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Effect of biopolymer clusters on the fouling property of sludge from a membrane bioreactor (MBR) and its control by ozonation

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Running Head: Ozonation of BPC for MBR fouling mitigation

Abstract

Organic substances in the liquid phase of the sludge in a membrane bioreactor (MBR) have a profound impact on membrane fouling. In this study, a single-fibre microfiltration apparatus was developed to investigate the fouling propensity of MBR sludge and the effectiveness of ozonation in membrane fouling mitigation. The results show that biopolymer clusters (BPC) in the MBR suspension had a significant influence on the fouling potential of the sludge. An increase in BPC concentration by 20% and 60% from around 3.5 mg/l in the mixed sludge liquor drastically increased the fouling rate by 120% and 300%, respectively. Ozonation of the BPC solution greatly reduced the detrimental role of BPC in membrane fouling. An ozone dose of 0.03 mg/mg TOC of BPC could reduce the mean BPC size from 38 to 27 μm , which was further reduced to 12 μm at 0.3 mg O_3 /mg TOC of BPC. In addition to BPC destruction, ozonation apparently also modified the surface properties of BPC, resulting in an increase in the filterable fraction and a decrease in the liquid viscosity. Based on the experimental findings, an approach for MBR membrane fouling control is proposed that applies ozonation to the supernatant containing BPC in a side-stream application.

Keywords: Biopolymer clusters (BPC); membrane bioreactor (MBR); membrane fouling; microfiltration; ozonation; soluble microbial products (SMP).

20 1. Introduction

21 Membrane bioreactors (MBRs) are increasingly being used as an advanced technology
22 for biological wastewater treatment and reuse. With the use of a membrane for sludge
23 filtration, the MBR ensures complete solid–liquid separation [1,2]. In MBRs, the sludge age
24 and concentration can be effectively manipulated, affording this type of bioreactors several
25 advantages over the conventional activated sludge (CAS) process [3]. At the same time,
26 however, because of the retention by the membrane, some of the soluble microbial products
27 (SMP) and other colloidal substances are unable to escape from the system with the effluent
28 [4,5]. The organic interception by membrane filtration results in the formation and
29 accumulation of organic foulants in the MBR sludge suspension, which in turn worsens the
30 membrane fouling problem.

31 The effect on membrane fouling of liquid–phase organic substances in the MBR sludge
32 mixture has long been recognised [6–10]. Recent research reveals the presence of a group of
33 large-sized organic solutes, termed biopolymer clusters (BPC), in MBR systems [11–13].
34 BPC are neither biomass flocs nor SMP or extracellular polymeric substances (EPS). They
35 can be larger than 10 μm in size, and are formed by the affinity clustering of SMP and loose
36 EPS on the membrane surface [14]. It has been suggested that BPC may facilitate sludge
37 deposition and the fouling layer formation on the membrane surface, and the detrimental role
38 of BPC in membrane fouling has been demonstrated qualitatively during the operation of
39 MBR systems [12,14]. However, more systematic studies remain to be conducted to
40 determine the correlation between the membrane fouling rate and the BPC content of the
41 sludge mixture. In addition, the effect of changes in BPC properties on the fouling potential

42 of the MBR sludge also merits investigation.

43 The reduction or modification of BPC in MBR sludge mixture is expected to be
44 beneficial for the control of membrane fouling. Removal of BPC and their precursors, such as
45 SMP and loose EPS, is an option, and indeed the use of adsorbents or coagulants in the MBR
46 mixed liquor has been found effective in decelerating membrane fouling [15-19]. However,
47 continuous addition of these chemicals may either be harmful to the membrane due to
48 physical abrasion, as is the case for granular activated carbon, or affect the MBR treatment
49 performance, as is the case with some coagulant metal ions (e.g., Fe(II) and Fe(III) that are
50 reportedly toxic to the nitrifying bacteria [20]). More recently, the ozonation of bulk sludge
51 has been tested as a means of membrane fouling control during continuous MBR operation
52 [21-24]. The results show that at appropriate doses the membrane fouling rate can be
53 effectively reduced, meanwhile, ozonation coupled with MBR appears to be an effective
54 method for sludge reduction and toxic organic wastewater treatment [23,24]. However, a
55 possible overdose of ozone and its impact on the biomass activity is a concern with direct
56 sludge ozonation. Moreover, the underlying mechanisms of sludge ozonation for membrane
57 fouling mitigation are not well understood.

58 There is thus a need to determine the effect of BPC in MBR sludge mixture on
59 membrane fouling, and to investigate the effectiveness of the ozonation of BPC in reducing
60 the fouling propensity of sludge. In this study, a lab-scale MBR was operated to supply both
61 biomass sludge and BPC dispersion. A newly designed single-fibre microfiltration (MF)
62 system was fabricated for the membrane filtration-fouling tests on different sludge–BPC
63 mixture samples under well-controlled hydrodynamic conditions. Ozonation was applied to

64 the BPC solution only, rather than the entire sludge mixture, before mixing into the sludge
65 suspension. The objectives of the experimental study were (1) to determine the fouling
66 propensity of MBR sludge with different BPC contents, and (2) to investigate the
67 effectiveness of the ozonation of BPC in minimising membrane fouling during sludge
68 filtration. The mechanism of sludge ozonation to mitigate fouling was also identified, and on
69 this basis a more reliable ozonation approach for MBR fouling control is proposed.

70

71 **2. Materials and Methods**

72

73 *2.1. Filtration setup and operation*

74 A single-fibre filtration apparatus was fabricated for the sludge filtration and fouling tests
75 (Fig. 1). The apparatus was made of a plexiglass tube 1.5 cm in internal diameter and 50 cm
76 in height. A polyethylene (PE) hollow-fibre MF membrane (pore size = 0.4 μm , diameter =
77 0.14 cm, working length = 40 cm, surface area = 16 cm^2 , Mitsubishi Rayon, Japan) was
78 installed along the centreline of the filtration tube. The sludge suspension in a feed tank was
79 pumped through the MF test tube by a helical pump (SELTZ-L40 II, Hydor, USA). A
80 constant cross-flow rate of 2 l/min (0.19 m/s) was applied by the recirculation of the sludge
81 suspension for continuous membrane surface cleaning. The permeate was drawn out through
82 the MF membrane by a suction pump (MasterFLEX, Cole-Parmer, USA) at a constant flux of
83 37.5 $\text{l/m}^2 \text{ h}$. An electronic balance (Arrw 60, OHAUS, USA) was used to record the permeate
84 production during the filtration-fouling tests. Unless sampled for analysis, the collected
85 permeate was returned manually to the feed tank at regular intervals to maintain the same

86 sludge concentration. A pressure sensor (PTX Ex-0129, Druck, USA) was installed before
87 the suction pump to record the trans-membrane pressure (TMP) during sludge filtration. Both
88 the permeate production and TMP data were transferred to a PC for continuous data
89 recording (Fig. 1). The membrane fouling rate was measured by the increase in TMP with the
90 amount of permeate produced (filtrate depth, L), or $\Delta\text{TMP}/\Delta\text{L}$. After each filtration-fouling
91 test, the membrane fibre was taken off the filtration tube and washed with 100 ml of DI water
92 at 40°C to recover all of the sludge and foulants deposited on the membrane surface. The
93 sludge and foulant dispersion was then settled for 2 h at 4°C and the supernatant was analysed
94 for total organic carbon (TOC) and chemical composition, including proteins (PN),
95 polysaccharides (PS) and humic-like substances (HS). The sludge in the dispersion was
96 collected on a filter, dried for 2 h at 105°C and then weighed to obtain the suspended solids
97 (SS) content.

98

99 2.2. *MBR activated sludge and biopolymer clusters*

100 The sample activated sludge (AS) and biopolymer substances for the filtration tests were
101 collected from a submerged MBR (SMBR). The laboratory SMBR had a working volume of
102 5 l and contained a submerged 0.4 μm polyethylene MF module (surface area = 0.2 m^2 ,
103 Mitsubishi Rayon, Japan). The SMBR system had been in stable operation for more than four
104 years before the present experiment [12,25]. The influent (feeding wastewater) to the SMBR
105 was a mixture of a glucose-based synthetic wastewater prepared according to the basic recipe
106 given in the Environmental Engineering Process Laboratory Manual of the AEESP [26] and
107 domestic sewage collected from the Stanley Sewage Treatment Works in Hong Kong. The

108 sewage fraction supplied around 10% of the total organic load in the influent. The wastewater
109 influent had a chemical oxygen demand (COD) of around 500 mg/l and a COD:N:P ratio of
110 100:9:3. NaHCO₃ was added to the influent at 50 mg/l or higher to maintain the pH of the
111 MBR suspension between 6.5 and 7.5. The biomass concentration, food-to-microorganism
112 (F/M) ratio, solid retention time (SRT) and hydraulic retention time (HRT) of the SMBR
113 system were 10 g/l, 0.2 g COD/g SS d, 25 d and 8 h, respectively.

114 The AS mixture collected from the SMBR was settled for 1 h, and the settled sludge was
115 then diluted with a 0.05% NaCl solution to a mixed liquor suspended solids (MLSS)
116 concentration of 3 g/l. Large organic substances, or biopolymer clusters, were obtained from
117 the cake sludge (CS) deposited on the surface of the membrane in the SMBR. When the
118 membrane was seriously fouled, the CS layer was scraped off the membrane using a spatula.
119 The CS was then re-suspended and dispersed by stirring it in a 0.05% NaCl solution. The CS
120 suspension was then separated by sedimentation at 4°C for 12 h and the supernatant was
121 collected. The organic substances in the CS supernatant were regarded as biopolymer clusters
122 [12,14]. The CS supernatant, or BPC solution, was analysed for TOC and PN, PS and HS
123 content.

124 The BPC solution was added into the AS suspension (3 g/l) at different doses. Each
125 sludge suspension was then tested for its fouling propensity using the single-fibre MF
126 filtration apparatus. In this way, the effect of the BPC content in the sludge mixture on the
127 fouling potential of the sludge during membrane filtration was determined.

128

129 *2.3. Ozonation of the BPC solution*

130 Ozonation was also applied to the BPC solution with an intention to modify the BPC
131 properties before their addition to the sludge. Ozonation was performed quantitatively by
132 adding ozone-containing water into the BPC solution. Ozone was generated in the gaseous
133 phase by an ozone generator (5000 BF, Enaly, China) that was supplied with pure oxygen. To
134 dissolve the ozone in water and prepare an ozone solution, 500 ml ultra-pure water
135 (Milli-Q-Advantage, Water Purification, Millipore, USA) was bubbled with the ozone gas at
136 4°C for 10 min or longer. The ozone concentration achieved in the ozone solution was about
137 8 mg/l. A pre-determined amount of the ozone solution was then added to 30 ml of the BPC
138 solution. The mixed solution was placed in the dark and stirred for 5 min at 60 rpm to ensure
139 complete ozonation. Similar to the previous sludge filtration tests, the ozonated BPC solution
140 was added into the AS suspension at different doses, and the sludge mixtures were then tested
141 for their fouling potential using the single-fibre filtration apparatus.

142

143 *2.4. BPC characterisation*

144 In the characterisation of the organic substances in the BPC solution, the fraction that
145 could not pass through a 0.4 µm membrane filter (polycarbonate, Osmonics, USA) was
146 defined as non-filterable BPC. The proportion of non-filterable BPC to the total organic
147 content in the BPC solution was termed as the BPC cut-off ratio [11]. The BPC solution
148 before and after ozonation and its filtrate were analysed to determine the TOC concentration
149 and the PN, PS and HS content.

150 The BPC size distribution was determined by using a laser diffraction particle analyser
151 (LS 13 320, Beckman Coulter, USA). Before the particle sizing and counting, the BPC in the

152 solution were stained with NanoOrange (Molecular Probes, Eugene, USA), which is a
153 fluorescent probe that targets proteins in organic polymers. Five millilitres of NanoOrange
154 dye solution was added to 30 ml of BPC solution for a final dye concentration of 20 mg/l, and
155 the mixture was kept in the dark for 30 min. After staining, the transparent BPC became
156 detectable by a laser particle analyser [14]. Moreover, both before and after ozonation, the
157 BPC were filtered on a membrane filter and examined directly under a confocal laser
158 scanning microscope (CLSM) (LSM Pascal, Zeiss, Thornwood, USA), following the
159 procedures described previously [5,27]. For the CLSM observations, BPC (actually
160 non-filterable BPC) and other solids collected on a 0.4 µm black polycarbonate membrane
161 (25 mm, Osmonics, USA) were stained using a combination of two probes: SYTO9 to target
162 the bacterial cells and ConA-TRITC to target the polysaccharides with D-glucose or
163 D-mannose [12].

164

165 *2.5. Analytical methods*

166 The TOC was measured by a TOC analyser (IL550 TOC-TN Analyzers, Lachat, USA)
167 using the high-temperature combustion method. The protein and humic concentrations were
168 determined via an UV/VIS spectrophotometer (Lambda 25, Perkin Elmer, USA) following
169 the modified Lowry method using albumin bovine (Sigma, Germany) and humic acid (Fluka,
170 Italy), respectively, as the standards [28]. The polysaccharide content was measured
171 according to the phenol method using glucose as the standard [29]. The MLSS concentration
172 of the sludge was measured in accordance with the Standard Methods [30]. The concentration
173 of dissolved ozone in the ozonated water was determined based on the UV absorbance as

174 measured by an UV/VIS spectrophotometer (Lambda 25, Perkin Elmer, USA) following the
175 conventional indigo method [31,32]. The liquid viscosity was measured by a vibration
176 viscometer (SV-10, A&D, Japan).

177

178 **3. Results and Discussion**

179

180 *3.1. Significance of BPC in membrane fouling*

181 The membrane fouling rate for the sludge samples was well indicated by the increase in
182 TMP during the filtration process (Fig. 2). For the filtration-fouling tests with the single-fibre
183 MF apparatus, the sludge suspension collected from the SMBR was kept at a SS
184 concentration of 3 g/l. At a constant filtration flux, the membrane fouling rate shown by the
185 TMP increase was reflective of the fouling propensity of the sludge samples. As all of the
186 conditions were identical except for the amount of BPC added to the sludge mixture, the
187 comparative results directly demonstrate the effect of BPC on membrane fouling, and clearly
188 show that an increase in BPC concentration in the MBR sludge mixture led to a significant
189 acceleration in membrane fouling during the sludge filtration. However, it should be pointed
190 out that a fixed signal fibre MF membrane was used in the present study to determine the
191 fouling rate during sludge filtration. The actual MBR situation is more complication with
192 aeration and membrane fibre movement. The membrane fibre movement caused by aeration
193 turbulence is expected to reduce the membrane fouling rate; however, the contact between
194 membrane fibres in a membrane module would reduce the fluid shear over the membrane
195 surface, worsening the fouling situation.

196 The control sludge (without extra BPC) had a background liquid-phase organic
197 concentration C_0 of around 3.5 mg/l. A small BPC addition of $0.2C_0$ resulted in a notable
198 increase in the membrane fouling rate (Fig. 2a), and a further addition of BPC beyond $0.6C_0$
199 increased the fouling rate dramatically. The membrane fouling rate during sludge filtration
200 increased almost linearly with the BPC content in the sludge suspension (Fig. 3). The results
201 of the well-controlled sludge filtration experiments thus prove that BPC are a crucial foulant
202 in MBR systems. BPC are a group of organic solutes formed by the affinity clustering of
203 soluble and colloidal substances on the membrane surface during MBR operation [11]. It is
204 believed that large-sized BPC in the MBR sludge mixture function as a “glue” that facilitates
205 sludge attachment and the formation of a fouling layer on the membrane surface [12–14].

206

207 *3.2. Reduction of the membrane fouling rate by BPC ozonation*

208 Ozonation was applied to the BPC solution before its addition into the sludge suspension.
209 No residual ozone was found in the ozonated BPC solutions. At the ozone dose employed,
210 which was less than 1 mg O_3 /mg TOC of BPC, the amount of BPC removed was minimal, as
211 shown below (Fig. 5 in Section 3.3) by the insignificant TOC reduction, but BPC destruction
212 by ozonation was expected. The fouling test results demonstrate that ozonation can greatly
213 reduce the detrimental effect of BPC on membrane fouling during sludge filtration. Upon
214 ozonation of the BPC solution, the membrane fouling rates of the sludge–BPC mixtures
215 decreased significantly compared with the identical test cases without ozonation (Fig. 2b).
216 The average fouling mitigation efficiency by BPC ozonation was over 70% (Fig. 3). Thus,
217 the ozonation of BPC may be an effective fouling control measure in SMBR systems.

218 After each filtration test, the fouling sludge layer on the single-fibre membrane was
219 collected and analysed for the solid and BPC content to determine the average deposition
220 rates of the solid matter and BPC on the membrane surface during sludge filtration. The
221 results show that the deposition rates of both the solids and BPC decreased as the ozone dose
222 applied to the BPC solution increased (Fig. 4). It should be noted that the biomass solids were
223 the predominant foulant material (over 95%) in the fouling (cake) layer on the membrane
224 surface. The BPC content in the sludge cake layer averaged around 14.2 mg TOC/g SS. The
225 proportional deposition of BPC and suspended solids suggests that BPC function as the
226 “glue” in cake layer formation. In comparison, BPC after ozonation apparently lost their
227 “gluing” capability to a great extent. However, the effectiveness of ozonation in reducing
228 foulant attachment on the membrane surface did not continue to increase with an increasing
229 amount of ozone. This implies that the improvement of the sludge filterability by BPC
230 ozonation has a limit. Fortunately, a small ozone dose is effective in reducing the fouling
231 potential of sludge.

232

233 *3.3. Destruction of BPC by ozonation*

234 As stated previously, ozonation did not result in significant BPC oxidation or organic
235 mineralization. The TOC concentration remained largely unchanged in the BPC solutions
236 after ozonation at different doses (Fig. 5). Moreover, according to the chemical analysis,
237 ozonation did not lead to a clear trend of change in the chemical composition of BPC in terms
238 of the polysaccharide, protein and humic content (Fig. 5). Apparently, oxidation of the
239 organic polymers by ozone at the doses applied did not reach the level of their component

240 units, that is, simple sugars for polysaccharides, amino acids for proteins and aliphatic or
241 phenolic acids for humic substances. The main change brought about by ozonation appeared
242 to be the breaking up of large BPC. This is demonstrated by the significant reduction in BPC
243 size after ozonation. According to the particle size analysis (Fig. 6), a small ozone dosage of
244 0.03 mg/mg TOC of BPC decreased the volume-based mean size of BPC from 38 μm to 27
245 μm . As the ozone dose increased to 0.30 mg/mg TOC of BPC, the mean BPC size decreased
246 to about 12 μm . However, further increases in the ozone dose resulted in little decrease in
247 BPC size. This indicates that larger BPC are more vulnerable than small BPC to break-up by
248 ozonation.

249 The breaking up of large BPC by ozonation was further confirmed by CLSM
250 examination (Fig. 7). BPC could be well observed by staining their polysaccharide
251 components with fluorescent ConA-TRITC. The CLSM images show that large BPC, many
252 of which were larger than 50 μm , disintegrated into smaller BPC after ozonation at small
253 ozone doses of 0.03 to 0.18 mg/mg TOC of BPC.

254 In addition to BPC break-up, ozonation at a low dose also altered the chemical properties
255 of the BPC. Ozone is a selective oxidant that reacts faster with some chemicals or functional
256 groups than with others [33]. Polymeric substances and their clusters contain several sites that
257 are reactive to ozone. For example, the glycosidic bonds inherent in chains of
258 polysaccharides can be easily cleaved by ozone attack, resulting in their breakdown into
259 short-chain polysaccharides or oligosaccharides [34,35]. Some reactive sites that are located
260 at the branches of polymeric substances are readily cut by ozonation from the main chains
261 [36]. This leads not only to the fragmentation of BPC, but also the modification of their

262 surface properties.

263 The viscosity of the BPC solution decreased as a result of ozonation (Fig. 8). A high
264 viscosity generally suggests a high fouling potential of the feed liquid [37,38]. The
265 significantly higher viscosity of the BPC solution than water is probably due to the
266 abundance of large BPC and their interaction. The decrease in viscosity of the BPC solution
267 after ozonation thus not only reflects the breaking up of the BPC, but also implies the
268 modification of their surface properties.

269

270 *3.4. Effect of BPC destruction on membrane fouling control*

271 Due to the size reduction and possible modification of the surface properties of BPC after
272 ozonation, the cut-off ratio of the BPC by filtration decreased (Fig. 8). In other words, the
273 portion of filterable BPC increased considerably after ozonation, although the total amount of
274 BPC hardly changed. The cut-off ratio provides an indication of the fouling propensity of
275 BPC dispersion [11], as the fouling resistance greatly depends on the amount of foulant
276 deposition. A reduction in size leads to a decrease in BPC retention due to the steric effect
277 [39], whereas surface property modification affects the gelling propensity of the polymeric
278 substances [40].

279 An SMBR is an almost completely enclosed system that does not allow the overflow of
280 loose sludge flocs or organic foulants from the system. As a result, fouling materials,
281 including SMP, loose EPS and colloidal organics, accumulate in the bioreactors. Sludge
282 filtration through a large membrane surface provides a unique condition for BPC formation
283 from polymeric organic substances [11]. BPC within the sludge cake deposited on the

284 membrane greatly increase the filtration resistance of the cake layer. The detachment of BPC
285 from the membrane by aeration turbulence brings BPC back into the sludge suspension,
286 which in turn worsens the fouling potential of the sludge [12,13]. It is therefore desirable to
287 remove or destruct BPC regularly in an MBR system.

288 Previous studies showed that direct sludge ozonation could practically reduce the
289 membrane fouling rate in SMBR [19]. Huang and Wu [20] demonstrated that in continuous
290 SMBR operation, ozonation of the bulk sludge at 0.25 mg O₃/g SS effectively controlled
291 membrane fouling. The experimental findings of this study indicate that the underlying
292 mechanism of ozonation for fouling minimisation reported in previous studies is probably the
293 effective destruction of BPC by ozonation. In future MBR applications, a side-stream could
294 be used with an intermediate sedimentation tank for simple liquid–solid separation to allow
295 the ozonation of the supernatant alone to destroy BPC. The advantage of such an approach
296 over direct ozonation of the entire bulk sludge mixture is that it maintains consistent
297 membrane fouling alleviation whilst avoiding the damage of possible ozone overdoses on the
298 biomass properties or MBR treatment performance.

299

300 **4. Conclusions**

- 301 • The single-fibre MF filtration system is a highly efficient testing device for the
302 determination of the fouling propensity of MBR sludge samples with different BPC
303 contents.
- 304 • Liquid-phase organic substances, particularly BPC, in MBR sludge suspension have a
305 profound impact on the fouling potential of the sludge. A small increase in BPC

-
- 306 concentration by 20% and 60% from a background level of about 3.5 mg/l in the mixed
307 sludge drastically increased the membrane fouling rate by 120% and 300%, respectively.
- 308 • Ozonation of BPC is shown to be an effective means of controlling membrane fouling
309 for MBR sludge. An ozone dose of only 0.18 mg/mg TOC of BPC can reduce the
310 membrane fouling rate by up to 70%. Ozone is able to destruct large BPC and modify
311 their surface properties, which increases the filterability of BPC and the sludge mixture.
312 It appears that ozonation causes BPC to lose their “gluing” capability, thereby serving as
313 an effective measure of membrane fouling mitigation.
 - 314 • A side-stream approach may be developed that allows the ozonation of the
315 BPC-containing supernatant before its return to the MBR. Such a technique can help
316 control membrane fouling in MBRs whilst avoiding possible adverse effects on biomass
317 properties and MBR treatment performance.

318

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427 **Figure Captions:**

428 **Fig. 1.** Schematic diagram of the single-fibre MF testing apparatus.

429 **Fig. 2.** Membrane fouling rate indicated by TMP increase during the filtration of the sludge
430 (SS concentration = 3 g/l) with different BPC dose ratios: (a) BPC without ozonation;
431 (b) BPC after ozonation at 0.18 mg O₃/mg TOC. C₀: background organic (TOC)
432 concentration in the sludge suspension; C_{BPC}: TOC of the BPC added.

433 **Fig. 3.** Membrane fouling rate of the sludge during MF filtration as a function of the BPC
434 content: comparison of the fouling effect between raw BPC and ozonated BPC.

435 **Fig. 4.** Changes in the sludge and BPC deposition rates in the fouling layer on the single-fibre
436 membrane during sludge filtration as a function of the ozone dose applied to the BPC
437 solution. The BPC dose ratio $C_{BPC}/C_0 = 1.6$.

438 **Fig. 5.** Comparison of the organic content and chemical composition of BPC before and after
439 ozonation at different ozone doses.

440 **Fig. 6.** Change in (a) the size distribution and (b) the mean size of BPC after ozonation at
441 different ozone doses.

442 **Fig. 7.** CLSM observation of BPC before and after ozonation: (a₁) and (a₂) before ozonation;
443 (b₁) and (b₂) ozone dose ratio = 0.03 mg O₃/mg TOC; (c₁) and (c₂) ozone dose ratio =
444 0.18 mg O₃/mg TOC. (red: polysaccharides in the BPC; green: bacterial cells)

445 **Fig. 8.** Changes in the viscosity and cut-off ratio of the BPC solution after ozonation.

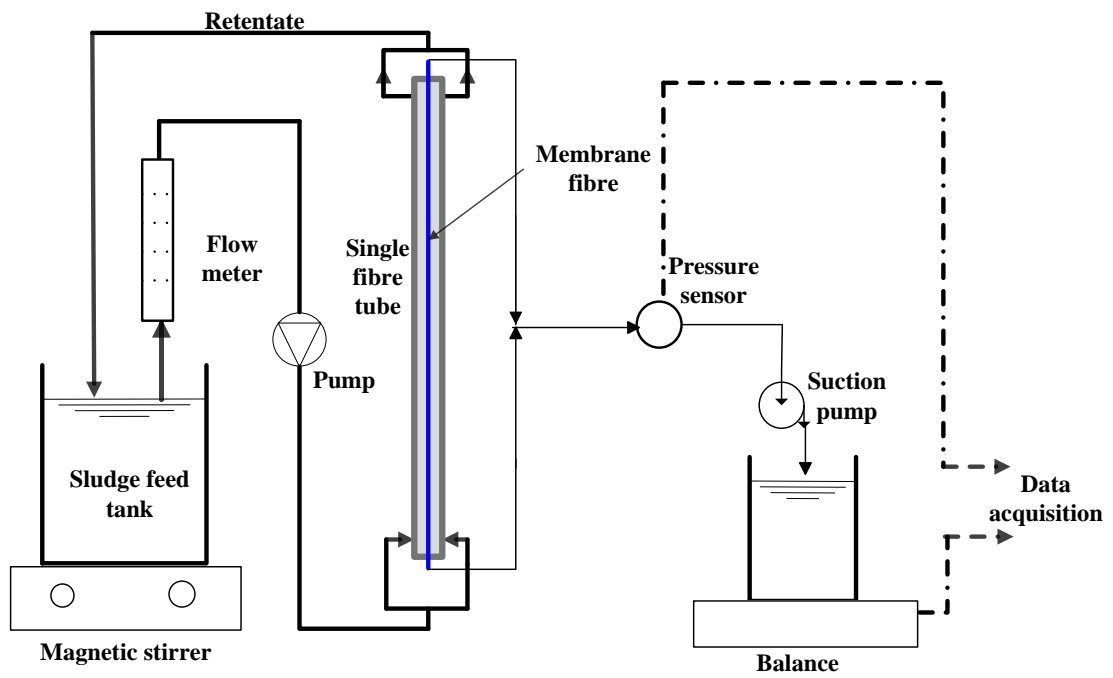


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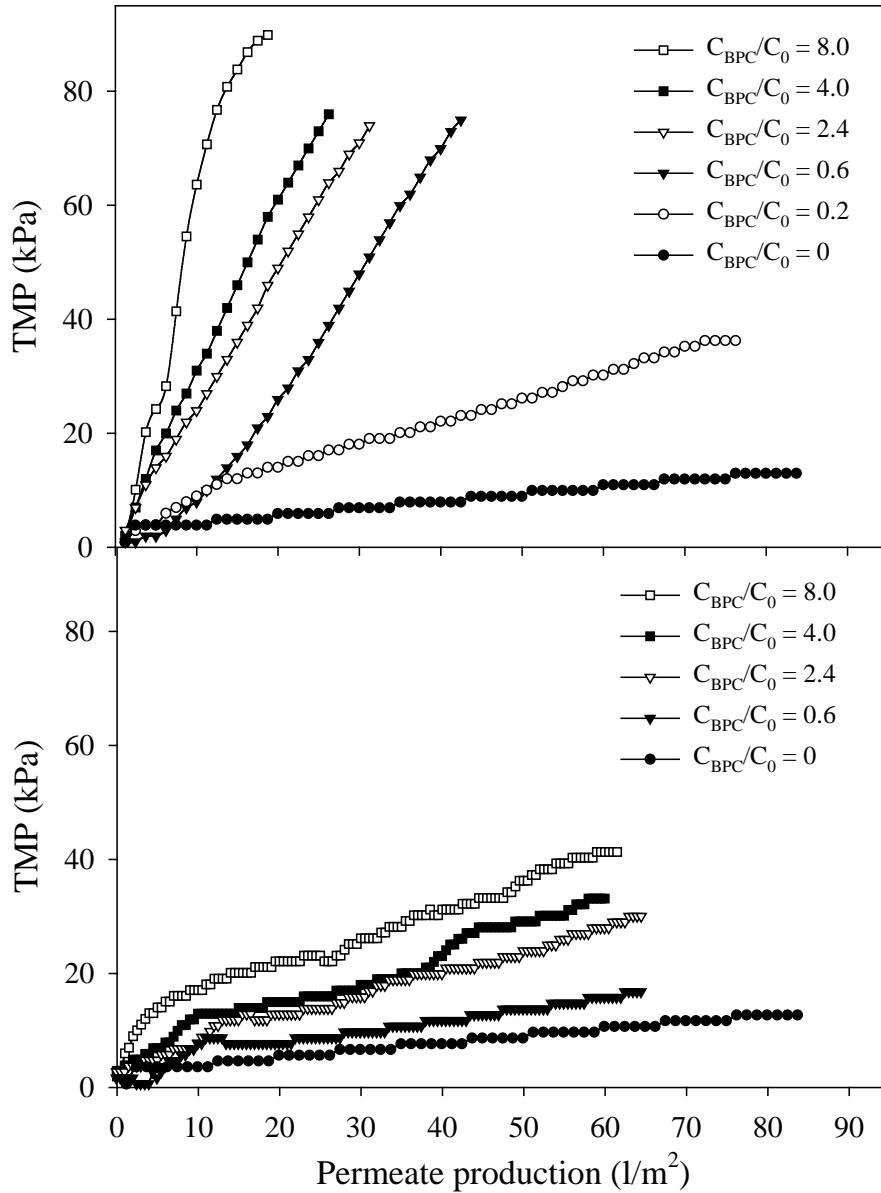


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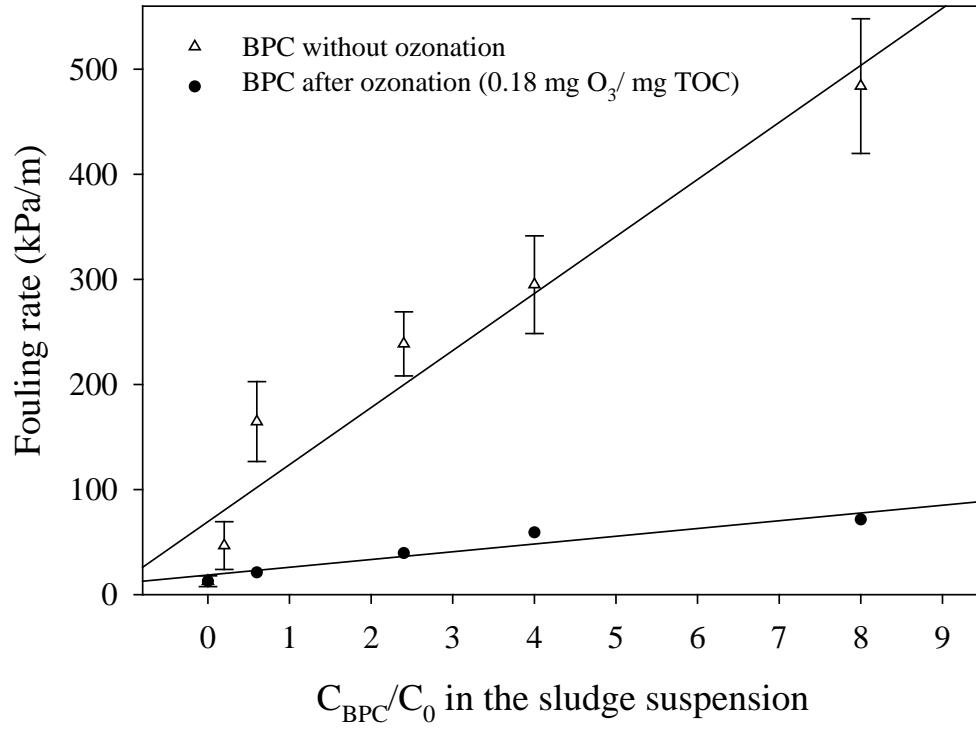


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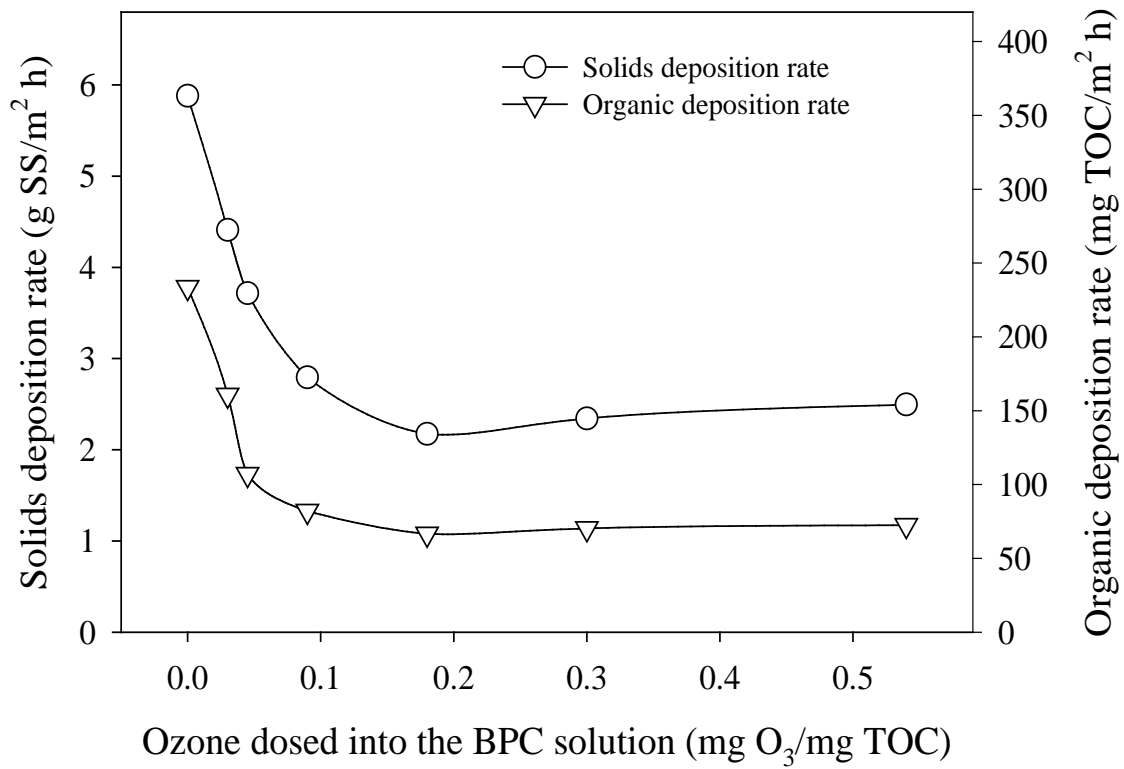


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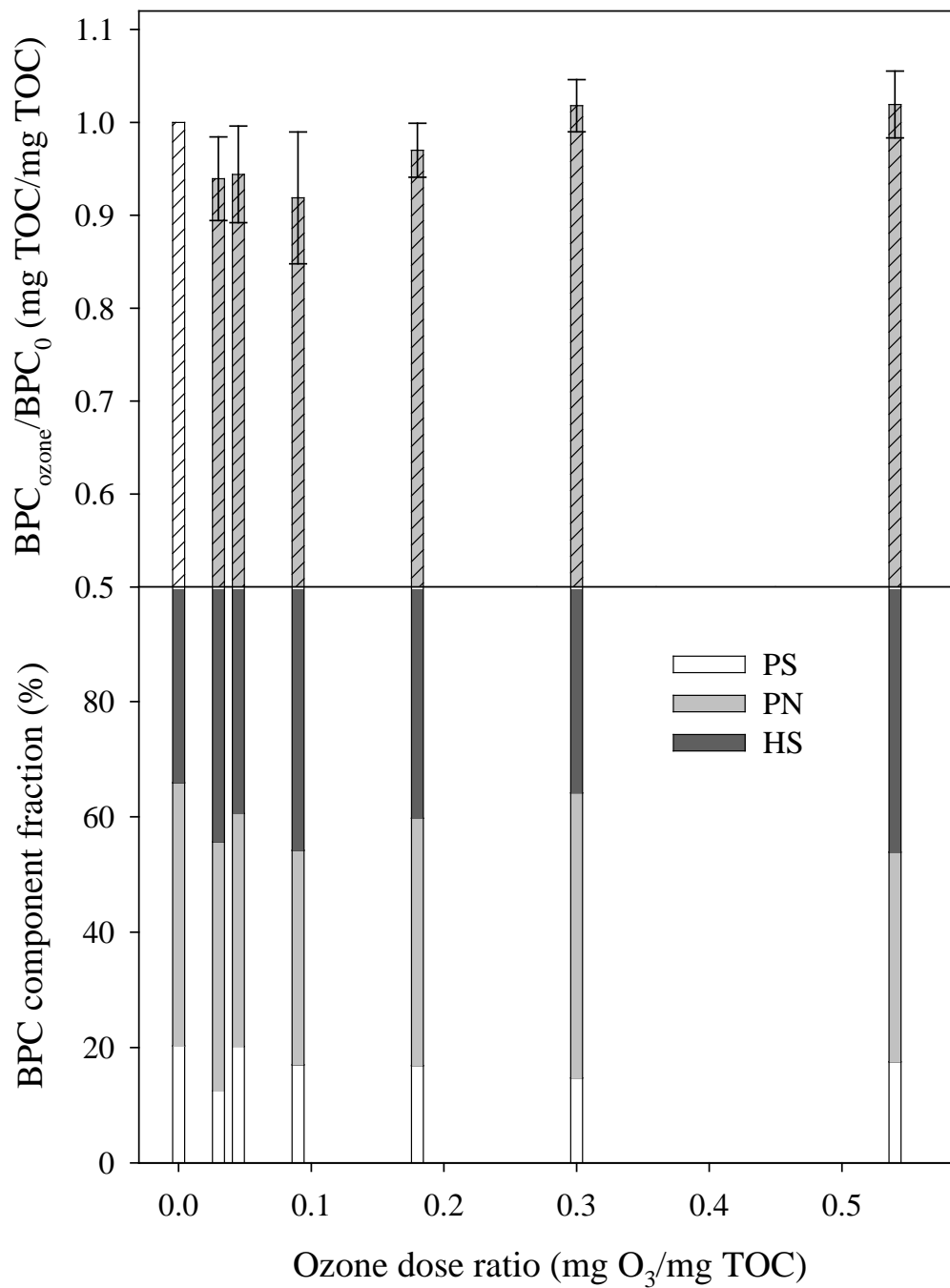


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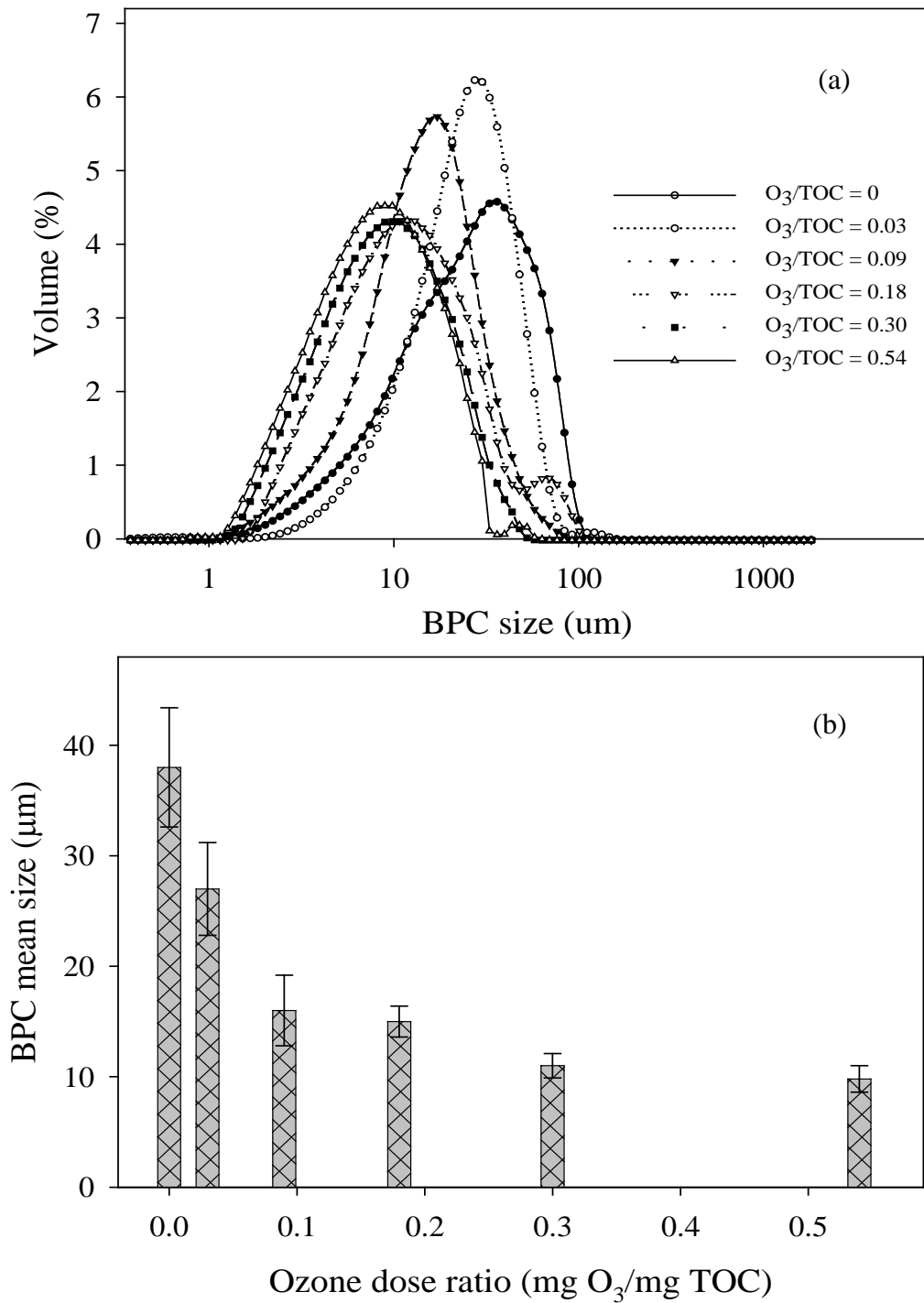


Fig. 6. Change in (a) the size distribution and (b) the mean size of BPC after ozonation at different ozone doses.

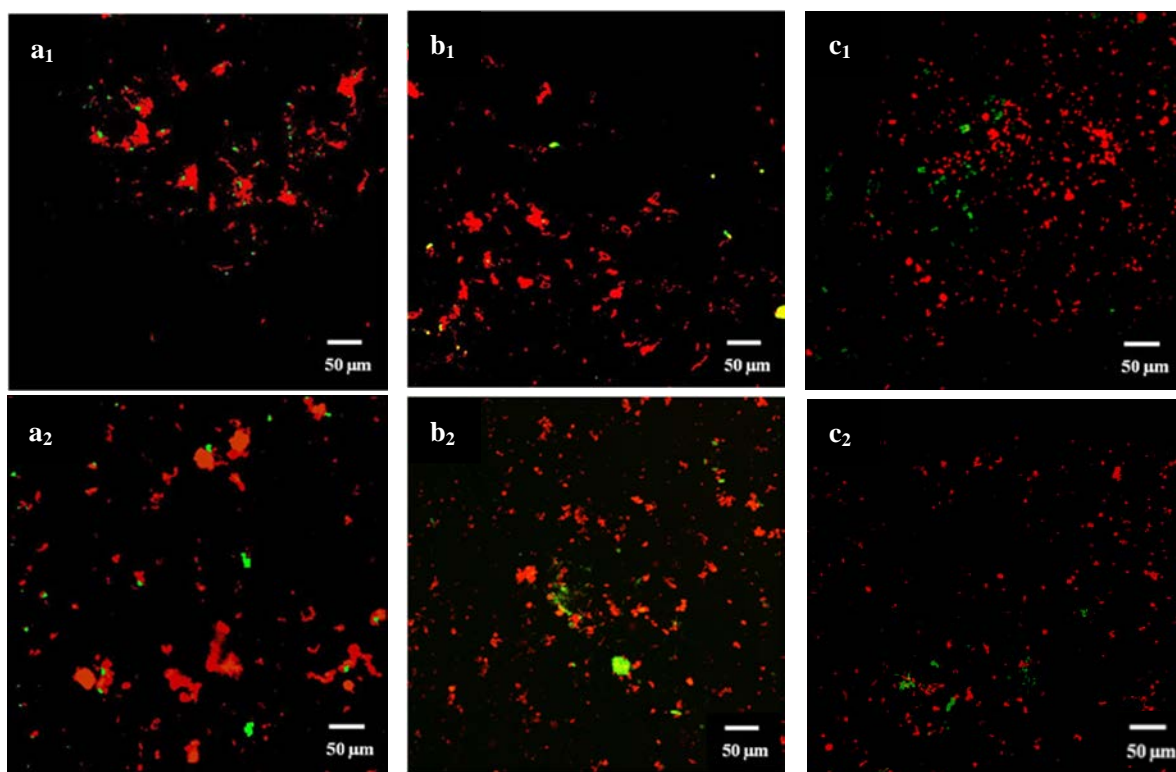


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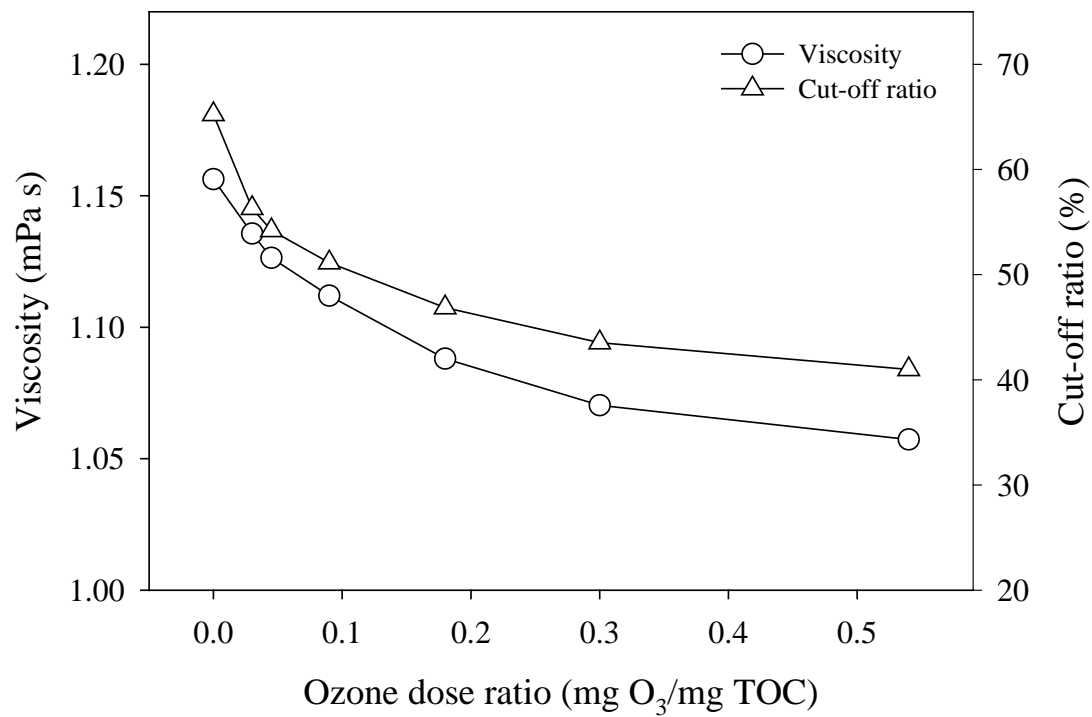


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