

Efficient removal of organic compounds from shale gas wastewater by coupled ozonation and moving-bed-biofilm submerged membrane bioreactor

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20 **Abstract:** Shale gas wastewater (SGW) with complex composition and high salinity  
21 needs an economical and efficient method of treatment with the main goal to remove  
22 organics. In this study, a coupled system consisting of ozonation and mov-  
23 ing-bed-biofilm submerged membrane bioreactor (MBBF-SMBR) was comprehensively  
24 evaluated for SGW treatment and compared with a similar train comprising ozonation  
25 and submerged membrane bioreactor (SMBR) without addition of carriers attaching  
26 biofilm. The average removal rates of MBBF-SMBR were 77.8% for dissolved organic  
27 carbon (DOC) and 37.0% for total nitrogen (TN), higher than those observed in SMBR,  
28 namely, 73.9% for DOC and 18.6% for TN. The final total membrane resistance in  
29 SMBR was 40.1% higher than that in MBBF-SMBR. Some genera that specifically  
30 contribute to organic removal were identified. Enhanced gene allocation for membrane  
31 transporters and nitrogen metabolism was found in MBBF-SMBR biofilm, implying  
32 that this system has significant industrial application potential for organics removal  
33 from SGW.

34

35 **Keywords:** Moving-bed-biofilm submerged membrane bioreactor (MBBF-SMBR);  
36 Shale gas wastewater (SGW); Ozonation; Microbial community; Submerged Membrane  
37 bioreactor (SMBR)

38

## 39 **1. Introduction**

40 The extraction of shale gas, one of the unconventional energy resources, is ex-  
41 pected to raise the global technically recoverable gas resources by over 40% [1]. In par-  
42 ticular, horizontal drilling and hydraulic fracturing (HF) techniques have promoted shale  
43 gas development [2]. However, HF causes some environmental problems, the most se-  
44 rious being arguably the production of hazardous shale gas wastewater (SGW). In the  
45 drilling and completion of horizontal wells, abundant flowback and produced water  
46 (FPW) returns to the surface as SGW [3]. In HF operations, pollutants including sus-  
47 pended solids, salt, organic chemicals, naturally occurring radioactive materials  
48 (NORMs), and heavy metals contaminate the SGW [4], resulting in environmental risks  
49 if this stream is not properly purified before discharge or reuse.

50 Typical techniques that have been applied to treat SGW include basic separation  
51 technologies, adsorption, advanced oxidation, low pressure membrane filtration, and  
52 desalination technologies [5]. Pre-treatment for desalination steps is usually required,  
53 the complex organic matters limiting the efficiency and durability of desalination. For  
54 example, organics cause membrane fouling and limit the application of  
55 high-pressure-driven membrane technologies in SGW desalination [6]. Thousands of  
56 organic compounds have been detected in SGW and can originate from the shale for-  
57 mation, chemical reactions underground, or from HF additives [7]. Low molecular  
58 weight hydrophilic organic compounds, which are considered readily or inherently bio-  
59 degradable, often represent the majority of dissolved organic matter (DOM) in SGW [7,  
60 8]. Therefore, biological treatment may have great potential as an economical method

61 for organic matter removal and pre-treatment of the SGW for subsequent polishing.

62 Some researchers have utilized biological methods to treat FPW from oil or gas  
63 production, by applying: MBR and its variant methods [9-11]; microbial mats [12];  
64 aerobic sludge granulation (ASG) [13]; biologically active filtration (BAF) [14, 15];  
65 plant-microbial synergism [16]. The reported organic carbon removal rates were gener-  
66 ally high by MBR and its variant methods for treating FPW with different quality [9-11].  
67 MBR operates with a large amount of biomass, an important advantage when treating  
68 effluents with complex organic matters, and needs small footprint, thus it is suitable for  
69 on-site reuse. High salinity, refractory biodegradation of SGW, and the toxicity of some  
70 HF additives are major challenges in its application [8]. Advanced oxidation, especially  
71 ozonation, has been used as pretreatment to enhance organics removal in biological  
72 processes by pre-oxidizing refractory organic compounds and toxic substances [17, 18].  
73 Ozonation coupled with BAF removed 83.2% DOC in SGW treatment [17]. Moreover,  
74 the coupling of ozonation and MBR has been proven to reduce membrane fouling and  
75 improving MBR performance [19], but this process has not yet been explored in SGW  
76 treatment.

77 MBBF-SMBR combines moving-bed-biofilm reactor (MBBR) with submerged  
78 membrane bioreactor (SMBR) [20] and it has several potential advantages: lower mem-  
79 brane fouling because of the interacting forces between the membrane and the carriers  
80 [21]; higher resistance of attached biomass to overloading and toxic compounds [22];  
81 higher nitrogen removal from simultaneous nitrification and denitrification [23]. Con-  
82 sidering the relatively low concentration of organic compounds and the medium-high

83 salinity of the SGW from the Sichuan Basin [5], in this study a MBBF-SMBR is applied  
84 for the biodegradation of organic matter and compared with a conventional SMBR.  
85 Ozonation is chosen as a pretreatment option to increase the biodegradability of SGW.  
86 Except for the addition of carriers, the two systems were operating in similar conditions.

87 Therefore, this study aims at evaluating the efficacy and feasibility of  
88 MBBF-SMBR and SMBR following ozone-based pre-oxidation for organics removal  
89 from SGW in the Sichuan Basin. Specifically, the main objectives of the present study  
90 are: (a) to evaluate and compare the removal of organic matters from SGW for the two  
91 systems; (b) to investigate and compare the component of membrane fouling; (c) to an-  
92alyze difference of the microbial community composition and predicted function in the  
93 treatment of SGW.

## 94 **2. Materials and methods**

### 95 **2.1 Pre-treatment of shale gas raw water (RW)**

96 The SGW was obtained from the Weiyuan shale gas play (Sichuan, China). It was  
97 stored in plastic containers in the dark at room temperature and was coagulated and pre-  
98 cipitated to remove large particles and colloids before other experiments. The resulting  
99 effluent is referred to as raw water (RW).

100 Pre-ozonation was applied to improve the biodegradability of the RW. In each  
101 batch, 900 mL RW was added into a 1000 mL glass bottle and treated with 100 mg  
102 ozone. Ozone, produced from dry oxygen (99%, v/v), was injected from the bottom  
103 through a gas diffuser with inlet concentration of  $17\pm 3$  mg/L, for 60 min of reaction  
104 time and 0.1 L/min of flow rate (see supplementary material). The temperature was 20

105  $\pm 1$  °C, maintained by a temperature-controlled water bath (HH-1, Xinrui Instrument  
106 Company, China) [24]. The concentration of ozone produced from laboratory ozone  
107 generator (Beijing Tonglin Co., Ltd., China) and that of the off-gas from the reaction  
108 bottle were measured by the indigo method. Before the bio-treatment, the water was set-  
109 tled for 2 h to sediment flocs formed after ozonation and the residual ozone in water was  
110 quenched by water bath heating for 30 min at 50 °C.

## 111 **2.2 Experimental set-up and operation**

112 Fig.1 presents the experimental setup of the biological processes. Both the MBR  
113 and the BFMBR tanks were made of glass with an effective volume of 300 mL. The  
114 water temperature of each bioreactor was maintained at 20-25 °C. An aeration disk was  
115 installed at the bottom to supply O<sub>2</sub> for microorganism growth at the flow rate of 20  
116 mL/min: the dissolved oxygen (DO) concentration was kept at 4-6 mg/L in both  
117 MBBF-SMBR and SMBR. The submerged membrane module comprised hollow-fiber  
118 membranes made of polyvinylidene fluoride (PVDF). Their active filtration area was 5.2  
119 cm<sup>2</sup>. Virgin polyurethane cubes (side length: 10 mm) were used as carriers with volume  
120 fraction of 20% in the MBBF-SMBR.

121 The SMBR and the MBBF-SMBR were started for acclimation of the biofilm and  
122 the sludge. The bioreactor of MBBF-SMBR was firstly filled by the carriers at a filling  
123 ratio of approximately 20%, and then inoculated with the activated sludge mixed liquor,  
124 which was taken from a municipal wastewater treatment plant (Chengdu, China), up to  
125 half of the bioreactor's effective volume. Afterwards, the bioreactor was fed with mu-  
126 nicipal wastewater from the same treatment plant, and run in batch mode to allow con-

127 tact with the biomass. Then, the wastewater was fed for 2 days in continuous mode. At  
128 last, the bioreactor was fed with the mixture of municipal wastewater and pre-ozonized  
129 RW in continuous mode: the proportion of pre-ozonized RW was increased gradually  
130 up to 100% while the biofilm growth stabilized. In the continuous stage, the feed water  
131 was circulated between the feed tank and the bioreactor [15]. The acclimation of SMBR  
132 was the same as MBBF-SMBR, except for the addition of carriers. The detailed start-up  
133 strategy is found **in supplementary material**.

134 After the biofilm was acclimated, the SMBR and the MBBF-SMBR were operated  
135 in continuous mode. The operating conditions of the two systems were similar. The av-  
136 erage membrane flux was  $12 \text{ L m}^{-2}\text{h}^{-1}$  (LMH). The nominal hydraulic retention time  
137 (HRT) was 48 h while the nominal solid residence time (SRT) was 30 d. The experiment  
138 lasted 110 days. Days 1 to 50 were the start-up period, while days 51 to 111 were the  
139 formal experimental operation period. In the following description, the first day of the  
140 formal experimental operation is described as day 0.

## 141 **2.3 Analytical methods**

### 142 **2.3.1 SGW quality analysis**

143 Water samples were collected from the feed and the effluent during the experi-  
144 ments. DO, pH, and turbidity were measured with a DO meter (JPBJ-610L, INESA,  
145 Shanghai, China), a pH meter (PB-10, Sartorius Scientific Instruments Co, Ltd.,  
146 Gottingen, Germany), and a turbidimeter (TL2310, Hach Company, Loveland, USA),  
147 respectively. An Ultrameter II 6PFC (Myron L Company, Carlsbad, USA) portable mul-  
148 tifunctional meter was utilized to determine the TDS. The DOC concentration was de-

149 terminated with a total organic carbon analyzer (TOC-L CPH, Shimadzu, Japan). Total  
150 nitrogen (TN) was quantified by alkaline potassium persulphate digestion-UV spectro-  
151 photometric method using the special reagent LH-NT (Lianhua Environmental Protec-  
152 tion Technology Co., Ltd., Lanzhou, China). A UV-Vis spectrophotometry (Orion Aq-  
153 uaMate 8000, Thermo Fisher Scientific Inc., MA, USA) was used to measure UV ab-  
154 sorbance at 254 nm.

155 A three-dimensional fluorescence excitation emission matrix (3D EEM) spectro-  
156 fluorometer (F-7000, Hitachi, Japan) was used to characterize the DOM fraction in the  
157 feed, effluent, and membrane fouling layer. Samples were filtered and diluted to a  $UV_{254}$   
158 of 0.05 to avoid inner filter effect. In this study, the EEM spectra were collected by  
159 scanning emission wavelengths from 200 nm to 550 nm at 1 nm increments and excita-  
160 tion wavelengths from 200 nm to 400 nm at 5 nm increments. The excitation and emis-  
161 sion slit width was set at 5 nm and the scanning speed was set at 12000 nm/min. The  
162 EEM spectrum of deionized (DI) water was collected as the blank and was subtracted  
163 from all the sample spectra to remove the influence of Raman scattering.

### 164 **2.3.2 UF membrane fouling analysis**

165 The surface morphology and elemental composition of the membrane fouling lay-  
166 ers were investigated by scanning electron microscopy (SEM) and energy dispersive  
167 spectroscopy (EDS) (Regulus 8230, Hitachi, Tokyo, Japan). Before SEM-EDS analysis,  
168 the membrane samples were coated with ~2 nm of gold using a magnetron sputter  
169 (MSP-2S, IXRF Systems, USA). The method of filtration resistance analysis was de-  
170 scribed in detail in previous study [25].

#### 171 **2.3.4 Biological analysis**

172 To assess the bacterial populations in the two bioreactors and analyze the function  
173 of the microorganisms, the RW, pre-ozonized RW, suspended sludge, and biofilm at-  
174 tached on carriers at the end of the experiment were collected and analyzed. DNA ex-  
175 traction, polymerase chain reaction (PCR) amplification, and Illumina Miseq sequenc-  
176 ing were conducted in the same way as in previous study [25]. Analyses of the alpha  
177 diversity (e.g., Chao, Shannon, Simpson, ACE, and Coverage), principal component  
178 (PCA), bacterial community composition, and metabolic functional prediction were  
179 performed using the online Majorbio I-Sanger Cloud Platform ([www.i-sanger.com](http://www.i-sanger.com)).

### 180 **3 Results and discussion**

#### 181 **3.1 Contaminant removal performance**

##### 182 **3.1.1 Organic matters and nitrogen removal**

183 The characteristics of RW, pre-ozonized RW, and the effluent of SMBR and  
184 MBBF-SMBR are listed in Table 1. The organic matter removal rates of SMBR and  
185 MBBF-SMBR treatments are presented in Fig 2. The DOC removal rates were as high  
186 as 87.8% (SMBR) and 87.9% (MBBF-SMBR), but as the running time increased, the  
187 DOC removal rate decreased to 59.5% (SMBR) and 70.1% (MBBF-SMBR), with the  
188 MBBF-SMBR showing an obviously higher removal of DOC after 45 days. Overall, the  
189 average DOC removal rates were 73.9% (SMBR) and 77.8% (MBBF-SMBR). In the  
190 middle of the experiment, the DOC of the influent stream decreased significantly, which  
191 may be caused by the volatilization of a fraction of organic matter due to frequent  
192 opening of the container in the summer or degradation by anaerobic bacteria in the raw

193 water. The DOC contents of the mixture liquid were 39.61 mg/L in SMBR and 36.94  
194 mg/L in MBBF-SMBR, higher than in the influent, indicating some accumulation of  
195 organic matter and possibly some toxic materials, such as biocides used as additive in  
196 the hydraulic fluid, which may inhibit the biological activity. The removal of  $UV_{254}$  was  
197 low both in the SMBR and in the MBBF-SMBR process, thus that the  $UV_{254}$  values of  
198 the effluent were sometimes higher than those of the influent water. Organic matters  
199 containing aromatic chromophores or unsaturated bonds (primarily humic substances)  
200 are related to the value of  $UV_{254}$  [26]. Considering the high DOC removal, some humic  
201 matter with high light absorption at 254 nm may have been produced by microorgan-  
202 isms as a byproduct of the degradation of other parent substances. As for TN, the  
203 MBBF-SMBR showed higher removal rates, which may be due to simultaneous nitrifi-  
204 cation and denitrification processes occurring on the carriers [23]. Because of the influ-  
205 ence of high salinity and pH value to nitrifying bacteria [27, 28], the average value of  
206 TN removal rate were only 18.6% (SMBR) and 37.0% (MBBF-SMBR), lower than that  
207 observed with low salinity wastewater in other studies (>60%) [29].

208 Compared with other systems treating high salinity wastewater, the MBBF-SMBR  
209 system showed a high removal rate of organic matters. According to Lester's study [30],  
210 the removal rate of dissolved chemical oxygen demand decreased from 90% to 60%  
211 when the TDS increased from 1500 mg/L to 45000 mg/L in their synthetic hydraulic  
212 fracturing flowback stream treated with an activated sludge mixed liquor. Wang [31]  
213 used an aerobic sludge (AS) system to treat FPW, and removed about 72% organics  
214 with TDS of 16087 mg/L, lower than that observed in this study using the

215 MBBF-SMBR.

### 216 **3.1.2 Fluorescence EEM spectra**

217 The EEM fluorescence spectra of the RW and of the effluents are presented in Fig  
218 2(c)-(f). In general, peaks in the excitation wavelength range from 200 to 250 nm and  
219 the emission wavelength range from 280 to 380 nm (Regions I: 280-330 nm; Regions II:  
220 330-380 nm) are associated with simple aromatic proteins, such as tyrosine and trypto-  
221 phan. Peaks located in the range of excitation wavelengths from 200 to 250 nm and the  
222 emission wavelengths from 380 to 550 nm represent fulvic acid-like substances (Region  
223 III). Peaks in the range from 250 to 400 nm excitation wavelengths and from 280 to 380  
224 nm emission wavelengths are related to soluble microbial by-product-like material (Re-  
225 gion IV). Peaks from 250 to 400 nm (excitation) and from 380 to 550 nm (emission) are  
226 related to humic acid-like organics (Region V) [32, 33]. The fluorescence regional inte-  
227 gration (FRI) has been used for semi-quantitative analysis of DOM in SGW, and EEM  
228 fluorescence intensity is proved to be positively correlated with the real concentration of  
229 soluble microbial by-product-like material and aromatic proteins in produced water [34].  
230 The distribution of FRI of DOM samples in the SMBR and MBBF-SMBR systems is  
231 shown in Fig 2 (g).

232 Soluble microbial by-product-like matters (region IV) was the dominant fraction in  
233 RW, and the ozonation removed most fluorescent fraction (75.9%) of the DOM. Soluble  
234 microbial by-product-like matters (region IV) and humic acid-like organics (Region V)  
235 increased after the SMBR unit (38.2%) or the MBBF-SMBR unit (70.6%). In particular,  
236 a significant peak appeared for soluble microbial by-product-like matters (region IV) in

237 the MBBF-SMBR effluent, indicating that the low molecular weight DOM produced by  
238 microorganisms in the bioreactor could not be rejected by membrane, and that the mi-  
239 crobial activity in the MBBF-SMBR might be stronger than SMBR. This result is con-  
240 sistent with the rationalization discussed above about the values of  $UV_{254}$  and their ap-  
241 parent uniformity between influent and effluents streams.

## 242 **3.2 Analysis of membrane fouling**

### 243 **3.2.1 Characteristics and elemental analysis of the cake layers**

244 The fouling cake characteristics of SMBR and MBBF-SMBR membranes were  
245 analyzed with EDS, as shown in Fig 3. Representative SEM micrographs can be ob-  
246 served in the supplementary material file. Compared with the pristine membrane, the  
247 fouled membrane surface was covered by a thick layer of foulants. The surface of the  
248 sample from the SMBR was rougher, while the cake on the sample from the  
249 MBBF-SMBR was more porous. Numerous spherical particles were observed on the  
250 surface of the SMBR membrane, which may be silica (see supplementary material).  
251 According to the EDS analysis, organic foulants (C, O) were dominant, but inorganic  
252 elements (Si, Cl, I, Fe, Ca, Al, Na, K, Mg, Mn) were also observed on the surface. The  
253 proportion of Si was significantly larger for the membrane used in the SMBR, suggest-  
254 ing that inorganic pollution on SMBR may be more severe, and consistent with SEM  
255 images. As a consequence, the proportion of C and N elements was higher on the  
256 MBBF-SMBR membrane surface, which might be also partly due to a larger proportion  
257 of proteins attached on the MBBF-SMBR membrane surface. The membrane organic  
258 foulants, mainly including proteins and polysaccharides, were likely extracellular poly-

259 meric substance (EPS). The increased ratio of proteins from EPS was reported to be an  
260 important reason for decreasing membrane fouling in previous studies, which would be  
261 consistent with the trend observed in the two different reactors and the larger fraction of  
262 proteins found on the MBBF-SMBR membrane sample [35].

### 263 **3.2.2 Organic composition of the cake layers**

264 The EEM fluorescence spectra of the cake layers were acquired to analyze their  
265 organic composition (Fig 4(a) - (c)). Three peaks were observed on the SMBR mem-  
266 brane: Ex/Em of 280/350 nm in region IV; 225/350 nm in region II; 275/455 nm in re-  
267 gion V. The peak in region V was not observed on MBBF-SMBR samples, suggesting  
268 that the macromolecular organic matters were degraded more significantly in the  
269 MBBF-SMBR system. Moreover, fouling components were lower for every region in  
270 the MBBF-SMBR sample with respect to SMBR membranes. The DOC values of the  
271 foulants were 110.9 mg/m<sup>2</sup> (SMBR) and 59.1 mg/m<sup>2</sup> (MBBF-SMBR), respectively,  
272 corroborating that the membrane fouling of SMBR was more significant and the result-  
273 ing cake layer thicker and/or denser.

274 The fouling resistance was determined at the end of the experiments and shown in  
275 Fig 4(d). The final total membrane resistance for SMBR and MBBF-MBR were  $12.50 \times$   
276  $10^{12} \text{ m}^{-1}$  and  $8.92 \times 10^{12} \text{ m}^{-1}$ , and the reversible portions were  $8.38 \times 10^{12} \text{ m}^{-1}$  and  $5.33$   
277  $\times 10^{12} \text{ m}^{-1}$ , representing 67.0 % and 59.7 % of the total resistance, respectively. These  
278 data indicate that most fouling could be removed by physical cleaning with water, and  
279 that the fouling in SMBR was more significant and lightly more reversible than that in  
280 MBBF-SMBR. The porosity observed for the MBBF-SMBR cake layer (Fig 3(c)) and

281 the different composition in the two reactors, related to a larger EPS fraction and to an  
282 improved biodegradation in the MBBF-SMBR, may explain the lower resistance quan-  
283 tified for this system compared to the SMBR system.

### 284 **3.3 Bacterial diversity**

285 The Simpson, Shannon, chao1, abundance-based coverage estimator (ACE) and  
286 coverage are shown in Table 2 to evaluate the microbial richness and diversity of sam-  
287 ples. The high coverage indicates that the analyses are robust and covered almost all  
288 sequences of samples. The microbial diversity (Shannon and Simpson indexes) and mi-  
289 crobial richness (ACE and Chao 1 indexes) from bioreactors on day 0 of the experi-  
290 mental test were significantly higher than those on day 60. This result suggests that the  
291 accumulation of toxicity and salinity with the operating time inhibited the growth of  
292 some bacteria originated from domestic sewage from the municipal treatment plant. In  
293 addition, ozonation inactivated some bacteria, with the richness and diversity of  
294 pre-ozonized RW being the lowest among all samples. The PCA analysis (see supple-  
295 mentary material) suggested that the community composition of samples from the bio-  
296 reactors became more similar and approached that of the pre-ozonized RW as the run-  
297 ning time increased.

#### 298 **3.3.1 Bacterial community in SMBR and MBBF-SMBR systems**

299 The bacterial community composition at the phylum level of RW, pre-ozonized  
300 RW, and samples obtained at different times (day 0 and day 60) from the two biological  
301 treatment systems is shown in Fig 5(a). A total of 43 bacterial phyla were identified.  
302 Seed sludge enriched the microbial community composition, so the quantity of phyla

303 was much larger than what previously detected in SGW [34, 36]. *Proteobacteria* was  
304 the most dominant bacterial in all samples (29.8%-87.9%), with the dominance becom-  
305 ing more obvious at the end of the experiments. In RW, *Desulfobacterota* (13.0%), *Bac-*  
306 *teroidota* (10.7%), *Chloroflexi* (10.3%) were also major phyla. Because members of  
307 *Thermotogota* are anaerobic [37], this phylum was not found in other samples except  
308 RW. *WPS-2*, living in oxygen-rich environments [38], was a dominant phylum only in  
309 pre-ozonized RW (32.3%), possibly due to the large amount of oxygen produced by  
310 ozone decomposition. The inactivation effects of ozonation were obviously observed on  
311 *Actinobacteriota*, *Chloroflexi*, *Firmicutes*, *Acidobacteriota*, *Patescibacteria*, and *Verru-*  
312 *comicrobiota*, causing the content of these phyla to drop rapidly in pre-ozonized efflu-  
313 ents. In samples obtained on day 0, *Actinobacteriota* (13.9%-27.5%), *Bacteroidota*  
314 (10.0%-11.8%), *Chloroflexi* (8.1%-17%) were the major phyla after *Proteobacteria*. In  
315 samples obtained on day 60, *Actinobacteriota* (3.2%-12.9%) was still a major phylum,  
316 but the third major group changed to *Gemmatimonadota* (2.1-6.2%). *Gemmatimonadota*  
317 was not found in significant fraction in RW and pre-ozonized RW, thus this phylum is  
318 hypothesized to originate from the seed sludge. These results indicate that the composi-  
319 tion on the phylum level changed and adapted during the experiment.

320 The community compositions were further analyzed at genus level taxonomy (Fig.  
321 5(b)). A total of 686 bacterial genera were recognized. *Norank\_o\_\_OPB41*, which was  
322 related to methanogenesis and is most likely anaerobic, was the major genus in RW.  
323 *Roseovarius*, which has been found in different oil reservoirs and water injection treat-  
324 ment systems [39], was also important in RW and especially pre-ozonized RW.

325 The dominant bacteria in the two bioreactors were similar, but very different com-  
326 pared to those observed in RW. Specifically, some bacteria related to the removal of ni-  
327 trogen and other contaminants were detected, including *Denitromonas*, *nor-*  
328 *ank\_f\_\_Xanthomonadaceae*, *norank\_f\_\_Gemmatimonadaceae*, *Paracoccus*, *Roseovari-*  
329 *us*, *norank\_f\_\_JG30-KF-CM45*, *Iodidimonas*, *Nocardia*, *Erythrobacter*, *Defluviimonas*,  
330 *Mycobacterium*, *Paracoccus*, *Muricauda*, *Thalassospira* and *norank\_f\_\_Rhodococcus*.  
331 The summary of functions of these genera and their reference sources can be found  
332 in supplementary materials. *Denitromonas* is a kind of denitrifying bacteria, and often  
333 discovered in hypersaline wastewater [40]. *Norank\_f\_\_Xanthomonadaceae*, *nor-*  
334 *ank\_f\_\_Gemmatimonadaceae*, *Defluviimonas* and *Paracoccus* also have the ability of  
335 denitrification. *Roseovarius* (also found in RW) and *norank\_f\_\_JG30-KF-CM45* are  
336 nitrifying bacteria. Most of the nitrifying bacteria live in slightly alkaline environments.  
337 Possibly due to the consumption of alkalinity and the decrease of pH value during oper-  
338 ation, the presence of denitrifying bacteria became much larger than that of nitrifying  
339 bacteria after 60 days. *Roseovarius* also belongs to sulfur-oxidizing prokaryotes, and  
340 has the ability to produce iodinated organic compounds [41]. *Iodidimonas* can oxidize  
341 organic iodine, and according to the EDS results of membrane fouling, the high iodine  
342 content in bioreactors might be their nutrient source. There might be a symbiotic rela-  
343 tionship between *Roseovarius* and *Iodidimonas* in bioreactor systems. *Nocardia* is  
344 common filamentous bacteria in activated sludge and can degrade and assimilate recal-  
345 citrant aliphatic hydrocarbons [42]. It has been reported that *Pseudomonas* can degrade  
346 and detoxify phenolic compounds, including phenol, pentachlorophenol, and p-cresol

347 [43]. *Erythrobacter* contribute to the degradation of hydrocarbons, especially aromatic  
348 substances in saline oily wastewater [44]. *Defluviimonas*, *Mycobacterium*, *Paracoccus*,  
349 *Muricauda*, and *Thalassospira* are related to the degradation of PAHs. *Nor-*  
350 *ank\_f\_\_Rhodococcus* can use hydroxylated derivatives of polychlorobiphenyls  
351 (HO-PCBs) as a source of carbon [45]. The MBBF-SMBR content of bacteria, mainly  
352 with the ability of denitrification and PAHs removal (*Denitromonas*, *Roseovarius*, *nor-*  
353 *ank\_f\_\_Caldilineaceae*, *Defluviimonas*, *Mycobacterium*, and *Paracoccus*) was obvi-  
354 ously higher than that in the SMBR. This different composition may be the principal  
355 reason for higher TN and DOC removal rates determined in the MBBF-SMBR system.

### 356 **3.3.2 Bacterial functional prediction in SMBR and MBBF-SMBR systems**

357 The metabolic functions of bacterial communities were predicted using the soft-  
358 ware package “Phylogenetic Investigation of Communities by Reconstruction of Unob-  
359 served States” (PICRUSt, v2.0.0) based on the Kyoto Encyclopedia of Genes and Ge-  
360 nome (KEGG) Orthology database. 24 functional pathways from 5 categories are listed  
361 in Fig 6. Amino acid, carbohydrate, energy, and nucleotide metabolism were found to  
362 be related to the transformation of the main nutrients [46], while membrane transport  
363 was reported to play a crucial role in the survival of microbes in adverse environments,  
364 including the hypersaline environment [47].

365 The functional profile of ABC transporters was found at a higher relative abun-  
366 dance in biofilms than in the sludge, and it decreased from 3.27-3.79% on day 0 to  
367 3.02-3.51% on day 60 of the experimental tests. Membrane transporters are related to  
368 the material exchange between bacteria and the environment, and might contribute to

369 biofilm formation [48]. They were also reported as directly or indirectly affecting fac-  
370 tors connected to hydrocarbon metabolism [49]. Previous research found that with the  
371 increase of salinity the content of ABC transporters increased [50], the opposite of what  
372 generally observed in this study. It is hypothesized that other factors like the acidic en-  
373 vironment may have slightly inhibited the expression of genes related to ABC trans-  
374 porters, causing their decrease after 60 days. Higher proportion of nitrogen metabolism  
375 was found in biofilm and it may be the cause of the higher TN removal rate in  
376 MBBF-SMBR. Overall, most of the pathways about metabolism were common among  
377 the eight samples, with some slight differences in the relative abundance of each meta-  
378 bolic pathway for the various samples. For example, the sequences related to nucleotide  
379 metabolism and alanine, aspartate and glutamate metabolism showed relative higher  
380 abundance in RW, and may suggest more energy consumption. Higher abundance of  
381 methane metabolism was found in RW indicating more anaerobic bacteria. The ability  
382 of starch and sucrose metabolism, citrate cycle (TCA cycle), glycolysis / gluconeogene-  
383 sis, and amino sugar and nucleotide sugar metabolism (included in carbohydrate metab-  
384 olism) were significantly lower after ozonation.

#### 385 **4. Conclusion**

386 The results of this study indicated that the addition of biofilm attached to carriers  
387 significantly improved the removal efficiency of DOC and TN in ozonation-SMBR  
388 systems by 3.9% and 18.4%, respectively. This work demonstrated the feasibility and  
389 potential of MBBF-SMBR following ozonation for SGW treatment, and identified the  
390 accumulation of bacteria related to organic and nutrient removal. These functional bac-

391 teria were found in larger amount in MBBF-SMBR, causing higher organics removal  
392 rate. The functional genes related to membrane transport and nitrogen metabolism were  
393 enhanced in biofilm, which may be one of the reasons for the higher organic removal in  
394 the MBBF-SMBR system.

#### 395 **Appendix A. Supplementary data**

396 E-supplementary data for this work can be found in e-version of this paper online.

#### 397 **Acknowledgment**

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403

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555 **Figure captions**

556 **Fig 1.** Schematic diagram of the SMBR and MBBF-SMBR.

557 **Fig 2.** Organic compounds removal: (a) DOC influent concentration (right axis), efflu-  
558 ent concentration (right axis), and removal rates (left axis) as a function of time during  
559 SMBR and MBBF-SMBR treatment. (b) Average values of DOC,  $UV_{254}$ , and TN re-  
560 moval rates (n=21). 3D EEM fluorescence spectra of: (c) RW; (d) pre-ozonized RW; (e)  
561 effluent of SMBR; (f) effluent of MBBF-SMBR. (g) FRI distribution of RW,  
562 pre-ozonized RW, effluent of SMBR, and effluent of MBBF-SMBR.

563 **Fig 3.** EDS analysis results for fouled membrane from (a) SMBR, (b) MBBF-SMBR  
564 (magnification: 1000× and 5000×).

565 **Fig 4.** Composition of the membrane fouling layer. 3D-EEM fluorescence spectra of  
566 membrane fouling from (a) SMBR and (b) MBBF-SMBR. (c) FRI distribution of mem-  
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569 ( $R_{ir}$ ) at the end time of SMBR and MBBF-SMBR tests.

570 **Fig 5.** Bacterial community composition of: RW; pre-ozonized RW; samples (sludge  
571 and biofilm) obtained from SMBR and MBBF-SMBR on day 0 and day 60. Analysis at:  
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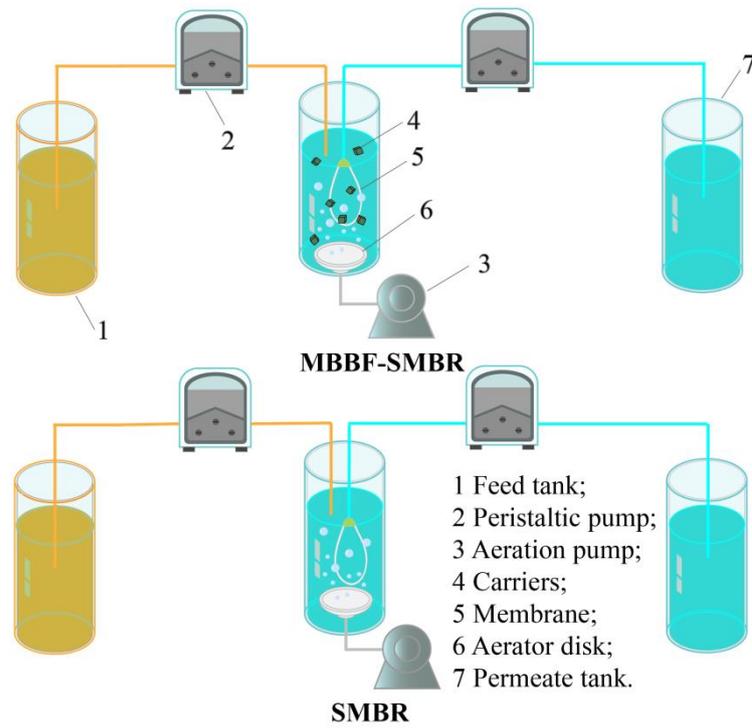
581

582 **Table captions**

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585 **Table 2.** Estimates of richness and diversity for operational taxonomic units (OTUs)  
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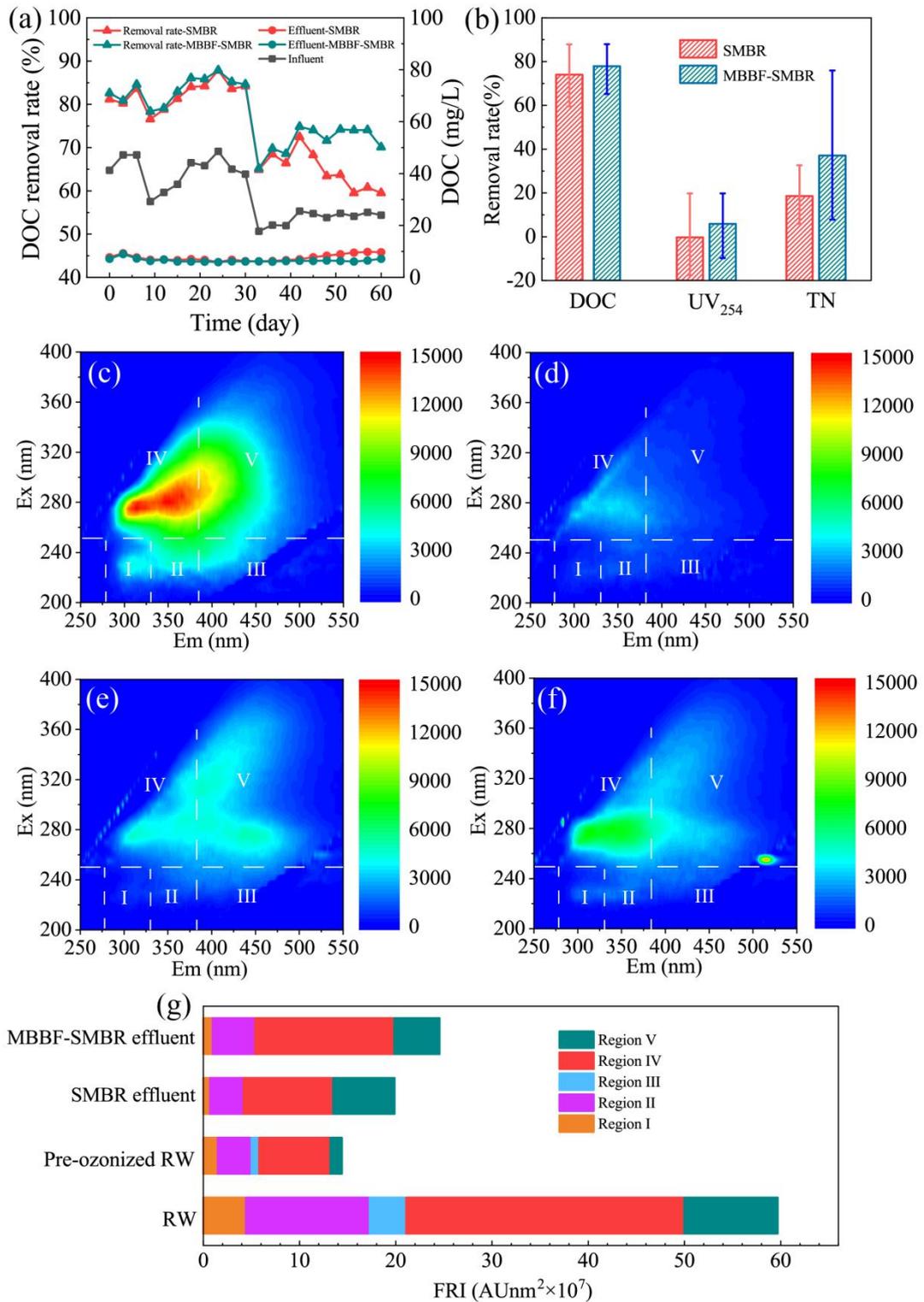
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593 **Fig 1.** Schematic diagram of the SMBR and MBBF-SMBR.

594



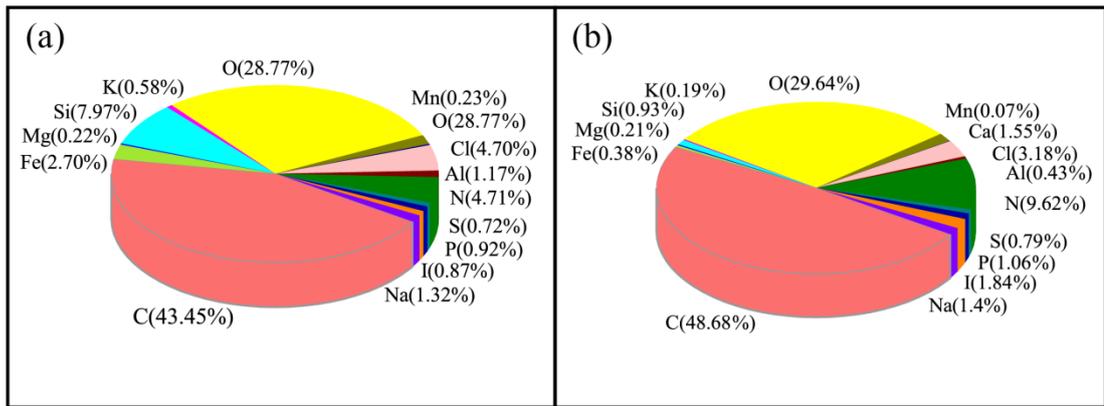
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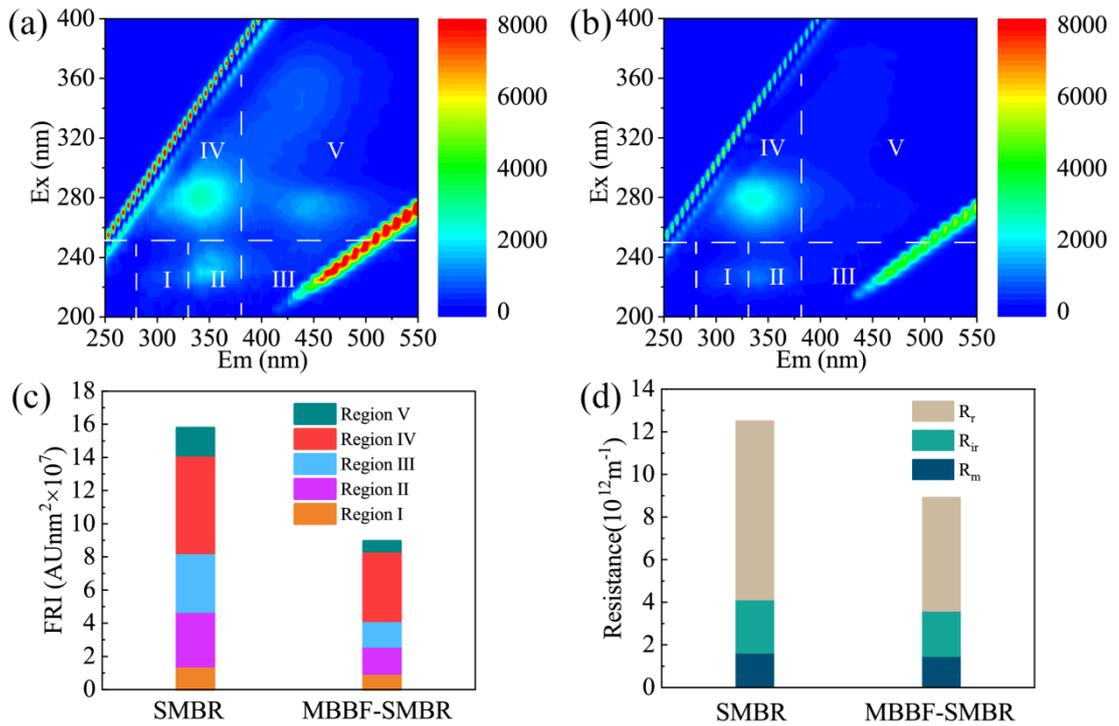


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607

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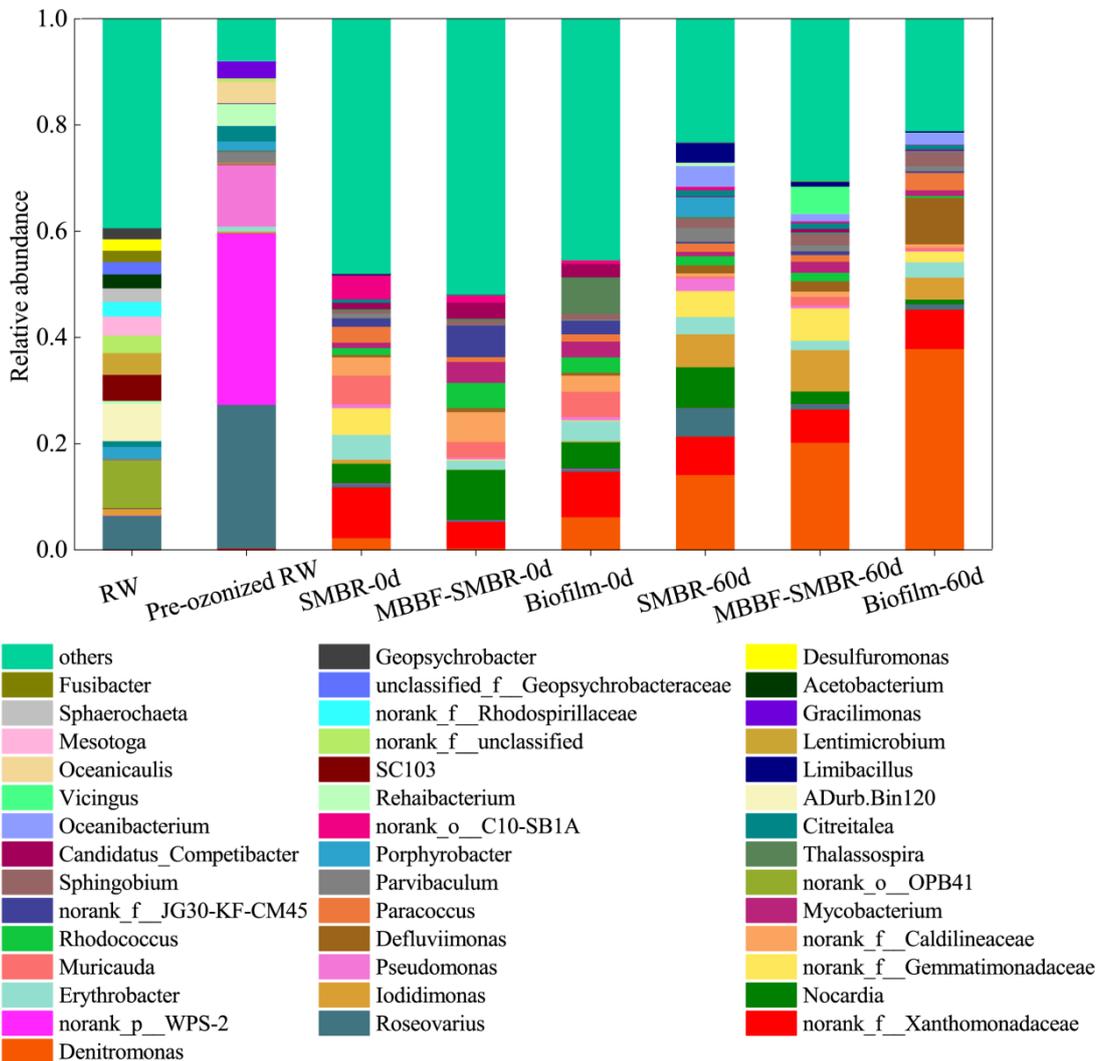
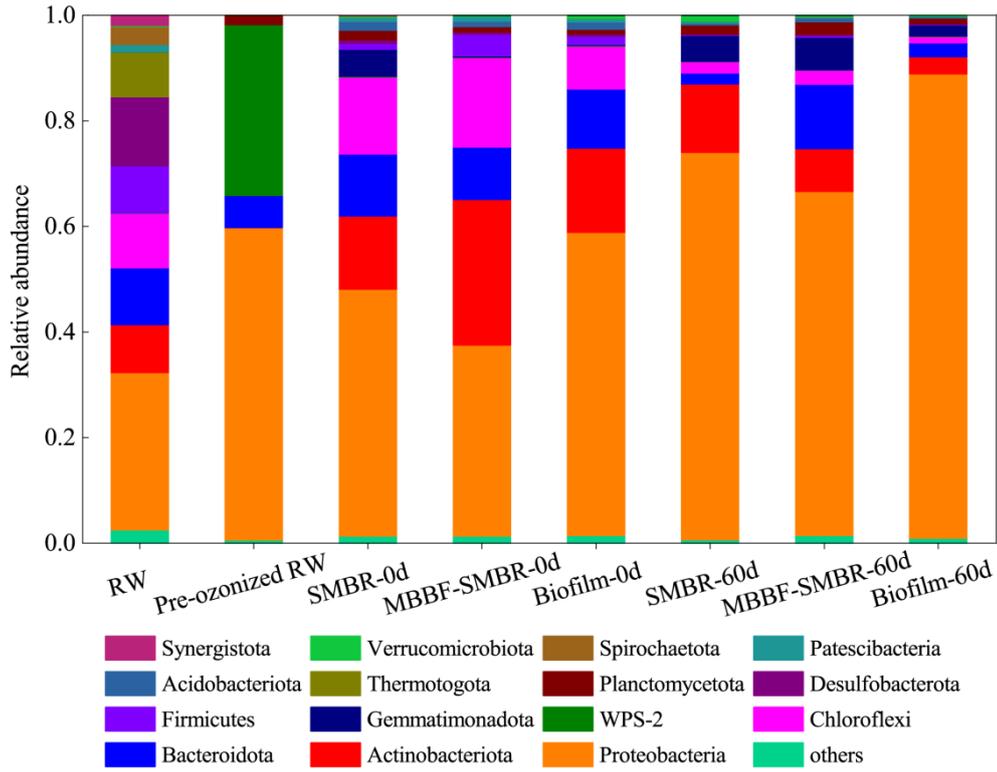
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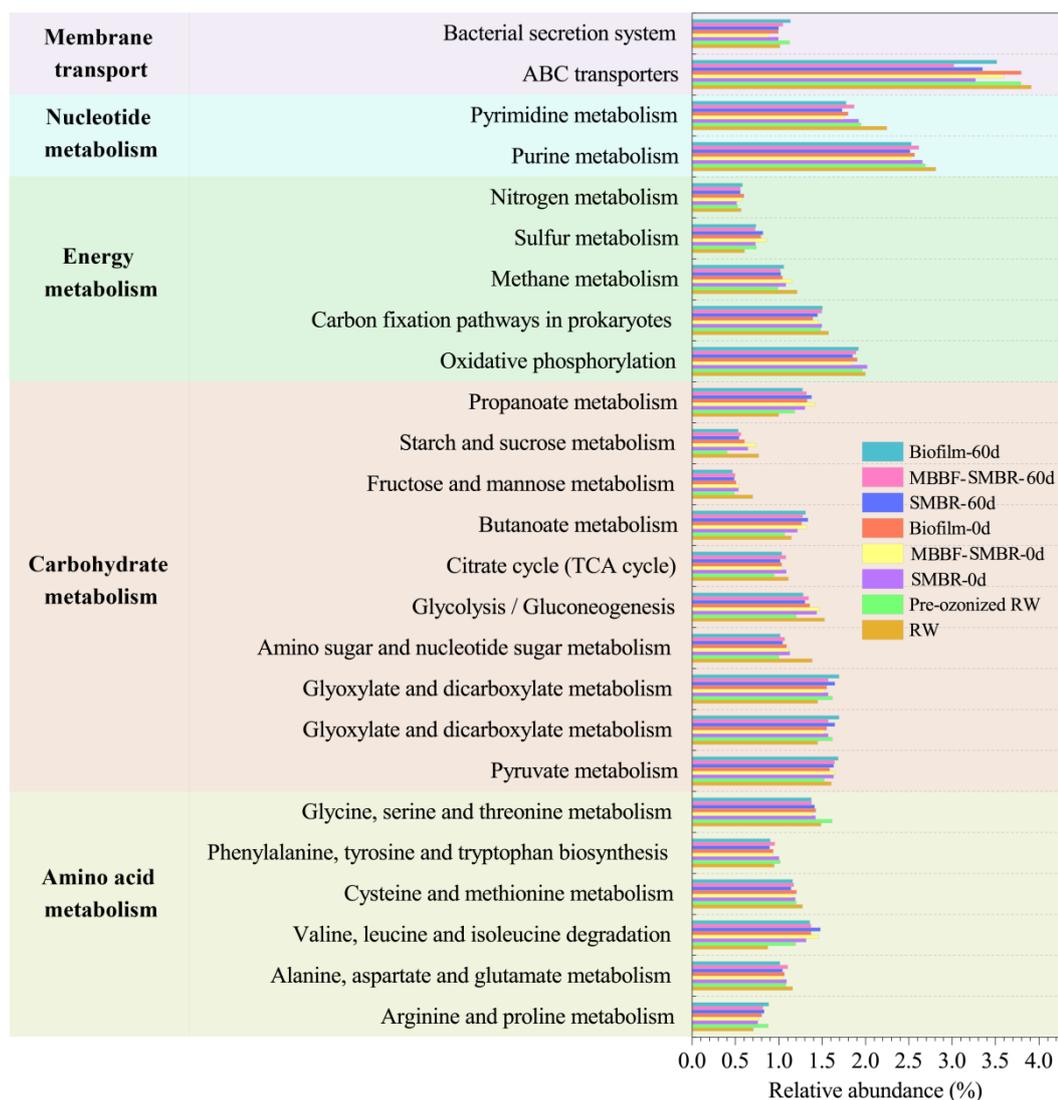
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613



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628

629 **Table 1.** Characteristics of RW, pre-ozonized RW, effluent of SMBR, and effluent of  
 630 MBBF-SMBR.

<b>Parameters</b>	<b>RW</b>	<b>Pre-ozonized RW</b>	<b>SMBR</b>	<b>MBBF-SMBR</b>
pH	7.28	7.77	5.65	5.23
TDS (g/L)	19.69	19.98	20.65	20.84
Electrical conductivity (mS)	32.34	32.73	33.28	33.79
Turbidity (NTU)	30	19.4	0.19	0.18
DOC (mg/L)	27.32	32.34	7.56	6.53
TN (mg/L)	64.51	46.56	37.77	29.45
UV <sub>254</sub> (cm <sup>-1</sup> )	0.115	0.087	0.087	0.082

631

632

633 **Table 2.** Estimates of richness and diversity for operational taxonomic units (OTUs)  
634 definition of 97% similarity for RW, pre-ozonized RW, and for samples (sludge and  
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637 and MBBF-SMBR systems, while “Biofilm” refers to the biofilm samples from the  
638 MBBF-SMBR system).

<b>Samples</b>	<b>Shannon</b>	<b>Simpson</b>	<b>Ace</b>	<b>Chao 1</b>	<b>Coverage</b>
RW	4.24	0.03	264.89	263.33	1.00
Pre-ozonized RW	2.70	0.13	185.30	165.33	1.00
SMBR -0d	4.82	0.02	1203.11	1180.38	0.99
MBBF-SMBR -0d	4.91	0.02	1228.62	1247.05	0.99
Biofilm-0d	4.70	0.03	1198.41	1200.22	0.99
SMBR -60d	3.90	0.05	831.78	827.67	1.00
MBBF-SMBR -60d	4.10	0.06	1076.83	1161.83	0.99
Biofilm-60d	3.13	0.16	773.38	751.52	1.00

639