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1 Ultrafiltration behaviors of alginate blocks at various calcium
2 concentrations

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21 **Abstract**

22 Alginate, a linear copolymer, is composed of 1,4-linked β -d-mannuronic acid (M) and
23 α -l-guluronic acid (G), which are combined into homopolymeric blocks (MM-block
24 and GG-block) and heteropolymeric block (MG-block). It has been widely used as a
25 model foulant in various studies of membrane fouling, thus this study investigated the
26 impacts of calcium ion on MG-, MM- and GG-blocks of alginate and the filtration
27 behaviors of the three types of alginate blocks at different concentrations of calcium
28 ion. Results showed that calcium ion had the most serious effects on GG-blocks and
29 significantly promotes the formation of transparent exopolymeric particles (TEP)

30 from GG-blocks which in turn led to rapid formation of thick cake layer on membrane
31 surface during the filtration of GG-blocks. As for MM-blocks, it was found that the
32 formation of TEP was proportional to the Ca^{2+} concentration in MM-blocks solution,
33 while the membrane fouling was enhanced by Ca^{2+} in the filtration of MM-blocks
34 solution. Unlike MM- and GG-blocks, MG-blocks were nearly not affected by
35 addition of calcium ion, as the result, there was no significant increase in TEP. The
36 initial fouling rates and the mass of foulants deposited on the membrane surfaces
37 further revealed a close correlation between the TEP concentration and the membrane
38 fouling propensity. The observations by field emission scanning electron microscope
39 (FESEM) and atomic force microscope (AFM) further confirmed the formation
40 process of the cake layer by TEP on the membrane surface. This study offers deep
41 insights into the development of membrane fouling by different alginate blocks in the
42 presence of calcium ion, and suggests that TEP formed from alginate blocks played a
43 very significant role in the fouling development.

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50 Keywords: alginate blocks, transparent exopolymeric particles (TEP), calcium ion,
51 membrane fouling

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56 1. Introduction

57 Membrane technology has been intensively employed worldwide for clean water

58 production and wastewater reclamation. However, membrane fouling has been known
59 as a factor leading to increased operation and maintenance costs (Shannon et al. 2008,
60 Elimelech and Phillip 2011, Zhang et al. 2014). Alginate as a typical model foulant
61 has often been used in study of membrane fouling under various conditions (Lee and
62 Elimelech 2006, van den Brink et al. 2009, Mi and Elimelech 2010, Katsoufidou et al.
63 2010). Indeed, alginate is a linear copolymer composed of 1,4-linked β -d-mannuronic
64 acid (M) and α -l-guluronic acid (G) which are combined into homopolymeric blocks
65 (i.e. MM- and GG-blocks) and heteropolymeric block (i.e. MG-blocks) (Draget et al.
66 2005). The combinations and sequences of these blocks in alginate may vary widely,
67 and determine the chemical and physical properties of alginate which in turn affect
68 their filtration behaviors (Draget et al. 2005, Lee and Mooney 2012, Meng and Liu
69 2013). Evidence also showed that the filtration behaviors of alginates were also
70 significantly influenced by the presence of divalent cations, e.g. calcium ion
71 (Katsoufidou et al. 2007, van de Ven et al. 2008, Listiarini et al. 2009). For example, a
72 strong correlation between calcium ion concentration and membrane fouling rate by
73 alginate had been reported by van den Brink et al. (2009). Such an observation can be
74 explained by the classic “egg-box” model according to which calcium ions tend to
75 bind preferentially to the GG-blocks of alginate and form an egg-box-like structure
76 with alginate molecules (Grant et al. 1973, Draget et al. 2005). In turn, the later may
77 lead to the development of alginate cake layer on membrane surfaces (Katsoufidou et
78 al. 2007, van den Brink et al. 2009). It should be realized that no study has explored
79 the effects of calcium ion on membrane fouling development in consideration of
80 alginate molecular composition. As MG-, MM- and GG-blocks of alginate have
81 distinguished chemical and physical characteristics, it is reasonable to consider that
82 these alginate blocks may possess highly different fouling propensities in the presence
83 of calcium ion.

84 Increasing evidence suggests that transparent exopolymeric particles (TEP) are
85 responsible for the development of membrane fouling (Berman and Holenberg 2005,
86 Meng et al. 2013, Bar-Zeev et al. 2015). TEP originally found in marine environment
87 is a class of transparent particulate acidic polysaccharides that can be stained by

88 alcian blue (Alldredge et al. 1993). Operationally, TEP has been defined as particles
89 retained by 0.4 μm polycarbonate filter that can be stained by alcian blue (Passow and
90 Alldredge 1995). This pore size 0.4 μm was chosen due to the fact that in
91 limnological research, substances passing through 0.2-0.4 μm filters are usually
92 considered as “soluble” (Discart et al. 2015). Concurrently, polysaccharide materials
93 stained by alcian blue and passing through 0.4 μm polycarbonate filters are
94 considered as precursors of TEP, which can further form TEP (Passow 2000).
95 Subsequently, to better understand the role of TEP in the development of membrane
96 fouling, Villacorte et al. (2009a) further fractionated TEP according to their sizes by
97 using in-series filtrations with filters of different pore sizes (i.e. 0.05 to 0.4 μm). So
98 far, the involvement of TEP in the fouling development in various membrane systems
99 including microfiltration (MF), ultrafiltration (UF) and reverse osmosis (RO) had
100 been reported (Villacorte et al. 2009a,b, 2010a,b, 2012). Alginate as a common and
101 widespread polysaccharide has been reported to generate TEP-like particles that can
102 be stained by alcian blue and was used as standard calibration substance for TEP
103 determination (Passow and Alldredge 1995, Thornton et al. 2007). Previous study
104 showed that TEP can form from alginate blocks (e.g. MG-, MM- and GG-blocks)
105 (Meng and Liu 2013). Therefore, it is reasonable to use alginate blocks as a model of
106 TEP and this study mainly aimed to explore the effects of calcium ion on the
107 formation of TEP from MG-, MM- and GG-blocks as well as the specific roles of the
108 formed TEP in the development of membrane fouling.

109 2. Materials and methods

110 2.1 Alginate fractionation

111 Sodium alginate (Wako, Japan) was fractionated according to the method used by
112 Leal et al. (2008). 10 g/L of sodium alginate solution was stirred for 2 hours at 1,000
113 rpm. 3 M of HCl solution was then slowly added into the above alginate solution to
114 reach a final concentration of 0.3 M HCl, and the later was heated at 100 $^{\circ}\text{C}$ in oil bath
115 for 30 min with slow stirring. The cooled solution was centrifuged at 13,420 g for 30
116 min. The supernatant was harvested and neutralized with 1 M NaOH solution and

117 then poured into the equal-volume ethanol solution, yielding a white precipitate. This
118 precipitate was collected by centrifugation at 13,420 g-force for 30 min and was
119 finally freeze-dried to obtain the fraction of MG-blocks. The insoluble fraction from
120 the first centrifugation was re-dissolved in 1 M of NaOH solution under rapid vortex
121 condition. The pH of this solution was then readjusted to 2.85 by addition of 1M of
122 HCl solution. At pH 2.85, a precipitate appeared and was further separated out by
123 centrifugation. After neutralization by 1 M of NaOH solution, the remaining soluble
124 fraction was precipitated out by adding ethanol, and resulting precipitate was
125 harvested by centrifugation and freeze-dried to obtain the fraction of MM-blocks.
126 Again, the insoluble fraction was similarly dissolved in 1M NaOH solution, then
127 neutralized by 1M HCl and finally was precipitated out by addition of ethanol to
128 obtain the fraction of GG-blocks. All the freeze dried alginate blocks were grinded
129 into powder form in an agate mortar and were then stored in a copper sulfate dryer to
130 prevent them from being exposed to the damp room condition. All the solutions used
131 were prepared with ultrapure Milli-Q water. The above fractionation was repeated
132 three times. Results showed that the sodium alginate used was composed of $13.8 \pm 1.9\%$
133 MG-blocks, $53.5 \pm 1.3\%$ MM-blocks and $32.7 \pm 0.6\%$ GG-blocks, respectively, which
134 was consistent with that reported by Draget et al. (2005).

135 2.2 TEP formation from alginate blocks at different levels of calcium ion

136 2.2.1 Determination of TEP

137 TEP formed in respective MG-, MM-, and GG-blocks solutions with and without the
138 presence of calcium ion were determined with the modified Passow and Alldredge's
139 method (Passow and Alldredge, 1995; Villacorte et al. 2009a). 50 mg/L of sample
140 solutions were prepared by dissolving the required amount of MG-, MM- and
141 GG-blocks into ultrapure Milli-Q water with continuous stirring for 2 hours. The ionic
142 strengths of all solutions were adjusted to 10 mM by NaCl (Wako, Japan), while
143 CaCl_2 (Sigma, USA) was used to make up solutions with different calcium ion
144 concentration. All sample solutions were freshly prepared just before the

145 determination of TEP. The above prepared sample solutions were first filtered at a
146 constant pressure of 0.2 bars through a series of polycarbonate filters (Whatman,
147 United Kingdom) of pore size 0.05, 0.10, 0.2 and 0.4 μm respectively (Villacorte et al.
148 2009a). The TEP retained on the filters were further stained using 1 mL of pre-filtered
149 (0.05 μm polycarbonate filter) alcian blue solution which contained 0.02% of alcian
150 blue 8 GX (Sigma, USA) in 0.06% acetic acid (pH 2.5). After 5-s reaction, excess dye
151 was removed by filtration of 1 mL of ultrapure water (Milli-Q) through each filter at a
152 pressure ≤ 0.2 bars. The washed filters were then transferred into a series of small
153 beakers and were soaked in 5 mL of 80% H_2SO_4 solution for 2 hours. The beakers
154 were gently swirled 3-5 times over 2 hours to ensure the complete dissolution of
155 alcian blue in the H_2SO_4 solution. Lastly, the absorbance of the H_2SO_4 solution in
156 each beaker with the eluted alcian blue was measured using a UV-Vis
157 spectrophotometer (Shimadzu UV-2501PC, Japan) at the wavelength of 787 nm in a 1
158 cm cuvette, with ultra-pure water as reference. Three to five measurements were
159 conducted for each sample and every sample was measured at least three times. The
160 average absorbance was then used to calculate the TEP concentration in each sample.
161 Gum xanthan (Sigma, USA) was used as a standard substance of TEP for calibration
162 and the TEP concentration was finally expressed as mg gum xanthan equivalent per
163 liter of water ($\text{mg X}_{\text{eq}}\cdot\text{L}^{-1}$). It should be noted that the term TEP used in this study was
164 subjective to the definition by Villacorte et al. (2009a), i.e. alcian blue-stainable
165 particles that can be retained by 0.05 μm polycarbonate filter.

166 2.2.2 Microscopic observation of TEP

167 The appearance of TEP derived from alginate blocks in the presence or absence of
168 calcium ion was observed by a microscope (Keyence, Japan). The fresh TEP solutions
169 were prepared prior to observation as described in Section 2.2.1. In order to visualize
170 TEP with the bright-field microscope, alginate block solutions with or without
171 presence of calcium ion were stained by freshly pre-filtered (0.05 μm polycarbonate
172 filter) alcian blue solution as presented above (Passow and Alldredge, 1995). Stained
173 samples were then observed under the microscope. For each sample, about 20 images

174 were randomly taken.

175 2.3 Field emission scanning electron microscope (FESEM) analysis and zeta potential 176 measurements

177 The changes in the microstructures of alginate blocks due to addition of calcium ion
178 were observed by an FESEM (Jeol JSM-7600F, Japan). For FESEM observation,
179 MG-, MM- and GG-blocks in the absence and presence of calcium ion were first
180 collected. 10-20 mL of the same sample solutions as used in above section were
181 filtered through 0.1 μm polycarbonate filters (Whatman, United Kingdom) at 0.2 bars
182 and then freeze dried completely in a freeze dryer (Christ, Germany) for further
183 examination. All samples were observed at least three times and each time 5-10
184 pictures were randomly recorded.

185 The zeta potentials of alginate blocks in the absence and presence of calcium ion
186 were also measured using a Malvern ZetaSizer Nano ZS. All samples were freshly
187 prepared prior to the measurements and each measurement was repeated at least five
188 times.

189 2.4 Cross-flow ultrafiltration experiments

190 The filtration behaviors of various alginate blocks solutions in the presence or absence
191 of calcium ion were examined in a standard laboratory-scale cross-flow ultrafiltration
192 module (Sterlitech, USA) at a constant pressure of 2 bars and a temperature of
193 25 ± 2 °C. Feed solutions were prepared as described in Section 2.2.1. A commercial
194 polyethersulfone (PES) UF membrane (AMFOR INC, Beijing) with molecular weight
195 cut off (MWCO) of 20 kDa was used in this study. The flat sheet PES membrane was
196 soaked in the Milli-Q water for at least 12 hours for membrane wetting prior to use.
197 Feed water was circulated in the close-loop module by a gear pump (Cole-Parmer,
198 USA) at a cross-flow velocity of 10 cm/s. Permeate was collected in a beaker which
199 was placed on an electronic balance. The weight of permeate was recorded at the time
200 interval of 15 s by the data logger system which was connected to a computer. Before
201 each filtration test run, Milli-Q water was first filtered for 24 hours to compact and
202 equilibrate the membrane. Filtration test of each sample was repeated at least three

203 times. Furthermore, in order to determine the rejection rate of MG-, MM- and
204 GG-blocks by the UF membrane, the total organic carbon (TOC) concentrations in the
205 feed and the permeate were analyzed by a TOC analyzer (Shimadzu, Japan). The
206 respective TOC concentrations in the feed water prepared from MG-, MM- and
207 GG-blocks were found to be 18.0 ± 0.2 mg/L, 18.0 ± 0.1 mg/L and 17.9 ± 0.2 mg/L.

208 2.5 Analyses of fouling rate and foulant mass

209 The total membrane filtration resistances were calculated according to Darcy's law:

$$210 \quad J = \frac{\Delta P}{\mu R_t} \quad (1)$$

211 where J is the permeate flux ($\text{m}^3 \text{m}^{-2} \text{s}^{-1}$); R_t is the total filtration resistance (m^{-1}); ΔP is
212 the applied pressure (Pa); and μ is the solution viscosity (Pa·s). Fouling rates were
213 determined from the slope of the plot of total resistance versus time, whereas the
214 initial fouling rate was calculated from the initial linear part in the plot of total
215 resistance against filtration time. In addition, total foulant mass deposited on the
216 membrane surface was measured as the difference between the initial amount of
217 alginate blocks in the feed and that left in the solution after the filtration.

218 2.6 Characterization of fouled membrane

219 Surface morphology and topography of the fouled membranes were examined by the
220 FESEM and a XE 100 atomic force microscope (AFM, Park Systems, Korea)
221 respectively. In order to track of the development of cake layer on the membrane
222 surface, membrane samples taken at different filtration times of 10 min, 30 min and
223 1,500 min were examined. The sampled membranes were completely dried in a freeze
224 dryer (Christ, Germany) prior to observation. For the purpose of comparison, samples
225 used for respective FESEM and AFM observations were cut from the same location of
226 fouled membranes. For each observation, at least 16-20 pictures were randomly
227 recorded.

228 3. Results and discussion

229 3.1 TEP derived from alginate blocks

230 Fig. 1 shows the concentrations of TEP derived from the MG-, MM- and
231 GG-blocks in the absence and presence of calcium ion. Without addition of calcium
232 ion, the MM-blocks exhibited greatest tendency of self-aggregation, as the result, the
233 highest concentration of TEP was recorded among three alginate blocks, whereas the
234 lowest-concentration TEP was observed in the solution of MG-blocks. These can be
235 reasonably explained by different cohesive interaction energies between alginate
236 block molecules that can be calculated by extended
237 Derjaguin-Landau-Verwey-Overbeek (XDLVO) theory (Meng and Liu 2013). For
238 example, the cohesive interaction energies of MG-, MM- and GG-blocks are all
239 positive and tend to decrease in the order of $MG > GG > MM$. A positive cohesion free
240 energy indeed reflects a repulsive force between interfacial surfaces. Therefore,
241 MG-blocks should have the lowest self-aggregation tendency for TEP formation,
242 whereas TEP would likely be easily formed from self-aggregation of MM-blocks as
243 observed.

244 It should be noted that the formation of TEP in the presence of calcium ion
245 appeared to be different from those observed in the cases without addition of calcium
246 ion. In sense of chemical coagulation, calcium ion can enhance the aggregation of all
247 the alginate blocks, leading to more TEP production as shown in Fig. 1. It was found
248 that the concentrations of TEP derived from alginate blocks tended to increase in the
249 order $MG < MM < GG$ in the presence of calcium ion. In fact, it was not surprising to
250 observe that GG-blocks generated the highest-concentration TEP. As documented in
251 the literature, there is a strong binding between GG-block and calcium ion, resulting
252 in a three dimensional network of alginate strands held together through ionic
253 interactions, known as “egg-box” structure (Grant et al. 1973, Draget et al. 2005). The
254 biaxially linked G residues in GG-blocks form a cavity that can accommodate
255 divalent calcium ion, i.e. GG-blocks can easily link together through the bridging of
256 calcium ion. As such, more TEP can develop from GG-blocks in the presence of
257 calcium ion as observed in Fig. 1. Although the TEP produced from MG- and
258 MM-blocks was also enhanced by addition of calcium ion, it was much lower than
259 that from GG-blocks. The binding mechanisms of MG- and MM-blocks with calcium

260 are currently unclear. It had been reported that hydrogels formed by alginates rich in
261 M residues were softer, less porous and unstable towards disintegration with time as
262 compared with those developed from alginates rich in G residues (De Vos et al. 1996,
263 Simpson et al. 2004). These imply that MG- and MM-blocks can't firmly bind to
264 calcium ion, evidenced by differences in the stiffness of alginate blocks which are in
265 the order $MG < MM < GG$ (Smidsrød, 1974, Vold et al. 2006). The highly flexible
266 MG-blocks preferably tend to bind to water molecules instead of forming complexes
267 with calcium ion (Smidsrød, 1974). Therefore, such behavior of MG-blocks indeed
268 unfavors the formation of TEP even in the presence of calcium ion. This is further
269 supported by the observation of that the TEP concentration was increased
270 insignificantly with increasing the calcium concentration from 1 mM to 2 mM (Fig. 1).
271 As the rigidity of MM-blocks was higher than that of MG-blocks, MM-blocks should
272 preferably react with calcium ion, as a result, more TEP was produced (Fig. 1). In
273 addition, the positive correlation between TEP in different size ranges and calcium
274 concentrations in solutions of MM-blocks are observed in Fig. 2, suggesting the
275 dependence of the TEP formation on the level of calcium ion in the solution
276 MM-blocks.

277 TEP formed from MG-, MM- and GG-blocks in the presence or absence of calcium
278 ion were visualized by a light-field microscope. Fig. 3 provides direct observation of
279 the morphology and structure of TEP derived from alginate blocks. In the absence of
280 calcium ion (Fig. 3 a-c), only few small TEP were observed. However, much more
281 and large TEP with complex structures were captured in the vision field of the
282 microscope in the presence of calcium ion, as indicated by strong blue color (Figs. 3
283 d-f). For example, both the concentration and the size of TEP derived from GG-blocks
284 were significantly increased in the presence of calcium ion. These indeed are in good
285 agreement with the TEP results presented in Fig. 1. As discussed above, GG-blocks
286 can generate more TEP through the formation of the complex "egg-box" structure
287 among GG-blocks and calcium ion.

288 3.2 Structural changes of alginate blocks

289 FESEM images revealed the changes in microstructure of alginate blocks induced by
290 calcium ion. The filament-type alginate blocks were observed in the absence of
291 calcium ion (Fig. 4 a-c), whereas much bigger and more compact structure was
292 developed for all the three alginate blocks after addition of calcium ion (Fig. 4 d-f).
293 Moreover, the microscopic morphologies of MG-, MM- and GG-blocks were
294 completely altered by calcium ion. Such changes in the microstructure of alginate
295 blocks are likely due to the binding with calcium ion as discussed above.

296 Table 1 summarizes the zeta potentials of MG-, MM- and GG-blocks solutions at
297 various concentrations of calcium ion. With the addition of calcium ion, the zeta
298 potentials of alginate blocks all tended to decrease, indicating that alginate blocks
299 became less stable and had greater tendency of aggregation among themselves.
300 Consequently, this may affect their fouling propensity during membrane filtration.

301 3.3 Filtration behaviors of alginate blocks at various calcium concentrations

302 3.3.1 Flux profiles of MG-, MM- and GG-blocks

303 As presented above, it is obvious that calcium ion has significant effects on the
304 physic-chemical properties and microstructures of MG-, MM- and GG-blocks,
305 subsequently their potentials to form TEP. Fig. 5a shows the permeate flux profiles
306 observed during the ultrafiltration of MG-blocks at various concentrations of calcium
307 ion. The higher of the calcium concentration, the severer membrane fouling is
308 observed. In the absence of calcium ion, the initial flux declines slowly. However, in
309 the presence of 1 mM and 2 mM calcium ion, a rapid decline was observed at the
310 initial phase. The TOC concentration of the permeate further revealed that the
311 rejection rate of MG-blocks (Table 2) by the membrane was only about 28.8%
312 without calcium ion, however it increased to 56.9% and 57.2% with the addition of 1
313 mM and 2 mM Ca^{2+} , respectively. These results indicate that most of MG-blocks were
314 smaller than the pores of membrane, thus could pass through the membrane. On the
315 contrary, with the addition of calcium ion, calcium ion promoted the self-aggregation
316 of MG-blocks (Fig. 1), leading to the formation of large MG-blocks complexes that
317 can be effectively retained by the ultrafiltration membrane used in this study.

318 Fig. 5b shows the filtration profiles of MM-blocks in the absence and presence of
319 calcium ion. Without addition of calcium ion, the flux decline during the ultrafiltration
320 of MM-blocks was much less than that observed for MG-block. The TOC
321 concentration of the permeate shows that 88.7% of MM-blocks was retained by the
322 ultrafiltration membrane used in this study. According to the filtration data of MM-
323 and MG-blocks, it is reasonable to consider that most of MM-blocks had a size bigger
324 than MG-blocks. This indeed is supported by the prediction by the XDLVO theory, i.e.
325 MM-blocks possess the highest self-aggregation potential to form bigger TEP in water
326 (Meng and Liu 2013). Furthermore, the addition of calcium ion significantly promotes
327 the self-aggregation of MM-blocks and the formation of TEP. As the result, much
328 severer decline in the permeate flux was observed. As can be seen in Fig. 5 b, the
329 severity of the flux decline is proportionally related to the concentration of calcium
330 ion added to the MM-block solutions. For example, the filtration flux of MM-block
331 solution in the absence of calcium ion only decreased to 82.7% of its initial flux after
332 48-hour ultrafiltration. However, with the addition of 1 mM and 2 mM calcium ion to
333 the MM-block solutions, the respective fluxes significantly dropped to 23.9% and
334 14.7% of the initial flux. In addition, the measured TOC concentrations in the
335 permeates further indicate that 96.0% and 97.2% of MM-blocks were rejected in the
336 presence of 1 mM and 2 mM of calcium ion, respectively. Previous study also showed
337 that the formation of cake layer on the membrane surface was the main cause of
338 membrane fouling in the filtration of MM-blocks with a 0.2- μm microfiltration
339 membrane (Meng and Liu 2013). Similarly, it is reasonable to consider that the
340 formation of cake layer by MM-blocks was mainly responsible for the observed
341 fouling during the ultrafiltration with a membrane of 20,000 Daltons. As shown in Fig.
342 1, the addition of 1 mM and 2 mM of calcium ion greatly promoted the formation of
343 TEP ($>0.05 \mu\text{m}$) in the MM-blocks solutions from 4.0 mg Xeq/L without calcium ion
344 to 9.6 and 17.1 mg Xeq/L) respectively. On the other hand, Fig. 3 also reveals that
345 TEP formed in the presence of calcium had more complex structure than TEP
346 developed in the absence of calcium. Obviously, these calcium-induced physical
347 changes to TEP inevitably expedite the formation of cake layer on the UF membrane.

348 Fig. 5c shows the effects of calcium ion on the ultrafiltration behaviors of
349 GG-blocks. Compared to the MG- and MM-blocks, calcium ion had a much greater
350 effect on the ultrafiltration of GG-blocks. The permeate flux of GG-blocks in the
351 absence of without calcium, only declined 89.7% of its initial value after 24-hour
352 filtration. However, in the presence of 1 mM and 2 mM of calcium, an extremely
353 quick drop in the respective filtration fluxes of GG-blocks was observed in the initial
354 phase, and only 6.8% and 5.6% of the initial flux remained after 24-hour
355 ultrafiltration. Such severe membrane fouling can be explained by the highest
356 concentration of TEP obtained in the GG-blocks solutions after the addition of
357 calcium (Fig. 1), as compared to MM- and MG-blocks. For example, only about 3.6
358 mg Xeq/L TEP > 0.05 μm was determined in the GG-block solution without addition
359 of calcium, whereas 71.1 and 72.2 mg Xeq/L were obtained at the calcium
360 concentrations of 1 mM and 2 mM respectively. Meanwhile, Fig. 3 showed the
361 structure of TEP derived from GG-blocks in the presence of calcium also became
362 larger and more complex. Furthermore, the TOC concentration in the permeate
363 showed 93.7% and 95.3% of GG-blocks were rejected after the addition of 1 mM and
364 2 mM calcium. Therefore, the increases in concentration and size of TEP derived from
365 the GG-blocks solutions in the presence of calcium are definitely responsible for the
366 observed quick and severe membrane fouling (Fig. 5c).

367 3.3.2 Analysis of fouling rate and foulant deposited on membrane surface

368 The initial fouling rate and total foulant mass on the membrane surface deposition
369 were calculated in order to establish the relationship between TEP concentration and
370 the fouling development. The ultrafiltration membrane used in this study has a
371 MWCO of 20 kDa, thus TEP measured with a size >0.05 μm should not result in the
372 pore blocking of membrane. Fig. 6 shows a positive correlation between the TEP
373 concentration (>0.05 μm) and the initial fouling rate. It appears that TEP plays a
374 critical role in the initial development of membrane fouling. These results also
375 suggest that TEP can adhere onto the membrane surface very quickly to form a
376 gel-like layer as also reported by Bar-Zeev et al. (2012).

377 The foulant mass deposited on the membrane surface offers direct insights into the
378 cake layer formed by TEP (TEP>0.05 μm). It can be seen in Fig. 7 that the amount of
379 foulants is proportionally correlated to the TEP concentration in the feed water. Figs.
380 6 and 7 offer a much clearer understanding of the role of TEP in the development of
381 membrane fouling. In fact, such an observation is supported by the study of Bar-Zeev
382 et al. (2012) showing that TEP preferably tend to attach to solid surfaces.

383 3.3.3 Development of cake layer at different TEP concentrations

384 As discussed above, the formation of cake layer on the membrane surfaces was of
385 critical importance in the ultrafiltration of MM- and GG-blocks. In the present study,
386 FESEM and AFM techniques were employed to visualize the membrane surfaces
387 fouled at two different TEP concentrations. Although TEP were freeze-dried prior to
388 microscopic observation, these visualization techniques could still provide direct
389 evidence of the TEP attachment on the membrane surfaces.

390 As can be seen in Fig. 8a, only few and small TEP were deposited on the membrane
391 surface after 10-minute filtration at the low TEP concentration, while remarkable
392 accumulation of TEP on the membrane surface was observed at the high concentration
393 (Fig. 8b). After 30-minute filtration, some membrane still remained blank in the
394 ultrafiltration of low-concentration TEP solution, but the entire membrane surface was
395 eventually completely covered by TEP in the filtration of the high-concentration TEP
396 solution (Figs. 8c and 8d). These observations indeed are in good agreement with the
397 results in Fig. 6 showing high-concentration TEP resulted in high initial fouling rate.
398 Figs. 8e and 8f further show the fouled membrane surface after 1500-minute filtration.
399 It should be noted that crafts in these two figures were created by the freeze dry
400 process in the preparation of the samples for FESEM. Fortunately, these crafts
401 revealed that the thicker cake layer was developed at the higher TEP concentration.

402 In addition, three dimensional AFM images (Fig. 9) also revealed the phenomena
403 similar to those by FESEM. The roughness was evaluated from AFM images on
404 different locations of the same fouled membrane and the average values were reported
405 in Fig 9. It appears that the roughness of the fouled membrane surfaces tended to

406 increase with the successive accumulation of TEP, and the changes in the surface
407 roughness was also related to the TEP concentration, e.g. the surface roughness was
408 found to increase rapidly during the filtration of high-concentration TEP solution.

409 3.4 Effects of calcium ion on the cross-linking of MG-, MM and GG-blocks

410 It is demonstrated above that despite derived from the same source of alginate, MG-,
411 MM- and GG-blocks behaved totally differently in the reactions with calcium ion. Fig.
412 10 schematically illustrates possible interactions between various alginate blocks and
413 calcium ion. As discussed above, most of MG-blocks are small enough to pass
414 through the membrane used in present study. After the addition of calcium ion, only
415 few TEP were form from MG-blocks, i.e. the interaction or reaction between
416 MG-blocks and calcium ion is insignificant. Unlike MG-blocks, the binding between
417 MM-blocks and calcium ion appeared to be much stronger and seemingly
418 Ca^{2+} -dependent. As a result, the TEP concentration in the MM-block solution was
419 much higher than that observed in the MG-block solution. The most notable reaction
420 was found between GG-blocks and calcium ion, and the addition of calcium ion to the
421 GG-block solution significantly enhanced the production of TEP, resulting in a quick
422 formation of cake later on the membrane surface.

423 So far, more than 200 kinds of commercial alginates derived from different sources
424 are available in the market and the MG-, MM and GG-blocks contents in alginates
425 vary largely (Tonnesen and Karlsen 2002; Lee and Mooney 2012). In the literature,
426 alginate from various suppliers has been commonly employed as a model foulant, but
427 with no consideration of possible differences in their molecular compositions and
428 structures. As shown in Fig. 10, MG-, MM- and GG-blocks indeed exhibit different
429 cross-linking structures with calcium ion which exists extensively in various water
430 and wastewater environments. Therefore, it appears to be necessary to characterize the
431 molecular compositions and structures of alginates from various sources which would
432 be useful for better interpretation of filtration results from different types of
433 membranes.

434 4. Conclusions

435 Although derived from the same source alginate, MG-, MM and GG-blocks exhibited
436 distinguished properties, and such differences were further intensified by the presence
437 of calcium ion. The reaction between calcium ion and GG-blocks was found to be
438 strongest, and more TEP was thus produced. As the result, ultrafiltration membrane
439 was quickly fouled by accumulated TEP on its surface. Although the formation of
440 TEP from MM-blocks was enhanced by the addition of calcium ion, it was not as
441 intensive as that observed for GG-blocks. As such, the development of membrane
442 fouling during the ultrafiltration of MM-block solution was slowed down. The effect
443 of calcium ion on MG-blocks was slightest, and only very limited amount of TEP was
444 produced. Thus, the membrane fouling by MG-blocks was not as serious as those
445 observed during the ultrafiltration of MM- and GG-blocks. More importantly, this
446 study provided experimental evidence showing the critical role of TEP in the
447 development of membrane fouling.

448

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452

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