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Role of membrane fouling substances on the rejection of N-nitrosamines by reverse osmosis

Abstract

The impact of fouling substances on the rejection of four *N*-nitrosamines by a reverse osmosis (RO) membrane was evaluated by characterizing individual organic fractions in a secondary wastewater effluent and deploying a novel high-performance liquid chromatography-photochemical reaction-chemiluminescence (HPLC-PR-CL) analytical technique. The HPLC-PR-CL analytical technique allowed for a systematic examination of the correlation between the fouling level and the permeation of *N*-nitrosamines in the secondary wastewater effluent and synthetic wastewaters through an RO membrane. Membrane fouling caused by the secondary wastewater effluent led to a notable decrease in the permeation of *N*-nitrosodimethylamine (NDMA) while a smaller but nevertheless discernible decrease in the permeation of *N*-nitrosomethylethylamine (NMEA), *N*-nitrosopyrrolidine (NPYR) and *N*-nitrosomorpholine (NMOR) was also observed. Fluorescence spectrometry analysis revealed that major foulants in the secondary wastewater effluent were humic and fulvic acid-like substances. Analysis using the size exclusion chromatography technique also identified polysaccharides and proteins as additional fouling substances. Thus, further examination was conducted using solutions containing model foulants (i.e., sodium alginate, bovine serum albumin, humic acid and two fulvic acids). Similar to the secondary wastewater effluent, membrane fouling with fulvic acid solutions resulted in a decrease in *N*-nitrosamine permeation. In contrast, membrane fouling with the other model foulants resulted in a negligible impact on *N*-nitrosamine permeation. Overall, these results suggest that the impact of fouling on the permeation of *N*-nitrosamines by RO is governed by specific small organic fractions (e.g. fulvic acid-like organics) in the secondary wastewater effluent.

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1 **Role of membrane fouling substances on the rejection of**
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22 **Abstract**

23 The impact of fouling substances on the rejection of four *N*-nitrosamines by a reverse
24 osmosis (RO) membrane was evaluated via a systematic characterisation of individual
25 organic fractions in a secondary wastewater effluent and the deployment of a novel high-
26 performance liquid chromatography-photochemical reaction-chemiluminescence (HPLC-PR-
27 CL) analytical technique. The HPLC-PR-CL analytical technique allowed for a systematic
28 examination of the correlation between the fouling level and the permeation of *N*-
29 nitrosamines in the secondary wastewater effluent and synthetic wastewaters through an RO
30 membrane. Membrane fouling caused by the secondary wastewater effluent led to a notable
31 decrease in the permeation of *N*-nitrosodimethylamine (NDMA) while a smaller but
32 nevertheless discernible decrease in the permeation of *N*-nitrosomethylethylamine (NMEA),
33 *N*-nitrosopyrrolidine (NPYR) and *N*-nitrosomorpholine (NMOR) was also observed. The
34 decrease in *N*-nitrosamine permeation became insignificant after membrane permeability
35 decreased by approximately 30%. Fluorescence spectrometry analysis revealed that major
36 foulants in the secondary wastewater effluent were humic and fulvic acid-like substances.
37 Analysis using the size exclusion chromatography technique also identified polysaccharides
38 and proteins as additional fouling substances. Thus, further examination was conducted using
39 solutions containing model foulants (i.e., sodium alginate, bovine serum albumin, humic acid
40 and two fulvic acids). Similar to the secondary wastewater effluent, membrane fouling with
41 fulvic acid solutions resulted in a decrease in *N*-nitrosamine permeation. In contrast,
42 membrane fouling with the other model foulants resulted in an increase in *N*-nitrosamine
43 permeation. Overall, these results suggest that the impact of fouling on the permeation of *N*-
44 nitrosamines by RO is governed by specific small organic fractions (e.g. fulvic acid-like
45 organics) in the secondary wastewater effluent.

46 **Keywords:** Fulvic acid; membrane fouling; *N*-nitrosamines; NDMA; reverse osmosis;
47 potable water reuse.

48

49 **1. Introduction**

50 Potable water reuse has become an attractive approach for augmenting fresh water sources in
51 drought stricken regions such as the southwestern USA, southern Europe and Australia.
52 Stringent quality assurance is required in potable water reuse to avoid adverse impacts on
53 public health. Aside from the need to mitigate acute microbial risks through multiple
54 treatment barriers and robust disinfection (CSWRCB, 2016), the occurrence of trace organic
55 chemicals is of particular concern due to their potential for chronic health effects (Murphy et
56 al., 2012; Villanueva et al., 2014). As a result, reverse osmosis (RO) has been widely used for
57 the removal of these trace organic chemicals in many water reclamation plants around the
58 world (Shannon et al., 2008; Verliefde et al., 2008). Removal efficiencies of most trace
59 organic contaminants of over 90% can be achieved by RO (Al-Rifai et al., 2011).

60 Of the many trace organic chemicals of concern, the removal of *N*-nitrosamines is arguably
61 the most challenging for potable water reuse. Several *N*-nitrosamines are probable
62 carcinogenic chemical (USEPA, 1993). In particular, unlike most other trace organic
63 chemicals, the rejection of *N*-nitrosodimethylamine (NDMA) by RO membranes is well
64 below 90% due to its small molecular size and uncharged property in aqueous solution
65 (Plumlee et al., 2008). NDMA and other *N*-nitrosamines can occur naturally in wastewater
66 and are not well removed by conventional treatment processes (Drewes et al., 2006). A more
67 important source of NDMA is the direct result of chloramination of secondary wastewater
68 effluent prior to RO treatment which is used to control biofouling on the RO membranes
69 (Shah and Mitch, 2011). Because NDMA is sometimes identified in RO permeate at
70 concentrations higher than the California regulatory notification level and Australian
71 Guidelines for Water Recycling value of 10 ng/L (CDPH, 2015; NRMCC et al., 2008) in
72 potable reuse schemes, additional water treatment such as an ultraviolet (UV) photolytic

73 process or UV-advanced oxidation process (AOP) is employed downstream of the RO
74 process (Fujioka et al., 2012a; Sharpless and Linden, 2003). This additional treatment process
75 ultimately increases the overall cost of potable water reuse. A high rejecting RO membrane
76 for the removal of NDMA could potentially reduce the capital and operating costs of the UV-
77 AOP. However, the large variation in NDMA rejection by RO (negligible to 80%) reported in
78 the literature (Farré et al., 2011; Plumlee et al., 2008; Sedlak and Kavanaugh, 2006) makes it
79 difficult to rely solely on RO for the removal of NDMA.

80 The underlying mechanisms of the observed variation in NDMA rejection by RO have been
81 elucidated in several recent studies. In addition to membrane properties (Fujioka et al.,
82 2013b) and RO feed solution temperature (Fujioka et al., 2012b), membrane fouling has been
83 shown to affect NDMA rejection (Fujioka et al., 2013a; Steinle-Darling et al., 2007).
84 However, the effects of membrane fouling on NDMA rejection in these previous studies did
85 not produce consistent results. Steinle-Darling et al. (2007) reported that membrane fouling
86 with model foulants (alginate) resulted in a reduction in the rejection of *N*-nitrosamines
87 including NDMA. In a subsequent study, Fujioka et al. (2013a) observed an increase in the
88 rejection of *N*-nitrosamines with tertiary wastewater effluent. It is noteworthy that Fujioka et
89 al. (2013a) also observed only negligible impact of fouling layer on *N*-nitrosamine rejection
90 when the membrane was fouled with large molecular weight model foulants (i.e., sodium
91 alginate, bovine serum albumin and humic acid). These previous results suggested that the
92 impact of membrane fouling could vary depending on the properties of the foulants, but the
93 major model foulants were unlikely to be representative of substances causing the increased
94 *N*-nitrosamine rejection.

95 In a well-controlled laboratory-scale study to evaluate the effects of membrane fouling on *N*-
96 nitrosamine rejection, bench-scale RO systems have the advantage of precise regulation of

97 the operating conditions. However, sample volumes required for their analysis can be
98 excessive. The standard method for the analysis of *N*-nitrosamines including NDMA
99 (McDonald et al., 2012; Munch and Bassett, 2004) is based on solid-phase extraction (SPE)
100 followed by gas chromatography and tandem mass spectrometry (GC-MS/MS) detection and
101 requires a sample volume of 0.2–1.0 L/sample. This limits the number of samples that can be
102 acquired, which has ultimately contributed to a lack of understanding of the dynamics of
103 NDMA rejection during RO treatment. Of a particular note, previous bench-scale studies
104 (Fujioka et al., 2013a; Steinle-Darling et al., 2007) have only evaluated *N*-nitrosamine
105 rejection by RO membranes under two sampling conditions—before and after membrane
106 fouling development.

107 Recently, a fast, high-throughput, and reliable high-performance liquid chromatography-
108 photochemical reaction-chemiluminescence (HPLC-PR-CL) analytical technique for the
109 quantitation of *N*-nitrosamines has been developed (Kodamatani et al., 2009). The analytical
110 method can be performed with a very small sample injection volume (20–200 μ L) and
111 requires no concentration steps, unlike the SPE-GC-MS/MS method (Munch and Bassett,
112 2004). In addition, this HPLC-PR-CL method can achieve more precise determination of
113 NDMA concentrations with method detection limits of 2 and 0.2 ng/L in UF-treated
114 wastewater and RO permeate, respectively (Fujioka et al., 2016). Thus, this newly established
115 HPLC-PR-CL analytical technique opens up new opportunities for a systematic examination
116 of the correlation between the fouling condition and *N*-nitrosamine rejection.

117 This work aimed to identify major foulants that influence *N*-nitrosamine rejection by an RO
118 membrane. A nanofiltration (NF) membrane was also used for comparison. The HPLC-PR-
119 CL analytical technique was modified for the determination of *N*-nitrosamines in the
120 secondary wastewater effluent and model foulant solutions, and was used to systematically

121 examine the correlation between fouling development and *N*-nitrosamine rejection.
122 Consequently, five model foulants were selected and four *N*-nitrosamines, including NDMA,
123 were selected for delineation of the mechanisms underlying the impact of membrane fouling
124 on *N*-nitrosamine rejection.

125 **2. Materials and methods**

126 *2.1. Chemicals*

127 Four analytical grade *N*-nitrosamines (Ultra Scientific, Kingstown, RI, USA) were used in
128 this study: NDMA, *N*-nitrosomethylethylamine (NMEA), *N*-nitrosopyrrolidine (NPYR) and
129 *N*-nitrosomorpholine (NMOR) (**Table 1**). A stock solution containing all four *N*-nitrosamines
130 was prepared at 1 µg/mL of each compound in pure methanol. Five model foulants – sodium
131 alginate, bovine serum albumin (BSA), humic acid and two fulvic acids – were also used.
132 Sodium alginate and humic acids were supplied by Sigma-Aldrich (St Louis, MO, USA).
133 BSA was purchased from Wako Pure Chemical Industries (Tokyo, Japan). Suwannee River
134 fulvic acid standard II and Pahokee Peat fulvic acid standard II were purchased from
135 International Humic Substances Society (IHSS, MN, USA). Analytical grade NaCl, CaCl₂,
136 NaHCO₃ and luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) were supplied from Wako
137 Pure Chemical Industries (Tokyo, Japan). Secondary wastewater effluent was collected from
138 a municipal wastewater treatment plant (WWTP) in Japan. The sampling point was before
139 chlorine disinfection and after screening, primary settling and activated sludge treatment.

140 *2.2. Membrane treatment system*

141 A low pressure RO membrane – ESPA2 – was supplied as flat sheet samples by
142 Nitto/Hydranautics (Osaka, Japan). The ESPA2 membrane is a composite polyamide RO
143 membrane that has been used widely in water reclamation applications (Fujioka et al., 2012a).

144 An NF membrane – ESNA1-LF – from Nitto/Hydranautics (Osaka, Japan) was also used in
145 this study. A bench-scale RO system with a cross-flow configuration was used (**Fig. S1**). The
146 treatment system includes a stainless steel membrane cell (Iwai Pharma Tech, Tokyo, Japan)
147 that can hold a circular flat sheet membrane coupon with effective surface area of 36.3 cm².
148 A high-pressure pump (KP-12, FLOM, Tokyo, Japan) was also used to transport feed
149 solution from a 2-L glass reservoir to the membrane cell. The feed solution temperature was
150 controlled in the reservoir with a stainless steel heat exchanging coil connected to a
151 temperature control unit (NCB-500, Tokyo Rikakikai, Tokyo, Japan).

152 *2.3. Experimental protocols*

153 Each experiment was initiated by conditioning the RO membranes with deionized water (Q
154 18.0 MΩcm) at 1,500 kPa until the permeate flux stabilised. The deionized water was then
155 replaced with 2 L of the secondary wastewater effluent or solutions of model foulant. The
156 model foulant solutions contained background electrolytes (20 mM NaCl, 1 mM NaHCO₃, 1
157 mM CaCl₂) and 30–50 mg/L of one of the model foulants in Milli-Q water. Each *N*-
158 nitrosamine was spiked into the RO feed at a concentration of 500 ng/L. The RO treatment
159 system was operated at constant flux of 60 or 80 L/m²h. During each experiment, both RO
160 feed and permeate were recirculated into the feed reservoir to maintain a constant
161 concentration of each solute and foulant in the RO feed. While full-scale RO systems in water
162 reclamation applications are typically designed and operated at the permeate flux of ~20
163 L/m²h (Fujioka et al., 2012a), the high flux was used in this study to accelerate membrane
164 fouling. The feed temperature was maintained at 20 °C and transmembrane pressure (TMP)
165 was recorded. RO feed and permeate samples were collected periodically in amber vials (1.5
166 mL). Concentrations of *N*-nitrosamines in the RO feed and permeate samples were used for
167 calculating their rejections. The RO permeate and feed sample volumes were negligible (i.e.

168 1.5 mL) as compared to 2 L of the initial feed volume; thus, *N*-nitrosamine concentration in
169 the RO feed was expected to be constant throughout the experiment. In addition, a previous
170 study (Fujioka et al., 2012b) has confirmed that changes in *N*-nitrosamine concentrations
171 from 250 to 1,500 ng/L had no impact on the rejection of *N*-nitrosamines. Overall, the
172 experimental condition of this study allowed for an accurate evaluation of *N*-nitrosamine
173 rejections without any interference from changes in their concentrations in the RO feed.

174 2.4. Analytical techniques

175 2.4.1. HPLC-photochemical reaction-chemiluminescence detection (HPLC-PR-CL)

176 *N*-nitrosamine concentrations were determined by HPLC-PR-CL. This method is based on
177 the chemiluminescence reaction between peroxyxynitrite with luminol. Peroxyxynitrite is formed
178 by the photochemical reaction of *N*-nitrosamines with UV irradiation at 254 nm after HPLC
179 separation. The HPLC separation was performed with an InertSustain AQ-C18 (5 μ m, 4.6 \times
180 250 mm) (GL Sciences, Tokyo, Japan) with an eluent of 5 mM phosphate buffer and
181 methanol (95:5 v/v). Further details of this method are provided elsewhere (Fujioka et al.,
182 2016; Kodamatani et al., 2016). A sample HPLC-PR-CL chromatogram of the separation of
183 NDMA, NMOR, NMEA and NPYR is shown in **Fig. S2**. Each sample from the RO feed was
184 pre-filtered with a 0.45 μ m hydrophilic PTFE syringe filter (Filtstar, Starlab Scientific,
185 China). The sample injection volume was from 20 to 200 μ L.

186 2.4.2. Fluorescence spectroscopy

187 Excitation emission matrix (EEM) fluorescence spectra (Aqualog, Horiba, Kyoto, Japan) of
188 the samples were obtained using a 1-cm quartz cuvette. The EEM spectra (EEMs) were
189 acquired with scanning emission spectra every 8 pixels from 245.21 to 827.61 nm by
190 changing the excitation wavelength from 220 to 800 nm at 1 nm step with a 4.60 nm CCD

191 bin increment at low gain and 1 s integration. All EEMs were corrected through blank
192 subtraction (ultrapure water – 18.2 MΩcm with 1 g/L methanol and humic acid) to reduce
193 scatter from the water Raman peak for instrument/spectral biases according to the emission
194 and excitation correction factors provided by the manufacturer.

195 2.4.3. Size exclusion chromatography

196 Organic carbon content in the water samples were characterised by a liquid chromatography-
197 organic carbon detection (LC-OCD) system (DOC-LABOR, Karlsruhe, Germany). Details of
198 the analysis can be found in previous published studies (Henderson et al., 2011; Huber et al.,
199 2011). The analysis was performed at 1.1 mL/min flow rate with a mobile phase of phosphate
200 buffer, 2.5 g/L KH₂PO₄ and 1.2g/L Na₂HPO₄. Samples was diluted 1:10 in Milli-Q water and
201 a volume of 2.0 mL of the sample was injected into the LC-OCD system.

202 **3. Results and discussion**

203 *3.1. Analysis in a secondary wastewater effluent*

204 The analysis of *N*-nitrosamines in the secondary wastewater effluent using HPLC-PR-CL was
205 validated through spike testing. Each *N*-nitrosamine was spiked into the secondary
206 wastewater effluent at a concentration of 50 ng/L for analyte recovery evaluation. Recovery
207 was calculated based with the ratio of the peak height of *N*-nitrosamine in the secondary
208 wastewater effluent to the peak height of *N*-nitrosamine in the pure water matrix. With the
209 injected sample volume of 200 μL, the peak height of NDMA at the retention time (*rt*) of
210 6.1 min (**Fig. 1a**) revealed 66% recovery relative to the pure water matrix. Recovery in the
211 range of 87 and 90% was observed for all other *N*-nitrosamines (**Table S3**). Impurities in the
212 secondary effluent could interfere with photochemical and/or chemiluminescence reaction,
213 leading to the low recovery observed here when a large injection volume was used. The

214 observed decreasing peak heights of *N*-nitrosamines were attributed to the reduction of
215 baseline chemiluminescence after 3 min as compared to the initial baseline
216 chemiluminescence. The impact was particularly strong around the NDMA peak (*rt* = 6.1
217 min) and gradually recovered to the original baseline as shown in **Fig. 1a**. Because the
218 baseline chemiluminescence is generated from the reaction of the eluent, the reduction of
219 baseline chemiluminescence after the sample injection substances in the secondary
220 wastewater effluent could have interfered with the photochemical reaction and/or
221 chemiluminescence reaction. Accordingly, the peak heights of *N*-nitrosamines may also have
222 reduced by the interference.

223 To reduce the presence of interfering substances, the sample injection volume was reduced
224 from 200 to 20 μL , which was successfully validated for NDMA in ultrafiltration-treated
225 wastewater in a previous study (Fujioka et al., 2016). With the smaller injection volume, the
226 chemiluminescence around the four *N*-nitrosamine peaks dropped to an intensity near the
227 initial baseline (*rt* = 0–2 min) (**Fig. 1b**). As a result, recovery of NDMA improved from 66%
228 (injection volume = 200 μL) to 96% (injection volume = 20 μL). Similarly, the other *N*-
229 nitrosamines generally revealed improved recoveries (96–106%) (**Table S3**). The method
230 detection limits (MDLs) for NDMA, NMEA, NPYR and NMOR in the secondary wastewater
231 effluent were 1.8, 3.7, 3.3 and 2.3 ng/L, respectively.

232 3.2. *N*-nitrosamine rejection associated with a secondary wastewater effluent

233 The fouling propensity of the ESPA2 RO membrane was identified for the secondary
234 wastewater effluent. Fouling development using the ESPA2 RO membrane with the
235 secondary wastewater effluent led to an increase in the rejection of all four *N*-nitrosamines
236 investigated (**Fig. 2**). In particular, NDMA rejection increased from 75.7 (*t* = 5 min) to 80.0%
237 (*t* = 200 min) with an increase in TMP from 1.6 to 2.5 MPa (approximately 30% increase in

238 TMP). Similar observations could be made with the other *N*-nitrosamines, although the
239 increase in their rejection was less significant compared to NDMA (**Fig. 2**). In response to the
240 fouling development from 5 to 200 min, the rejections of NMEA, NPYR and NMOR also
241 increased from 93.3 to 95.1%, from 97.5 to 98.2% and from 99.2 to 99.6%, respectively. The
242 results suggest that membrane fouling at full-scale applications can lead to a gradual decrease
243 in the permeation of NDMA, meaning that the prolonged operation could result in an increase
244 in NDMA rejection. It should be noted that the accelerated membrane fouling protocol
245 applied here could only show the behaviour of NDMA rejection during fouling development
246 and the rejection values do not directly simulate the actual impact of fouling in full scale.

247 Treated wastewater contains a diverse range of organics. It is essential to identify individual
248 organic fractions most responsible for the variation in *N*-nitrosamine rejection. Thus, further
249 investigation was performed by characterising the secondary wastewater effluent and
250 conducting RO studies using model foulants.

251 *3.3. Characterisation of organics in the RO feed*

252 *3.3.1. LC-OCD*

253 Organic constituents in the secondary wastewater effluent were characterised by LC-OCD
254 and were separated into four main fractions – biopolymers (>20,000 Da), humics
255 (approximately 1,000 Da), building blocks (300–500 Da) and low molecular weight (LMW)
256 acids and neutrals (<350 Da) (Henderson et al., 2010; Huber et al., 2011) (**Table S4**). The
257 fraction identified as biopolymers can be polysaccharides and proteins, and the fraction of
258 building blocks includes breakdown products during the degradation of humic substances
259 (Huber et al., 2011). The secondary wastewater effluent contained a wide distribution of
260 organic fractions (**Fig. 3a**). The distribution of dissolved organic matter was biopolymers
261 (8%), humic substances (43%), building blocks (14%) and LMW neutrals (21%).

262 Biopolymers can be represented by model organic foulants including sodium alginate (i.e.
263 polysaccharide) and BSA (i.e. protein) (**Fig. 3b and 3c**), and humic substances can be
264 represented by humic acids (**Fig. 3d**) and fulvic acids (**Fig. 3e and 3f**), thus, these model
265 foulants were selected for further investigation of this study. In contrast, there were no model
266 foulants that were readily available for the other small organics including building blocks and
267 LMW neutrals.

268 3.3.2. EEM spectroscopy

269 The organics in the secondary wastewater effluent were also characterised by EEM
270 fluorescence spectroscopy. EEM peaks can be classified as protein-like, fulvic-like and
271 humic-like fluorophores. A strong peak in the EEM spectrum of the secondary wastewater
272 effluent was observed at the excitation/emission (Ex/Em) wavelengths of 350/425 nm which
273 was designated as C (Liu et al., 2011) in **Fig. 4a** and indicates a humic acid-like fluorophore
274 as suggested in the literature (Chen et al., 2003; Coble, 1996; Nam and Amy, 2008). Another
275 peak at the Ex/Em of 220/416-427 nm was designated as A in **Fig. 4a** indicating the presence
276 of fulvic acid-like fluorophore (Chen et al., 2003). It is noted that humic and fulvic acid-like
277 fluorophore could coexist in these EEM regions (i.e., A and C) and their presence cannot be
278 distinguished from each other (Rosario-Ortiz and Korak, 2017). Two other small peaks at the
279 Ex/Em of 220/325-334 nm (aromatic amino acid) and 270/310-320 nm (tryptophan, amino
280 acid) which were designated as T₁ and T₂ in **Fig. 4a**, respectively. The EEM spectroscopy
281 results (**Fig. 4a**) imply the presence of proteins and humic organics, which is consistent with
282 the findings attained through the LC-OCD chromatography (**Fig. 3a**).

283 Solutions of individual model foulants were also characterised using fluorescence
284 spectroscopy to compare to the organics in the secondary wastewater effluent. The EEM of
285 the sodium alginate solution revealed negligible peaks in the spectrum (**Fig. 4b**), which was

286 expected since polysaccharide-like substances do not contain molecular structure sensitive to
287 photon excitation. A peak of protein-like substance was identified with the BSA solution at
288 the Ex/Em of 265/325-350 nm and 223/334-348 nm which are designated as T₁ and T₂,
289 respectively (**Fig. 4c**). These peaks were also identified in the secondary wastewater effluent.
290 The EEM spectrum of the humic acid solution (**Fig. 4d**) revealed a peak at the Ex/Em of
291 225/415-435 nm (A₁) and 250/435-449 nm (A₂), and they were also identified at the
292 secondary wastewater effluent (**Fig. 4a**). The EEM spectrum of the fulvic acid solution (**Fig.**
293 **4e and 4f**) showed two peaks – a strong peak at the Ex/Em of 250/430-460 nm (A) and a
294 weak peak at the Ex/Em of 350/425 nm (C). This is consistent with a previous study (Chen et
295 al., 2003) where the same source of Suwannee River fulvic acid was examined. These two
296 peaks (A and C) observed in the fulvic acid solution were also identified in the secondary
297 wastewater effluent. The characterisation performed above indicate that the secondary
298 wastewater effluent contains humic acid- and fulvic acid-like substances as major sources of
299 fluorophores.

300 3.4. *N*-nitrosamine rejection by model foulants

301 Further examination using model foulants (i.e., sodium alginate, BSA, humic acid and two
302 fulvic acids) was conducted to identify fouling substances in the secondary effluent that
303 govern the variation in the permeation of *N*-nitrosamines. Overall, initial NDMA rejections
304 with the solutions containing one of the five model foulants (63–70%) were lower than the
305 initial NDMA rejection with the secondary effluent (76%). This indicates that the difference
306 in organic and inorganic constituents in the feed solution could affect the permeation of
307 NDMA through RO.

308 Membrane fouling with three model foulant (sodium alginate, BSA and humic acid) resulted
309 in negligible impact on the permeation of *N*-nitrosamines through the RO membrane (**Fig. 5**

310 **and S5**). Membrane fouling with sodium alginate decreased NDMA rejection from 70.3 to
311 59.5% despite the considerable increase in TMP from 1.6 ($t = 0$ min) to 2.7 MPa ($t = 45$ min)
312 (**Fig. 5a**). Likewise, sodium alginate fouling caused decreased rejections of NMEA, NPYR
313 and NMOR from 91.3 to 85.3%, from 95.9 to 93.3% and from 98.4 to 97.5%, respectively.
314 Similar observations were identified for membrane fouling with BSA and humic acid
315 solutions. Membrane fouling with BSA lead to a reduction in NDMA rejection from 64.0
316 (TMP = 1.6 MPa, $t = 0$ min) to 57.7% (TMP = 2.0 MPa, $t = 80$ min) (**Fig. 5b**). Membrane
317 fouling with humic acid caused a minor reduction of NDMA rejection from 62.9 (TMP = 1.7
318 MPa, $t = 0$ min) to 59.7% (TMP = 2.6 MPa, $t = 70$ min) (**Fig. 5c**).

319 In contrast, membrane fouling with fulvic acid solutions caused a slight increase in *N*-
320 nitrosamine rejection (**Fig. 6 and S6**). When the TMP increased from 1.64 ($t = 0$ min) to 1.78
321 MPa ($t = 360$ min) by the fouling development with Suwannee River fulvic acid solution,
322 NDMA rejection increased from 64.7 to 69.4% (**Fig. 6a**). In response to the fouling
323 development, the rejections of NMEA, NPYR and NMOR also increased from 88.9 to 91.2%,
324 from 93.6 to 95.3% and from 98.2 to 98.9%, respectively. Another fouling test with Pahokee
325 Peat fulvic acid solution also revealed the trend of increasing *N*-nitrosamine rejection;
326 NDMA rejection increased from 67.5 to 73.6% when the TMP increased from 1.6 ($t = 0$ min)
327 to 2.5 MPa ($t = 240$ min) (**Fig. 6b**). In conjunction with fulvic acid fouling development,
328 NMEA, NPYR and NMOR revealed increased rejection from 91.0 to 91.3%, from 95.4 to
329 95.8% and from 98.7 to 99.1%, respectively.

330 The trend of reducing the permeation of *N*-nitrosamines with a fouling layer of small
331 molecular weight foulants (i.e. fulvic acids) was also observed with the ESNA1-LF NF
332 membrane (**Fig. S7**). Membrane fouling with Pahokee Peat fulvic acid solution caused an
333 increase in *N*-nitrosamine rejection only after reaching as high TMP as those used for the

334 ESPA2 RO membrane. For example, NDMA remained almost zero for the increase in TMP
335 from 0.13 ($t = 0$ min) to 0.57 MPa ($t = 90$ min) but thereafter increased from 0.9 (TMP = 0.75
336 MPa, $t = 120$ min) to 5.8% (TMP = 1.85 MPa, $t = 155$ min) (**Fig. S7a**). The rejection of the
337 other *N*-nitrosamines also increased from 4 to 10–11% for the TMP increase from 0.75 to
338 1.85 MPa. In contrast, only negligible increase in NDMA rejection by up to 2% occurred
339 with membrane fouling caused by a solution containing a larger model foulant – humic acid –
340 even after reaching the high TMP (i.e. >1.5 MPa) at 40 min (**Fig. S8**). Considering that the
341 ESNA1-LF membrane itself has almost no *N*-nitrosamine rejection capacity, the mechanism
342 behind the increased rejection with fulvic acid can be hypothesized that the fouling layer of
343 the small molecular weight fulvic acid foulants can function as an additional barrier of *N*-
344 nitrosamine transport to the membrane. Another plausible mechanism is the restriction of
345 permeation pathway of *N*-nitrosamine in the membrane structure by these small foulants
346 (Steinle-Darling et al., 2010), resulting in less permeation through the RO membrane.

347 3.5. Proposed mechanisms

348 The compounds with uncharged and hydrophilic properties including *N*-nitrosamines are
349 essentially rejected by size exclusion as previously suggested in the literature (Bellona et al.,
350 2004; Fujioka et al., 2012b). Size exclusion in RO treatment is based on the relationship
351 between compound size and the size of pathway within the RO membrane (e.g. free-volume
352 holes) (Fujioka et al., 2013b). As a result, the main focus of the impact of fouling substances
353 on the permeation of *N*-nitrosamines is on the size of pathway inside the fouling layer formed
354 on the RO membrane surface and the size of the internal pathway of the RO membrane.

355 The formation of the fouling layer with large molecular weight model foulants (sodium
356 alginate, BSA and humic acid) resulted in a negligible decrease in *N*-nitrosamines rejection
357 (**Fig. 6a-c**). Considering that fouling of the RO membranes progresses with cake layer

358 formation, the fouling layer is sufficiently porous such that *N*-nitrosamines can readily
359 permeate from the bulk solution through the fouling layer and to the membrane surface,
360 which could explain the negligible impact on the permeation of *N*-nitrosamines.

361 In contrast to the effects of high molecular weight model foulants, membrane fouling with the
362 secondary wastewater effluent (containing a diverse range of molecular weight organics, **Fig.**
363 **3**) led to decreased permeation of *N*-nitrosamines (**Fig. 2**). It is important to note that similar
364 observations were also identified with the low molecular weight model foulants (i.e. fulvic
365 acids) in this study (**Fig. 6**). The secondary wastewater effluent and fulvic acid solutions both
366 contain fractions of low molecular weight organics (**Fig. 3**). Thus, these organics can form a
367 densely packed cake layer that functions as an additional sieving barrier (Ang et al., 2011) or
368 can obstruct the pathway of solutes (Steinle-Darling et al., 2010). Thus, it can be suggested
369 that low molecular weight organics in the secondary effluent allow less solutes to permeate
370 through RO membranes, leading to the enhanced rejection of *N*-nitrosamines. The results also
371 suggest that the identification of fractions of low molecular weight organics using LC-OCD
372 technique could allow for changes in the permeation of *N*-nitrosamines during long-term
373 plant operation.

374 **4. Conclusions**

375 A high throughput HPLC-PR-CL analytical technique was used to examine the correlation
376 between the type of foulant and *N*-nitrosamine rejection by an RO membrane. Membrane
377 fouling with a secondary wastewater effluent led to a decrease in the permeation of NDMA
378 and the other *N*-nitrosamines (i.e. NMEA, NPYR and NMOR), although the membrane
379 fouling (accelerated at a high permeate flux) only provided a trend of *N*-nitrosamine rejection
380 during fouling development. Examination by LC-OCD chromatography revealed that the
381 major constituents in the secondary wastewater effluent were biopolymers (e.g.

382 polysaccharides and proteins) and humic substances (e.g. humic acid and fulvic acid). Further
383 investigation with fluorescence spectrometry also identified humic acid-like organics, fulvic
384 acid-like organics and proteins. Thus, the effects of membrane fouling on *N*-nitrosamine
385 rejection were also evaluated using solutions of these compounds as model foulants.
386 Membrane fouling with these model foulant solutions with the exception of fulvic acids
387 generally resulted in a negligible impact on the permeation of *N*-nitrosamines. In contrast,
388 membrane fouling with fulvic acids led to a notable decrease in the permeation of *N*-
389 nitrosamines, which was similar to that observed with the secondary wastewater effluent.
390 Secondary wastewater effluent and fulvic acid solutions contain low molecular weight
391 organics, thus, can form a densely packed fouling layer formed on the RO membrane surface
392 or can obstruct the pathway of solutes in the RO membrane structure. They can reduce the
393 permeation of *N*-nitrosamines through RO membranes. The results indicate that specific
394 foulants in reclaimed wastewater (e.g. fulvic acid-like substances) could play an important
395 role in the variation of *N*-nitrosamine rejection over long-term RO system operation. Future
396 work is necessary to isolate individual organic fractions from reclaimed wastewater to
397 identify substances influencing *N*-nitrosamine rejection.

398 **5. Acknowledgements**

399 We thank Hydranautics/Nitto for providing NF and RO membrane samples for this
400 investigation. We also thank Organo Corporation for their assistance of LC-OCD analysis.

401 **6. References**

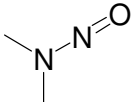
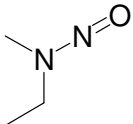
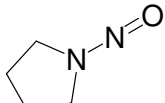
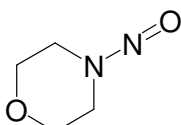
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521 **Table 1** Physicochemical properties of the selected *N*-nitrosamines.

Compound	Structure	Molecular formula	Molecular weight [Da]	Low <i>D</i> at pH 8 ^a	pK _a ^a
NDMA		C ₂ H ₆ N ₂ O	74.1	0.04	3.5
NMEA		C ₂ H ₈ N ₂ O	88.1	0.40	3.4
NPYR		C ₄ H ₈ N ₂ O	100.1	0.44	3.3
NMOR		C ₄ H ₈ N ₂ O ₂	116.1	-0.18	3.1

522 ^a Chemicalize (<http://www.chemicalize.org>).

523 **LIST OF FIGURES**

524 **Fig. 1** – Analysis of *N*-nitrosamine concentrations in the secondary wastewater effluent using
525 the HPLC-PR-CL analysis with sample injection volume of (a) 200 μL and (b) 20 μL .

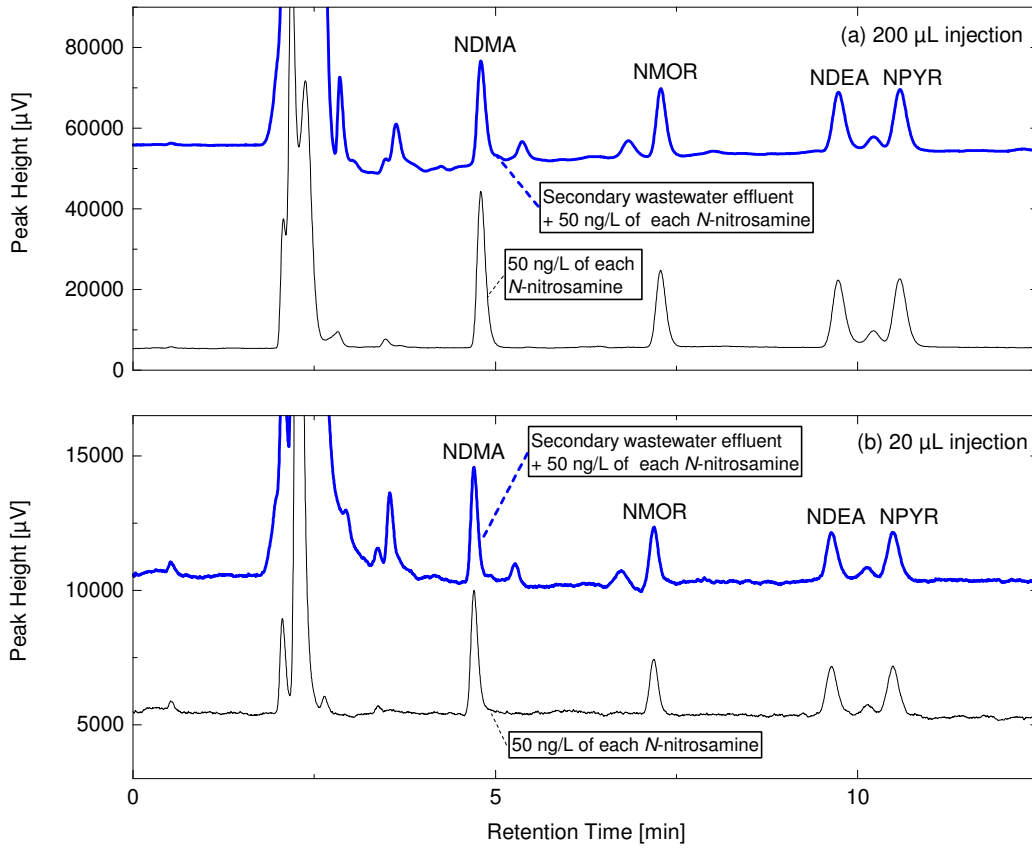
526 **Fig. 2** – Changes in *N*-nitrosamine rejection and TMP during RO treatment of the secondary
527 wastewater effluent with ESPA2 membrane (permeate flux = 80 $\text{L}/\text{m}^2\text{h}$, feed solution
528 temperature = 20 $^\circ\text{C}$, pH = 8). Values here are the average and range of duplicate results.

529 **Fig. 3** – LC-OCD chromatogram of the (a) secondary wastewater effluent and solutions
530 containing (b) sodium alginate, (c) BSA, (d) humic acid, (e) Suwannee River fulvic acid and
531 (f) Pahokee Peat fulvic acid.

532 **Fig. 4** – EEM fluorescence spectrum of (a) secondary effluent, solutions containing (b)
533 sodium alginate, (c) BSA, (d) humic acid, (e) Suwannee River fulvic acid and (f) Pahokee
534 Peat fulvic acid.

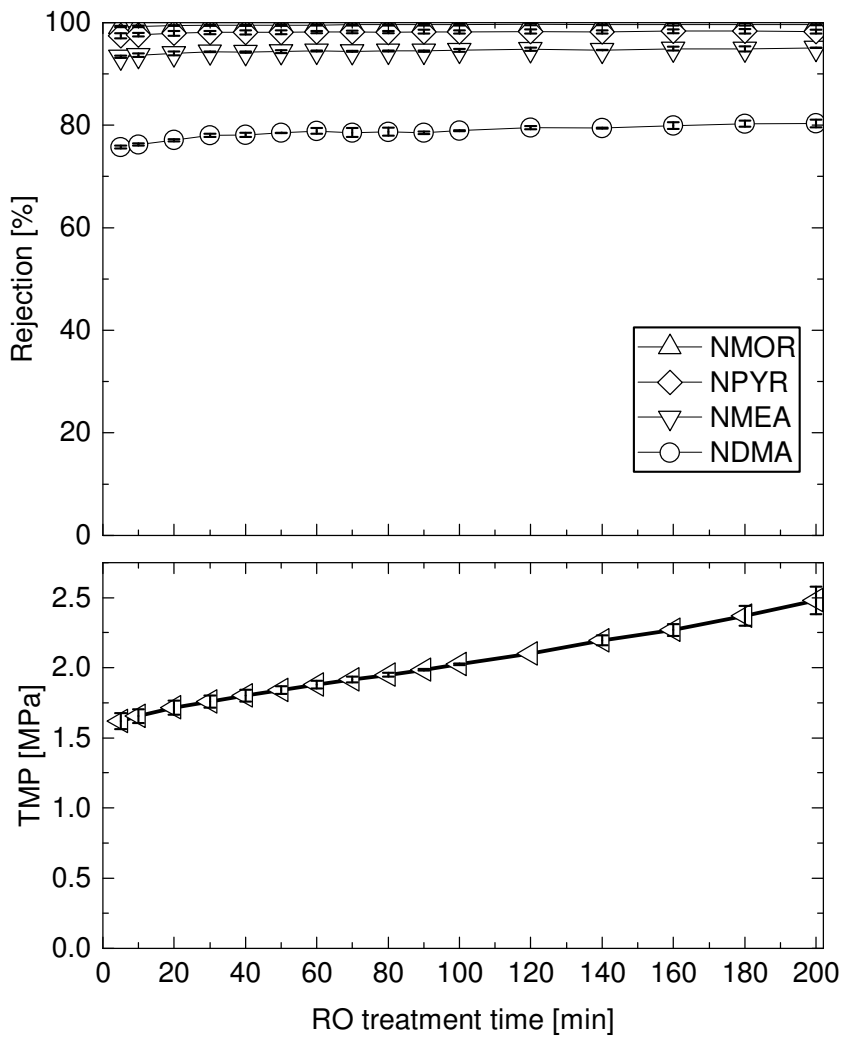
535 **Fig. 5** – Changes in *N*-nitrosamine rejection and TMP during RO treatment of solutions
536 containing 50 mg/L of (a) sodium alginate, (b) BSA and (c) humic acid with ESPA2
537 membrane (20 mM NaCl, 1 mM NaHCO_3 , 1 mM CaCl_2 , feed temperature = 20.0 ± 0.1 $^\circ\text{C}$,
538 permeate flux = 80 $\text{L}/\text{m}^2\text{h}$).

539 **Fig. 6** – Changes in *N*-nitrosamine rejection and TMP during RO treatment of solutions
540 containing 30 mg/L of (a) Suwannee River fulvic acid and (b) Pahokee Peat fulvic acid with
541 ESPA2 membrane (20 mM NaCl, 1 mM NaHCO_3 , 1 mM CaCl_2 , feed temperature = $20.0 \pm$
542 0.1 $^\circ\text{C}$, permeate flux = 80 $\text{L}/\text{m}^2\text{h}$).



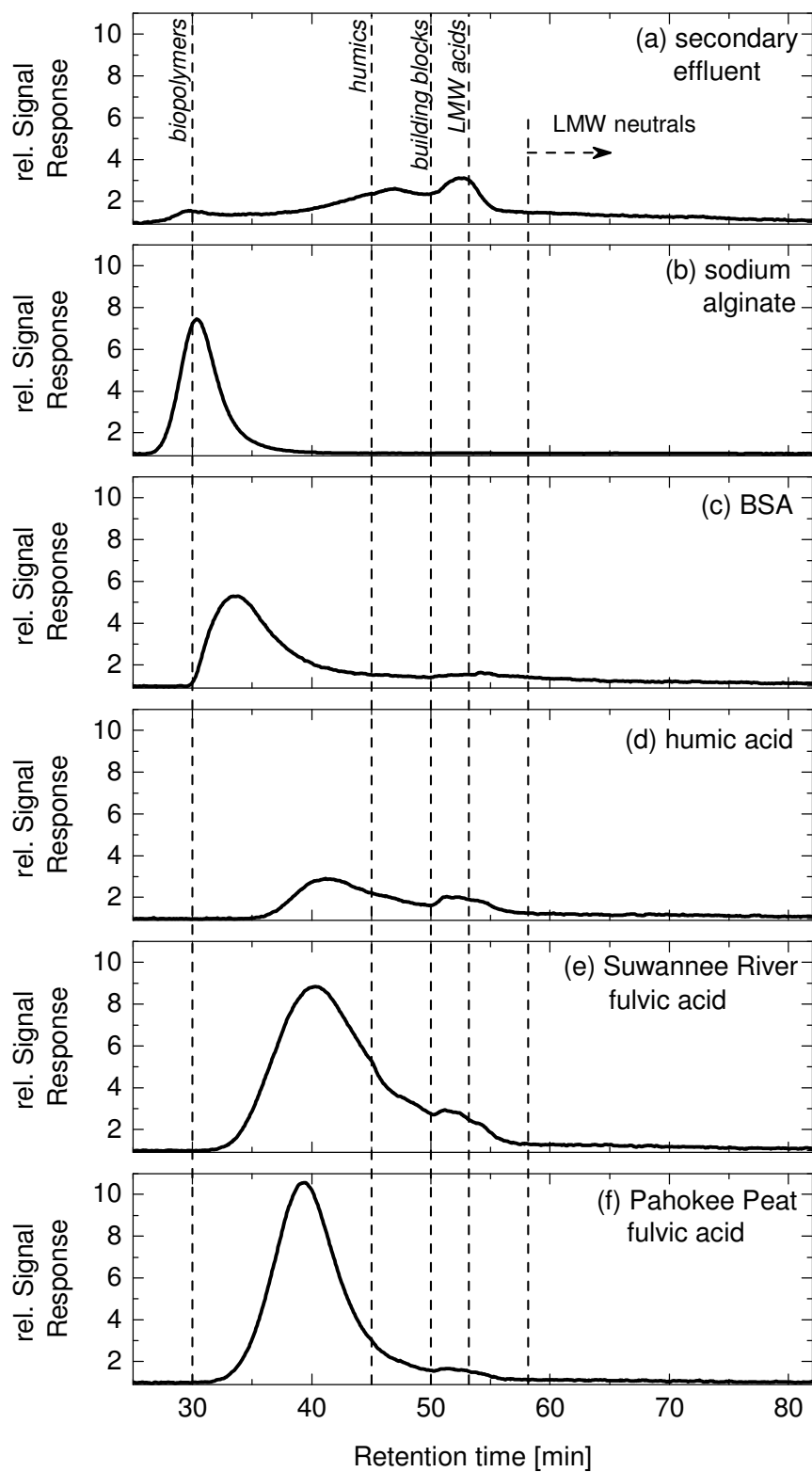
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544 **Fig. 1**



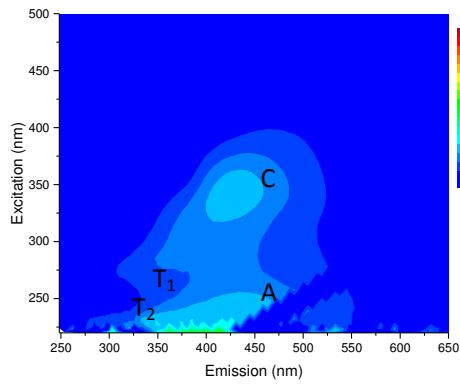
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546 **Fig. 2**

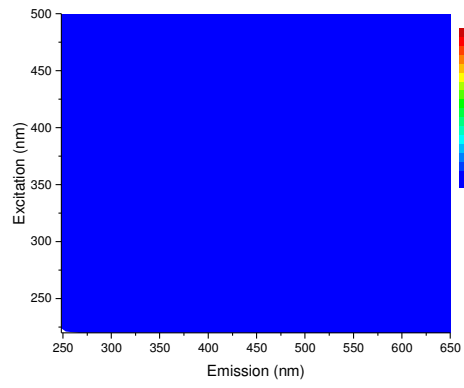


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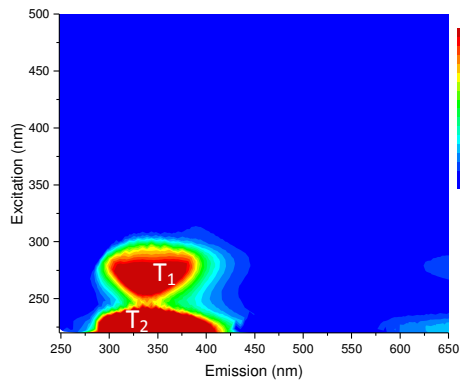
548 **Fig. 3**



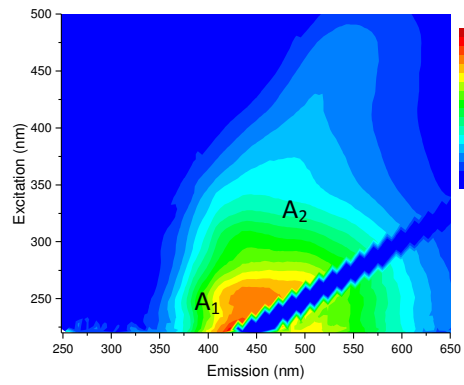
(a) secondary effluent



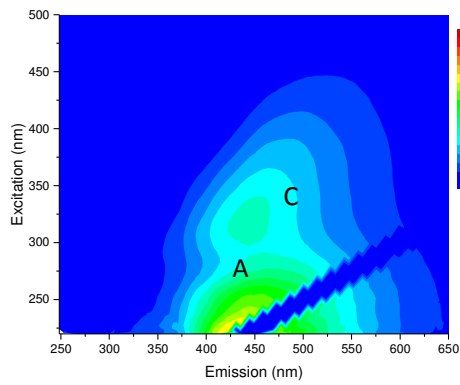
(b) sodium alginate



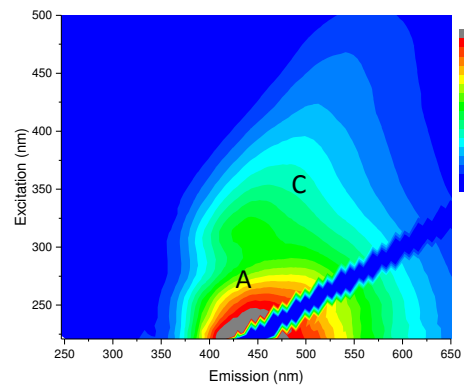
(c) BSA



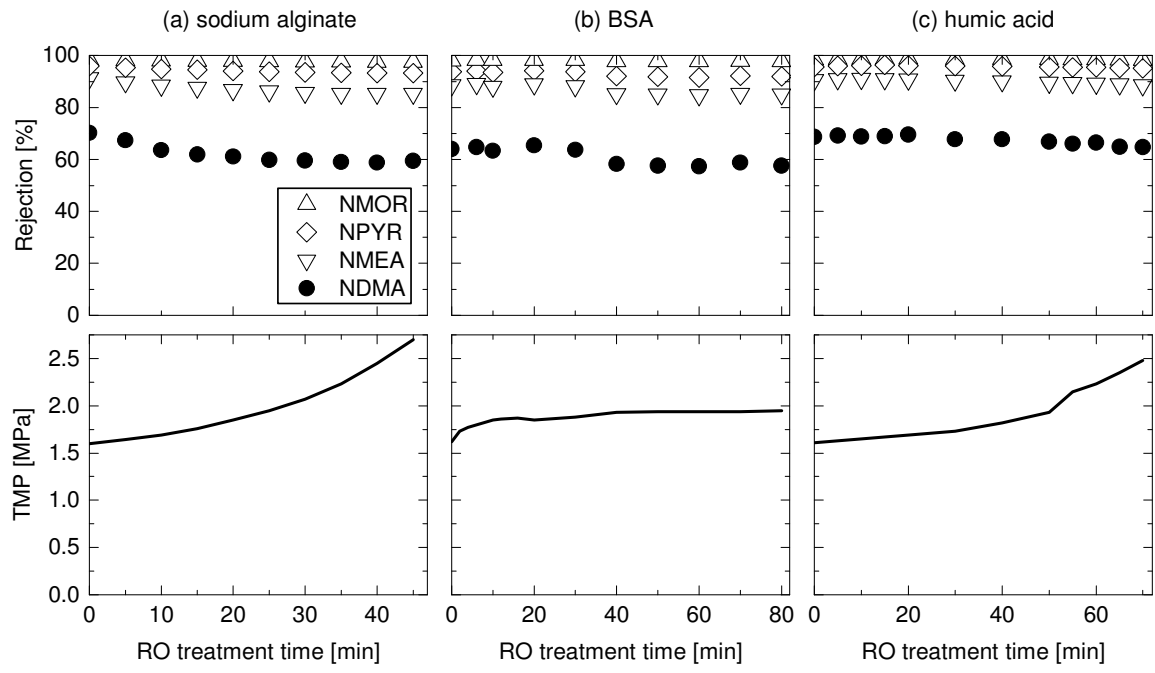
(d) humic acid



(e) Suwannee River fulvic acid

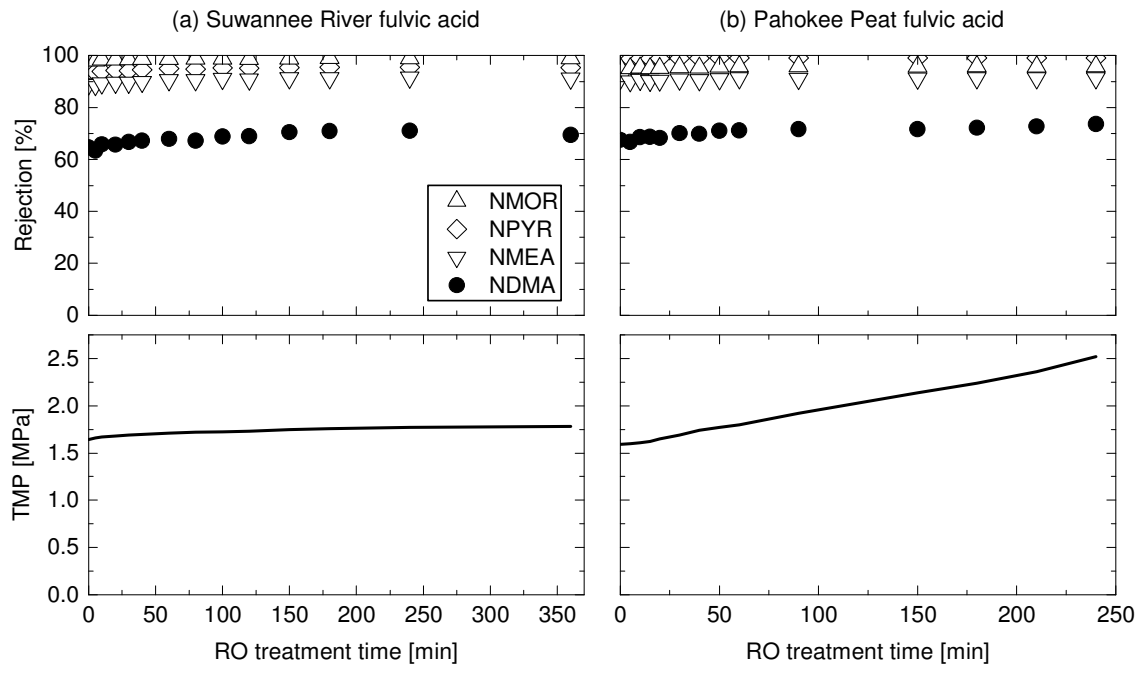


(f) Pahokee Peat fulvic acid



550

551 **Fig. 5**



552

553 **Fig. 6**

1 **Role of membrane fouling substances on the rejection of**
2 ***N*-nitrosamines by reverse osmosis**

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17 **SUPPLEMENTARY MATERIAL**

18 _____
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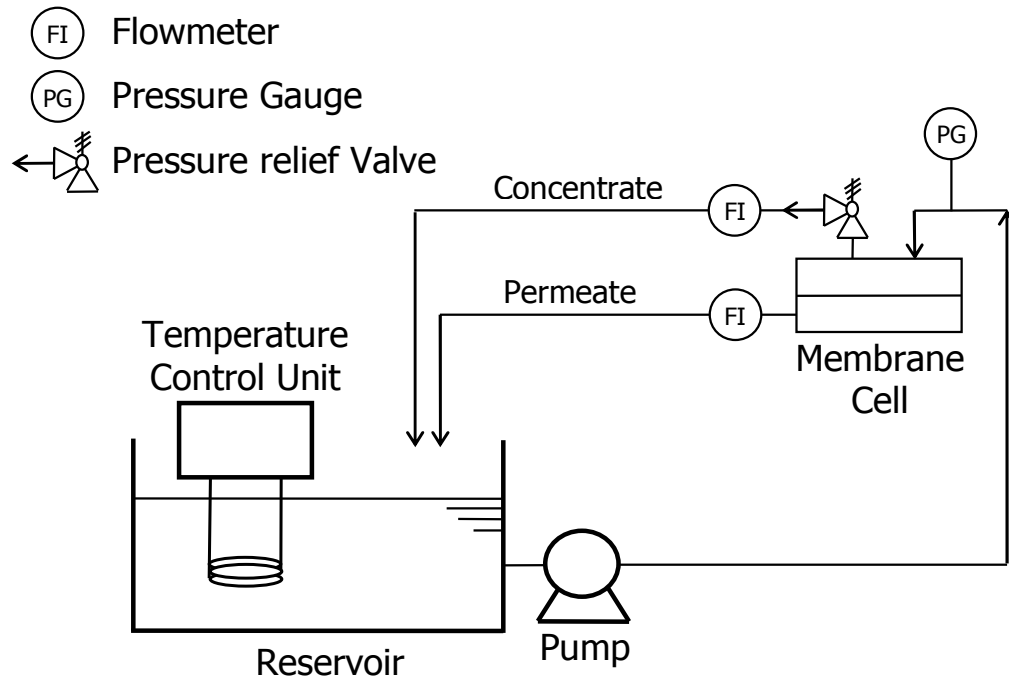


Fig. S1 – Schematic diagram of the cross-flow RO filtration system.

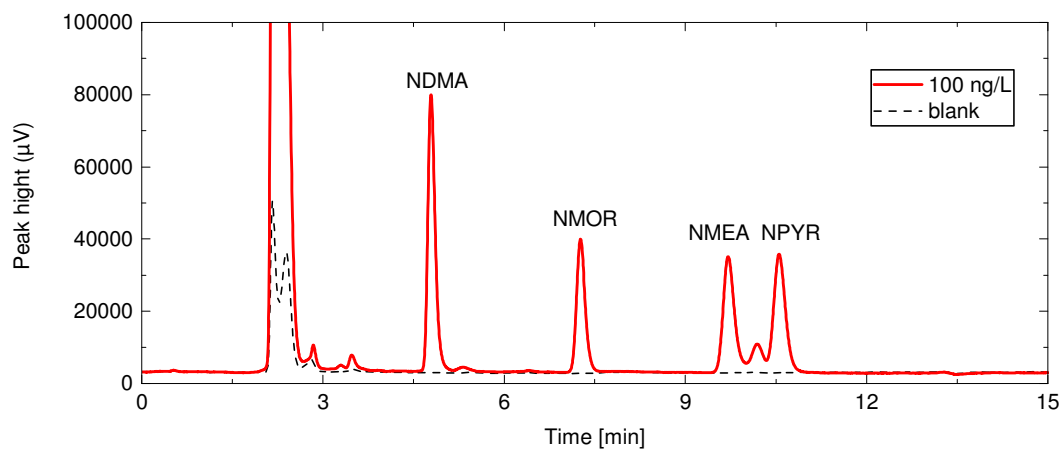


Fig. S2 – HPLC-PR-CL chromatogram of NDMA, NMOR, NMEA and NPYR.

Table S3 – HPLC-PR-CL peak height and recovery for each *N*-nitrosamine. *N*-nitrosamines were not detected in the secondary wastewater effluent sample prior to spiking of NDMA into the wastewater samples.

Chemical	Injection volume [μ L]	Peak height of 50 ng/L dose [μ V]		Recovery [%]
		Secondary wastewater effluent	Clean water matrix	
NDMA	200	25526	38820	66
	20	4352	4537	96
NMEA	200	14545	16709	87
	20	1802	1847	98
NPYR	200	15297	16994	90
	20	1827	1911	96
NMOR	200	16733	19026	88
	20	2163	2049	106

Table S4 – Description of LC-OCD fractions.

Fraction	Molecular weight range	Properties	Description
Biopolymers	> 20,000 Da	Hydrophilic, Not UV-absorbable	Polysaccharides and proteins
Humics	~1,000 Da	Hydrophobic, UV-absorbable	Calibration based on humic acid and fulvic acids of Suwannee River standards from IHSS.
Building blocks	350–500 Da	UV-absorbable	Breakdown products of humics.
Low molecular weight acids	< 350 Da	Negatively charged at neutral pH, Aliphatic	All aliphatic low molecular mass organic acids, Small amount of humics
Low molecular weight neutrals	< 350 Da	Weakly charged, hydrophilic or slightly hydrophobic (i.e. amphiphilic)	Alcohols, aldehydes, ketones, amino acids, biogenic organic matter

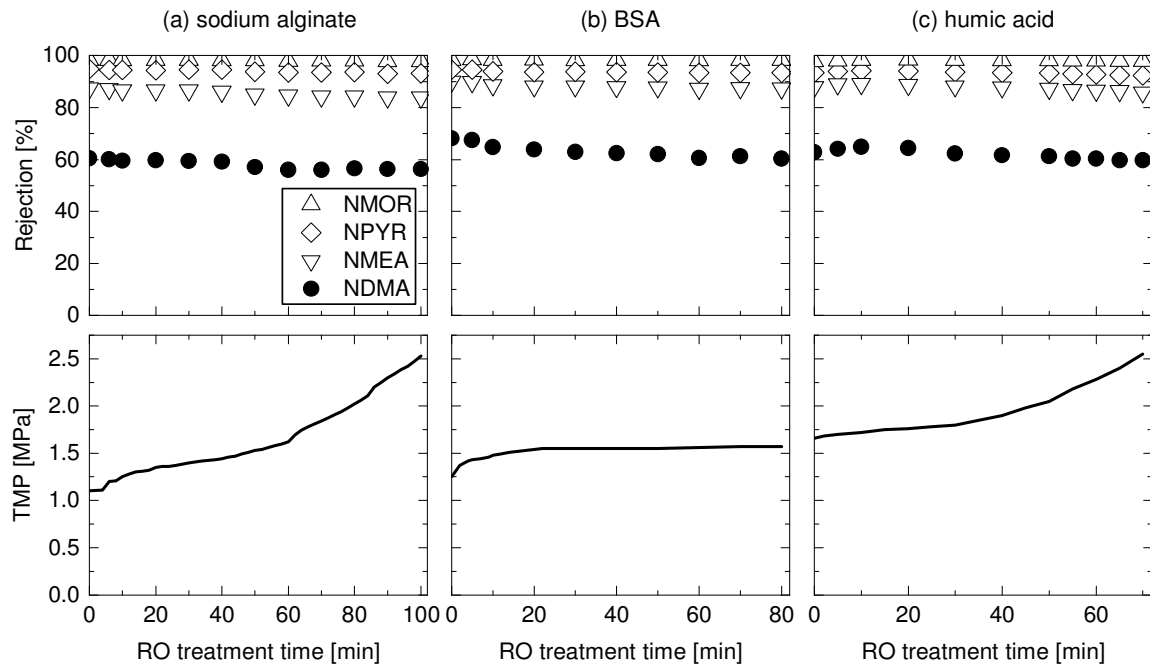


Fig. S5 – Changes in *N*-nitrosamine rejection and TMP during RO treatment of solutions containing (a) 50 mg/L of sodium alginate, (b) 100 mg/L of BSA and (c) 50 mg/L of humic acid with ESPA2 membrane (20 mM NaCl, 1 mM NaHCO₃, 1 mM CaCl₂, feed temperature = 20.0 ± 0.1 °C, permeate flux = (a)–(b) 60 L/m²h and (c) 80 L/m²h).

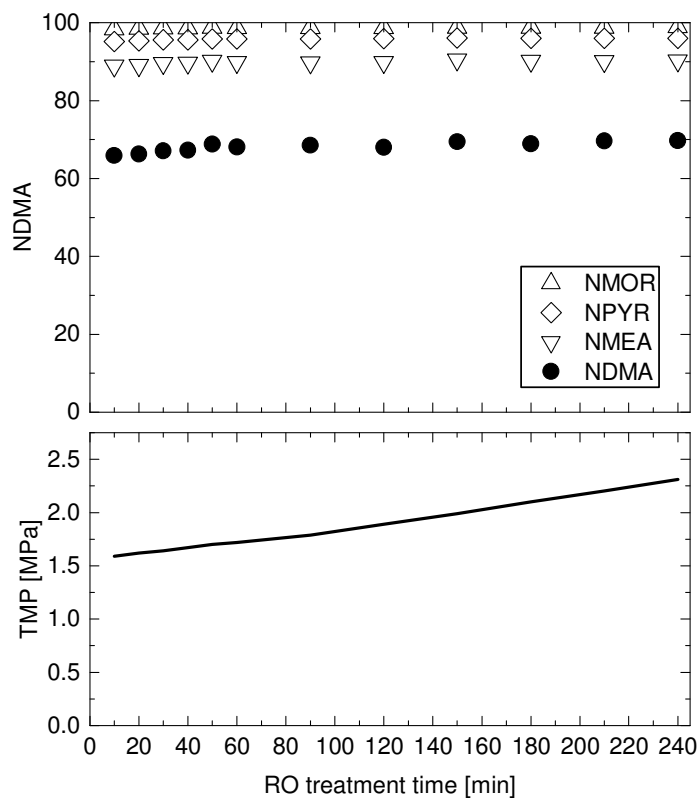


Fig. S6 – Changes in *N*-nitrosamine rejection and TMP during RO treatment of solutions containing 20 mg/L of Pahokee Peat fulvic acid with ESPA2 membrane (20 mM NaCl, 1 mM NaHCO₃, 1 mM CaCl₂, feed temperature = 20.0 ± 0.1 °C, permeate flux = 80 L/m²h).

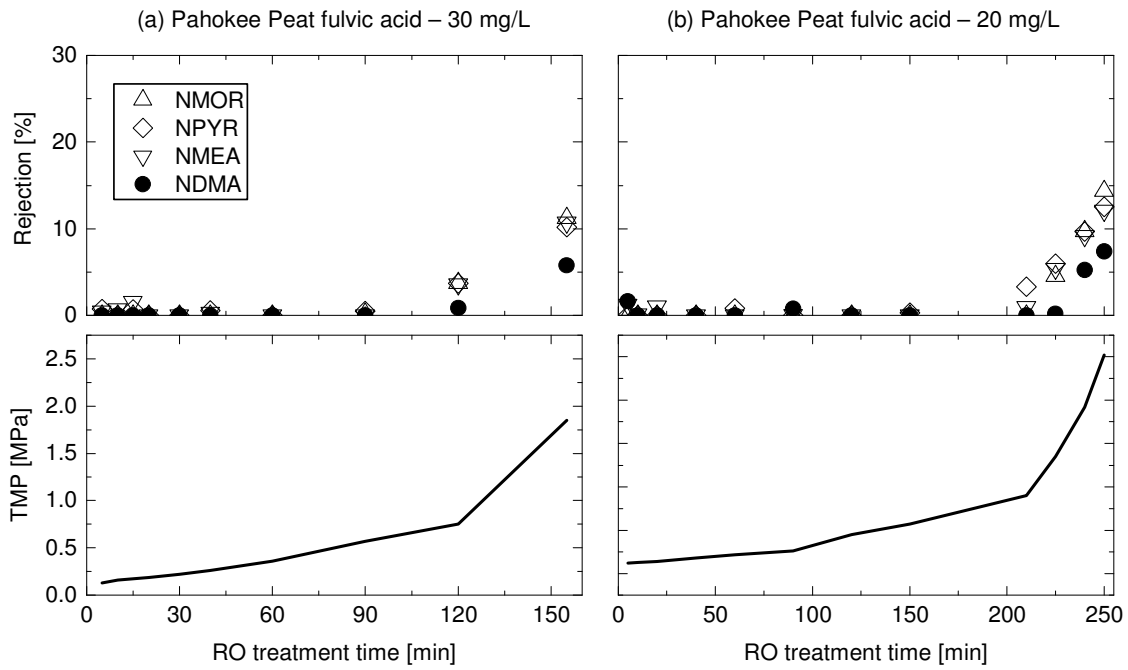


Fig. S7 – Changes in *N*-nitrosamine rejection and TMP during NF treatment of solutions containing (a) 30 mg/L and (b) 20 mg/L of Pahokee Peat fulvic acid with ESPA1-LF membrane (20 mM NaCl, 1 mM NaHCO₃, 1 mM CaCl₂, feed temperature = 20.0 ± 0.1 °C, permeate flux = 120 L/m²h).

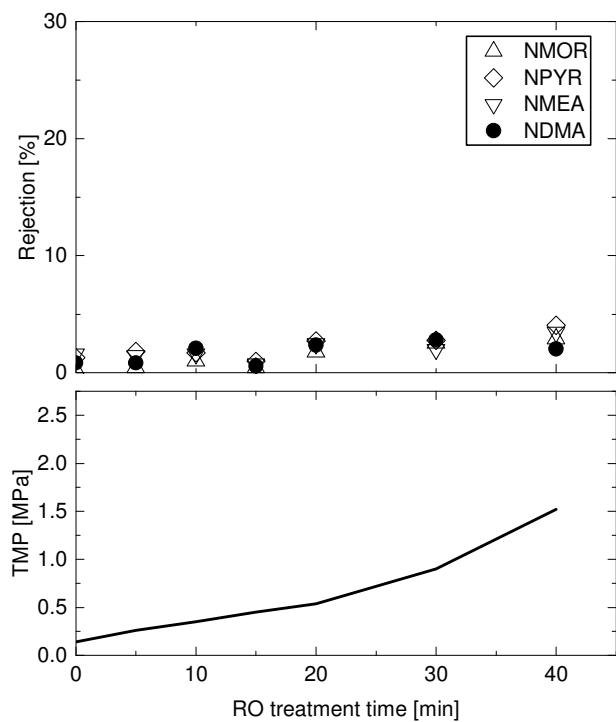


Fig. S8 – Changes in *N*-nitrosamine rejection and TMP during NF treatment of solutions containing 50 mg/L of humic acid with ESPA1-LF membrane (20 mM NaCl, 1 mM NaHCO₃, 1 mM CaCl₂, feed temperature = 20.0 ± 0.1 °C, permeate flux = 120 L/m²h).