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Measuring transparent exopolymer particles (TEP) as indicator of the (bio)fouling potential of RO feed water

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ABSTRACT

Transparent exopolymer particles (TEP) are an abundant type of EPS in surface waters which were recently regarded as major initiators of biofilm formation on reverse osmosis (RO) membranes. The goal of this study was to monitor the presence of these substances in RO feed water and its removal by pre-treatment with MF/UF. Modifications were applied on the existing TEP determination method to measure both particulate (>0.4 μm) and colloidal (0.05–0.40 μm) TEP. Results show colloidal TEPs (c-TEP) were more abundant than particulate TEP (p-TEP) in both fresh and sea water samples, affirming the importance of measuring this often neglected colloidal fraction. Higher TEP concentrations were recorded in seawater than in freshwater samples and a significant variation of TEP concentration was observed in seawater samples collected during the spring season. In two integrated membrane systems (IMS), 70–75% of TEP removal was recorded by MF and UF with in-line coagulation. However, significant amounts of TEP (mostly c-TEP) remained after MF/UF pretreatments, and this may potentially cause organic and/or biological fouling in the RO system downstream.

Keywords: Transparent exopolymer particles; Reverse osmosis; Biofouling; Integrated membrane systems

1. Introduction

Organic and biological fouling may occur at the same time in reverse osmosis (RO) membranes. This is primarily due to the abundance of natural organic matter (NOM) in the feed water of most RO systems. NOM is an extremely complex mixture of organic substances which are present in all water sources. It can serve as a substrate for biological growth in water treatment processes and affects the behaviour of colloidal matter by binding to the colloidal surfaces [1]. The deposition of these substances on membranes will serve as a "conditioning film" for bacterial growth and re-growth. Over the years, there have been increasing reports of membrane fouling due to the deposition of biopolymers (polysaccharides and proteins), an abundant form of organic matter that belongs to the most labile fraction of NOM [2,3].

Biopolymers, specifically the sticky extracellular polymeric substances (EPS), have long been considered notorious in causing organic and biological fouling, as it is

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known to have a major role in microbial growth and particle aggregation on membrane surfaces. An important discovery in the last decade of a formerly unknown but abundant form of EPS called transparent exopolymer particles (TEP), led to a better understanding of the role of EPS in the carbon cycle and biological life in aquatic systems. TEP was found in abundance in both fresh and marine waters, and has been characterised as transparent, sticky and amorphous particles comprise mainly of polysaccharides [4–6]. In aquatic systems, some of these particles were found in flexible fibrillar form originating from biological detritus and release of aquatic organisms. Majority of TEP were formed abiotically from dissolved precursors of fibrils 1–3 nm in diameter by 100s of nanometers long and can pass through 8 kDa pore size membranes [7]. Hence, in integrated membrane systems (IMS), MF/UF pretreatments may not provide a complete barrier for TEP colloidal precursors from potentially fouling the RO systems downstream. Just recently, TEP was regarded by some experts as the "major initiator" of biofilm formation in membrane systems and could potentially lead to biofouling [8,9]. Studying the presence and behavior of these substances is therefore necessary to understand its role in membrane fouling.

TEP has been extensively studied in the field of oceanography and limnology but there is very little information on its relevance to membrane systems used for surface water treatment. A number of techniques are already available to quantify and monitor these previously elusive particles, involving simple spectrophotometric measurements [4,10–12]. Although these techniques had been around for some time now, their application to water treatment monitoring is still in its initial stage [13]. The main goal of this study was to modify the existing method of measuring TEP, use it to assess TEP removal by different pretreatments and monitor TEP in the RO feed water of integrated membrane systems (IMS).

2. Materials and methods

This study was carried out in three phases. The first phase was to modify the existing TEP method for IMS applications. The second phase was to verify its application for different types of water (fresh and marine). The last phase was to collect water samples from IMS plants to monitor TEP concentrations along the pre-treatment steps and the RO feed water.

2.1. Apparatus and materials

Filtrations were carried out in a pump-controlled Sartorius vacuum filtration system (50 mm Ø) using Whatman Nuclepore 47 mm Ø polycarbonate filters (0.40, 0.20, 0.10 and 0.05 μm pore sizes). Absorbance was measured using a Shimadzu UV-Vis spectrophotometer (UV-2501PC) and TOC was measured through a Shimadzu TOC-V_{CPN} total organic carbon analyser.

The staining solution was prepared to contain 0.02% of Alcian Blue 8 GX Standard Fluka (C.I.N. 74240) in 0.06% aqueous acetic acid buffer solution maintained at pH 2.5. The standard solution was prepared using a Gum Xanthan (Sigma; CAS 11138-66-2) and homogenised using a Dounce tissue grinder tube and pestle (100 ml volume; Sigma-Aldrich). All aqueous solutions and reagents were prepared using ultra-pure water from a Millipore Milli-Q Advantage water system.

2.2. Modified TEP determination

The TEP method used was based on the spectrophotometric technique (TEP >0.40 μm) originally developed by Passow and Alldredge [10]. The technique mainly involves staining with Alcian Blue, a dye that specifically binds to acidic polysaccharides. The dye is used to quantify TEP in surface waters. For this research, some modifications were introduced in order to measure both particulate (>0.40 μm) and colloidal (0.05–0.40 μm) TEP for IMS applications.

2.3. Calibration of staining solution

In staining with Alcian Blue, the weight of the substrate (e.g. TEP) is directly proportional to the amount of stain binding to it [14]. However, Alcian Blue may react differently under different conditions (e.g. concentration, pH) while TEPs of different origins (species of planktons or bacteria) also react differently with the stain [10]. Therefore, each batch of staining solution needs to be pre-calibrated using a known type of polysaccharide — Gum Xanthan. A standard solution was prepared by mixing 20 mg of Gum Xanthan in 200 ml of ultra-pure water and then homogenised using a tissue grinder. Consequently, 40 ml volumes of 4–5 dilutions of the standard solution were prepared. For each dilution, 20 ml was filtered through 0.2 μm polycarbonate filters under a vacuum of 0.2 bars. The filtrate and remaining 20 ml of the feed were set aside for TOC measurements. The retained Gum Xanthan on the polycarbonate filter was stained with 1 ml of pre-filtered (0.05 μm polycarbonate filter) Alcian Blue staining solution. The excess stain was removed by applying a vacuum (0.2 bars) through the filter and rinsed by filtering 1 ml of ultra-pure water. The stained filter was transferred to a 50 ml beaker then soaked in 6 ml of 80% H_2SO_4 solution to elute the Alcian Blue bound to Gum Xanthan and those adsorbed by the filter. After 2 h, the absorbance of the acid solution was measured using a

spectrophotometer. Absorbance was measured at 787 nm wavelength using a 1-cm cuvette and ultra-pure water as reference. The calibration factor (f_x) was computed by relating the weight of Gum Xanthan to the absorbance of the eluted stain in the acid solution following the equation:

$$f_x = \overline{W}_x \left(\overline{X}_{787} - B_{787}\right)^{-1} \text{ [mg X}_{eq}\text{]}$$

where $\overline{W}_x$ is the average retained dry weight for different Gum Xanthan dilutions in mg; $\overline{X}_{787}$ is the average absorbance of stain eluted from Gum Xanthan and the filter; and B_{787} the average absorbance of eluted stain from stained blank filters. The average retained dry weight of Gum Xanthan (W_x) was estimated by computing the retained TOC as the difference between the feed TOC (TOC_{feed}) and filtrate TOC (TOC_{filt}) per unit volume of filtrate (V_f), and then converted to equivalent weight of Gum Xanthan based on its molecular composition ($C_{35}H_{49}O_{29}$):

$$\overline{W}_x = \frac{V_f}{0.45 n_{max}} \sum_{n=1}^{n=n_{max}} TOC_{feed} - TOC_{filt} \qquad \text{[mg X]}$$

where n = number of Xanthan dilutions.

To minimise the effects of organic carbon contamination on the TOC results, acid cleaning and ultra-pure water flushing of polycarbonate filters (16% HCl) and filtration equipment (80% H_2SO_4) were performed before filtration. After each cleaning, ultra-pure water was filtered through the filter using the vacuum filtration system set at the same settings as in the calibration. The filtrate was then collected for TOC measurement. TOC results should be less than 0.1 mg-C.L^{-1}; otherwise, cleaning was repeated until the required TOC was reached. In this study, the computed calibration factor (f_x) was 0.476 mg X_{eq} per unit absorbance at 787 nm. This was about four times higher than the f_x values reported by Passow and Alldredge [10]. The higher f_x value can be attributed to lower concentration of Alcian Blue stain, which was a result of pre-filtering the staining solution with 0.05 μm polycarbonate filter instead of a 0.2 μm filter used in the original method. However, no tests were performed to directly compare the two methods. Hence, TEP concentrations reported in this study are considered relative until further verifications are made.

2.4. TEP measurement

To measure TEP, 40–200 ml of water samples were filtered through a series of polycarbonate filters (0.40, 0.20, 0.10 or 0.05 μm) under constant vacuum of 0.2 bars. The retained TEP on the filter was stained with 1 ml of pre-filtered (0.05 μm) Alcian Blue stain. The excess stain was removed and then the filter was rinsed as in the calibration. The filter was transferred into a 50-ml beaker and soaked in 6 ml of 80% H_2SO_4 for about 2 h. The beaker was gently swirled 3–5 times within this period. After soaking, the absorbance (at 787 nm) of the acid solution was measured using a spectrophotometer.

Absorbance corrections due to stain adsorption to the filter media and interference due to high turbidity were also measured using two clean polycarbonate filters. Filter blank staining with Alcian Blue was performed on one of the filters (filter blank) and a volume (consistent with the sample measurement) of water sample was filtered through the second filter (turbidity). Both filters were soaked in acid solution for 2 h and the absorbance was measured thereafter as previously described. The absorbance of the sample was corrected by subtracting the corrections due to turbidity and filter blank. Using the results of the calibration, TEP concentration in terms of milligram Gum Xanthan equivalent per liter (mg X_{eq}.L^{-1}) was computed following the equation:

$$TEP = (A_{787} - B_{787} - T_{787}) \times f_x \times (V_f)^{-1} \quad \text{[mg X}_{eq}\text{.L}^{-1}\text{]}$$

where A_{787} is the absorbance of the stain eluted from TEP and the filter; B_{787} is the average absorbance of stain eluted from stained blank filters; T_{787} is the absorbance correction due to turbidity; f_x is the calibration factor of the staining solution in mg X_{eq} per unit absorbance at 787 nm; and V_f is the filtered volume of the sample in liters. Three replicates were performed for each of the samples tested.

Concentration of TEP fractions was based on serial filtration using different pore size filters. Particulate TEP or p-TEP refers to TEP retained on 0.4 μm polycarbonate filters while colloidal TEP or c-TEP refers to TEP that passed through 0.4 μm polycarbonate filters but retained on 0.05 μm polycarbonate filters. Passow [7] reported part of c-TEP as dissolved TEP-precursors (<0.2 μm). However, this size fraction belongs to the colloidal size range (1–0.001 μm) based on the IUPAC definition. Hence, it is referred to in this study as "colloidal" rather than "dissolved".

3. Results and discussion

3.1. TEP size distribution in seawater and freshwater

To determine the size distribution of TEP in different source waters, serial filtrations were performed using polycarbonate filters of different pore sizes (0.4 > 0.2 > 0.1 > 0.05 μm). TEP retained in each filtration were measured using the modified spectrophotometric method. Water samples were collected from four locations in the Netherlands: (1) Scheveningen coast, North Sea; (2) Eemshaven

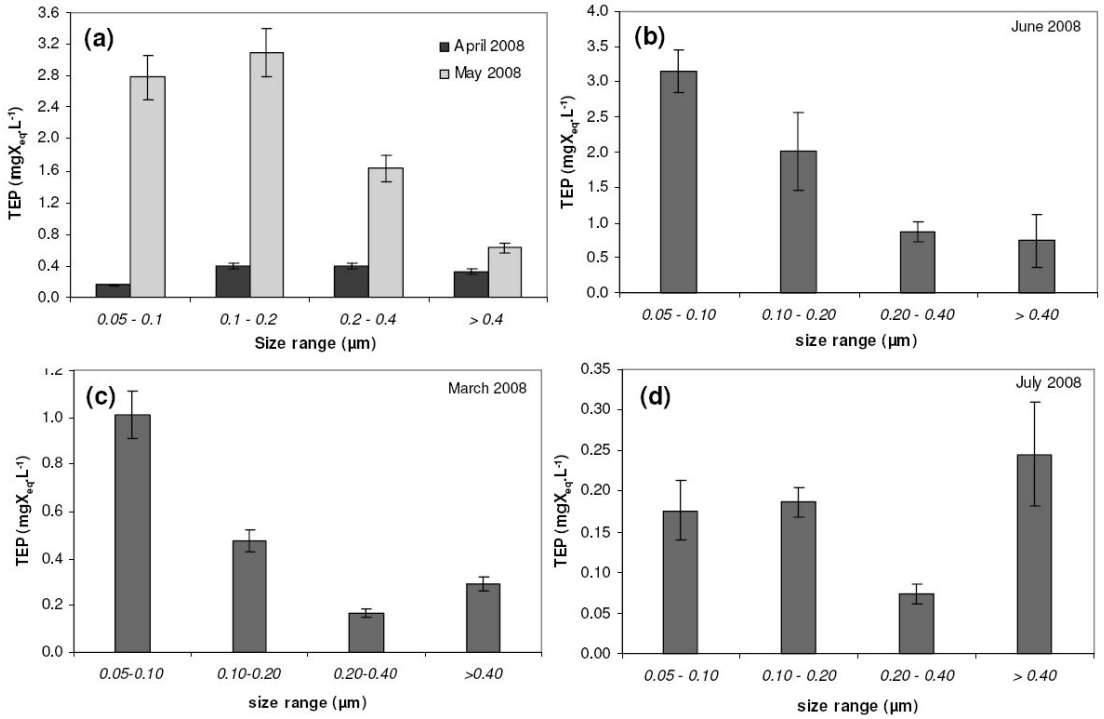

Fig. 1. Size distribution of TEP in seawater and freshwater: (a) Scheveningen coast (North Sea); (b) Eemshaven coast (Wadden Sea); (c) Westvest canal, Delft; and (d) transported reservoir water from De Biesbosch (River Meuse).

coast, Wadden Sea; (3) Westvest canal, Delft; and (4) transported reservoir water from De Biesbosch, River Meuse. Samples were collected in mid-spring and late spring for Site 1, early summer for Site 2, early spring for Site 3 and early summer for Site 4. Fig. 1 shows the size distribution of TEP larger than 0.05 μm of the four sampled sites.

Among the four sites sampled, the recorded total TEP concentration was highest in seawater of 8.1 mg $X_{eq}.L^{-1}$ and lowest in reservoir water (3–4 months residence time) of 0.7 mg $X_{eq}.L^{-1}$. However, in terms of p-TEP (>0.40 μm), the concentrations were all below 1 mg $X_{eq}.L^{-1}$ for all types of water measured. These were within the TEP concentration range reported by Passow [6]. Although this study found significantly higher amounts of TEP in seawater than in fresh water, TEP can be of great concern for both seawater and freshwater RO plants because a number of studies reported TEP concentrations in freshwater which are comparable or sometimes higher than what was reported in seawater [5,15,16], especially during algal blooming seasons.

Coastal seawater from the North Sea showed a significant increase of TEP concentration (six times in magnitude) between samples collected in mid-spring (8°C average temperature) and in late spring (12°C average temperature). This can be attributed to massive release of TEP during phytoplankton blooms which normally occurs in spring and summer seasons. The significant increase of TEP during these seasons generally coincides with the high incidence of fouling in most RO plants. This is an indication that TEP could in fact initiate fouling in RO, especially during phytoplankton blooms.

Results of the size distribution revealed that c-TEP (<0.40 μm) were more abundant than p-TEP (>0.40 μm). Percentage of TEP in the range of 0.05 to 0.40 μm with respect to total TEP (>0.05 μm) were 75–92% for the North Sea samples, 89% for the Wadden Sea sample, 85% for the Delft canal sample, and 65% for the River Meuse sample. Most previous studies of TEP only measure particulate TEP and usually neglected the contribution of smaller TEPs, as this requires the use of smaller pore size filters to measure. The results of the TEP size distribution suggested the importance of measuring the smaller TEP (c-TEP) since it is likely more abundant than particulate TEP (p-TEP), and with its smaller size, can be more resistant to pre-treatment.

3.2. Pretreatment removal of TEP in integrated membrane systems (MF/UF–RO)

TEP was monitored through the treatment lines of two integrated membrane systems (IMS). Plant A is a pilot plant treating seawater from coastal Wadden Sea with a production capacity of 3 $m^3.h^{-1}$. Pre-treatment was by microfiltration (nominal pore size = 100 nm) without in-line coagulation. Plant B is a full-scale IMS plant treating water from a reservoir fed by River Meuse and with a

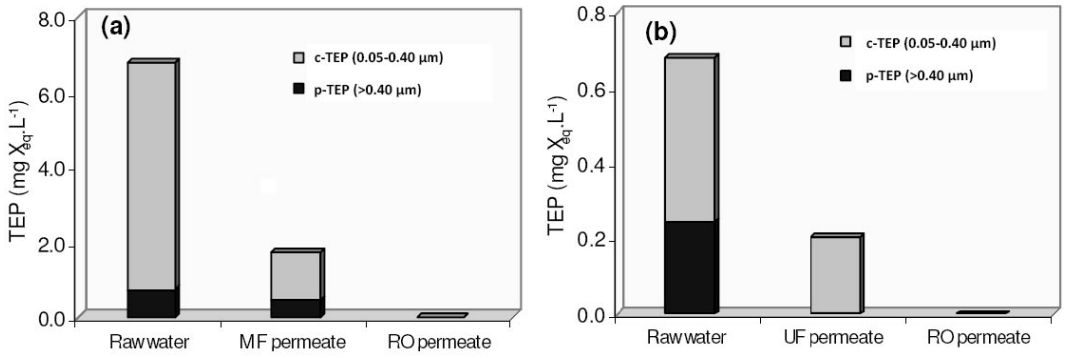

Fig. 2. TEP monitoring through the treatment lines of two IMS: (a) Plant A, seawater pilot plant; (b) Plant B, freshwater full-scale treatment plant.

production capacity of 155 $m^3.h^{-1}$. Pre-treatment consisted of in-line coagulation by $FeCl_3$ (0.3 mg $Fe^{3+}.L^{-1}$) followed by ultrafiltration (nominal pore size = 30 nm). Results of TEP measurements for the two IMS are shown in Fig. 2.

Total TEP concentration of the raw water in Plant A was 6.8 mg $X_{eq}.L^{-1}$. TEP composition of the raw water was about 11% p-TEP (>0.40 μm) and 89% c-TEP (0.05–0.40 μm). Pre-treatment by microfiltration removed about 75% of TEP, which comprised of removal of 35% p-TEP and 80% c-TEP. In spite of the nominal pore size (100 nm) of MF, TEP larger than 0.4 μm were able to pass through the membrane. This can be attributed to the fibrillar nature of some TEP, which are flexible enough to pass through smaller pores if under pressure [7].

For Plant B, total TEP concentration of the raw water was 0.7 mg $X_{eq}.L^{-1}$. TEP composition of the raw water was about 36% p-TEP and 64% c-TEP. Pre-treatment by in-line coagulation followed by utrafiltration removed about 70% of total TEP with 100% removal of p-TEP and 54% of c-TEP. The 30 nm nominal pore size of the UF membrane and the application of coagulant in the raw water provided the complete removal of p-TEP. However, some c-TEP were able to pass through the UF membrane, confirming an earlier study by Passow [7] where it was demonstrated that low pressure membranes (MF/UF) are not complete barriers of small colloidal TEPs.

TEP concentrations in the RO feed water were 1.7 and 0.2 mg $X_{eq}.L^{-1}$ after pre-treatment by MF and UF, respectively. Although MF alone had higher TEP removal than in-line coagulation plus UF, TEP concentrations in the MF permeate was relatively higher. Apparently, in-line coagulation followed by UF was more effective than MF alone in limiting TEP in the RO feed water.

As expected, TEP was totally rejected by RO, which means that TEPs from the feed water was either in the RO concentrate or deposited on the RO membrane. The latter scenario could eventually result in accumulation of more colloidal particles (organic or inorganic), because TEP may act like "natural glue" that promotes adhesion of colloids present in the RO feedwater to the membrane. A major reason for concern is biofilm initiation by TEP. Some bacteria, which are attached to TEP [17] and/or eventually attached to TEP after the latter had accumulated on the membrane, may feed on dissolved biodegradable nutrients (C, P, N) from the feedwater, and grow exponentially within the biofilm matrix dominated by TEP [8]. This may then lead to biological fouling, causing unwanted performance decline in the RO system. Further studies are necessary to better understand the removal of TEP from the RO feedwater and its role in membrane fouling.

4. Conclusions

This study was able to present the following:

- The existing TEP method was modified and extended to measure the colloidal (0.05–0.40 μm) fraction of TEP in surface water. This fraction was more abundant than the widely studied particulate fraction (>0.40 μm) by about 2–5 times in magnitude. This affirms the importance of measuring colloidal TEP as they are likely more abundant than particulate TEP in surface waters, and with its smaller size, can be more resistant to pre-treatment.
- TEP was found higher in seawater than in fresh water samples tested. However, TEP variations at different seasons may produce different results. Thus, TEP could be significant in both fresh and sea water sources and therefore should be monitored in membrane water treatment plants.
- In two IMS installations, significant amounts of TEP were removed by microfiltration (75%) and ultrafiltration with in-line coagulation (70%). However, neither were absolute barriers for TEP from entering the RO system. Seasonal monitoring is recommended to better understand pretreatment removal at different raw water TEP concentrations.
- Since pre-treatment by MF/UF did not remove all TEP, there was enough reason for concern that TEP is

present in the RO feed water and that it may adhere to RO membranes and eventually cause fouling. Further studies are therefore necessary to monitor the presence of TEP in the RO feed water and to understand its role in organic and biological fouling of RO systems.

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