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

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Abstract

The impact of fouling substances on the rejection of four *N*-nitrosamines by a reverse osmosis (RO) membrane was evaluated via a systematic characterisation of individual organic fractions in a secondary wastewater effluent and the deployment of 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. The decrease in *N*-nitrosamine permeation became insignificant after membrane permeability decreased by approximately 30%. 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 an increase in *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.

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

1. Introduction

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

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

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

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

In a well-controlled laboratory-scale study to evaluate the effects of membrane fouling on *N*-nitrosamine rejection, bench-scale RO systems have the advantage of precise regulation of the operating conditions. However, sample volumes required for their analysis can be excessive.

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

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

This work aimed to identify major foulants that influence *N*-nitrosamine rejection by an RO membrane. A nanofiltration (NF) membrane was also used for comparison. The HPLC-PR-CL analytical technique was modified for the determination of *N*-nitrosamines in the secondary wastewater effluent and model foulant solutions, and was used to systematically examine the correlation between fouling development and *N*-nitrosamine rejection. Consequently, five model foulants were selected and four *N*-nitrosamines, including NDMA, were selected for

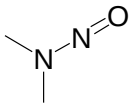
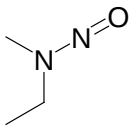
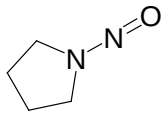
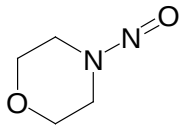
delineation of the mechanisms underlying the impact of membrane fouling on *N*-nitrosamine rejection.

2. Materials and methods

2.1. Chemicals

Four analytical grade *N*-nitrosamines (Ultra Scientific, Kingstown, RI, USA) were used in this study: NDMA, *N*-nitrosomethylethylamine (NMEA), *N*-nitrosopyrrolidine (NPYR) and *N*-nitrosomorpholine (NMOR) **(Table 1)**. A stock solution containing all four *N*-nitrosamines was prepared at 1 µg/mL of each compound in pure methanol. Five model foulants – sodium alginate, bovine serum albumin (BSA), humic acid and two fulvic acids – were also used. Sodium alginate and humic acids were supplied by Sigma-Aldrich (St Louis, MO, USA). BSA was purchased from Wako Pure Chemical Industries (Tokyo, Japan). Suwannee River fulvic acid standard II and Pahokee Peat fulvic acid standard II were purchased from International Humic Substances Society (IHSS, MN, USA). Analytical grade NaCl, $CaCl_2$, $NaHCO_3$ and luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) were supplied from Wako Pure Chemical Industries (Tokyo, Japan). Secondary wastewater effluent was collected from a municipal wastewater treatment plant (WWTP) in Japan. The sampling point was before chlorine disinfection and after screening, primary settling and activated sludge treatment.

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

Compound	Structure	Molecular formula	Molecular weight [Da]	Low *D* at pH 8[a]	pK_a[a]
NDMA		$C_2H_6N_2O$	74.1	0.04	3.5
NMEA		$C_2H_8N_2O$	88.1	0.40	3.4
NPYR		$C_4H_8N_2O$	100.1	0.44	3.3
NMOR		$C_4H_8N_2O_2$	116.1	-0.18	3.1

[a] Chemicalize (http://www.chemicalize.org).

2.2. Membrane treatment system

A low pressure RO membrane – ESPA2 – was supplied as flat sheet samples by Nitto/Hydranautics (Osaka, Japan). The ESPA2 membrane is a composite polyamide RO membrane that has been used widely in water reclamation applications (Fujioka et al., 2012a). An NF membrane – ESNA1-LF – from Nitto/Hydranautics (Osaka, Japan) was also used in this study. A bench-scale RO system with a cross-flow configuration was used (**Fig. S1**). The treatment system includes a stainless steel membrane cell (Iwai Pharma Tech, Tokyo, Japan) that can hold a circular flat sheet membrane coupon with effective surface area of 36.3 cm^2. A high-pressure pump (KP-12, FLOM, Tokyo, Japan) was also used to transport feed solution from a 2-L glass reservoir to the membrane cell. The feed solution temperature was controlled in the reservoir with a stainless steel heat exchanging coil connected to a temperature control unit (NCB-500, Tokyo Rikakikai, Tokyo, Japan).

2.3. *Experimental protocols*

Each experiment was initiated by conditioning the RO membranes with deionized water (Q 18.0 MΩcm) at 1,500 kPa until the permeate flux stabilised. The deionized water was then replaced with 2 L of the secondary wastewater effluent or solutions of model foulant. The model foulant solutions contained background electrolytes (20 mM NaCl, 1 mM $NaHCO_3$, 1 mM $CaCl_2$) and 30–50 mg/L of one of the model foulants in Milli-Q water. Each *N*-nitrosamine was spiked into the RO feed at a concentration of 500 ng/L. The RO treatment system was operated at constant flux of 60 or 80 L/m^2h. During each experiment, both RO feed and permeate were recirculated into the feed reservoir to maintain a constant concentration of each solute and foulant in the RO feed. While full-scale RO systems in water reclamation applications are typically designed and operated at the permeate flux of ~20 L/m^2h (Fujioka et al., 2012a), the high flux was used in this study to accelerate membrane fouling. The feed temperature was maintained at 20 °C and transmembrane pressure (TMP) was recorded. RO feed and permeate samples were collected periodically in amber vials (1.5 mL). Concentrations of *N*-nitrosamines in the RO feed and permeate samples were used for calculating their rejections. The RO permeate and feed sample volumes were negligible (i.e. 1.5 mL) as compared to 2 L of the initial feed volume; thus, *N*-nitrosamine concentration in the RO feed was expected to be constant throughout the experiment. In addition, a previous study (Fujioka et al., 2012b) has confirmed that changes in *N*-nitrosamine concentrations from 250 to 1,500 ng/L had no impact on the rejection of *N*-nitrosamines. Overall, the experimental condition of this study allowed for an accurate evaluation of *N*-nitrosamine rejections without any interference from changes in their concentrations in the RO feed.

2.4. Analytical techniques

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

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

2.4.2. Fluorescence spectroscopy

Excitation emission matrix (EEM) fluorescence spectra (Aqualog, Horiba, Kyoto, Japan) of the samples were obtained using a 1-cm quartz cuvette. The EEM spectra (EEMs) were acquired with scanning emission spectra every 8 pixels from 245.21 to 827.61 nm by changing the excitation wavelength from 220 to 800 nm at 1 nm step with a 4.60 nm CCD bin increment at low gain and 1 s integration. All EEMs were corrected through blank subtraction (ultrapure water – 18.2 MΩcm with 1 g/L methanol and humic acid) to reduce scatter from the water Raman peak for instrument/spectral biases according to the emission and excitation correction factors provided by the manufacturer.

2.4.3. Size exclusion chromatography

Organic carbon content in the water samples were characterised by a liquid chromatography-organic carbon detection (LC-OCD) system (DOC-LABOR, Karlsruhe, Germany). Details of the analysis can be found in previous published studies (Henderson et al., 2011; Huber et al., 2011). The analysis was performed at 1.1 mL/min flow rate with a mobile phase of phosphate buffer, 2.5 g/L KH_2PO_4 and 1.2g/L Na_2HPO_4. Samples was diluted 1:10 in Milli-Q water and a volume of 2.0 mL of the sample was injected into the LC-OCD system.

3. Results and discussion

3.1. Analysis in a secondary wastewater effluent

The analysis of *N*-nitrosamines in the secondary wastewater effluent using HPLC-PR-CL was validated through spike testing. Each *N*-nitrosamine was spiked into the secondary wastewater effluent at a concentration of 50 ng/L for analyte recovery evaluation. Recovery was calculated based with the ratio of the peak height of *N*-nitrosamine in the secondary wastewater effluent to the peak height of *N*-nitrosamine in the pure water matrix. With the injected sample volume of 200 µL, the peak height of NDMA at the retention time (*rt*) of 6.1 min **(Fig. 1a)** revealed 66% recovery relative to the pure water matrix. Recovery in the range of 87 and 90% was observed for all other *N*-nitrosamines **(Table S3)**. Impurities in the secondary effluent could interfere with photochemical and/or chemiluminescence reaction, leading to the low recovery observed here when a large injection volume was used. The observed decreasing peak heights of *N*-nitrosamines were attributed to the reduction of baseline chemiluminescence after 3 min as compared to the initial baseline chemiluminescence. The impact was particularly strong around the NDMA peak (*rt* = 6.1 min) and gradually recovered to the original baseline as shown in **Fig. 1a**. Because the baseline chemiluminescence is generated from the reaction of

the eluent, the reduction of baseline chemiluminescence after the sample injection substances in the secondary wastewater effluent could have interfered with the photochemical reaction and/or chemiluminescence reaction. Accordingly, the peak heights of *N*-nitrosamines may also have reduced by the interference.

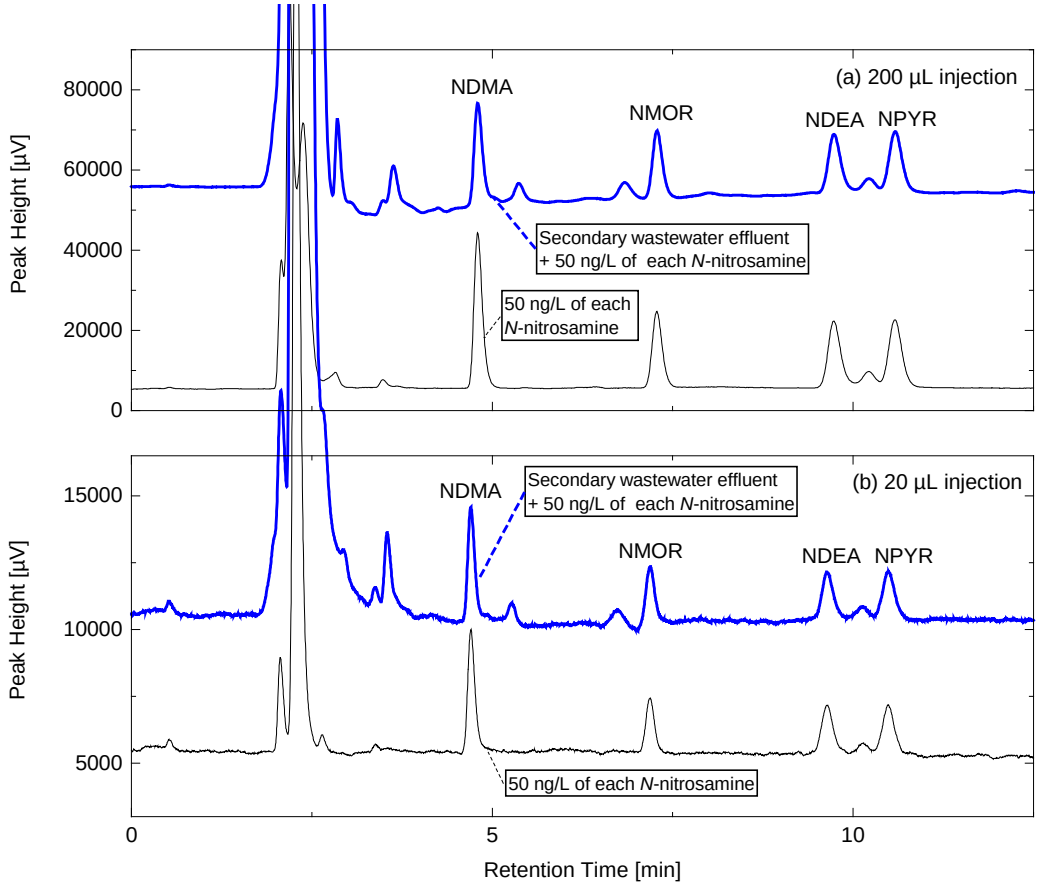


Fig. 1 – Analysis of *N*-nitrosamine concentrations in the secondary wastewater effluent using the HPLC-PR-CL analysis with sample injection volume of (a) 200 µL and (b) 20 µL.

To reduce the presence of interfering substances, the sample injection volume was reduced from 200 to 20 µL, which was successfully validated for NDMA in ultrafiltration-treated wastewater in a previous study (Fujioka et al., 2016). With the smaller injection volume, the chemiluminescence around the four *N*-nitrosamine peaks dropped to an intensity near the initial baseline (*rt* = 0–2 min) **(Fig. 1b)**. As a result, recovery of NDMA improved from 66% (injection volume = 200 µL) to 96% (injection volume = 20 µL). Similarly, the other *N*-nitrosamines generally revealed improved recoveries (96–106%) **(Table S3)**. The method

detection limits (MDLs) for NDMA, NMEA, NPYR and NMOR in the secondary wastewater effluent were 1.8, 3.7, 3.3 and 2.3 ng/L, respectively.

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

The fouling propensity of the ESPA2 RO membrane was identified for the secondary wastewater effluent. Fouling development using the ESPA2 RO membrane with the secondary wastewater effluent led to an increase in the rejection of all four *N*-nitrosamines investigated **(Fig. 2)**. In particular, NDMA rejection increased from 75.7 (t = 5 min) to 80.0% (t = 200 min) with an increase in TMP from 1.6 to 2.5 MPa (approximately 30% increase in TMP). Similar observations could be made with the other *N*-nitrosamines, although the increase in their rejection was less significant compared to NDMA **(Fig. 2)**. In response to the fouling development from 5 to 200 min, the rejections of NMEA, NPYR and NMOR also increased from 93.3 to 95.1%, from 97.5 to 98.2% and from 99.2 to 99.6%, respectively.

The results suggest that membrane fouling at full-scale applications can lead to a gradual decrease in the permeation of NDMA, meaning that the prolonged operation could result in an increase in NDMA rejection. It should be noted that the accelerated membrane fouling protocol applied here could only show the behaviour of NDMA rejection during fouling development and the rejection values do not directly simulate the actual impact of fouling in full scale.

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

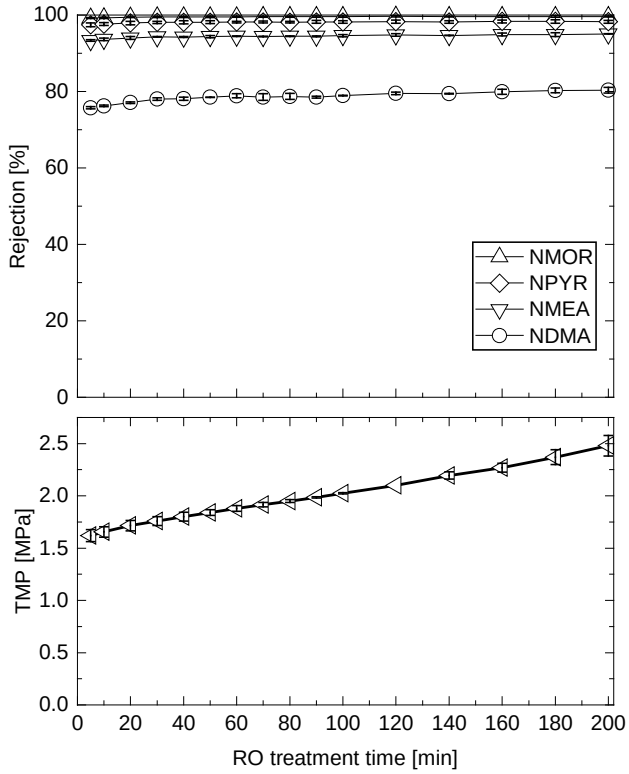


Fig. 2 – Changes in *N*-nitrosamine rejection and TMP during RO treatment of the secondary wastewater effluent with ESPA2 membrane (permeate flux = 80 L/m^{2}h, feed solution temperature = 20 °C, pH = 8). Values here are the average and range of duplicate results.

3.3. Characterisation of organics in the RO feed

3.3.1. LC-OCD

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

building blocks (14%) and LMW neutrals (21%). Biopolymers can be represented by model organic foulants including sodium alginate (i.e. polysaccharide) and BSA (i.e. protein) **(Fig. 3b and 3c)**, and humic substances can be represented by humic acids **(Fig. 3d)** and fulvic acids **(Fig. 3e and 3f)**, thus, these model foulants were selected for further investigation of this study. In contrast, there were no model foulants that were readily available for the other small organics including building blocks and LMW neutrals.

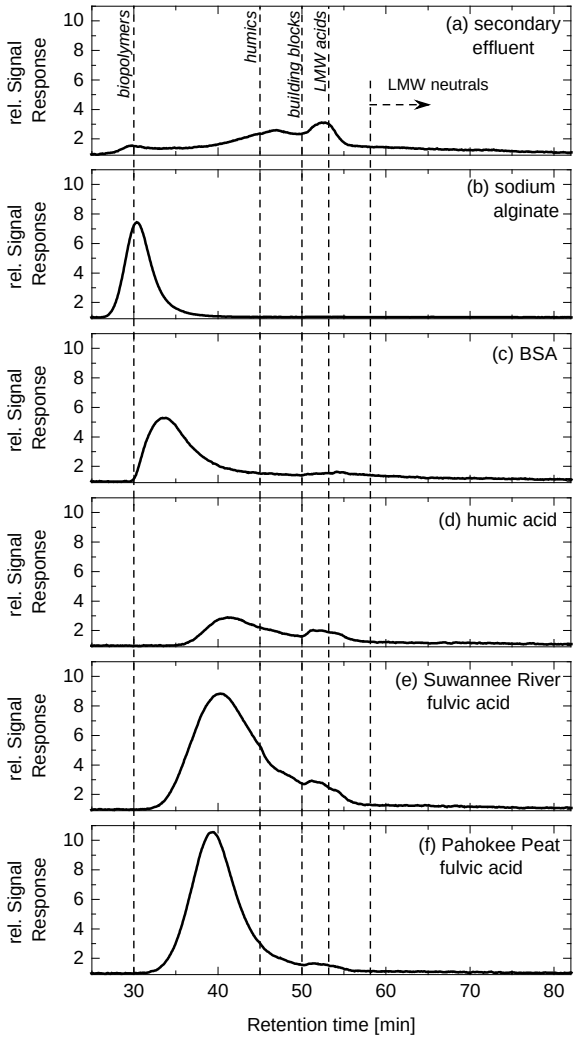
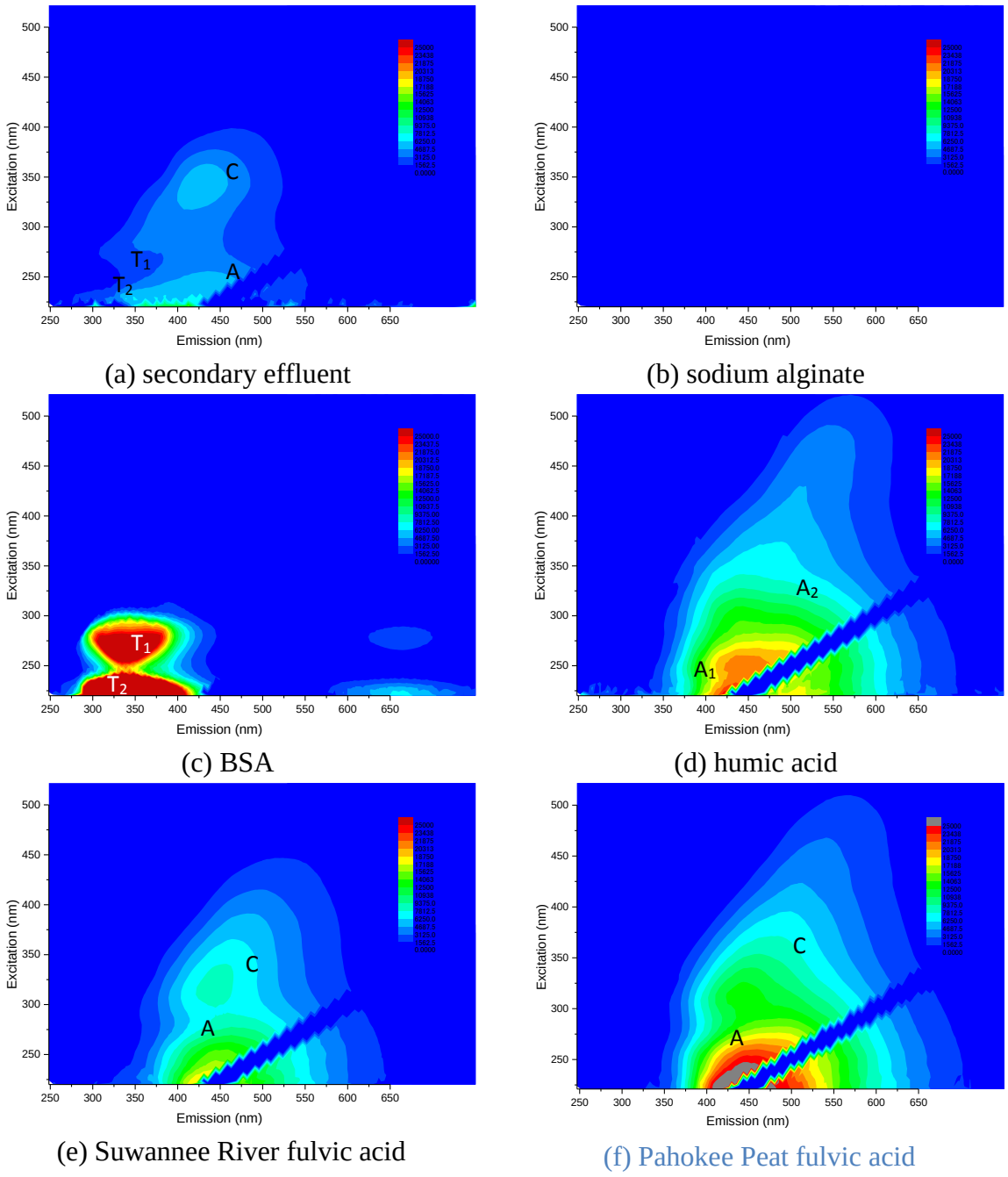


Fig. 3 – LC-OCD chromatogram of the (a) secondary wastewater effluent and solutions containing (b) sodium alginate, (c) BSA, (d) humic acid, (e) Suwannee River fulvic acid and (f) Pahokee Peat fulvic acid.

3.3.2. EEM spectroscopy

The organics in the secondary wastewater effluent were also characterised by EEM fluorescence spectroscopy. EEM peaks can be classified as protein-like, fulvic-like and humic-like fluorophores. A strong peak in the EEM spectrum of the secondary wastewater effluent was observed at the excitation/emission (Ex/Em) wavelengths of 350/425 nm which was designated as C (Liu et al., 2011) in **Fig. 4a** and indicates a humic acid-like fluorophore as suggested in the literature (Chen et al., 2003; Coble, 1996; Nam and Amy, 2008). Another peak at the Ex/Em of 220/416-427 nm was designated as A in **Fig. 4a** indicating the presence of fulvic acid-like fluorophore (Chen et al., 2003). It is noted that humic and fulvic acid-like fluorophore could coexist in these EEM regions (i.e., A and C) and their presence cannot be distinguished from each other (Rosario-Ortiz and Korak, 2017). Two other small peaks at the Ex/Em of 220/325-334 nm (aromatic amino acid) and 270/310-320 nm (tryptophan, amino acid) which were designated as T_1 and T_2 in **Fig. 4a**, respectively. The EEM spectroscopy results (**Fig. 4a**) imply the presence of proteins and humic organics, which is consistent with the findings attained through the LC-OCD chromatography (**Fig. 3a**).



(a) secondary effluent (b) sodium alginate

(c) BSA (d) humic acid

(e) Suwannee River fulvic acid (f) Pahokee Peat fulvic acid

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

Solutions of individual model foulants were also characterised using fluorescence spectroscopy to compare to the organics in the secondary wastewater effluent. The EEM of the sodium alginate solution revealed negligible peaks in the spectrum **(Fig. 4b)**, which was expected since polysaccharide-like substances do not contain molecular structure sensitive to photon

298 excitation. A peak of protein-like substance was identified with the BSA solution at the Ex/Em
299 of 265/325-350 nm and 223/334-348 nm which are designated as T_1 and T_2, respectively **(Fig.**
300 **4c)**. These peaks were also identified in the secondary wastewater effluent. The EEM spectrum
301 of the humic acid solution **(Fig. 4d)** revealed a peak at the Ex/Em of 225/415-435 nm (A_1) and
302 250/435-449 nm (A_2), and they were also identified at the secondary wastewater effluent **(Fig.**
303 **4a)**. The EEM spectrum of the fulvic acid solution **(Fig. 4e and 4f)** showed two peaks – a
304 strong peak at the Ex/Em of 250/430-460 nm (A) and a weak peak at the Ex/Em of 350/425
305 nm (C). This is consistent with a previous study (Chen et al., 2003) where the same source of
306 Suwannee River fulvic acid was examined. These two peaks (A and C) observed in the fulvic
307 acid solution were also identified in the secondary wastewater effluent. The characterisation
308 performed above indicate that the secondary wastewater effluent contains humic acid- and
309 fulvic acid-like substances as major sources of fluorophores.

310 ### *3.4. N-nitrosamine rejection by model foulants*

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

318 Membrane fouling with three model foulant (sodium alginate, BSA and humic acid) resulted
319 in negligible impact on the permeation of *N*-nitrosamines through the RO membrane **(Fig. 5**
320 **and S5)**. Membrane fouling with sodium alginate decreased NDMA rejection from 70.3 to
321 59.5% despite the considerable increase in TMP from 1.6 (t = 0 min) to 2.7 MPa (t = 45 min)

(Fig. 5a). Likewise, sodium alginate fouling caused decreased rejections of NMEA, NPYR and NMOR from 91.3 to 85.3%, from 95.9 to 93.3% and from 98.4 to 97.5%, respectively. Similar observations were identified for membrane fouling with BSA and humic acid solutions. Membrane fouling with BSA lead to a reduction in NDMA rejection from 64.0 (TMP = 1.6 MPa, *t* = 0 min) to 57.7% (TMP = 2.0 MPa, *t* = 80 min) **(Fig. 5b)**. Membrane fouling with humic acid caused a minor reduction of NDMA rejection from 62.9 (TMP = 1.7 MPa, *t* = 0 min) to 59.7% (TMP = 2.6 MPa, *t* = 70 min) **(Fig. 5c)**.

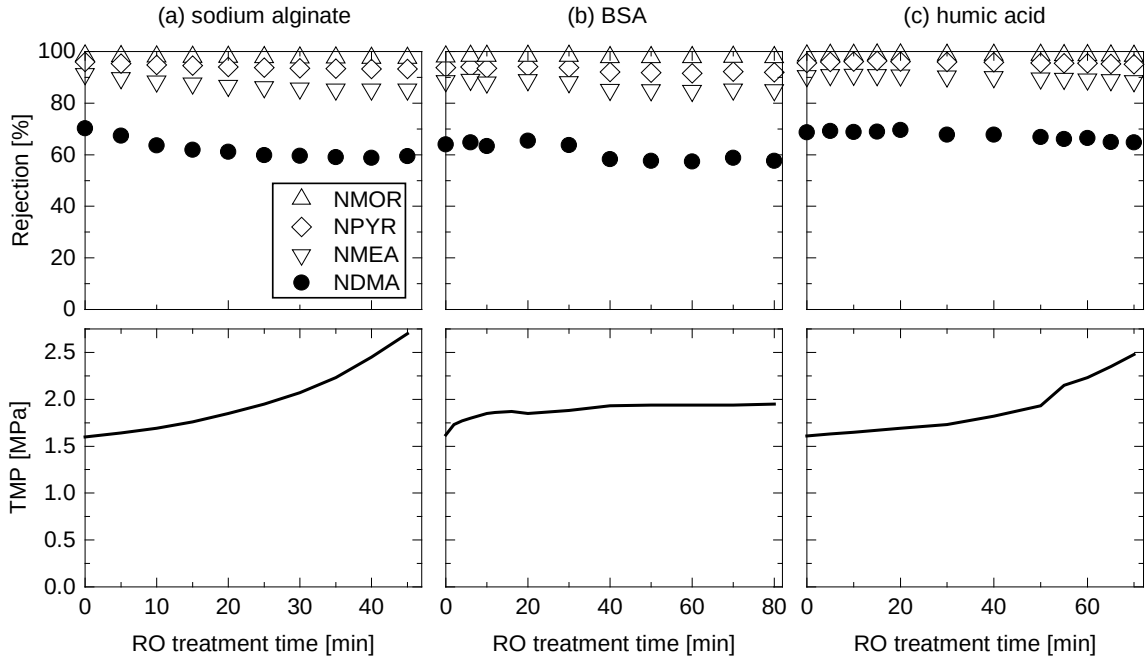


Fig. 5 – Changes in *N*-nitrosamine rejection and TMP during RO treatment of solutions containing 50 mg/L of (a) sodium alginate, (b) BSA and (c) humic acid with ESPA2 membrane (20 mM NaCl, 1 mM $NaHCO_3$, 1 mM $CaCl_2$, feed temperature = 20.0 ± 0.1 ºC, permeate flux = 80 L/m^{2}h).

In contrast, membrane fouling with fulvic acid solutions caused a slight increase in *N*-nitrosamine rejection **(Fig. 6 and S6)**. When the TMP increased from 1.64 (*t* = 0 min) to 1.78 MPa (*t* = 360 min) by the fouling development with Suwannee River fulvic acid solution, NDMA rejection increased from 64.7 to 69.4% **(Fig. 6a)**. In response to the fouling development, the rejections of NMEA, NPYR and NMOR also increased from 88.9 to 91.2%,

from 93.6 to 95.3% and from 98.2 to 98.9%, respectively. Another fouling test with Pahokee Peat fulvic acid solution also revealed the trend of increasing *N*-nitrosamine rejection; NDMA rejection increased from 67.5 to 73.6% when the TMP increased from 1.6 (t = 0 min) to 2.5 MPa (t = 240 min) **(Fig. 6b)**. In conjunction with fulvic acid fouling development, NMEA, NPYR and NMOR revealed increased rejection from 91.0 to 91.3%, from 95.4 to 95.8% and from 98.7 to 99.1%, respectively.

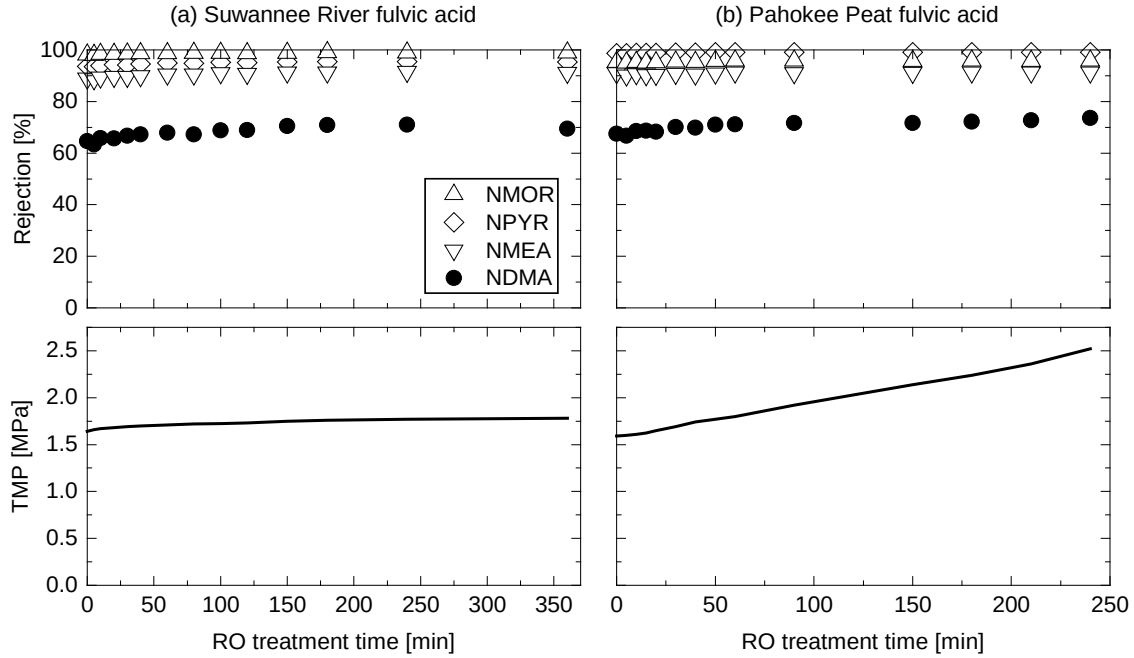


Fig. 6 – Changes in *N*-nitrosamine rejection and TMP during RO treatment of solutions containing 30 mg/L of (a) Suwannee River fulvic acid and (b) Pahokee Peat fulvic acid with ESPA2 membrane (20 mM NaCl, 1 mM $NaHCO_3$, 1 mM $CaCl_2$, feed temperature = 20.0 ± 0.1 ^oC, permeate flux = 80 L/m^2h).

The trend of reducing the permeation of *N*-nitrosamines with a fouling layer of small molecular weight foulants (i.e. fulvic acids) was also observed with the ESNA1-LF NF membrane **(Fig. S7)**. Membrane fouling with Pahokee Peat fulvic acid solution caused an increase in *N*-nitrosamine rejection only after reaching as high TMP as those used for the ESPA2 RO membrane. For example, NDMA remained almost zero for the increase in TMP from 0.13 (t =

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

3.5. Proposed mechanisms

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

The formation of the fouling layer with large molecular weight model foulants (sodium alginate, BSA and humic acid) resulted in a negligible decrease in *N*-nitrosamines rejection **(Fig. 6a-c)**. Considering that fouling of the RO membranes progresses with cake layer formation, the fouling layer is sufficiently porous such that *N*-nitrosamines can readily permeate from the bulk

solution through the fouling layer and to the membrane surface, which could explain the negligible impact on the permeation of *N*-nitrosamines.

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

4. Conclusions

A high throughput HPLC-PR-CL analytical technique was used to examine the correlation between the type of foulant and *N*-nitrosamine rejection by an RO membrane. Membrane fouling with a secondary wastewater effluent led to a decrease in the permeation of NDMA and the other *N*-nitrosamines (i.e. NMEA, NPYR and NMOR), although the membrane fouling (accelerated at a high permeate flux) only provided a trend of *N*-nitrosamine rejection during fouling development. Examination by LC-OCD chromatography revealed that the major constituents in the secondary wastewater effluent were biopolymers (e.g. polysaccharides and proteins) and humic substances (e.g. humic acid and fulvic acid). Further investigation with fluorescence spectrometry also identified humic acid-like organics, fulvic acid-like organics

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

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