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2 **Safe Purification of Rural Drinking Water by Biological Aerated**
3 **Filter Coupled with Ultrafiltration**

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15 **ABSTRACT**

16 In many rural areas, lakes or reservoirs represent typical water resources. Some of these sources
17 have poor water qualities compared with groundwater or river water, which are commonly used
18 as the main drinking water sources in large modern cities. Drinking water supply in remote rural
19 areas usually suffers from various challenges, such as the high cost of construction and
20 maintenance of centralized drinking water treatment plants and pipe networks due to the
21 dispersed nature of villages, which are often located in varied and complex topographies. In this
22 study, a combined process comprising biological aerated filter (BAF) combined with
23 ultrafiltration was developed to treat polluted reservoir water. Organic matter indexes, turbidity,
24 and chroma were used as indicators for the evaluation of the system performance. In a long-term
25 experiment lasting 260 days, the combined process was tested under different values of critical
26 operational parameters, including filler types and empty bed contact time (EBCT). Furthermore,
27 the microbial communities in different BAF reactors were carefully evaluated at different times,
28 finding that microorganisms with specific functions were enriched in the various BAF reactors.
29 The combined process reached 85.5% removal rate of DOC with an EBCT of 45 min and using
30 granule active carbon (GAC) as filler. Most of the effluents of BAF reactors met the
31 requirements for drinking water in China. The combined system showed practical potential for
32 polluted water treatment in some rural areas.

33

34 **KEYWORDS:** Biological aerated filter; Ultrafiltration; Combined system; Rural drinking water
35 treatment; Microbial community.

36

37 **1. Introduction**

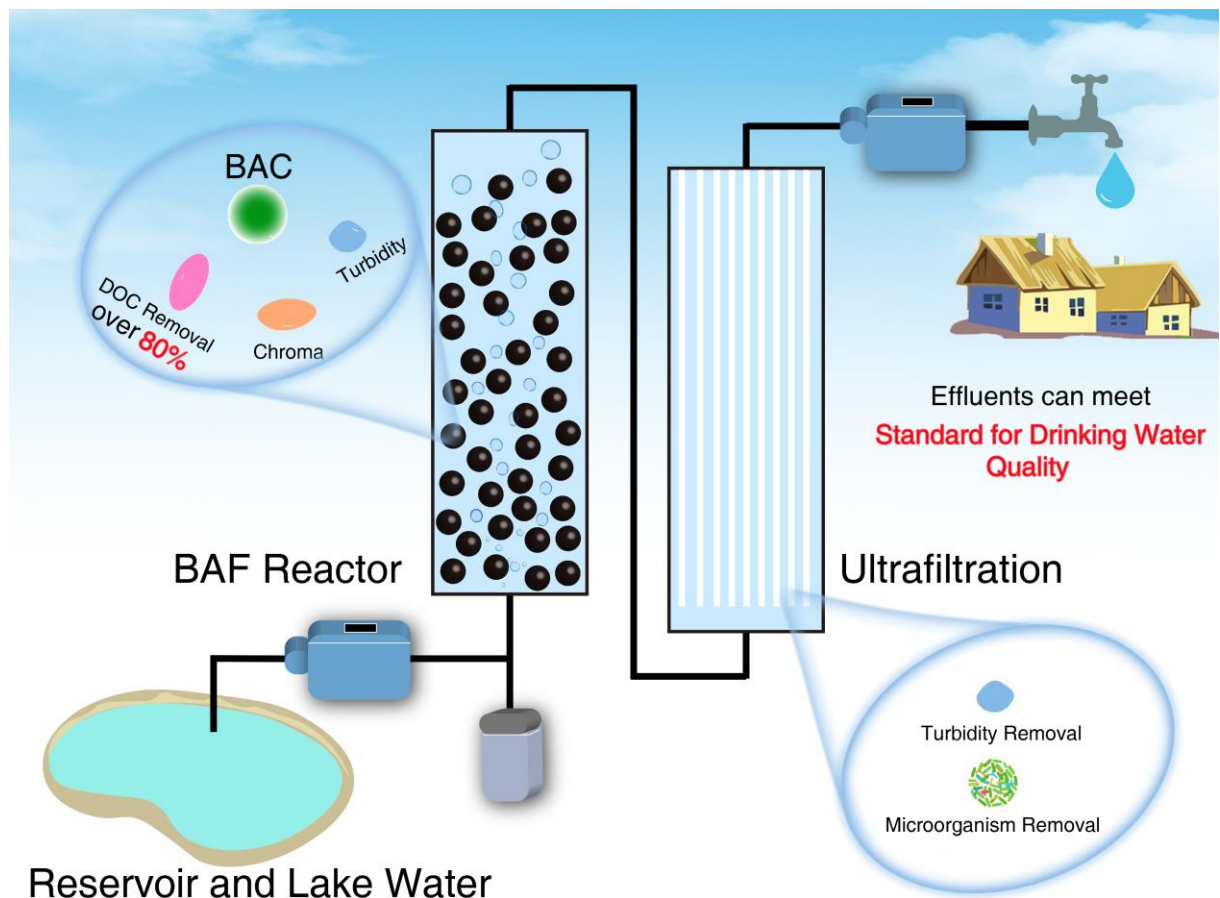
38 With the rapid growth of industrialization and population, access to adequate and safe
39 drinking water is an increasing challenge (Li and Wu, 2019; Pedro-Monzonis et al., 2015).
40 Traditional drinking water treatment processes, commonly consisting of coagulation,
41 sedimentation, filtration and disinfection, are capable of meeting the requirements for water
42 quality in modern cities in most cases, and advanced treatment processes are commonly applied
43 in urban water treatment plants to ensure a drinking water of high quality (Shen et al., 2020;
44 Wang, 2016). However, currently around 2.2 billion people still suffer from the lack of safely
45 managed drinking water, especially in remote villages, such as in mountainous, pastoral, and
46 forest areas (Li and Wu, 2019; Song et al., 2020; UNICEF, 2019; WHO, 2018). In fact, rural
47 drinking water access and safety is a global concern, with complex natural and economic
48 conditions, such as related to geography, water source quality, costs and labor issues, limiting the
49 widespread implementation of decentralized drinking water treatment plants (DWTPs). For
50 example, the territory in China counts numerous villages that are dispersedly located. In addition,
51 some reservoirs and lakes in rural areas that serve as the main water source are contaminated due
52 to inadequate environmental protection, thus increasing the challenges of water treatment
53 approaches aimed at ensuring water safety. Science and technology advances should tackle the
54 development of simple and effective water treatment techniques, suitable for local conditions,
55 characterized by low construction and maintenance costs and by the ability to serve areas of
56 flexible sizes (Li and Qian, 2018). Given that a single treatment process can hardly meet the
57 standards for drinking water quality in rural areas of China, combined processes coupling
58 different approaches represent a promising approach to reduce water stress in those areas (Scheili
59 et al., 2015).

60 Membrane filtration, especially ultrafiltration, is an easily maintainable water treatment
61 technology able to intercept most suspended matter, microorganisms, and large macromolecules
62 in water (Yu et al., 2021; Yu et al., 2020). More importantly, membrane technologies can be
63 widely applied even in rugged terrain. However, dissolved substances and small molecules also
64 exist in polluted water sources, such as odor compounds, ammonia nitrogen, and other organic
65 micropollutants, which cannot be significantly removed using porous membrane processes (Kim
66 et al., 2018; Teodosiu et al., 2018). Moreover, pollutants usually accumulate on the membrane
67 surface and result in membrane fouling, consequently reducing the filtration efficiency,
68 shortening the service life of the membrane, and increasing the operating costs (Shi et al., 2014;
69 Tian et al., 2018). A pretreatment or a synergic process, such as flocculation, advanced oxidation,
70 or biological treatment, is essential to improve the efficiency of overall treatment and the quality
71 of the final effluent. (Gao et al., 2011). In particular, inexpensive biological methods may be
72 suitable for remote areas: they include drip filtration, moving bed biofilm reactors, and
73 membrane bioreactors. Here, it is hypothesized that the biological aerated filter (BAF) is superior
74 to all the competing biological technology due to lower construction costs, easier operability, and
75 the ability to guarantee a continuous and long-term efficiency pollutant removal (Neoh et al.,
76 2016; Udomkittayachai et al., 2021).

77 BAF is commonly applied in wastewater treatment to remove organic matter, nitrogen, and
78 phosphorus, taking advantage of the activity of microorganisms attached on a filler (Tang et al.,
79 2021). Abu Hasan et.al improved the removal rates of ammonia, Mn(II), and chemical oxygen
80 demand (COD) by investigating the different operation parameters in a BAF system as an
81 additional treatment in DWTP (Abu Hasan et al., 2011). The efficiency of the BAF process is
82 influenced by numerous factors, including the empty bed contact time (EBCT), the properties of

83 the fillers, as well as the activity of various microorganisms, which may form communities on
84 the surface of the fillers and determine the removal efficacy for pollutants in the raw water. Until
85 now, very few studies have investigated the combination of the BAF reactor and ultrafiltration
86 for small-scale drinking water treatment in actual rural areas. For this reason and for the
87 advantages described above, such combined process was applied for rural drinking water
88 treatment in this study, with a schematic diagram of the system shown in Figure 1.

89 Specifically, in this work we discuss the impact of filler properties, EBCT, and microbial
90 communities on the effluent quality. Also, the treatment efficiency, productivity, and fouling
91 behavior of the ultrafiltration process are evaluated. The stability of the whole system in long-
92 term tests is presented, together with the potential for the application of the process to provide
93 safe drinking water starting from polluted water in rural areas.



94

95 **Figure 1.** The installation diagram of the combined system contains the BAF and ultrafiltration.

96

97 **2. Materials and Methods**

98 *2.1. Materials*

99 The polluted water was collected from a reservoir in Zhongxian county (Chongqing, China),
100 which serves as the water source for rural villages and towns nearby, and used as raw water.
101 Information regarding the quality of as-collected water is listed in Supporting Information (Table
102 S1). Granular activated carbon (GAC, 12 × 40 mesh, 0.425~1.70 mm) with an iodine number of
103 950 mg/g was purchased from Calgon Carbon corporation (USA). New and exhausted
104 polyvinylidene difluoride (PVDF) hollow fiber membranes were obtained from Litree Purifying
105 Technology Co., Ltd (Hainan, China), and the exhausted sample was then cut into pieces with 5-
106 10 mm in length and used as filler in one of the BAF reactors. Plexiglass reactors with an inner
107 diameter of 2.0 cm and a height of 30 cm were custom-made and used as the BAF reactors; a
108 schematic diagram of the reactors is shown in Figure S1. Peristaltic pumps (BT-300-2 J, Longer
109 Pump, China) were used to feed the raw water from the tanks to the BAF reactors, and feed the
110 effluents of the reactors to the ultrafiltration hollow fiber membrane modules. Glucose was
111 purchased from a local pharmacy. The LH-D/E reagents used for COD_{Cr} measurement were
112 purchased from Lianhua technology (Beijing, China): LH is the abbreviation for the company
113 name, while D and E are product codes. LH-D is potassium dichromate and LH-E is silver
114 sulfate. Deionized water (DI water) used in this work was produced by an ultrapure water system
115 from Ulupure (Chengdu, China).

116

117 *2.2. Methods*

118 2.2.1. Operation of BAF Reactors

119 Undisturbed sediment samples of about 1 kg from shallow water areas in the reservoir were
 120 collected and stored in a 5 L culture tank with glucose (1 g) and raw water (4 L) for microbe
 121 enrichment. A volume of roughly 2 L of culture solution was taken from the tank every 3 days,
 122 followed by replenishment with glucose (0.5 g) and raw water (2 L). The solution collected from
 123 the culture tank was mixed with raw water as the influent during the domestication period of the
 124 reactor systems to facilitate rapid growth and adaptation of microorganisms in the BAF reactors.
 125 The mixing ratios of collected culture solution and raw water varied from 1:1 to 1:14 over time,
 126 until the water quality of the BAF reactor effluents were stable. Owing to the presence of culture
 127 solution, the microorganisms accumulated in the BAF reactors and a biofilm formed on the
 128 fillers. The domestication period lasted about 45 days. Peristaltic pumps were used to pump the
 129 raw water into the BAF reactors and then pump the effluents into the ultrafiltration membrane
 130 modules. Meanwhile, the reactors were continuously aerated by aeration pumps with a gas/water
 131 ratio equal to 5/1. The BAF reactors were backwashed for 15 minutes with gas and water every
 132 15 days. The influent rate during the domestication and running periods was 0.12 L/h and the
 133 aeration rate was 0.6 L/h. For backwashing, the influent rate was 1.5 L/h and the aeration rate
 134 was 1.5 L/h. Information of different BAF reactors are listed in Table 1. The water quality of the
 135 effluents during domestication is listed in Figure S2 and S3 of the Supporting Information.

136 **Table 1.** The operation parameters of BAF reactors in this work.

Operators ID	Fillers	EBCT (min)	Gas-water ratio	Running Time (d)
BAF-60-AC	GAC	60	5:1	285
BAF-60-M	Shredded hollow fiber membrane	60	5:1	285
BAF-45-AC	GAC	45	5:1	260
BAF-30-AC	GAC	30	5:1	260

137 Note: The running time of the reactors does not include the domestication phase. The reactors were not started
138 simultaneously.

139

140 2.2.2. Characterizations and Analyses of Influent and Effluent Streams

141 The raw water, effluents of BAF reactors, and effluents of membrane modules were
142 collected to monitor turbidity, pH, and total dissolved solid (TDS) directly during the entirety of
143 the experiments. Dissolved organic carbon (DOC), chemical oxygen demands (COD_{Cr}), UV
144 absorbance at 254 nm (UV₂₅₄), and fluorescence excitation-emission matrix (EEM) of the
145 samples were determined after filtration through 0.45 µm acetate fiber membrane. A turbidimeter
146 (TL2310, Hach company, USA), a PB-10 pH meter (Sartorius Scientific Instruments Co., Ltd.,
147 Germany), an Ultrameter II 6PFC portable multifunctional meter (Myron L Company, USA), a
148 total organic carbon (TOC) analyzer (TOC-L CPH CN200, Shimadzu, Japan), a UV-vis
149 spectrophotometer (Orion AquaMate 8000, Thermo Fisher Scientific INC., MA, USA), and a
150 fluorescence spectrophotometer (F-7000, Hitachi, Japan) were used for analyses. The metal ions
151 were detected using inductively coupled plasma-mass spectrometry (ICP-MS, NexION 1000 G,
152 Perkin Elmer, Waltham, MA) and the anions were detected by Dionex Intergration HPLC ion
153 chromatography (Thermo Fisher Scientific Inc., USA, with AS11-HC chromatographic columns).
154 The ammonia nitrogen was measured using an ammonia nitrogen meter (HI96715, Hanna
155 Instruments Co., Ltd., Italy). Detailed methods can be found in our previous studies and in Text
156 S1 of the Supporting Information (Tang et al., 2020; Tang et al., 2021).

157

158 2.2.3. Microbial Diversity Sequencing Analysis

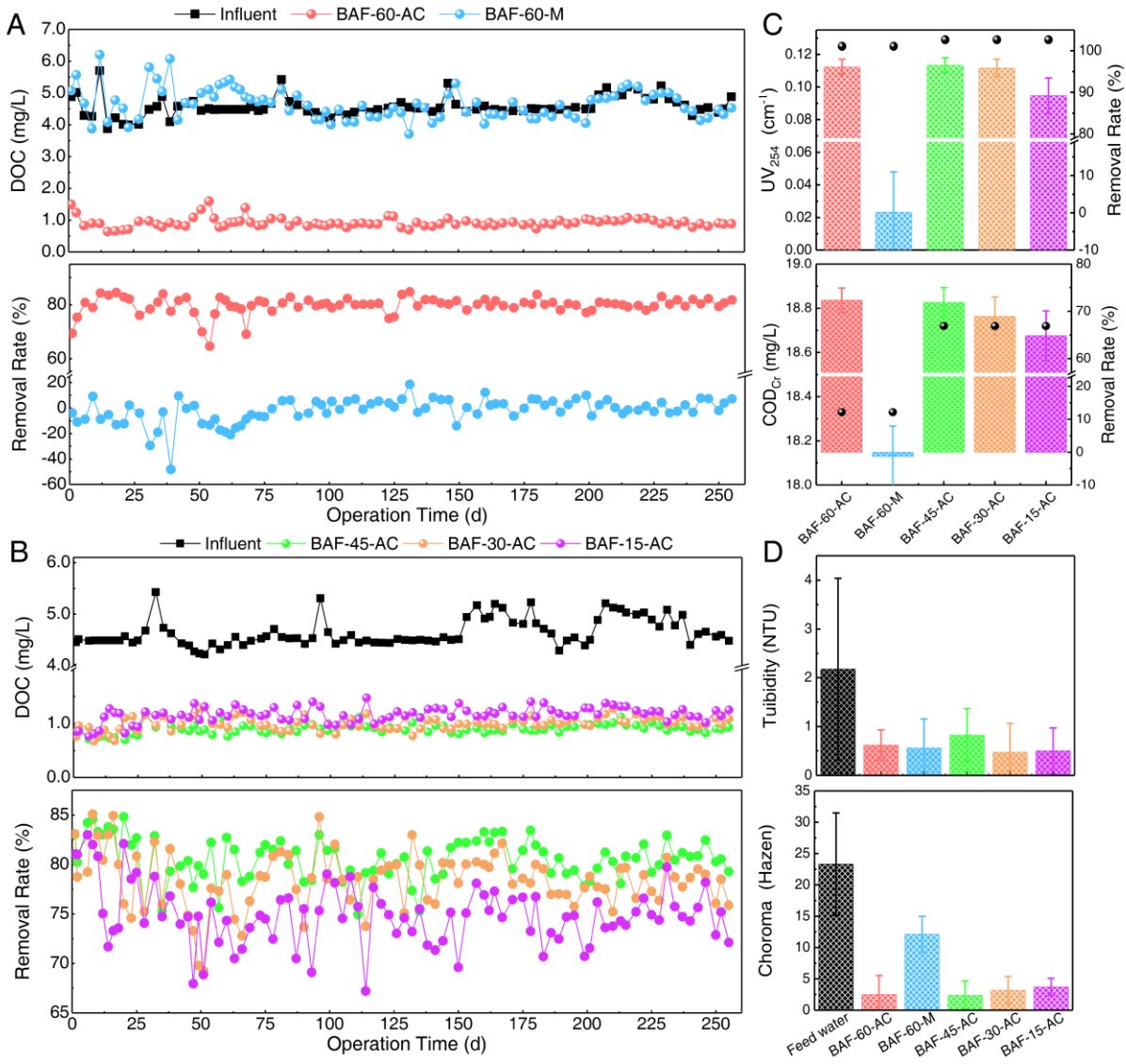
159 The 16s rRNA gene is the DNA sequence corresponding to the coding rRNA on bacteria. It
160 exists in the genome of all bacteria. The conserved regions and variable regions of 16s-rRNA
161 reflect the relationship and differences among bacteria, respectively. In this study, 2 g filler
162 samples in the BAF reactors were collected at about 5 cm depth from the top, and the same
163 amount of fillers was backfilled immediately after withdrawing. The variation of microbial
164 communities and the dominant functional microorganisms over time were analyzed through the
165 microbial diversity sequencing of the fillers in the continuous-mode BAF reactors at different
166 times using 16s-rRNA. The sequencing of 16s rRNA took place on the 10th, 60th and 110th day
167 after the end of the domestication stage. Details for the microbial diversity sequencing analysis
168 are presented in Text S2.

169

170 **3. Results and Discussion**

171 *3.1. Removal of Organic Matters in Raw Water by BAF Process*

172 In this work, DOC, UV₂₅₄, and COD_{Cr} values were measured to characterize the organic
173 matter in water samples, and EEM was applied to determine the removal rate of various types of
174 organic pollutants from the raw water. Considering the variation of water quality of influents and
175 effluents of the BAF reactors over time, it can be concluded that the fillers in the BAF reactors
176 reached a stable state. A certain amount of microorganisms were attached on the surface of GAC,
177 transforming this material into biological activated carbon (BAC) during the domestication
178 period (Zhang et al., 2011).



179

180 **Figure 2.** Water quality indexes of influent and effluent streams of different BAF reactors during the operation

181 period. A: DOC values of influent and effluent streams of BAF reactors with different fillers and their

182 corresponding removal rates; B: DOC values of influent and effluent streams of BAF reactors with different

183 EBCTs and their corresponding removal rates; C: Average UV₂₅₄ and COD_{Cr} values (black dots referring to

184 the left axis) of influent streams and removal rates (bars referring to the right axis) of different reactors; D:

185 Average turbidity and chroma values of influent streams and removal rates of different reactors.

186

187 Figure 2A presents the DOC removal efficiency values achieved in the BAF reactors with

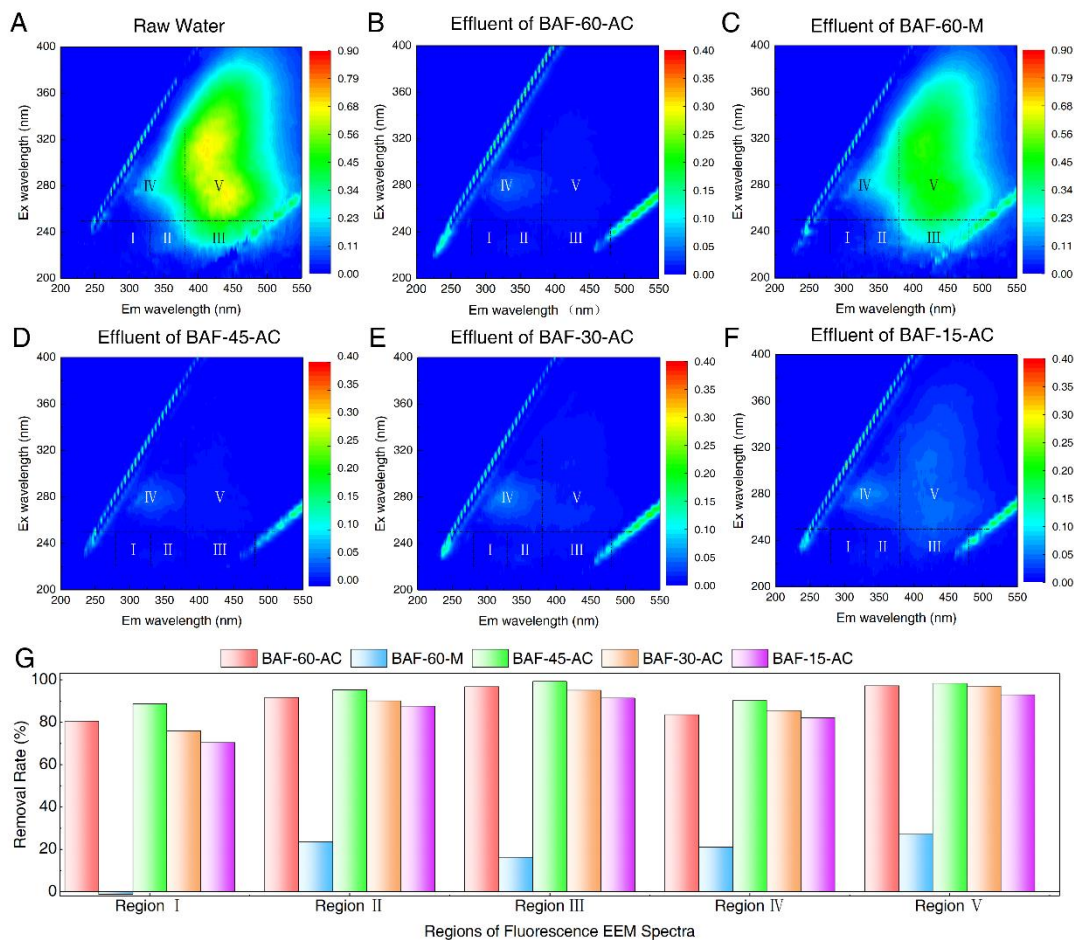
188 different fillers: the DOC removal rate of BAF-60-AC was about 80%, higher than that of BAF-

189 60-M, indicating that the filler type is a critical parameter influencing the efficiency of the
190 biological degradation activity. Figure 2B shows the effect of EBCT on the DOC removal
191 efficiency by the BAF reactors with GAC as the fillers. The average DOC removal rates
192 increased from $75.0 \pm 3.7\%$ to $79.5 \pm 4.0\%$ with increasing EBCT from 15 to 60 min, indicating
193 that the EBCT also has an important role in ensuring high biological degradation rate of organic
194 matters in the BAF reactors. The results suggest that the BAF reactors with GAC as fillers can
195 adsorb and degrade a significant portion of organic matter in the streams within 15 minutes. In
196 addition, for each 15-minute extension in EBCT, the removal rate of DOC increased by an
197 average of 2%. Higher EBCT values endowed the BAF reactors with higher stability for DOC
198 removal even with varied DOC concentrations in the influent solution.

199 The UV_{254} parameter can be used to represent the amount of some organic matters that
200 contains C=C double bonds and benzene ring structures, including humic macromolecular
201 substances and aromatic compounds (J.C. Crittenden, 2016). As illustrated in Figure 2C, the
202 removal rates of UV_{254} were 96.1% (BAF-60-AC), 0.17% (BAF-60-M), 96.5% (BAF-45-AC),
203 95.9% (BAF-30-AC), and 89.2% (BAF-15-AC). The average removal rates of BAF reactors for
204 COD_{Cr} were instead 72.3% (BAF-60-AC), -1.29% (BAF-60-M), 71.9% (BAF-45-AC), 68.9%
205 (BAF-30-AC), and 64.8% (BAF-15-AC). Using GAC as the filler, the BAF reactors showed
206 consistent removal rates for COD_{Cr} and could remove organic matters that contain C=C double
207 bonds and benzene ring structures effectively, while the BAF-60-M reactor (containing
208 membrane pieces as fillers) exhibited a negligible effect on organics. Consistent with results
209 observed for DOC, increasing EBCT also enhanced the performance of BAF reactors in terms of
210 UV_{254} and COD_{Cr} removal.

211 Turbidity, chroma, pH, TDS, and the concentration of ions that are listed in local drinking
212 water standard were used as indicators related to drinking water quality. As presented in Figure
213 2D, the average turbidity of the effluents from BAF reactors was always lower than 1.0 NTU,
214 with BAF-60-M resulting in comparable ability of removing turbidity of other reactors,
215 indicating that fillers had negligible impacts on turbidity removal, as opposed to organic matter
216 removal. Note that even when the turbidity of raw water reached values up to 100 NTU, the BAF
217 reactors reduced turbidity to single digit values (1-3 NTU). The chromas of water samples were
218 measured using a Pt/Co-based method (Chang et al., 2020). The BAF process demonstrated a
219 suitable performance in terms of chroma removal rate and showed a significantly better
220 performance when using GAC as the filler. The highest chroma of raw water reached 200 hazen,
221 while all effluent chroma values were less than or equal to 15 hazen.

222 The influent pH was in the range 7.4-8.0, while the pH of the effluents was always in the
223 range 7.5-8.1 (Figure S4). After treatment in the BAF reactors, the pH of the effluent stream was
224 generally higher than that of influent, with an increase of 0.1-0.4, attributed to the presence of
225 extracellular polymeric substance and small organic matters in the effluent (Ugalde et al., 2013).
226 The TDS of raw water was in the range 130-150 ppm. When treated by BAF reactors, the TDS
227 of effluents was 110-140 ppm, thus decreasing of roughly 10-20 ppm due to adsorption of ions
228 by the microorganisms for growth. Some hazardous ions that were detected during the
229 experiments included arsenic, cadmium, chromium, and lead. The concentrations of these ions
230 and of ammonia in influent and effluent streams of different BAF reactors are reported in Table
231 S2. The removal rates of ammonia in BAF reactors were nearly 100%. The BAF reactors showed
232 a negligible effect on the removal of some ions, such as metal ions, indicating that these ions
233 were not required for microbial growth at significant amounts.



234

235 **Figure 3.** Fluorescence EEM spectra of influent and effluent solutions of BAF reactors: A) Influent; B) BAF-

236 60-AC effluent; C) BAF-60-M effluent; D); BAF-45-AC effluent; E) BAF-30-AC effluent; F) BAF-15-AC

237 effluent; G) Removal rates of different BAF reactors. (Region I: (Ex/Em = 220-250/280-330 nm, tyrosine

238 protein-like substances), region II: (Ex/Em=220-250/330-380 nm, tryptophan protein-like substances), region

239 III: (Ex/Em = 220-250/380-480 nm, fulvic acid-like matters), region IV: (Ex/Em = 250-440/280-380 nm,

240 soluble microbial by-product-like matters), and region V: (Ex/Em = 250-400/380-540 nm, humic acid-like

241 components))

242

243 The difference in dissolved organic matter between raw water and effluents of different BAF

244 reactors was also investigated by fluorescence EEM spectroscopy to identify the removal of

245 different components. The EEM spectra can be divided into five regions using fluorescence

246 regional integration (FRI) (Hu et al., 2021; Tang et al., 2020). As shown in Figure 3A, the
247 dissolved organic matter in raw water was mainly composed of three categories, namely, fulvic
248 acid-like matters (region III), soluble microbial by-product-like matters (region IV), and humic
249 acid-like components (region V). Figure 3B-F present EEM spectra of effluents from various
250 BAF reactors, while Figure 3G illustrated the removal rates of each component. When GAC was
251 used as the filler, the removal rates were roughly the same, regardless of the EBCT, while a large
252 difference was observed for BAF-60-M, in which removal rates were not satisfying. The average
253 removal rates of various components when GAC was used were 78.8% (region I), 91.1% (region
254 II), 95.6% (region III), 85.3% (region IV) and 96.4% (region V). For BAF-60-M, the rates were
255 were -1.26% (region I), 23.6% (region II), 16.2% (region III), 21.1% (region IV), and 27.0%
256 (region V). It is well known that the amino acid-like and humic acid-like components are the
257 main components of aquatic humus substances, hence of natural organic matter in surface water
258 (Nerger et al., 2015; Peng et al., 2022). The BAF reactors containing GAC as fillers
259 demonstrated suitable performance in the removal of two such components, thus showing
260 promise in the purification of surface water like reservoirs and rivers in rural areas.

261 As aforementioned, the BAF-60-M exhibited negligible performance in the removal of
262 organic matter compared with other reactors using GAC as fillers, which is mainly attributed to
263 the different surface roughness of the fillers and the size of the pores on the filler surface.
264 Compared with the shredded hollow fiber membranes, GAC is rougher and rich in micropores,
265 which are more suitable for the attachment and growth of microorganisms (Lu et al., 2022; Lu et
266 al., 2020; Xu et al., 2021). The size of micropores of GAC is larger than that of the ultrafiltration
267 membrane, and the cavities connected to the micropores may have provided a suitable place for
268 the attachment of microorganisms, leading to more biomass growth on GAC. In addition, in the

269 long-term experiments, the microorganisms attached on the GAC would partially degrade the
270 adsorbed organic matters in the micropores, rendering the active sites of GAC that were
271 previously occupied newly available and allowing recovery of the adsorption capacity of GAC
272 fillers (Amini et al., 2018; Smolin et al., 2020). The adsorption capability of the GAC and the
273 biodegradation mediated by the microorganisms attached on the fillers contributed together to
274 the removal of organic pollutants. Moreover, considering the different filling modes, the GAC-
275 filled reactor was tighter and the packed bed more regular than that of the reactor filled with
276 shredded hollow fiber membrane pieces. When the raw water flows in the BAF reactors with
277 aeration, tighter and more regular fillers result in a greater chance of contact between the
278 contaminants in the influent and the fillers, also consistent with the explanation by the van
279 Deemter model used in chromatography (Blanc et al., 2015; Gritti and Guiochon, 2013).

280 The BAF reactor is the core process of the water treatment in the combined system. The
281 average removal rates for DOC, COD_{Cr} and UV₂₅₄ of BAF reactors with GAC as filler were
282 80%, 69% and 94%, respectively. The BAF-45-AC performed best with a DOC removal rate of
283 85.5%. The BAF-60-M showed a low organic matters removal rate. All reactors presented an
284 adequate performance in terms of turbidity and chroma removal.

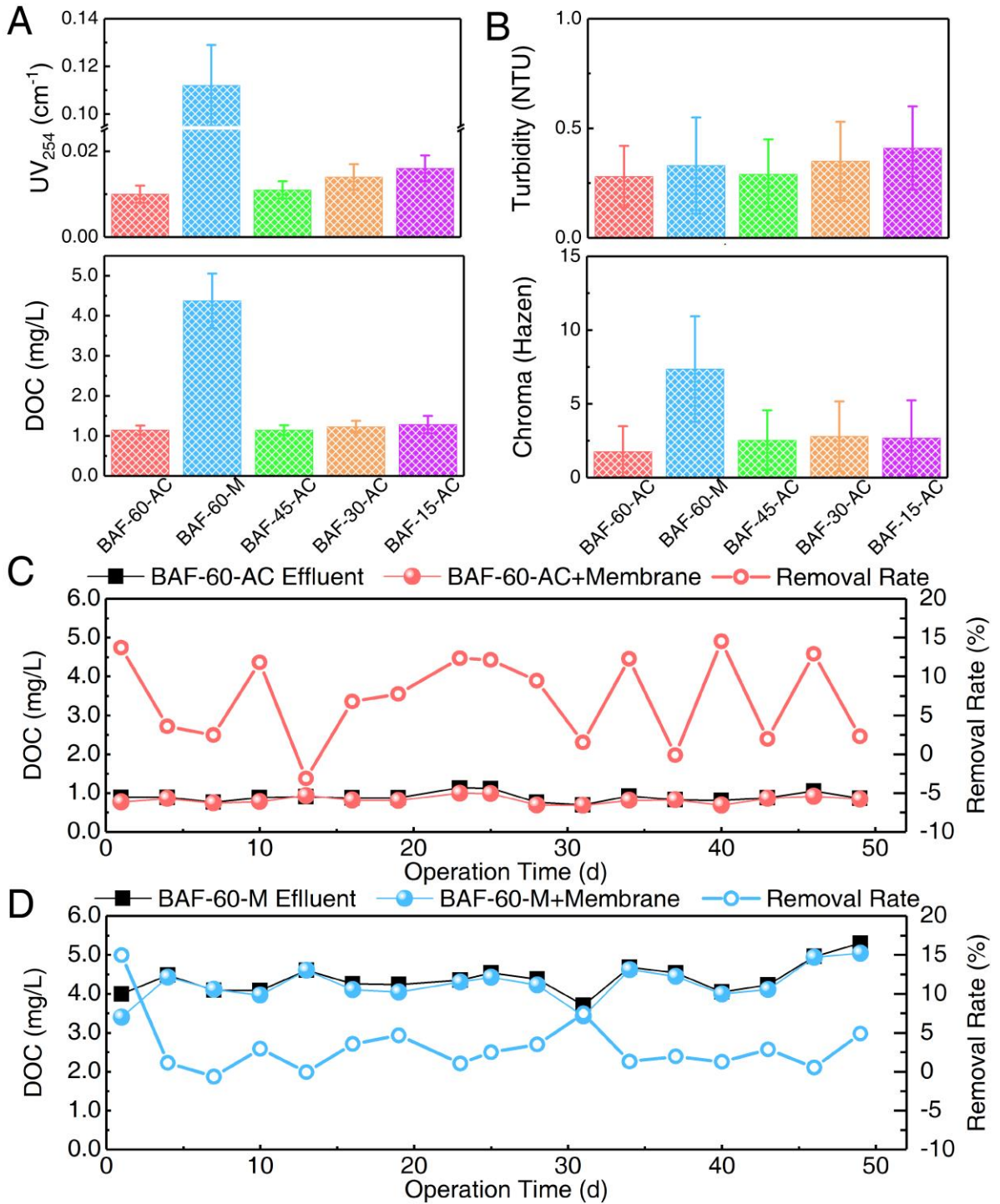
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286 *3.2. Ultrafiltration for BAF Effluents.*

287 The effluents of BAF reactors were pumped to the ultrafiltration membrane modules with
288 the same flow rates as the raw water. The quality of effluents in different combined systems is
289 presented in Figure 4A and B. The removal rates of ultrafiltration membranes for DOC in the last
290 50 days can be seen in Figure 4C and D with the detailed results of other reactors in Figure S5.
291 DOC and UV₂₅₄ were used to evaluate the amounts of organic matter in the effluents. Compared

292 with BAF reactors, the ultrafiltration membrane did not change the UV₂₅₄ or DOC significantly.
293 The average removal rates of DOC for the ultrafiltration stage in different systems were 7.2 ± 5.7%
294 (BAF-60-AC), 3.2 ± 3.7% (BAF-60-M), 6.0 ± 4.7% (BAF-45-AC), 6.6 ± 5.3% (BAF-30-AC)
295 and 7.0 ± 5.8% (BAF-15-AC). The organic matters blocked by the membranes were likely
296 untreated macromolecular organic matter and detached biofilms, demonstrating that organic
297 matters in the raw water could be almost completely removed by the BAF reactors, and that
298 backwashing was able to effectively control the amount of biofilm in the systems. Meanwhile,
299 the ultrafiltration membranes can achieve the near-complete removal of microorganisms
300 (including bacteria and viruses), which has been widely observed by common engineering
301 practice. Therefore, the UF process in the combined system can block pathogens, such as *E. coli*.
302 The turbidity and chroma of BAF effluent were instead further reduced during the ultrafiltration
303 stage, by 0.3 ± 0.12 NTU and 1.4 ± 0.41 hazen, respectively. Therefore, the ultrafiltration step
304 further increased the quality of the effluent as a double barrier treatment and allowed meeting of
305 the standard quality values, indicating the excellent performance of the combined process.

306 The UF process is a second step in the combined dual-barrier system proposed in this study.
307 The average removal rate of the UF process for DOC was 6%. No significant removal was
308 observed for COD_{Cr} or UV₂₅₄. The turbidity and chroma of the effluent were further reduced by
309 0.3 NTU and 1.4 hazen on average. Therefore, the effluents of the combined system met the
310 local drinking water standard based on the parameters measured in this study.



311

312

313

314

Figure 4. Water quality indexes of effluents of the ultrafiltration membrane modules for different BAF reactors during the operation period. A): Average UV₂₅₄ and DOC values of effluents; B): Average turbidity and chroma values of effluents; C): DOC values of influent and effluent solutions of membrane combined

315 with BAF-60-AC in the last 50 days; D): DOC values of influent and effluent solutions of membrane
316 combined with BAF-60-M in the last 50 days.

317

318 *3.3. Microbial Community Structure in the BAF Reactors.*

319 The microbial community in different BAF reactors was analyzed via the 16s-rRNA gene
320 sequencing. The number of effective sequences, operational taxonomic units (OTUs), alpha
321 diversity indexes for microbial communities in different BAF reactors at different times along
322 the experiments are presented in Table 2. Representative SEM pictures of the treated fillers of
323 each BAF reactors are reported in Figure S6. The coverage indexes (Table 2) and the rarefaction
324 curves of raw water and BAF reactors (Figure 5A) suggest that the amount of biological
325 detection of the microorganism was appropriate and that the sequencing results were reliable
326 (Fang et al., 2018; Tang et al., 2021).

327 The community richness index (chao) and the diversity index (shannon) in BAF reactors
328 decreased with increasing EBCT, as well as the average OTU number of different BAF reactors,
329 indicative of more bacterial species in the microbial communities in the reactors characterized by
330 shorter EBCT. As shown in Figure 5B, the principal component analysis (PCA) at the OTUs
331 level of the microbial communities of raw water and different BAF reactors indicates that the
332 variations in the proportion of communities were mainly ascribed to the adaptation of the
333 microbial community to the reactor environment under long-term operation conditions. Large
334 differences can be observed between the microbial community of raw water and those of BAF
335 reactors. Figure 5B also suggests that the microbial community structures varied for different
336 running times and BAF reactors, with the BAF-60-M being the reactor with the largest
337 difference compared to the other systems. The membrane-based filler was not suitable for
338 microbial attachment and growth, and it could not absorb substances when compared with GAC.

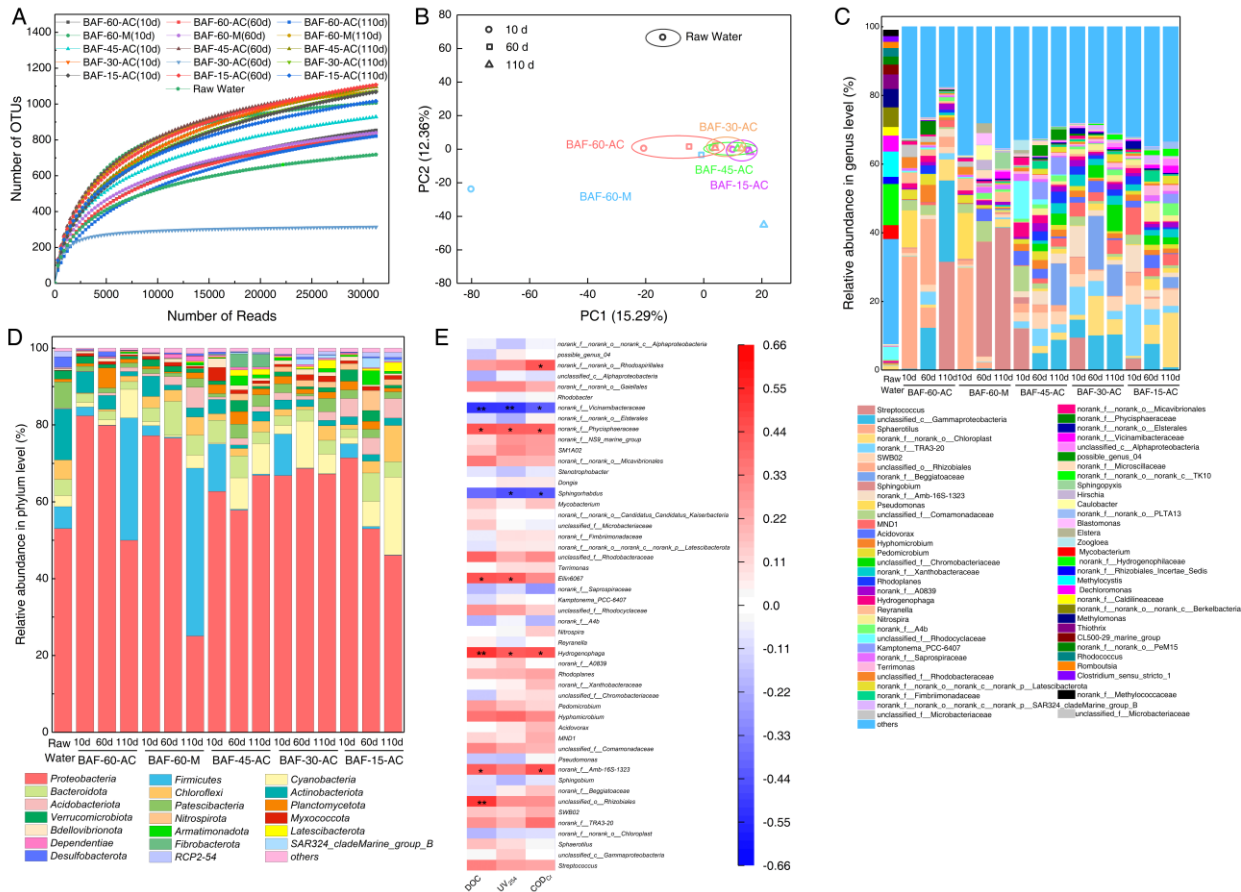
339 Hence, the chance for microorganisms to treat organic matter in water was low. On the other
340 hand, the microbial community structure of different BAF reactors with GAC as filler was
341 similar even at different operating times, as can be observed from Figure 5B, demonstrating the
342 importance of the environment to the microbial community in the reactor under long-term
343 operating conditions.

344 In more detail, the composition of the microbial communities in the raw water and different
345 BAF reactors in terms of phylum level and genus level are shown in Figure 5C and D,
346 respectively. The *Proteobacteria* dominated the phylum during early stages of all BAF reactors
347 and accounted for 72.1% on average; for instance, in the BAF-60-AC, the *Proteobacteria* was
348 82.4 % of the microbial community. Other major phyla in the early stages of the BAF reactors
349 included *Firmicutes* (6.34% for average), *Bacteroidota* (3.27% for average), *Chloroflexi* (1.56%
350 for average) and *Actinobacteriota* (3.80% for average), constituting higher than 82% of the entire
351 community. A similarly large proportion of *Proteobacteria* in the microbial communities were
352 detected in other drinking water treatment processes (Mahajna et al., 2022; Wang et al., 2017).
353 Differences can be observed compared with the microbial community of the raw water. The five
354 phyla mentioned above also contributed to about 81% of the entire community, while the
355 *Proteobacteria* only accounted for 53.0% and *Chloroflexi* accounted for more than 13.3% in that
356 of raw water. As shown in Figure 5C, The *norank_f__Hydrogenophilaceae* (12.00%),
357 *Methylocystis* (7.37%), *norank_f__norank_o__norank_c__Berkelbacteria* (5.76%), and other 12
358 kinds of microorganisms accounted for about 60.74% of the microbial community in raw water,
359 while these microorganisms were not a significant fraction of the microbial communities of BAF
360 reactors. The results suggest that adaptation of the microorganisms has taken place in the BAF
361 reactors and the microbial communities on the BAF fillers remained stable over time. In addition

362 to the difference between the microbial community in raw water and those of BAF reactors, the
363 microbial community of the same BAF reactor also changed. The proportion of *Proteobacteria*
364 (from 72.1% to 51.0%) and *Actinobacteriota* (from 3.80% to 1.89%) decreased over time, while
365 that of *Cyanobacteria* (from 1.29% to 8.81%) and *Chloroflexi* (from 1.56% to 4.44%) increased
366 instead. Meanwhile, the proportion of *Firmicutes* slightly decreased at first and then increased in
367 BAF-60-AC and BAF-60-M reactors (from 2.47% to 0.20% and then 37.8%), while the
368 *Firmicutes* decreased in the other BAF reactors (from 8.91% to 0.56%). The results suggest that
369 the *Proteobacteria* were dominant in all systems: as gram-negative bacteria, *Proteobacteria*
370 contain a variety of metabolic species, most of which are heterotrophic and are facultative or
371 obligate anaerobic. (Ahmad et al., 2022; Guarin and Pagilla, 2021). Specifically, *Alpha-*
372 *Proteobacteria* and *Gamma-Proteobacteria* were the main *Proteobacteria* in the BAF reactors.
373 They are facultative anaerobe, which take organic matter in raw water as the carbon source and
374 carry out respiration and fermentation metabolism at the same time (Tian et al., 2014). Among
375 the 42 genera presented in Figure 5C, 32 belonged to *Proteobacteria*, with
376 *norank_f__norank_o__Rhodospirillales* and *Ellin6067* having significant correlation with the
377 removal of organic matter in the experiments. This result implies that *Proteobacteria* played an
378 important role in organic matter removal. Besides, ammonia oxidizing bacteria also belong to
379 *Proteobacteria* phylum and may play a role in total nitrogen removal based on the ammonia
380 removal shown in Table S5. The second common phylum in the other three reactors was
381 *Cyanobacteria*, the prokaryotic microorganism that can produce oxygen through photosynthesis.
382 Some *Cyanobacteria* are harmful and their metabolites often contaminate water resources (Park
383 et al., 2017). The increase in the proportion of *Firmicutes* and *Cyanobacteria* in some reactors
384 with running time as the temperature and the nutrients in the raw water decreased due to the

385 seasonal change, led to an increase in the proportion of microorganisms with strong viability and
 386 autotrophic character.

387



388
 389 **Figure 5.** Results of the microbial community analysis in different BAF reactors: A) Rarefaction curves of
 390 OTUs in raw water and different BAF reactors at different operation times. B) PCA analysis at OTUs level
 391 of microbial communities in raw water and different BAF reactors at different operation times. C) Bacterial
 392 community compositions at phylum level (> 1%) in raw water and different BAF reactors at different
 393 operation times. D) Correlation analysis between microbial community at genus level (top 50) and
 394 environmental variables (organic matter removal rate). Here, “*” represents a value of $p < 0.05$ and “**”
 395 represents a value of $p < 0.01$. E) Bacterial community compositions at genus level (> 1.5%) in raw water
 396 and different BAF reactors at different operation times.

397

398 **Table 2.** Number of effective sequences, operational taxonomic units (OTUs), alpha diversity indexes for
 399 microbial communities in raw water and different BAF reactors at different times.

Sample ID	Number of effective Sequences	OTU at 97% identity	shannon	chao	coverage
Raw Water	38989	718	4.415047	850.784	0.994849
BAF-60-AC (10d)	48955	854	3.73616	850.784	0.992449
BAF-60-AC (60d)	41025	822	4.142537	1046.569	0.993601
BAF-60-AC (110d)	44233	821	3.225351	966.203	0.991969
BAF-60-M(10d)	40303	1006	4.215275	1046.719	0.996193
BAF-60-M(60d)	62034	840	4.18883	1076.919	0.993697
BAF-60-M(110d)	46179	1071	3.96469	981.956	0.994273
BAF-45-AC (10d)	54286	928	4.761545	1178.642	0.992641
BAF-45-AC (60d)	40519	1096	5.145886	1135.362	0.993057
BAF-45-AC (110d)	50466	1097	4.693617	1251.205	0.990594
BAF-30-AC (10d)	52352	1106	4.677481	1364.522	0.990114
BAF-30-AC (60d)	34857	314	4.140391	1382.663	0.999488
BAF-30-AC (110d)	53385	1072	4.768929	1326.517	0.990018
BAF-15-AC (10d)	56771	1068	4.64183	1393.298	0.990018
BAF-15-AC (60d)	31255	1107	5.113004	1369.342	0.991265
BAF-15-AC (110d)	50703	1016	4.878137	1361.301	0.991585

400 Note: Due to the low biomass, samples of BAF-60-M were tested again for BAF-60-M (10d) and BAF-
 401 60-M (60d).

402
 403 The linear discriminant analysis effect size (LEfSe) method was used to determine the
 404 microorganisms with significant abundance differences among the different BAF reactors (Wu et

405 al., 2018; Zhou et al., 2021). As shown in Figure S7, statistically significant differences appeared
406 among the 18 bacterial clades when the LDA (linear regression analysis) threshold was 3.2.
407 Except for BAF-60-AC, each reactor had its own characteristic microorganisms with
408 corresponding abundance was higher than that in other reactors regardless of the number of
409 bacterial clades. The *o__norank_c_WWE3* bacterial clades and *o_0319-6G20* bacterial clades
410 were enriched in BAF-45-AC, *Vicinamibacteria* bacterial clades and
411 *o_Candidatus_Peregrinibacteria* bacterial clades were enriched in BAF-30-AC and BAF-15-AC,
412 respectively. *Brevundimonas* was enriched in BAF-60-M, which might be the bacterial branches
413 existing in raw water of the reservoir. The higher the LDA score obtained by LDA analysis, the
414 greater the impact of corresponding bacterial clades abundance on the difference of the microbial
415 communities of the BAF reactors (Qiu et al., 2020). Based on the experiment, the difference
416 among bacterial branches in the different BAF reactors may be related to the existence of
417 different biodegradation states at the same sampling depth with different EBCT.

418 In order to further study the activity of microorganisms on the organic matter removal,
419 correlation analysis was conducted between the removal rate of organic matter indexes and
420 microbial community at the genus level (top 50 genus in abundance); see Figure 5E. It was found
421 that *norank_f_Phycisphaeraceae* and *Hydrogenophaga* were correlated with all indicators for
422 organic matter removal. The unclassified_*o_Rhizobiales* and *Hydrogenophaga* ($P < 0.01$),
423 *norank_f_Amb-16S-1323*, *Ellin6067* and *norank_f_Phycisphaeraceae* ($P < 0.05$) were
424 significantly correlated with DOC removal rates. The *norank_f_Amb-16S-1323*, *Ellin6067*
425 genera correlated with COD_{Cr} and organic matters that contain C=C double bonds and benzene
426 ring structure removal, respectively, while *norank_f_norank_o_Rhodospirillales* was only
427 correlated with COD_{Cr} removal. The *norank_f_Vicinamibacteraceae* and *Sphingorhabdus* were

428 negatively correlated with organic removal rate. Among the bacteria with correlation with
429 organic matter removal, only the *norank_f__Phycisphaeraceae* had an average proportion for
430 0.47% in the whole process, indicating that the quantity of the key bacteria is not a key factor for
431 organic removal. Due to the complex relationships between the different bacterial species in the
432 microbial communities, this analysis only presents a probable assessment, which would need
433 further testing and corroboration.

434 In summary, after the completion of the domestication stage, the microbial community in the
435 different BAF reactors changed in response to the variation of raw water, which is corroborated
436 by the results shown in Figure 5B, C and D. After the long-term operation of the systems,
437 *Proteobacteria* was the dominant phylum in all communities. Only the fillers with strong
438 biological attachment ability seem to be able to domesticate a stable microbial community,
439 reliably degrade contaminating substances in raw water, and play a role in water treatment. The
440 shorter the EBCT, the more complex the composition of microbial communities in the BAF
441 reactors. Moreover, several microorganisms were found to be strongly associated with the
442 removal of certain pollutants in the raw water, including association of
443 *norank_f__Phycisphaeraceae* and *Hydrogenophaga* with organic matters removal, as well as
444 *norank_f__Amb-16S-1323*, *Ellin6067* with the removal of COD_{Cr} and organics containing C=C
445 double bonds and benzene ring structures. These microorganisms may have a great potential for
446 treating the corresponding type of water.

447

448 **4. Conclusion**

449 In this work, a combined system consisting of a BAF reactor and an ultrafiltration
450 membrane was used to treat polluted surface water in a rural area, and the practical potential of

451 the combined technology was evaluated by investigating the variation of organic matter,
452 turbidity, chroma, and other indicators of water qualities in long-term operation. The BAF step
453 removed organic matter, chroma, turbidity and ammonia effectively. In the ultrafiltration
454 membrane stage for treatment of the effluents of the BAF reactors, the ultrafiltration membrane
455 would efficiently block the microorganisms in the effluents. The turbidity and chroma of the
456 water were further reduced by the membrane filtration, but not organic matter at a significant rate.

457 The combined technology showed suitable performance for some rural water treatment, and
458 possesses potential advantages compared to other techniques, such as convenient operation and
459 maintenance, certain practicality, and economy. However, it is still unclear whether the process
460 can adapt to different water sources in different rural areas. It is worth noting that the
461 experiments were conducted at laboratory scale, and that the full-scale applicability of this
462 approach in rural areas needs to be evaluated in further experiments conducted at a larger scale.

463

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471

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