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1 **Efficient removal of organic matters and typical odor substances in**
2 **rural drinking water using Ozone-BAC-UF combined system to meet**
3 **new water quality standards in China**

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13 **ABSTRACT**

14 China has just implemented new drinking water quality standards, canceling the provisions
15 detailed in the previous standards that allowed relatively lenient water quality standards in rural
16 areas, and unifying the water quality requirements for urban and rural areas. In rural areas of China,
17 lakes are important sources of drinking water, but their water typically contain high concentrations
18 of organic pollutants. In addition, removal odorous substances from such sources is challenging. In
19 response to these issues, an ozone-biological activated carbon-ultrafiltration process is proposed
20 and its efficiency in drinking water treatment is explored. The results indicate that O₃-BAC
21 pretreatment significantly improved the efficiency of the membrane process in removing organic
22 and odor substances. At an ozone dosage of 2 mg/L, with granular activated carbon as filler and
23 an EBCT of 25 min, the ozone-BAC pretreatment allowed removal rates in the membrane system
24 equal to 71.5% of COD_{Mn}, 84.2% of UV₂₅₄, as well as 92.2% and 92.5% of GSM and 2-MIB,
25 respectively, the latter being typical odor compounds. In addition, the microbial communities in
26 the reactors were evaluated under different pretreatment conditions at different times:
27 microorganisms with specific functions were enriched in different membrane reactors, and some
28 microorganisms contributing to the removal of organic and odorous substances were identified.
29 The effluent from the combined O₃-BAC-UF process consistently met the new requirements for
30 drinking water in China, implying that the combined system has significant potential for practical
31 applications for drinking water treatment in rural areas.

32

33 **KEYWORDS:** Ozone; Biological activated carbon (BAC); Ultrafiltration; Geosmin (GSM); 2-
34 methylisoborneol (2-MIB); Rural drinking water.

35

36 **1. Introduction**

37 As urbanization continues to progress globally, the development of urban areas has become
38 a focus of attention, while rural areas, especially in developing countries, are facing significant
39 challenges in terms of infrastructure and sanitation facilities (Du et al., 2021). The purity and safety
40 of rural drinking water is a major issue related to the health of the majority of township residents,
41 but also an important indicator of the improvement of rural living standards. In April 1, 2023,
42 China officially implemented the “Standards for Drinking Water Quality” (GB 5749-2022). In
43 these new standards, geographical limits have been eliminated, and the water quality requirements
44 for small centralized water supply and decentralized water supply are no longer divided by the
45 concept of "rural" and "urban" water quality standards. The unification of urban and rural standards
46 means that the improvement of rural drinking water quality faces new challenges and opportunities.
47 Currently, urban drinking water treatment technologies are well-established; they usually combine
48 advanced treatment processes with traditional treatment processes to ensure high quality of urban
49 drinking water (Wu et al., 2023). However, compared to urban areas, rural water supply is
50 generally characterized by difficulties, such as poor water quality of drinking water sources,
51 remote project locations, inadequate project scale, and weak operation and management (Qin et
52 al., 2021). Therefore, providing clean and safe drinking water in rural areas should start with
53 developing reliable, operable, and affordable water treatment systems (Du et al., 2021).

54 In rural areas of China, lakes serve as an important source of drinking water. However, as the
55 issue of eutrophication in lakes becomes more prevalent worldwide, taste and odor (T&O)
56 contamination resulting from the secondary metabolism of microorganisms has become a serious
57 and widespread issue (Zhang et al., 2019). For the average consumers, T&O represents the main
58 indicator of drinking water safety; therefore, the removal of odor substances is crucial for

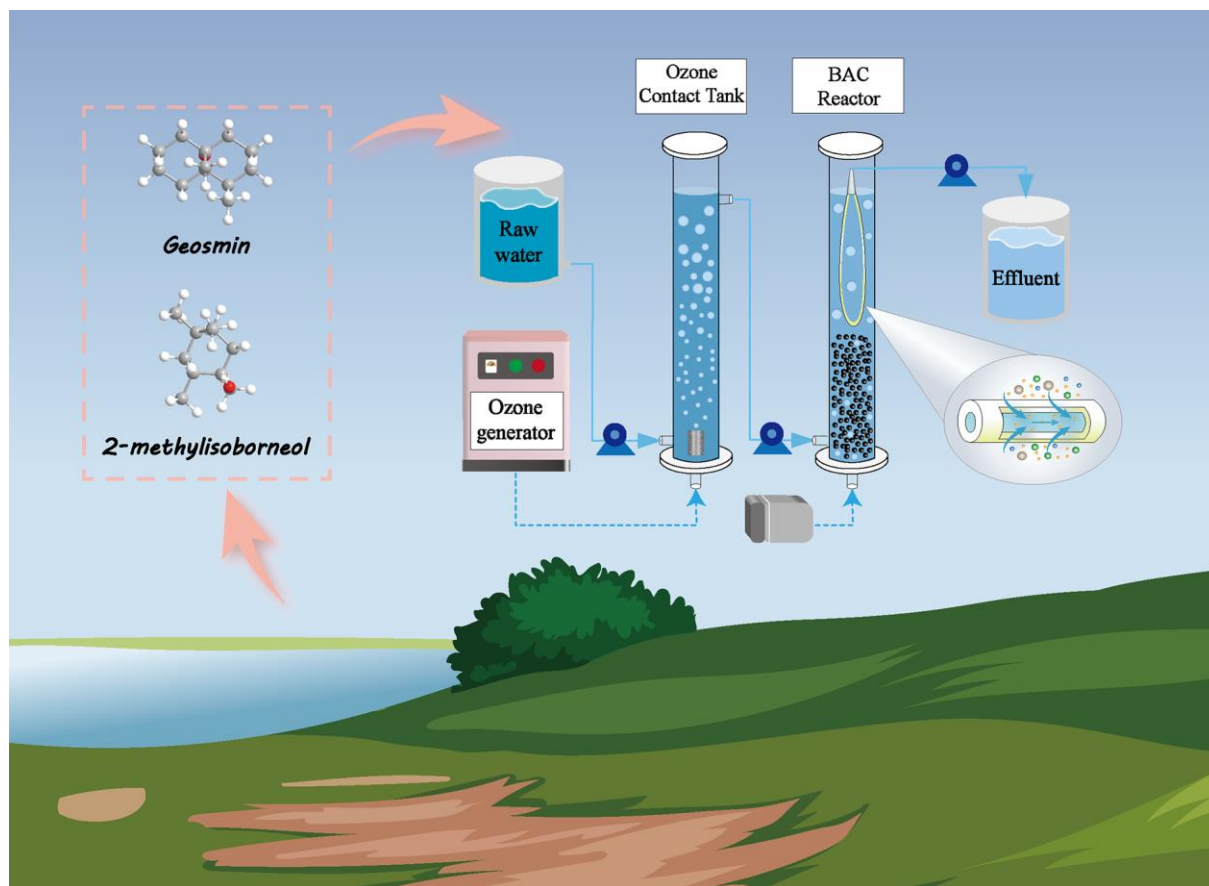
59 successful drinking water treatment and supply (Srinivasan and Sorial, 2011). The primary sources
60 of T&O are cyanobacteria (prokaryotes) and eukaryotic microorganisms, commonly known as
61 "algae", which produce a variety of volatile metabolites with different chemical structures and
62 odor characteristics (Christophoridis et al., 2021; Pivokonsky et al., 2021). Geosmin (GSM) and
63 2-methylisoborneol (2-MIB) are the most commonly detected T&O compounds in drinking water.
64 They are both saturated cyclo-tertiary alcohols formed as secondary metabolites of actinomycetes
65 and cyanobacteria (Abd El-Hack et al., 2022; Zhang et al., 2020). The odor threshold levels for
66 GSM and 2-MIB are 1.3-4.0 ng/L and 6.3-15 ng/L, respectively (Wert et al., 2014), and are
67 specified in many national drinking water standards. In China, the threshold concentration for both
68 odor compounds in drinking water is 10 ng/L. The main challenges associated with GSM and 2-
69 MIB are their extremely low odor threshold concentration and their persistence in conventional
70 water treatment processes (Srinivasan and Sorial, 2011).

71 The main objectives of conventional water treatment processes, such as coagulation,
72 sedimentation, and sand filtration, are to reduce the turbidity and the pathogenicity of water, and
73 retain large molecules of organic matter, while also providing some removal of GSM and 2-MIB.
74 Generally, due to the extreme resilience of GSM and 2-MIB to conventional water treatment
75 processes, their removal efficiency is relatively poor (Montiel, 1983; Srinivasan and Sorial, 2011).
76 Kim et al. (Kim et al., 1997) showed that after coagulation-sedimentation-filtration of raw water,
77 the effluent GSM decreased to 13.8 ng/L with a removal rate of 11.5%, while 2- MIB decreased
78 to 65 ng/L with a removal rate of 20.7%. These results imply that conventional processes are not
79 ideal for T&O removal. Membrane technology, especially ultrafiltration (UF), has shown suitable
80 prospects in rural drinking water treatment because of its high efficiency, small footprint, and ease
81 of operation and maintenance (Du et al., 2021). Due to the porous nature of the UF membrane,

82 soluble carbon and nutrients, such as ammonia and phosphorus, can pass through the membrane
83 and reach the permeate product, which may result in poor microbial stability of the treated water
84 and poor organic matter removal (Wu, 2019). Furthermore, studies have shown that membrane
85 filtration is not highly effective in removing GSM and 2-MIB (Liato and Aider, 2017). Membrane
86 separation is largely a size-exclusion mechanism, and contaminants that collect on the membrane
87 surface will eventually lead to membrane contamination, reducing filtration efficiency and
88 shortening membrane lifespan (Obotey Ezugbe and Rathilal, 2020). Therefore, proper
89 pretreatment or synergistic processes are necessary to improve the removal efficiency of organic
90 and odoriferous substances from drinking water and to optimize the membrane process.

91 Biological treatment is a cost-effective and efficient method for removing contaminants from
92 drinking water in rural areas (Neoh et al., 2016; Tang et al., 2022b). Biological activated carbon
93 (BAC) is a popular method for removing micropollutants, organic compounds, taste and odor
94 compounds, and halogenated hydrocarbons (Hamid et al., 2019). The removal of organic matters
95 is achieved through a combination of activated carbon adsorption and biodegradation by
96 microorganisms attached to the carbon. BAC is preferred over granular activated carbon (GAC)
97 as it does not require the disposal of waste carbon and can be used in several reactivation cycles
98 (Hamid et al., 2019; Tak and Vellanki, 2020). BAC has shown to be effective (>80%) in removing
99 GSM and 2-MIB, but its performance is influenced by water temperature, empty bed contact time
100 (EBCT), and the accumulation of attached microbial biomass (Beniwal et al., 2018). BAC
101 primarily targets the biodegradable fraction of dissolved organic carbon (DOC), but has difficulty
102 metabolizing complex, refractory, and non-biodegradable carbon. Therefore, when BAC is used
103 as a separate treatment technology, its efficiency in removing DOC is not satisfactory (Tak and
104 Vellanki, 2020).

105 To improve the biodegradation of organic matter in BAC treatment, incorporating a chemical
106 oxidation process prior to treatment is recommended (Lee et al., 2009). Ozone, a highly efficient
107 and cost-effective oxidant commonly used in drinking water treatment, has been shown effective
108 in breaking down organic matter into simpler forms and increasing the biodegradable fraction of
109 dissolved organic carbon (Guo et al., 2014). Application of ozone prior to biofiltration has been
110 shown effective in removing GSM, 2-MIB, and DBP precursors and improving biostability
111 (Beniwal et al., 2018; Wang et al., 2019). GSM and 2-MIB have some antioxidant properties due
112 to their saturated cyclic tertiary alcohol structure. Some oxidants such as chlorine, chlorine dioxide,
113 and potassium permanganate are not effective in removing GSM and 2-MIB (Rosenfeldt et al.,
114 2005). Ozone oxidation is more effective than other oxidants (Liato and Aider, 2017), since it act
115 by breaking the double bonds of odor compounds, forming aldehydes, acids and ketones, and
116 disrupting the metabolism of algae (Yuan et al., 2013). In addition, an ozone-BAC pretreatment
117 process can mitigate membrane contamination and extend membrane lifespan by improving water
118 quality and removing suspended matter (Tang et al., 2022b). In this study, the ozone-BAC and the
119 UF processes are combined and applied to rural drinking water treatment testing real raw water
120 from natural sources. The combined process shown in Fig. 1 is evaluated for the removal of organic
121 matter and odor substances, while the influence of filler characteristics and microbial community
122 on the effluent quality is also discussed. In addition, the mitigation of UF membrane fouling under
123 different pretreatment conditions is evaluated.



124

125 **Figure 1.** Diagram of the combined system comprising ozonation, BAC, and ultrafiltration.

126

127 **2. Materials and methods**

128 *2.1. Chemical and materials*

129 The raw water used in this study collected from a rural drinking water source reservoir in
 130 Zhongxian containing contaminants at low-medium concentrations (Chongqing, China). The
 131 standards of the target analytes 2-MIB and GSM were purchased from Sigma-Aldrich (USA).
 132 Ozone was generated using an ozone generator (Beijing Tonglin Co., Ltd., China) from dry oxygen
 133 (99%, v/v). The granular activated carbon (GAC) had a particle size of 12 × 40 mesh (0.425~1.70
 134 mm) and an iodine value of >950 mg/g, and it was purchased from Calgon Carbon Corporation
 135 (USA). The UF system employed a submerged polyvinylidene fluoride (PVDF) hollow fiber UF

136 membrane module (Litree Purifying Technology Co., Ltd., China). Ultra-pure water (18.25 MΩ
137 cm) (Ulupure, Chengdu, China) was used in this study.

138

139 2.2. *Methods*

140 2.2.1. *Experimental setup*

141 The target feed water concentration for 2-MIB and GSM spiking tests was 100 ng/L. A
142 continuous flow ozone oxidation experiment was performed in a plexiglass contact column with a
143 height of 80 cm and an inner diameter of 4 cm. The ozonated raw water was delivered to a BAC
144 system using a peristaltic Pump (BT-300-2 J, longer Pump, China). The BAC system employed a
145 granular activated carbon column with a height of 15 cm, an inner diameter of 2 cm, and an
146 effective packing height of 4 cm; a schematic diagram of the reactors is shown in Fig. S1 of the
147 Supporting Information (SI). The BAC was operated in up-flow mode to achieve a selected empty
148 bed contact time (EBCT) of 25 minutes. During the BAC column domestication process, the raw
149 water was gradually enriched in the activated carbon carrier by continuous batch feed and gradient
150 dilution, resulting in biofilm formation on the activated carbon over 30 days. Details of the BAC
151 system domestication process can be found in the SI ([Text S1](#)). The UF system employed a
152 submerged PVDF hollow fiber UF membrane module. The effective membrane area was 30 cm²,
153 and the membrane pore size was 11.3-28.8 nm ([Tang et al., 2022a](#)). As a first step, UF treatment
154 was conducted using raw water for 2-MIB and GSM removal without pretreatment. Three
155 pretreatment scenarios were then tested: 1) UF receiving the effluent from the ozone treatment
156 only (O₃-UF), 2) UF receiving the effluent from BAC treatment only (BAC-UF), 3) UF receiving
157 combined O₃-BAC effluent (O₃-BAC-UF).

158 On the 30th day of the operation of the combined system, control experiments were conducted
159 to measure the removal efficiency of T&O compounds at different process stages using a feed
160 water GSM/2-MIB concentration of 100 ng/L. An aeration treatment was performed to determine
161 the removal of T&O compounds from raw water due solely to aeration without pretreatment or
162 ultrafiltration membrane. Similarly, an aeration treatment was performed on the raw water after
163 different pretreatments without ultrafiltration, to determine the aeration-related removal after each
164 pretreatment. The effluent from each process stage was collected to determine the removal rate of
165 T&O compounds. In all cases, the 2-MIB/GSM concentrations in the inlet and outlet water were
166 measured thrice, and the average concentrations of inlet and outlet were calculated to determine
167 the average removal rates of 2-MIB and GSM.

168 2.2.2. *Water quality analysis*

169 The dissolved ozone concentration was determined by the indigo method (Bader and Hoign
170 é, 1981). To measure the concentrations of 2-MIB and GSM, gas chromatography-mass
171 spectrometer and tandem mass spectrometer (GC-MS/MS 8890-7010B, Agilent, USA) coupled
172 with solid phase micro-extraction (SPME) fiber holder and SPME fiber assembly-
173 divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) were employed. The GC/MS
174 was equipped with an HP-5 MS capillary column (30 m × 0.25 mm × 0.25 mm film thickness).
175 The detailed method is described in SI (Text S2 and Table S1). Standard chromatograms and 1-
176 200 ng/L 2-MIB/GSM standard curves are shown in SI, Figure S2. COD_{Mn} was determined based
177 on the protocol described in Water quality-Determination of permanganate index (GB 11892-89).
178 The concentration of total organic carbon (TOC) was measured using a TOC analyzer (TOC-L
179 CPH CN200, Shimadzu, Japan). The pH of the solution was measured by a pH-10 meter
180 (Sartorius Scientific Instruments Co., Ltd., Germany). The values of turbidity and TDS were

181 determined using a turbidimeter (TL2100 Q, Hach Company, USA) and a portable
182 multifunctional meter (Ultrameter II 6PFC, Myron L Company, USA), respectively. The
183 fluorescent organics were determined by a three-dimensional excitation-emission matrix (3D EEM)
184 fluorescence spectrum (F-7000, Hitachi, Japan). UV absorbance was measured at 254 nm in a
185 quartz cuvette with a UV-vis spectrophotometer (Orion AquaMate 8000, Thermo Fisher Scientific
186 INC., MA, USA). Depending on the varied affinities between different resins and organics,
187 nonionic macroporous resins can be used for the fractionation of hydrophobic/hydrophilic organics
188 (Liu et al., 2021). Herein, XAD-8 resin (Supelite, USA) and XAD-4 resin (Amberlite, USA) were
189 used to adsorb hydrophobic and hydrophilic organics, respectively. Water samples were adjusted
190 to pH = 2.0 with 0.1 M HCl and then underwent adsorption in XAD-8 and XAD-4 column resins
191 at a flow rate of 15 times the bed volume per hour (BV/h). Soaking and rinsing were performed
192 with 0.1 M NaOH, 0.1 M HCl, methanol, and ultrapure water, respectively.

193 *2.2.3. Microbial diversity sequencing analysis*

194 Spatial and temporal changes in microbial communities and dominant functional
195 microorganisms were analyzed by sequencing microbial diversity on the BAC or membrane
196 surfaces of the four systems at different times during continuous operation mode. Specifically, 16S
197 rRNA sequencing was performed at day 15 and day 30 after the domestication phase ended. The
198 sequencing was conducted on 2 g of filler samples collected from the BAC-UF reactor and O₃-
199 BAC reactor, located approximately 5 cm from the top (backfilled with an equal amount of filler
200 immediately after removal), and about 2 cm of membrane samples from the UF reactor and O₃-
201 UF reactor (Liu et al., 2022; Tang et al., 2021b; Wu et al., 2023). Details of the microbial diversity
202 sequencing analysis can be found in the SI (Text S3).

203

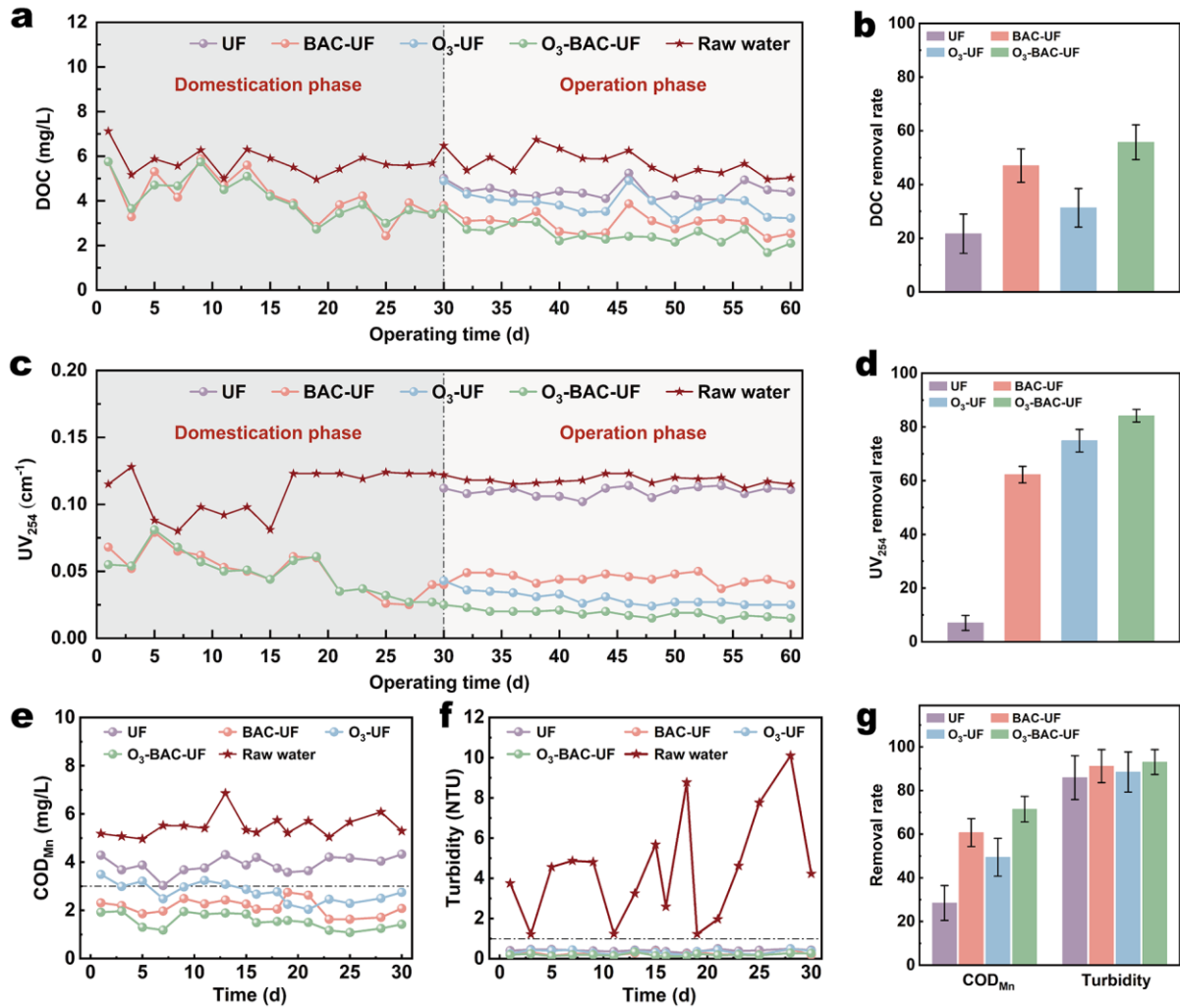
204 **3. Results and Discussion**

205 *3.1. Removal rates of organic matters*

206 During the GAC domestication process, microorganisms presented in water adhere to the
207 GAC surface and form biofilms. The GAC unit process can then effectively remove organic
208 pollutants from water through both adsorption and biodegradation mechanisms, thus potentially
209 prolonging the service life of the GAC material (Yuan et al., 2022a; Yuan et al., 2022b). In this
210 work, DOC, UV₂₅₄, and COD_{Mn} values of the raw water and effluent streams were measured to
211 characterize the organic matter present in water samples, and EEM was employed to determine the
212 removal rate of various types of organic pollutants. As discussed hereforth, consistent results were
213 obtained in the removal of the three organic matter indicators, overall suggesting the synergistic
214 effect of pre-oxidation, BAC adsorption and biodegradation, and surface retention by membranes.

215 In detail, Fig. 2a and 2b present the DOC values and their average removal rates during the
216 operation of the four investigated systems; please refer to chapter 2.2.1 for a description of the
217 systems. The BAC-UF and O₃-BAC-UF systems gradually reached stabilization during the
218 domestication period. During the operational period, the membrane filtration alone was not
219 effective in removing DOC, with an average removal rate of only 21.7%, and the effluent quality
220 fluctuated significantly in correlation with changes in raw water DOC. When BAC was as
221 pretreatment, with or without ozone treatment, the average DOC removal rate in the system
222 improved by 9.6% and 25.3%, respectively. On the other hand, pre-oxidation by ozone alone did
223 not significantly improve DOC removal of the membrane filtration, likely due to the increased
224 solubility of suspended organic matter from ozonation (Tang et al., 2020). Compared to BAC
225 pretreatment alone, the combined pretreatment showed a synergistic effect in organic removal, and
226 the O₃-BAC-UF system achieved an average DOC removal rate of 55.7% with stable effluent

227 quality. These results suggest that pre-oxidation can improve the removal efficiency of organics
 228 in the subsequent BAC-UF system, which may be due to the breakdown of recalcitrant organics
 229 such as aromatic compounds into simple and biodegradable organics (aldehydes, ketones, and
 230 carboxylic acids) by ozone (Loganathan et al., 2022; Loh et al., 2021), thereby improving their
 231 biochemical properties (Tang et al., 2020), and making them easily degradable in the BAC column.



232
 233 **Figure 2.** Water quality indexes of influent and effluent streams in different reactors during the operation period.
 234 (a) DOC values; (b) Corresponding DOC removal rates; (c) UV₂₅₄ values; (d) Corresponding UV₂₅₄ removal
 235 rates; (e) COD_{Mn} values; (f) Turbidity values; (g) Removal rates of COD_{Mn} and turbidity.

236

237 Fig. 2c and 2d present analogous results in terms of UV₂₅₄, a parameter that indicates the
238 fraction of organic substances containing C=C double bonds and benzene ring structures, including
239 humic macromolecules and aromatic compounds (Wu et al., 2023). The removal rates of UV₂₅₄
240 were 7.0% (UF), 62.3% (BAC-UF), 74.9% (O₃-UF), and 84.2% (O₃-BAC-UF), respectively.
241 Consistent with DOC analyses, the O₃-BAC-UF system is the most effective in removing UV₂₅₄.
242 Results graphed in Fig. 2e indicate that membrane filtration alone did not result in sufficient
243 removal of COD_{Mn} from the raw water to achieve the drinking water sanitation standard limit (3
244 mg/L). Pre-oxidation, although it improved removal, did not stabilize the effluent COD_{Mn} below
245 3 mg/L. The trend of COD_{Mn} removal was similar to that of DOC and UV₂₅₄ removal, with average
246 removal rates of 28.5% (UF), 60.7% (BAC-UF), 49.4% (O₃-UF), and 71.5% (O₃-BAC-UF) for the
247 the four systems. In particular, the O₃-BAC-UF stabilized the effluent COD_{Mn} below 2 mg/L, thus
248 greatly improving the efficiency of membrane treatment (Du et al., 2021; Obotey Ezugbe and
249 Rathilal, 2020).

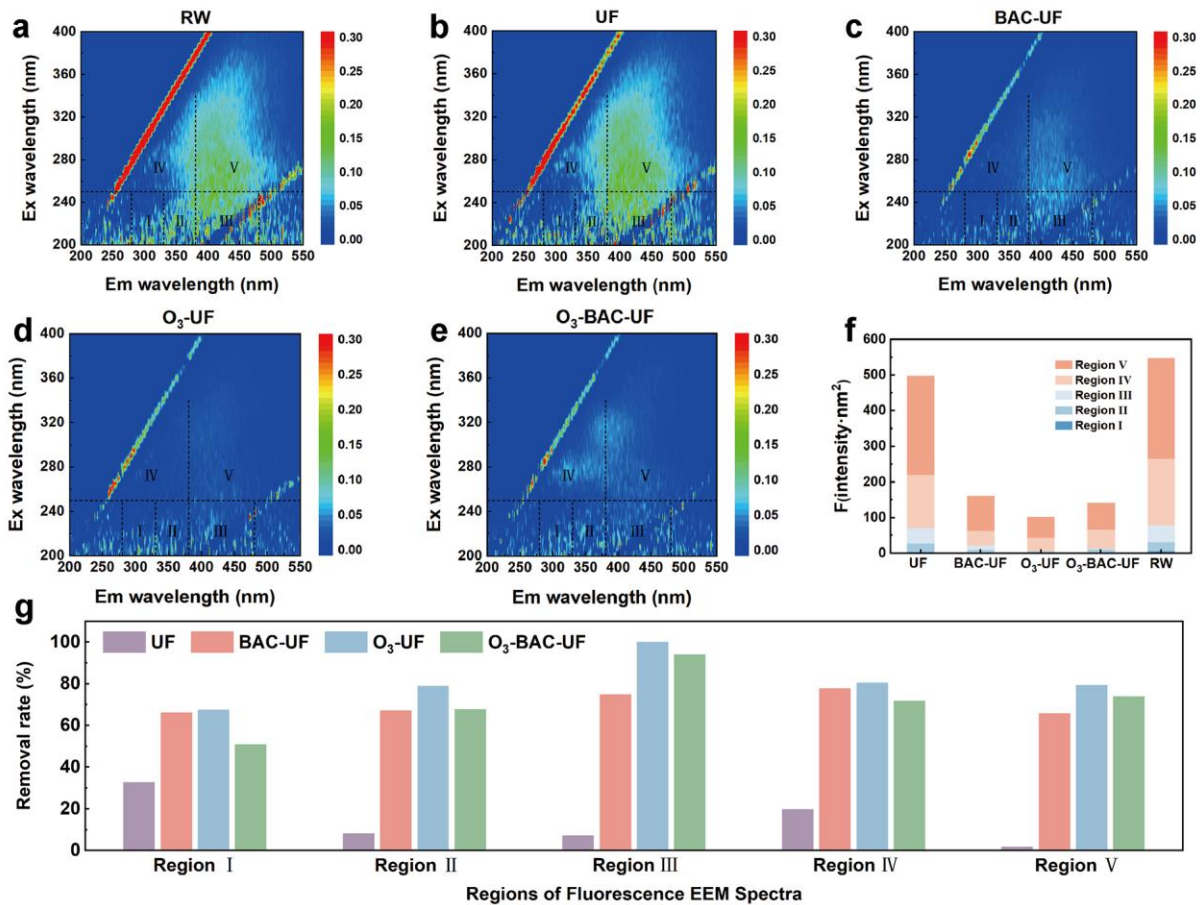
250 In addition, turbidity is another contamination indicator, related to the amount of suspended
251 matter in water. The raw water turbidity ranged from 1.2 NTU to 10.1 NTU (as shown in Fig. 2f).
252 Regardless of the raw water fluctuations, the effluent turbidity was significantly reduced in all four
253 systems of all four systems to levels between 0.12 and 0.50 NTU), hence below the drinking water
254 sanitation standard limit (1 NTU). Moreover, the average removal rates of the four systems were
255 as follows: 85.9% (UF), 91.w% (BAC-UF), 88.5% (O₃-UF), and 93.1% (O₃-BAC-UF). These
256 results demonstrate that all four systems were effective in intercepting particulate matter, which
257 was mainly achieved through the effective removal of suspended particulate matter by the
258 ultrafiltration membranes. The O₃-BAC-UF process also significantly demonstrated suitable
259 performance in chromaticity removal with a removal rate of 94% (Fig. S3 of the SI).

260 3.2 Removal mechanism of organic compounds

261 To further explore the impact of different pretreatments on changes of organic matter in
262 membrane filtration systems, fluorescence excitation-emission matrix (EEM) analysis was
263 performed raw water and treated effluents from the different systems, and the maximum
264 fluorescence intensity (F_{\max}) of each component in the water before and after treatment was
265 calculated (Fig. 3). The fluorescence spectra were categorized into five regions based on
266 fluorescence region integration: region I and II are associated with aromatic proteins (AP1 and
267 AP2). Region III, IV, and V are related to fulvic-like substances (FA), soluble microbial products
268 (SMP), and humic-like substances (HA), respectively. The total removal rates of fluorescent
269 organic matters were 6.6% with UF alone, 70.6% with BAC-UF, 81.9% with O₃-UF, and 74.3%
270 in the O₃-BAC-UF system, respectively.

271 HA (region V) dominated the fluorescent organic matter in raw water. Membrane filtration
272 alone showed inadequate removal of FA or HA. All the pretreatments showed a significant
273 increase on membrane filtration efficiency for the removal of these substances (74.8% - 100 %
274 and 65.7% - 79.4%, respectively). Region IV exhibited the second highest abundance of
275 fluorescent organic compounds. Soluble microbial products (SMP), encompassing substances
276 such as polycyclic aromatic hydrocarbons (PAHs), amino acids, polysaccharides, and hydrophobic
277 organic acids were previously identified within this region (Liu et al., 2021). While all pretreatment
278 combinations also allowed higher removal of substances in region IV, interestingly a lower
279 removal rate for SMP was observed compared to humic substances. SMP produced and
280 accumulated by microorganisms on BAC may flow into the effluent with microorganisms (Im et
281 al., 2019; Wang et al., 2022). Furthermore, according to Nguyen and Roddick (Nguyen and

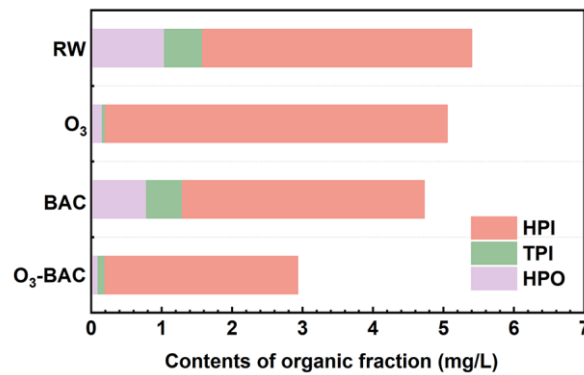
282 Roddick, 2010), the microorganisms under the effect of residual ozone may lead to an increase in
 283 SMP through the release of intracellular substances.



284
 285 **Figure 3.** Fluorescence EEM spectra of influent and effluent streams in different reactors. (a) Raw water; (b):UF
 286 effluent; (c): BAC-UF effluent; (d): O₃-UF effluent; (e) O₃-BAC-UF effluent; (f): F_{max} calculated from
 287 fluorescence EEM spectra of influent and effluent streams; (g): Removal rates of different reactors. (Region I:
 288 (Ex/Em = 220-250/280-330 nm, tyrosine protein-like substances), region II: (Ex/Em = 220-250/330-380 nm,
 289 tryptophan protein-like substances), region III: (Ex/Em = 220-250/380-480 nm, fulvic acid-like matters), region
 290 IV: (Ex/Em = 250-440/280-380 nm, soluble microbial by-product-like matters), and region V: (Ex/Em = 250-
 291 400/380-540 nm, humic acid-like components)).

292
 293 As shown in Fig. 4, the majority of organic compounds in the raw water were found within
 294 the hydrophilic (HPI) fraction (70.8%). In addition, pre-oxidation led to a further increase in the

295 content of HPI, likely because ozonation promoted the formation of oxygen-containing functional
 296 groups, such as hydroxyl groups, aldehyde groups, and ketones (Loganathan et al., 2022). Indeed,
 297 it has been reported that pre-treatment with ozone result in an increase in the hydrophilic character
 298 of dissolved organic matter (DOM), making the removal of DOM by conventional processes
 299 relatively unsatisfactory (Yang et al., 2023). In this work, the HPO removal by BAC treatment
 300 alone was not ideal (24.4%), but significantly increased after pre-oxidation (88.4%), consistent
 301 with the oxidation activity of ozone and with the results presented in Figure 4.

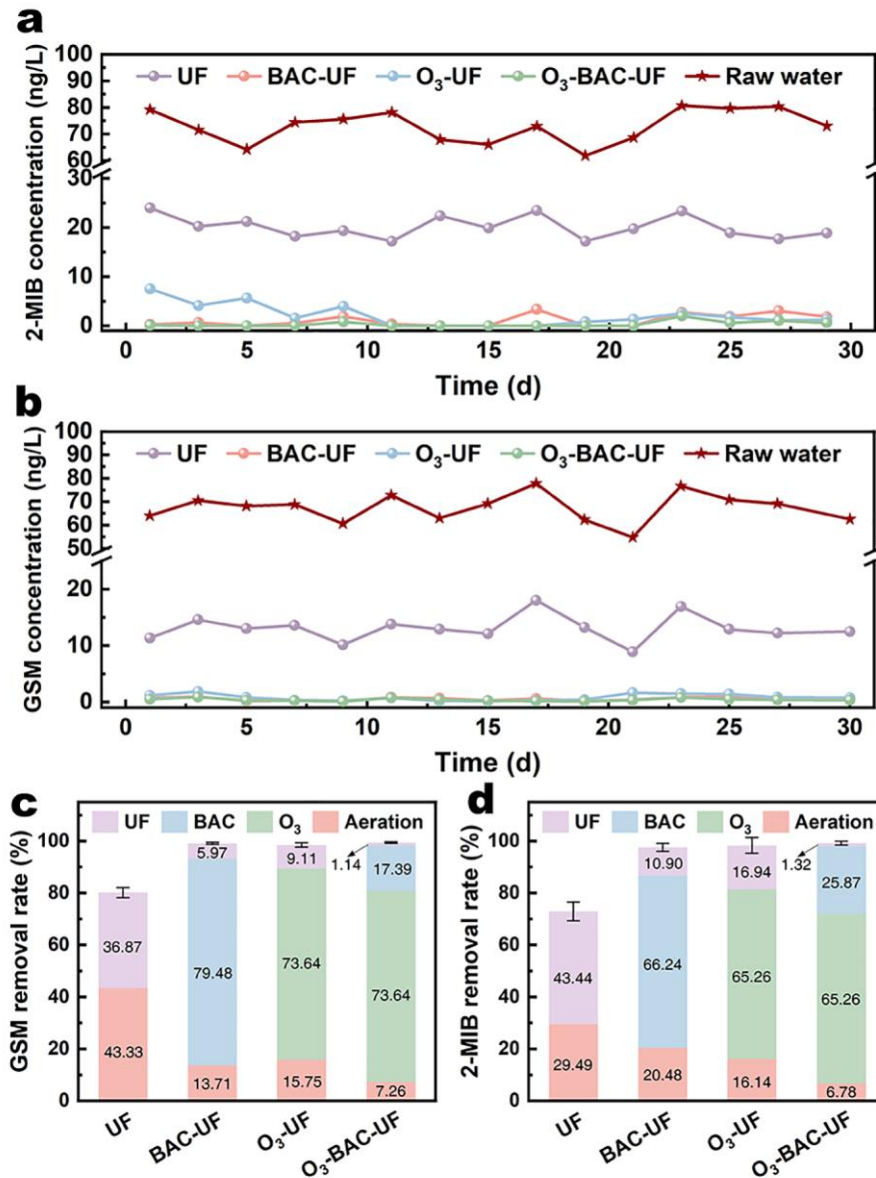


302
 303 **Figure 4.** Distribution of organic fractionation by XAD chromatographic column. Illustration of hydrophilic
 304 (HPI), transphilic (TPI), and hydrophobic (HPO) fractions in raw water before and after pretreatment.

305
 306 *3.3. GSM and 2-MIB removal rates and mechanisms*

307 During continuous operation, the concentrations of GSM and 2-MIB in the influent water
 308 fluctuated between 81.1-115ng/L and 86.4-112 ng/L, respectively. The experimental setup was in
 309 a non-fully sealed state (when in a fully sealed state, the pressure inside the reactor rises due to
 310 continuous aeration, and the resistance to water flow increases, resulting in a lower discharge rate),
 311 and a fraction of the odor compounds was lost due to natural volatilization. Control experiments
 312 indicated that natural volatilization accounted for 32.5% and 28.3% of the losses for GSM and 2-
 313 MIB, respectively, when the starting concentrations were 100 ng/L. These losses were subtracted

314 from the calculation of raw water concentration. As shown in the results graphed in Figs. 5a and
 315 5b, pretreatment significantly improved the efficiency of odor removal in the membrane treatment
 316 system, and the GSM and 2-MIB concentrations in the effluent after pretreatment met the drinking
 317 water quality standards.



318
 319 **Figure 5.** Removal of typical T&O substances: (a) GSM concentration in effluents of different reactors; (b) 2-
 320 MIB concentration in effluents of different reactors; (c) Contribution of different process stages to GSM removal
 321 rate; (d) Contribution of different process stages to 2-MIB removal rate.

322

323 [Figs. 5c](#) and [5d](#) present the contribution of each process stage to the removal of GSM and 2-
324 MIB in different systems. After deducting the loss due to volatilization, the total removal rates of
325 GSM for the different systems were O₃-BAC-UF (92.2%), O₃-UF (82.8%), BAC-UF (85.5%), and
326 UF (36.9%), and the total removal rates of 2-MIB were O₃-BAC-UF (92.5%), BAC-UF (82.2%),
327 O₃-UF (77.1%), and UF (43.4%). Note that the volatilization effect was significantly higher in the
328 individual UF system, with respect to all other systems, because without pretreatment the
329 membrane was subjected to more severe fouling, resulting in a slower effluent rate that lengthened
330 the filtration time, hence the volatilization time. The other factor contributing to odor compounds
331 removal by UF membranes may instead be associated with cake layer filtration from the fouling
332 layer and microorganisms attached and accumulated on the membrane surface during both the
333 domestication process and the long-term continuous operation.

334 GSM and 2-MIB are hydrophobic compounds with logK_{ow} values of 3.57 and 3.31,
335 respectively. Ozone oxidation had a slightly better degradation effect on GSM, partly attributed to
336 its lower water solubility with respect to 2-MIB and its consequent easier transfer from the native
337 liquid phase to the gas phase to react with ozone ([Yuan et al., 2013](#)). Similarly, the BAC treatment
338 alone showed better removal efficiency for GSM. According to [Doederer et al. \(Doederer et al.,](#)
339 [2019\)](#), adsorption is the main removal mechanism at the early stages of contact between the
340 odourant and the BAC, beyond which biodegradation becomes the primary removal process.
341 Compared to 2-MIB, GSM molecules have a more lipophilic and flatter structure, facilitating their
342 interaction with GAC and biofilms, and encountering less direct competition with NOM molecules
343 ([Cook et al., 2001](#)). It should be pointed out that, after ozone pre-oxidation, the subsequent BAC
344 stage exhibited higher removal efficiency for 2-MIB compared to GSM. This result is consistent

345 with the discussion above, as higher oxidation of GSM would produce more hydrophilic products,
346 which have less affinity with GAC. Also, Saadoun and El-Migdadi (Saadoun and El-Migdadi,
347 1998) suggested that GSM is more recalcitrant to degradation by Gram-negative bacteria when
348 used as the sole carbon source, in comparison to 2-MIB. Ozone treatment increased the proportion
349 of Gram-negative bacteria in water, which may be one of the reasons for the above phenomenon.

350 *3.4 Microbial diversity analysis*

351 *3.4.1 Microbial diversity*

352 The abundance and diversity of microorganisms in the four reactors were analyzed with the
353 Illumina sequencing method. A total of 32,133-56,154 valid sequences were obtained.
354 Bioinformatics analysis of the valid sequences was performed at a 97% similarity level, and the
355 results are summarized in Table 1. All samples had Coverage index values above 0.99 (Table 1),
356 and the sparsity curve tended to increase with increasing sequence number (Fig. 6a), indicating
357 that the sequence number was adequately large to represent nearly all microbial communities in
358 each sample. Shannon and Chao index results showed that pretreatment increased microbial
359 community diversity. Specifically, the Shannon index of the O₃-BAC-UF system was higher
360 compared to the unoxidized BAC-UF system, which may be due to the increased availability of
361 biodegradable organic carbon after ozone oxidation (Sauter et al., 2023; Tang et al., 2021a). Fig.
362 6b shows the number of OTUs that were unique and identical between the bacterial communities.
363 The number of OTUs across all four reactors was 372, while the numbers for UF, BAC-UF, O₃-
364 UF, and O₃-BAC-UF were 264, 96, 49, and 58, respectively. These results suggest that
365 pretreatment significantly altered the community structure.

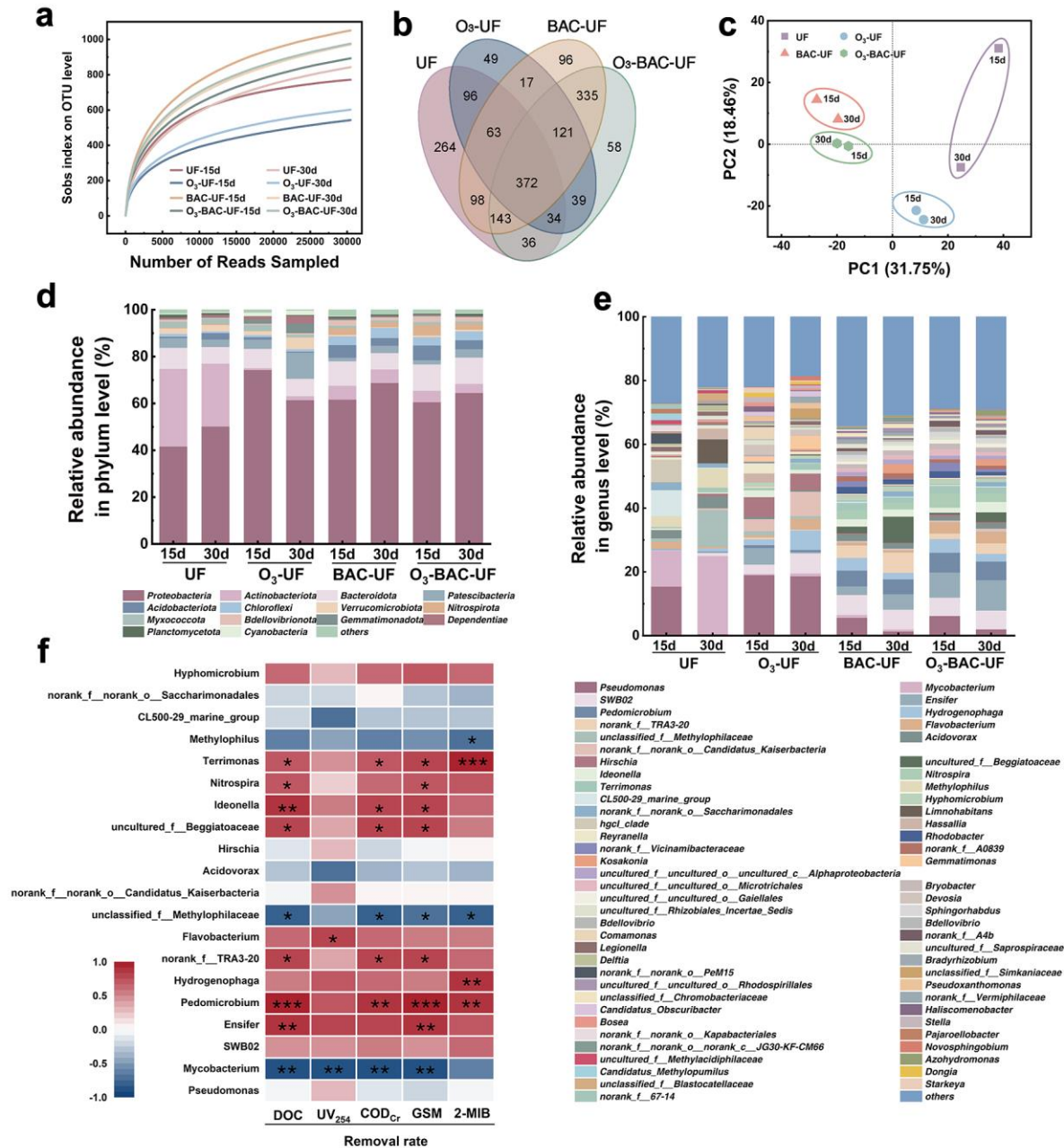
366

367

368 **Table 1.** Number of effective sequences, operational taxonomic units (OTUs), alpha diversity indexes for
 369 microbial communities in different reactors at different periods

Sample	Number of effective Sequences	OTU at 97% identity	Shannon	Chao	Coverage
UF-15d	32133	772	4.47	829.0	0.9963
UF-30d	44738	843	3.98	1038.9	0.9928
BAC-UF-15d	42960	1051	5.19	1238.4	0.9922
BAC-UF-30d	56154	971	5.02	1210.0	0.9913
O ₃ -UF-15d	47290	543	4.16	700.6	0.9950
O ₃ -UF-30d	51794	602	4.15	733.3	0.9950
O ₃ -BAC-UF-15d	44849	893	4.91	1102.5	0.9925
O ₃ -BAC-UF-30d	53216	976	5.05	1197.9	0.9920

370
 371 Principal component analysis (PCA) is an important method for comparing bacterial diversity
 372 and assessing the similarity of microbial community composition. The closer two sample points
 373 are, the more similar would be the species composition of the two samples. The PCA at the level
 374 of microbial community OTUs (Fig. 6c) shows that the microbial community structure differed
 375 between the microbial communities of different reactors under long-term operating conditions.
 376 This result indicates that new dominant organisms were formed within the pretreated membrane
 377 reactor. In addition, it can be observed that the structure of the biotope did not differ significantly
 378 over time within the same reactor.



379

380 **Figure 6.** Results of microbial community characterization of water from different reactors at different operation

381 times. a): Rarefaction curves of OTUs. b): Venn diagram at OTUs level; c): PCA analysis at OTUs level of

382 microbial communities; d): Bacterial community compositions at the phylum level (> 0.1%); e): Bacterial

383 community compositions at the genus level (> 0.12%). f): Correlation analysis between microbial community at

384 the genus level (top 20) and environmental variables (organic matter removal rate, typical odor substances

385 removal rate). Here, “*” represents a value of $p < 0.05$, “**” represents a value of $p < 0.01$, “***” represents a
386 value of $p < 0.001$.

387 3.4.2 Composition of the microbial community

388 Figs. 6d and 6e show the bacterial community composition at the phylum level and at the
389 genus level for samples obtained at different times (day 15 and day 30) from raw water (RW),
390 water undergone ozone pretreatment, and water undergone both biological treatment systems. At
391 the phylum level (Fig. 6d), 14 bacterial phyla with relative abundances $\geq 1\%$ were identified.
392 Among all samples, the five most dominant phyla were *Proteobacteria* (41.68% - 74.32%),
393 *Actinobacteriota* (0.81% - 33.16%), *Bacteroidota* (6.98% - 11.23%). These three phyla accounted
394 for roughly 80% of the total bacterial abundance.

395 The ozone treatment significantly influenced the inactivation of *Actinobacteriota*,
396 *Acidobacteriota*, *Nitrospirota*, and *Planctomycetota*, resulting in a notable reduction in the levels
397 of these bacterial clades in the effluent after ozone oxidation. Specifically, the most significant
398 reduction was observed for *Actinobacteriota* as a Gram-positive actinomycete, with the relative
399 content decreasing from 33.2% to 0.81%. In addition, an increase in the relative content of the
400 bacterial phyla *Gemmatimonadota*, *Cyanobacteria*, and *Dependentiae* was also observed. These
401 results indicate that oxidative processes have a discernible impact on the bacterial community,
402 leading to changes and adaptations in the composition of the phylum level.

403 The BAC-UF system exhibited an increase in both the abundance and diversity of bacteria in
404 the effluent water, which also showed spatial and temporal variation. At the phylum level, after 30
405 days of operation, the proportion of *Proteobacteria*, *Chloroflexi*, and *Acidobacteriota* in the
406 biomass of the BAC filter column increased by 27.1%, 3.13%, and 2.65%, respectively, compared
407 to the raw water. *Proteobacteria* include a variety of as Gram-negative metabolizing bacteria that
408 can play an important role in DOM decomposition and are the main bacteria for the removal of

409 dissolved biodegradable organic compounds (Du et al., 2020; Wang et al., 2022). Previous studies
410 have also reported the prevalence of *Proteobacteria* in GAC or BAC filters in DWTPs (Chang et
411 al., 2022; Du et al., 2020; Jiang et al., 2022). *Acidobacteria* and *Chroloflexi* can utilize a variety
412 of carbohydrates (Wang et al., 2022), which could explain the better removal performance of DOC
413 observed in the BAC treatment. In addition, a significant decrease in the relative content of
414 *Actinobacteriota* (by 27.4%) was observed, a similar result for ozone oxidation. The biotope
415 structure of the O₃-BAC-UF system differed from that of the BAC-UF system, although they
416 shared similar core organisms at the phylum level. O₃-BAC-UF system had the highest relative
417 content of *Bacteroidota* (11.2%) compared to the raw water and other treatment systems.

418 At the genus level, pre-oxidation caused a change in the bacterial community structure on the
419 BAC filter. With continuous operation of the system, a large number of bacterial genera with low
420 abundance in the raw water were gradually enriched on the BAC filter, such as
421 *uncultured_f__Beggiatoaceae* (8.2%), *norank_f__TRA3-20* (6.3%), *SWB02* (6.2%), *Ensifer*
422 (4.8%), and *Pedomicrobium* (4.7%). The genus *SWB02* is a potential symbiotic bacterium that can
423 co-degrade volatile fatty acids with methanogenic bacteria (Feng et al., 2023; Lee et al., 2019). In
424 contrast, after pre-oxidation, the relative content of *Ensifer* and *Pedomicrobium* on the BAC filter
425 increased to 9.5% and 5.8%, respectively. Both bacteria belong to the *Alphaproteobacteria*, which
426 are parthenogenic anaerobes that use organic matter in raw water as a carbon source while carrying
427 out respiration and fermentation metabolism (Tian et al., 2014). In addition, *Flavobacterium* (3.6%)
428 emerged as a new important genus in the ozone-BAC-UF system, belonging to the *Bacteroidota*,
429 which can be involved in protein degradation and amino acid fermentation, with a strong
430 metabolism of complex organic compounds and lipids (Jiang et al., 2022).

431 Fig. 5f shows the correlation analysis between bacterial genera (top 20 in abundance) and
432 environmental variables (organic matter removal rate, odorant substance removal rate). The
433 analysis identified several bacterial genera associated with DOC removal, namely, *Pedomicrobium*,
434 *Ensifer*, and *Ideonella*. Among them, *Pedomicrobium* with an abundance of 0.4%-6.2% was
435 significantly and positively associated with DOC removal ($P < 0.001$). Some studies reported that
436 *Pedomicrobium* may be associated with the biodegradation of phenolic compounds (Deng et al.,
437 2021). Significant correlations were also observed between *Pedomicrobium* and COD_{Mn} ($P < 0.01$),
438 2-MIB ($P < 0.01$), and GSM ($P < 0.001$) removal. *Ensifer* was associated with GSM removal.
439 Bacterial genera that were significantly and positively correlated with 2-MIB removal included
440 *Terrimonas* ($P < 0.001$) and *Hydrogenophaga* ($P < 0.01$).

441

442 4. Conclusion

443 The combined O_3 -BAC-UF system was employed for the treatment of drinking water in rural
444 areas, and its practical application potential was assessed by examining changes in water quality
445 indicators (e.g., organic matter, turbidity) and odor compound indicators (GSM, 2-MIB) during
446 continuous operation. Ozone oxidation altered the biochemical properties of organic matter,
447 facilitating the subsequent BAC process to efficiently remove organic matter and odorous
448 substances from the water. The ultrafiltration membrane effectively blocked microorganisms from
449 the BAC-treated effluent and reduced the turbidity of the water. During continuous operation after
450 the domestication phase, the combined O_3 -BAC-UF process was able to achieve a COD_{Mn} removal
451 rate of 71.5% and a UV_{254} removal rate of 84.2%. In terms of T&O removal, the combined process
452 also showed a stable removal capacity with an average removal rate of 92.2% for GSM and 92.5%

453 for 2-MIB. Ozone oxidation and BAC degradation were the primary removal mechanisms, with
454 air stripping and membrane filtration providing additional removal of T&O compounds.

455 The combined O₃-BAC-UF process exhibited promising applicability for the treatment of
456 rural drinking water with high organic concentrations and significant odor issues. However, it is
457 important to note that the presented results were obtained at the laboratory scale. Further large-
458 scale experiments are necessary to evaluate the broader applicability and feasibility of this process
459 for drinking water treatment in rural areas.

460

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