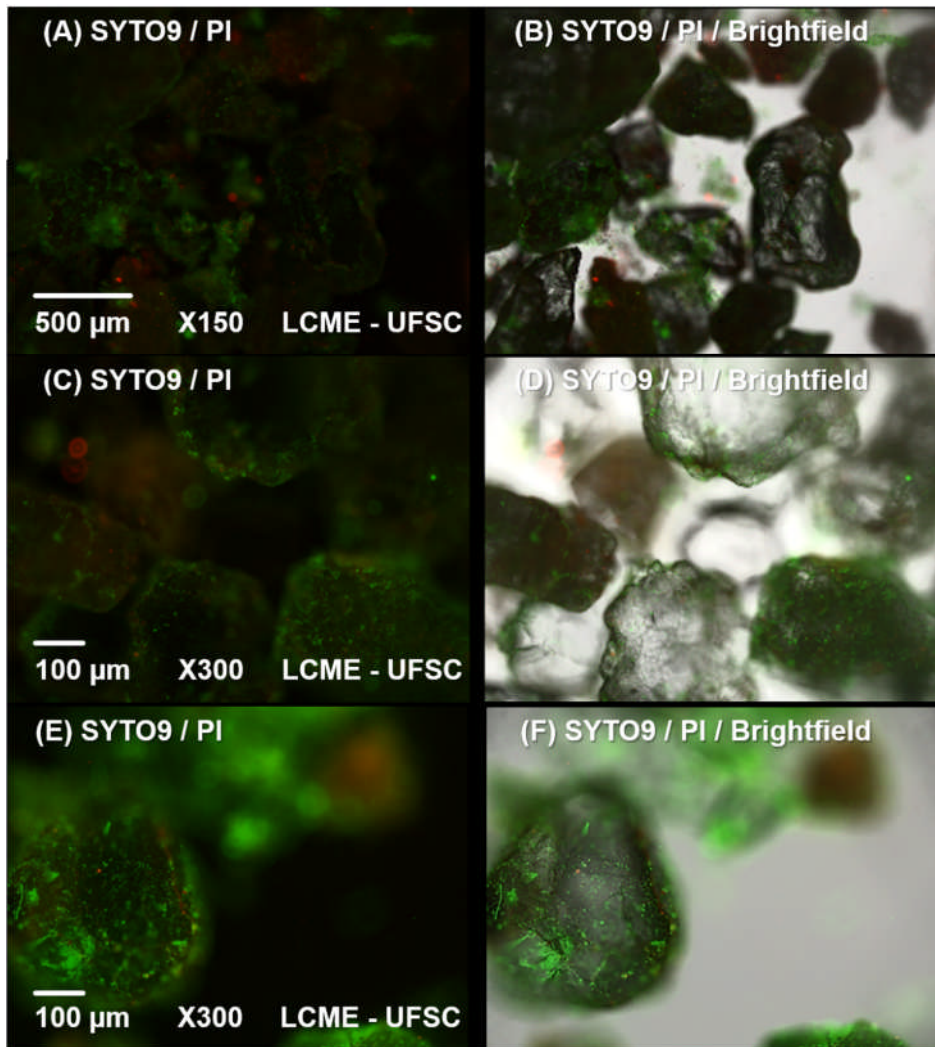
318   Figure 5 – Fluorescent microscopy micrographs showing *schmutzdecke* and the top sand layers before

319   cleaning of ScSF (A;B) and BSF (E;F), and after cleaning of ScSF (C;D). Fluorescence microscopy shows the

320   viable bacteria stained green by SYTO9 and the unviable bacteria stained yellow-red by SYTO9 and

321   Propidium Iodide (PI). Brightfield microscopy (B; D; and F) also give an idea of the surface of the sand

322   grains.

323   Microscope images similar to biomass results show that cleaning was not sufficient

324 to remove all of the attached material (Figure 4, **Error! Reference source not found.** E and

325 F), especially on BSF (**Error! Reference source not found.** E and F). Furthermore, by

326 fluorescent microscopy it was possible to see viable bacteria attached to the sand before

327   and after cleaning, especially in the aggregates on sand cavities. Viable bacteria were

328   observed, even after scraping which removed biomass significantly (**Error! Reference**

329   **source not found.** E and F). This is an evidence that although biomass was mostly

330   removed, bacteria were still attached on the sand grains right after cleaning. This

331   suggests that immediately resanding, if necessary, could be a good option for SSF

332   maturation (Barret et al., 1991; Huisman and Wood, 1974).

333   3.4   16S rRNA SEQUENCING OF THE DYNAMICS OF MICROBIAL COMMUNITY

334   For a more specific characterization of bacterial biomass, the microbial community

335   dynamics of the sand samples were investigated by 16S rRNA sequencing, as well as the

336   identification of the main genera found in the microbiota and their relative abundance.

337   This aimed to study the influence of backwashing on SSF at bacterial community structure

338   level.

339   A total of 790k sequences were retrieved from 16 samples of the high-throughput

340   16S rRNA Illumina MiSeq™ sequencing. After quality control by *QIIME2*™, and the

341   removal of chimera and low-quality reads (Phred<24), 555k high-quality sequences

342   remained for further analysis (Table S4).

343   **3.4.1   Bacterial Community Identification and Relative Abundance**

344   At phylum level, the most abundant bacteria were: Proteobacteria (42%-80%),

345   Acidobacteria (3%-22%), Verrucomicrobia (5%-16%), Chloroflexi (3%-15%), Bacteroidetes

346   (4%-12%), Actinobacteria (2%-6%), Nitrospirae (0%-6%), Chlorobi (1%-6%), and

347   Cyanobacteria (0%-2%) (Figure S6).

348   These phylum relative abundances were similar to those found in other studies,

349 with Proteobacteria, Nitrospirae, Planctomycetes, Actinobacteria, Bacteroidetes, and

350 Chloroflexi, being the most common (D'Alessio et al., 2015; Haig et al., 2015; Hwang et

351 al., 2014; Lautenschlager et al., 2014; Liao et al., 2015; Oh et al., 2018). Proteobacteria

352 are usually predominant on SSF due to the availability and variability of this phylum

353 metabolism in the environment. Its presence is related to the degradation of diverse

354 organic compounds on biofilters (D'Alessio et al., 2015; Haig et al., 2015; Lautenschlager

355 et al., 2014; Liao et al., 2015).

356   Other organic matter degradation associated bacteria phylum were

357 Verrucomicrobia, Chloroflexi, Bacteroidetes, and Actinobacteria (Sangwan et al., 2004;

358 Servin et al., 2008; Speirs et al., 2019; Thomas et al., 2011). Chloroflexi is usually present

359 in the sand bed rather than the *schmutzdecke* (D'Alessio et al., 2015; Haig et al., 2015).

360 However, these phylum did not change after scraping as previously reported by Haig et

361 al. (2015). Bacteroidetes were reported in other studies with decreasing abundance in

362 *schmutzdecke* with time (Haig et al., 2014; Zhao et al., 2019). On the other hand,

363 Nitrospirae phylum bacteria are indicative of the nitrification process on SSF and they are

364 more common in deeper layers (Lautenschlager et al., 2014).

365   The relative abundances of identified bacteria genera are shown on Figure 6. In

366 decreasing order, the most abundant identified genera were *Geobacter* (1% - 23%),

367 *Nitrospira* (1% - 9%), *Anaeromyxobacter* (0% - 8%), *Hyphomicrobium* (1% - 10%),

368 *Candidatus* Solibacter (0% - 9%), *Rhodoplanes* (1% - 6%), *Mycobacterium* (0% - 6%), and

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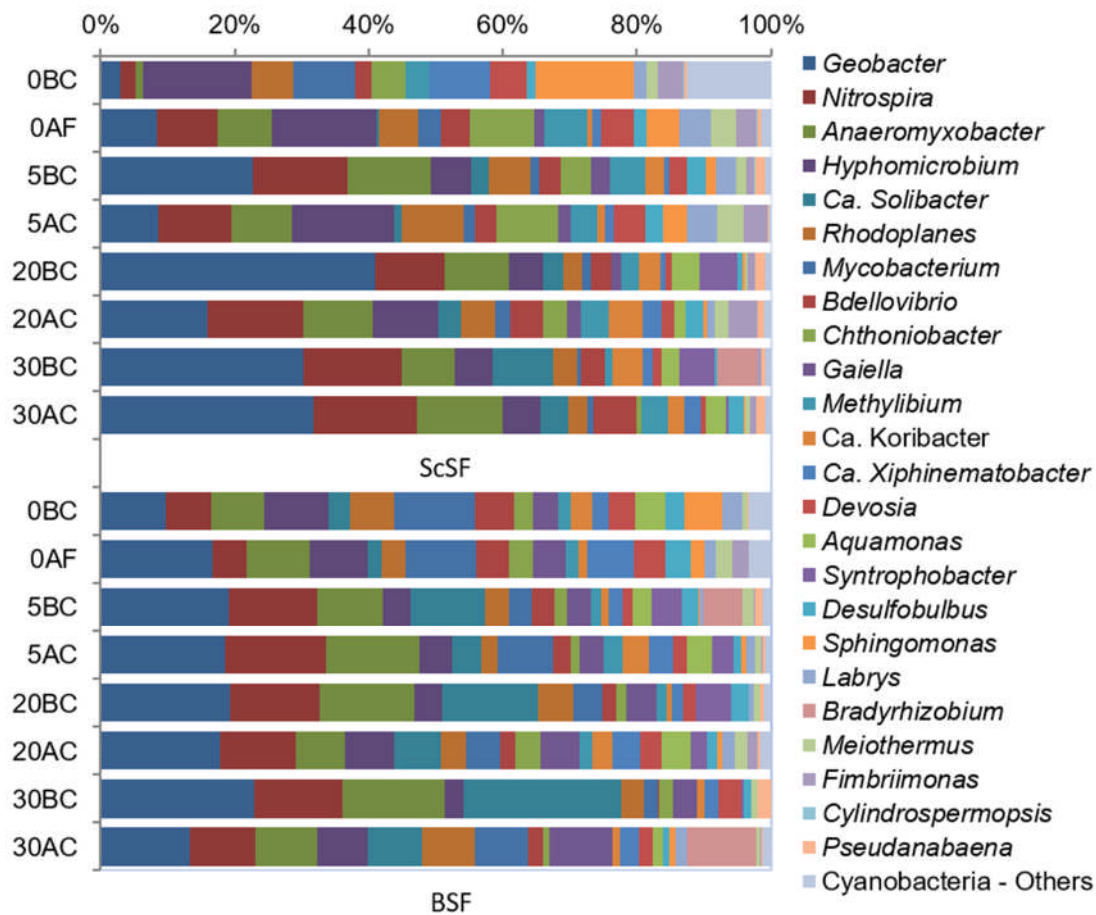
Figure 6 – Relative abundance at the genus level found in the datasets from ScSF and BSF, through filter depth, and before (BC) and after cleaning (AC). Numbers are indicating depth (0, 5, 20 and 30 cm)

Geobacter (Proteobacteria) was the most abundant identified 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 in 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.,

381 2011), then the associated bacteria presence would be expected (Tekerlekopoulou et al.,

382 2013).

383 *Nitrospira* (Nitrospirae phylum) was the second most occurring genus and is known

384 for its role in complete nitrification process (Daims et al., 2015; Palomo et al., 2016). This

385 genus was also found to be dominant in other SSF studies and is associated with the

386 nitrogen cycle (Oh et al., 2018; Wang et al., 2014). Other nitrogen cycle related genera (

387 *Anaeromyxobacter, Hyphomicrobium, Rhodoplanes* and *Candidatus* Solibacter) and

388 phylum (Verrucomicrobia and Planctomycetes) were also identified (Gupta et al., 2012;

389 Hiraishi and Ueda, 1994; Pearce et al., 2012; Sanford et al., 2002; Urakami et al., 1995;

390 Van Teeseling et al., 2015; Wang et al., 2019), and were reported in other drinking water

391 studies (Demir, 2016; Kaarela et al., 2015; Lautenschlager et al., 2014; Liao et al., 2013;

392 Oh et al., 2018; Vandenabeele et al., 1995; Wang et al., 2018)*.* The presence of nitrogen

393 cycle organisms confirms the complexity of bacterial activities on SSF, which have already

394 been reported as capable of complete nitrification (Aslan and Cakici, 2007; Nakhla and

395 Farooq, 2003).

396 ### 3.4.2   Bacterial Community Spatial Distribution and Alterations Due Cleaning Process

397 Overall, as relative abundances show, samples closer to the surface were more

398 influenced by the cleaning processes (Figure 6). Bacterial relative abundance also

399 changed due the different cleaning process and sand depth. *Spearman* correlations

400 between relative abundance and depth were significant ($p < 0.05$) in ScSF for the most

401 abundant genera, excluding *Anaeromyxobacter*, and most of the phylum, such as

402 Proteobacteria, Acidobacteria and Nitrospirae. These correlations were less obvious in

403 BSF (Table S5), and are probably related to bacterial characteristics and their attachment

404   strength to grains (Haig et al., 2015; Lautenschlager et al., 2014; Oh et al., 2018). Also,

405   they indicate the complexity and the different roles bacteria may have as SSF ultimately

406   relies upon biological treatment, which is affected by factors such as food availability,

407   nutrients and filter operation (Haig et al., 2015; Lautenschlager et al., 2014; Oh et al.,

408   2018).

409   Principal coordinate analysis (PCoA) based on the *Bray–Curtis* distance metric

410   showed differences between the BSF and ScSF groups (Figure 7, a and b). Pairwise

411   PERMANOVA also highlighted statistical differences between overall bacterial community

412   diversity ($p = 0.001$) in both filters.
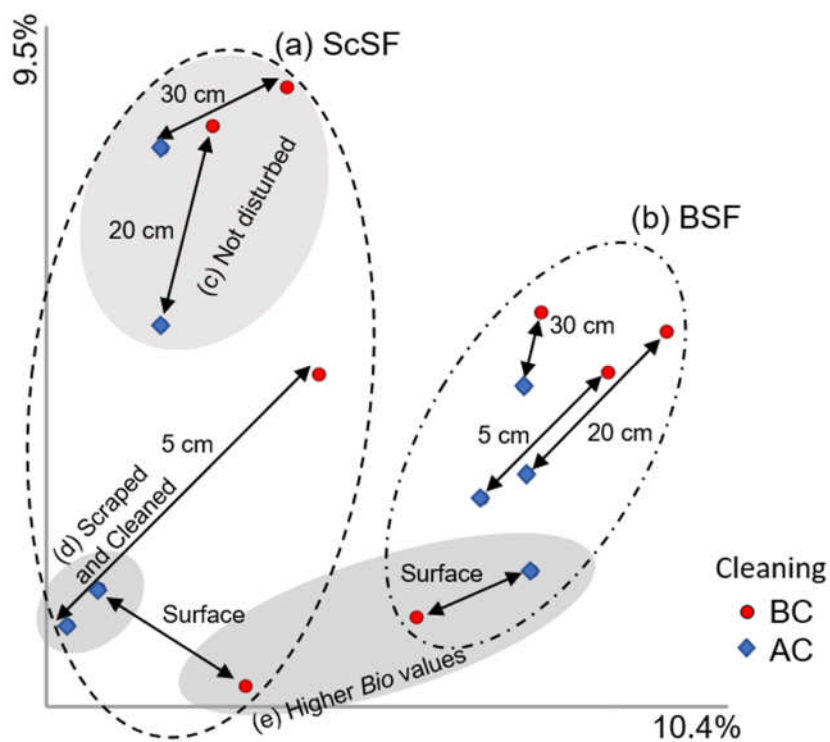
Figure 7- Principal coordinate analysis (PCoA) plot using *Bray-Curtis* distances for ScSF and BSF samples from different sand depths, before (BC) and after (AC) cleaning process. (a) ScSF sample group. (b) BSF sample group. (c) Samples not disturbed by any cleaning process. (d) Samples cleaned after scraping. (e) Samples at the top layer presenting higher biomass as *Bio*.

BSF samples coordinates appear closer to each other (Figure 7b), showing less variation in bacterial community diversity. It is also remarkable that sample coordinates from the top sand layers were near to each other, indicating a similarity with *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, 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).

25

427   These results agree well with the biomass distribution in sand layers (Figure 2),

428   confirming the differences in filtration mechanisms between the ScSF and BSF. Other

429   studies also reported differences between bacterial communities in the raw water (not

430   assessed in this study), and deeper sand layers (D'Alessio et al., 2015; Lautenschlager et

431   al., 2014; Oh et al., 2018; Pfannes et al., 2015).

432   Based on the alpha diversity indexes (*Shannon* and *Evenness*) and the number of

433   OTUs, the BSF samples were considered more uniform than ScSF, and were more diverse

434   on top layers (Table S4). Dalahmeh et al. (2014) also reported similar results. They argued

435   that the low genetic diversity in *schmutzdecke* bacteria was due to the high food chain

436   complexity on the sand surface, while competition and predation by other organisms

437   decreased with depth. Food is also less available at the lower layers, making the bacterial

438   community more homogenous. However, in BSF these indexes became more uniform

439   due to the sand fluidization, indicating backwashing mixture and bacteria selectivity

440   (Table S4).

441   3.5   OTHER MICRORGANISMS COMPOSING BIOMASS

442   Overall, as reported in other studies, *schmutzdecke* was visually the most diverse

443   layer, forming a complex food chain with microcrustaceans, midge larvae, nematodes,

444   rotifers, algae, and bacteria (Hurley and Wottom, 2006; Joubert, 2008; Law et al., 2001;

445   D. McNair et al., 1987; Nakamoto, 2014; Ranjan and Prem, 2018). This diversity was

446   mainly observed in the BSF but not in the ScSF. Algae were also visible under fluorescence

447   (Figure 8), but their viability cannot be related to the BVK due to chlorophyll-a natural

448   fluorescence (in red) (Reavie et al., 2010). *A. ambigua*, other diatoms, and filamentous

449   cyanobacteria were the most common microorganisms present (Figure 3, 3 and 8). These

450   microorganisms appearance is not surprising since they are common on the *Lagoa do*

451   *Peri* water and were previously reported as filter clogging phytoplankton (de Souza et al.,

452   2017; Saavedra del Aguila and Di Bernardo, 2003; Saupe and Mosimann, 2003).
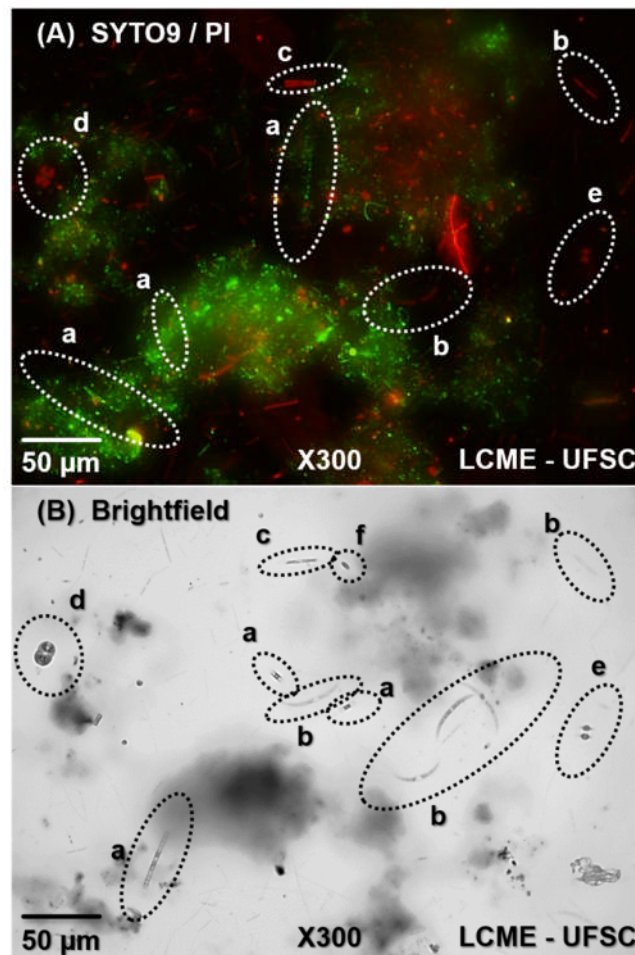
453

454   Figure 8 – Miscellaneous materials composing *schmutzdecke* with microalgae, especially diatoms,

455   cyanobacteria, bacteria and protozoa: (a) *Aulacoseira sp.*; (b) *Closterium sp.*; (c) *Fragilaria sp.*; (d)

456   *Cosmarium sp.*; (e) *Staurastrum sp.*; (f) Rotifer.

457   Cyanobacteria are highlighted in this study because of their abundance in the raw

458   water, especially the genera *Cylindrospermopsis* and *Pseudanabaena*. They are related to

459   filter clogging, are usually removed by SSF, and explain filtration runs of 15 days on

460   average (de Souza et al., 2017; Mondardo, 2009; Pereira et al., 2012; Pizzolatti et al.,

461   2014; Schöntag et al., 2015). Both the *Cylindrospermopsis* (0.00%-0.18%) and

462 *Pseudanabaena* (0.00%-2.10%) genera were identified in both ScSF and BSF, being more

463 abundant in the *schmutzdecke* (0.88%-12.67%), and were visible at microscope (Figure 3

464 and 4). Their presence on the ScSF surface can also be related to this filter faster headloss

465 development. Moreover, their presence along the depth (Figure 6) may explain their

466 breakthrough to effluent water (Table S2). The more significant chlorophyll-*a* removal is

467 probably due to diatom trapping at the surface because of their size (Figure 8 and Table

468 S2).

469   The presence of these different organisms is an example of *schmutzdecke*

470 complexity (Nakamoto, 2014). Its diversity preservation by BSF, as observed by

471 microscopy, is a promising result regarding this cleaning method. Lower disturbances of

472 *schmutzdecke* are recommended by some SSF researchers for a better preservation of

473 biological filtration mechanisms (Hurley and Wottom, 2006; Iwase et al., 2006;

474 Nakamoto, 2014, 2011).

475   These results show that regular scraping also preserves bacteria. However, other

476 organisms were not observed (e.g.: diatoms, nematodes, microcrustaceans), and

477 biomass decreased significantly after scraping (Figure 2). Such differences were not

478 observed on BSF, demonstrating that backwashing preserves the biomass diversity in the

479 filter bed as speculated by other studies (de Souza et al., 2016; Marnoto et al., 2008). On

480 the other hand, when scraping was used, biomass decreased. This suggests that scraping

481 disturbs the microbial community in ScSF, explaining the need for a filter maturation

482 period (Barret et al., 1991; Huisman and Wood, 1974).

483 3.6   BIOMASS CONSIDERATIONS REGARDING BACKWASHING

484   In this study, backwashing alone was not sufficient to significantly remove biomass

485 from sand surface. This may be explained by the backwashing hydrodynamics itself. The

486 lower d_{10} results in lower fluid/media tension, since water velocity is low (Cleasby et al.,

487 1977; Fitzpatrick, 1998; Valencia and Cleasby, 1979) . This probably resulted in less *Bio*

488 detachment from sand grains, although there is evidence of variation of the backwash

489 water turbidity in other studies (de Souza et al., 2016; Pizzolatti et al., 2014). In

490 backwashing, friction forces between grains are dominant at the beginning of the bed

491 expansion. After bed fluidization, the major forces acting on the sand grains are the drag

492 tension between the media and the fluid, pulling attached material out of the filter.

493 (Fitzpatrick, 1998; Valencia and Cleasby, 1979).

494 On average, a fluidized bed with 40% expansion is adopted for backwashing, based

495 on recommendations for rapid filter, although other values might be suggested for rapid

496 filters (Cleasby et al., 1977; Crittenden et al., 2012). However, expansion vary throughout

497 the backwashing duration; at the beginning of backwashing, dirt is usually removed due

498 to initial friction forces, higher velocities, and porosity augmentation liberating interstitial

499 trapped material (de Souza et al., 2016; Fitzpatrick, 1993; Pizzolatti et al., 2014).

500 Nevertheless, due to the smaller grain size used in the BSF, backwashing flow rates are

501 lower than the ones in rapid sand filters, leading to smaller drag tension between water

502 and sand grains after complete fluidization (de Souza et al., 2016; Fitzpatrick, 1998;

503 Valencia and Cleasby, 1979).

504 The lower tension may explain the differences in *Bio* and VS attached to the sand

505 media before and after backwashing. *Bio* and VS have different compositions (Figure 2).

506 While *Bio* represents cell biomass that can be strongly attached to the sand surface by

507 exopolymer substances, VS represents any organic material attached to the filter media

508 that might be easily removed when the sand bed is expanding. The nature of trapped

509   material (i.e. organic and non-organic) and their separate ways of attachment on grains

510   surface (e.g.: attachment mechanisms, position on the sand grains surface, size, and

511   shape) may have led to different detachment modes and, consequently, initial headloss

512   recovery. This *Bio* preservation may also be confirmed by the low variability of the

513   bacterial community and the microscopy observations (Figure 3, 4 and 5).

514   3.7   BIOMASS CONSIDERATIONS REGARDING BSF OPERATION

515   The results about biomass in BSF may be considered preliminary, due to the short

516   period of the study and its pioneering status. However, these results are promising for

517   introducing backwashing in small and medium-scale slow sand filters.

518   Considering the importance of biological degradation of certain compounds in SSF,

519   biomass preservation can be considered an advantage of BSF (Summers, 2014). Biofilm

520   preservation on the filter could maintain the microbial community despite consecutive

521   cleanings, reducing the ripening period. This requires further investigation, but it was

522   previously reported that ripening period could be eliminated due biomass preservation

523   in biosand filters (Ikhlef and Basu, 2017). A concern about this is that the maintenance of

524   biomass could result in an outbreak of persistent pathogens if they are present in the

525   sand bed or *schmutzdecke* (Hwang et al., 2014; Karon et al., 2011).

526   Despite possible advantages and concerns, initial headloss was recovered after

527   cleaning (Figure 2), indicating that it was mostly due to the interstitial or non-organic

528   materials which were removed during backwashing. Furthermore, headloss was lower in

529   BSF than in ScSF at the end of operation (Figure 2), which allows a longer operational time

530   and higher productivity (de Souza et al., 2016).

531   The characterization of bacterial communities by 16S rRNA gene sequencing is also

532   promising as Figure 6, 7 and S6 show that there is bacterial community stratification in

533   ScSF with depth (less evident on BSF), specially at genera level. Also, it shows that scraping

534   changes the bacterial community on sand, which may have a higher impact on ScSF

535   ripening than on BSF. Few studies have used 16S rRNA sequencing for bacteria

536   community characterisation on SSF, and the technique is promising since bacteria

537   degradation pathways could be better understood in SSF in the future (Haig et al., 2011).

538   In the BSF case, specific conditions as the maintenance of bacteria community may be an

539   indication of bacteria selectivity after consecutive backwashing, which could also lead to

540   a faster ripening, favouring the removal of target contaminants (Flemming et al., 2016;

541   Ikhlef and Basu, 2017). Other studies have reported the importance of bacterial

542   degradation on SSF for removal of target contaminants such as organic compounds and

543   nutrients, and the selectivity of specific bacteria due to these contaminants over

544   operational time or treatment process (Aslan and Cakici, 2007; D'Alessio et al., 2015; Li

545   et al., 2018, 2017; Liu et al., 2019; Miltner et al., 1995; Summers, 2014; Zearley and

546   Summers, 2012).

547   These results are representative of the complexity of SSF biological mechanisms.

548   Bacterial activity should be further studied in future research. Long-term studies could

549   investigate the possible backwashing role in selecting specific and better attached

550   bacteria for biofilm preservation and if this can reduce the filter maturation period in BSF.

551   **4   CONCLUSIONS**

552   The main conclusions of this study are:

31

- 553   • Biomass was developed in the sand bed differently depending on filter depth and
- 554   cleaning process. The top sand layers and *schmutzdecke* developed more biomass
- 555   in terms of *Bio* (99.7-114.1 µg-*Bio*/g-sand) and VS (5.43-9.04 mg-VS/g-sand). Also,
- 556   biomass stratification was more evident in ScSF deeper layers than in BSF,
- 557   resulting in a faster ScSF clogging.

- 558   • Microscopy observations confirmed the biomass quantification results, showing
- 559   biomass diversity preservation on BSF. The different techniques, i.e. SEM and
- 560   Fluorescence Microscopy, highlighted different aspects of the filter media
- 561   biomass and overall attached material. SEM analyses were able to show the
- 562   material attached to the sand grain surfaces, and Fluorescence Microscopy
- 563   showed that viable bacteria were spread across the *schmutzdecke* and sand
- 564   media, even after backwashing and scraping.

- 565   • *High-throughput* 16S rRNA sequencing complement the indirect biomass
- 566   quantification, being a useful tool for bacterial community structure
- 567   characterization. In this study, bacterial communities changed significantly due to
- 568   the cleaning process, indicating microbial selectivity of fluidization process.

- 569   • Proteobacteria was the predominant identified phylum (42%-80%). Meanwhile,
- 570   *Geobacter* (1%-23%) and *Nitrospira* (1%-9%) were the most prevalently identified
- 571   genera, being respectively associated with the iron and nitrogen cycles. Other
- 572   significant identified genera were associated with organic matter degradation,
- 573   demonstrating the complexity of SSF bacteria activity across the filter depth.

- 574   • Differences in biomass across the filter depth helped to understand the
- 575   differences between ScSF and BSF water qualities. Both filters had acceptable

576   efficiencies, according to WHO recommendations for drinking water standards,

577   especially turbidity (<1.0 NTU) and total coliforms (>1 Log).

578   • Overall, scraping and backwashing affected differently both slow sand filters,

579   resulting in distinct biomass accumulation and bacterial communities. Both filters,

580   ScSF and BSF, were able to improve water quality, but BSF was simpler to operate.

581   Therefore, BSF is recommended for small and community-scale filters as an

582   alternative to conventional SSF to produce good effluent quality with less

583   laborious cleaning processes.

584   • Further studies on biological pathways are recommended to better understand

585   the SSF bacterial purification mechanisms and possible selectivity. In addition, the

586   speed up of BSF maturation period, especially for removal of target contaminants

587   such as iron, nitrogen, and other biodegradable compounds, could be

588   investigated.

589 **5   SUPPLEMENTARY MATERIAL**

590   *Supplementary Material* presents additional methodology and data to support the

591 authors' statements (Table S and Figure S). The SEM original size micrographs are also

592 included for better observation.

593   All SEM micrographs taken from SSF samples used for this work are available at:

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

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928

The influence of slow sand filter cleaning process type on filter media biomass: scraping vs. backwashing – Supplementary Material

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Summary

Abbreviations

|	
AC	After cleaning
Bio	Cell Biomass
BC	Before cleaning
BSSF	Backwash Slow Sand Filter
BVK	*Live/Dead® BacLight Invitrogen*™ Viability Kit
C_{otg}	Extractable Organic Carbon
CSSF	Conventional Slow Sand Filter
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
FBF	Filtered Water from BSSF
FCF	Filtered Water from CSSF
FM	Filtration Media
HMDS	Hexamethyldisilazane
LAPOA	Drinking Water Laboratory
LCME	Electron Microscopy Central Laboratory
LIMA	Environmental Integrated Laboratory
LP	*Lagoa do Peri* Lake
Micro	Optic Microscopy
RFW	Rough Filtered Water
RW	Raw Water
SEM	Scanning Electron Microscopy
SSF	Slow Sand Filtration
SUVA	*Specific Ultraviolet Absorbance*
TOC	Total Organic Carbon
TOC_F	TOC Fumigated Samples
TOC_{NF}	TOC Non-Fumigated Samples
UFCS	Federal University of Santa Catarina
URF	Upflow Rough Filter with Layers
VS	Volatile Solids
WHC	Water Holding Capacity
WL_{max}	Maximum Water Level
WL_{min}	Minimum Water Level
WTP	Water Treatment Plant

1. SLOW SAND FILTER SCHEMES
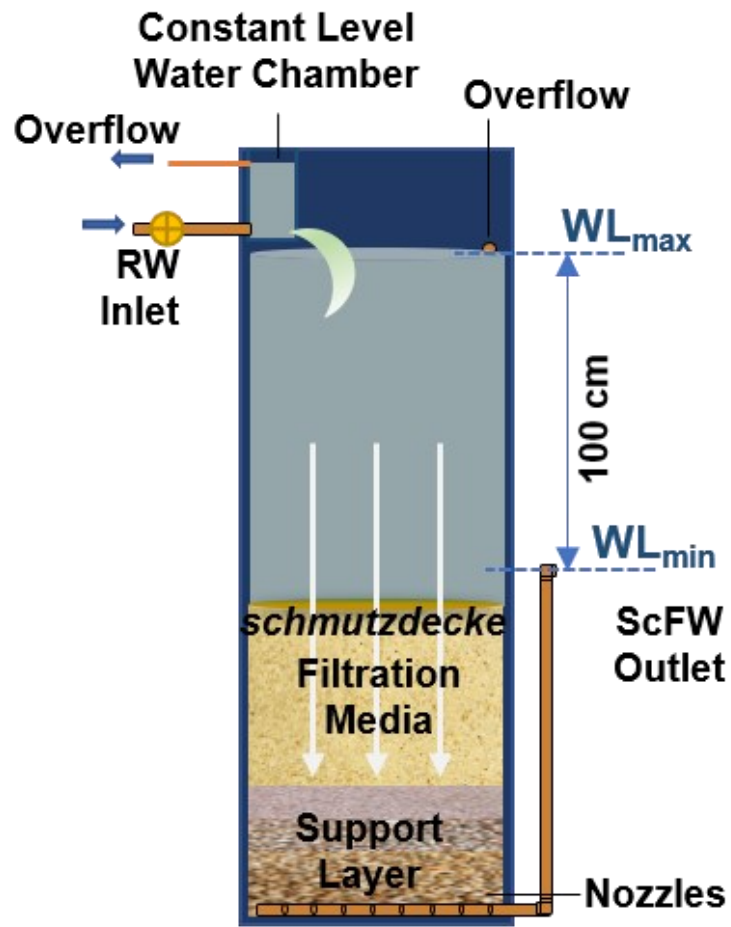
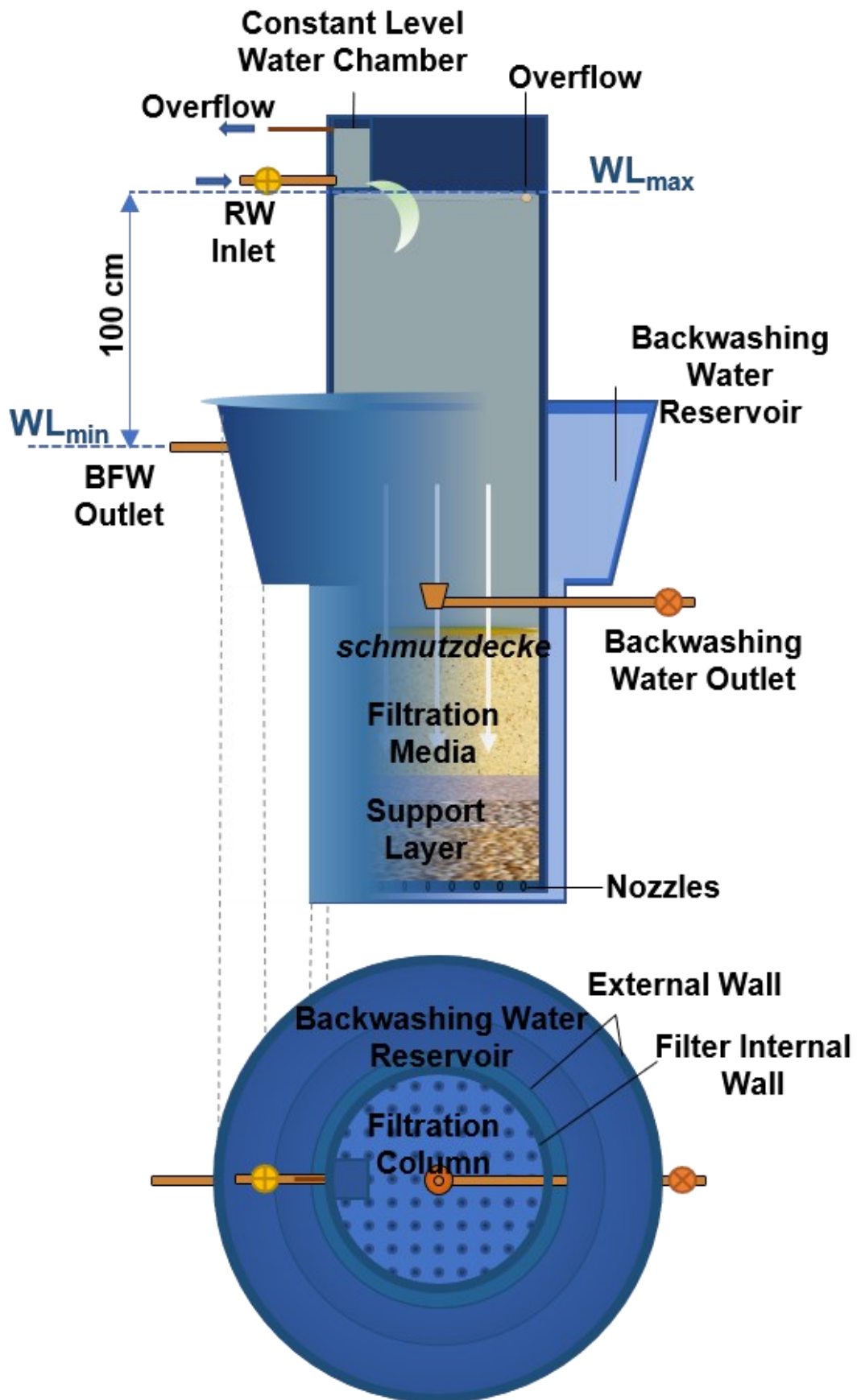Figure S1 – Community-scale Conventional Slow Sand Filter cleaned by scraping.

Figure S2 – Community-scale Backwashed Slow Sand Filter cleaned by filter bed fluidization.

2. SAMPLING AND ANALYSIS: DETAILED METHODOLOGIES

2.1. WATER SAMPLE PREPARATION

Water samples were filtered in 0.45 µm binder free Glassfiber membranes (*Machery-Nagel* GF-5, Germany) for true colour, DOC, and 254 nm absorbance determination. Firstly, membranes were washed with 500 mL of analytical water, then they were saturated with 50 mL of the sample. This filtrate was discharged, and 300 mL of the sample was filtered before undergoing the described analysis. Membranes were used for chlorophyll-*a* extraction with 80% ethanol (Ko et al., 2007; Liao et al., 2015, 2013). For effluent DO measurement, probes were used directly on the filter exit before coming into contact with air to avoid reaeration. SUVA was calculated as Equation S1:

Equation S1

$$SUVA = \frac{A \times 100}{DOC}$$

Where:
SUVA – Specific ultraviolet absorbance (L.mg^{-1}.m^{-1});
A –254 nm Absorbance (cm^{-1});
DOC – Dissolved Organic Carbon (mg/L);

2.2. WATER QUALITY PARAMETERS

Table S1 – Water quality parameters, equipment, and analysis frequency.

Parameter	Equipment and consumables	Frequency
Colour	• HACH DR2100 and DR2800 Spectrophotometer	1 day/week
Turbidity	• *HACH* 2100N Turbidimeter	5 days/week
Coliforms	• ONPG-MUG *COLILERT*® Substrate • *Quanti-tray*®/2000 Trays	1 day/week
DOC	• *Shimadzu* Toc5000A Analyser	1 day/week
Filamentous cyanobacteria	• Sedgewick chambers • *Olympus* BX40 Optic Microscope	1 day/week
Chlorophyll-*a*	• *HACH* DR2800 Spectrophotometer	1 day/week
Electrical conductivity	• *HACH HQ40D*	1 day/week
pH	• *HACH HQ40D*	1 day/week
DO	• AT 160 SP *Alfakit Oxymeter*	5 days/week
254 nm Absorbance	• OptizenPop 3000W Spectrophotometer	1 day/week
SUVA	• Shimadzu Toc5000A Analyser • OptizenPop 3000W Spectrophotometer	1 day/week
Ammonium	• HACH DR2800 Spectrophotometer	1 day/week

2.3. SCHMUTZDECKE AND FILTRATION MEDIA SAMPLING

Schmutzdecke and sand was sampled as shown on Figure S3 for different analyses: (A) biomass (*Bio*), (A) volatile solids (VS), (B) DNA extraction, (B) optical microscopy (Micro), and (B) scanning electron microscopy (SEM). The samples were taken from three different parts of the filter area with a tubular collector, using suction. Four different depths were separated, and three samples were taken from each depth and combined as one (at least 80 g).
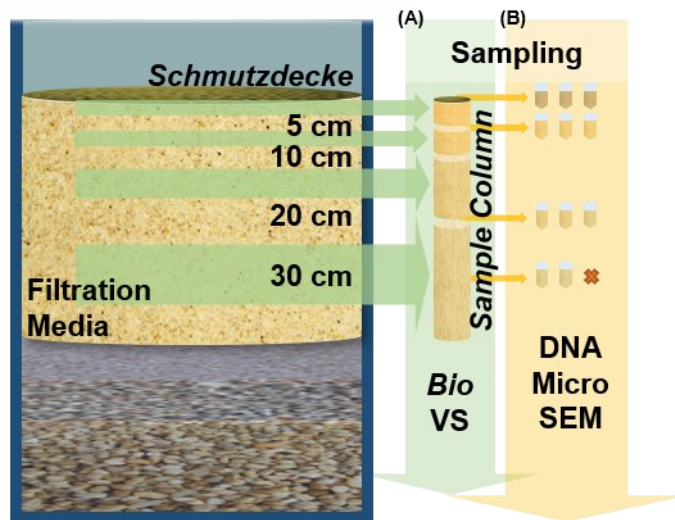
Figure S3 – *Schmutzdecke* and filtration media sampling strategy.

A weight of 10 g from each sample was first oven dried at 105°C for 24h to determine the dry weight and water holding capacity (WHC), then was burned in a muffle furnace at 550°C for 1h and reweighed for volatile solids (VS) determination.

From the column samples, three aliquots of roughly 1 g were collected from the *schmutzdecke* with the superficial sand, sand at 5 cm, 20 cm, and 30 cm. Samples were stored in 1.5 mL *Eppendorf* microtubes for observation by SEM, light microscopy (brightfield and fluorescence emission), and DNA extraction. No samples were taken for SEM at 30 cm depth since a previous analysis found that no significant attached material could be observed.

2.4. BIOMASS AND VOLATILE SOLIDS QUANTIFICATION

Sand biomass determination followed Campos et al. (2002) methodology. Two 25 g portions of sand samples had their water holding capacities adjusted from 40% to 50%, and one of them was fumigated with Chloroform P.A. for 24h at 25°C. Then both the fumigated and non-fumigated portions were extracted with 50 mL de K_2SO_4 0,1 M solution at 200 rpm rotational agitation for 30 min. The extraction products were filtered in 0.45 µm binder free glass fibre membranes (*Macherey-Nagel* GF-5) and were acidified with HCl 4 M at pH<3, before being stored at 4°C for total organic carbon determination (TOC). A *Shimadzu* Toc5000A was used for TOC determination by the high combustion method.

The different TOC concentrations from same samples were converted in cell

biomass (**Bio**) per sand weight as follows (Equation S2 and S3) (Campos et al., 2002):

Equation S2

$$C_{org} = TOC_F - TOC_{NF}$$

C_{org} = Extractable organic carbon (mg/L);
TOC_F= TOC from fumigated samples (mg/L);
TOC_{NF}= TOC from non-fumigated samples (mg/L).

Equation S3

$$Bio = 2.22 \, C_{org} \times 0{,}002$$

2.5. SCANNING ELECTRON MICROSCOPY

Around 0.5 g of sand samples were fixed with 1.5 mL of 5% Glutaraldehyde Solution for 1h, then were washed three times with 0.1 M Phosphate Buffer Solution. Sequence increased alcohol concentrations washing (50%, 60%, 80%, 90%) was used for dehydration. The samples were washed three times with Ethanol P.A. and were placed in filter paper to dry with a couple of drops from Hexamethyldisilazane Solution (HMDS) prior to SEM stubs preparation (Pinto et al., 2014; Proctor and Hammes, 2015). The samples were fixed on stubs with carbon tape and covered with gold for SEM observation. A model *JEOL JSM-6390LV Scanning Electron Microscope* was operated at 10 kV and X25 to X5000 amplifications for sample observation.

2.6. BRIGHTFIELD AND FLUORESCENCE OPTICAL MICROSCOPY

Bacterial availability was analysed with Microscopy *Live/Dead® BacLight Invitrogen™* Kit (BVK). BVK is composed of two different solutions: Solution A, with 1.67 mM *SYTO9* and 1.67 mM propidium iodide (PI) in 300 µL of dimethyl sulfoxide (DMSO), and Solution B, with 1.67 mM *SYTO9* and 18.3 mM propidium iodide (PI) in 300 µL of DMSO (All provided manufactured). Following manufacturing instructions, Solution AB was prepared using 3 µL of Solution A plus 3 µL of Solution B in 8 mL of 0.085% NaCl in analytical water. For 0.25 cm³ of sand, 1 mL of Solution AB staining was enough for fluorescent microscopy observations. Samples were prepared, were protected from light, and were kept at room temperature for at least 15 min prior to microscopy. Microscope slides were prepared with stained samples immediately before

observation in a *Leica DM5500 B* microscope system. Images were prepared using *Leica Application Suite*/LAS 3.3.

3. DATA DISTRIBUTION

3.1. SYSTEM EFFICIENCY

Table S2 – Water quality results summary. RW – Raw water; RFW – Rough filtered water; ScFW –ScSF filtrate; BFW – BSF filtrate; N – sample count; A – average; SD- Standard deviation; Min. – Minimum; M. – Median; Max. – Maximum; Rem. – average removal from last step; Statistically compared using Tuckey (^{T}) or Kruskal-Wallis (^{KW}) test; Rem. *p* – *p*-value from Tuckey test; $^\#$ No statistical difference to conclude variation from previous sample; * Statistically lower; $^+$ Below quantification limit; $^{++}$ Below detection limit; N.D. – Not detected; (-) not possible to calculate.

Parameter	Sample	N	A.^{T}	SD	Min.	M.^{KW}	Max.	Rem.	Rem. *p*
Turbidity	RW	77	9.4	4.6	3.1	7.8	23.8		
(NTU)	RFW	79	3.0	0.8	1.6	3.0	4.8	68%	0.000
	SCFW	72	0.64	0.45	0.22	0.47*	2.0	79%	0.000
	BFW	76	0.83	0.53	0.28	0.70	3.4	73%	0.000
Apparent Colour	RW	10	150	56	103	124	255		
(Pt-Co)	RFW	10	59	13	43	58	84	60%	0.000
	SCFW	10	11	7	2	9	26	82%	0.007
	BFW	8	15	6	6	14	26	75%	0.018
True Colour	RW	14	12	8	5	10	33		
(Pt-Co)	RFW	13	10$^\#$	4	6	9$^\#$	17	17%	0.683
	FLC	13	7$^\#$	2	4	7*	14	27%	0.488
	BFW	12	9$^\#$	2	6	9$^\#$	12	13%	0.902
DO	RW	20	7.64	1.45	3.50	7.99	9.99		
(mg/L)	RFW	23	4.56	2.53	1.20	4.68	9.50	40%	0.000
	ScFW	24	3.23	1.70	0.32	3.15	7.40		
	BFW	23	2.71	1.84	0.34	2.38	6.91		
254 nm Abs./cm	RW	14	0.056	0.009	0.041	0.056	0.071		
	RFW	14	0.052$^\#$	0.008	0.038	0.052$^\#$	0.065	7%	0.773
	ScFW	14	0.050$^\#$	0.014	0.035	0.042$^\#$	0.077	5%	0.913
	BFW	13	0.050$^\#$	0.010	0.034	0.052$^\#$	0.062	6%	0.894
COD	RW	7	6.291	0.412	5.758	6.248	7.048		
(mg/L)	RFW	10	6.143$^\#$	1.33	3.351	6.059$^\#$	8.059	2%	0.992
	ScFW	8	4.936$^\#$	0.688	4.076	4.652	5.792	20%	0.094
	BFW	8	5.323$^\#$	1.285	4.056	4.757$^\#$	7.727	13%	0.368
pH	RW	11	6.94	0.14	6.65	6.91	7.16		
	RFW	11	6.62	0.11	6.48	6.61	6.83	5%	0.000
	ScFW	11	6.42	0.16	6.19	6.42	6.73	3%	0.010
	BFW	11	6.42	0.17	6.15	6.4	6.68	3%	0.011
Electrical Conductivity	RW	11	71.11	1.08	68.7	71.30	72.5		
(µS/cm)	RFW	10	73.64$^\#$	1.30	71.8	73.85	75.3	-4%	0.222
	ScFW	10	72.95$^\#$	5.39	66.5	71.85$^\#$	82.8	1%	0.953
	BFW	10	73.97$^\#$	1.98	70.5	74.35$^\#$	76.9	0%	0.994
Clorophyll-*a*	RW	12	21.27	6.45	9.5	23.46	30.46		
(µg/L)	RFW	13	8.57	5	2.25	9.32	20.49	60%	0.000
	ScFW	13	0.710	0.498	0.111	0.586	1.912	92%	0.000
	BFW	12	0.546	0.554	0.06	0.489	2.214	94%	0.000
SUVA	RW	8	0.862	0.172	0.632	0.898	1.059		
L.mg-1.m-1	RFW	11	0.866$^\#$	0.363	0.490	0.765$^\#$	1.82	0%	1000
	ScFW	9	1.007$^\#$	0.392	0.566	0.875$^\#$	1.84	-16%	0.749
	BFW	8	0.930$^\#$	0.241	0.554	0.983$^\#$	1.296	-7%	0.971
Total Coliforms	RW	8	4.118	0.525	2.972	4.205	4.714		
(Log10[NMP/100mL])	RFW	11	3.901$^\#$	0.92	2.301	4.150$^\#$	5.298	0.217	0.964
	ScFW	13	2.391	1.235	0.477	2.195	4.991	1.510	0.003
	BFW	9	2.611	0.942	1.299	2.488	4.352	1.290	0.029

Parameter	Sample	N	A.[T]	SD	Min.	M.[KW]	Max.	Rem.	Rem. p
Filamentous Cyanobacteria (cell/mL)	RW	16	3.7×10^5	1.1×10^5	2.0×10^5	3.9×10^5	5.6×10^5		
	RFW	16	1.8×10^5	8.7×10^4	6.3×10^5	1.8×10^5	3.2×10^5	51%	0.000
	ScFW	16	6.6×10^3	4.1×10^3	5.0×10^2	8.2×10^3	1.2×10^4	96%	0.000
	BFW	16	8.4×10^3	5.9×10^3	3.0×10^3	6.8×10^3	1.9×10^4	95%	0.000
Ammonium (mg/L)	RW	16	0.1[+]	0.1[+]	N.D.[++]	0.0[++]	0.3[+]	-	-
	RFW	16	0.0[++]	0.1[+]	N.D.[++]	0.0[++]	0.3[+]	-	-
	ScFW	16	0.2[+]	0.6	N.D.[++]	0.0[++]	2.3	-	-
	BFW	16	0.0[++]	0.1[+]	N.D.[++]	0.0[++]	0.3[+]	-	-

3.2. SLOW SAND FILTER BIOMASS

Table S3 – Cell biomass (*Bio*) and total volatile solids (VS) on SSF FM before (BC) and after cleaning (AC). ; N – sample count; A – average; SD- Standard deviation; Min. – Minimum; Q1 – 1st Quartile; M. – Median; Q3 – 3rd Quartile; Max. – Maximum. * Statistically different from the deeper layer. # Statistically different after cleaning.

Biomass	SSF	Condition	Depth	N	A.	SD	Min.	Q1	M.	Q3	Max.
Bio (ug/g)	BSF	BC	0	5	99.7	60.0	25.6	49.5	80.3	159.6	178.0
			5	5	57.3	29.2	19.2	30.3	54.2	85.7	92.0
			10	5	62.0	73.4	3.4	9.8	41.2	124.5	186.8
			20	4	62.3	46.8	20.5	26.6	49.8	110.5	129.1
		AC	0	4	99.0	33.4	68.4	69.9	94.3	132.6	138.8
			5	4	75.5	41.5	15.2	31.8	90.7	104.0	105.4
			10	5	65.7	32.5	35.7	36.1	65.9	95.3	114.1
			20	5	48.5	29.6	13.4	17.6	57.5	74.9	82.0
	ScSF	BC	0	5	114.1*#	39.5	80.5	85.3	103.4*#	148.3	180.5
			5	5	49.7	23.0	12.9	27.3	61.1	66.3	69.2
			10	4	36.98	15.63	15.04	20.41	42.04	48.49	48.80
			20	5	25.18	11.73	5.83	14.80	27.65	34.31	34.96
		AC	0	4	38.02	16.32	16.44	20.93	42.05	51.09	51.56
			5	5	42.04	21.94	8.79	22.72	43.08	60.84	67.28
			10	5	28.58	10.14	13.73	19.38	28.55	37.79	39.81
			20	5	22.71	11.64	3.30	11.96	28.47	30.58	31.94
VS (mg/g)	BSF	BC	0	5	9.04*	2.37	6.70	6.90	8.91*	11.24	12.57
			5	5	5.897	1.210	4.423	4.961	5.848	6.857	7.772
			10	5	4.996	1.597	3.273	3.743	4.280	6.606	7.284
			20	5	4.934	0.719	4.245	4.352	4.608	5.679	5.953
		AC	0	5	7.203	1.549	5.510	5.770	7.097	8.690	9.366
			5	5	6.496	1.746	5.064	5.142	6.073	8.062	9.372
			10	5	5.518	1.251	3.471	4.503	5.786	6.398	6.872
			20	5	4.958	1.572	2.991	3.460	5.346	6.263	7.101
	ScSF	BC	0	5	5.431	1.684	3.956	4.121	4.665	7.125	8.000
			5	5	4.938	0.802	3.847	4.183	4.991	5.667	5.934
			10	5	4.726	1.520	2.138	3.420	5.173	5.808	5.858
			20	5	4.914	1.301	3.370	3.546	5.299	6.089	6.254
		AC	0	5	4.578	0.757	3.760	3.839	4.615	5.299	5.570
			5	4	3.878	1.240	2.109	2.569	4.276	4.790	4.852
			10	5	4.803	0.700	3.720	4.120	5.072	5.353	5.478
			20	5	5.152	0.811	3.836	4.408	5.457	5.744	5.940

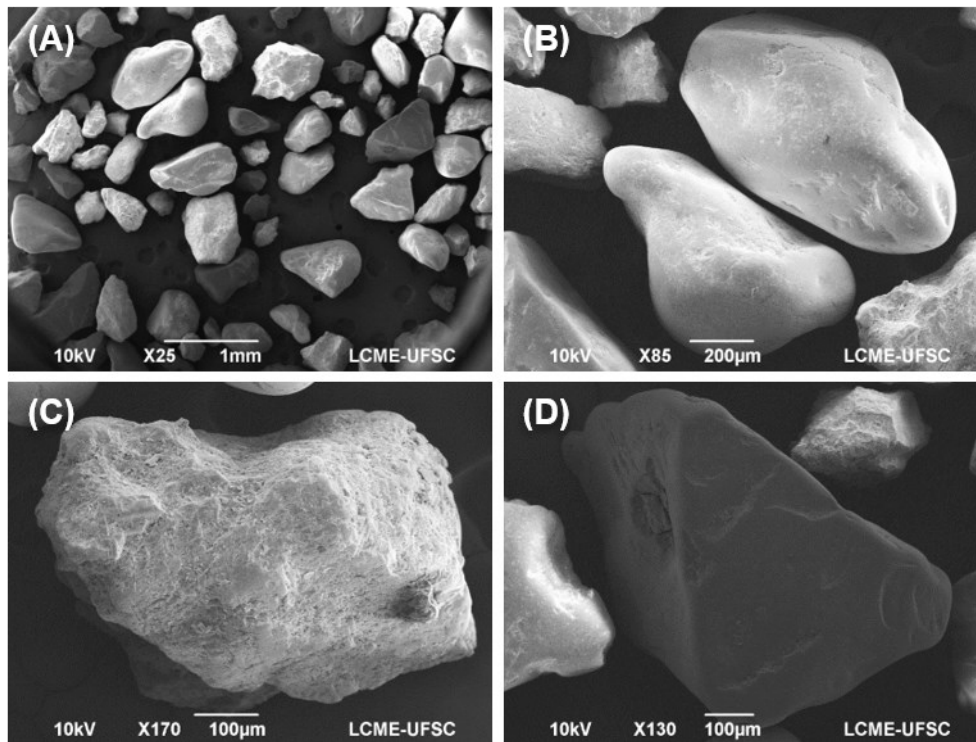## 4. GRAIN CHARACTERISTICS AND SIZE DISTRIBUTION

Figure S4 – SEM micrographs showing sand used as media. (A) general view at X25; (B) Smooth surface and round shaped sand grain at X85; (C) Rough surface and angular shaped sand grain at X170; and (D) Smooth surface and angular shaped sand grain at X130.
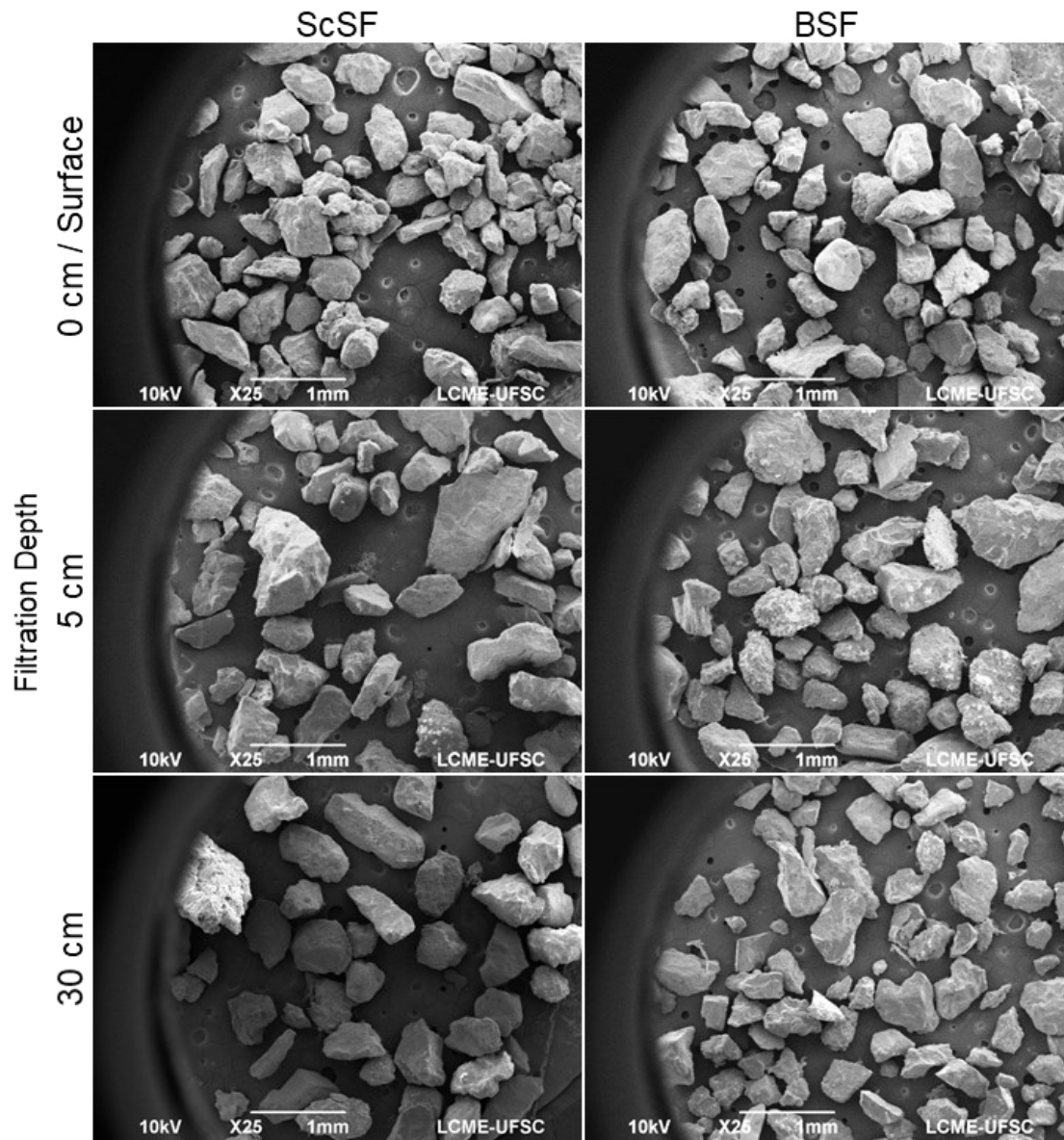
Figure S5 – ScSF and BSF filter media along depth showing filter media grain size with no apparent grain size stratification.

5. HIGH-THROUGHPUT DNA SEQUENCING

Table S4 – Numbers of sequences analysed, OTU richness, Shannon diversity index, and Evenness.

Filter	Cleaning	Depth	Raw reads	Removed [a]	Effective	Norm[b] Reads	Norm[b] OTUs	Norm[b] Shannon	Norm[b] Evenness
ScSF	BC	0	43711	29267	29255	10200	440	7.76	0.90
		5	35470	26181	26137	10200	537	8.24	0.91
		20	62675	44640	44459	10200	675	8.57	0.91
		30	59408	40233	40233	10200	627	8.53	0.91
	AC	0	38787	28020	27950	10200	503	8.12	0.90
		5	46225	31797	31797	10200	468	7.95	0.89
		20	54876	40400	40400	10200	671	8.61	0.91
		30	51643	37483	37459	10200	629	8.49	0.91
BSF	BC	0	55155	39230	39230	10200	628	8.50	0.91
		5	56042	41417	41412	10200	780	8.80	0.91
		20	50970	35496	35489	10200	621	8.48	0.91
		30	13659	10312	10312	10200	352	7.82	0.88
	AC	0	53652	35607	35587	10200	622	8.47	0.91
		5	63980	43464	43181	10200	638	8.55	0.91
		20	48766	33740	33740	10200	616	8.50	0.91
		30	55046	38815	38815	10200	597	8.43	0.91

(a) Low-quality reads and chimaera

(b) Normalized
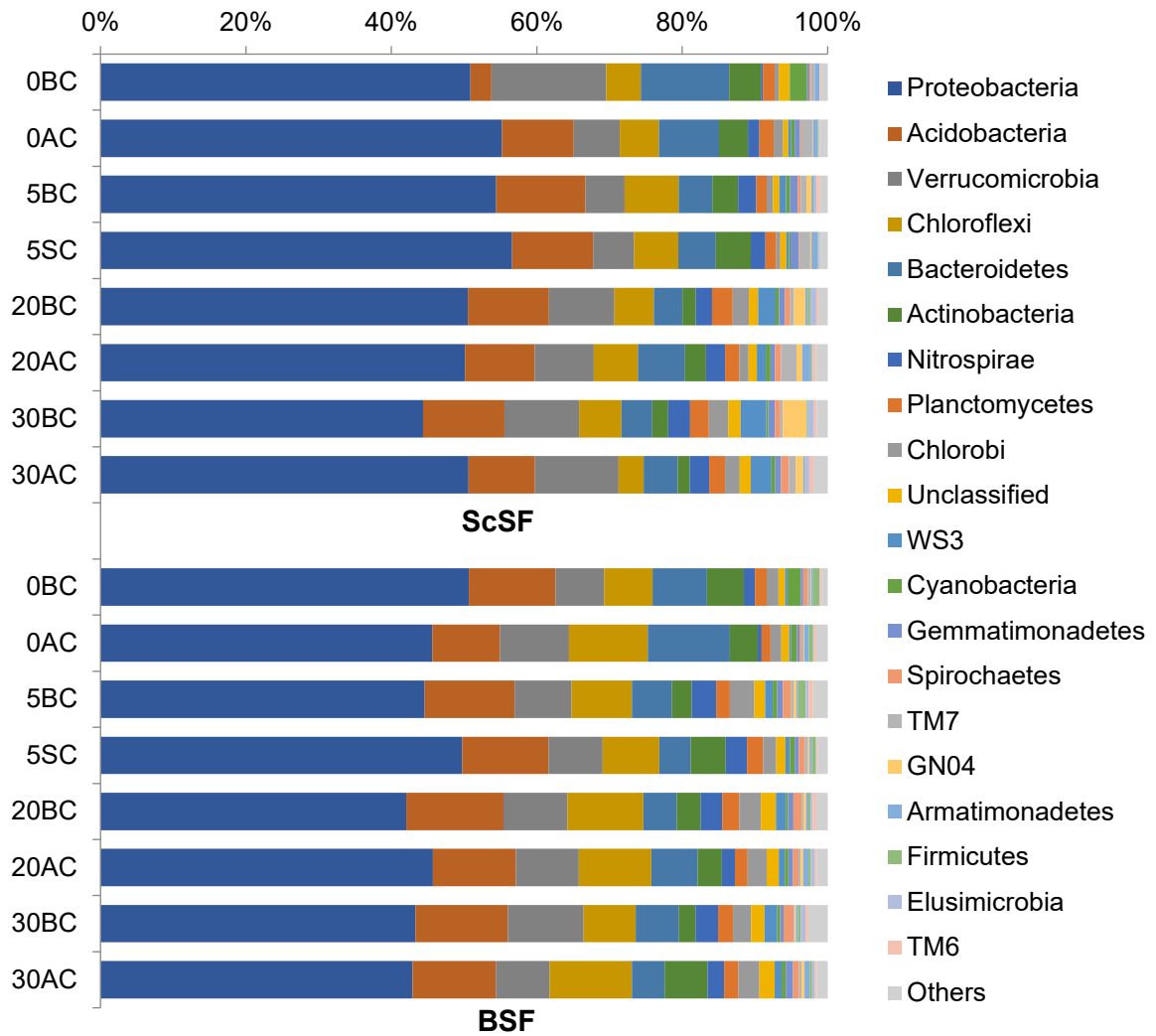
Figure S6 – Relative abundance at the phylum level found in the datasets from ScSF and BSF, through filter depth, and before (BC) and after cleaning (AC). Numbers are indicating depth (0, 5, 20 and 30 cm).

Table S5 – Spearman correlations and p-values between depth and relative abundance of bacteria phylum and genera.

Phylum	Spearman (ρ) ScSF	BSF	p-value (p) ScSF	BSF	Genera	Spearman (ρ) ScSF	BSF	p-value (p) ScSF	BSF
Proteobacteria	-0,732	-0,634	0,039	0,091	Geobacter	0,830	0,439	0,011	0,276
Acidobacteria	0,195	0,146	0,643	0,729	Nitrospira	0,878	0,390	0,004	0,339
Verrucomicrobia	0,293	0,098	0,482	0,818	Anaeromyxobacter	0,439	0,293	0,276	0,482
Chloroflexi	0,000	0,098	1,000	0,818	Hyphomicrobium	-0,830	-0,537	0,011	0,170
Bacteroidetes	-0,683	-0,586	0,062	0,127	Ca. Solibacter	0,976	0,781	0,000	0,022
Actinobacteria	-0,83	-0,293	0,011	0,482	Rhodoplanes	-0,732	0,146	0,039	0,729
Nitrospirae	0,927	0,439	0,001	0,276	Mycobacterium	-0,878	-0,683	0,004	0,062
Planctomycetes	0,586	0,342	0,127	0,408	Bdellovibrio	0,488	-0,927	0,220	0,001
Chlorobi	0,683	0,537	0,062	0,170	Chthoniobacter	-0,859	-0,390	0,006	0,339
Unclassified	0,683	0,634	0,062	0,091	Gaiella	-0,200	0,098	0,635	0,818
WS3	0,927	0,634	0,001	0,091	Methylibium	-0,439	-0,736	0,276	0,037
Cyanobacteria	-0,293	-0,781	0,482	0,022	Ca. Koribacter	0,732	-0,537	0,039	0,170
Gemmatimonadetes	0,244	0,488	0,560	0,220	Ca. Xiphinematobacter	0,000	-0,293	1,000	0,482
Spirochaetes	0,781	0,586	0,022	0,127	Devosia	-0,927	-0,293	0,001	0,482
TM7	-0,293	-0,488	0,482	0,220	Aquamonas	0,832	-0,250	0,010	0,550
GN04	0,927	0,488	0,001	0,220	Syntrophobacter	0,727	0,150	0,041	0,723
Armatimonadetes	-0,537	0,244	0,170	0,560	Desulfobulbus	-0,195	-0,830	0,643	0,011
Firmicutes	0,342	-0,683	0,408	0,062	Sphingomonas	-0,976	-0,565	0,000	0,145
Elusimicrobia	0,878	0,586	0,004	0,127	Labrys	-0,850	-0,342	0,008	0,408
TM6	0,634	0,146	0,091	0,729	Bradyrhizobium	0,507	0,319	0,200	0,441
					Meiothermus	-0,683	-0,439	0,062	0,276
					Fimbriimonas	-0,634	-0,250	0,091	0,550
					Cylindrospermopsis	-0,598	*	0,117	*
					Pseudanabaena	0,488	0,537	0,220	0,170

ρ -1   1

| p-value | p > 0,10 | p < 0,10 | p < 0,05 | p < 0,01 |

ρ – Spearman's rho

* - Not enough data. The bacterium was not present in all layers.
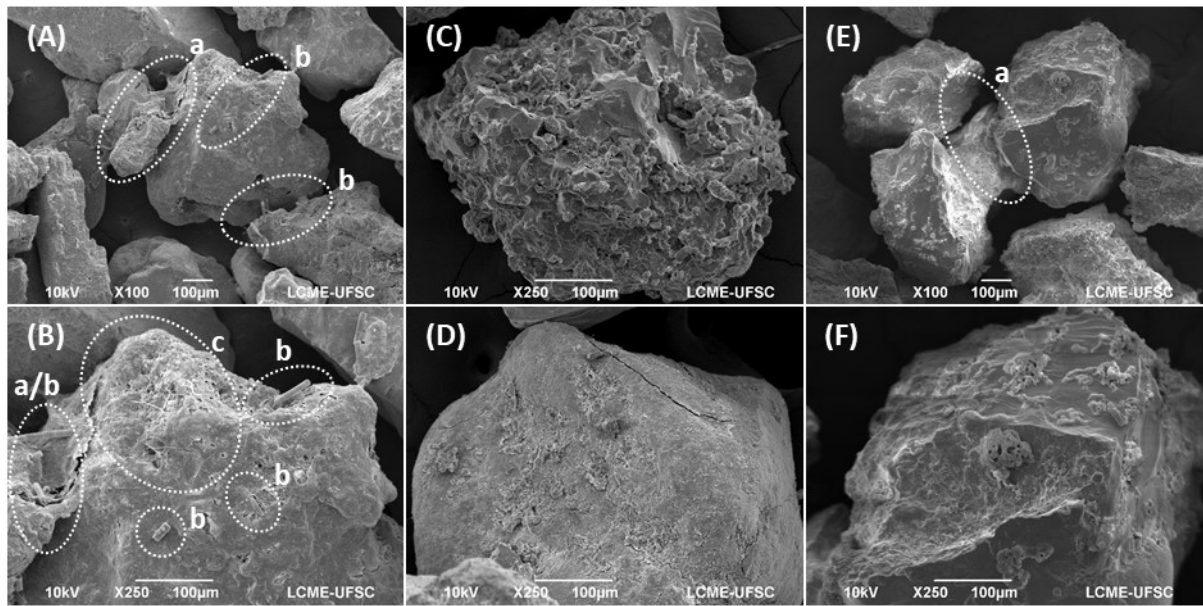
6. ORIGINAL SEM MICROGRAPHS

Figure 3 – SEM micrographs showing ScSF FM before and after cleaning. (A) FM mixed with *schmutzdecke* at X100; (B) Sand grain uniformly covered by biomass, X250; (C) Sand grain with cavities covered by biomass, X250; (D) Sand grain with uniform discreet biomass cover, X250; (E) FM after manual external cleaning, X100; (F) FM after manual external cleaning, X250; (a) Biomass and grains cohesion; (b) diatoms; (c) filamentous cyanobacteria agglomerate.
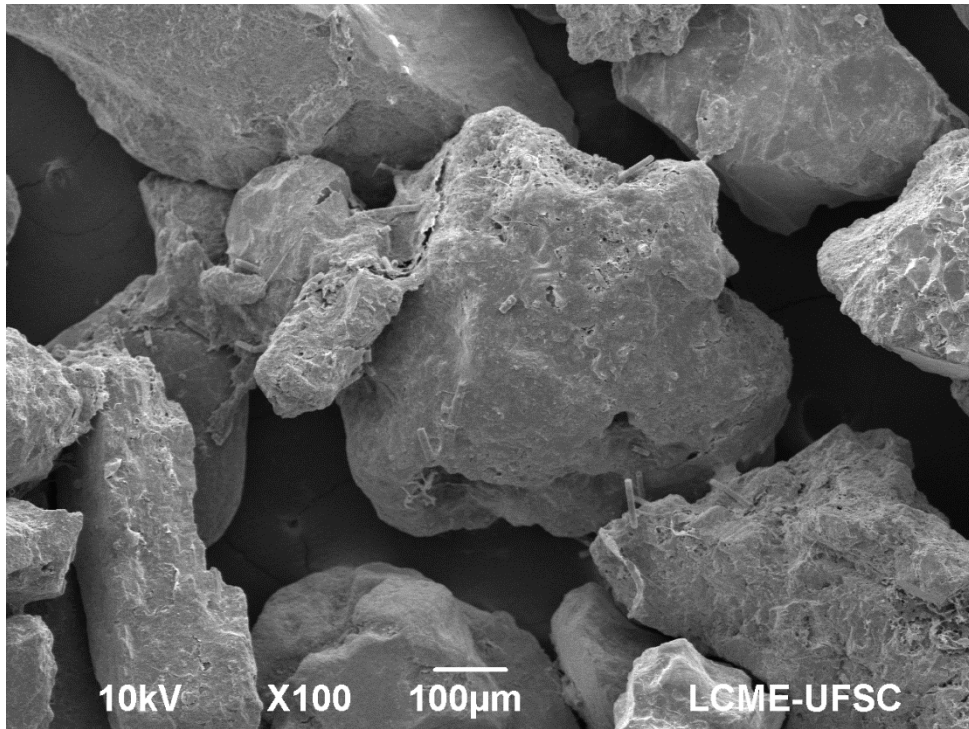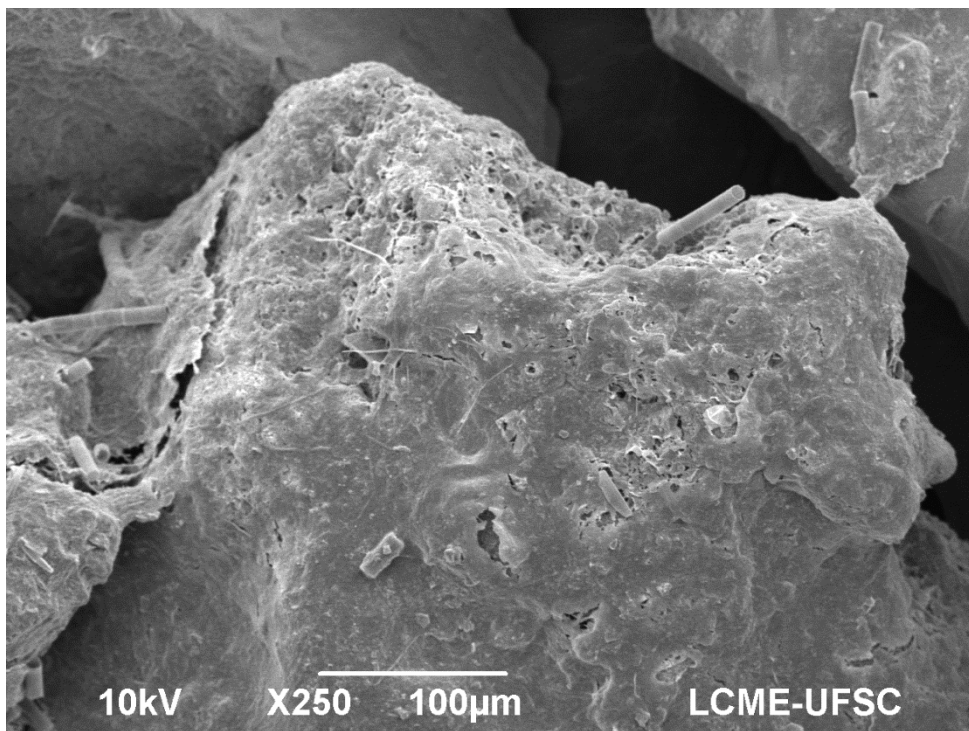
Figure 4 (A)

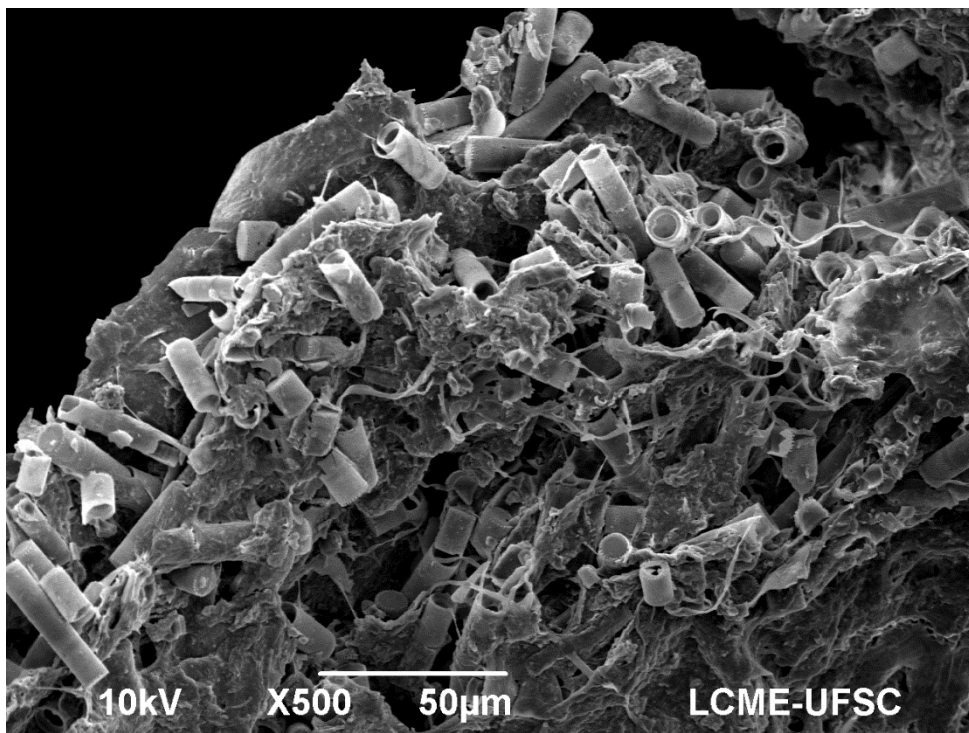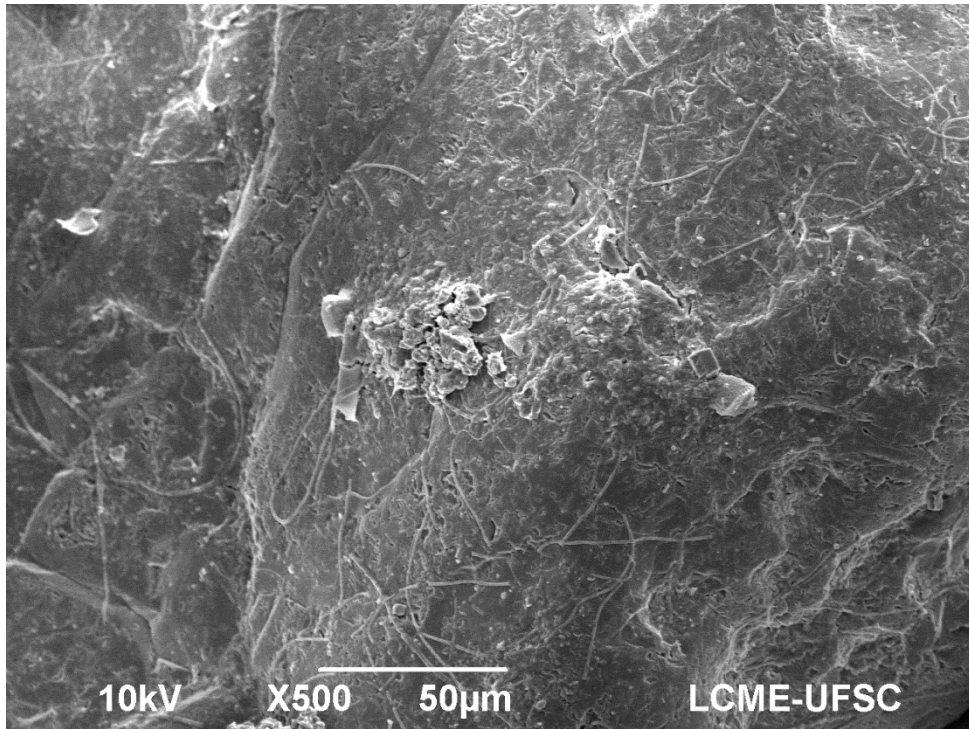
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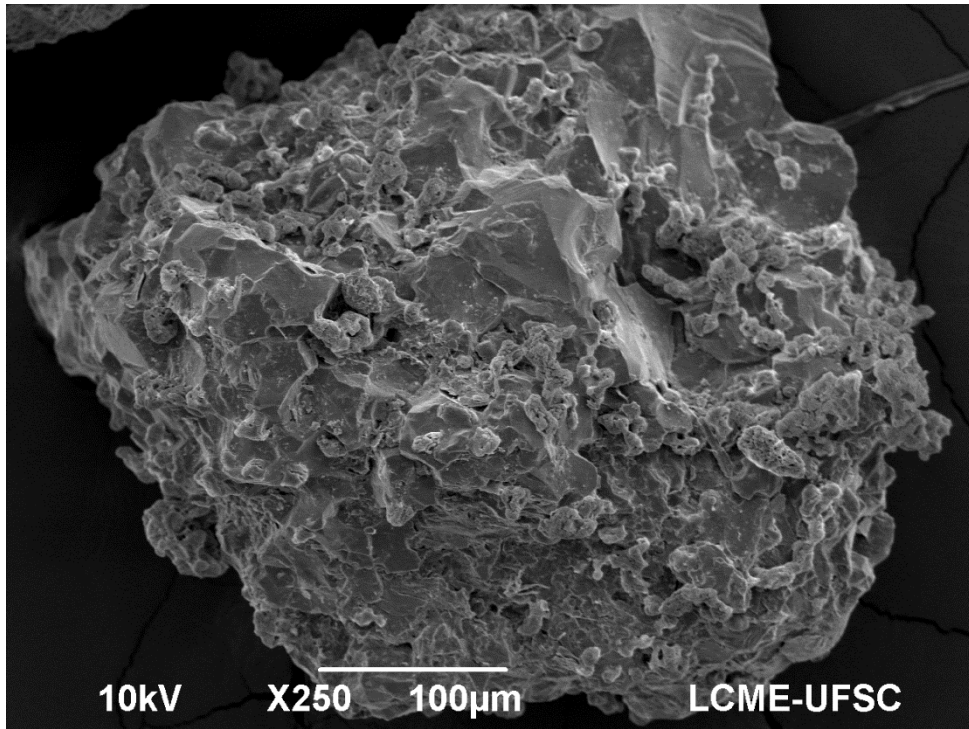
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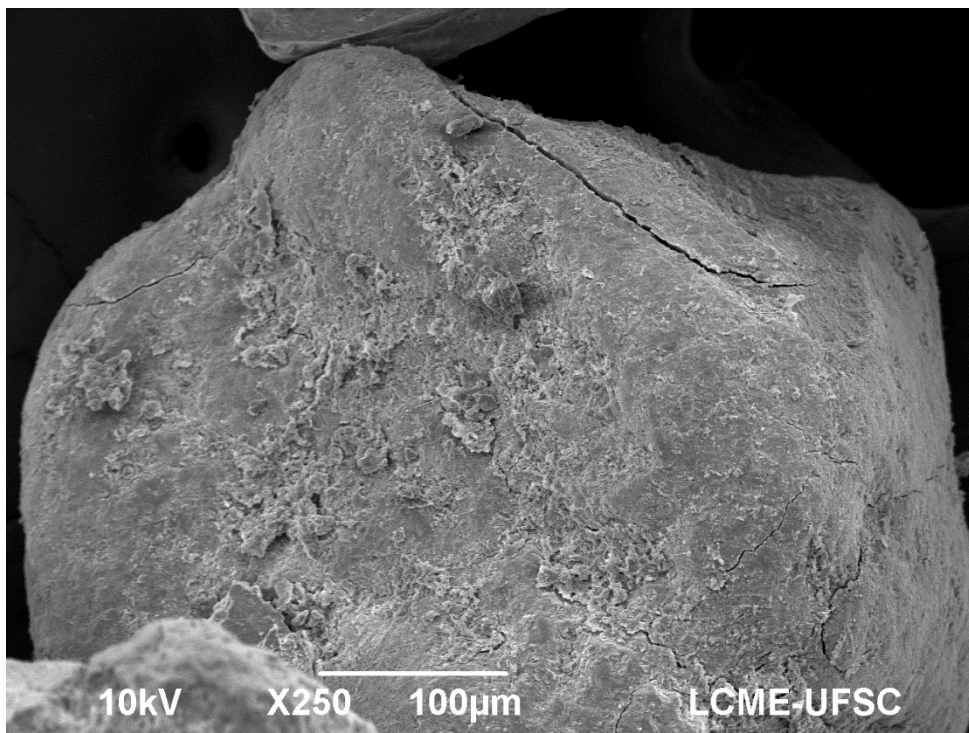
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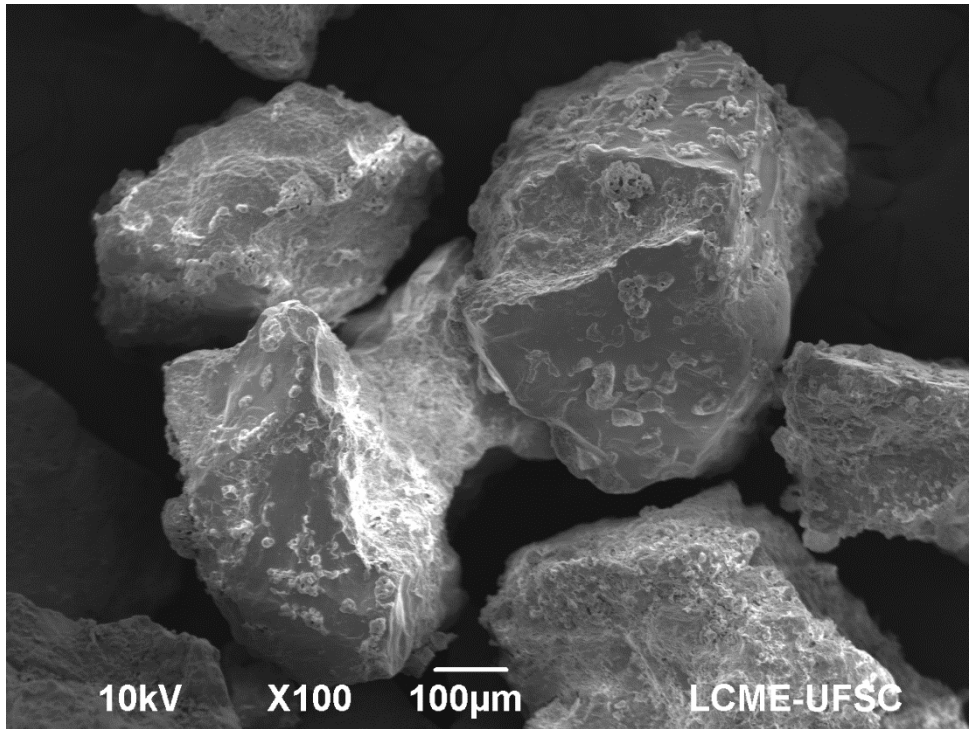
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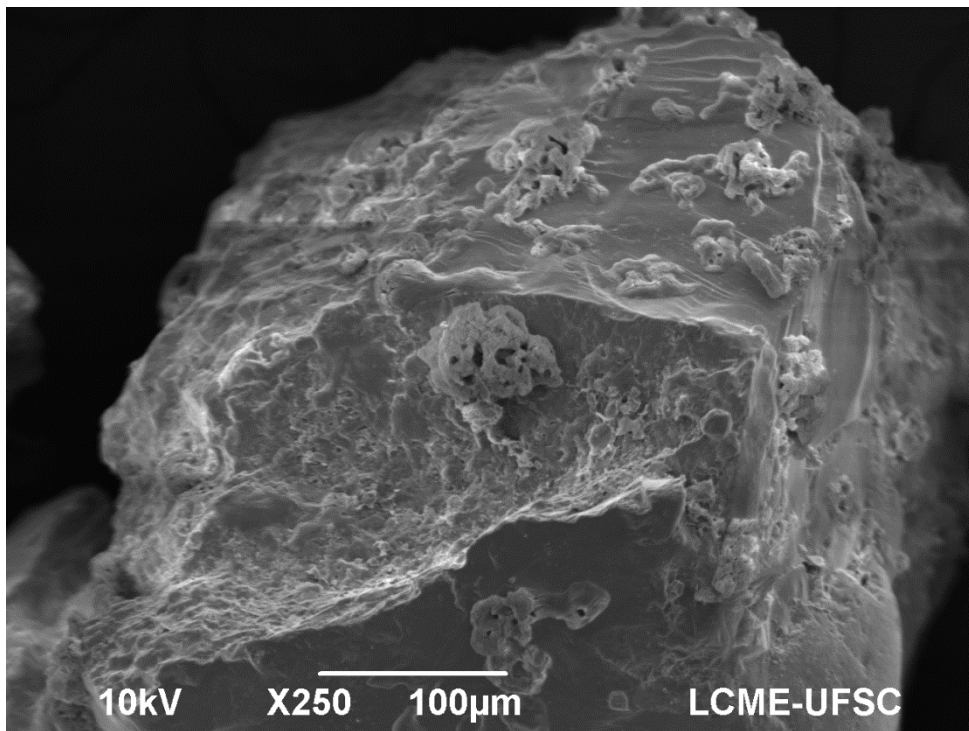
Figure 4 (F)
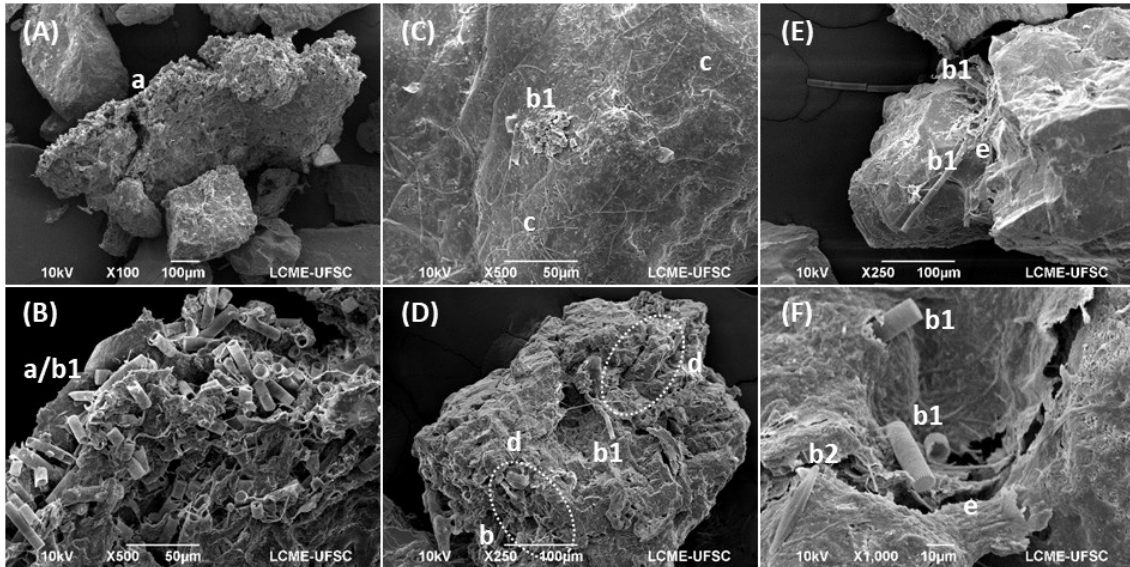
Figure 4 – SEM micrographs of BSF FM before and after cleaning. (A) *Schmutzdecke* and FM, X100; (B) Diatoms forming a cohesive *schmutzdecke*, X500; (C) Sand grain surface covered by filamentous Cyanobacteria, X250; (D) Sand grain covered by biomass, X250; (E) FM after cleaning, X250; (F) FM after cleaning, X1000; (a) *Schmutzdecke* biomass agglomerate; (b) diatoms, (1) *Aulacoseira ambígua* and (2) *Navicula* sp.; (c) filamentous cyanobacteria; (d) filamentous cyanobacteria agglomerate; and (e) biomass maintaining grains cohesion.
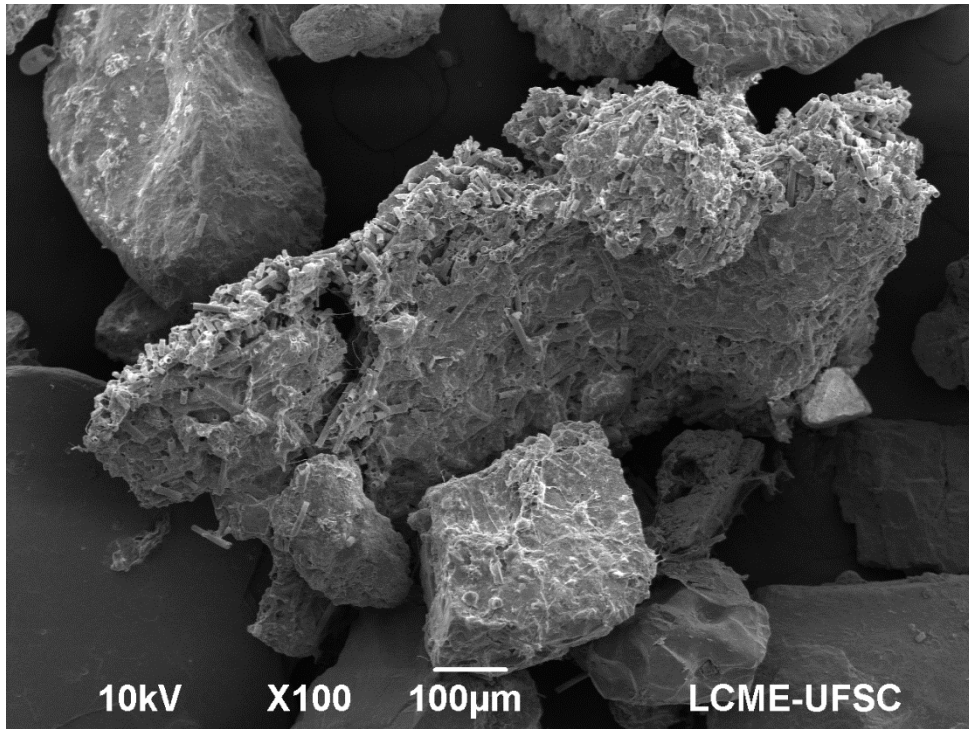
Figure 5 (A)

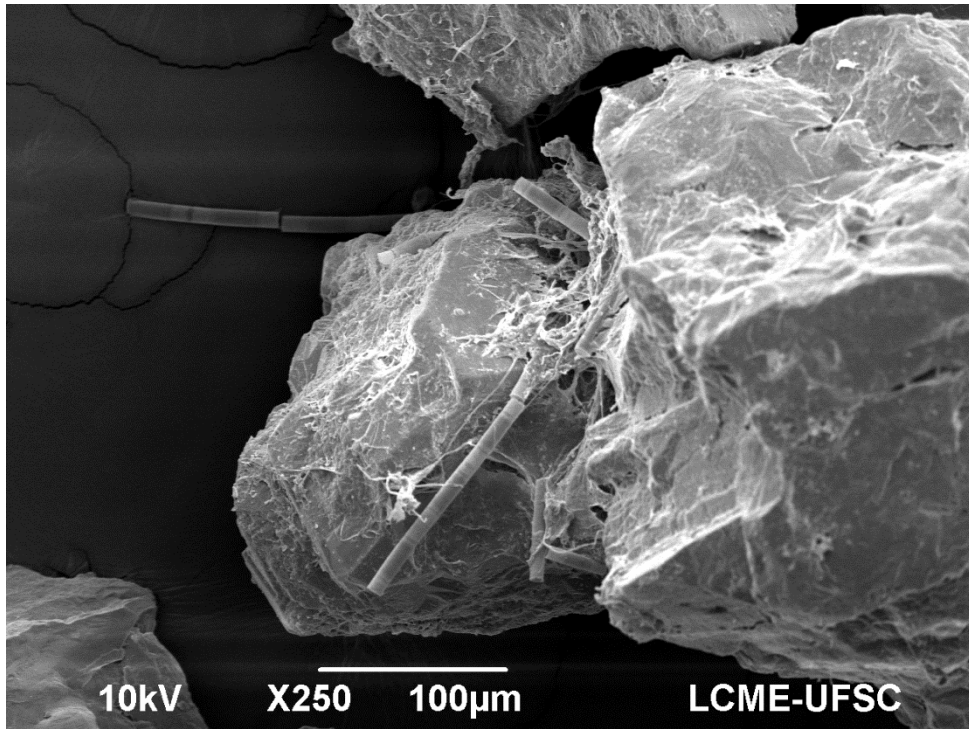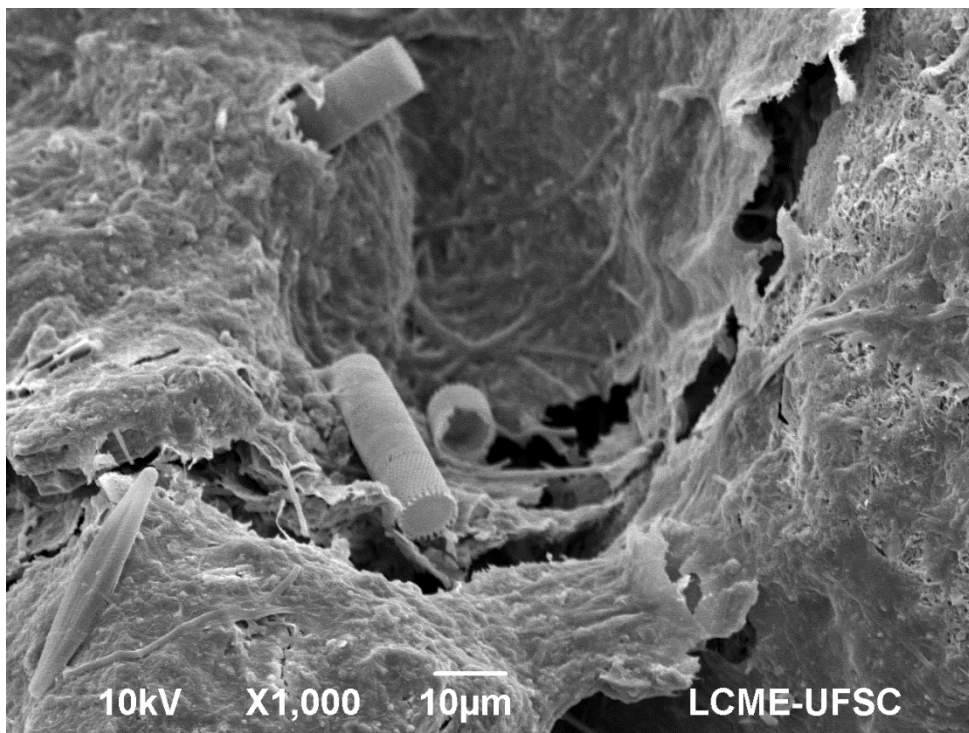Figure 5 (B)

Figure 5 (C)
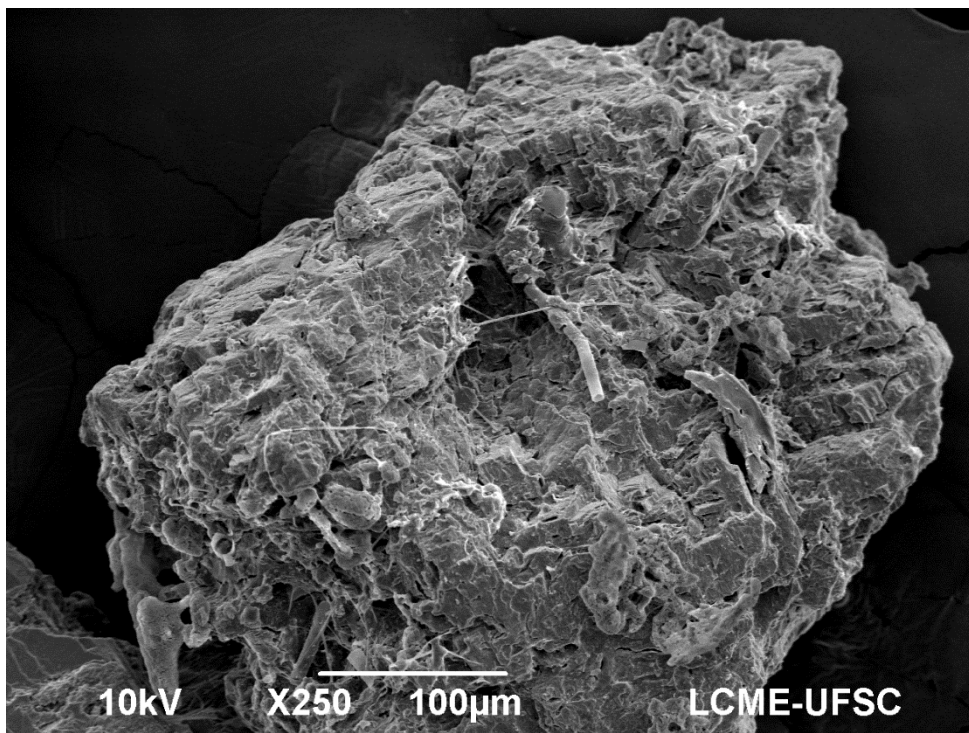
Figure 5 (D)

Figure 5 (E)

Figure 5 (F)

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