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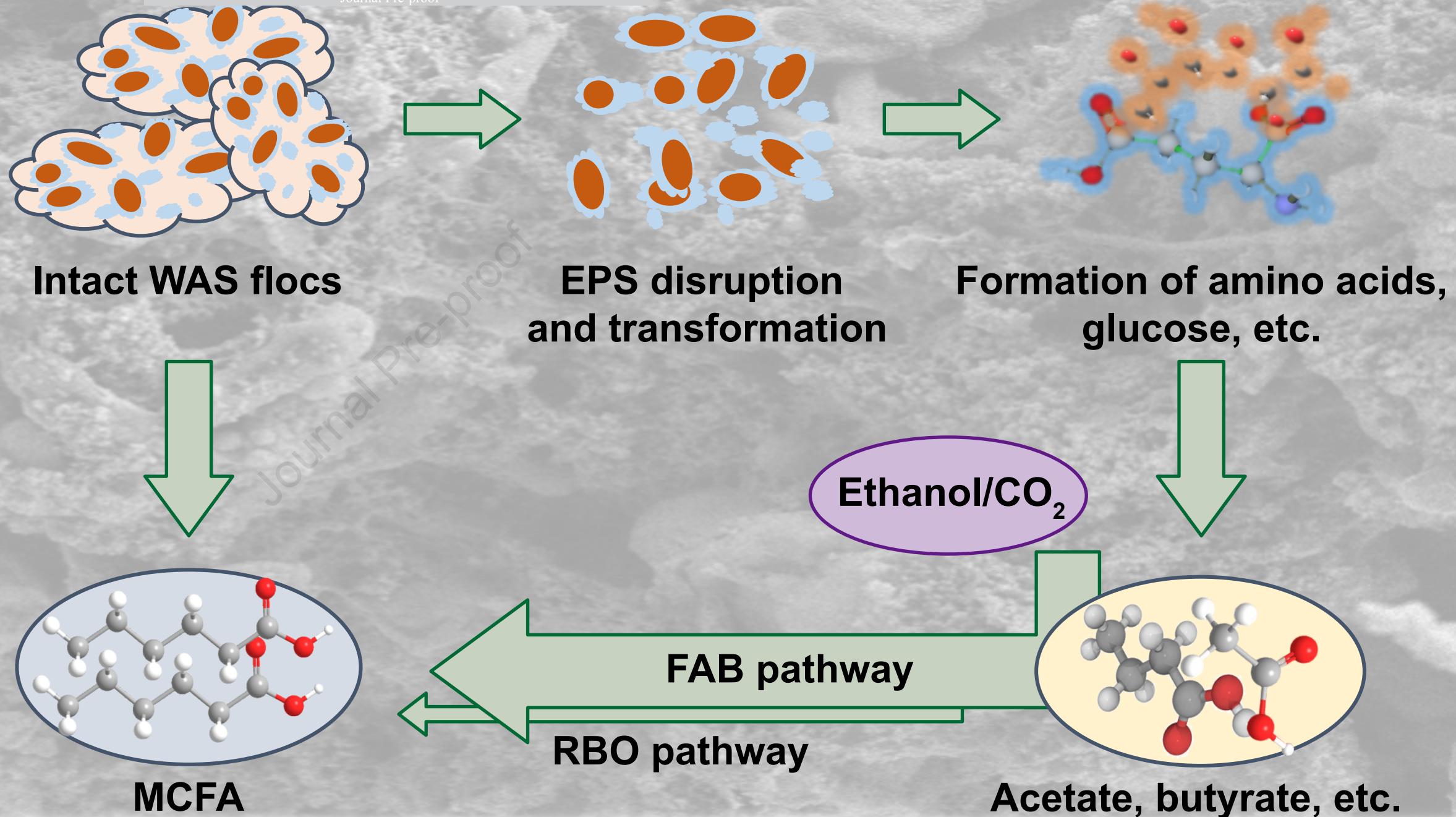
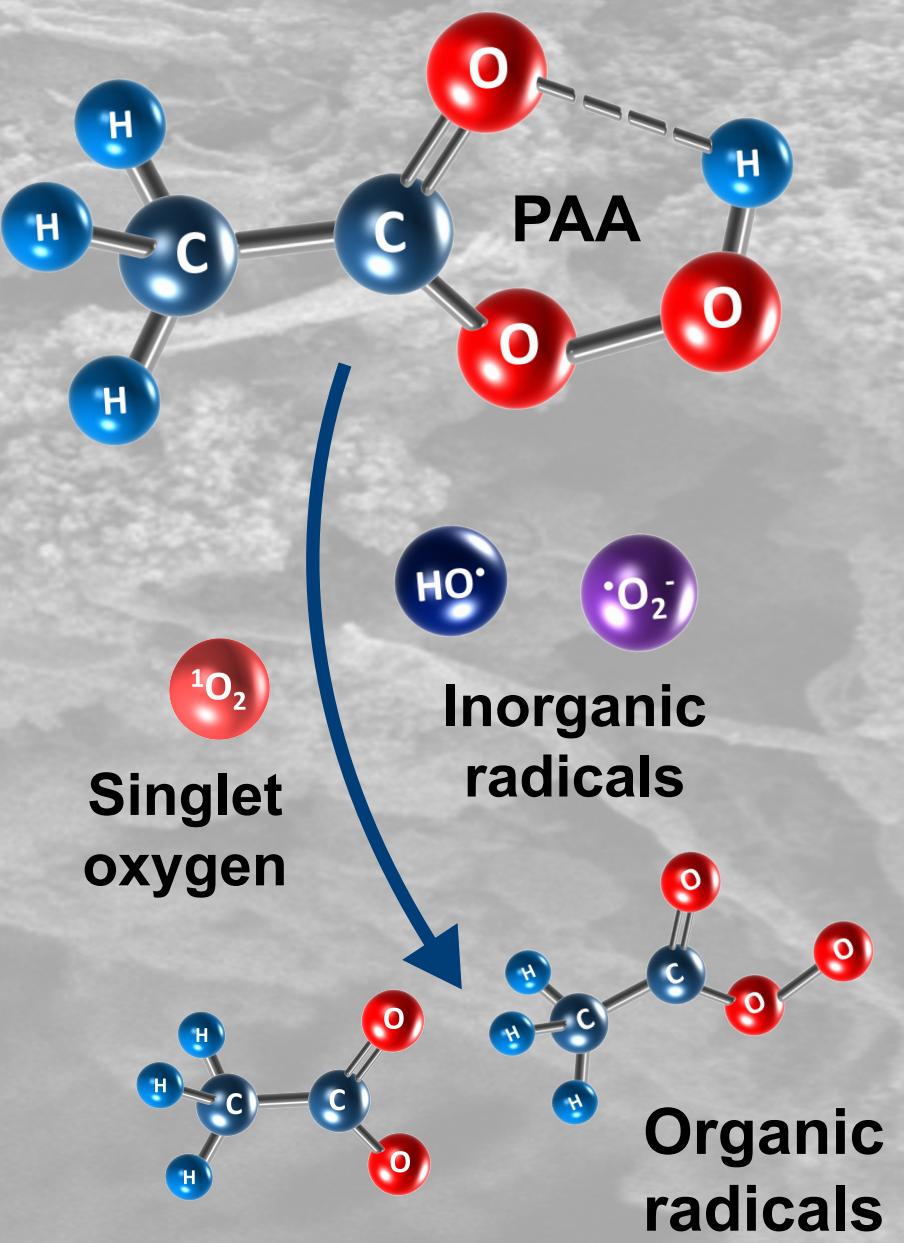
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Peracetic acid (PAA)-based pretreatment effectively improves medium-chain fatty acids (MCFAs) production from sewage sludge

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1 **Abstract:** Peracetic acid (PAA), known for its environmentally friendly properties as a
2 oxidant and bactericide, is gaining prominence in decontamination and disinfection
3 applications. The primary product of PAA oxidation is acetate that can serve as an
4 electron acceptor (EA) for the biosynthesis of medium-chain fatty acids (MCFAs) via
5 chain elongation (CE) reactions. Hence, PAA-based pretreatment is supposed to be
6 beneficial for MCFAs production from anaerobic sludge fermentation, as it could
7 enhance organic matter availability, suppress competing microorganisms and furnish
8 EA by providing acetate. However, such a hypothesis has rarely been proved. Here we
9 reveal that PAA-based pretreatment leads to significant exfoliation of extracellular
10 polymeric substances (EPS) from sludge flocs and disruption of proteinic secondary
11 structures, through inducing highly active free radicals and singlet oxygen. The
12 production of MCFAs increases substantially to 11,265.6 mg COD L⁻¹, while the
13 undesired byproducts, specifically long-chain alcohols (LCAs), decrease to 723.5 mg
14 COD L⁻¹. Microbial activity tests further demonstrate that PAA pretreatment stimulates
15 the CE process, attributed to the up-regulation of functional genes involved in fatty acid
16 biosynthesis pathway. These comprehensive findings provide insights into the
17 effectiveness and mechanisms behind enhanced MCFAs production through PAA-
18 based technology, advancing our understanding of sustainable resource recovery from
19 sewage sludge.

20

21 **Keywords:** Sewage sludge; Medium-chain fatty acids (MCFAs); Extracellular
22 polymeric substances (EPS); Peracetic acid (PAA); Metabolic activity.

23 **1. Introduction**

24 The recycling of sewage sludge, including primary sludge and waste activated
25 sludge (WAS), which are inherent byproducts of biological wastewater treatment, is an
26 important part of achieving carbon neutrality at wastewater treatment plants (WWTPs)
27 [1-3]. As a low-cost and simple technology, anaerobic fermentation can effectively
28 reduce the contamination properties of sludge and convert organic fractions into value-
29 added products [4, 5]. Compared to short-chain fatty acids (SCFAs, i.e., traditional
30 fermentation products), medium-chain fatty acids (MCFAs) carry higher energy
31 densities. They are more hydrophobic and can be easily separated from fermentation
32 liquor [6-8]. MCFAs, denoting straight-chain monocarboxylates containing 6–12
33 carbon atoms, primarily encompass caproate, heptylate, and caprylate when produced
34 through biotechnological means [9]. MCFAs can be employed in diverse industries,
35 such as food additives and antimicrobial agents [6]. They are also precursors to
36 manufacture industrial products, e.g., surfactants, lubricants, plasticizers, etc., or to
37 synthesize jet fuel and diesel [10]. Consequently, MCFAs have a higher economic and
38 practical value.

39 Sewage sludge is a suitable and cheap substrate for MCFAs production. On one
40 hand, most organics in sludge can be biodegraded into acetate-dominated SCFAs. Chain
41 elongation (CE) microorganisms prefer to initiate CE with acetate as the initial electron
42 acceptor (EA) [11]. Meanwhile, sewage sludge is rich in microorganisms and
43 extracellular hydrolytic enzymes, e.g., acid-producing bacteria, CE microorganisms,
44 and protease, benefiting hydrolysis/acidification of substrates and SCFAs upgrading

[12]. When electron donor (ED, commonly ethanol) is sufficient, CE microorganisms will likely be enriched to upgrade SCFAs to MCFAs. For example, it was reported that 6615 mg COD per L MCFAs were produced from anaerobic WAS fermentation due to the stimulation of 170 mM ethanol [13]. When WAS alkaline fermentation liquid was applied as EA for CE, with different concentrations of ethanol provided as ED, MCFAs production was elevated from 5570 to 7670 mg COD L⁻¹ [6]. However, EA provided by sewage sludge is often limited since the organic components in hard cell walls and extracellular polymeric substance (EPS) are resistant to biological hydrolysis [12, 14], resulting in hindered MCFAs production [15, 16]. Meanwhile, potential competing microbes in sewage sludge allow the conversion of ED and EA to byproducts, e.g., long-chain alcohols (LCAs) and methane, leading to dispersive carbon fluxes and incomplete CE [10]. To accelerate sludge hydrolysis and eliminate competitive inhibiting processes, sludge pretreatment is essential before anaerobic fermentation. Yin et al. proved that Fenton-persulfate oxidation could promote sludge acidification and suppress microorganisms capable of producing LCAs, increasing MCFAs production from 2367.7 to 3445.9 mg COD L⁻¹ [16]. Wang et al. indicated that after free ammonia (FA) pretreatment for 24 h, MCFAs production from WAS was promoted from 1400 to 8300 mg COD L⁻¹ [15]. Nevertheless, adding persulfate may induce a sulfate-reducing process, and fermentation broth reflux would increase sludge volume. Therefore, finding an economical and eco-friendly pretreatment technology is urgently needed to scale up the application of MCFAs recovery from WAS.

Peracetic acid (PAA, E⁰ = 1.81 eV) is a wide-spectrum antimicrobial agent for

67 many industries, e.g., medical and food, without byproducts generation. Also, the PAA-
68 based advanced oxidation process (AOP) has exhibited excellent functions in
69 chemical/microbiological micropollutant removal from water/wastewater [17].
70 Recently, the inhibition of PAA in the biological wastewater treatment process and its
71 effect on WAS dissolution have attracted much attention. It was reported that PAA could
72 inhibit the enzyme activity associated with nitrification and decrease nitrate production
73 rates [18]. Appels et al. reported that PAA oxidation caused effective solubilization of
74 sludge organics and increased methane production from anaerobic WAS digestion by
75 21% [19]. It was also unveiled that PAA could efficiently dissolve sludge cells and EPS
76 into soluble components [20]. Additionally, Li et al. demonstrated that PAA suppressed
77 non-functional microorganisms, i.e., hydrogen-consuming microorganisms, during
78 WAS dark fermentation for hydrogen production, resulting in H₂ cumulation increasing
79 to 14.2 mL per g VSS with considerable SCFAs as liquid-phase products [21]. Although
80 PAA-based oxidation has been demonstrated feasible for sludge solubilization and
81 primary fermentation, its enhancement effect on MCFAs production from WAS and the
82 related mechanisms have not been elucidated. Therefore, we propose employing PAA
83 oxidation as a pretreatment strategy for sludge disintegration and MCFAs production.
84 Our hypotheses are based on the following premises: (1) PAA can be activated *in situ*
85 within sludge that is rich in iron [22] and accelerate organic components solubilization
86 and conversion, providing more soluble materials as precursors for EA (i.e., SCFAs)
87 formation. (2) The only byproduct of PAA oxidation is acetic acid, which can be
88 consumed by CE microorganisms as EA to produce MCFAs. (3) PAA has bactericidal

89 or bio-inhibitory effects that may inhibit the production of LCAs and increase electron
90 transfer efficiency from ED to MCFAs.

91 This work aims to explore the feasibility and corresponding mechanism of PAA
92 pretreatment in enhancing MCFAs production from WAS. Firstly, WAS disintegration
93 performance based on PAA oxidation is elaborated from three aspects: soluble substrate
94 production, changes in sludge properties, and extracellular organics transformation.

95 Subsequently, the details of different doses (i.e., 0–25 mg per g TSS) of PAA
96 pretreatment on MCFAs biosynthesis from sludge are assessed based on ED
97 consumption, MCFAs accumulation, byproduct (i.e., LCAs) production, and product
98 distributions. Meanwhile, community succession of MCFAs-forming bioreactors
99 mediated by PAA pretreatment is elucidated by 16S rRNA sequencing. Furthermore,
100 mechanisms of enhanced MCFAs production were clarified from three perspectives:
101 reaction mechanisms of PAA oxidation for WAS disintegration, metabolic activities of
102 key bioprocesses, and variations in microbial functions and functional gene expression.

103 The findings observed in this work would fill the knowledge gap of PAA-based AOP
104 for enhanced MCFAs production from WAS and expand the understanding of the role
105 of PAA in sludge resource recovery.

106 **2. Material and methods**

107 **2.1 Main parameters of WAS and PAA**

108 WAS applied for the experiments was from a local WWTP in Tianjin, China. After
109 impurities were removed and concentrated, WAS was stored at 4 °C. The key
110 characteristics of the WAS were as follows: total suspended solids (TSS) of 26.34 ±

111 0.46 g L⁻¹, volatile suspended solids (VSS) of 15.86 ± 0.28 g L⁻¹, a total COD (TCOD)
112 of 24540 ± 917 mg L⁻¹, total carbohydrates of 2862 ± 123 mg COD L⁻¹, and total
113 proteins of 14756 ± 225 mg COD L⁻¹. The inoculum was cultured by WAS with ethanol
114 as ED. The key properties of this inoculum were characterized by total solids (TS) of
115 17.69 ± 0.13 g L⁻¹, volatile solids (VS) of 9.79 ± 0.12 g L⁻¹, a TCOD of 16800 ± 591
116 mg L⁻¹, total carbohydrates of 1853 ± 81 mg COD L⁻¹, and total proteins of 9401 ± 157
117 mg COD L⁻¹. The commercial PAA solution was purchased from Tianjin Damao
118 Chemical Reagent Factory, and the PAA content in the solution was 12%.

119 **2.2 WAS disintegration by PAA pretreatment**

120 Effective sludge flocs disintegration is the essential precondition for the
121 acidogenic fermentation of WAS. In this batch experiment, we employed five serum
122 bottles as reactors, each containing 150 mL of WAS. Subsequently, a certain volume of
123 PAA solution was dosed to these reactors to reach a concentration of 0, 10, 15, 20, or
124 25 mg PAA per g TSS, respectively. This pretreatment process was conducted over a
125 24-hour period to ensure complete peroxidation [19, 23]. These tests were conducted in
126 triplicates. After pretreatment, we analyzed the contents of soluble organic components
127 (such as proteins and carbohydrates). Furthermore, changes in extracellular substances
128 and excitation-emission matrix (EEM) fluorescence intensities were determined to
129 expound the impacts of PAA oxidation on WAS properties. Fourier transform infrared
130 (FTIR) and X-ray photoelectron spectroscopy (XPS) were used to assess the structure
131 variations of extracellular organic components induced by PAA oxidation.

132 **2.3 Bioreactor operation for MCFAs production**

133 To evaluate the performance of MCFAs production from PAA pretreated WAS, we
134 employed five serum bottles ($V = 300$ mL) as anaerobic bioreactors. Briefly, 135 mL
135 pretreated WAS was employed as substrate, and 15 mL inoculum was applied as
136 inoculum. The procedures and PAA dosage for sludge pretreatment were the same as in
137 Section 2.2. The inoculated sludge (i.e., inoculum) was acclimatized by fermentation
138 of WAS with 50 mM 2-bromoethanesulfonate as methane inhibitor and 160 mM ethanol
139 as ED [13]. The initial pH values for all reactors were 6.5 and adjusted to 6.0 on day 6
140 when the pH dropped to about 5.5. To trigger effective MCFAs production, 160 mM
141 ethanol and 25 mM 2-bromoethanesulfonate were employed as ED and methanogen
142 inhibitors, respectively. Before anaerobic fermentation, all reactors were flushed with
143 N_2 , capped by butyl rubber stoppers, and wrapped with sealing films. All trials were
144 performed in triplicates. These reactors were operated for 14 days under 35 °C, during
145 which MCFAs, SCFAs, LCAs, and ethanol were determined every two days.

146 **2.4 Influence of PAA pretreatment on individual steps associated with MCFAs
147 production**

148 Three key bio-steps, i.e., hydrolysis, acidification, and CE, are associated with
149 MCFAs production when sewage sludge serves as substrate. PAA pretreatment could
150 result in soluble substrates increment and cause microbe damage to some degree. Thus,
151 how does PAA pretreatment affect microbial activities that determine MCFAs
152 production? To clarify the potential impacts, several standard substances were used to
153 simulate these processes and differentiate the response of each step to PAA pretreatment.
154 Three batch tests were conducted, i.e., hydrolysis, acidification, and CE tests, and the

155 sludge withdrawn from the above anaerobic reactors (described in section 2.3) was used
156 as inoculum. Before experiments, the residual substances in the inocula were removed
157 by washing with tap water. Each trial was performed in triplicates, as depicted in
158 Supplementary Information (SI).

159 **2.5 Microbial evolution and prediction of functional genes**

160 Microbial evolution was characterized by 16S rRNA sequencing, with 338F and
161 806R as primers [6]. Functional gene prediction was performed according to research
162 conducted by Huang et al. [24]. Sludge samples were taken from four bioreactors,
163 including the control, 15, 20, and 25 mg PAA per g TSS, for microbial analysis and
164 prediction of functional genes at the end of anaerobic fermentation (i.e., on day 14).

165 **2.6 Analytic methods and statistical assessment**

166 TS, VS, TSS, and VSS of sludge were detected by standard procedures [25]. COD,
167 proteins, and carbohydrates were determined using HACH reagents, Lowry-Folin, and
168 anthrone-sulfuric methods [22]. A meter (PHS-3E, Leici) was used to measure the
169 oxidation reduction potential (ORP) of sludge. The zeta potential of sludge samples was
170 detected by a zeta potential analyzer (Litesizer 500). A gas chromatography (GC2014,
171 SHIMADZU), equipped with a flame ionization detector (FID), was employed to detect
172 SCFAs, MCFAs, ethanol, and LCAs (i.e., butanol and hexanol) contents [26]. Protease
173 and α -glucosidase activities were analyzed according to research conducted by Xie et
174 al. [12]. The changes in the biodegradability of sewage sludge were assessed by
175 quantities of biodegradable substances due to PAA pretreatment using EEM
176 fluorescence technology. FTIR spectroscopy and XPS were determined according to

177 Wang et al. [27]. Free radicals and singlet oxygen were analyzed by an electron spin
178 resonance (ESR) spectrometer (JEOL JES-FA200) using DMPO (5,5-dimethyl-1-
179 pyrroline-N-oxide) and TEMP (2,2,6,6-tetramethyl-4-piperidine) as trapping agents.
180 Sludge morphology was detected by scanning electron microscopy (SEM), with
181 element content determined by energy dispersive spectroscopy (EDS). Experimental
182 data were analyzed using the SPSS software, with $p < 0.05$ statistically significant.

183 **3. Results and discussion**

184 **3.1 WAS disintegration and organics transformation triggered by PAA oxidation**

185 **3.1.1 Soluble substrates production**

186 The forms of proteins and carbohydrates, as the primary organic fractions
187 presented in WAS, are significantly influenced by PAA pretreatment (Fig. 1). Their
188 contents in the supernatant gradually increase with time and PAA dose within 24 h of
189 reaction (Fig. 1a–b). The release rates of proteins and carbohydrates are significantly
190 greater in the first 6 h, indicating that PAA can rapidly oxidize sludge flocs and
191 effectively convert particulate organic matter to soluble substrates. After pretreatment,
192 soluble proteins and carbohydrates concentrations reach 850.2 and 208.1 mg COD L⁻¹
193 for the 25 mg PAA per g TSS group (Fig. 1c), which are respectively elevated by 212.9%
194 ($p < 0.001$) and 133.0% ($p < 0.001$). Similarly, Yang et al. reported that after
195 pretreatment with 90 mg per g TSS peroxymonosulfate for 24 h, dissolved proteins and
196 carbohydrates increased to about 630 and 160 mg COD L⁻¹, respectively, and SCFAs
197 were eventually accumulated to 4894 mg COD L⁻¹ [28]. This suggests that PAA and
198 PMS have similar oxidative and destructive abilities on sludge flocs, thus providing

199 more precursors for SCFAs formation that can act as EA for MCFAs production.

200 EEM spectra of pretreated WAS can further disclose the enhanced effect of PAA

201 oxidation on WAS dissolution. As displayed in Fig. 1d, two fluorescence peaks, peak 1

202 and peak 2, appear on the spectrogram, and their color reflects the changes in organic

203 matter content [29]. The fluorescence intensity is enhanced by PAA pretreatment at all

204 doses compared to the control, implying more soluble material release. Peak 1

205 represents soluble microbial byproducts like organics, and they are widely considered

206 biodegradable substrates, whose significant increase indicates the improvement of

207 biochemical degradability [30]. Interestingly, the peak fluorescence intensity decreases

208 when the PAA dose exceeds 10 mg per g TSS (Fig. 1d), inconsistent with elevated

209 proteins and carbohydrates releases. It may be attributed to the higher level of strong

210 oxidative species (such as ·OH) triggered by increased PAA dose, which could react

211 with the dissolved organics in sludge and destroy their fluorescent groups.

Fig. 1.

3.1.2 Changes in sludge properties

212 Previous studies have shown that PAA could cause cell inactivation and inhibit

213 functional enzyme activities [17, 31]. However, details of PAA pretreatment on EPS

214 structure have been neglected [32]. As displayed in Fig. 2a–b, EPS is predominantly in

215 the tightly bound (TB) state for the control, explaining the low rate of sludge

216 solubilization. Proteins and polysaccharides contents in TB-EPS decrease gradually

217 with the increase of PAA dose. For loosely bound (LB)-EPS, the control group has

218 fewer proteins than raw WAS, while PAA groups all have higher proteinic organics than

221 raw WAS. It implies that PAA oxidation leads to decreased EPS and migration of TB-
 222 EPS to LB-EPS [1, 33]. Whereas the variation of polysaccharides is not in accord with
 223 proteins, which may be due to its low content in LB-EPS (i.e., 5.4–9.8 mg COD L⁻¹).
 224 The EEM spectra further support the variation of protein contents in EPS (Fig. S1 and
 225 Fig. 2c). Apparently, the fluorescence peak intensity of TB fraction decreases
 226 significantly with PAA dose, while that of LB fraction in the control is lower than those
 227 of PAA groups.

228 Furthermore, Figure 2d reflects the infrared spectra information of different EPS
 229 fractions extracted from the control and PAA-pretreated WAS. The infrared peak about
 230 1580–1670 cm⁻¹ or near 1410 cm⁻¹ represents the stretching vibrations of C–N and
 231 C=O, or C=O symmetric stretching of –COOH groups in proteins, respectively [34].
 232 The bond at 1070 cm⁻¹ stands for the vibration of C–OH and C–O–C groups in organics
 233 (i.e., carbohydrates or polysaccharides) [34]. Generally, PAA pretreatment increases the
 234 infrared peak intensities of these organic component groups in LB fraction (e.g., C–OH
 235 and C–O–C) while significantly reducing their intensities in TB fraction (e.g., C–N,
 236 C=O, C–OH, and C–O–C), consistent with Fig. 2a and 2b. It implies the disruption,
 237 migration, and dissolution of sludge EPS. As a response, some organic groups at bonds
 238 1580–1670 cm⁻¹ are increased in the soluble fraction after PAA pretreatment. In
 239 addition, the alteration of sludge structure by PAA can be visualized by SEM images
 240 (Fig. 2e–f). After pretreatment, sludge flocs become loose, and EPS is reduced,
 241 exposing more microorganisms. Accordingly, PAA destroys the protective barrier of
 242 microbial cells against extreme environments (i.e., oxidative stress) and facilitates the

243 rupture of sludge cells. Chen and Pavlostathis [31] indicated that PAA affected the
 244 biological nitrogen removal system by causing cell lysis and enzyme inhibition. Ao et
 245 al. [17] reported that PAA disrupted the membrane and cell wall lipids and attacked the
 246 DNA and RNA in microorganisms. These results above illustrate that PAA disrupts the
 247 dense sludge floc structures by causing the disruption and migration of EPS fractions,
 248 thus boosting the dissolution of EPS and intracellular organics. EPS destruction is
 249 conducive to the contact of hydrolases and functional microorganisms with released
 250 organic components. Subsequently, the soluble organics produced by sludge
 251 disintegration would be rapidly and efficiently converted to SCFAs, supplying more
 252 directly accessible EA for CE bacteria.

253 **Fig. 2.**

254 **3.1.3 Degradation and transformation of extracellular macromolecular organics**

255 As the primary organic substrate in WAS, proteins occupy 60.1% of TCOD, whose
 256 extracellular transformation can be reflected by conformational structure alterations.
 257 Hence, for characterizing the change of proteinic conformation, FTIR spectra with
 258 wavelengths ranging from 1600–1700 cm⁻¹ were subjected to deconvolution and curve-
 259 fitting analysis (Fig. 3a) [34]. The relative proportions of proteinic secondary structures
 260 in the soluble, LB, and TB fractions are significantly altered after PAA pretreatment.
 261 Particularly, the proteinic conformation in the soluble fraction is significantly disrupted
 262 by PAA oxidation, as only aggregated strands are found, i.e., the band at 1610–1625 or
 263 1680–1695 cm⁻¹. It has been indicated that proteinic stability is mostly determined by
 264 α -helix, β -sheet, and random coil, and a low ratio of α -helix/(β -sheet + random coil)

265 could make the proteinic structure looser [35]. In Fig. 3c, the proportion of α -helix/(β -
266 sheet + random coil) is largely reduced by PAA, with LB fraction from 33.1% to 24.6%
267 and TB fraction from 72.3% to 46.9%. These observations demonstrate that PAA
268 pretreatment changes the secondary structures of extracellular proteins, promoting their
269 decomposition and transformation [36].

270 XPS C 1s spectra can be divided into four peaks, as shown in Fig. 3b. The peak at
271 284.8 eV (C1) is associated with C–(C,H), primarily ascribed to polysaccharides, lipids,
272 and side chains of amino acids [36]. The ratio of C1 increases from 40.3% to 72.6%
273 after PAA pretreatment, which proves the efficient WAS disintegration and particulate
274 organics solubilization (Fig. 1). The peak at 286.1 eV (C2) belongs to C–(O,N) bonds
275 from amines, amides, alcohols and ethers groups, while that at 288.0 eV (C3) is
276 associated with O–C–O and C=O primarily existed in acetal, hemiacetal, amide,
277 carboxylic or carbonyl groups [37]. The apparent decrease of C2 (from 43.9% to 13.4%)
278 and C3 (from 15.8% to 0) might be attributed to the effective decomposition of
279 macromolecular substances or intermediates by PAA pretreatment. Accordingly, the
280 peak at 288.5 eV (C4) increases from 0 to 14.0%, attributing to O=C–OR and O=C–OH
281 in ester or carboxyl groups. It implies that PAA results in considerable substrate
282 transformation and SCFAs accumulation during the pretreatment step [37]. N1 at 399.6
283 eV is closely related to the N–C–R bond in amino acids of proteins or peptidoglycans
284 [38]. N2 at 400.4 eV reflects the presence of the H–N–C=O bond, i.e., the pivotal
285 structure in proteins and peptide chains [38]. The increase of N1 (from 35.7% to 57.1%)
286 and N2 (from 22.0% to 37.8%) further supports the elevation of protein levels in the

287 aqueous phase and the destruction of proteins and peptidoglycans induced by PAA
 288 pretreatment, concurrently (Fig. 3b). The peak at 402.0 eV represents the existence of
 289 inorganic N, and its increase from 0 to 5.1% intuitively indicates the promoted proteins
 290 and peptidoglycans degradation and the production of ammonia nitrogen [39].
 291 Additionally, peaks at 164.0 eV (S1) and 169.0 eV (S2) in S 2p spectra, respectively,
 292 belong to the S–S bond and inorganic S (Fig. 3b) [36]. The decrease of the S–S bond
 293 and increase of inorganic S hints that PAA promotes the rupture of proteinic spatial
 294 structure and strengthens the degradation and transformation of extracellular
 295 macromolecular organics (e.g., proteins) in WAS [40].

296 **Fig. 3.**

297 **3.2 MCFAs production from PAA-pretreated sludge through anaerobic
 298 fermentation**

299 Figure 4 displays ethanol consumption results and product generation from sewage
 300 sludge. Ethanol (i.e., ED) is completely utilized by CE microorganisms (Fig. 4a), and
 301 accumulative MCFAs stop rising at the end (Fig. 4b), suggesting the completion of the
 302 CE process. Caproate is the only MCFAs for all experiments, similar to previous studies
 303 using sewage sludge to produce MCFAs [11, 13]. The highest MCFAs production is
 304 7173.1 mg COD L⁻¹ for the control, similar to a previous study in which MCFAs
 305 production was 6115 mg COD L⁻¹ with sewage sludge as substrates and 170 mM
 306 ethanol as ED [13]. For experimental groups, MCFAs peak values are dependent on
 307 PAA dosage. PAA pretreatment with a dosage from 10–15 mg per g TSS only shortens
 308 the optimal fermentation time rather than further improves the maximal MCFAs

309 production ($p > 0.05$). In contrast, the maximal MCFAs concentration is boosted from
 310 8859.8 to 11265.6 mg COD L⁻¹ when the PAA dose increases from 20 to 25 mg per g
 311 TSS, respectively increasing by 23.5% ($p = 0.002$) and 57.1% ($p = 0$) than the control.
 312 The maximal MCFAs production attained in this work is higher than that of pretreated
 313 sludge with free ammonia (8300 mg of COD L⁻¹) [15]. Besides, the optimal time
 314 required for MCFAs accumulation in the PAA-pretreated groups is shortened from 12
 315 to 10 days. These facts prove that PAA pretreatment promotes MCFAs production and
 316 accelerates MCFAs generation rate.

317 LCAs, as the main byproducts of CE, usually occur with MCFAs formation. The
 318 maximum concentration of LCAs (including butanol and hexanol) is 4629.0 mg COD
 319 L⁻¹ for the control, similar to that of 10 or 15 mg PAA per g TSS reactors (Fig. 4c).
 320 Whereas, PAA pretreatment at 20 or 25 mg per g TSS significantly decreases LCAs
 321 production ($p = 0$). Similarly, Yin and Wang reported that LCAs production from
 322 sewage sludge was repressed by AOP pretreatment [16]. Wang et al. found that FNA
 323 pretreatment largely promoted MCFAs production while decreasing LCAs
 324 accumulation in the sludge fermentation system supplemented with ethanol [41]. In
 325 contrast, Fe₃O₄ addition was revealed to concurrently promote MCFAs and LCAs
 326 generation [8]. Thereby, it could be speculated that PAA pretreatment suppresses the
 327 microbes that produce LCAs due to its microbiocidal effect, reducing the conversion of
 328 organic carbons to byproducts. Due to PAA pretreatment, MCFAs proportion increases
 329 from 44.4% to 56.9%, while LCAs proportion reduces from 28.6% to 4.0% (Fig. 4d).
 330 This indicates that PAA pretreatment promotes the carbon specificity towards MCFAs,

331 and thus greatly avoids electron equivalents loss of ED.

332 **Fig. 4.**

333 **3.3 Microbial community evolutions**

334 Figure 5 elucidates the community succession of MCFAs-forming bioreactors
 335 mediated by PAA pretreatment. Rarefaction curves indicate the reliability of sequencing
 336 results (Fig. S2). Alpha diversity analysis suggests that PAA pretreatment has a
 337 negligible effect on community richness but increases community diversity (Table S1).

338 As displayed in Fig. 5a, Firmicutes is the predominant phylum in experimental groups,
 339 whose abundance is positively associated with PAA dose, increasing from 20.3% (i.e.,
 340 the control) to 45.0%. Firmicutes can hydrolyze and convert complex macromolecular
 341 organics into small molecules, thus promoting substrate transformation and providing
 342 EA for CE [12]. Firmicutes were also enriched in anaerobic systems for MCFAs
 343 generation [11].

344 Microbial composition at a genus level was further analyzed (Fig. 5b). PAA
 345 pretreatment significantly enriches functional microorganisms, i.e., those associated
 346 with hydrolysis, acidification, and CE, with their abundances rising from 18.3% to
 347 39.7%. *Paraclostridium*, positively associated with substrate hydrolysis and MCFAs
 348 production [16, 42], dominates in experimental groups, i.e., 12.2% for 20 mg PAA per
 349 g TSS and 12.9% for 25 mg PAA per g TSS, versus 0.5% in the control. *Clostridium* is
 350 a common SCFAs producer and a typical CE microorganism [43], whose abundance
 351 significantly increases with PAA dose, i.e., from 10.0% to 17.9%. Besides,
 352 *NK4A214_group* is related to butyric acid production [44], and *Romboutsia* is positive

353 to SCFAs generation [11]. Their abundances are also enhanced in experimental groups.
 354 On the contrary, some non-functional bacteria (e.g., denitrifying bacteria) are
 355 significantly reduced. The enrichment of functional microorganisms is mainly
 356 attributed to PAA oxidation, which leads to the removal/inhibition of non-functional
 357 microbes and stimulates the metabolism of functional microorganisms by providing
 358 more soluble organics. Specifically, sludge floc is fragmented, inhibiting initial
 359 microbial activity due to PAA pretreatment. Meanwhile, substantial amounts of
 360 dissolved organics are directly available to anaerobes, triggering the revival and
 361 enrichment of hydrolytic and SCFAs-producing bacteria that are usually more tolerant
 362 to extreme environments [45, 46]. Driven by ethanol, EA from sludge and PAA
 363 decomposition are elongated to MCFAs by CE microorganisms. Consequently,
 364 functional microorganisms are enriched by PAA pretreatment, and more substrates are
 365 converted to MCFAs.

366 **Fig. 5.**

367 **3.4 Mechanisms of enhanced MCFAs production from WAS by PAA pretreatment**

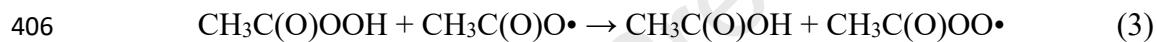
368 **3.4.1 Reaction mechanisms of PAA oxidation for WAS disintegration**

369 Previous studies reported that PAA led to pollutants degradation mainly by
 370 inducing the generation of inorganic radicals (i.e., $\cdot\text{OH}$, $\cdot\text{O}_2^-$), organic radicals ($\text{R}-\text{O}\cdot$
 371 (i.e., $\text{CH}_3\text{COO}\cdot$, $\text{CH}_3\cdot$, $\text{CH}_3\text{C}(\text{O})\text{O}\cdot$), and $\text{CH}_3\text{C}(\text{O})\text{OO}\cdot$) and singlet oxygen ($^1\text{O}_2$),
 372 which are strongly oxidative to WAS flocs [47, 48]. In Fig. 6a–c, the signal peaks of
 373 three adducts, i.e., DMPO–OH, PMPO– O_2^- , and TEMP– $^1\text{O}_2$, are found, with
 374 intensities detected at 10 min stronger than those at 5 min of reaction. This implies that

375 •OH, •O₂⁻, and ¹O₂ play important roles in the PAA-based sludge pretreatment system
 376 [1, 33]. It was indicated the DMPOX signal could be accompanied by DMPO-OH,
 377 which might be formed via DMPO oxidation by R-O• and thus closely correlated with
 378 the presence of R-O• [49]. Since CH₃OO• and CH₃• are significantly weaker than
 379 CH₃C(O)O• and CH₃C(O)OO• in terms of oxidation capacity, the former is usually
 380 considered to be negligible for PAA-induced AOP [50]. It was recorded that DMPO
 381 could be oxidized by R-O• to generate a DMPOX signal [51]. As shown in Fig. 6a,
 382 weak DMPOX signals coexist with DMPO-OH adduct, consistent with previous
 383 studies, which proves the presence of CH₃C(O)O• and CH₃C(O)OO• during the
 384 pretreatment stage [49]. Figure 6d reflects the dynamic variations of the redox state in
 385 the sludge pretreatment system. The ORP is largely enhanced by PAA-based AOP in
 386 the first 20 min, e.g., 493 mV at 25 mg per g TSS, illustrating the strong oxidative
 387 capacity of PAA. Then, it gradually decreases and finally approaches 0 or below after
 388 24 h of pretreatment, implying the completion of oxidation reactions. Hence, the
 389 introduced PAA may not harm the subsequent fermentation stage.

390 How does PAA lead to active species production without external iron addition?
 391 Iron is the commonly existing transition metal in sewage sludge, ranging from 2.9 to
 392 39.7 mg Fe per g TS [22]. It was demonstrated that Fe²⁺ could activate PAA to promote
 393 the degradation of naproxen, bisphenol-A, and methylene blue [52]. EDS results
 394 illustrate iron as the dominant transition metal in the control and PAA-pretreated sludge
 395 (Table S2). Iron content on the surface of PAA-induced WAS is lower than that of the
 396 control (Fig. 6d), attributing to the fact that PAA-induced AOP causes Fe²⁺ release from

397 EPS and its redistribution in sludge flocs through disrupting sludge structure. [53]. Thus,
 398 Fe^{2+} is likely to be the vital substance for PAA activation by WAS. The zeta potential
 399 results further indicate that PAA pretreatment increases the positive charge of the
 400 centrate liquid (Fig. S3). It can be further inferred that after PAA enters sludge, it is
 401 activated by Fe^{2+} in sludge, inducing a sequence of chemical reactions and strong
 402 oxidizing materials. Thereby, relative reaction mechanisms for Fe^{2+} /PAA AOP could be
 403 proposed in equations (1)–(7) [48, 49, 52].



411 Accordingly, these reactive species cause oxidative stress to microorganisms in
 412 sludge and lead to the leakage of intracellular organic components [4]. Meanwhile, they
 413 disintegrate sludge structure and cause the decomposition and transformation of EPS,
 414 which provides abundant soluble substrates directly accessible to anaerobic bacteria
 415 and creates looser EPS for further microbial metabolism, facilitating rapid EA (i.e.,
 416 SCFAs) generation for CE process in the subsequent fermentation stage [15].

417 **Fig. 6.**

418 **3.4.2 Variation in metabolic activities of key bioprocesses**

419 The organic matter released from sludge disintegration undergo hydrolysis,
420 acidification, and CE processes to achieve carbon chain upgrading [8]. Is the microbial
421 metabolic activity of these key processes affected by PAA pretreatment? Thus, the
422 results of synthetic wastewater experiments reveal the potential impact at a macroscopic
423 level (Fig. 7).

424 After four days' fermentation, the degradation extent of BSA is respectively 32.1%
425 ($p = 0.002$) and 35.7% ($p = 0.001$) in the experimental groups of 20 and 25 mg PAA per
426 g TSS, largely greater than the control (i.e., 26.9%) (Fig. 7a). Dextran is almost
427 completely degraded for all groups on day 4, while PAA groups exhibit a lower
428 degradation extent during the initial two-day fermentation (Fig. S4a). These results can
429 be supported by protease and α -glucosidase activities in anaerobic reactors (Fig. S4b).
430 It is implied that PAA might stimulate the metabolic activity of protein-like substrates
431 and inhibit the degradation of polysaccharide-like substrates but generally behave as a
432 facilitator of hydrolytic processes. Although PAA inhibits substrate acidification on day
433 1, glucose and amino acids of all groups could be completely metabolized after the
434 three-day fermentation (Fig. 7b), indicating the slight effect of PAA pretreatment on the
435 microbial activity of acidification [21].

436 In contrast, PAA pretreatment promotes ethanol and acetate consumption while
437 increasing butyrate and caproate production (Fig. 7c–d). On day 8, butyrate and
438 caproate are respectively 4984.2 and 3432.1 mg COD L⁻¹ for the control, which are
439 significantly lower than the 25 mg per g TSS group, i.e., 6176.2 ($p = 0.006$) and 4886.8
440 ($p = 0.001$) mg COD L⁻¹. Meanwhile, the yield of LCAs in the experimental group is

441 deducted, suggesting a reduced conversion to byproducts and an improved carbon
 442 specificity towards MCFAs [54].

443 **Fig. 7.**

444 **3.4.3 Variation in microbial functions and functional gene expression**

445 The microbial potential functions were predicted by PICRUSt software (Fig. 7e),
 446 further elaborating on the changes in microbial metabolism at the genetic level.
 447 Generally, 11 of the selected 2-level KEGG pathways are stimulated by PAA
 448 pretreatment. The potential functions related to substrate degradation, i.e.,
 449 carbohydrates and amino acids, are upregulated in PAA-pretreated systems. The higher
 450 abundances of carbohydrate and amino acid metabolisms indicate efficient conversion
 451 of organics to EA [43]. Additionally, functions of cell motility, membrane transport, and
 452 replication and repair affiliated to cellular processes, environmental information, and
 453 genetic information are enhanced in PAA reactors.

454 Upgrading of EA (i.e., SCFAs) is achieved by CE microorganisms via two cyclic
 455 processes, i.e., fatty acid biosynthesis (FAB) and reverse β -oxidation (RBO) (Fig. 8a)
 456 [10]. RBO is started by acetyl-CoA directly, while FAB is initiated by malonyl-CoA,
 457 derived from acetyl-CoA carboxylation with ATP expense [16]. The generation of
 458 acetyl-CoA and the entire CE pathways involve the participation of functional enzymes
 459 [7]. Generally, the genes encoding functional enzymes in the FAB pathway are
 460 strengthened in experimental groups and higher than in the RBO pathway (Table S3).
 461 Figure 8b presents the main predictions of functional gene abundances encoding
 462 specific enzymes. Acetyl-CoA might be generated from ethanol and H₂/CO₂, and the

463 former is the main pathway due to its higher abundance in functional enzymes. EC
 464 1.1.1.1 (adh) is significantly enriched, indicating increased ethanol consumption in
 465 experimental groups, consistent with the synthetic wastewater experiment (Fig. 7d).
 466 Besides, the abundances of EC 1.2.7.4 (cdhA) and EC 2.1.1.245 (cdhD) are increased,
 467 and they are associated with acetyl-CoA production from H₂/CO₂. It implies more
 468 endogenous electron donors likely to be produced due to PAA pretreatment. Specifically,
 469 there is an increase in EC 6.4.1.2 (ACACA), EC 2.3.1.39 (fabD), EC 2.3.1.80 (fabH),
 470 and EC 1.3.1.9 (fabV), which are respectively functional enzymes involved in the
 471 generation of malonyl-ACP, and initiation/termination of CE in FAB pathway. It is
 472 hinted that PAA facilitates SCFAs upgrading through stimulating ethanol
 473 transformation and FAB pathway. These facts expound the positive influence of PAA
 474 pretreatment on microbiological metabolism in sludge fermentation systems, further
 475 revealing the mechanism of enhanced MCFAs production at a microscopic level.

476 Fig. 8.

477 3.5. Implications

478 Since MCFAs have longer carbon chains, they possess higher energy density and
 479 are more hydrophobic compared to SCFAs [6], thus suitable for large-scale industrial
 480 production [26]. Accordingly, CE has recently become a growingly attractive anaerobic
 481 technology to convert primary fermentation products of WAS, i.e., SCFAs, into MCFAs
 482 with ethanol as ED [8]. However, the organic matter of WAS is well protected by EPS
 483 and hard cell walls [15]. Thus, pretreatment techniques are usually required. As a green
 484 oxidizer and disinfectant, PAA is widely available and easily accessible. PAA-based

485 AOP has been proven powerful for WAS cracking, accumulation of SCFAs, methane,
486 and hydrogen from sludge, sludge dewatering, and pollutants degradation [17, 19-21,
487 23]. Nevertheless, these studies only focused on the traditional fermentation model or
488 chemical oxidation-based PAA and neglected the underlying mechanisms. This study
489 aims to fill the knowledge gap and broaden the understanding and applicability of PAA-
490 based AOP in CE.

491 Results show that PAA significantly promotes sludge disruption and increases
492 dissolved material quantities. Further characterization of WAS structure and property
493 illustrates that PAA oxidation causes substantial EPS exfoliation from microbiological
494 indications, disrupts the secondary structure of EPS and solution layer proteins, and
495 enhances the degradation and conversion of macromolecular organic matter (e.g.,
496 proteins and polysaccharides). It is confirmed that PAA pretreatment creates a looser
497 EPS and provides abundant accessible substrates for subsequent sludge fermentation.
498 Consequently, MCFAs production is promoted to 11265.6 mg COD L⁻¹, while the
499 byproducts, i.e., LCAs, are decreased to 723.5 mg COD L⁻¹. Mechanistic studies show
500 that the production of free radicals (i.e., •OH, •O₂⁻, CH₃C(O)O•, and CH₃C(O)OO•)
501 and singlet oxygen (¹O₂) are the oxidation mechanisms for WAS disruption. However,
502 the oxidative stress induced by PAA largely disappears after 24 h, rather than causing a
503 negative influence on the subsequent bioprocesses. Further, hydrolytic enzyme tests and
504 synthetic wastewater experiments demonstrate at a macroscopic level that PAA
505 stimulates protease activity and enhances the metabolic activity of protein-like substrate
506 degradation and CE process while it causes a slight effect on that of acidification.

507 Additionally, KEGG pathways and functional gene analysis microscopically illustrate
508 that PAA pretreatment up-regulates the functional genes related to substrate degradation
509 and cellular activity, and increases the functional enzyme abundances in ethanol
510 conversion and FAB pathway. These findings provide a systematic and comprehensive
511 understanding of the effectiveness and mechanism for enhanced MCFAs generation by
512 PAA-based AOP.

513 In this work, PAA is activated by Fe^{2+} contained in WAS without extra activator
514 addition, and relative reactions occur under mild pH ranges, avoiding the increase of
515 iron-bearing sludge at WWTPs (Fig. S5). The main product of PAA oxidation is acetic
516 acid, which could serve as EA for MCFAs formation. This indicates that PAA has the
517 dual role of cracking sludge and replenishing EA, posing no environmental risk. In
518 contrast, other oxidant-induced AOP, such as calcium peroxide and persulfate [16, 55],
519 might cause great risks to the equipment and final sludge disposal due to the detrimental
520 byproduct formation. It could be speculated that PAA would probably contribute to
521 killing potential pathogenic bacteria and degrading micropollutants in WAS due to its
522 powerful oxidizing property, further reducing the environmental risk of sludge. Besides,
523 LCAs concentration is reduced to $723.5 \text{ mg COD L}^{-1}$, clearly inferior to the current
524 reported values [15, 27]. It might be attributed to the potential inhibitions of PAA to
525 LCAs microorganisms [16]. Inversely, functional microorganisms are enriched, the
526 metabolic activity of key processes is heightened, and functional enzyme abundances
527 in CE are enhanced. This suggests that the antimicrobial properties of PAA could
528 selectively inhibit LCAs microorganisms, decreasing electron flow to byproducts but

improving the conversion of EA and ED to MCFAs. In comparison, MCFAs production triggered by the PAA technique (i.e., 70.4 mg COD per mM ED) is significantly higher than that of other strategies, e.g., Fe₂O₃ (i.e., 53.9 mg COD per mM ED) [26], Fe₃O₄ (i.e., 46.8 mg COD per mM ED) [11], combined alkaline fermentation with CE (i.e., 20.2 mg COD per mM ED) [6], free ammonia (i.e., 59.3 mg COD per mM ED) [15], and ferrate pretreatment (i.e., 47.7 mg COD per mM ED) [27]. Despite the multiple advantages of PAA-based technology, this study is currently a proof of concept. Therefore, endeavors should be dedicated to driving the engineering application of this technology, such as combining it with other technologies, e.g., ultrasound radiation, thermal pretreatment, or pH adjustment, to enhance sludge solubilization and reduce PAA dosage [30, 48]. The effectiveness of PAA-based AOP for inactivating pathogens and degrading contaminants in fermented sludge also warrants future investigations.

4. Conclusion

This work discloses the positive functions of PAA-based AOP on MCFAs production from sewage sludge. Results exhibit that PAA pretreatment largely disrupts sludge structure and promotes EPS decomposition. Consequently, this leads to a substantial increase in MCFAs production, accompanied by a notable reduction in the formation of undesired byproducts, such as LCAs. Microbial analysis proves functional microbe enrichment. Mechanistic investigations illustrate that sludge disruption is closely related to the generation of free radicals (i.e., •OH, •O₂⁻, CH₃C(O)OO•, and CH₃C(O)O•) and singlet oxygen (¹O₂) through PAA activation by Fe²⁺ in WAS. Furthermore, microbial activity tests demonstrate the positive impacts of PAA

551 pretreatment on protein-like substrate degradation and the CE process. Additionally, CE
552 improvement might be ascribed to functional gene up-regulation in ethanol
553 transformation and FAB pathway.

554

555 **CRediT authorship contribution statement**

556 **Yufen Wang:** Investigation, Methodology, Data Curation, Writing - Original Draft,
557 Software, Writing - Review & Editing. **Haixiao Guo:** Formal Analysis, Investigation,
558 Visualization. **Xuecheng Li:** Investigation, Methodology, Formal Analysis. **Xueming**
559 **Chen:** Conceptualization, Validation. **Lai Peng:** Writing - Review & Editing,
560 Validation. **Tingting Zhu:** Methodology, Supervision, Validation. **Peizhe Sun:**
561 Conceptualization, Supervision, Methodology. **Yiwen Liu:** Conceptualization,
562 Resources, Writing - Review & Editing, Supervision, Project Administration, Funding
563 Acquisition.

564

565 **Declaration of competing interest**

566 The authors declare that they have no known competing financial interests or personal
567 relationships that could have appeared to influence the work reported in this paper.

568

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573

574 **Supporting Information**

575 Additional details about materials and methods used in this study, including one
576 section of experimental procedures, five figures (Figs. S1–S5), and three tables (Tables
577 S1–S3).

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Figure captions

Fig. 1. **a–b**, Changes of soluble proteins (**a**) and carbohydrates (**b**) within 24 h of PAA oxidation. **c**, Soluble proteins and carbohydrates contents after pretreatment with significance test results. **d–h**, EEM spectra after pretreatment: Control (**d**), 10 (**e**), 15 (**f**), 20 (**g**), and 25 mg PAA per g TSS (**h**). **i**, Peak fluorescence intensity of EEM spectra. “*” represents $p < 0.05$, “**” represents $p < 0.01$, “***” represents $p < 0.001$. Error bars represent standard deviations of triplicate experiments.

Fig. 2. **a–b**, Contents of proteins (**a**) and polysaccharides (**b**). **c**, Peak fluorescence intensity of extracted EPS. **d**, FTIR spectra of soluble and bound EPS fractions. **e–f**, changes of sludge structure reflected by SEM, i.e., the control (**e**) and PAA group (**f**).

Fig. 3. **a**, The second-derivative fitting curves of FTIR spectra from 1700 to 1600 cm^{-1} , with samples being the soluble, LB, and TB fractions of control and PAA-pretreated WAS. **b**, XPS C 1s, N 1s, and S 2p analysis of aqueous phase obtained from the control and PAA-pretreated WAS. **c**, The area (%) of secondary structures determined by second-derivative fitting.

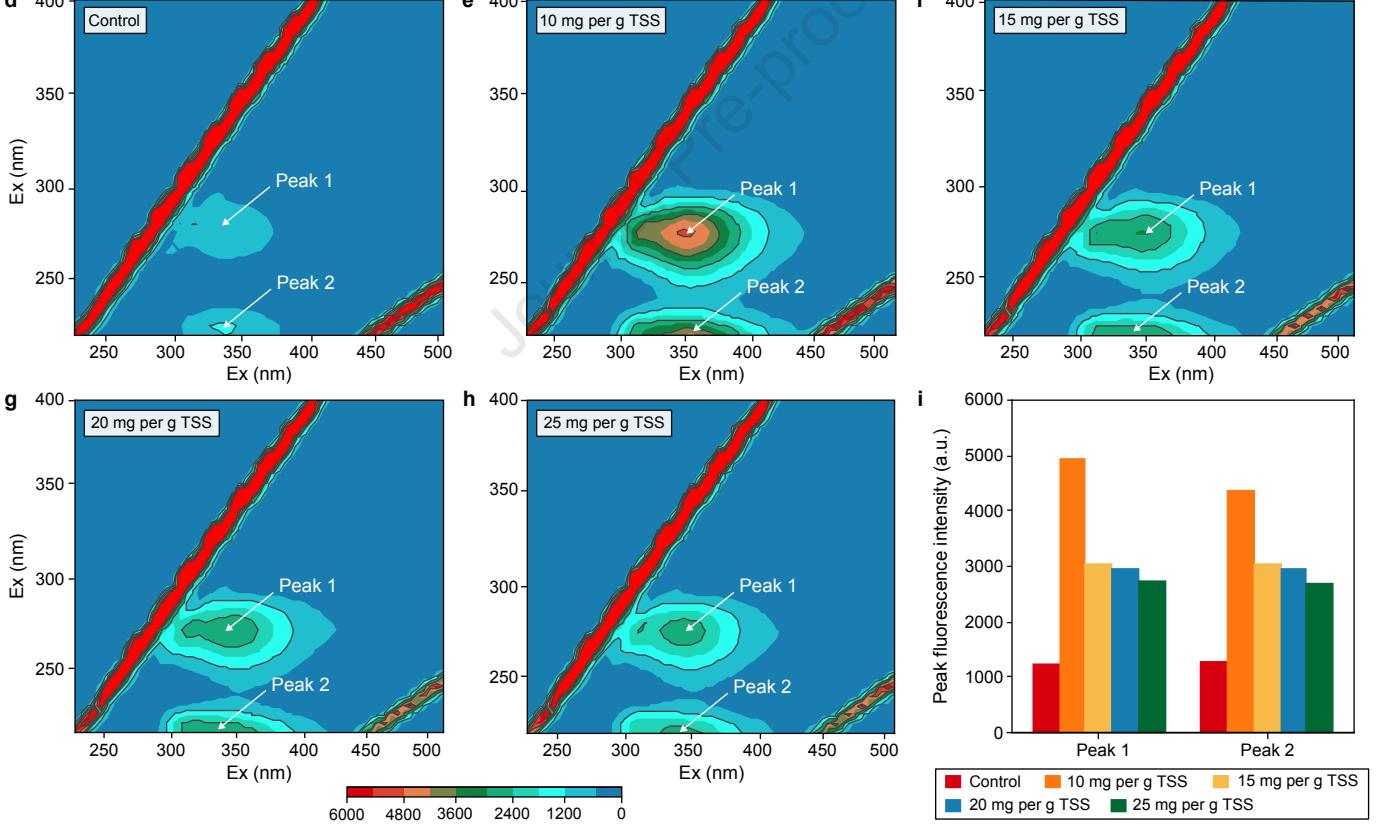
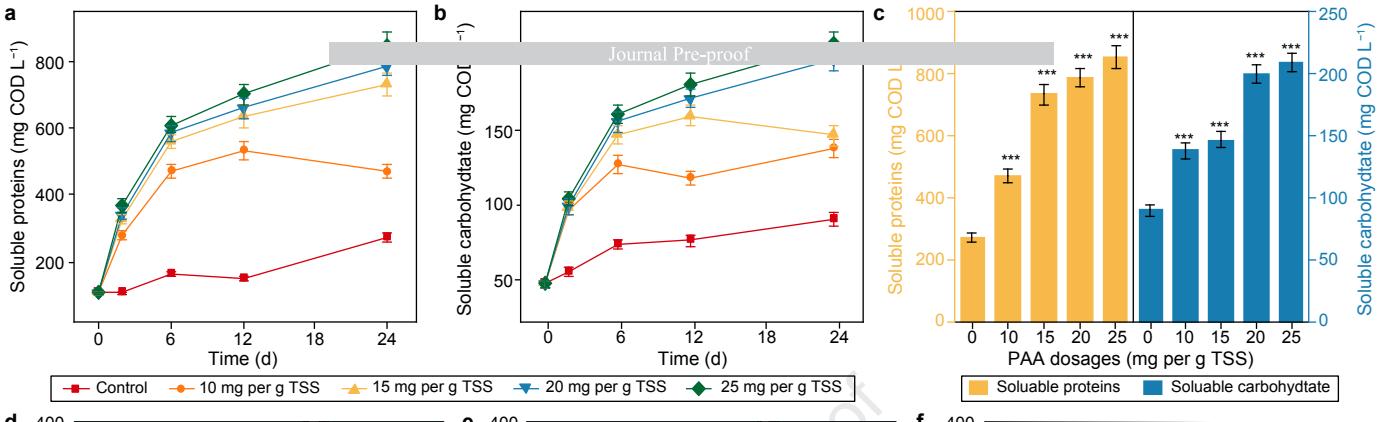
Fig. 4. Concentrations evolution of ethanol (**a**), MCFAs (**b**), LCAs (**c**), and product distribution (**d**) in the control and PAA-pretreated reactors during anaerobic sewage sludge fermentation with ethanol as ED. “*” represents $p < 0.05$, “**” represents $p < 0.01$, “***” represents $p < 0.001$. Error bars represent standard deviations of triplicate experiments.

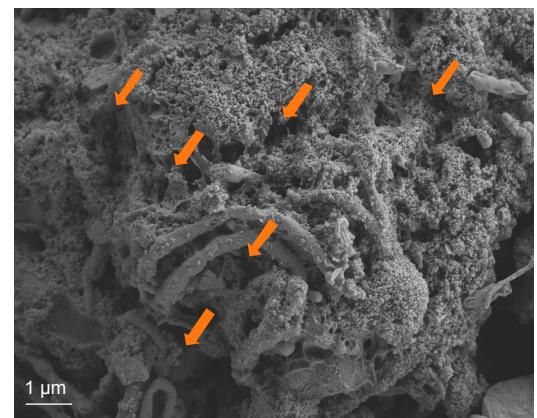
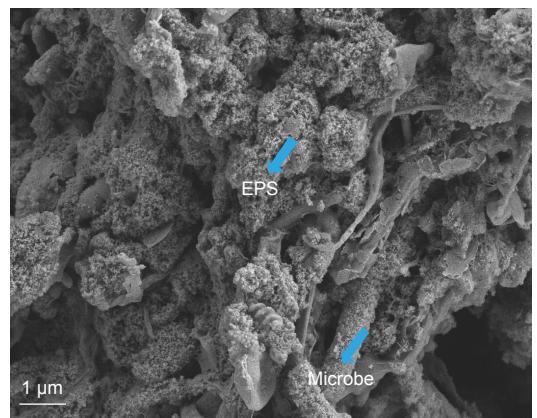
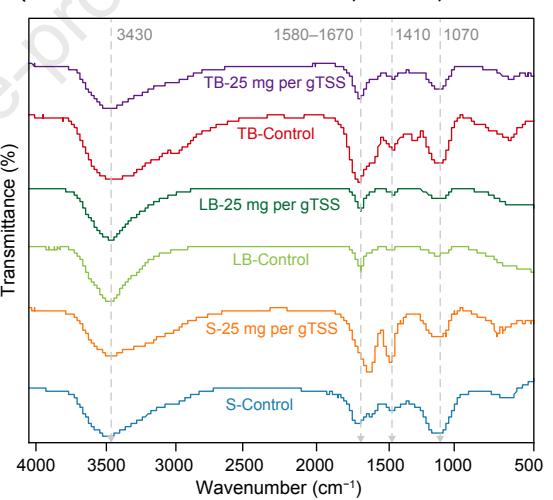
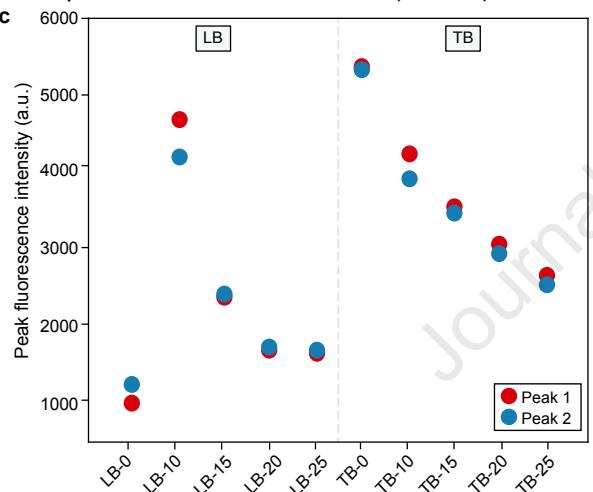
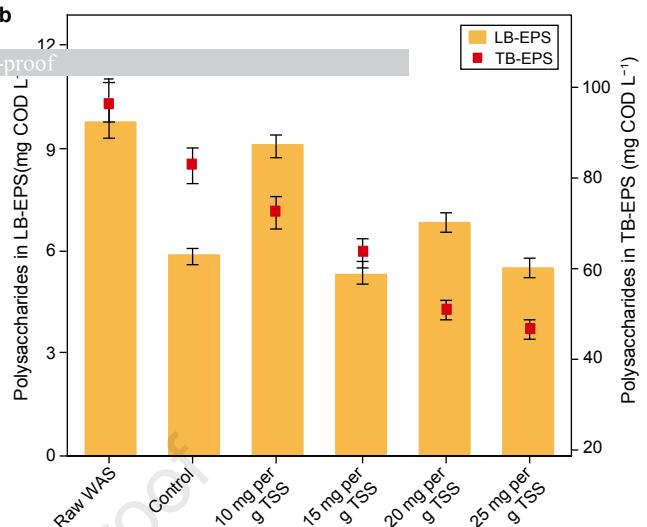
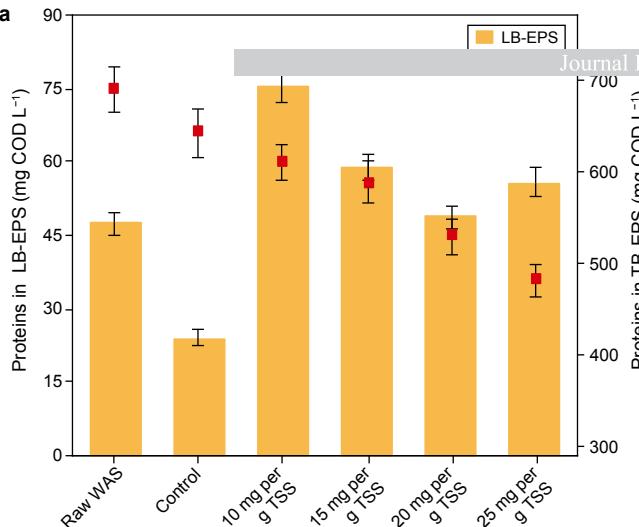
Fig. 5. Microbial community evolutions of the experimental (i.e., 15, 20, and 25 mg PAA per g TSS) and control reactors. **a**, Microbial abundance analysis at a phylum level. **b**, Heatmap of functional microorganisms and typical non-functional bacteria based on a genus level.

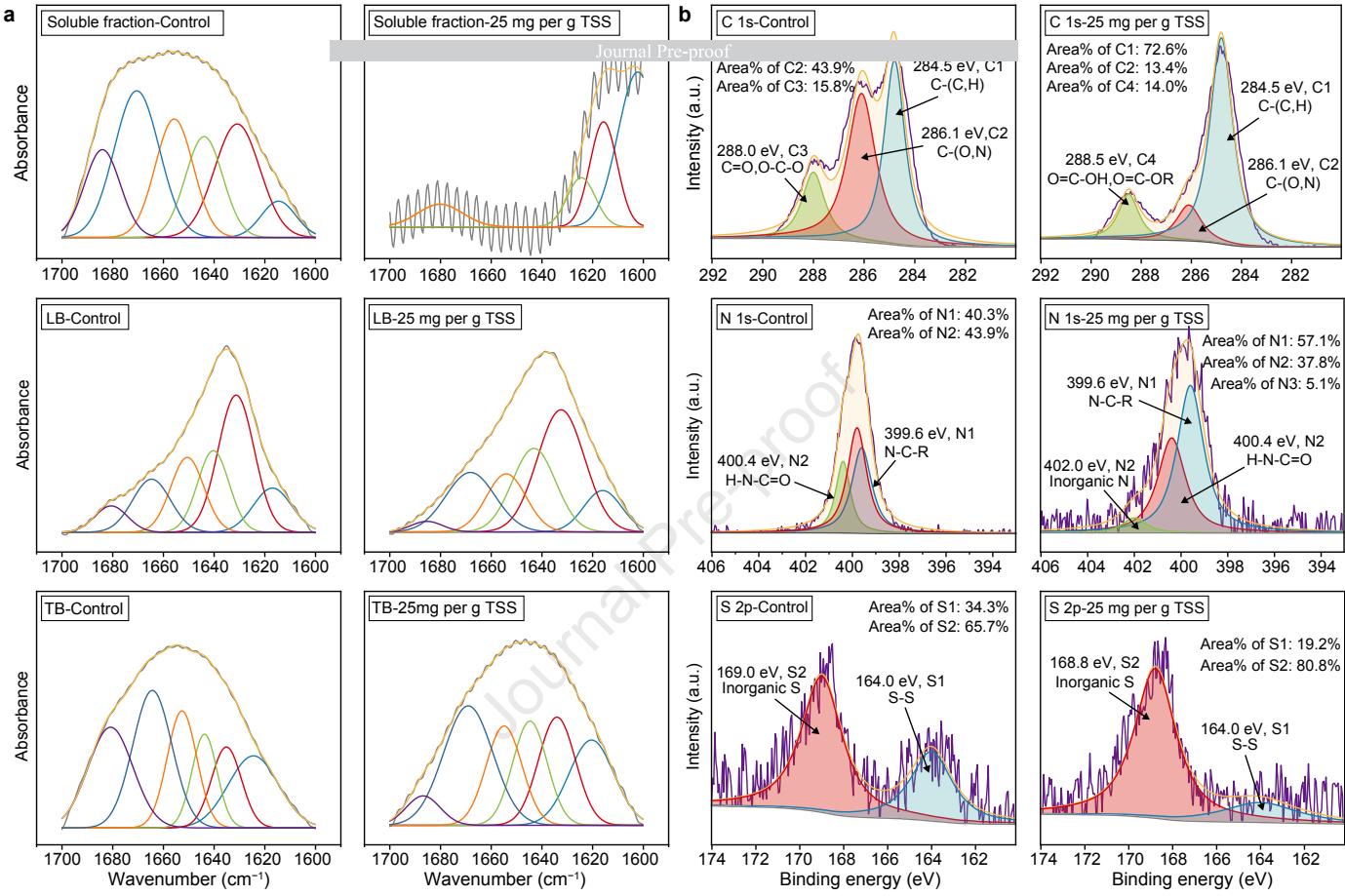
Fig. 6. **a–c**, ESR spectra detected from the PAA group (i.e., 25 mg per g TSS), including DMPO-OH (**a**), DMPO- O_2^- (**b**), and TEMP- $^1\text{O}_2$ (**c**). Note: * and • stand for DMPOX and DMPO-OH adducts. **d**, ORP variations for the control and PAA groups during pretreatment, with the inner bar chart representing Fe element content detected by EDS.

Fig. 7. **a**, Degradation of BSA during a four-day fermentation. **b**, Accumulative SCFAs production during a three-day fermentation. **c–d**, MCFAs production with ethanol and acetate as substrates during an eight-day fermentation for the control (**c**) and experimental (**d**) groups. **e**, Predictions of microbial functions in the control and PAA systems. Error bars represent standard deviations of triplicate experiments.

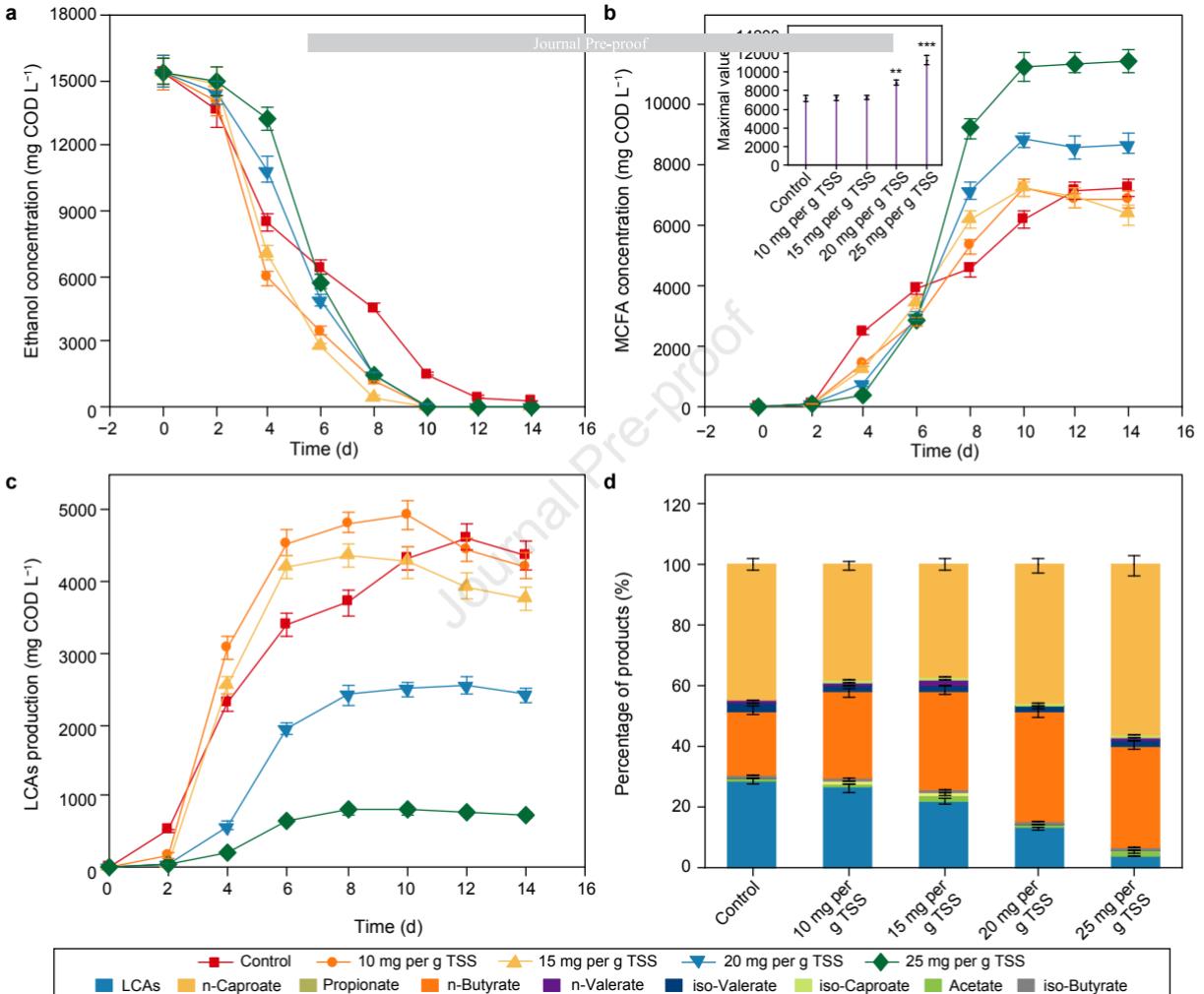
Fig. 8. **a**, Proposed metabolic pathways for WAS degradation and CE with ethanol as ED, with functional enzyme numbers listed and upgrading functional enzymes marked in red. **b**, Predicted functional enzyme abundances of CE microorganisms from the control, 15, 20, and 25 mg PAA per g TSS groups.

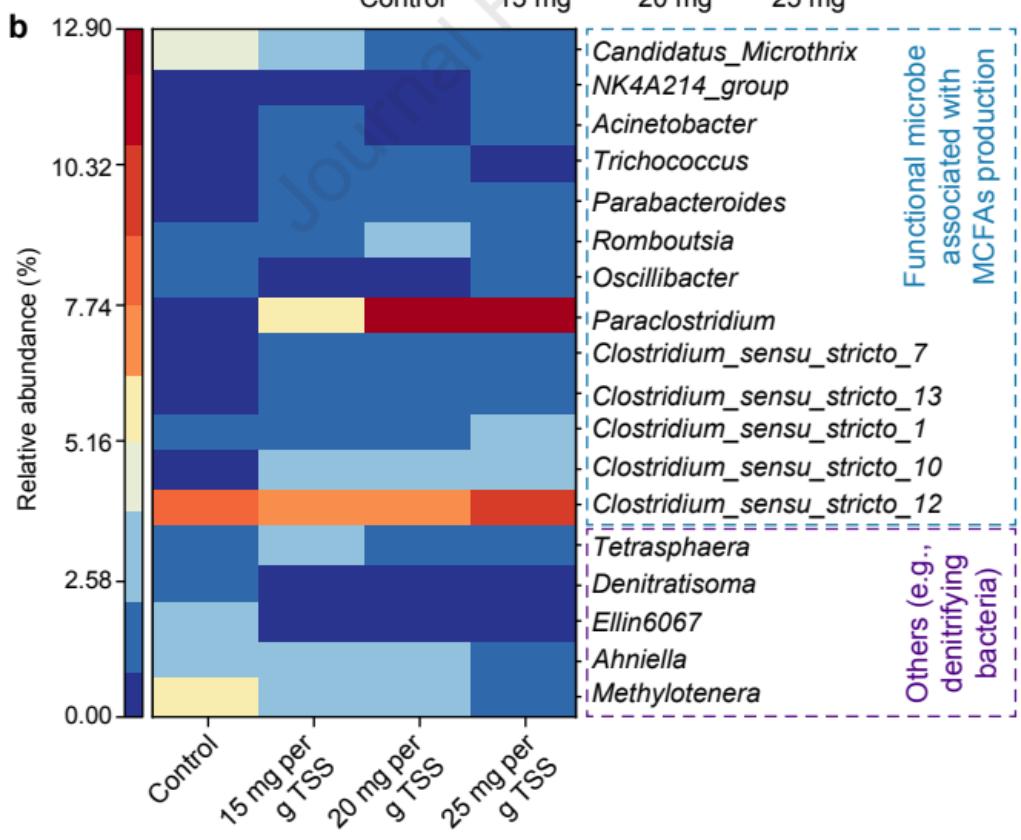
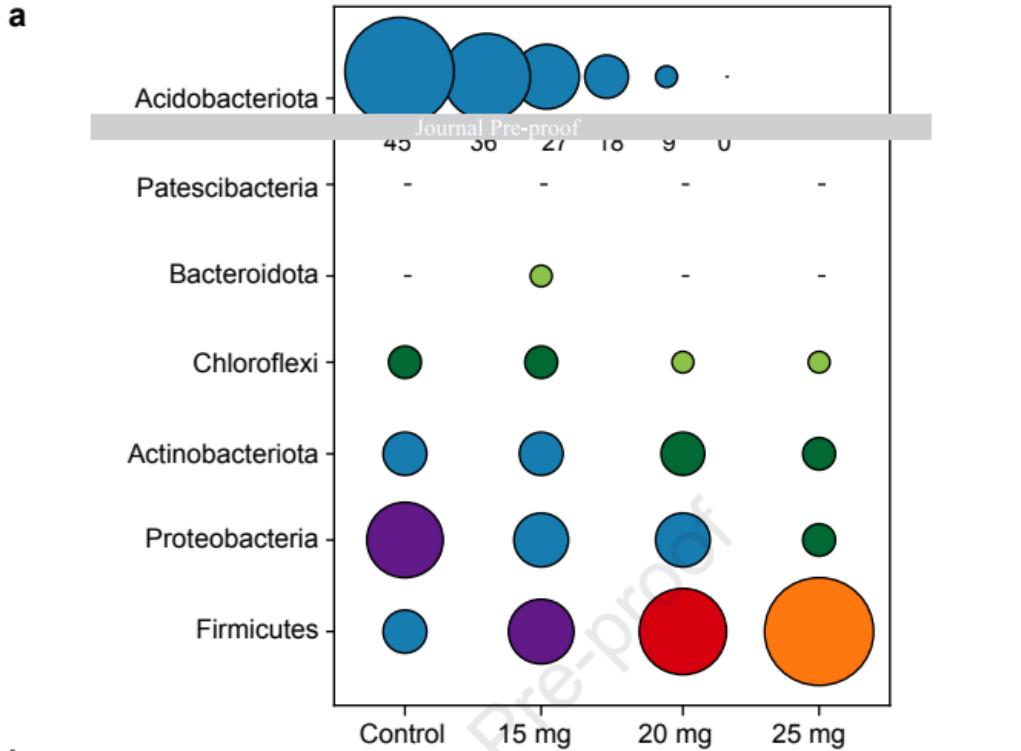


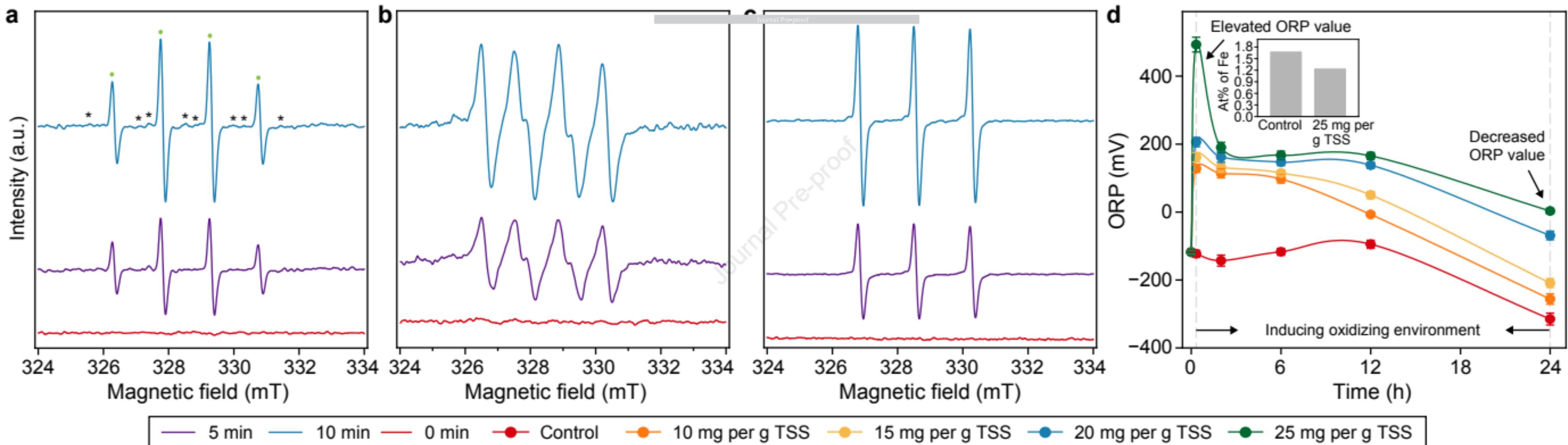


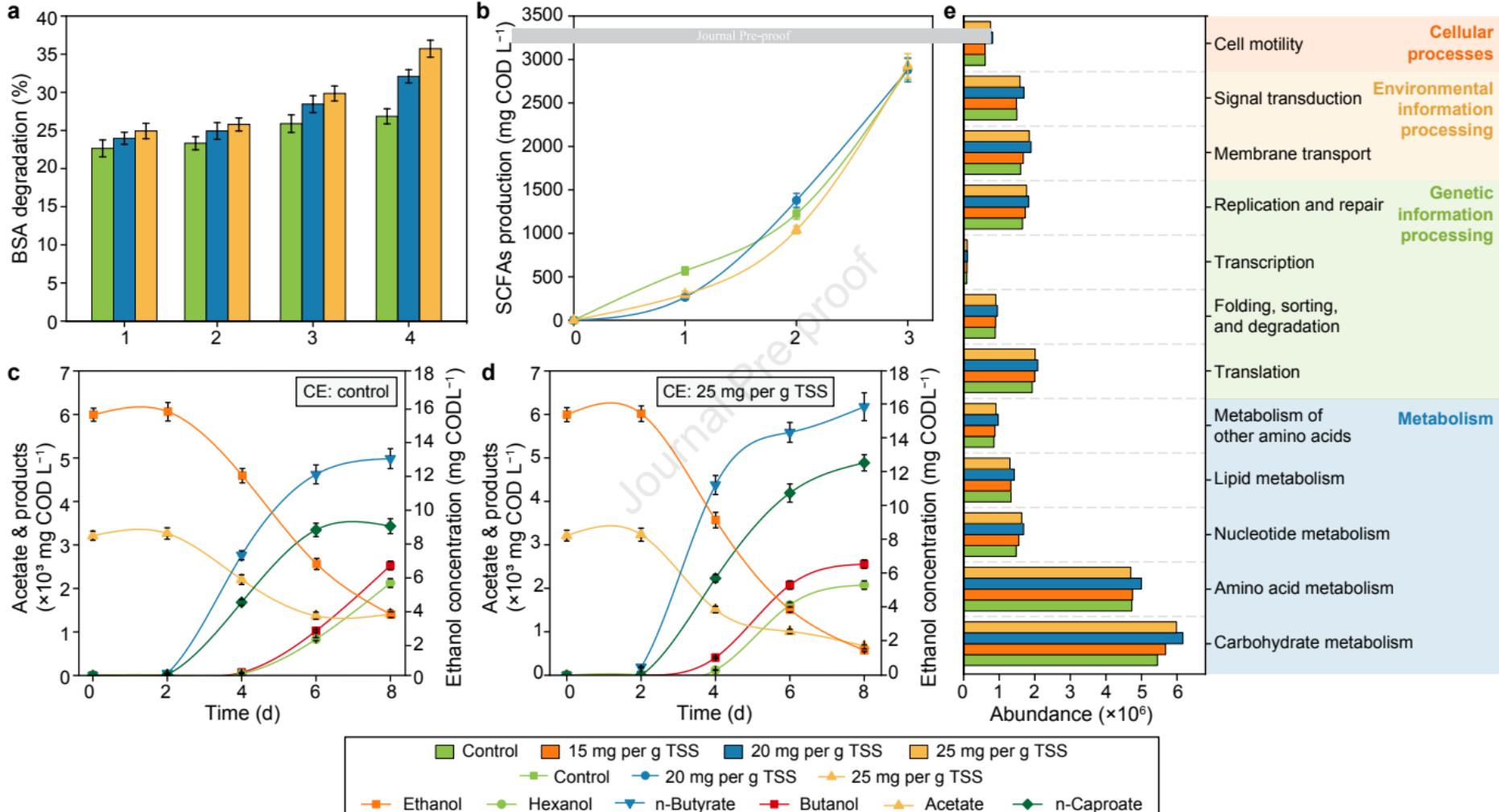


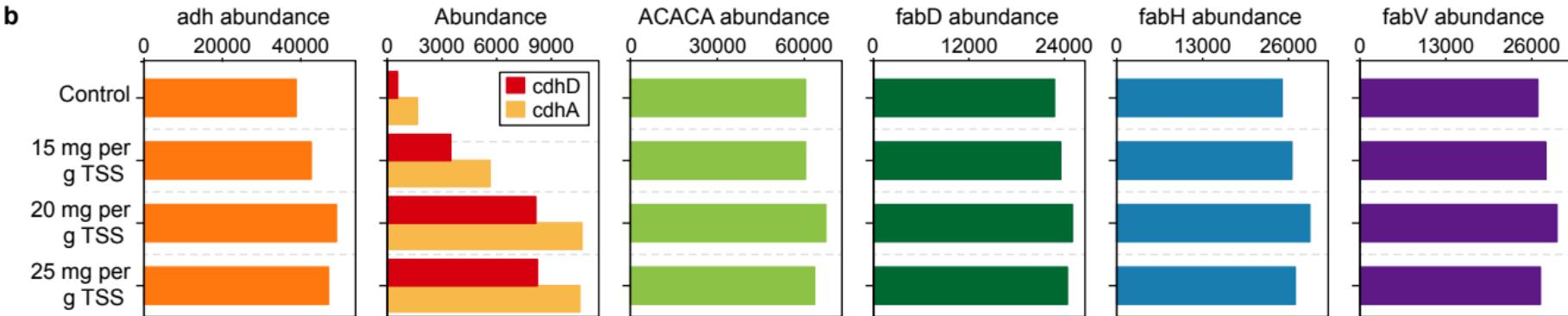
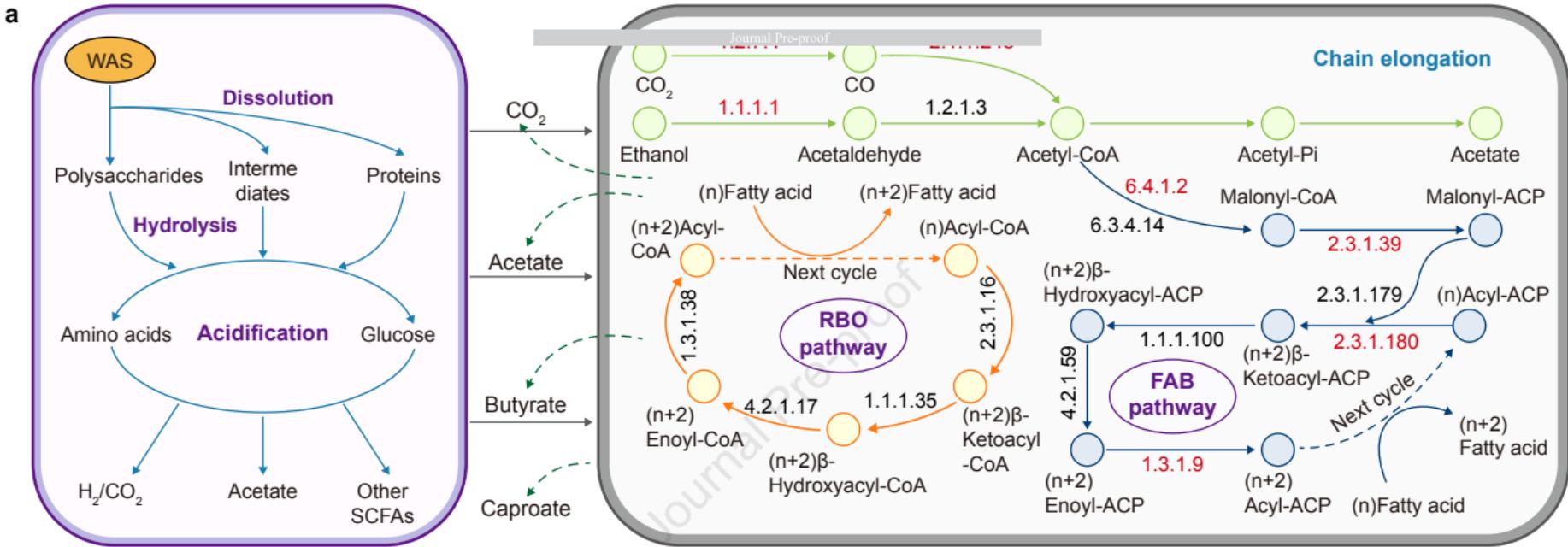
Samples	PAA level (mg per g TSS)	Secondary structures (%)						α -helix/ (β -sheet + random coil)
		Aggregated strands (1610–1625)	β -sheet (1630–1640)	Random coil (1640–1645)	α -Helix (1648–1657)	3-Turn helix (1659–1672)	Antiparallel B- sheet/aggregated strands (1680–1695)	
Soluble layer	0	5.1	21.1	15.4	18.4	27.1	12.9	50.6
	25	46.9	0	0	0	0	11.3	-
LB-EPS	0	11.6	34.0	17.6	17.1	13.8	5.9	33.1
	25	9.5	34.9	21.9	14.0	17.7	2.0	24.6
TB-EPS	0	16.6	11.1	10.9	15.9	24.7	20.7	72.3
	25	17.5	18.3	16.4	16.2	27.0	4.6	46.9











Highlights

- Peracetic acid (PAA) can boost sludge disruption and provide electron acceptors.
- Medium-chain fatty acids (MCFAs) are promoted to 11,265.6 mg COD L⁻¹ by PAA.
- Free radicals and singlet oxygen are the reactive species for PAA oxidation.
- PAA promotes protein-like substrate hydrolysis and chain elongation.
- PAA induces profitable microbial communities and metabolic pathways.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

