Highlights

- Water with low mineral ion concentrations caused filter media leaching.
- I-HSSF showed low efficiency in BPA removal and C-HSSF did not remove it.
- HSSF as a single treatment was not efficient in organic compound removal.
- Strategies to improve the HSSF hydraulic performance are not required.
- Biological layer role in the HSSF must be better understood.

Highlights

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1   *Household slow sand filters in intermittent and continuous flows to treat*

2   *water containing low mineral ion concentrations and Bisphenol A*

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13   Household slow sand filter (HSSF) has been used as an alternative to drinking

14   water treatment in rural communities worldwide; however, its performance to

15   treat influent water with quality similar to rainwater still needs further studies.

16   Rainwater presents low pH and slight mineral ion concentrations, an aspect that

17   can modify the filter media and consequently the HSSF efficiency. Furthermore,

18   house roofs used in rainwater harvesting can be made of plastic. Therefore, it

19   can introduce chemicals such as Bisphenol A (BPA) in the water. In this context,

20   two pilot-scale HSSFs operated in continuous and intermittent flows were

21   evaluated to treat water containing BPA and low mineral ion concentrations in

22   order to assess the filter performance. Filter media leaching was noticed in the

23   trials; thus, filter media and construction material selection must be carefully

24   evaluated to eliminate risks of pollutant occurrence in drinking water.

25   Operational differences between continuous and intermittent flows influenced

26   the HSSF efficiency for BPA and DOC removals; even so, the filters'

27   performance was low probably due to the slow *schmutzdecke* development.

28   According to tracer test results, HSSF can be classified as a plug flow reactor

29   and strategies to improve its hydraulic performance are not required.

30   Keywords: biosand filter; decentralised treatment; drinking water;

31   endocrine disruptor; rainwater

32 **1. Introduction**

33   Access to drinking water in rural communities is a problem because they usually

34 have a regional diffuse distribution that limits technically and/or economically the

35 interconnection with water supply networks. Therefore, they need a decentralised

36 supply solution. Research for efficient, easy-to-implement, operate and maintain low-

37 cost technological solutions are essential to the success of water projects in these

38 overlooked communities. According to WHO (2012) until reliable, safe, and piped

39 water is accessible to every household, temporary actions, such as household water

40 treatment and safe storage (HWTS) are needed to reduce waterborne diseases. In this

41 context, household slow sand filter (HSSF) has acquired importance worldwide due to

42 its efficiency and simplicity (Cawst, 2012; Sobsey et al., 2008). Real-scale HSSF has

43 been reported in 69 countries and there are more than 300,000 units in operation

44 worldwide (Cawst, 2012).

45

1.1. HSSF basic concepts

46

47 The worldwide requirement for a low-cost HWTS, which is simple to maintain and has

48 safe water production, led to the development of the household slow sand filter (HSSF)

49 in the 1990s. HSSF is a small filter that can work in intermittent or continuous flows,

50 making it appropriate for homes (Cawst, 2012; Terin and Sabogal-Paz, 2019; Young-

51 Rojanschi and Madramootoo, 2014). HSSF is made of concrete or plastic and it is filled

52 with layers of sand and gravel that are carefully prepared (Cawst, 2012). The

53 development of the biological layer (*schmutzdecke*) on top of the fine sand is required to

54 obtain the highest efficiency. HSSF has similar limitations to SSF when removing solids

55 and organic compounds. High concentration of suspended material in the influent water

56 obstructs the intergranular voids causing a reduction in the filter run and an increase in

57 the frequency of cleaning (Souza Freitas and Sabogal-Paz, 2019). However, solids and

58 organic compound removals are easily enhanced by using pre-treatment (e.g. coagulant

59 dosage or sedimentation) and/or post-treatment (e.g. adsorption). Influent water quality

60 and efficiency reported by some authors are shown in Table 1.

61

62 [Table 1 near here]

63

64   The maximum turbidity for HSSF is up to 50 NTU, according to Cawst (2012);

65 however, for countries with more restrictive drinking water standards, this value must

66 be reduced to 10 NTU.

67

1.2. HSSF in intermittent and continuous flows

68

69 HSSF is a modified SSF which works with a higher filtration rate (up to 29 times) and a

70 smaller sand layer (up to 50% less) than the conventional filter. HSSF cleaning

3

71   processes do not require removing the top of the filter media (Cawst, 2012) and it has

72   reduced the scale, compatible with a household water treatment (WHO, 2016). A single

73   user can build an HSSF with easily accessible materials (Faria Maciel and Sabogal-Paz,

74   2018) and it can operate with intermittent flow, an operational aspect not possible in

75   conventional SSF. Furthermore, HSSF can improve its performance by installing a non-

76   woven synthetic fabric on the top of the filter media (Faria Maciel and Sabogal-Paz,

77   2018), which can be easily positioned and fixed because the filter has a small superficial

78   area, usually, up to 0.1 m^2.

79   HSSF in intermittent flow (I-HSSF) can operate with filtration rates up to 29

80   m^3.m^{-2}day^{-1} (1.2 m/h), depending on the hydraulic head (Elliott et al., 2006). Water to

81   be treated has to rest in the pores of the filter media for a period of 1 to 48 h (i.e. pause

82   period) between each batch operation (Cawst, 2012). This pause period is important to

83   allow physico-chemical and biological processes to act on the *schmutzdecke* to treat

84   water. The pause period is a design parameter directly related to the HSSF efficiency

85   and its establishment (1 to 48 h) is not yet fully understood. The user feeds the I-HSSF

86   manually with 15-20 L directly into the unit after the pause period. The treated volume

87   corresponds to the water that is retained in the filter media; consequently, a unit can

88   usually produce up to 80 L day^{-1} according to the pause period adopted (Schmidt and

89   Cairncross, 2009). The I-HSSF area occupied inside the residence is around 0.1 m^2.

90   HSSF in continuous flow (C-HSSF) usually works with lower filtration rates, up

91   to 9.6 m^3.m^{-2}day^{-1} (Faria Maciel and Sabogal-Paz, 2018). The filter can produce up to

92   200 L day^{-1} of filtered water, depending on filter configuration. C-HSSF can be fed by

93   gravity (using an elevated tank) or by direct pumping. This filter needs a filtration rate

94   control and may require more area inside the home (±1.0 m^2) as it demands an external

95   supply unit (i.e. elevated tank or pump).

1.3. HSSF hydraulic behaviour

HSSF flow characterisation is an important operational parameter (e.g. it can define the water sampling time) and few studies have considered this aspect. Bradley et al. (2011), Elliott et al. (2008) and Lynn et al. (2013) have evaluated I-HSSF hydraulic behaviour and classified it as a plug flow reactor. The C-HSSF has been also classified as a plug flow reactor by Faria Maciel and Sabogal-Paz (2018), Terin and Sabogal-Paz (2019), and Young-Rojanschi and Madramootoo (2015). However, relatively little attention has been given to the hydrodynamics of these filters.

1.4. HSSF versus emerging contaminants

Various studies have been conducted on the application of SSF and HSSF for the removal of pharmaceutical and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) from water and wastewater (D'Alessio et al., 2015; Haig et al., 2016; Katayama-Hirayama et al., 2010; Li et al., 2018; Pompei et al., 2017). These authors evaluated filtration rates between 0.02 and 4.8 $m^3 m^{-2} day^{-1}$ and the mean removal efficiencies were between 11 to 92% for the target compounds. Nevertheless, there has been relatively little understanding of the fundamental mechanisms operating during SSF.

1.5. Bisphenol A, risk and detection

Bisphenol A (BPA, CAS n. 80-05-7) was synthesised in 1905 from phenol and acetone and it is mainly used to generate polycarbonate and epoxy (95% of the production) and the rest (5%) is transformed into resins, antioxidants, fungicides, paints and can coating (Huang et al., 2012).

5

120   BPA is an endocrine disruptor; hence, it is an exogenous agent that interferes

121   with the synthesis, production, secretion, release, transport, binding, action or

122   elimination of natural hormones responsible for homeostasis, reproduction,

123   development and behaviour (Kavlock et al., 1996; Zoeller et al., 2012).

124   It has been detected in drinking water and food and has been banned from plastic

125   containers in Europe and Canada (Rogers et al., 2013). BPA in drinking water may arise

126   from its contact with polycarbonate plastics and epoxy resins (FAO and WHO, 2011) or

127   contaminated raw water. According to Vom Saal and Hughes (2005), 115 *in vivo*

128   studies were published regarding the effects of low BPA dosages and 94 indicated

129   significant effects. In addition, in 31 publications on vertebrates and invertebrates,

130   endocrine changes were found with apparently safe dosages (<50 μg kg^{-1}day^{-1}). An

131   estrogenic effect was confirmed by *in vitro* tests with disruption of cell function

132   (Beausoleil et al., 2018; Vom Saal and Hughes, 2005). Finally, the above authors

133   reported that there is a need to consider the health risk based on the scientific literature

134   relating adverse effects on animals in dosages considered safe.

135   Regarding biological treatment, bacteria and fungi can degrade BPA (Kang et

136   al., 2006) and this opens up space to treat water affected by endocrine disruptors by

137   HSSF. However, BPA metabolites generated after treatment may have estrogenic

138   effects (Huang et al., 2012; Kang et al., 2006).

139   BPA detection in environment matrixes has generated the development of

140   chromatographic techniques. Methods based on high performance liquid

141   chromatography (HPLC) have usually been used for BPA analyses (Rodriguez-Mozaz

142   et al., 2004). HPLC may be impracticable in developing countries due to high cost and

143   technical complexity. Therefore, simpler methods that can detect BPA are needed to

144   assess the drinking water risk. From this perspective, UV absorbance of BPA can be

145   measured with a spectrophotometer, allowing its quantification in ppm (Cao et al.,

146   2014).

147

148   **1.6. Rainwater harvesting and treatment**

149   Rainwater is slightly acidic and has very low dissolved mineral concentration. Thus, it

150   is relatively aggressive and it can dissolve metals and impurities from catchment and

151   storage tanks, resulting in unacceptably high pollutant concentrations in the water

152   (WHO, 2017). BPA may be present in plastic materials, pipes, fittings and tanks (Huang

153   et al., 2012) that can be used for rainwater harvesting, an aspect that needs more studies.

154   Slow sand filtration is a technology that may be used to treat rainwater in developing

155   countries (Helmreich and Horn, 2009) and its performance should be better understood.

156   Bearing in mind the lack of research about the endocrine disruptor's removal

157   from rainwater, this paper aims to investigate the potential of two pilot-scale HSSFs

158   (operating in intermittent and continuous flow regimes) in the BPA removal from water

159   containing low mineral ion concentrations.

160

161   **2. Materials and Methods**

162   **2.1. HSSF characteristics**

163   Two pilot-scale HSSFs were constructed in acrylic with a 98 mm inside diameter (cross

164   sectional area = 0.0075 m²). One HSSF was designed to operate intermittently (I-HSSF)

165   and the other to operate continuously (C-HSSF). The filters were covered to protect

166   them from light. HSSFs schemes can be found in Fig. 1.

167

168   [Figure 1 near here]

169

170　　　　The HSSF filter media was a 55 cm fine sand layer (0.09 mm to 0.5 mm) with an

171　　effective size (D_{10}) of 0.18 mm and uniformity coefficient (UC) of 1.64. Fine sand used

172　　(CH52, Minerals Marketing, UK) presented the following chemical composition: SiO_2

173　　= 97.3%, Fe_2O_3 = 0.1%, Al_2O_3 = 1.37%, K_2O = 0.83% and loss-on-ignition = 0.25%.

174　　Support media consisted of a 5 cm layer of coarse sand (1 to 3 mm), 5 cm layer of fine

175　　gravel (3 to 6 mm) and 7.5 cm layer of coarse gravel (10 to 12 mm). The average

176　　porosity of the filter materials was 32%. Fine sand and support media were washed in

177　　tap water prior to their introduction inside each unit. Acrylic columns were filled with

178　　tap water before inserting the filter media to avoid air pocket formation and to allow

179　　fine sand stratification as well.

180　　　　Finally, a non-woven synthetic fabric (specific gravity: $\pm$ 0.2 g cm^{-3},

181　　composition: 100% polyester, and thickness = 2.8 mm with 25 μm fibres) was

182　　positioned at the filter media top. After the HSSF assembling, deionised water

183　　continuously fed each filter by 24 h to remove the chlorine from the tap water.

184　　　　Water from Regent's Park Lake (London, UK) was used as a ripening agent (i.e.

185　　agent to accelerate the filter maturation in a simple way) and was only added at the

186　　beginning of the HSSF operation. The filter volume (i.e. sum of standing water volume,

187　　outlet pipe volume and filter media and support layer pore volumes) was introduced

188　　twice to each HSFF (i.e. 2 L from Regent's Park Lake) and it was left for one day before

189　　starting off the operation with influent water. Regent's Park water quality comprised

190　　total coliforms of 1.8×10^4 CFU 100 mL^{-1}, *Escherichia coli* of 200 CFU 100 mL^{-1},

191　　turbidity of 2.02 NTU, conductivity of 1158 μS m^{-1}, pH of 7.69, temperature of 23 $^{\circ}$C,

192　　dissolved oxygen (DO) of 4.34 mg L^{-1} and dissolved organic carbon (DOC) of 19.7 mg

193　　L^{-1}. Filtered water samples were collected one day after the maturation process, when

194   the filters started the operation with influent water, to assess the efficiency of the

195   HSSFs.

196   HSSFs were cleaned when they reached the maximum hydraulic head.

197   Maintenance consisted of removing the synthetic fabric, scraping off the top and

198   draining the supernatant without removing the sand from the top. The fabric was

199   washed in deionised water and it was then placed back on the filter.

200

201   **2.2. HSSF operation**

202   HSSFs were operated for 90 continuous days. Influent water was prepared weekly by

203   diluting BPA (Alfa Aesar ®, 97%) stock solution in deionised water to simulate

204   rainwater contaminated by endocrine disruptor (Table 2).

205

206   [Table 2 near here]

207

208   HSSF filtration rates were calculated considering a daily production of 2.9 ± 0.9

209   L for the C-HSSF and 2.6 ± 0.8 L for the I-HSSF. The flow rate in the C-HSSF was

210   controlled by a peristaltic pump (Watson-Warlow, MHRE 100) producing a filtration

211   rate of $0.38 \pm 0.13 \ m^3 \ m^{-2} \ day^{-1}$. The I-HSSF hydraulic head was variable generating a

212   filtration rate between 0 to 21 $m^3 \ m^{-2} \ day^{-1}$. The I-HSSF was filled with 1.0 L (filter

213   volume) three times per day by a submersible pump (Jeneca ®, HM 5063) controlled

214   with a valve and timer, causing an 8-hour pause period.

215   **2.3.Tracer tests**

216   HSSF flow characterisation was carried out using 200 mg L^{-1} sodium chloride

217   (NaCl) solution as a tracer, prepared with tap water (the tests were performed in

9

218 triplicate). Electric conductivity variation in the filtered water was detected using a

219 conductivity probe (*Vernier*, USA) situated in the outlet hose. Data was collected by

220 *Logger Lite* software (*Vernier*, EUA) and it was processed by Excel 2013 (*Microsoft*,

221 EUA) and Origin 8.6 (*OriginLab*, EUA). In each tracer test, the HSSFs were cleaned

222 with tap water until the salt solution from the previous test was completely removed.

223 NaCl solution was applied to the C-HSSF as a step input and the probe allowed a

224 correlating conductivity variation with tracer concentration. The filtration rate was kept

225 on 0.5 $m^3 m^{-2} day^{-1}$ and the hydraulic retention time (HRT) was determined. The flow

226 pattern was adjusted into three hydrodynamic mathematical models: dispersion models

227 (low and high dispersion) and N-continuous stirred tank reactors (N-CSTRs), as

228 reported by Levenspiel (1999).

229 The first filling to the I-HSSF was carried out with NaCl solution and the

230 subsequent feedings were with tap water. The filtration rate declined to zero when the

231 hydraulic head reached the lowest level, at which time a new water charging was

232 performed (V = 1.0 L). Salt concentration versus filter volume curves produced a

233 positive step followed by a negative step (increased and decreased concentrations).

234 Afterwards, the Morrill Dispersion Index (MDI) and the modified MDI (mMDI) were

235 calculated as described by Tchobanoglous et al. (2003) and Lynn et al. (2013),

236 respectively.

237

238 **2.4. BPA detection**

239 BPA was measured by UV-Vis spectrophotometer (Shimadzu UV 2600, Japan). UV

240 absorbance for six BPA concentrations (0 to 12 mg L^{-1}) was measured from 200 to 1000

241 nm wavelengths, in triplicate, in order to identify the characteristic absorbance peak (it

242 was detected at 224 nm). Afterwards, the BPA standard curve was made from data

243   obtained at 224 nm. The relationship between UV absorbance and BPA concentration

244   was established [UV absorbance = 0.0748 x BPA concentration (mg L^{-1})]. The

245   calibrated curve showed r^2 of 0.94, detection limit of 0.03 mg L^{-1} and limit of

246   quantification of 0.10 mg L^{-1}.

247

248   **2.5. *Schmutzdecke* evaluation**

249   Scanning electron microscope (SEM) and flow cytometry (FC) were used to evaluate

250   the biological layer (*schmutzdecke*) at the end of the HSSF operating period.

251   SEM with energy dispersive x-ray spectroscopy (EDS) (JEOL JSM-6480LV,

252   Japan) was used to capture photomicrographs and chemical compositions from

253   synthetic fabric and fine sand of dried samples at room temperature. Samples were

254   analysed at different magnifications, variable pressure analytical scanning electron

255   microscope with secondary electron imaging (SEI) and backscattered electron imaging

256   (BEI) detectors and with an accelerating voltage of 15 kV. Individual particles and

257   compacted samples were rigidly mounted on a specimen stub and they were coated with

258   an ultrathin gold layer. EDS did more than a hundred spot analyses.

259   Bacteria cells (alive and dead) were determined by flow cytometry using

260   Guava® easyCyte 5HT Benchtop Flow Cytometer (Millipore, UK). Samples from the

261   biological layer for I-HSSF and C-HSSF at the end of the filter operation were collected

262   and stored at 4 ºC before processing. LIVE/DEAD BacLight Bacterial Viability kit

263   (Thermo Fisher Scientific, UK), with propidium iodide dye and SYTO® 9 dye, was

264   prepared and applied according to the manufacturer´s instructions. 20 µL of sample

265   (*schmutzdecke*) and controls (*E. coli* strain K-12 and deionised water) were added to

266   180 µL of the prepared stock staining into 1.5 mL microcentrifuge tubes.

267   *E. coli* was diluted before measuring in the flow cytometer in filtered deionised

268   water (0.22 mm; PTFE Syringe, Gilson scientific). It was used as a biological positive

269   control, and filtered deionised water was utilised as a control for background

270   fluorescence. All prepared samples were incubated at room temperature in the dark for

271   15 min. The bacteria acquisition gate was determined according to forward scatter

272   (FSC) and side scatter (SSC) channels to eliminate background noise and debris.

273

274   **2.6. Sample collection and analysis**

275   Influent water and filtered water samples were collected and analysed daily, according

276   to the water sampling time defined by the tracer tests. The water quality parameters

277   analysed were turbidity (Hach 2100N, USA), DO (Jenway 9200, USA), conductivity,

278   temperature and pH (Mettler Toledo, S47K, USA), DOC (TOC-L, Shimadzu, Japan),

279   cations and anions (IC1100, Dionex, USA and Varian ICP-AES 720-ES, USA), and

280   coliforms (m-ColiBlue24®, Hach, USA). Standard methods defined by APHA, AWWA

281   and WEF (2012) were followed to evaluate the above parameters. Head loss was

282   measured daily in both filters.

283   **2.7.Statistical analyses**

284   Statistical analyses were performed using PAST 3 software (PAlaeontological

285   STatistics) created by Hammer et al. (2018). The Kruskal-Wallis test was used to

286   compare data from the filtered water samples among each other and with influent water

287   (95% confidence interval). When statistical analyses showed that the mean values were

288   significantly different, the Mann-Whitney test was selected to define which sample was

289   different from another (95% confidence interval).

290

291   **3. Results and Discussion**

292   **3.1. Tracer tests**

293   Tracer test results for the I-HSSF are shown in Fig. 2a. Tracer concentration increased

294   from 0 mg L^{-1} up to 182 mg L^{-1} and this 9% difference relative to the initial

295   concentration (200 mg L^{-1}) can be attributed to the filter's hydraulic head, which may

296   have diluted the tracer solution (Terin and Sabogal-Paz, 2019).

297

298   [Figure 2 near here]

299

300   According to the results, two feedings were required before collecting samples

301   for the I-HSSF performance evaluation. Salt concentration decreased from the third

302   filter volume and after the fifth feeding, the tracer left the filter (Fig. 2a). Similar

303   behaviour was described by Bradley et al. (2011), Faria Maciel and Sabogal-Paz (2018)

304   and Terin and Sabogal-Paz (2019), characterising a plug flow reactor for HSSF.

305   I-HSSF MDI was 1.54 ± 0.01, lower than the one observed by Young-Rojanschi

306   and Madramootoo (2015), who found an MDI value of 1.8 and slightly higher than the

307   ones reported by Elliot et al. (2008) and Bradley et al. (2011) of 1.3 and 1.4,

308   respectively. As stated by USEPA (1986) and Tchobanoglous et al. (2003), this MDI

309   characterises the I-HSSF as a plug flow reactor (MDI up to 2).

310   I-HSSF mMDI was 0.95 ± 0.1, lower than the one found by Lynn et al. (2013),

311   who reported values of 2.86 and 3.01. According to Lynn et al. (2013), the calculated

312   mMDI did not change significantly over time, which was a phenomenon noticed in our

313   study. Consequently, additional strategies to improve the I-HSSF hydraulic performance

314   in comparison to the ideal plug-flow reactor are not required.

315   Tracer test results for the C-HSSF are shown in Fig. 2b and Table 3. HRT was

316   857 ± 21 min and it was used to determine the sample collection time. The N-CSTR

317   model showed a better adjustment with an r^2 of 0.75 and N of 17. As indicated by

318   Levenspiel (1999), a high N value also designates a plug flow reactor.

319

320   [Table 3 near here]

321

322   In the plug flow reactor, the fluid passes through the reactor (filter) with no

323   mixing of earlier and later entering fluid (no overtaking). The necessary and sufficient

324   condition for plug flow condition is that the residence time in the reactor must be the

325   same for all elements of fluid (Levenspiel, 1999). In this context, a HSSF evaluated by

326   Elliott et al. (2008) showed a minimal effect of dispersion by flow paths through the

327   porous media, a result analogous to our study for both filters. Therefore, from the

328   perspective of the biological layer development and microbial removal processes, the

329   results suggest the same time is available for all portions of water that enter the HSSF,

330   helping the water treatment.

331

332   **3.2. HSSF operation**

333   Filtered water quality and removal or variation rates are shown in Table 4.

334   Turbidity removal showed a negative value for both filters (i.e. filtered water presented

335   74-76% higher turbidity) and there was no removal improvement over time (Fig. 3),

336   contradicting the literature.

337

338   [Table 4 near here]

339

14

340   [Figure 3 near here]

341

342   Turbidity removal within the range of 70% to 96% in laboratory and field

343   studies has been described worldwide with influent water turbidity up to 58 NTU

344   (Cawst, 2012; Frank et al., 2014; Jenkins et al., 2011). However, according to Frank et

345   al. (2014), HSSF generally has greater turbidity removal when influent levels are

346   higher. This may explain the performance found in our study, since the influent water

347   turbidity was only 0.37 ± 0.11 NTU (Table 2).

348   Another possible explanation for the increased filtered turbidity may be

349   attributed to the filter media leaching. Thiry et al. (1988) reported this phenomenon,

350   when the effect of groundwater in sands was analysed. This can be confirmed by the ion

351   concentration increase in the filtered water for both filters (Table 4). It should be noted

352   that the sands used in HSSF in real scale are washed only with water; therefore, it is not

353   possible remove all the minerals prior to use. On the other hand, the HSSFs produced

354   most of the time filtered water with turbidity below 1.0 NTU and this value is

355   associated with 1-2 log and 2.5-3 log reduction of viruses and protozoa, respectively

356   (WHO, 2017). There was no significant statistical difference between filter efficiencies

357   when turbidity was evaluated ($p = 0.972$).

358   It is important to highlight that HSSF accepts a maximum turbidity of 50 NTU,

359   according to Cawst (2012); however, high turbidity values often generate cleanliness of

360   the unit, reducing the filter efficiency when the overall performance is evaluated. In this

361   context, influent water with low turbidity is always desired.

362   Conductivity drastically increased in the filtered water with a statistically

363   significant difference for I-HSSF ($p = 0.001$). However, the value was always below 50

364     $\mu S \, m^{-1}$ for both filters. Conductivity depends on ion concentration (i.e. phosphate,

15

365   chloride, sulphate, nitrate, silicon, aluminium, calcium, iron, magnesium, sodium, etc.)

366   and most of the time all these ions increased considerably after filtration (Table 4), and

367   this may explain our findings. Likewise, Young-Rojanschi and Madramootoo (2015)

368   noticed an increase in the conductivity and pH from filtered water and this anomaly was

369   intensified when the influent water stayed longer in contact with the filter media (i.e.

370   longer residence period) and they attributed this phenomenon to the filter media

371   leaching. Therefore, this finding may explain why the conductivity was higher for the

372   C-HSSF in our study (mean HRT = 14.3 h).

373   Increased pH (2-4%) in the filtered water was observed in both filters, a similar

374   fact also reported by Young-Rojanschi and Madramootoo (2015). Murphy et al. (2010)

375   attributed the increased pH to the calcium carbonate leaching from concrete-built HSSF

376   walls. As the filters were acrylic fabricated in our study, the leaching from filter media

377   may better explain this phenomenon. No significant statistical difference between filters

378   was found for this parameter ($p = 0.061$).

379   There was a slight temperature variation (1.0%) throughout the tests with around

380   22 $^{\circ}$C in the filtered water. However, no significant statistical difference between filters

381   was found ($p = 0.860$). Arnold et al. (2016) stated that HSSF could be effective at any

382   temperature above freezing; nevertheless, the biological layer needs time to adapt to

383   changes in the temperature. They also indicated that HSSF should be kept at warmer

384   temperatures since the coldest temperatures have less bacteria removal in the

385   operational beginning. In this context, this parameter was not pointed out as a limiting

386   factor for the HSSF efficiency in our study.

387   DO reductions were detected in filtered water (60-66%); however, anoxic

388   conditions were not noticed. No significant statistical difference between HSSFs was

389   identified ($p = 0.181$). DO consumption is expected in HSSF due to the biological layer

16

390   development (Young-Rojanschi and Madramootoo, 2015). According to Kennedy et al.

391   (2012), both pH and DO decreased during the operation of their tested HSSFs and this

392   phenomenon was most likely due to carbon oxidation. Young-Rojanschi and

393   Madramootoo (2014) found anoxic conditions in HSSF and this condition is not desired

394   since nitrate reduction may occur to nitrite, as observed by Murphy et al. (2010). Based

395   upon our experimental results, DO cannot be considered as a restrictive factor for HSSF

396   efficiency.

397   I-HSSF showed statistically significant BPA removal efficiency than the C-

398   HSSF ($p = 0.001$). However, mean PBA removal was low (3%) and on some occasions,

399   the PBA concentration was higher in the filtered water than the influent water (Fig. 4).

400   BPA removal in the I-HSSF may be explained by biosorption from bacteria, as

401   described by Vecchio et al. (1998), who evaluated heavy metal biosorption by bacterial

402   cells, and by Vijayaraghavan and Yun (2008), who published a review about the status

403   of biosorption technology.

404

405   [Figure 4 near here]

406

407   There was an unexpected BPA increase in the C-HSSF filtered water.

408   Nonetheless, this may be explained by PBA desorption from the sand surface, as

409   reported by Tran et al. (2002) for cadmium. In addition, this could be caused by BPA

410   accumulation inside the living cells and when they die, the accumulated BPA may enter

411   the water again, as reported by Terin and Sabogal-Paz (2019) for cyanobacteria and

412   consequent microcystin production. Katayama-Hirayama et al. (2010) evaluated a lab-

413   scale SSF efficiency to treat river water with tetrabromobisphenol A. They found low

414   removal (20%) at the initial concentration of 100 μg L^{-1} throughout the experimental

17

415   period (18 days). According to these authors, bisphenol removal by SSF may be related

416   to the type of chemical structure, since hydroxylation of a phenol ring is an early step in

417   microbial aromatic degradation. An attached group next to a hydroxyl group may inhibit

418   phenol hydroxylation and this may explain the results obtained in our study.

419   Both filters showed low DOC removal (7 to 12%), however the C-HSSF had

420   statistically significant DOC reduction efficiency ($p = 0.003$). This result agrees well

421   with D'Alessio et al. (2015) and Terin and Sabogal-Paz (2019) who found TOC

422   removals up to 11% in the filtered water. Contrary to other research, DOC in the

423   influent water was higher (132.92 ± 15.50 mg L^{-1}) once Elliott et al. (2015) reported

424   TOC values up to 12.5 mg L^{-1} in influent water to HSSFs.

425   According to PBA and DOC removals, HSSF as a single treatment was not

426   effective in terms of eliminating organic compounds; therefore, activated carbon

427   adsorption as an HSSF's post-treatment is recommended for generating safe water in

428   rural communities. Li et al. (2018) obtained promising results when using granular

429   activated carbon sandwich slow sand filtration to remove pharmaceutical and personal

430   care products.

431   Both HSSFs did not show a significant statistical difference in the reduction of

432   total coliforms ($p = 0.686$), with the mean in the range of 0.78 to 0.84 log. This

433   efficiency was lower than the ones reported by Lynn et al. (2013) and Pompei et al.

434   (2017) with 1.2 log and 2.0 log, respectively. Coliform removal depends on

435   *schmutzdecke* development and a slow ripening may be responsible for the low

436   reduction rate. The filters in our study needed frequent cleaning (vertical lines indicate

437   maintenance activity in Fig. 3 and Fig. 4), since both HSSFs reached their maximum

438   hydraulic head quickly, a fact that may have influenced the complete development of

439   the biological layer.

440   Filtered water presented an increase in phosphate, chloride, sulphate, nitrate,

441   silicon, aluminium, calcium, iron, potassium, magnesium and sodium concentrations for

442   both HSSFs (Table 4). This indicates that there was a mineralisation in the filtered

443   water. There was a higher calcium and magnesium increase in the C-HSSF ($p = 0.004$

444   and $p = 0.036$, respectively) and, on the other hand, for the other ions there were no

445   significant statistical differences between filters.

446   The presence of some of these ions may be a result of sand leaching, a fact that

447   can be confirmed, since the fine sand presented SiO_2, Fe_2O_3, Al_2O_3 and K_2O in its

448   composition, according to the supplier's information. The influent water (which

449   simulated rainwater) was slightly acidic and had low mineral ion concentrations.

450   Therefore, it was relatively aggressive and could dissolve some compounds from the

451   filter media. WHO (2017) established guideline values for some of the above ions, and

452   for those regulated, the drinking water recommendations were met.

453   Both filters removed fluoride (55 to 88%) as stated by Devi et al. (2008), who

454   reached an 85.6% reduction by an HSSF. There was a significant statistical difference

455   between filters in our study ($p = 0.045$) showing a better performance for the I-HSSF.

456   According to the WHO (2017), the guideline value is 1.5 mg L^{-1} in drinking water;

457   therefore, the filtered water in our study met this recommendation.

458

459   **3.3. *Schmutzdecke* analysis**

460   SEM photomicrographs and chemical compositions from synthetic fabric and fine sand

461   are shown in Fig. 5. Potassium, silicon, aluminium, calcium, sodium, chloride and iron

462   were detected in the original fine sand (Fig. 5a), an already expected composition, as

463   discussed above. Potassium was not found in the original synthetic fabric (Fig. 5b). C-

464   HSSF biofilm presented mainly silicon, potassium, magnesium and aluminium in its

19

465 chemical composition (Fig. 5 c and Fig. 5 d); however, magnesium was not detected in

466 the I-HSSF biofilm (Fig. 5 e and Fig. 5 f). Evidently, all the above ions helped the

467 development of the biological layer in the filters (Fig. 6), providing essential nutrients.

468 As established by Faria Maciel and Sabogal-Paz (2018), the increase of nutrients in

469 HSSFs accelerates the filter maturation process.

470

471 [Figure 5 near here]

472

473 [Figure 6 near here]

474

475   Flow cytometry assay results are shown in Fig. 7. C-HSSF showed a high

476 number of live and dead cells; however, I-HSSF presented slightly higher live cell

477 percentages (99.7% vs 98.9%).

478

479 [Figure 7 near here]

480

481   According to Chan et al. (2018), flow cytometry with DNA staining can be used

482 to study the microbial dynamics in both treatment and distribution of drinking water

483 and, in the case of our study, the technique may evaluate the state of the biological layer

484 in relation to the presence of live microorganisms, which can help the water treatment.

485   As reported by Hall-Stoodley et al. (2004), biofilms are structurally complex,

486 dynamic systems with attributes of both primordial multicellular organisms and

487 multifaceted ecosystems. Biofilm formation is a protected mode of growth that allows

488 cells to colonise new niches or survive in adverse environments. Optimising nutrient

489 and waste-product exchange provides the first link between form and function of the

20

490 biofilm in both natural and fabricated environments. In addition, this theory can be

491 applied to the *schmutzdecke* development in both filters of our study. Evidently, there is

492 still a need to understand how the microorganisms grow in the HSSF biofilm, therefore,

493 further research is recommended.

494 **4. Conclusions**

495 • Water with low mineral ion concentrations generated sand leaching, increasing

496   the values of turbidity, conductivity, pH, phosphate, chloride, sulphate, nitrate,

497   silicon, aluminium, calcium, iron, potassium, magnesium and sodium in the

498   filtered water. In this context, when making the analogy with rainwater, care

499   must be taken in relation to the selection of filter media and construction

500   materials in order to reduce the risk of introducing pollutants in drinking water.

501 • Operational differences related to continuous and intermittent flow showed

502   influence in the filter efficiency for BPA and DOC for the I-HSSF and C-HSSF,

503   respectively, although the mean performance was low. Consequently, HSSF as a

504   single treatment was not effective for the removal of organic compounds,

505   possibly by the slow *schmutzdecke* development in both filters.

506 • Activated carbon adsorption as an HSSF's post-treatment must be researched to

507   improve BPA and DOC removals in drinking water for rural communities.

508 • Strategies to improve the HSSF hydraulic performance compared to ideal plug

509   flow reactor are not required. However, more research is needed to understand

510   the role of the HSSF biological layer in water treatment.

514   **Statement**

515   The authors hereby declare previous originality check, no conflict of interest and open

516   access to the repository of data used in this paper for scientific purposes.

517   **5.   Bibliography**

518   Arnold, N., Archer, A., Barkdoll, B. 2016. Bacterial adaptation and performance of

519   household biosand water filters in differing temperatures. Water Sci Tech Water

520   Supply, 16(3), 794-801. https://doi.org/10.2166/ws.2015.192

521   APHA, AWWA, WEF, 2012. Standard Methods for Examination of Water and

522   Wastewater, twenty-second ed. American Public Health Association, Washington,

523   ISBN 978-087553-013-0, p. 1360.

524   Beausoleil, C., Emond, C., Cravedi, J. P., Antignac, J. P., Applanat, M., Appenzeller, B.

525   R., ...Chevrier, C. 2018. Regulatory identification of BPA as an endocrine

526   disruptor: context and methodology. Mol Cell Endocrinol, 475, 4-9.

527   https://doi.org/10.1016/j.mce.2018.02.001

528   Bradley, I., Straub, A., Maraccini, P., Markazi, S., Nguyen, T.H., 2011. Iron oxide

529   amended biosand filters for virus removal. Water Res. 45, 4501–4510.

530   https://doi.org/10.1016/j.watres.2011.05.045

531   Chan, S., Pullerits, K., Riechelmann, J., Persson, K. M., Rådström, P., Paul, C. J. 2018.

532   Monitoring biofilm function in new and matured full-scale slow sand filters using

533   flow cytometric histogram image comparison (CHIC). Water Res. 138, 27-36.

534   https://doi.org/10.1016/j.watres.2018.03.032

535   Cao, G., Zhuang, Y., Liu, B. 2014. Simultaneous determination of bisphenol A and

536   bisphenol S in environmental water using ratio derivative ultraviolet spectrometry.

537   S. Afr. J. Chem. 67(1), 99-103. https://hdl.handle.net/10520/EJC157112

538   Cawst, 2012. Biosand Filter Construction Manual, Centre for Affordable Water and

539   Sanitation Technology. Canada, Calgary.

540   D'Alessio, M., Yoneyama, B., Kirs, M., Kisand, V., Ray, C. 2015. Pharmaceutically

541   active compounds: Their removal during slow sand filtration and their impact on

542   slow sand filtration bacterial removal. Sci Total Environ, 524, 124-135.

543   https://doi.org/10.1016/j.scitotenv.2015.04.014

544   Devi, R., Alemayehu, E., Singh, V., Kumar, A., Mengistie, E. 2008. Removal of

545   fluoride, arsenic and coliform bacteria by modified homemade filter media from

546   drinking water. Bioresour Technol., 99(7), 2269-2274.

547   https://doi.org/10.1016/j.biortech.2007.05.002

548   Elliott, M., Stauber, C.E., Digiano, F.A., de Aceituno, A.F., Sobsey, M.D., 2015.

549   Investigation of E. coli and virus reductions using replicate, bench-scale biosand

550   filter columns and two filter media. Int. J. Environ. Res. Public Health 12, 10276–

551   10299. https://doi.org/10.3390/ijerph120910276

552   Elliott, M.A., Stauber, C.E., Koksal, F., DiGiano, F.A., Sobsey, M.D., 2008. Reductions

553   of *E. coli*, echovirus type 12 and bacteriophages in an intermittently operated

554   household-scale slow sand filter. Water Res. 42, 2662–2670.

555   https://doi.org/10.1016/j.watres.2008.01.016

556   Elliott, M. A., Stauber, C. E., Koksal, F., Liang, K. R., Huslage, F. A., DiGiano, F. A.,

557   Sobsey, M. D. 2006. The operation, flow conditions and microbial reductions of an

558   intermittently operated, household-scale slow sand filter. In: Recent Progress in

559   Slow Sand and Alternative Biofiltration Processes (R. Gimbel, N. J. D. Graham &

560   M. R. Collins, eds). IWA, London.

561   Faria Maciel, P.M., Sabogal-Paz, L.P., 2018. Household slow sand filters with and

562   without water level control: continuous and intermittent flow efficiencies. Environ.

563   Technol. https://doi.org/10.1080/09593330.2018.1515988

564   Food and Agriculture Organization, World Health Organization. 2011. Toxicological

565   and Health Aspects of Bisphenol A: Report of Joint FAO/WHO Expert Meeting

566   and Report of Stakeholder Meeting on Bisphenol A. ISBN 978 92 14 156427 4.

567   Frank, T. E., Scheie, M. L., Cachro, V., Muñoz, A. S. 2014. The effect of increasing

568   grain size in biosand water filters in combination with ultraviolet disinfection. J

569   Water Sanit Hyg Dev., 4(2), 206-213. https://doi.org/10.2166/washdev.2013.171

570   Haig, S. J., Gauchotte-Lindsay, C., Collins, G., Quince, C. 2016. Bioaugmentation

571   mitigates the impact of estrogen on coliform-grazing protozoa in slow sand filters.

572   Environ Sci Technol., 50(6), 3101-3110. https://doi.org/10.1021/acs.est.5b05027

573   Hall-Stoodley, L., Costerton, J. W., Stoodley, P. 2004. Bacterial biofilms: from the

574   natural environment to infectious diseases. Nat. Rev. Microbiol., 2(2), 95.

575   https://doi.org/10.1038/nrmicro821

576   Hammer O., Harper D.A., Ryan, P.D. 2018. PAST3palaeontological statistics, ver. 3.22

577   Paleontological Museum, University of Oslo, Norway.

578 Helmreich, B., Horn, H. 2009. Opportunities in rainwater harvesting. Desalination,

579   248(1-3), 118-124. https://doi.org/10.1016/j.desal.2008.05.046

580 Huang, Y. Q., Wong, C. K. C., Zheng, J. S., Bouwman, H., Barra, R., Wahlström, B., ...

581   Wong, M. H. 2012. Bisphenol A (BPA) in China: a review of sources,

582   environmental levels, and potential human health impacts. Environ Int. 42, 91-99.

583   https://doi.org/10.1016/j.envint.2011.04.010

584 Jenkins, M.W., Tiwari, S.K., Darby, J., 2011. Bacterial, viral and turbidity removal by

585   intermittent slow sand filtration for household use in developing countries:

586   Experimental investigation and modeling. Water Res. 45, 6227–6239.

587   https://doi.org/10.1016/j.watres.2011.09.022

588 Kang, J. H., Katayama, Y., Kondo, F. 2006. Biodegradation or metabolism of bisphenol

589   A: from microorganisms to mammals. Toxicology, 217(2-3), 81-90.

590   https://doi.org/10.1016/j.tox.2005.10.001

591 Kavlock, R.J., Daston, G.P., DeRosa, C., Fenner-Crisp, P., Gray, L.E., Kaattari, S.,

592   Lucier, G., Luster, M., Mac, M.J., Maczka, C., Miller, R., Moore J, Rolland, R.,

593   Scott, G., Sheehan, D.M., Sinks, T., Tilson, H. A., 1996. Research needs for the

594   risk assessment of health and environmental effect of endocrine disruptors: a report

595   of the USEPA-sponsored workshop. Environ Health Perspect 104 (Suppl 4):715–

596   740. https://doi.org/10.1289/ehp.96104s4715

597 Katayama-Hirayama, K., Suzuki, A., Mukaiyama, S., Kaneko, H., Hirayama, K.,

598   Akitsu, T. 2010. Removal of tetrabromobisphenol A by slow sand filtration and a

599   high-performance UV lamp system. Sustain. Environ. Res. 20(4), 221-225.

600 Kennedy, T. J., Hernandez, E. A., Morse, A. N., Anderson, T. A. 2012. Hydraulic

601 loading rate effect on removal rates in a biosand filter: a pilot study of three

602 conditions. Water Air Soil Pollut, 223(7), 4527-4537.

603 https://link.springer.com/article/10.1007/s11270-012-1215-4

604 Levenspiel, O., 1999. Chemical Reaction Engineering, Industrial & Engineering

605 Chemistry Research. https://doi.org/10.1021/ie990488g

606 Li, J., Zhou, Q., & Campos, L. C. 2018. The application of GAC sandwich slow sand

607 filtration to remove pharmaceutical and personal care products. Sci Total Environ,

608 635, 1182-1190. https://doi.org/10.1016/j.scitotenv.2018.04.198

609 Lynn, T. J., Wanjugi, P., Harwood, V. J., Ergas, S. J. 2013. Dynamic performance of

610 biosand filters. J Am Water Works Assoc, 105(10), E587-E595.

611 https://doi.org/10.5942/jawwa.2013.105.0116

612 Murphy, H. M., McBean, E. A., Farahbakhsh, K. 2010. A critical evaluation of two

613 point-of-use water treatment technologies: can they provide water that meets WHO

614 drinking water guidelines?. J Water Health, 8(4), 611-630.

615 https://doi.org/10.2166/wh.2010.156

616 Rodriguez-Mozaz, S., de Alda, M. J. L., Barceló, D. 2004. Monitoring of estrogens,

617 pesticides and bisphenol A in natural waters and drinking water treatment plants by

618 solid-phase extraction–liquid chromatography–mass spectrometry. J Chromatogr

619 A., 1045(1-2), 85-92. https://doi.org/10.1016/j.chroma.2004.06.040

620 Rogers, J. A., Metz, L., Yong, V. W. 2013. Review: Endocrine disrupting chemicals

621 and immune responses: A focus on bisphenol-A and its potential mechanisms. Mol

622 Immunol. 53(4), 421-430. https://doi.org/10.1016/j.molimm.2012.09.013

623   Schmidt, W. P., Cairncross, S. 2009. Household water treatment in poor populations: is

624   there enough evidence for scaling up now? Environ Sci Technol., 43(4), 986-992.

625   https://doi.org/10.1021/es802232w

626   Sobsey, M.D., Stauber, C.E., Casanova, L.M., Brown, J.M., Elliott, M. a., Elliot, M.A.,

627   2008. Point of Use Household Drinking Water Filtration: A Practical, Effective

628   Solution for Providing Sustained Access to Safe Drinking Water in the Developing

629   World. Environ. Sci. Technol. 42, 4261-4267. https://doi.org/10.1021/es702746n

630   Souza Freitas, B. L., Sabogal-Paz, L. P. 2019. Pretreatment using *Opuntia*

631   *cochenillifera* followed by household slow sand filters: technological alternatives

632   for supplying isolated communities. Environmental technology, 1-12.

633   https://doi.org/10.1080/09593330.2019.1582700

634   Pompei, C. M., Ciric, L., Canales, M., Karu, K., Vieira, E. M., Campos, L. C. 2017

635   Influence of PPCPs on the performance of intermittently operated slow sand filters

636   for household water purification. Sci Total Environ. 581, 174-185.

637   https://doi.org/10.1016/j.scitotenv.2016.12.091

638   Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2003. Wastewater Engineering:

639   Treatment and Reuse. McGraw-Hill Higher Education, New York.

640   Terin, U. C., Sabogal-Paz, L. P. 2019. Microcystis aeruginosa and microcystin-LR

641   removal by household slow sand filters operating in continuous and intermittent

642   flows. Water Res. 150, 29-39. https://doi.org/10.1016/j.watres.2018.11.055

643   Thiry, M., Ayrault, M. B., Grisoni, J. C. 1988. Ground-water silicification and leaching

644   in sands: Example of the Fontainebleau Sand (Oligocene) in the Paris Basin. Geol

645   Soc Am Bull. 100(8), 1283-1290. https://doi.org/10.1130/0016-

646   7606(1988)100<1283:GWSALI>2.3.CO;2

647 Tran, Y. T., Barry, D. A., Bajracharya, K. 2002. Cadmium desorption in sand. Environ

648   Int. 28(6), 493-502. https://doi.org/10.1016/S0160-4120(02)00077-6

649 US Environmental Protection Agency, 1986. Design manual, municipal wastewater

650   disinfection, Cincinnati, OH, EPA/625/ 1-86/021

651 Vecchio, A., Finoli, C., Di Simine, D., Andreoni, V. 1998. Heavy metal biosorption by

652   bacterial cells. Fresenius J Anal Chem, 361(4), 338-342.

653   https://doi.org/10.1007/s002160050899

654 Vijayaraghavan, K., Yun, Y. S. 2008. Bacterial biosorbents and biosorption. Biotechnol

655   Adv., 26(3), 266-291. https://doi.org/10.1016/j.biotechadv.2008.02.002

656 Vom Saal, F. S., Hughes, C. 2005. An extensive new literature concerning low-dose

657   effects of bisphenol A shows the need for a new risk assessment. Environ Health

658   Perspect. 113(8), 926. https://doi.org/10.1289/ehp.7713

659 World Health Organization, 2017. Guidelines for Drinking- water Quality: fourth

660   edition incorporating the first addendum. World Health Organization. Geneva,

661   Switzerland.

662 World Health Organization. 2016. Results of round I of the WHO international scheme

663   to evaluate household water treatment technologies. World Health Organization.

664   Geneva, Switzerland.

665 World Health Organization. 2012. A toolkit for monitoring and evaluating household

666   water treatment and safe storage programmes. World Health Organization. WHO

667   Press. Geneva, Switzerland.

668   Young-Rojanschi, C., Madramootoo, C., 2015. Comparing the performance of biosand

669   filters operated with multiday residence periods. J. Water Supply Res. Technol. 64,

670   157. https://doi.org/10.2166/aqua.2014.027

671   Young-Rojanschi, C., Madramootoo, C., 2014. Intermittent versus continuous operation

672   of biosand filters. Water Res. 49, 1–10.

673   https://doi.org/10.1016/j.watres.2013.11.011

674   Zoeller, R. T., Brown, T. R., Doan, L. L., Gore, A. C., Skakkebaek, N. E., Soto, A. M.,

675   Woodruff, T. J., Vom Saal, F. S. 2012 . Endocrine-disrupting chemicals and public

676   health protection: a statement of principles from The Endocrine Society.

677   Endocrinology, 153(9), 4097-4110. https://doi.org/10.1210/en.2012-1422

Table 1. Influent water quality and HSSF efficiency without pre or post treatment.

Author	Influent Water	Turbidity		*Escherichia coli*	
		Initial value (NTU)	Removal (%)	Initial value (CFU/100 mL)	Reduction (log)
Elliott et al. (2008)	Raw water	1.86-8.96	74.8(a)*	255 ± 33	0.5-1.9*
Faria Maciel and Sabogal-Paz (2018)	Mixture of well water with kaolinite	10.92-11.75 (a)	85(a)	3,969 - 5,021(a)	1.26-2.29(a)
Frank et al. (2014)	Mixture of tap water with sewage	19.9 (a)	75.4(a)	39,400(a)	1.88(a)*
Lynn et al. (2013)	Mixture of raw water with sewage	7.5(a)	86(a)	1.11×10^4(a)	1.7(a)
Murphy et al. (2010)	Deep well	10.4(a)	98(a)*	109(a)	0.92(a)*
	Surface water	9.4(a)	90(a)*	5,842(a)	1.74(a)*
Young-Rojanschi and Madramootoo (2014)	Raw water	12.6 ± 7.3	87-96(a)	410 ± 60	1.67-3.71

Notes: the greatest efficiency happens when the biological layer is formed; the symbol

(a) means average; *values calculated by the authors with the data available in the

manuscript.

Table 2. Influent water quality

Parameter	Mean value ± Standard deviation	Parameter	Mean value ± Standard deviation
Turbidity (NTU)	0.37 ± 0.11	Sulphate (mg L^{-1})	1.06 ± 1.63
Conductivity (μS m^{-1})	3.24 ± 0.78	Nitrate (mg L^{-1})	0.22 ± 0.71
pH	6.10 ± 0.14	Silicon (mg L^{-1})	0.002 ± 0.002
Temperature (^{o}C)	22.2 ± 1.6	Aluminium (mg L^{-1})	0.006 ± 0.004
DO (mg L^{-1})	3.25 ± 0.53	Calcium (mg L^{-1})	0.196 ± 0.07
BPA (mg L^{-1})	2.35 ± 0.41	Iron (mg L^{-1})	0.002 ± 0.002
DOC (mg L^{-1})	132.9 ± 15.5	Calcium (mg L^{-1})	0.465 ± 0.66
Total coliforms (CFU/100 mL)	11.8 ± 4.3	Sodium (mg L^{-1})	0.04 ± 0.03
Phosphate (mg L^{-1})	0.64 ± 2.01	Potassium (mg L^{-1})	0.035 ± 0.06
Fluoride (mg L^{-1})	0.57 ± 0.71	Magnesium (mg L^{-1})	0.008 ± 0.004

Deionised water quality used to prepare influent water: turbidity (NTU) = 0.07 ± 0.01; conductivity (μS m^{-1}) = 0.85 ± 0.18; pH = 6.0 ± 0.1; temperature (^{o}C) = 21.4 ± 1.3; DO (mg L^{-1}) = 3.6 ± 0.3; DOC (mg L^{-1}) = 0.1 ± 0.03; chloride (mg L^{-1}) = 0.03 ± 0.01; measured and undetected: coliforms, lithium, ammonium, phosphate, potassium, bromide, fluoride, sulphate, nitrite, nitrate, silicon, aluminium, calcium, iron, magnesium and sodium.

Table 3. Tracer test results for the C-HSSF

Statistic	HRT	N-CSTR		Small dispersion model		High dispersion model	
		N	r^2	D/uL	r^2	D/uL	r^2
Mean	857	17	0.75	0.032	0.67	0.028	0.66
Standard deviation	21	5	0.02	0.008	0.03	0.007	0.03

Notes: N-CSTR: N-continuous stirred tank reactors model; N: number of stirred tank reactors; D/uL: dimensionless group characterising the spread in the whole reactor (close to zero denote negligible dispersion, hence, plug flow reactor); HRT: hydraulic retention time; and r^2: coefficient of determination.

Table 4. Filtered water quality and removal or variation rates for I-HSSF and C-HSSF

Parameter	C-HSSF		I-HSSF		*p*-value
	Value (M ± SD)	Removal (R) or variation (V) (%) (M ± SD)	Value (M ± SD)	Removal (R) or variation (V) (%) (M ± SD)	
Turbidity (NTU)	0.62 ± 0.3	-74 ± 73 (R)	0.62 ± 0.2	-76 ± 53(R)	0.972
Conductivity (µS m^{-1})	36.2 ± 10.4	-1063 ± 386 (V)	30.0 ± 8.0	-868 ± 313 (V)	0.001 SS
pH	6.2 ± 0.3	-2 ± 5 (V)	6.3 ± 0.4	-4 ± 6 (V)	0.061
Temperature (^{o}C)	22.1 ± 1.6	1 ± 1 (V)	22.1 ± 1.6	1 ± 1 (V)	0.860
DO (mg L^{-1})	1.2 ± 0.8	60 ± 28 (V)	1.1 ± 0.6	66 ± 22 (V)	0.181
BPA (mg L^{-1})	2.65 ± 0.37	-14 ± 16 (R)	2.26 ± 0.31	3 ± 8 (R)	0.001 SS
DOC (mg L^{-1})	115.9 ± 14.8	12 ± 9 (R)	123.2 ± 12.3	7 ± 6 (R)	0.003 SS
Total coliforms (CFU 100mL^{-1})	2.3 ± 1.7	0.78 log ± 0.3 log (R)	1.92 ± 1.38	0.84 log ± 0.25 log (R)	0.686
Phosphate (mg L^{-1})	1.86 ± 3.10	-12 ± 58 (V)	2.50 ± 3.67	-18 ± 60 (V)	0.501
Fluoride (mg L^{-1})	0.44 ± 0.84	55 ± 65 (R)	0.11 ± 0.18	88 ± 17 (R)	0.045 SS
Chloride (mg L^{-1})	0.17 ± 0.20	-86 ± 202 (V)	0.17 ± 0.17	-60 ± 141 (V)	0.785
Sulphate (mg L^{-1})	1.70 ± 2.17	-18 ± 26 (V)	1.58 ± 2.24	-10 ± 74 (V)	0.844
Nitrate (mg L^{-1})	0.28 ± 0.87	-43 ± 141 (V)	0.38 ± 1.09	-89 ± 174 (V)	0.712
Silicon (mg L^{-1})	0.46 ± 0.05	-38804 ± 19491 (V)	0.43 ± 0.07	-36820 ± 20359 (V)	0.412
Aluminium (mg L^{-1})	0.01 ± 0.003	-533 ± 873 (V)	0.01 ± 0.002	-846 ± 1678 (V)	0.675
Calcium (mg L^{-1})	4.18 ± 0.32	-2271 ± 894 (V)	3.39 ± 0.30	-1883 ± 940 (V)	0.004 SS
Iron (mg L^{-1})	0.01 ± 0.004	-345 ± 372 (V)	0.01 ± 0.003	-349 ± 190 (V)	0.930
Potassium (mg L^{-1})	0.19 ± 0.10	-1523 ± 1275 (V)	0.13 ± 0.01	-919 ± 558 (V)	0.174
Magnesium (mg L^{-1})	0.92 ± 0.08	-14577 ± 8155 (V)	0.80 ± 0.07	-12644 ± 7717 (V)	0.036 SS
Sodium (mg L^{-1})	0.11 ± 0.06	-316 ± 289 (V)	0.10 ± 0.04	-312 ± 361 (V)	0.764

Notes: M: mean; SD: standard deviation; statistically significant difference (SS) when p-value < 0.05
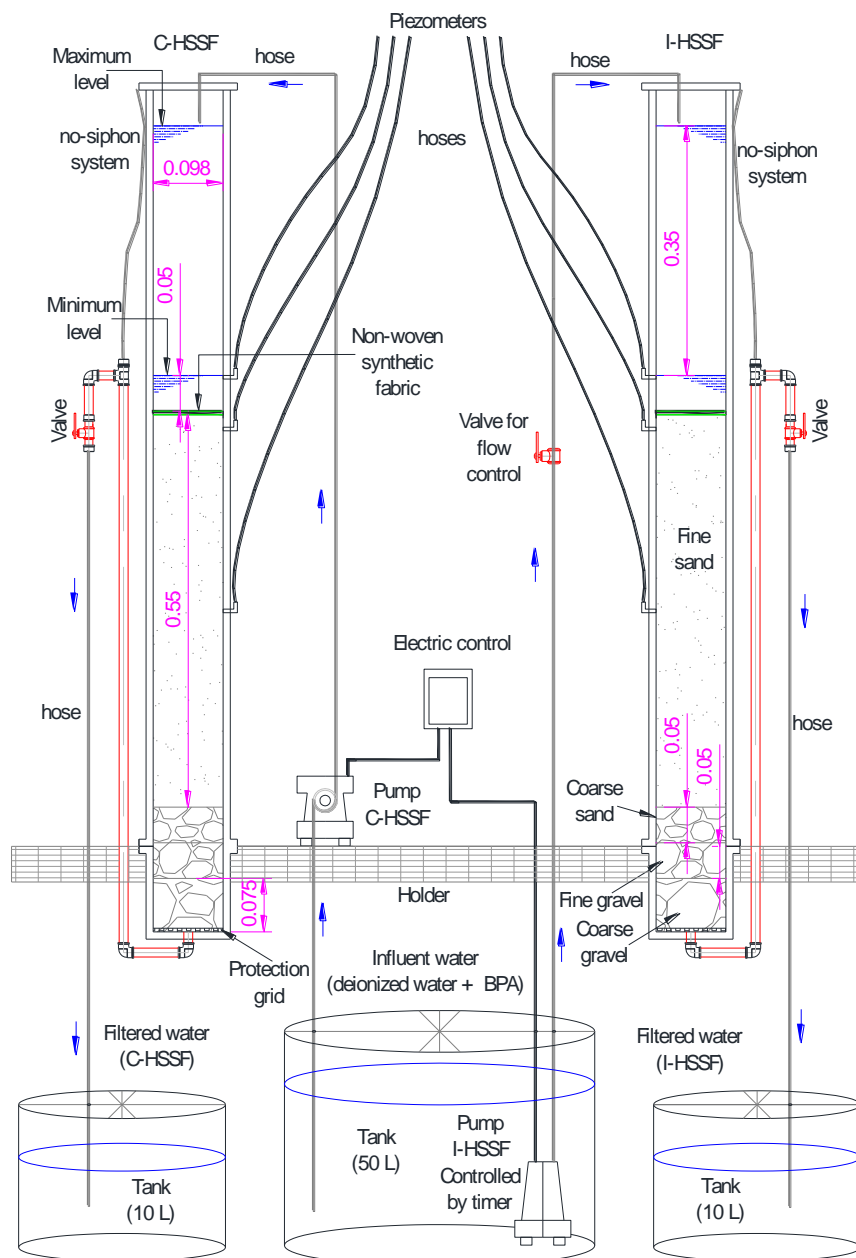
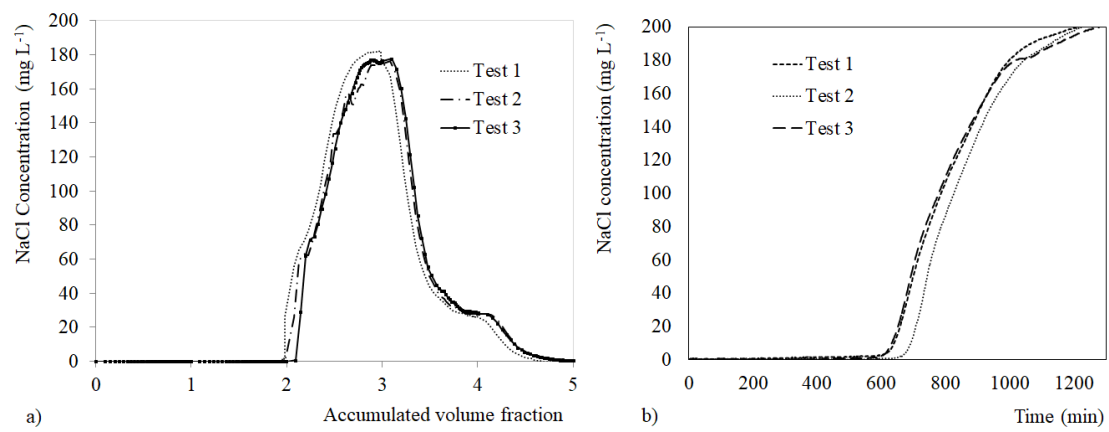**Fig. 1.** Cross-section of pilot-scale HSSFs (units in meters)

Fig. 2. Tracer tests results for I-HSSF (a) and C-HSSF (b)
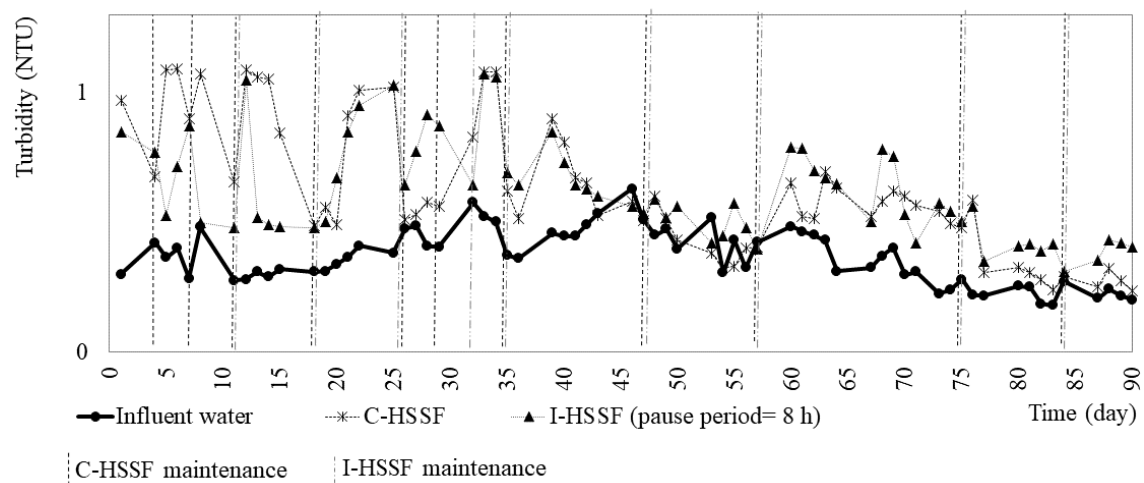
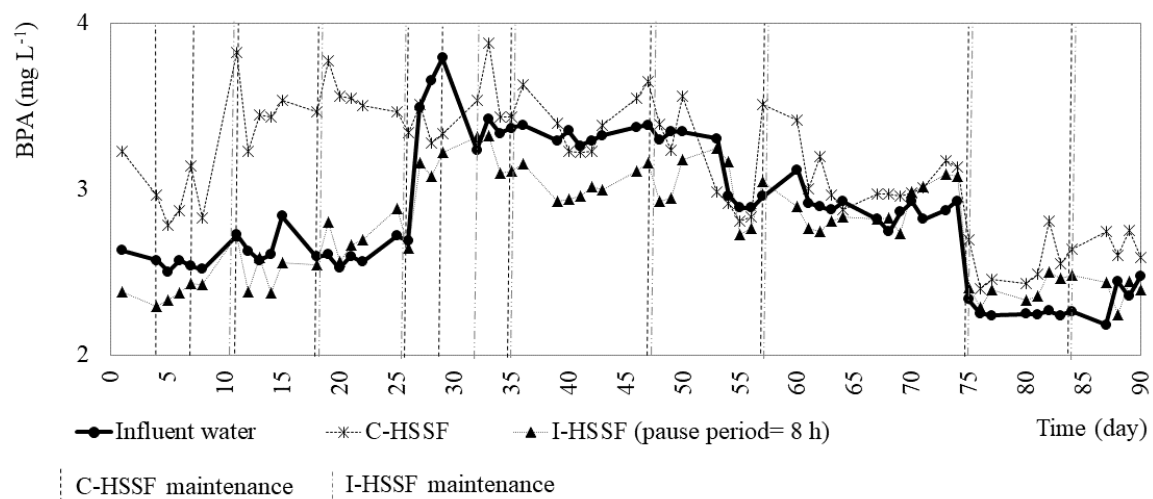**Fig. 3.** Turbidity variation for the influent water and filtered water over time for I-HSSF and C-HSSF

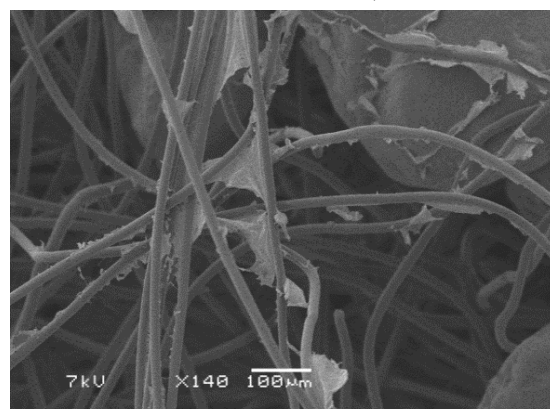**Fig. 4.** BPA in the influent water and filtered water over time for I-HSSF and C-HSSF
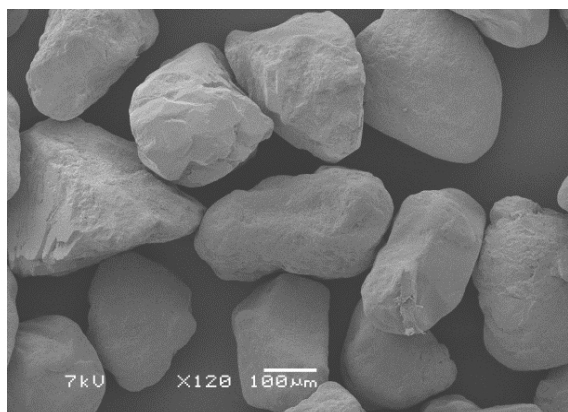
a) original fine sand (K, Si, Al, Ca, Na, Cl and Fe were detected)
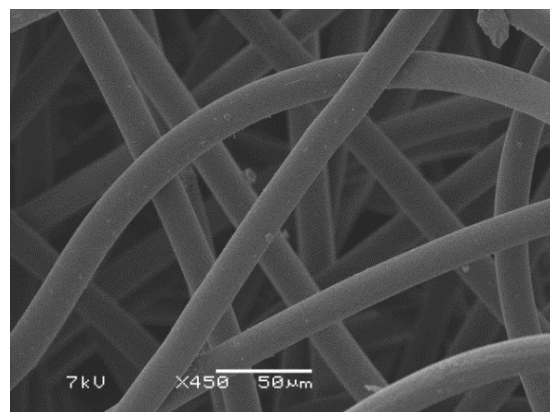
b) original synthetic fabric (Si, Al, Ca, Na, Cl and Fe were detected)
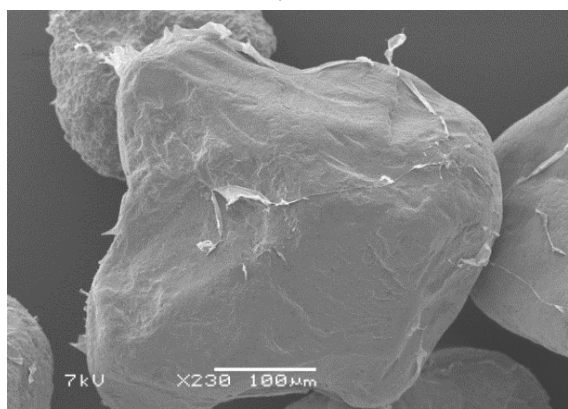
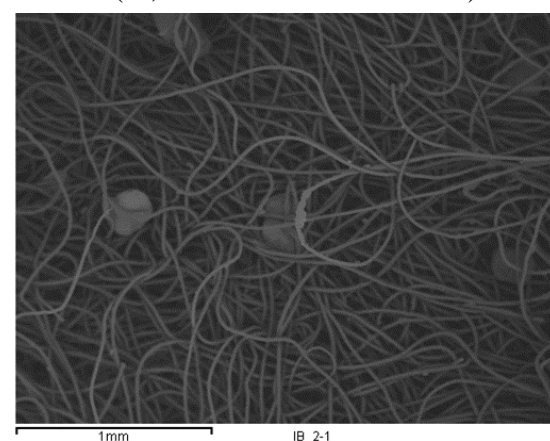c) C-HSSF fine sand with formed biofilm (Si, K, Mg and Al were detected)

d) C-HSSF synthetic fabric with formed biofilm (Si, K and Al were detected)
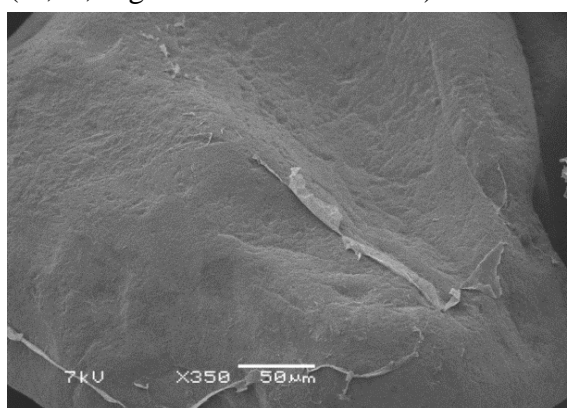
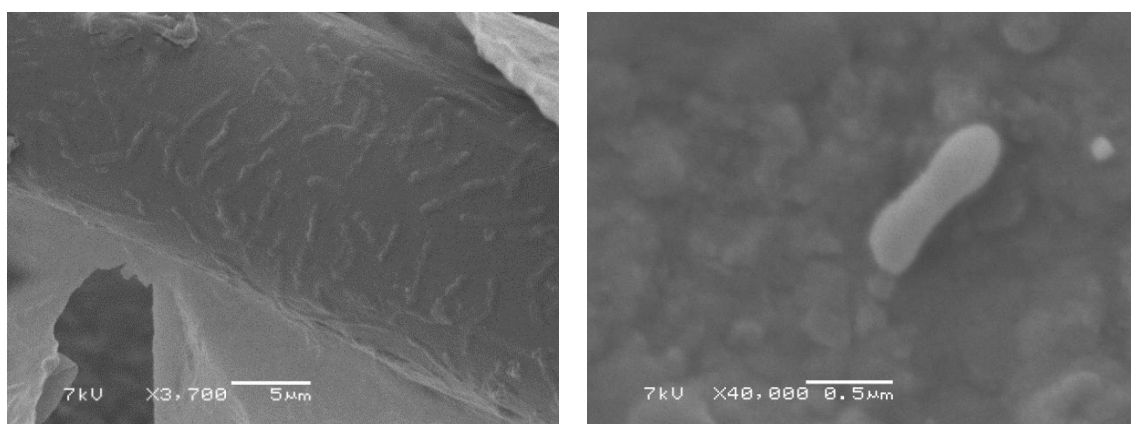e) I-HSSF fine sand with formed biofilm (Si, Mg and Al were detected)

f) I-HSSF synthetic fabric with formed biofilm (Si, Al and Na were detected)

Fig. 5. SEM photomicrographs and chemical compositions from the synthetic fabric and fine sand.

a) C-HSSF biofilm (synthetic fabric)   b) I-HSSF biofilm (fine sand)

Fig. 6. Microorganisms visualised in the biofilms by SEM photomicrographs

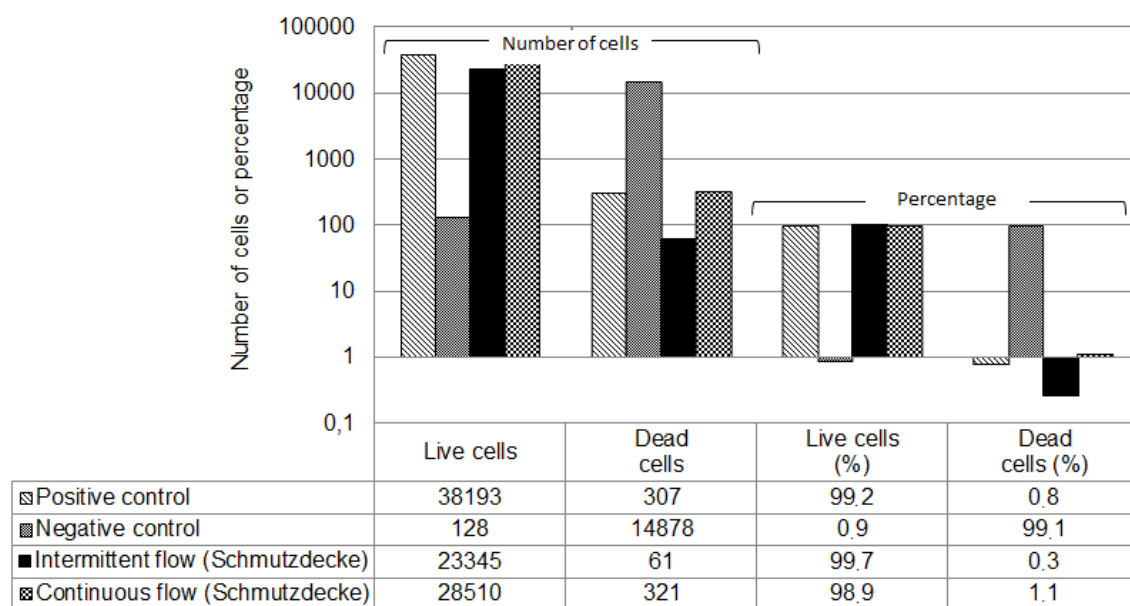|	Live cells	Dead cells	Live cells (%)	Dead cells (%)
Positive control	38193	307	99.2	0.8
Negative control	128	14878	0.9	99.1
Intermittent flow (Schmutzdecke)	23345	61	99.7	0.3
Continuous flow (Schmutzdecke)	28510	321	98.9	1.1

Fig. 7. Flow cytometry results for samples from the biological layer collected at the end of the HSSF operation.

1 **Statement**

2 The authors hereby declare previous originality check, no conflict of interest and open

3 access to the repository of data used in this paper for scientific purposes.