

Efficiency and Mechanisms of Biochar Aerogel-Assisted Biodegradation of Taste and Odor Compounds in a One-Step Membrane Bioreactor for Rural Drinking Water Production

*Original*

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4 Efficiency and mechanisms of biochar  
5 aerogels-assisted biodegradation of taste &  
6 odor compounds in a one-step membrane  
7 bioreactor for rural drinking water production

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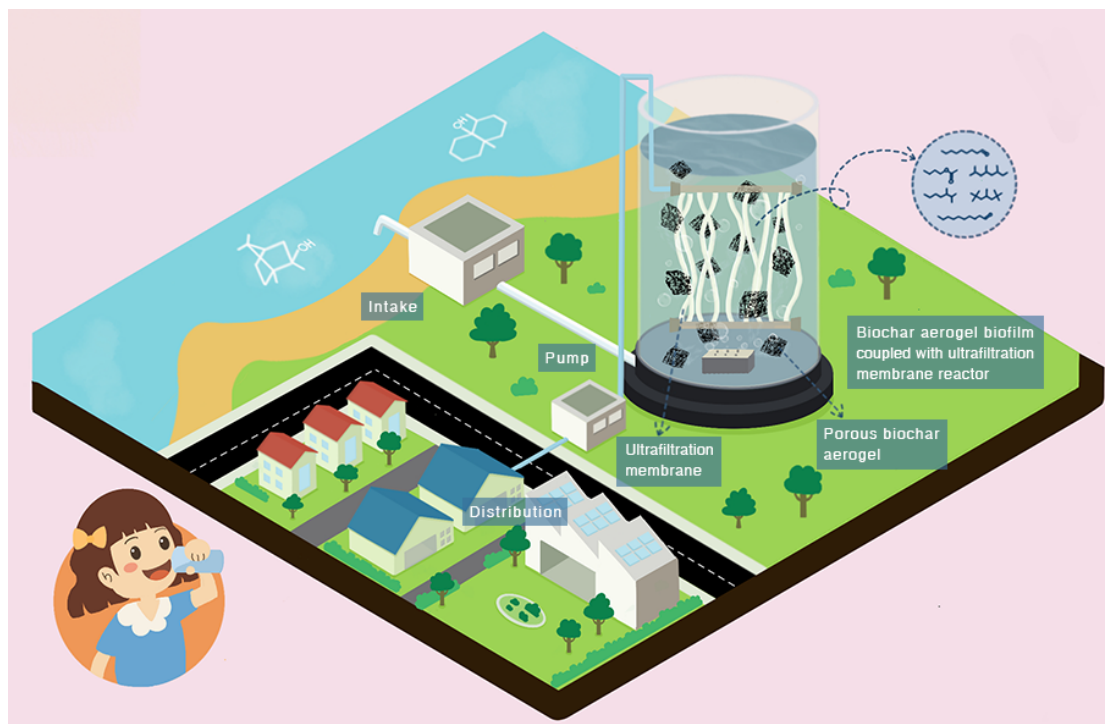
17

18 **Abstract:** In many rural areas, lakes and reservoirs represent common sources for  
19 drinking water, but these water bodies are more likely affected by taste & odor problems  
20 that cause discomfort to consumers. Taste & odor compounds, especially 2-  
21 methylisobornol (2-MIB) and geosmin (GSM), are not easily removed using  
22 conventional water treatment processes, and this problem is exacerbated in rural areas  
23 where treatment systems are often sparser, modest, and dated compared to typical urban  
24 water sources. Herein, a combined process deploying biochar aerogels-supported  
25 biofilms and performed in an ultrafiltration reactor (BAB-UF) was evaluated and  
26 investigated to treat rural water polluted with 2-MIB and GSM. During a 40-day  
27 experiment, the system performance was analyzed at different values of the empty bed  
28 contact time, while the microbial communities in different BAB-UF reactors were  
29 examined extensively. The process proved to be effective in removing 2-MIB and  
30 GSM, predominantly through biodegradation. Specifically, using biochar aerogels as  
31 suspended fillers in the reactor and an EBCT of roughly 1 h, the removal rate of 2-  
32 MIB/GSM was higher than 95%, and the effluent satisfied the requirements for  
33 domestic drinking water. Microorganisms with specific functions were enriched in  
34 different BAB-UF reactors and governed the transformation process, highlighting the  
35 importance of system tuning for achieving the desired biological function, hence  
36 product water quality.

37 **Keywords:** Biochar aerogel; Geosmin (GSM); 2-methylisobornol (2-MIB); Microbial  
38 community; Rural drinking water treatment.

39 **Synopsis:** This study proposes and analyzes a combined process of biotransformation  
40 and selective filtration to produce safe drinking water from rural reservoir water  
41 polluted with taste & odor compounds.

42 **TOC/Abstract Art:**



43

44

## 45 1. Introduction

46 Drinking water quality in rural areas is a global concern. Currently, approximately 2.2  
47 billion people, particularly those living in remote rural areas, e.g., mountainous,  
48 pastoral, and forest areas, do not have access to safely managed or high-quality  
49 drinking water.<sup>1,2</sup> For example, according to a national Chinese survey, about 80% of  
50 water bodies in China have taste & odor issues, particularly, lakes and reservoirs that  
51 serve as main water sources in rural areas.<sup>3</sup> Human life depends on access to clean,  
52 safe drinking water, and consumers instinctively avoid water that tastes or smells  
53 unpleasant because this characteristic may be an indicator of a harmful supply.<sup>4-6</sup> The  
54 terpenes, *2-methylisobornyl* (2-MIB, C<sub>11</sub>H<sub>20</sub>O) and geosmin (GSM, *trans-1,10-*  
55 *dimethyl-trans-9 decanol*-C<sub>12</sub>H<sub>22</sub>O), originate from the metabolism and  
56 biodegradation activity of certain types of cyanobacteria<sup>7,8</sup> and they have been  
57 identified in water supplies as key odorants of the musty/earthy group.<sup>9</sup> The  
58 concentrations of 2-MIB and GSM in water can be perceived by the human nose at  
59 concentrations as low as 6 ng/L<sup>4</sup> and 4 ng/L<sup>10</sup>, respectively.

60 Studies have shown that 2-MIB/GSM are not removed to concentration values  
61 below their odor threshold concentrations by conventional drinking water treatment  
62 processes consisting of coagulation-flocculation, sedimentation, and filtration.<sup>8, 11, 12</sup>  
63 Treatment methods that have been successfully employed by centralized water  
64 treatment plants to remove 2-MIB/GSM include granular activated carbon (GAC)  
65 adsorption and advanced oxidation processes (AOP)<sup>13</sup>. Several researchers have

66 demonstrated the use of oxidants, such as ozone and hydrogen peroxide, and the use of  
67 ultraviolet light (UV) for the removal of 2-MIB/GSM<sup>14-16</sup>. However, these methods  
68 have limitations for rural water treatment. For instance, activated carbon has a limited  
69 adsorption capacity and needs to be replaced frequently for capacity regeneration. In  
70 addition, adsorption is strongly influenced by the presence of natural organic material  
71 (NOM) in natural water.<sup>17</sup> NOM is generally present in much higher concentrations  
72 (mg/L) compared to T&O compounds (ng/L). The various fractions of NOM occupy  
73 some adsorption sites and cause inhibition of adsorption of target odor substances. Also,  
74 large NOM molecules may block the diffusion pathway of odorants and thwart  
75 transport toward the adsorption sites<sup>13</sup>. As a result, high doses of activated carbon are  
76 required for successful removal of the T&O compounds. Most rural drinking water  
77 supply systems have lower capacity than urban systems, are managed by operators with  
78 typically less professional experience compared to those operating in urban areas,<sup>1</sup> and  
79 the addition of costly chemicals or complicated operating procedures associated with  
80 adsorption and advanced oxidation processes would require sustained management and  
81 maintenance that are not compatible with such rural systems. A simpler approach,  
82 characterized by less complexity and requiring minimum skilled oversight, would  
83 benefit water supply in rural areas.

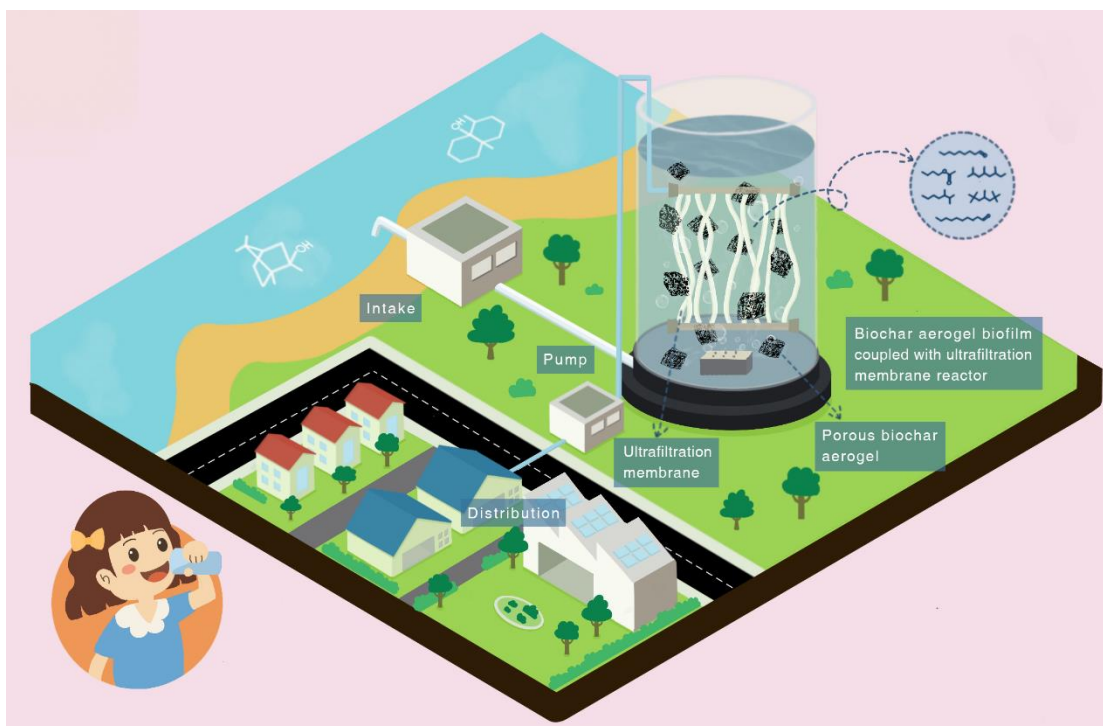
84 Biological processes play an important role in the future of drinking water  
85 treatment, especially for developing countries, due to their environmental friendliness.  
86 Biological drinking water treatment relies on the activity of non-pathogenic bacteria for

87 biochemical degradation, and to the ability of these microbes to produce a biologically  
88 stable water that prevents the growth of other pathogenic microorganisms in the water  
89 distribution system.<sup>18</sup> These processes remove pollutants with minimal technological  
90 complexity, require the addition of little or no chemicals, and limit the creation of by-  
91 products that may be toxic or harmful for humans or the environment. Common  
92 biological technologies include slow and rapid sand biofiltration (SSF/RSF), biological  
93 activated carbon (BAC), and biological aerated filter (BAF).<sup>7, 19</sup> For example, BAC  
94 consists of microbes attaching and growing on the GAC surface to form a biofilm,  
95 which removes contaminants as water filters through the media, thus exploiting  
96 adsorption and biodegradation simultaneously, and extending the GAC service life.<sup>20,</sup>  
97 <sup>21</sup> Biosand and BAC depth filtration, as well as their aerated counterpart (BAF), are  
98 effective methods for removing low molecular weight organic compounds and are  
99 becoming widespread processes for advanced water treatment in large urban water  
100 supply systems also in developed countries (such as Japan, the Netherlands, the United  
101 States).<sup>22, 23</sup>

102 A possible biological treatment alternative consists of a combined process  
103 whereby biological activated carbon is used within a membrane bioreactor. In such a  
104 configuration, the biologically-active media are suspended as fillers in the aerated raw  
105 water and the product water is extracted through membrane filtration. It has been  
106 suggested that such system would improve the quality of treated water and also help  
107 alleviating membrane fouling.<sup>24, 25</sup> Since membrane filtration can intercept most of the

108 suspended solids, microorganisms, and macromolecules in water,<sup>26, 27</sup> and since it can  
109 be applied in rugged mountain areas to meet drinking water treatment needs of remote  
110 areas,<sup>28</sup> the hypothesis of this study is that the combination of biological treatment and  
111 ultrafiltration should provide a **simple and easy-to-operate** way to solve odor problems  
112 associated with 2-MIB and GSM in rural drinking water systems. To date, little or no  
113 research has been conducted on these processes, resulting in a substantial lack of  
114 knowledge related both to the engineering and to the underlying mechanisms  
115 characterizing the related systems.

116 This study investigates the use of activated carbon-assisted biological degradation  
117 combined with an ultrafiltration bioreactor to address T&O issues in rural drinking  
118 water. To reduce the adsorption of NOM on the support carbon material, biochar  
119 aerogels were used as suspended biological fillers to form an aerogel-supported biofilm  
120 (BAB). **A schematic diagram of the combined one-step process (BAB-UF reactor) is**  
121 **shown in Figure 1.** Specifically, this study examines: (1) the treatment performance of  
122 rural reservoir water containing 2-MIB and GSM via four different BAB-UF reactors;  
123 (2) the biodegradation rates of 2-MIB and GSM; (3) the possible pathways of  
124 biodegradation of 2-MIB and GSM; (4) the efficiency and potential of the system for  
125 drinking water treatment; (5) the microbial communities developing in the reactor and  
126 their role on treatment performance.



127

128 **Figure 1.** The schematic diagram of the combined one-step process (BAB-UF reactor)

129

## 130 2. Material and Methods

### 131 2.1. Materials.

132 Water from a reservoir in Zhong county (Chongqing, China) was used as  
 133 experimental water. Wahaha brand bottled water was used for the establishment of 2-  
 134 MIB/GSM standard curves and for blank control experiments. A standard mixture of 2-  
 135 MIB (trace CERT<sup>®</sup>) and GSM (trace CERT<sup>®</sup>) was purchased from Sigma-Aldrich <sup>13</sup>.  
 136 Sodium chloride (NaCl, 98% purity), potassium hydroxide (KOH, 90%), acetic acid  
 137 (CH<sub>3</sub>COOH, 99.5% purity), and sodium azide (NaN<sub>3</sub>, 99%) were provided by Agilent  
 138 Technologies (China) Co., Ltd. Chitosan was purchased from Jinan Haidebe Marine  
 139 Biological Engineering Co., Ltd. (China), and glucose was purchased from Chengdu  
 140 Kelong Chemical Reagent Factory (China). The ultrafiltration hollow fiber membranes

141 used in this study were provided by Litree Purifying Technology Co., Ltd (Hainan,  
142 China). Peristaltic pumps (BT-300-2 J, Longer Pump, China) were used to feed water  
143 from the tanks to the BAB-UF reactors, as well as to extract effluents from the  
144 ultrafiltration hollow fiber membrane modules.

## 145 **2.2. Methods**

### 146 **2.2.1. BAB-UF reactors set-up.**

147 The shallow sediment of the reservoir was collected for microbial enrichment, and  
148 a 28-day acclimation process was required to allow microbial aggregation and biofilm  
149 formation on the suspended fillers. **Specific details regarding the acclimation process**  
150 **and associated data can be found in the Supporting Information (SI, Text S1 and Figure**  
151 **S1-2).** Following the 28-day acclimation period, degradation experiments were initiated.  
152 **Actual water from the reservoir mixed with 2-MIB and GSM at a concentration of 100**  
153 **ng/L was used as the raw water (RW), which was supplied to all reactors simultaneously.**  
154 Because odor problems are seasonal<sup>29</sup>, with outbreaks typically lasting about 1 to 2  
155 months during the warm weather season, the study was conducted in the laboratory for  
156 40 days. Basic information of the different BAB-UF reactors is shown in **Table 1.**<sup>4</sup>  
157 Concentrations of 2-MIB and GSM were measured every three days for 33 days in the  
158 influent and effluent streams from the BAB-UF reactors. After the systems achieved  
159 stability, the concentration of odorant was gradually increased from 100 ng/L to 200  
160 ng/L.

161 **Table 1.** Operating parameters of the BAB-UF reactors in this work.

Operators ID	Suspended Fillers	Flow rate (ml/min)	EBCT (min)	Gas-water ratio	Running Time (d)
UF	-	0.524	-	3:1	40
BAB-20-UF	BA	0.524	18	3:1	40
BAB-40-UF	BA	0.524	36	3:1	40
BAB-60-UF	BA	0.524	54	3:1	40

162 Note: The running time of the reactors only includes the degradation experiments period.

163

### 164 2.2.2. Experimental setup comprising biodegradation, adsorption and aeration in

#### 165 BAB-UF

166 The following three control experiments were conducted on the 40th day of the  
167 experiment when the influent 2-MIB/GSM concentration was 200 ng/L. In one control  
168 experiment, only aeration was applied while the fillers and ultrafiltration membranes  
169 were not present, to understand the rate of volatilization. In a second control experiment,  
170 Wahaha purified water containing 300 mg/L sodium azide was used to soak the filler in  
171 the BAB-UF device for 2 h, which inhibited the biological activity of the biofilm  
172 without affecting other functions (e.g., adsorption).<sup>20</sup> A third control test was conducted  
173 in which the membrane reactors operated simultaneously under the same conditions but  
174 without aeration and without the presence of the ultrafiltration membrane. The four full  
175 BAB-UF reactors were instead run at different empty bed contact time (EBCT) of 18,  
176 36, 54 min and contained 20%, 40%, and 60% fillers, respectively. In all cases, we  
177 measured the influent 2-MIB/GSM concentrations and the effluent concentrations three

178 times, and for each setup we calculated the average effluent concentration as a  
179 percentage of the influent concentration to obtain the removal rate of 2-MIB and GSM.  
180 The biodegradation contribution in the full BAB-UF reactors was obtained by  
181 subtracting the aeration and adsorption rates from the total BAB-UF removal rate.

182

### 183 **2.2.3. Preparation of biochar aerogel fillers for biofilm support**

184 The biochar adsorbent powder was prepared using the sol-gel method;<sup>30-32</sup> refer to  
185 the SI (**Text S2**) for a detailed description of the method. The method of BAB aerogel  
186 production was adapted from previous research<sup>33</sup> and comprised with the following  
187 steps: chitosan powder with a mass concentration of 4.0 wt% was dissolved in 1.0 wt%  
188 aqueous glacial acetic acid solution by stirring, resulting in a yellow transparent  
189 homogeneous solution. The previously prepared 0.1 wt% biochar adsorbent powder was  
190 added to the chitosan solution and stirred for 30 min to ensure even dispersion. The  
191 mixture was then injected quickly **with a syringe into a cylindrical mold about 7 mm**  
192 **height and about 5 mm in diameter**, and then rapidly frozen at  $-20^{\circ}\text{C}$  before being  
193 freeze-dried for 24 h. The resulting dried granular aerogel was removed from the mold  
194 and placed in a 4 wt% aqueous NaOH solution, where it was shaken for 2 h at room  
195 temperature to fully react and neutralize the acid in the aerogel. Finally, the pellet  
196 aerogels were washed with pure water until neutral pH to obtain the final BAB supports.

## 197 **2.3. Analytical methods**

### 198 **2.3.1. 2-MIB and GSM analysis**

199 The concentrations of 2-MIB and GSM were analyzed using a gas  
200 chromatography-mass spectrometry (GC-MS) system, which was coupled with a solid  
201 phase micro-extraction (SPME) fiber holder and SPME fiber assembly-  
202 divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). An Agilent 8890  
203 Series gas chromatograph equipped with an HP-5 MS capillary column (30 m × 0.25  
204 mm × 0.25 mm film thickness) interfaced to an Agilent 7010B mass selective detector  
205 was used. To quantify the 2-MIB/GSM, 35 mL of water sample and 3.5 g of NaCl baked  
206 at 300 °C in a muffle for 2 h were mixed evenly into a 40 mL brown headspace bottle.  
207 The bottle was placed into a 65 °C water bath for preheating, and extraction was carried  
208 out for 15 min. The extraction head was then inserted into a GC injection port for 5 min  
209 for quantitative analysis of 2-MIB/GSM. The carrier gas was high purity He (99.999%,  
210 Rising Corp., China) at a column flow rate of 1 mL/min. The injection port operated in  
211 a separation mode with split ratio of 20:1 while the temperature maintained at 250 °C.  
212 The column oven initial temperature was set at 60 °C for 2.5 min, increased to 250 °C  
213 at a rate of 10 °C/min, and then maintained for 5 min. The MS ion source temperature,  
214 the four-stage rod temperature, and the auxiliary heating zone temperature were  
215 maintained at 230 °C, 150 °C, and 280 °C respectively. The solvent delay was set to 7.5  
216 min. The MS ionization energy was set to 70 eV, and the scanning mode was set at  
217 multiple reaction monitoring (MRM). The quantitative ion pair parameters are 95/67

218 for 2-MIB,112/97 for GSM. Standard chromatograms and 1-200 ng/L 2-MIB/GSM  
219 standard curves are presented in the SI (**Figure S4**).

220

### 221 **2.3.2. Effluent quality analysis**

222 A portable multifunctional Ultrameter II 6PFC (Myron L Company, Carlsbad,  
223 USA) was utilized to determine the total dissolved solids (TDS). COD<sub>Mn</sub> was  
224 determined based on the permanganate index (GB 11892-89). DOC and UV<sub>254</sub> values  
225 were obtained, respectively, with a TOC analyzer (TOC-L CPH, Shimadzu, Japan) and  
226 with a UV-vis spectrophotometer (Orion AquaMate 8000, Thermo Fisher, USA). The  
227 pH was measured using a pH meter (PB-10, Sartorius Scientific Instruments Co, Ltd.,  
228 Gottingen, Germany), while turbidity was measured using a turbidimeter (2100Q, Hach  
229 Company, Loveland, USA). A three-dimensional excitation-emission matrix (3D EEM)  
230 fluorescence spectrum (F-7000, Hitachi, Japan) was used to characterize the DOM  
231 components in the effluent and on the membrane contaminated layers. For detailed  
232 information on the related methods, please refer to our previous studies.<sup>34,35</sup>

### 233 **2.3.3. 16S-rRNA analysis**

234 The microbial diversity of the biofilm formed on the suspended fillers in the  
235 continuous mode BAB-UF reactor at different times was sequenced using 16S-rRNA  
236 to analyze changes in microbial communities and dominant functional microorganisms  
237 over time. In the four BAB-UF reactors, a total of six BAB filler samples and two  
238 sections of ultrafiltration membrane samples were collected on days 10 and 40. For

239 each BAB filler sample, 2 g of packing material from approximately the middle depth  
240 of the reactor was taken and placed into a sterilized 10 mL polyethylene bottle. The  
241 sample was then transported to the analytical laboratory on ice packs, while an equal  
242 volume of packing was backfilled immediately after removal. The detailed operation  
243 method of 16S-rRNA can be found in the SI (**Text S4**).

#### 244 **2.3.4. Membrane fouling analysis**

245 The membrane fouling layer morphology was examined with scanning electron  
246 microscopy (SEM, Regulus 8230, Hitachi, Japan) on membrane samples after their use  
247 in the reactor. Before SEM analysis, the dried sample surfaces were coated with Au by  
248 a model MS-2S gold coating instrument (IXRF Systems Inc., USA), reaching a  
249 thickness of approximately 2 nm.

250

### 251 **3. Results and Discussion**

#### 252 **3.1. Biodegradation is the dominant removal mechanism of 2-MIB and GSM**

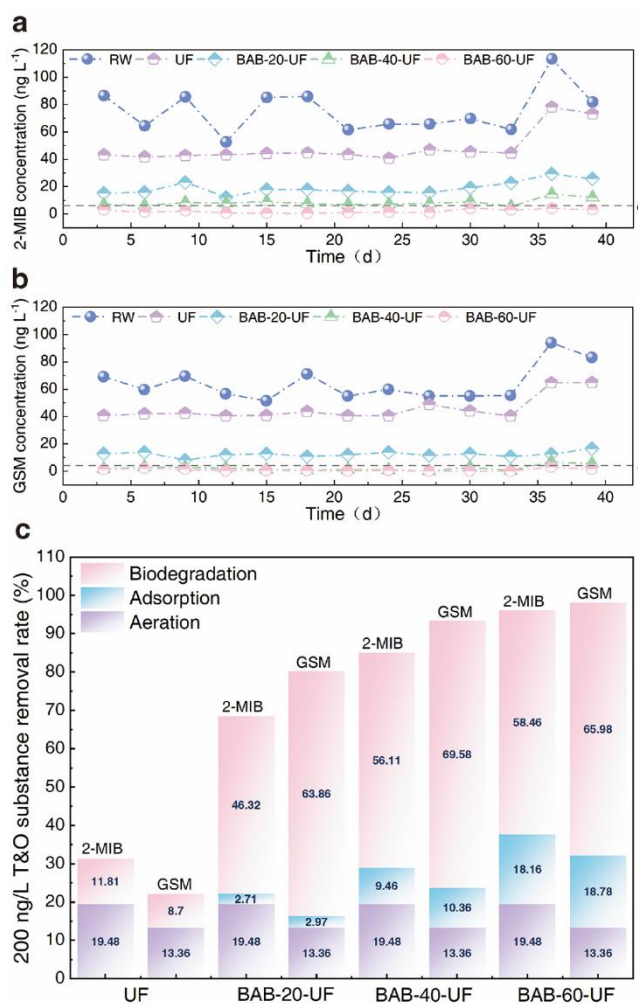
253 In this study, a fraction of 2-MIB/GSM was lost due to natural volatilization since  
254 the experimental device could not be kept sealed (due to continuous aeration, the  
255 pressure in the reactor would increase if it was completely sealed, resulting in increased  
256 water flow resistance and reduced water flow velocity). The results of the control  
257 experiments suggested that the fractions of volatilized 2-MIB and GSM were 32.4%  
258 and 42.3%, respectively, at a starting concentration of 100 ng/L, while 56.25% and

259 61.01%, were lost at a starting concentration of 200 ng/L. These losses were accounted  
260 for when computing raw water concentrations and in the following calculations.

261 By observing the influent and effluent concentrations 2-MIB/GSM from the four  
262 BAB-UF reactors throughout 40 day of testing, it is clear that the removal efficiency  
263 increased with EBCT; see **Fig. 2a, b**. For example, the effluent 2-MIB/GSM  
264 concentrations were 6 and 4 ng/L, respectively, from BAB-40-UF and BAB-60-UF,  
265 hence lower than the human nose perception threshold and attaining values that respect  
266 the local standards for drinking water quality (GB 5749-2022). Note that the removal  
267 rate obtained in the control experiments comprising the UF membrane only was around  
268 or lower than 30%, due to the small molecular size of the two T&O compounds that  
269 cannot be excluded from entering and moving across the membrane pores. **Such**  
270 **removal rates recorded in the control experiment are attributed to the presence and**  
271 **activity of bacteria on the ultrafiltration membrane. These bacteria were remnants from**  
272 **the initial acclimation phase conducted prior to the commencement of the control**  
273 **experiment.**

274 The contribution of aeration, adsorption, biodegradation to the overall 2-  
275 MIB/GSM removal rate is presented in **Fig. 2c**. Aeration was responsible for 19.5%  
276 and 13.4% of the total removal of the two substances. The adsorption removal rates  
277 were instead 2.7%, 9.5%, and 18.2% for 2-MIB and, quite similarly, 3.0%, 10.4%, and  
278 18.8% for GSM, from the three reactors characterized by EBCT 18, 36, 54,  
279 respectively. Indeed, the contribution of biodegradation was dominant and estimated as

280 follows: UF only (11.8%, 8.7%), BAB-20-UF (46.3%, 63.9%), BAB-40-UF (56.1%,  
 281 69.6%), BAB-60-UF (58.5%, 66.0%). The findings indicate that the process of  
 282 adsorption did not provide substantial removal, possibly due to the chemical nature and  
 283 to the low concentrations of 2-MIB and GSM. On the other hand, biodegradation  
 284 contributed to around 60% of the overall removal rate, which aligns with the  
 285 conclusions drawn by earlier research<sup>7</sup>, and represented the dominant removal  
 286 mechanism in the examined system.



287  
 288 **Figure 2.** Removal rates of T&O compounds: (a) 2-MIB concentrations and  
 289 corresponding removal rates in different reactors: the grey line indicates the 2-MIB

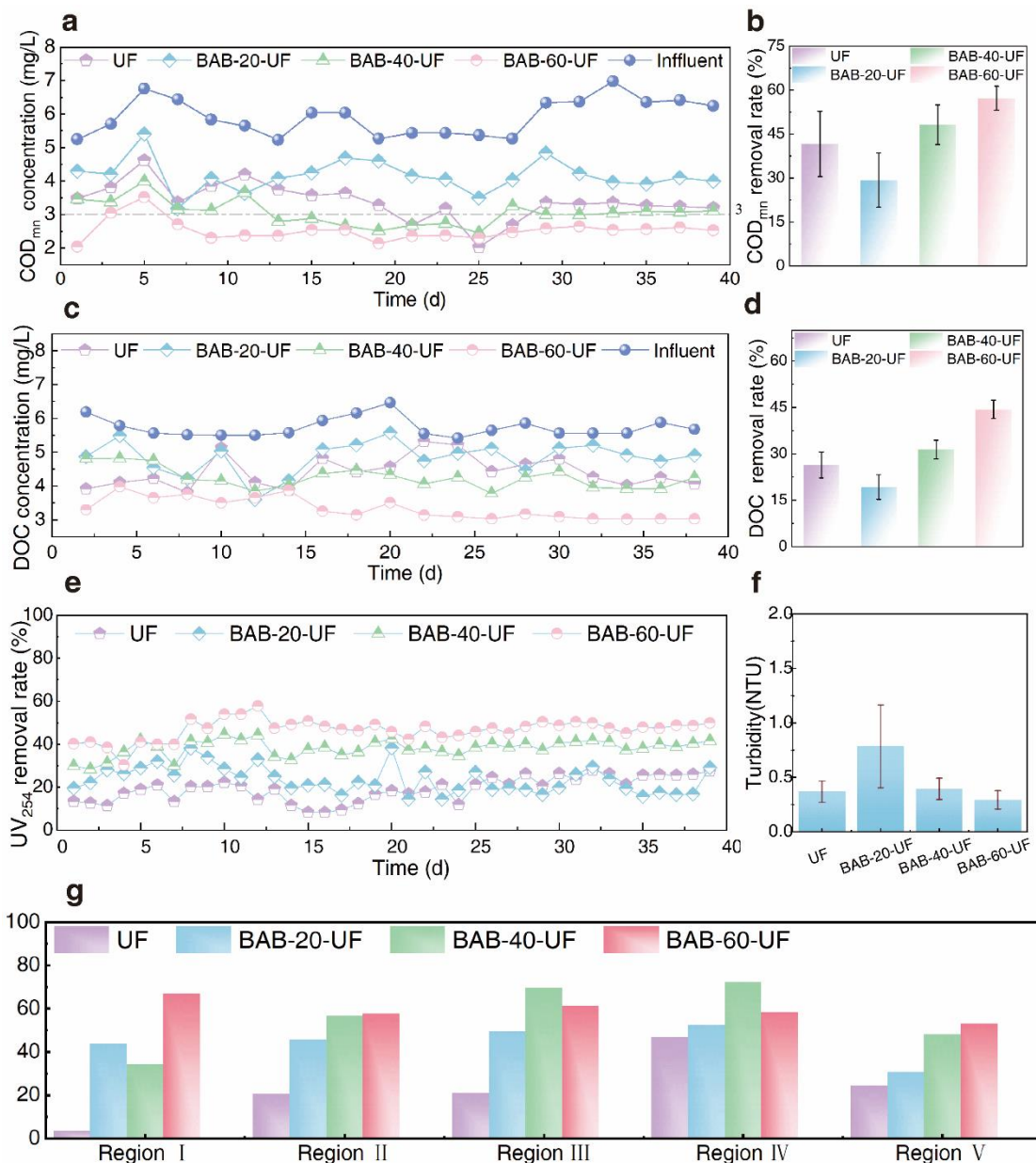
290 odor threshold in water at 6 ng/L; (b) GSM concentrations and corresponding removal  
291 rates in different reactors: the grey line indicates the GSM odor threshold in water at 4  
292 ng/L; (c) contributions to removal rates from different mechanisms. RW refers to the  
293 reservoir water containing 2-MIB/GSM.

294

### 295 3.2. The effluent from the BAB-UF systems met local drinking water standards

296 The water quality parameters of influent and effluent streams from different BAB-  
297 UF reactors are displayed in **Fig. 3**. The  $\text{COD}_{\text{Mn}}$  of effluents from BAB-60-UF and  
298 BAB-40-UF were below 3 mg/L, thus meeting local drinking water standards  
299 (GB5749-2022); see **Fig. 3a, b**. Specifically, the  $\text{COD}_{\text{Mn}}$  removal efficiency was higher  
300 than that of the UF device alone, suggesting that BAB adsorbed and/or biodegraded  
301 part of organic compounds. Considering DOC, the average removal rate increased by  
302 roughly 12% with each doubling of EBCT, achieving approximately 44% in BAB-60-  
303 UF (**Fig. 3c, d**). Other than removal rate itself, the stability of removal was apparently  
304 higher for reactors characterized by longer EBCT. A similar trend was also observed  
305 for the  $\text{UV}_{254}$  parameter, which can be used to quantify the amount of dissolved  
306 organic pollutants with unsaturated bonds in the raw water. As shown in **Fig. 3e**, the  
307 removal of  $\text{UV}_{254}$  was between approximately 19.0 (BAB-20-UF) and 46% (BAB-60-  
308 UF). The fact that the removal rate of  $\text{UV}_{254}$  was higher than that of DOC indicates that  
309 the BAB-UF reactor more efficiently degraded unsaturated organic and aromatic  
310 compounds.

311 Turbidity and TDS were used as additional indicators related to the quality of  
 312 drinking water. As shown in **Fig. 3f** and consistent with the removal behavior of  
 313 organics, the removal rates generally increased with EBCT and the average turbidity of  
 314 the effluent of BAB-UF reactors was lower than 0.5 NTU, indicating higher than 90%  
 315 removal rate of turbidity in all cases. Finally, the TDS did not change considerably,  
 316 converting from 120-140 ppm in the RW to 100-130 ppm in the treated effluents, the  
 317 minimal removal likely due to uptake by microorganisms for growth.



318

319 **Figure 3.** Water quality parameters of influent and effluent streams from different  
320 BAB-UF reactors during 40-days. (a)  $\text{COD}_{\text{Mn}}$  concentrations; (b) corresponding  
321 average removal rates of  $\text{COD}_{\text{Mn}}$ : here, the grey dotted line marks the local drinking  
322 water standard limits for  $\text{COD}_{\text{Mn}}$  in the GB5749-2022 legislation; (c) DOC  
323 concentrations; (d) corresponding average removal rates of DOC; (e)  $\text{UV}_{254}$  removal  
324 rates; (f) average turbidity concentrations in the effluents. (g) Removal rates of  
325 Fluorescence EEM spectra computed for different BAB-UF reactors.

326

327 Beside overall removal rates, the changes in the composition of DOC were  
328 examined with fluorescence EEM spectroscopy. The EEM spectrum may be classified  
329 into five regions: Region I : ( $\text{Ex/Em} = 220\text{-}250/280\text{-}330$  nm, tyrosine protein-like  
330 substances), region II : ( $\text{Ex/Em}=220\text{-}250/330\text{-}380$  nm, tryptophan protein-like  
331 substances), region III: ( $\text{Ex/Em} = 220\text{-}250/380\text{-}480$  nm, fulvic acid-like matters),  
332 region IV: ( $\text{Ex/Em} = 250\text{-}440/280\text{-}380$  nm, soluble microbial by-product-like matters),  
333 and region V: ( $\text{Ex/Em} = 250\text{-}400/380\text{-}540$  nm, humic acid-like components).<sup>34, 36</sup> As  
334 shown in **Fig. S5e**, fulvic acid-like matters (region III), soluble microbial by-product-  
335 like matters (region IV), and humic acid-like components (region V) were the main  
336 organic constituents in the raw water, far more than tyrosine protein-like substances  
337 (regions I and II). This observation is attributed to the fact that, similar to previous  
338 studies, fulvic acid-like matters (region III) and humic acid-like components (region V)  
339 are the major components of NOM in surface waters.<sup>37</sup> The removal efficiency of BAB-

340 UF for regions III and IV were the highest and most consistent for the different systems,  
341 with removal rates higher than 50% observed for the two higher EBCT values for  
342 regions II, III, IV, and V, consistent with the removal efficiency of COD<sub>Mn</sub> and UV<sub>254</sub>;  
343 see **Fig. 3g**. This result implies that the BAB-UF reactors are especially apt to the  
344 purification of hydrophilic low and medium molecular weight organic components,  
345 which actually represent the largest fraction of organics in rural surface water sources,  
346 such as lakes and reservoirs.

347

### 348 **3.3. Adaptation of the microbial community in BAB-UF systems**

349 All analyzed samples had coverage values greater than 0.99 (see **Text S4.2**) ,  
350 indicating that the sequencing depth was sufficient to cover most microorganisms, even  
351 some rare species.<sup>35, 38</sup> The flattening of the rarefaction curve in **Fig. 4a** also indicates  
352 sufficient sequencing depth. The combined result imply that the community richness  
353 and diversity of the 40th day samples was higher than that of the 10th day samples.

354 **Fig. 4b** and **c** show the microbial community composition at the genus level  
355 (relative abundance > 0.05% in all samples) and at the phylum level (relative abundance  
356 > 0.1% in all samples), respectively. Similar to previous studies using BAF or other  
357 methods for drinking water treatment,<sup>28,39</sup> *Proteobacteria* were the most abundant and  
358 broad in all samples (in this study, 40%-88% of the samples on the 10th day, and 51%-  
359 93% of the samples on the 40th day). Another dominant phylum was *Bacteroidetes*,  
360 which are well-known degraders of organic matter.<sup>40</sup> The above two phyla, together

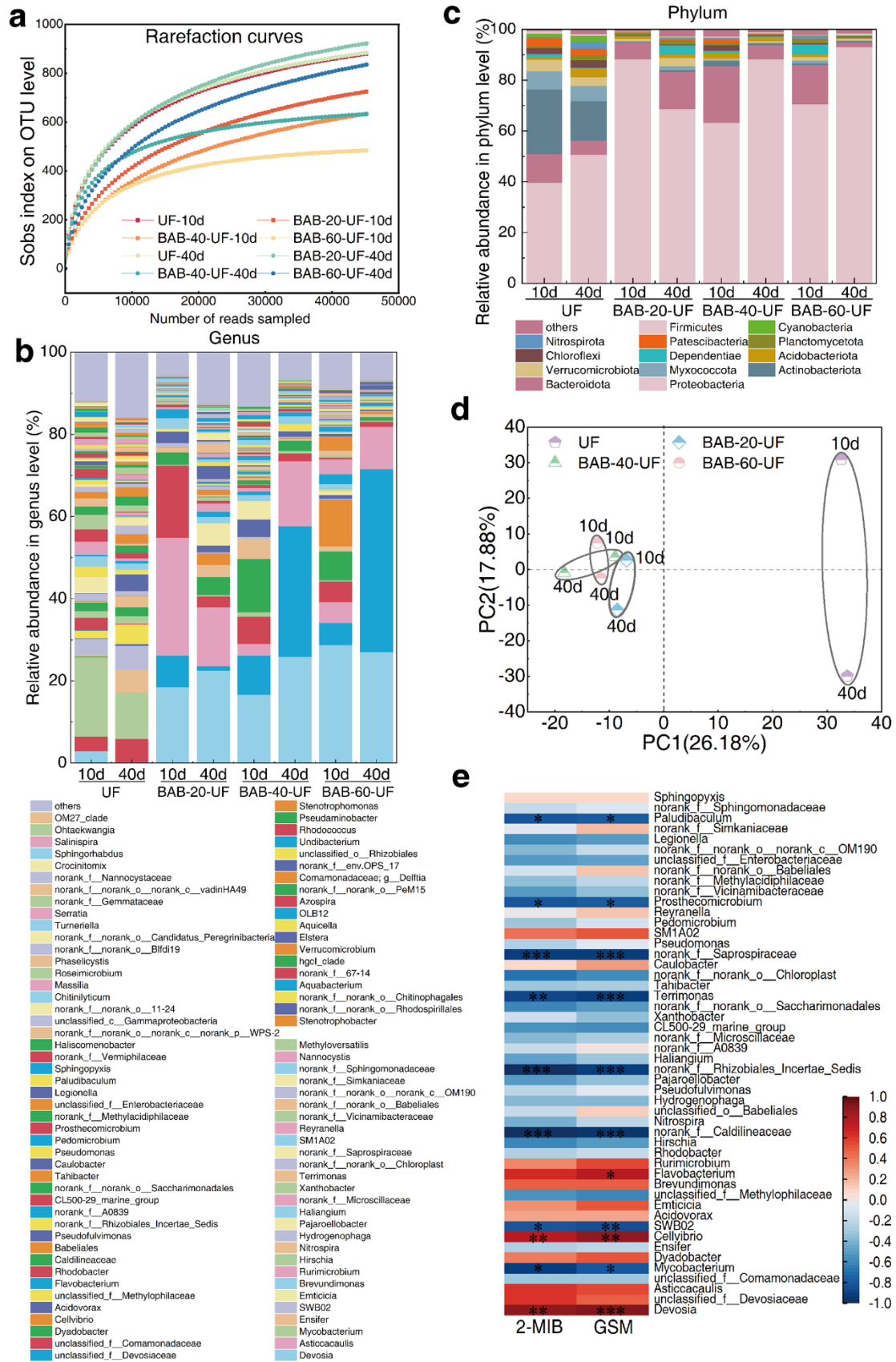
361 with *Actinobacteriota*, *Myxococcota* and *Verrucomicrobiota*, accounted for more than  
362 81% of the community.

363 Principal component analysis (PCA) of OTUs levels of microbial communities in  
364 different BAB-UF reactors (**Fig. 4d**) provides information on the similarity of the  
365 bacterial community structure between the four reactor samples and the effect of  
366 different operating times on the bacterial community structure. The bacterial  
367 community structures of BAB-20-UF, BAB-40-UF, and BAB-60-UF were similar,  
368 whereas the bacterial community structures of the reactors using ultrafiltration alone  
369 showed substantial differences. This result may be mainly attributed to the fact that the  
370 ultrafiltration membrane is not suitable for microbial attachment and growth compared  
371 to fillers and that it has a relatively poor ability to absorb substances from the influent  
372 water. The results also imply that materials with strong bioattachment capabilities may  
373 play an important role in acclimating stable microbial communities. While there were  
374 some differences in the bacterial community structure across the three BAB-UF  
375 reactors, the essential microorganisms were comparable. According to the data plotted  
376 in Fig. 4c, these core microbes were *Devosia*, *unclassified\_f\_Devosiaceae*,  
377 *Asticcacaulis*, *Unclassified\_f\_Comamonadaceae*, *Dyadobacter*, *Ensifer* and *Cellvibrio*,  
378 all belonging to *Proteobacteria* and *Bacteroidetes*, indicating that a large number of  
379 microorganisms potentially capable of degrading organic matter were adapted and  
380 retained in the biofilm-rich systems.

381 To further investigate microorganisms activity for 2-MIB/GSM removal,  
382 correlation analysis (**Fig. 4f**) was performed between the 2-MIB/GSM removal rate  
383 and the genus-level microbial community (top 50 genus in abundance). The results  
384 suggest that *Devosia* with abundance of 0.2% to 26% was positively correlated with  
385 substance removal ( $p < 0.01$  and  $p < 0.001$  for 2-MIB and GSM, respectively). It has been  
386 reported that *Devosia* isolated from enriched bacterial cultures showed effective  
387 degradation of alcohols, e.g., deoxynivalenol, whose molecular structure is similar to  
388 that of GSM.<sup>41</sup> Degradation enzymes in *Devosia* may belong to the aldo-keto reductase  
389 (AKR) family.<sup>42</sup> *Cellvibrio* (0.9%-13%) were also positively correlated with the  
390 removal rates of 2-MIB and GSM ( $p < 0.05$  and  $p < 0.01$ , respectively), but its  
391 mechanistic contributions requires additional research. Finally, *Flavobacterium* (0.2%-  
392 2.4%) had a significant correlation with the GSM removal rate ( $p < 0.05$ ). Yuan *et al.*  
393 reported that the ability of *Flavobacterium* to remove 2-MIB with an initial  
394 concentration of 515 ng/L reached 96.3%.<sup>7, 19</sup> According to the correlation analysis  
395 results of this study, *Flavobacterium* may also contribute to the degradation of GSM,  
396 but further studies are needed to prove this hypothesis. It is worth noting that  
397 *Pseudomonas*, *Sphingomonas sp.* were found in the four groups of BAB-UF reactors,  
398 and it has been pointed out that these two bacteria are likely to participate in the  
399 biodegradation of 2-MIB and GSM.<sup>7, 19</sup> Indeed, the abundance of *Pseudomonas* and  
400 *Sphingomonas* in the BAB-UF reactors at 40 days was an order of magnitude higher  
401 than that measured at 10 days, and also it increased with BAB filler amount,

402 corroborating previous reports. Note that the correlation analysis discussed above is  
403 hypothetical and based on data obtained from the 16s rRNA assay. Additional  
404 investigations will be conducted in future studies to test this hypothesis and the  
405 relationships with other water elements will also be taken into consideration. Specific  
406 microbiological tests will be conducted for each distinct bacterium in the water at the  
407 appropriate 2-MIB and GSM concentrations.

408 In conclusion, after the completion of the acclimation phase, the microbial  
409 communities in the different BAB-UF reactors were adapted to the raw water. After  
410 long-term operation, *Proteobacteria* was dominant in all communities. It appears that  
411 suspended fillers with robust biological affinity were able to acclimate stable microbial  
412 communities, which in turn effectively broke down contaminants in water. In addition,  
413 several microorganisms were found have a strong correlation with 2-MIB/GSM  
414 removal, including *Devosia*, *Cellvibrio*, *Flavobacterium*, *Pseudomonas*, as well as  
415 *Sphingomonas*. These correlations shall be further evaluated and quantified by future  
416 studies.



417

418 **Figure 4.** Microbial community analysis in four BAB-UF reactors: a) OTUs-level

419 rarefaction curves at different operation times. Bacterial community compositions of  
420 (b) genus (> 0.05%), (c) the phylum (> 0.1%) and (d) principal component analysis. (e)  
421 are the correlation analysis of the above genus-level (top 50) microbial community and  
422 2-MIB/GSM removal rate variables (“\*” represents a value of  $p < 0.05$  , “\*\*”  
423 represents a value of  $p < 0.01$  and “\*\*\*” represents a value of  $p < 0.001$ ).

424

### 425 **3.4 Biochar aerogel fillers slightly improved the membrane fouling behavior**

426 The morphology of the fouling layer on the membrane surface in various BAB-  
427 UF reactors was analyzed with SEM; see representative micrographs and results in **Fig.**  
428 **S8**. Surfaces were covered with a thick fouling layer. Interestingly, the fouling layer of  
429 BAB-60-UF membranes was the thinnest among the aerogel-assisted reactors,  
430 suggesting that EBCT ought to be no less than roughly 54 min to control fouling in the  
431 investigated system. Therefore, the impact of aerogel was minor but not negligible, and  
432 may deserve further study to understand how the presence of fillers might change the  
433 interaction of microorganisms and organic matter with the membrane.

434

## 435 **ASSOCIATED CONTENT**

### 436 **Supporting Information**

437 Additional experimental materials, methods, and procedures for data processing;  
438 discussion on DTPs and comparative analysis; and a number of other supporting  
439 materials are provided in Texts S1–S5, Tables S1–S4, and Figures S1–S8.

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456

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