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1 Organics Removal from Shale Gas Wastewater  
2 by Pre-oxidation Combined with Biologically  
3 Active Filtration

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20 **ABSTRACT:** Biological treatment technology is increasingly explored in shale gas  
21 wastewater (SGW) treatment owing to its cost effectiveness and requires efforts to  
22 improve its efficacy. In this work, ozone and ferrate(VI) oxidation pre-treatment were  
23 evaluated to enhance the performance of the subsequent biologically active filtration  
24 (BAF) in the removal of organic contaminants. The oxidation improve the SGW  
25 biodegradability and organic composition in the presence of high salinity (~20 g/L).  
26 Due to the degradation activity of microorganisms, the organics removal efficiency in  
27 the BAF system was observed to gradually improve and then reaching stability in  
28 long-term continuous-mode operation. The removal rate of dissolved organic carbon  
29 (DOC) of the ozone-BAF (O<sub>3</sub>-BAF) and the ferrate(VI)-BAF (Fe(VI)-BAF) systems  
30 was 83.2% and 82.8%, respectively, higher than that of BAF alone (80.9%). This  
31 increase was attributed to higher activity and content of microorganisms in O<sub>3</sub>-BAF  
32 and Fe(VI)-BAF systems. The presence of uncultured bacteria of genus  
33 *Rehaibacterium* with high abundance of 7.2-21.0% was significantly correlated with  
34 DOC removal. Also, uncultured bacteria of genus *Methyloversatilis* (2.24-22.31%)  
35 were significantly correlated with fluorescent organics removal. Results suggest that  
36 these two bacterial species have strong ability to degrade organics. More research is  
37 needed to understand whether the species were new and their specific function. This  
38 study provides valuable suggestions for extracting safe water from SGW with an  
39 efficient treatment train.

40 **KEYWORDS:** Shale gas wastewater; Biologically active filtration; Ozone; Ferrate

41 (VI); Microbial community

42

## 43 1. INTRODUCTION

44 With the fast development of the shale gas industry, associated shale gas  
45 wastewater (SGW) streams caused by hydraulic fracturing increasingly threaten water  
46 ecosystem and human health. In 2016, 6 million tons and 131 million tons of SGW  
47 were produced in the Sichuan Basin of China and in the United States, respectively.<sup>1,2</sup>  
48 SGW contains high concentrations of salt, heavy metals, microorganisms, and  
49 refractory organics released from the shale formation or as chemical residue of  
50 compounds added to enhance hydraulic fracturing.<sup>3, 4</sup> Effectively managing SGW  
51 produced from shale gas extraction has become an urgent environmental and  
52 engineering issue.

53 Membrane technologies are usually considered as the most effective tertiary  
54 treatment for SGW, but they require significant energy and face serious fouling  
55 challenges.<sup>4,5</sup> A limited number of studies has investigated hybrid systems that apply  
56 biological treatment to reduce membrane fouling, and found that biological treatment  
57 processes can efficiently control fouling in ultrafiltration and nanofiltration.<sup>6</sup>  
58 Furthermore, cost-effective biological treatment technology is regarded as a  
59 high-potential treatment technology for SGW, because the large amount of organics  
60 present in SGW is mostly biodegradable.<sup>7,8</sup> In the past few years, activated sludge,<sup>9</sup>  
61 sequencing batch reactor,<sup>10,11</sup> membrane bioreactor,<sup>12-14</sup> microbial mats,<sup>15,16</sup> moving  
62 bed biofilm reactor,<sup>17</sup> biologically active filtration (BAF)<sup>6,18-21</sup> and bioelectrochemical  
63 system<sup>22-26</sup> have been evaluated for SGW treatment.

64 BAF exploits the biofilm attached to filter media to degrade and adsorb organics  
65 from the wastewater.<sup>3</sup> Limited research has shown that BAF can remove organics  
66 (72-90% COD and 72-92% DOC) from six different SGW generated in basins of USA  
67 with varying salinity (10.5-31.2 g/L TDS) and organics content (85-6360 mg/L COD  
68 and 36-2170 mg/L DOC).<sup>6, 19</sup> The BAF efficiency under different operating conditions  
69 (aeration rate, temperature, empty bed contact time, and type of activated carbon) was  
70 systematically studied.<sup>19-21</sup> However, analysis of the microbial community  
71 composition and function in BAF is still scarce. Concurrently, the feasibility of BAF  
72 in treating SGW needs further evaluation and the mechanism of pollutant migration  
73 and transformation in BAF needs more detailed study.

74 At present, the combination of ozonation and BAF (O<sub>3</sub>-BAF) has been widely  
75 used in water treatment, because ozone can degrade refractory pollutants and improve  
76 the performance of the subsequent biological process.<sup>27, 28</sup> Also, biological processes  
77 can effectively remove ozonation by-products. This system has never been tested for  
78 the treatment of shale gas wastewater and its feasibility is still unclear for this  
79 application. Ferrate(VI) or Fe(VI) has been also successfully used in water and  
80 wastewater treatment as a new type of green oxidant.<sup>29</sup> The redox potential of Fe(VI)  
81 is +0.7-+2.2 V.<sup>29</sup> The redox potential of ozone is slightly higher than that of Fe(VI) in  
82 solutions of basic pH.<sup>29</sup> SGW typically contains high concentrations of Cl<sup>-</sup> and Br<sup>-</sup>,  
83 which seriously weaken the oxidation ability and the safety of ozonation.<sup>30</sup> On the  
84 contrary, Fe(VI) has no known reactivity with halogens,<sup>29</sup> indicating that Fe(VI) might

85 have a certain advantage in oxidizing shale gas wastewater. Similar to ozone, Fe(VI)  
86 can degrade refractory pollutants and improve their biodegradability. Nevertheless,  
87 there are only a few studies on the combination of Fe(VI) and biological  
88 processes.<sup>31-33</sup> Ma et al.<sup>32</sup> found that Fe(VI) (1 mg/L) pretreatment significantly  
89 increased the removal rate of COD<sub>Mn</sub>, UV<sub>254</sub>, NH<sub>4</sub> by BAF in treating river water.  
90 Besides, a simple comparison of Fe(VI)-BAF and O<sub>3</sub>-BAF showed that the COD<sub>Mn</sub>  
91 removal rate of Fe(VI)-BAF was slightly lower than that of O<sub>3</sub>-BAF, and the NH<sub>4</sub><sup>+</sup>  
92 removal rate of Fe(VI)-BAF was much higher than that of O<sub>3</sub>-BAF. In general,  
93 Fe(VI)-BAF has shown interesting potential, but a more systematic and  
94 comprehensive assessment of Fe(VI)-BAF is needed, especially in the treatment of  
95 SGW.

96 Therefore, the objectives of this study are to (i) evaluate the effect of Fe(VI) and  
97 O<sub>3</sub> pre-oxidation on organics removal; (ii) assess the effect of Fe(VI) and O<sub>3</sub>  
98 pre-oxidation on the performance of BAF systems; (iii) analyze the composition and  
99 evolution of microbial community in such hybrid systems, and explore the dominant  
100 and functional microorganisms; (iv) comprehensively analyze the feasibility of  
101 Fe(VI)-BAF in treating SGW compared with that of O<sub>3</sub>-BAF.

102

## 103 **2. EXPERIMENTAL SECTION**

104 **2.1. Water Samples and Water Quality Analysis.** SGW samples were collected  
105 from the Changning shale gas play (Sichuan Basin, China). Due to the high turbidity (>

106 1000 NTU) of SGW, SGW was pre-treated with coagulation-sedimentation before the  
107 subsequent processing. Therefore, the raw water in the article referred to the SGW  
108 after coagulation. Aluminum sulfate was chosen as flocculant and the dose was 600  
109 mg/L according to our previous work.<sup>34</sup> The coagulation step was divided into three  
110 stages: rapid mixing at 200 rpm for 1 minute, then slow mixing at 40 rpm for 20 min,  
111 and settling for 30 min.<sup>34</sup> The water quality parameters of SGW were summarized in  
112 [Table S2](#) of the Supporting Information (SI). The methods for the quantification of  
113 dissolved organic carbon (DOC), turbidity, pH, chemical oxygen demands (COD),  
114 biochemical oxygen demand (BOD<sub>5</sub>), UV absorbance at 254 nm (UV<sub>254</sub>), total  
115 dissolved solid (TDS), and fluorescence excitation-emission matrix (EEM) can be  
116 found in our previous articles<sup>30, 35</sup> and in [Text S1](#) of the SI.

117 **2.2. Experimental Setups and Procedures of Pre-oxidation.** Ferrate (VI)  
118 treatment was one of pre-oxidation method. The dosage of Fe(VI) was 40 mg/L. The  
119 recrystallization method was used in this study to increase the purity of potassium  
120 ferrate (K<sub>2</sub>FeO<sub>4</sub>) to >90%.<sup>36-38</sup> The Fe(VI) pre-oxidation experiment consisted of three  
121 stages: rapid mixing at 200 rpm for 2 min, then slow mixing at 40 rpm for 20 min, and  
122 settling for 30 min. The supernatant was used as feed water for the subsequent BAF  
123 process.

124 Ozonation was another pre-oxidation method. The dosage of ozone was 80 mg/L,  
125 according to our previous work.<sup>30</sup> Treatment of raw water by pre-ozonation was  
126 carried out in batch experiment. In each batch experiment, 1 L raw water was added

127 into a reactor and oxidized by ozone produced from ozone generator (Beijing Tonglin  
128 Co., Ltd., China) at desired flow rate. Before the subsequent BAF treatment, the  
129 residual ozone in water was quenched by water bath heating with 30 min at 50 °C.

130 **2.3. Experimental Setup and Protocol of BAF Tests.** In the BAF column  
131 acclimation process, microorganisms in shale gas wastewater were gradually enriched  
132 in activated carbon carrier through sequential batch influent and gradient dilution of  
133 raw water, thus forming biofilm on activated carbon. Operation parameters and steps  
134 of BAF column acclimation can be found in [Table S1 \(SI\)](#). A carbon source (sodium  
135 acetate anhydrous) was added to adjust the C:N ratio to 3.5:1 of the raw water, which  
136 was beneficial to the growth of microorganisms. BAF systems were operated in  
137 batch-mode at influent flow rate of 0.14 L/h and aerated at a rate of 50 mL/min. The  
138 volume of circulation feed tank was 0.55 L, and 0.5 L raw water was changed every  
139 two days. The inner diameter and height of the BAF reactor were 1.4 cm and 80 cm,  
140 respectively. The filling height of activated carbon (CPG LH 12×40, Calgon Carbon  
141 Co., Ltd., USA) was 45 cm, and the filling mass was about 30 g. The corresponding  
142 data and analysis during the acclimation stage are summarized in [Figure S2](#) and  
143 [Figure S3 \(SI\)](#).

144 In the continuous-mode BAF systems, the raw water pre-oxidized by ozone or  
145 Fe(VI) was used as BAF influent to investigate the treatment effect of combined  
146 processes of ozone-BAF (O<sub>3</sub>-BAF) and Fe(VI)-BAF. The raw water was also used as  
147 BAF influent for comparison. Three BAF systems were thus operated in

148 continuous-mode at influent flow rate of 0.014 L/h and aerated at a rate of 10 mL/min.  
149 The backwashing frequency, backwashing flow rate, and backwashing time were 20  
150 days, 0.14 L/h, and 10 min, respectively.

151 **2.4. Analysis of Biofilm on GAC.** At the end of BAF operation, a certain  
152 amount of granular activated carbon (GAC) samples were collected from each BAF  
153 column to evaluate the activity, relative concentration and growth of the biofilm on  
154 GAC through measurement of the oxygen uptake rate (OUR), extracellular polymeric  
155 substances (EPS), GAC static adsorption, and using scanning electron microscopy  
156 (SEM) combined with energy dispersive X-ray spectroscopy (EDS) analysis. GAC  
157 samples were collected from ~15 cm bed-depth (from the top).

158 A mass equivalent to 0.3 g GAC was placed into a 150 mL conical flask on a  
159 magnetic stirrer. The conical flask was then filled with raw water (about 8 mg/L  
160 dissolved oxygen). A rubber plug with a dissolved oxygen probe was installed on the  
161 conical flask to ensure that there were no bubbles. Then, the dissolved oxygen was  
162 measured as a function of time under stirring (400 rpm) at 20 °C. The final result was  
163 expressed in mg of dissolved oxygen consumed per g of activated carbon per hour  
164 ( $\text{mgO}_2/(\text{g}_{\text{GAC}}\cdot\text{h})$ ).

165 Extraction and detection of EPS, which was defined as the sum of  
166 polysaccharides and proteins, were conducted using the standard methods, while the  
167 specific detection of polysaccharides and proteins was undertaken using the  
168 anthrone/ $\text{H}_2\text{SO}_4$  and Bradford methods, respectively.

169 Static adsorption experiments were performed on new GAC, used GAC, and  
170 used GAC after sterilization to distinguish the adsorption and microbial degradation in  
171 each BAF systems. A high pressure steam sterilizer was used to sterilize the used  
172 GAC. The operating temperature was 125 °C and the sterilization time was 20 min. In  
173 the static adsorption experiment at 20 °C, the dosage of GAC was 2 g/L, the stirring  
174 speed was 400 rpm and the running time was 72 h. Reaction mixtures were withdrawn  
175 at specific time intervals to measure the variation of DOC and UV<sub>254</sub>.

176 GAC samples were analyzed using SEM (FE-SEM, Regulug-8230, Hitachi,  
177 Japan) and EDS (X-MAX Extreme, Oxford-Instruments, UK) to detect physical and  
178 chemical changes on the GAC surface and observe the morphology of biofilm. GAC  
179 was prepared for SEM imaging by fixation with 2% glutaraldehyde, dehydration in  
180 20–100% ethanol, and drying in a freeze vacuum dryer. EDS was applied in tandem  
181 with SEM to map and evaluate the deposition of elemental content on the GAC  
182 surface throughout BAF treatment.

183 **2.5. Microbial diversity analysis.** Through the microbial diversity sequencing  
184 of the raw water, the GAC at the end of BAF column acclimation, and the GAC at  
185 different times of continuous-mode BAF systems, the temporal and spatial variation  
186 of the microbial community and the dominant functional microorganisms were  
187 analyzed. Details about microbial diversity sequencing and analysis are presented in  
188 [Text S2](#) of the SI and in our previous study.<sup>35, 39</sup> Note that the same amount of GAC  
189 was filled into the reactor after sampling.

190

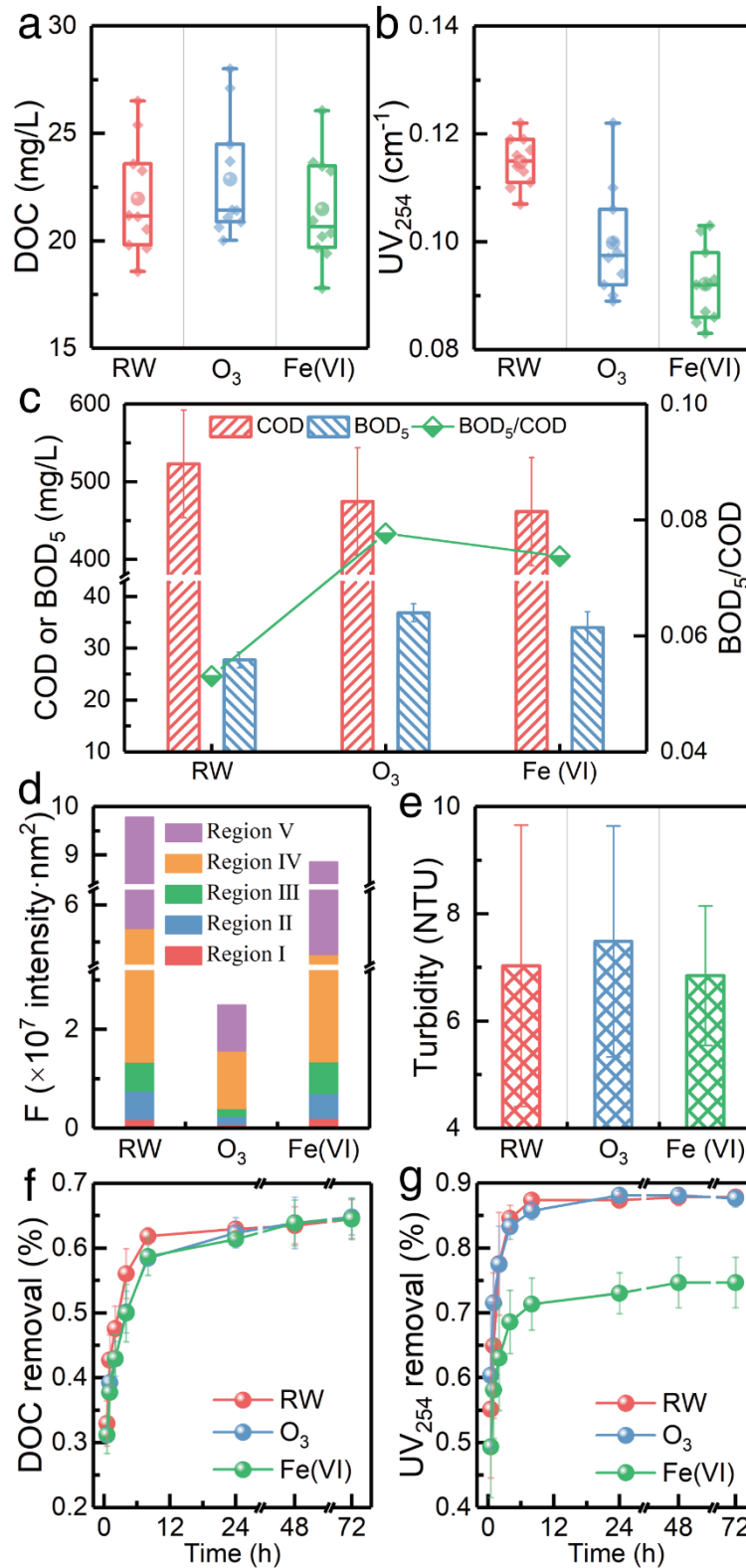
### 191 3. RESULTS AND DISCUSSION

192 **3.1. Effect of Pre-oxidation on Water Quality.** Pre-oxidation had a negligible  
193 effect on the DOC parameter, shown in [Figure 1a](#). The DOC change upon Fe(VI)  
194 treatment was  $-2.1\%$ , while that upon  $O_3$  treatment was  $+4.1\%$ , caused by the  
195 competing effect of organic matter mineralization and solubilization. More explicitly,  
196 pre-oxidation only partly mineralizes the organic content, which would translate into a  
197 decrease of DOC values. However, this reaction simultaneously increases the  
198 solubility of suspended organic matter and promotes the release of intracellular  
199 organic substances from sterilized bacteria, with the effect of increasing the DOC.<sup>30</sup>  
200 The effect of oxidation was instead directly associated with the reduction of other  
201 parameters related to organic composition. The  $UV_{254}$  removal rates by  $O_3$  and Fe(VI)  
202 were  $13\%$  and  $23\%$ , respectively ([Figure 1b](#)). As shown in [Figure 1c](#),  $O_3$  and Fe(VI)  
203 removed part of the COD ( $9.2\%$ - $11.8\%$ ) and, most importantly, increased the  
204 concentration of  $BOD_5$  ( $33.2\%$ - $22.7\%$ ), as well as the value of  $BOD_5/COD$   
205 ( $47.2\%$ - $39.6\%$ ), suggesting that pre-oxidation significantly improved the  
206 biodegradability of SGW. The composition and relative content of fluorescent organic  
207 matters in SGW were obtained through fluorescence EEM spectra and FRI analysis  
208 method ([Figure S4](#) and [Figure 1d](#)).<sup>40</sup> The soluble microbial by-product-like matters  
209 (region IV) and humic acid-like matters (region V) were the dominant fluorescent  
210 organic components in SGW.  $O_3$  had excellent removal effect on all kinds of

211 fluorescent organic matters (74.5%), while Fe(VI) only slightly removed fluorescent  
212 organic matters (9.4%), mainly acting on soluble microbial by-product-like matters  
213 and humic acid-like matters. In summary, the mineralization of organic matters by O<sub>3</sub>  
214 or Fe(VI) was limited, while pre-oxidation mainly changed the properties of organic  
215 matters.

216 Some interesting phenomena were found in GAC static adsorption experiments  
217 of pre-oxidized SGW. In the first 24 h of adsorption, the adsorption rate of DOC in  
218 pre-oxidized SGW was practically the same of that measured in raw SGW, as shown  
219 in [Figure 1f](#). The same trend was observed for UV<sub>254</sub> before and after ozonation  
220 (Figure 1g). On the contrary, the adsorption rate and equilibrium adsorption capacity  
221 of GAC for UV<sub>254</sub> in SGW treated by Fe(VI) was lower than that assessed in raw  
222 SGW. The value of UV<sub>254</sub> mainly represents the content of low molecular weight  
223 aromatic compounds.<sup>41, 42</sup> This result might indicate that, as this fraction of organic  
224 matter was removed more efficiently by Fe(VI) than O<sub>3</sub> (Figure 1b), the affinity or the  
225 kinetics of adsorption for other fractions was higher upon oxidation with ferrate. In  
226 general, these tests suggested a complex effect of pre-oxidation on organic content  
227 and composition. Also, the data evidently indicate that pre-oxidation would not  
228 translate into better water quality if followed by simple GAC adsorption in the  
229 absence of microorganisms. However, the analysis of organic matter biodegradability  
230 upon oxidation suggests that the performance of BAF systems may be improved  
231 compared to a raw water not subjected to this pre-treatment step, and this effect is

232 discussed below.



233

234 **Figure 1.** Effect of preoxidation on (a-e) water quality, (f) DOC adsorption on GAC,

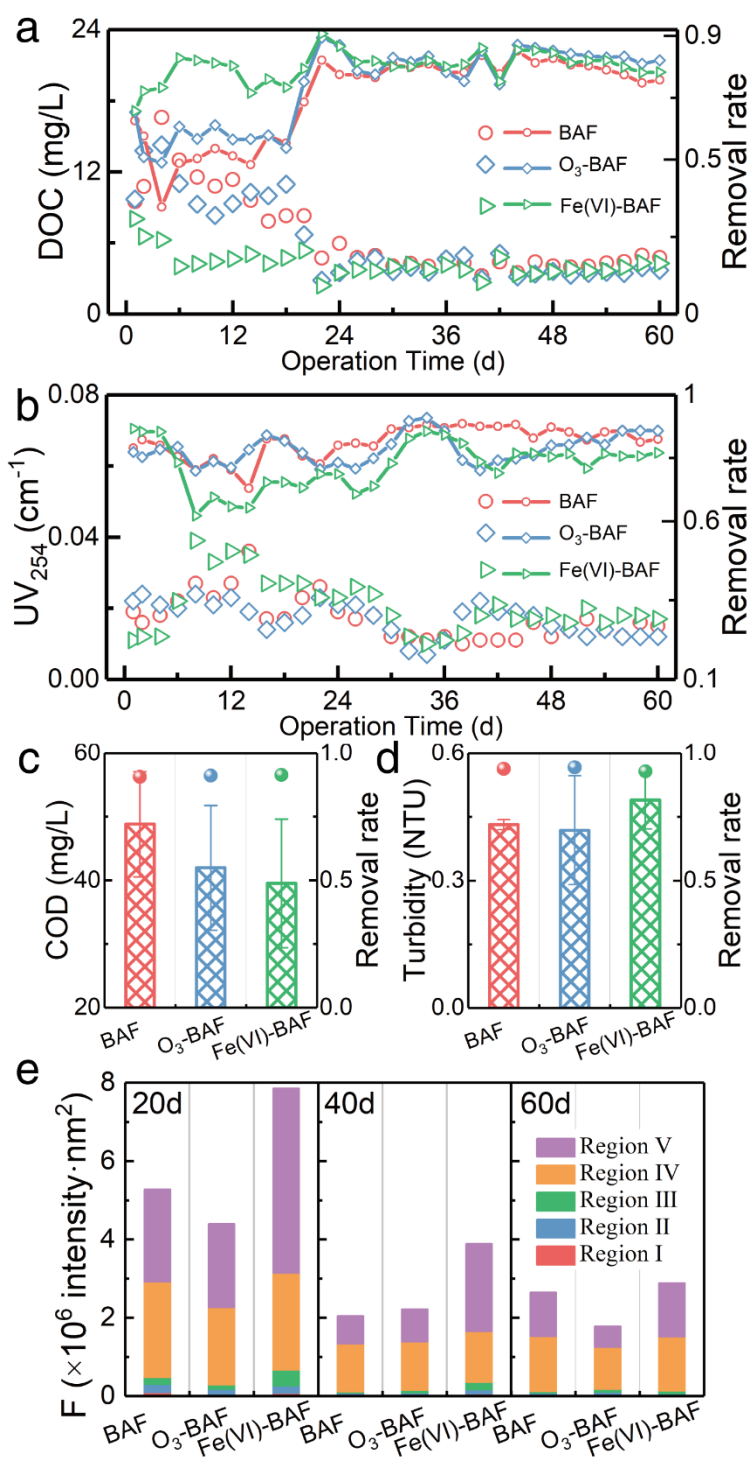
235 and (g) UV<sub>254</sub> adsorption on GAC. The dosages of O<sub>3</sub> and Fe(VI) were 80 mg/L and

236 40 mg/L, respectively. The GAC dosage was 2 g/L.

237 **3.2. Performance of BAF, O<sub>3</sub>-BAF, and Fe(VI)-BAF.** The variation of DOC  
238 and UV<sub>254</sub> in three BAF systems effluent measured during 60 days of continuous  
239 operation is shown in [Figure 2a](#) and [Figure 2b](#). In the first 18 days of continuous  
240 operation, the DOC removal rate of the three BAF systems was somewhat erratic with  
241 the water pre-treated by Fe(VI) oxidation showing the highest value. With the  
242 operation and related strengthening of microbial activity, the removal rate of DOC in  
243 all three systems increased and reached stability at values around 80%, specifically  
244 with rates that decreased in the order O<sub>3</sub>-BAF > Fe(VI)-BAF > BAF. Similarly, the  
245 removal rate of UV<sub>254</sub> by three BAF systems was more inconsistent during the initial  
246 stage of BAF experiments and reached stability toward the end. Generally, the UV<sub>254</sub>  
247 removal rate of BAF was the highest, while that of Fe(VI)-BAF was the lowest,  
248 consistent with what reported in [Figure 1g](#) for static adsorption tests. However, the  
249 efficiency of UV<sub>254</sub> removal by Fe (VI)-BAF was gradually improved during  
250 operation, which may be attributed to the continuous enrichment of microorganisms.

251 Overall, the BAF systems had high COD removal rates (90.7%-91.4%) ([Figure](#)  
252 [2c](#)). In particular, the COD content in effluent from BAF, O<sub>3</sub>-BAF, and Fe(VI)-BAF  
253 was 48.8, 42.0, and 39.5 mg/L, respectively. The turbidity (0.42-0.49 NTU) of the  
254 effluents was also low ([Figure 2d](#)). The composition and relative content of  
255 fluorescent organic compounds were measured in the effluents of the three BAF  
256 systems on the 20th day, 40th day, and 60th day of operation, and the results are

257 shown in Figure 2e and Figure S4. BAF systems efficiently removed fluorescent  
 258 organic compounds, and the removal rate increased gradually with the operation. The  
 259 soluble microbial by-product-like matters (region IV) and humic acid-like matters  
 260 (region V) were the dominant fluorescent organic components in the effluents.



261

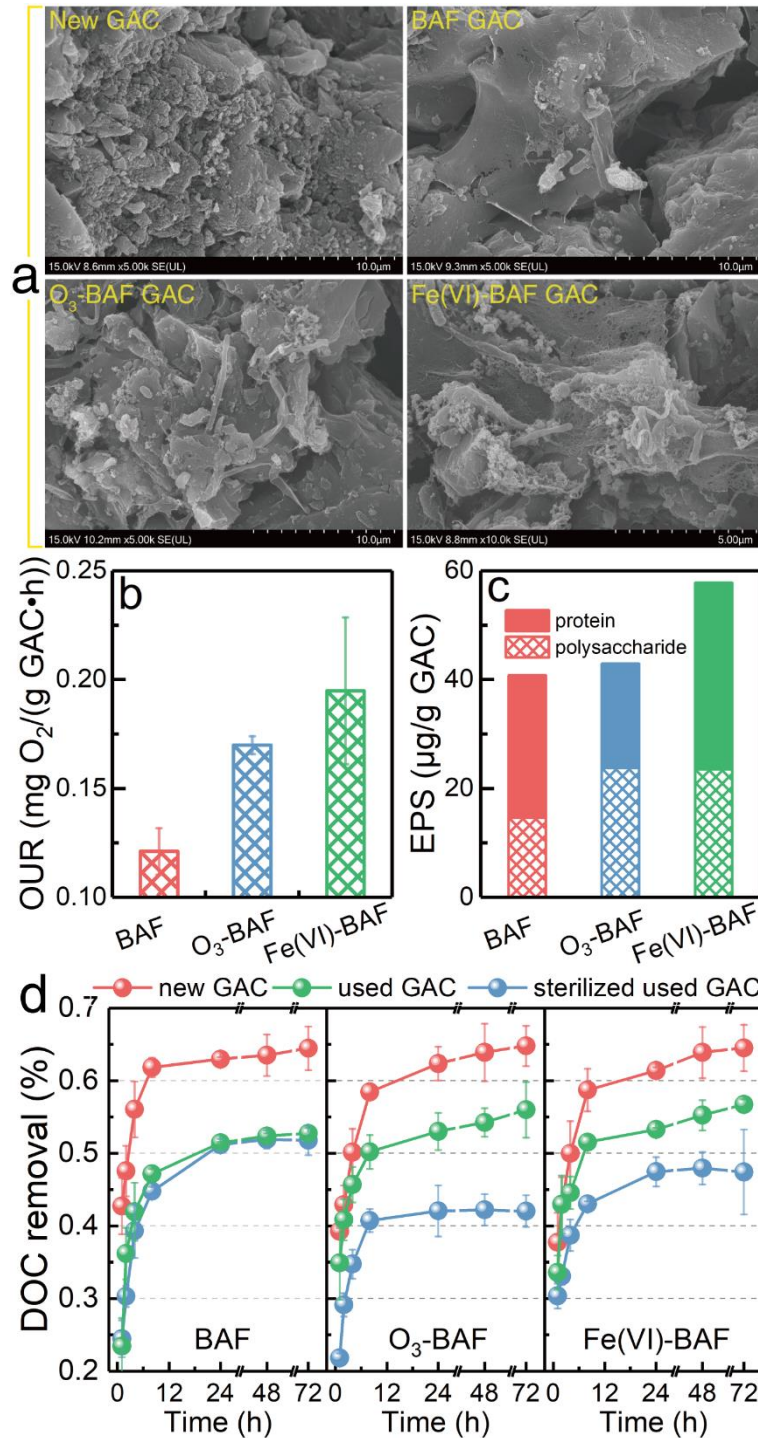
262 **Figure 2.** Quality of the effluents from the BAF systems. **(a-b)** DOC and UV254  
263 parameters as a function of time during operation: here the larger data points refer to  
264 the concentrations (left axis) and the small data points connected by lines to the  
265 removal rate (right axis). **(c-d)** COD and turbidity values of the effluent: here, the bars  
266 refer to the value of each parameter (left axis) and the circles to the average removal  
267 rate (right axis). **(e)** Fluorescent organic components at three moments of the BAF  
268 operation.

269

270 **3.3. Biofilm Morphology and Microbial Activity.** The results in [Figure 3a](#) and  
271 [Figure S6](#) indicate the presence of a large amount of microorganisms and of some  
272 microbial micelleons on the GAC surface from the three BAF systems. The  
273 microorganisms were mainly bacilli, cocci, and filamentous bacteria. Clearly, the  
274 microorganisms observed in samples from O<sub>3</sub>-BAF and Fe(VI)-BAF reactors were far  
275 more than those observed in the BAF reactor. As shown in [Figure 3b](#) and [Figure 3c](#),  
276 the OUR of microorganisms and the concentration of EPS decreased in the order  
277 Fe(VI)-BAF > O<sub>3</sub>-BAF > BAF, corroborating that the activity of the microorganism in  
278 BAF systems was higher upon pre-oxidation.<sup>43, 44</sup>

279 The adsorption performance of GAC after long-term operation in BAF systems  
280 was always lower than that of the new GAC, as presented in [Figure 3d](#). This result is  
281 rationalized with the larger density of available sites for adsorption on pristine GAC.  
282 In real operation, the lower adsorption of biologically-enhanced GAC would be

283 compensated by the concurrent degradation of organic matter, which is the main target  
284 of this treatment. Interestingly, sterilization of the GAC used in the BAF system  
285 without pre-oxidation did not change its DOC removal performance compared to the  
286 material analyzed after use and without sterilization. On the contrary, both the  
287 adsorption rate and the equilibrium adsorption capacity of sterilized used GAC for  
288 DOC were significantly lower than that of used GAC from O<sub>3</sub>-BAF and Fe(VI)-BAF  
289 systems. This analysis, combined with the observations from Figure 3a-d, suggests  
290 that microorganisms in O<sub>3</sub>-BAF and Fe(VI)-BAF systems were highly functional and  
291 played an important role in the removal of organic matter. This result is attributed to  
292 the better biodegradability of pre-oxidized SGW, which helped sustaining a healthier  
293 and more active microbial community in the BAF systems.



294

295 **Figure 3.** (a) Surface micrographs of new GAC and used GAC from three BAF

296 systems. (b) OUR of microorganisms and (c) EPS concentration in the used GAC

297 biofilm. (d) Variation of DOC removal in static adsorption experiment with new GAC,

298 used GAC, and sterilized used GAC as adsorbents. The used GAC was collected on

299 the 60th day of continuous operation of the three BAF systems. The magnification of  
300 surface micrographs is 5k × or 10k ×. In static adsorption experiment, the GAC  
301 dosage was 2 g/L.

302

303 **3.4. Microbial Community Analysis.** The number of effective sequences, OUTs,  
304 alpha diversity indexes, and rarefaction curves for microbial communities in raw  
305 water and three BAF systems at different operation times are presented in [Table S4](#)  
306 and [Figure S8](#). The richness and diversity of microbial communities in raw water  
307 were higher than those in the three BAF reactors. The coverage values and rarefaction  
308 curves suggested the sequencing depth were sufficient.<sup>45, 46</sup> Through principal  
309 component analysis (PCA) at OUT level ([Figure S9](#)), the affinity relationships of  
310 microbial community between raw water and three BAF reactors, as well as among  
311 the three BAF reactors are revealed. The microbial community in the raw water was  
312 vastly different from that in the BAF reactor at 0 day after acclimation process,  
313 indicating new dominant microorganisms had been formed in BAF reactors. Also, the  
314 microbial communities in the same reactor at different times were similar, and O<sub>3</sub>  
315 seemed to have an effect in affecting more pronounced changes of the microbial  
316 community compared to ferrate pre-treatment.

317 [Figure 4a](#) and [Figure 4b](#) show in details the microbial community composition at  
318 the phylum and genus level, respectively. *Proteobacteria* (30.4%), *Actinobacteriota*  
319 (18.1%), *Bacteroidota* (14.7%), *Firmicutes* (12.3%), *Desulfobacterota* (9.5%), and

320 *Synergistota* (7.0%) were the major phyla and constituted 92% of bacteria in raw  
321 water. Through acclimation, *Proteobacteria* (98.1%) became the absolute dominant  
322 microbial phylum in BAF reactors. Similarly, the major genera in raw water, which  
323 also were widely detected in SGW from shale gas wells,<sup>46-48</sup> were  
324 *norank\_f\_Coriobacteriaceae* (18.0%), *Marinobacterium* (8.7%), *Lentimicrobium*  
325 (6.0%), *Roseovarius* (5.0%), and *Desulfovibrio* (4.6%). Family *Coriobacteriaceae* is  
326 an anaerobic fermentative bacteria within phylum *Actinobacteriota*.<sup>23</sup>  
327 *Marinobacterium* is a strict aerobe microorganism capable of utilizing a wide range of  
328 carbon sources.<sup>49, 50</sup> *Lentimicrobium* is a strictly anaerobic bacterium with the function  
329 of hydrolyzing organics.<sup>51, 52</sup> Almaraz et al.<sup>18</sup> reported *Roseovarius* as an iodine  
330 oxidation bacterium, which can promote the formation of large amounts of iodinated  
331 organic compounds that would cause serious negative implications to the water  
332 environment. *Desulfovibrio* as a sulfate-reducing bacterium is widely detected in  
333 shale gas wastewater, and is associated with the risk of corrosion to shale gas  
334 production facilities.<sup>47, 53, 54</sup>

335 A large amount of relatively low abundance microorganisms were enriched upon  
336 acclimation, with the major genus components being *Methyloversatilis* (39.1%),  
337 *Rhizobium* (20.0%), *Rehaibacterium* (10.4%), *Acinetobacter* (6.3%), *Pseudomonas*  
338 (4.5%), and *Acidovorax* (2.9%). During the BAF tests, the communities adapted  
339 differently based on the presence and type of oxidation pre-treatment. Consistent with  
340 PCA analysis, the microbial communities in the same BAF reactor at different

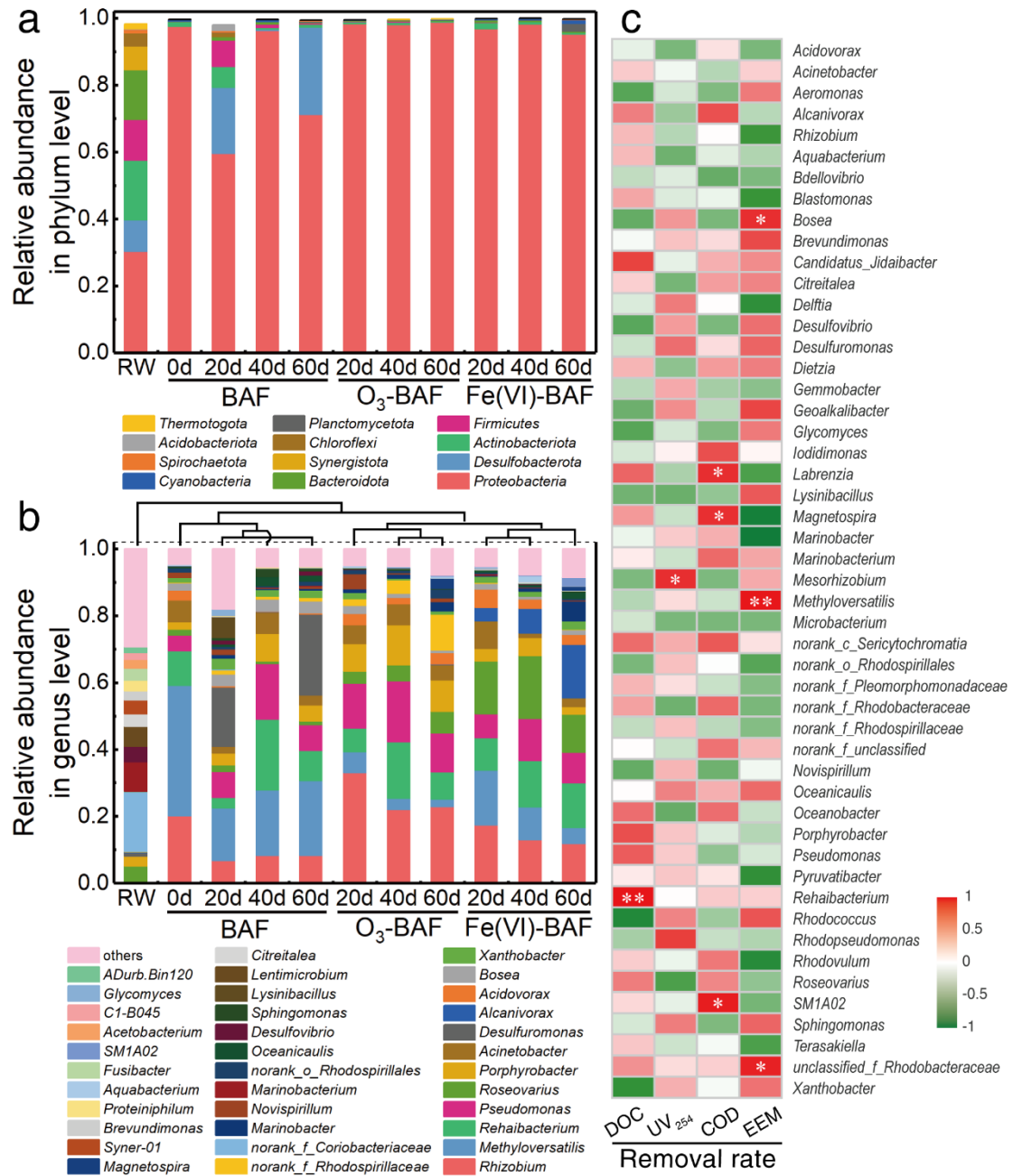
341 operation times were similar. Although there were some differences in the microbial  
342 community structure of the three BAF systems, the core microorganisms were similar.  
343 These were *Rehaibacterium*, *Methyloversatilis*, *Pseudomonas*, *Rhizobium*,  
344 *Porphyrobacter*, *Acinetobacter*, *Bosea*, *Roseovarius*, *Acidovorax* and *Xanthobacter*.  
345 *Methyloversatilis* is a salinity tolerant bacterium with the ability of denitrification and  
346 organics degradation.<sup>55, 56</sup> *Rhizobium* are typical denitrifying bacteria, which widely  
347 exists in activated sludge, soil, and wastewater.<sup>35, 57, 58</sup> Genus *Acinetobacter* is related  
348 rich functions, such as degradation of organics, denitrification, phosphorus removal,  
349 and oxidation of heavy metals.<sup>59-62</sup> Members of *Pseudomonas* can degrade organics  
350 like toluene and chloroform.<sup>63</sup> Some research shows that members of *Acidovorax*  
351 could conduct heterotrophic denitrification.<sup>64, 65</sup> In general, SGW contains a large  
352 number of microorganisms with ability of degrading organic matter, removing  
353 nitrogen, and oxidizing heavy metals. Efficiently taking advantage of these  
354 microorganisms for biological treatment has great prospects in SGW management.  
355 Furthermore, a large number of anaerobes were eliminated during BAF acclimation  
356 process, and new dominant microorganisms were formed with the variation of  
357 environmental factors (aeration, variation of TDS, and the addition of sodium  
358 acetate).

359 In order to determine the microorganisms with significant abundance differences  
360 between the three BAF systems, we performed biomarker analysis using the linear  
361 discriminant analysis effect size (LEfSe) method. As shown in [Figure S10](#), 9 bacterial

362 clades presented statistically significant differences with an LDA threshold of 3.4.  
363 Each reactor had its own characteristic microorganisms whose abundance was higher  
364 than that of other reactors. Specifically, *norank\_o\_Bacteroides\_VC2\_1\_Bac22* and  
365 *norank\_f\_Vermiphilaceae* were enriched in the BAF reactor without pre-oxidation.  
366 *Norank\_f\_Rhodospirillaceae*, *Gemmobacter*, and *Rhizobium* were enriched in  
367 O<sub>3</sub>-BAF reactor. Instead, *Dietzia*, *Roseovarius*, *norank\_o\_*  
368 *Gammaproteobacteria\_Incertae\_Sedis*, and *norank\_f\_Rhodobacteraceae* were  
369 enriched in the Fe(VI)-BAF reactor.

370 The correlation analysis between microbial community at genus level (top 50)  
371 and environmental variables (organic matter removal rate) is shown in [Figure 4c](#). It  
372 was found that *Rehaibacterium* with the high abundance of 3.0-21.1% was  
373 significantly correlated with DOC removal rate ( $P < 0.01$ ). In a previous study,  
374 *Rehaibacterium terrae*, a thermotolerant and strictly aerobic bacterium was found in  
375 geothermally heated soil of Rehai National Park, China.<sup>66</sup> *Rehaibacterium terrae* can  
376 survive under the conditions of 0-30 g/L NaCl solution and 30-55 °C and degrade  
377 some organics.<sup>66</sup> One species in the genus *Rehaibacterium* was detected in our study  
378 but could not be defined: the base pair fragments were different from those of  
379 *Rehaibacterium terrae*. This result might indicate that a new species of genus  
380 *Rehaibacterium* was present with the strong ability of degrading DOC. Of course,  
381 more research is needed to study this hypothesis and to understand the new functions  
382 of this putative species. *Mesorhizobium* (0.01-0.20%) were correlated with UV<sub>254</sub>

383 removal rate ( $P < 0.05$ ). Research studies reported that *Mesorhizobium* members are  
384 halotolerant potential denitrifying bacteria and organics degrading bacteria.<sup>67, 68</sup> In  
385 addition, *Labrenzia* (0.01-1.29%), *Magnetospira* (0.01-3.04%), and *SMIA02*  
386 (0.01-2.42%) were correlated with COD removal rate ( $P < 0.05$ ). *Bosea* (0.75-3.58%)  
387 and *unclassified\_f\_Rhodobacteraceae* (0.02-1.25%) were correlated with EEM  
388 removal rate ( $P < 0.05$ ). *Methyloversatilis* (2.24-22.31%) was significantly correlated  
389 with EEM removal rate ( $P < 0.01$ ).



390

391 **Figure 4.** Bacterial community compositions at (a) the phylum (> 1%) and (b) the

392 genus level (> 1.5%) in raw water and the three BAF systems at different operation

393 times. (c) Correlation analysis between microbial community at genus level (top 50)

394 and environmental variables (organic matter removal rate). Here, “\*” represents a

395 value of  $p < 0.05$  and “\*\*” represents a value of  $p < 0.01$ .

396

397       **Implications.** O<sub>3</sub> and Fe(VI) pre-oxidation can effectively improve the removal  
398 efficiency of organics in BAF, which is attributed to higher activity and content of  
399 microorganisms in O<sub>3</sub>-BAF and Fe(VI)-BAF systems compared with BAF. In our  
400 experiments, the removal rate of organic matters by BAF systems gradually increased  
401 and stabilized, owing to the enhancement of the microbial degradation function, with  
402 the enrichment of a large number of microorganisms with specific functions, such as  
403 organic matter degradation, nitrogen removal, heavy metals oxidation. For example,  
404 *Rehaibacterium* is significantly correlated with DOC removal rate (P < 0.01). Besides,  
405 *Methyloversatilis* is significantly correlated with fluorescent organics removal (P <  
406 0.01).

407       The oxidation behavior of O<sub>3</sub> is different from that of Fe(VI), but both processes  
408 can effectively improve the biodegradability of wastewater. However, the  
409 mineralization rate and the improvement of organic quality in the effluent of systems  
410 upon pre-oxidation with O<sub>3</sub> and Fe(VI) were still limited. Combination with other  
411 oxidants (such as H<sub>2</sub>O<sub>2</sub>) or with electrooxidation may further improve the oxidation  
412 efficiency and the efficiency of the BAF process. Ozonation has feasibility and  
413 application value in the treatment of SGW, already at this stage of its technological  
414 development. Compared with pre-ozonation, Fe(VI) pre-oxidation has the potential  
415 advantages of easy operation and maintenance, but its application is still under  
416 development and should be optimized.<sup>32</sup> The in-situ Fe(VI) synthesis in wastewater  
417 treatment plant through wet chemical or electrochemical method is expected to further

418 reduce the chemical cost.<sup>69</sup> It should be noted that the results presented here were  
419 obtained at the lab scale, while further studies are needed to evaluate the relevant  
420 systems at the pilot and full scales in long-term operation.

421

## 422 **ASSOCIATED CONTENT**

### 423 **Supporting Information**

424 The Supporting Information is available free of charge on the ACS Publications  
425 website.

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### 432 **Notes**

433 The authors declare no competing financial interest.

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440

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