Transparent exopolymer particles (TEP) and their precursors in reverse osmosis (RO) systems: quantification, fouling potential and cleaning

Xu Li

This thesis is presented for the degree of

Doctor of Philosophy

School of Engineering and Information Technology,
Murdoch University, Western Australia

2018
STATEMENT OF ORIGINALITY

I declare that this thesis is my own account of my research and includes no material previously published or written by another person, except where other sources are acknowledged. The thesis contains its main content work which has not previously been submitted for a degree at any other institution.

Xu Li
October, 2018
## STATEMENT OF CONTRIBUTION

### Title of Paper
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### Principal Author
**Name of Principal Author (Candidate):** Xu Li

**Contribution to the Paper:** Conducted all lab work, analysed data, wrote manuscripts, edited and finalised the paper for submission

**Overall percentage (%):** 80

**Signature:**

**Date:** 5/01/2018

### Co-Author Contributions
By signing the Statement of Contribution, each author certifies that:

i. the candidate’s stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate to include the publication in the thesis; and

iii. the sum of all the co-author contributions is equal to 100% less the candidate’s stated contribution.

**Name of Co-Author:** Lucy Skillman

**Contribution to the Paper:** Supervised the study and edited conclusion

**Overall percentage (%):** 6

**Signature:** L.Skillman@murdoch.edu.au

**Date:** 7/11/18

**Name of Co-Author:** Dan Li

**Contribution to the Paper:** Supervised the study and edited conclusion

**Overall percentage (%):** 4

**Signature:** D.Li@murdoch.edu.au

**Date:** 8/11/2018

**Name of Co-Author:** Wendell P. Ela

**Contribution to the Paper:** Supervised the study, edited and revised manuscript

**Overall percentage (%):** 10

**Signature:** W.Ela@murdoch.edu.au

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## Statement of Contribution

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### Principal Author

**Name of Principal Author (Candidate):** Xu Li

**Contribution to the Paper:** Conducted all lab work, analysed experimental, statistical data, and membrane characterization data, wrote manuscripts, edited and finalised the paper

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**Signature:** [Redacted] **Date:** 1/11/2018

### Co-Author Contributions

**By signing the Statement of Contribution, each author certifies that:**

i. the candidate’s stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate to include the publication in the thesis; and

iii. the sum of all the co-author contributions is equal to 100% less the candidate’s stated contribution.

**Name of Co-Author:** Ross S. Bowden

**Contribution to the Paper:** Supervised statistical analysis and edited manuscript

**Overall percentage (%):** 70

**Signature:** [Redacted] **Date:** 1/1/2018

**Name of Co-Author:** Brenton R. Clarke

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**Name of Co-Author:** Dan Li

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Name of Principal Author (Candidate)
Xu Li

Contribution to the Paper
Conducted all lab work, analysed experimental, statistical, and membrane characterization data, wrote manuscripts, edited and finalised the paper

Overall percentage (%) 80

Signature

Date: 5/11/2018

Co-Author Contributions
By signing the Statement of Contribution, each author certifies that:

i. the candidate’s stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate to include the publication in the thesis; and
iii. the sum of all the co-author contributions is equal to 100% less the candidate’s stated contribution.

Name of Co- Author
Lucy Skillman

Contribution to the Paper
Supervised experimental work

Overall percentage (%) 1%

Signature

L.Skillman@murdoch.edu.au

Date: 7/11/18

Name of Co- Author
Dan Li

Contribution to the Paper
Supervised experimental work.

Overall percentage (%) 5

Signature

L.Li@murdoch.edu.au

Date: 8/11/18

Name of Co- Author
Wendell P. Ela

Contribution to the Paper
Supervised the study and edited manuscript

Overall percentage (%) 5

Signature

W.Ela@murdoch.edu.au

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ABSTRACT

Transparent exopolymer particles (TEP) and their precursors have been identified as critical causal factors in fouling of desalination and other water treatment membranes. TEP and the fractions thereof are composed of a wide variety of organic constituents and size fractions and have conventionally been only quantified by an operational method, specifically, Alcian Blue measurement. For fouling control and mitigation purposes, it is important to accurately measure the amount of both TEP and TEP precursors, their fouling capacity, and their response to cleaning. However, these issues are not well understood nor are there generally accepted methods for undertaking comparative studies to address this lack of understanding. In this study, a three-stage research study was undertaken to evaluate (1) TEP and TEP precursors quantification methods, (2) TEP and TEP precursors fouling potential on seawater reverse osmosis (SWRO) membranes, and (3) hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) cleaning performance on TEP fouled SWRO membranes. It was found that the concentrations of TEP may only represent ~10% of the total mass; while TEP precursors represent ~80% of the total TEP. This highlights the importance of measuring, reporting, and operationally considering both TEP and TEP precursors for membrane biofouling studies. A reliable, adaptable statistical method using the T-test was developed to quantify and develop a criterion for differentiating fouling behaviour of different TEP surrogates and TEP fractions. Similar fouling potential was measured with total TEP, TEP, and TEP precursors at the same concentrations. Therefore, TEP concentrations rather than TEP sizes were correlated to membrane fouling. It highlighted the importance of developing new advanced pre-treatment techniques for TEP precursors removal. H\textsubscript{2}O\textsubscript{2} cleaning enhanced water flux, possibly due to re-compaction/re-attachment of xanthan gum on
the membrane surface following its breakdown into smaller fragments. The hypothesis of membrane degradation due to exposure to H$_2$O$_2$ during cycles of fouling and cleaning-in-place (CIP) was not supported by attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), salt flux, and salt rejection results. The work strongly suggests peroxide cleaning be further evaluated with field trials as an alternative to commonly used, but more destructive, cleaning techniques.
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LIST OF MANUSCRIPTS AND PUBLICATIONS


### ABBREVIATIONS

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<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&lt;sub&gt;filter blank&lt;/sub&gt;</td>
<td>Absorbance of Alcian blue on a negative control filter (abs/cm)</td>
</tr>
<tr>
<td>A&lt;sub&gt;m&lt;/sub&gt;</td>
<td>Effective membrane area (m²)</td>
</tr>
<tr>
<td>A&lt;sub&gt;sample&lt;/sub&gt;</td>
<td>Absorbance of Alcian blue stained sample (abs/cm)</td>
</tr>
<tr>
<td>A&lt;sub&gt;sample blank&lt;/sub&gt;</td>
<td>Absorbance of unstained sample filter (abs/cm)</td>
</tr>
<tr>
<td>AA</td>
<td>Alginic acid</td>
</tr>
<tr>
<td>AB</td>
<td>Alcian blue</td>
</tr>
<tr>
<td>AOX</td>
<td>Absorbable organic halogen</td>
</tr>
<tr>
<td>ATR-FTIR</td>
<td>Attenuated total reflectance - fourier transformed infrared spectroscopy</td>
</tr>
<tr>
<td>C&lt;sub&gt;b&lt;/sub&gt;</td>
<td>Bulk concentration of solutes (mol/L)</td>
</tr>
<tr>
<td>C&lt;sub&gt;filter&lt;/sub&gt;</td>
<td>Total carbohydrate concentrations of the re-suspended filter solution</td>
</tr>
<tr>
<td>C&lt;sub&gt;filtrate&lt;/sub&gt;</td>
<td>Total carbohydrate concentrations of the filtrate solution</td>
</tr>
<tr>
<td>C&lt;sub&gt;initial&lt;/sub&gt;</td>
<td>Total carbohydrate concentrations of the initial surrogate solution</td>
</tr>
<tr>
<td>CA</td>
<td>Cellulose acetate</td>
</tr>
<tr>
<td>CEB</td>
<td>Chemically enhanced backflush</td>
</tr>
<tr>
<td>CIA</td>
<td>Cleaning-in-air</td>
</tr>
<tr>
<td>CIP</td>
<td>Cleaning-in-place</td>
</tr>
<tr>
<td>ClO₂</td>
<td>Chlorine dioxide</td>
</tr>
<tr>
<td>CLSM</td>
<td>Confocal laser scanning microscopy</td>
</tr>
<tr>
<td>d</td>
<td>Polysaccharide depth of each image</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>D</td>
<td>Density</td>
</tr>
<tr>
<td>DDI</td>
<td>Distilled deionized water</td>
</tr>
<tr>
<td>DI</td>
<td>Deionized water</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved organic carbon</td>
</tr>
<tr>
<td>EPS</td>
<td>Extracellular polymeric substances</td>
</tr>
<tr>
<td>F&lt;sub&gt;filter&lt;/sub&gt;</td>
<td>The percentage of recovered carbohydrates in the surrogate retained on the membrane filters</td>
</tr>
<tr>
<td>F&lt;sub&gt;filtrate&lt;/sub&gt;</td>
<td>The percentage of recovered carbohydrates in the filtrate</td>
</tr>
<tr>
<td>f&lt;sub&gt;610&lt;/sub&gt;</td>
<td>Calibration factor</td>
</tr>
<tr>
<td>f&lt;sub&gt;787&lt;/sub&gt;</td>
<td>Calibration factor</td>
</tr>
<tr>
<td>FI</td>
<td>Fouling index</td>
</tr>
<tr>
<td>FTF</td>
<td>Filter-transfer-freeze</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>HOCl</td>
<td>Hypochlorous acid</td>
</tr>
<tr>
<td>I</td>
<td>Mean intensity for entire 3-D stacks</td>
</tr>
<tr>
<td>I&lt;sub&gt;n&lt;/sub&gt;</td>
<td>Mean intensity for layer n</td>
</tr>
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<td>J</td>
<td>Corrected water flux (LMH/Pa)</td>
</tr>
<tr>
<td>J&lt;sub&gt;0&lt;/sub&gt;</td>
<td>Corrected initial flux</td>
</tr>
<tr>
<td>J&lt;sub&gt;t&lt;/sub&gt;</td>
<td>Corrected flux at time t (h)</td>
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<td>J&lt;sub&gt;r&lt;/sub&gt;</td>
<td>Raw water flux</td>
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<td>J&lt;sub&gt;s&lt;/sub&gt;</td>
<td>Salt flux</td>
</tr>
<tr>
<td>J&lt;sub&gt;ss,0&lt;/sub&gt;</td>
<td>Initial water flux of sea salt baseline</td>
</tr>
<tr>
<td>J&lt;sub&gt;t&lt;/sub&gt;</td>
<td>Water flux at time t (h)</td>
</tr>
<tr>
<td>LMH</td>
<td>Liters per square meter per hour (flow rate measurement)</td>
</tr>
</tbody>
</table>
LOD  Limits of detection

\( m_{610} \)  Slope of the calibration curve at 610 nm wavelength 
\[ \text{[(abs/cm)/(mg surrogateeq/L)]} \]

\( m_{787}^{0.1\mu m} \)  Slope of the calibration curves for 0.1 \( \mu m \) fractions at 787 nm wavelength 
\[ \text{[(abs/cm)/\( \mu g \) surrogateeq]} \]

\( m_{787}^{0.4\mu m} \)  Slope of the calibration curves for 0.4 \( \mu m \) fractions at 787 nm wavelength 
\[ \text{[(abs/cm)/\( \mu g \) surrogateeq]} \]

\( \Delta m_w \)  Mass of permeate (g) collected during time \( \Delta t \) (h)

MBR  Membrane bioreactor

MF  Microfiltration

NDMA  N-nitrosodimethylamine

NF  Nanofiltration

\( \text{NH}_2\text{Cl} \)  Monochloramine

Norm J  Corrected water flux divided by initial flux (Chapter 4)

Norm J  Corrected water flux divided by flux of initial sea salt baseline (Chapter 5)

Norm \( J_{1,24} \)  Normalized water flux at the end of 24-h fouling operation at xanthan gum fouling cycle 1

Norm \( J_{n,24} \)  Normalized water flux at the end of 24-h fouling operation at xanthan gum fouling cycle \( n \) (\( n = 1 \) to 5)

P  Applied pressure (Pa)

PA  Polyamide

PN  Pectin

POC  Particulate organic carbon

\( Q_w \)  Permeate flowrate (L/h)
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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</thead>
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<tr>
<td>RO</td>
<td>Reverse osmosis</td>
</tr>
<tr>
<td>TEP</td>
<td>Transparent exopolymer particles</td>
</tr>
<tr>
<td>cTEP</td>
<td>Colloidal TEP</td>
</tr>
<tr>
<td>pTEP</td>
<td>Particulate TEP</td>
</tr>
<tr>
<td>R</td>
<td>TEP recovery (Chapter 3)</td>
</tr>
<tr>
<td>R</td>
<td>Universal gas constant (0.083145 L·bar/mol·K) (Chapter 4 &amp; 5)</td>
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<td>R (%)</td>
<td>Salt rejection (Chapter 5)</td>
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<tr>
<td>R$_1$</td>
<td>Salt rejection at the end of xanthan gum fouling cycle 1</td>
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<td>R$_n$</td>
<td>Salt rejection at the end of xanthan gum fouling cycle n</td>
</tr>
<tr>
<td>R$_{\text{projected}}$</td>
<td>Projected TEP recovery</td>
</tr>
<tr>
<td>SWRO</td>
<td>Seawater reverse osmosis</td>
</tr>
<tr>
<td>T</td>
<td>Thermodynamic (absolute) temperature (K)</td>
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<tr>
<td>TCF</td>
<td>Temperature correction factor</td>
</tr>
<tr>
<td>TEP$_{0.4\mu m}$</td>
<td>TEP equal and greater than 0.4 µm, also denoted as pTEP</td>
</tr>
<tr>
<td>TEP$_{0.1\mu m}$</td>
<td>TEP greater than 0.1 µm but smaller than 0.4 µm, also denoted as cTEP</td>
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<td>TEP$_{10kDa}$</td>
<td>TEP and their precursors</td>
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<td>TEP$_{\text{pre-stain}}$</td>
<td>AB staining prior to filtration by 0.4 µm PC filter</td>
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<tr>
<td>THMs</td>
<td>Trihalomethanes</td>
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<td>TOC</td>
<td>Total organic carbon</td>
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<tr>
<td>UF</td>
<td>Ultrafiltration</td>
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<tr>
<td>v$_n$</td>
<td>Voxel for layer n</td>
</tr>
<tr>
<td>V</td>
<td>Volume of filtered sample</td>
</tr>
<tr>
<td>V$_F$</td>
<td>Final re-suspended sample volume</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>$V_{\text{filter}}$</td>
<td>Volume of the re-suspended filter solution</td>
</tr>
<tr>
<td>$V_{\text{filtrate}}$</td>
<td>Volume of filtrate solution</td>
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<tr>
<td>$V_{\text{initial}}$</td>
<td>Volume of initial surrogate solution</td>
</tr>
<tr>
<td>XG</td>
<td>Xanthan gum</td>
</tr>
<tr>
<td>XG$_{\text{eq}}$</td>
<td>Xanthan gum equivalent</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Solution density (g/L)</td>
</tr>
<tr>
<td>$\varphi$</td>
<td>Osmotic coefficient</td>
</tr>
<tr>
<td>$\sigma_f$</td>
<td>Conductivity of feed solution</td>
</tr>
<tr>
<td>$\sigma_p$</td>
<td>Conductivity of permeate</td>
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<tr>
<td>$\pi_b$</td>
<td>Osmotic pressure at the bulk (Pa)</td>
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<tr>
<td>$\pi_p$</td>
<td>Osmotic pressure on the permeate side (Pa).</td>
</tr>
<tr>
<td>$\Delta \pi_m$</td>
<td>Transmembrane osmotic pressure (Pa)</td>
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CHAPTER 1

Introduction
1.1 Research background

The water crisis is one of the major global issues in 21st century due to increasing demand and limited freshwater resources. Along with climate change, it sounds an alarm of worldwide freshwater scarcity, and this is especially true for Western Australia (WA), the driest place in Australia. Freshwater is crucial to human community and all nations. It is one of the limiting factors for the growth of agriculture, industry, population (human resources and labour cost) and economies through the entire nation. In turn, these facts stimulate widespread development of desalination plants (Gude 2016). However, the major operational challenges for desalination are irreversible membrane fouling, membrane deterioration, and permeate flux decline, causing problems during operation and leading to high capital cost (Jiang et al. 2017; Shi et al. 2014). Among all the fouling problems (inorganic fouling/scaling, colloidal fouling, organic fouling and biofouling), membrane biofouling is the most common issue found in membrane systems (Jiang et al. 2017; Shi et al. 2014). It not only affects separation performance and causes flux decline, but also affects the lifetime of the membrane (Mansouri et al. 2010).

Over the past decade, interest has steadily risen in potential foulant transparent exopolymer particles (TEP) which are a subset of extracellular polymeric substances (EPS) and are released from microbial aquatic organisms (Bar-Zeev et al. 2009a; Berman 2005; Berman et al. 2011; de la Torre et al. 2008). Despite their microbial origin, they contribute to biofouling of membranes even when no viable microorganisms are present (e.g., after disinfection) (Bar-Zeev et al. 2015). Moreover, using conventional (i.e. coagulation and sand filtration) or advanced pre-treatment technology (i.e. microfiltration (MF) and ultrafiltration (UF)), it’s unlikely to
CHAPTER 1

completely remove TEP and TEP precursors from reverse osmosis (RO) feed water (Bar-Zeev et al. 2015). Therefore, membrane fouling caused by TEP and their precursors is unavoidable. Considering their natural abundance in seawater (Alldredge et al. 1993), it is crucial to develop accurate TEP and TEP precursors quantification method, understand their fouling potential in terms of different size fractions, and find cleaning/disinfection agents for TEP and TEP precursors removal from membrane surfaces.

The first TEP quantification method was developed two decades ago (Passow and Alldredge 1995), while quantification methods of TEP in smaller size fractions (i.e. colloidal TEP (0.05 - 0.4 µm) and TEP precursors) were developed since then (Villacorte et al. 2009a, b; Villacorte et al. 2015). They are all Alcian blue (AB) based methods which are indirect measurements of TEP stained by AB and exhibit poor reproducibility due to variable staining ability of AB. Although AB based TEP quantification method is the most commonly used in membrane systems, its accuracy and recovery in different matrices (i.e. seawater, brackish water, and freshwater) are not clear. Other issues regarding TEP fouling potential and cleaning efficiency haven’t been well studied as well.

1.2 Research questions

The first and fundamental question originates from TEP quantification methods. Unlike other polysaccharide substances which are named after their chemical compositions or chemical structures, TEP is a technical term based on specific filtration and staining processes (Passow and Alldredge 1995). To quantify TEP in water systems, TEP surrogates (i.e. xanthan gum (XG) and alginic acid (AA)) are usually used for
calibration (Fatibello et al. 2004; Passow and Alldredge 1995; Thornton et al. 2007; Villacorte et al. 2015). These facts elicit several questions that need to be answered. The first questions arise from the need to quantify TEP and the use of surrogates as a component in this effort. They are:

a. How well do the surrogates mimic TEP including size variants? How well does the behaviour of different surrogates' compare one to another in terms of TEP filtration processes?

b. To what interferences or conditions are the measurements sensitive?

c. How well are the TEP surrogates recovered and size fractionated in the various conditions and methods commonly utilized by researchers?

The second questions, which arise from gaps in understanding TEP fouling behaviour, are:

a. Is there a difference in fouling behaviour (flux decline rate) between different size fractions of TEP in seawater reverse osmosis (SWRO) systems?

b. Is there a difference in fouling behaviour (flux decline rate) between different surrogates of TEP in SWRO systems?

c. How can the differences in behavior be quantified?

The third questions, which are motivated by industry's point of view toward application, are:

a. What is the cleaning performance of hydrogen peroxide on TEP fouled SWRO membranes?
b. At what concentration does hydrogen peroxide best restore membrane permeation flux without causing undue membrane degradation, and what is the trajectory of membrane performance impairment over the course of multiple cleaning cycles on a SWRO membrane?

1.3 Objectives

To answer those questions, the objectives of this research are:

a. To determine the accuracy and recovery of the currently used AB based TEP measurement of XG, pectin (PN), and AA in different size fractions under fresh, brackish and seawater conditions.

b. To quantify the fouling behaviour of TEP surrogates (XG, PN and AA) and the different size fractions of XG (total XG, < 0.4 μm XG and ≥ 0.4 μm XG) on an SWRO membrane.

c. To develop a statistical method for quantification of the fouling potential difference between the three most common TEP surrogates (XG, PN and AA), and between different size fractions of XG (total XG, < 0.4 μm XG and ≥ 0.4 μm XG).

d. To evaluate the cleaning performance of hydrogen peroxide (H₂O₂) on a XG fouled RO membrane.

1.4 Thesis structure

The thesis includes the following chapters:

Chapter 1 presents a brief introduction to general research background, key questions
arising from the background and research gaps, thesis objectives, and the thesis structure.

*Chapter 2* reviews recent literatures addressing (1) significant roles played by TEP in desalination systems; (2) quantification methods of TEP; (3) fouling behaviour of TEP; and (4) membrane cleaning.

*Chapter 3* investigates TEP quantification method recovery for different fractions of TEP using three surrogates (XG, PN and AA) in fresh, brackish and seawater. The surrogates are compared to a natural seawater TEP material and the seawater TEP is also analyzed to compare with previous TEP studies using different calibration factors. The work highlights how the variability of standards and matrix conditions compromise the accuracy of transparent exopolymer particles (TEP) analysis methods. The work concludes that total carbohydrate analysis methods more accurately quantify TEP surrogates and avoid some measurement errors of the AB based TEP method.

*Chapter 4* focuses on the fouling behaviour difference between different size fractions of XG and between different TEP surrogates. Fractionated XG, namely total XG, < 0.4 μm XG and ≥ 0.4 μm XG are used for simulating total TEP, TEP and TEP precursors, respectively. A statistical method is developed for fouling potential difference quantification. An hour-by-hour T-test at one-hour intervals clearly identifies a significant fouling behaviour difference between AA and XG, and between AA and PN. No significant difference is observed between three size fractions of XG. This highlights the importance of TEP precursors in desalination systems, and the need for a new pre-treatment technique for TEP precursors removal.
Chapter 5 investigates cleaning efficiency of H$_2$O$_2$ at concentrations of 0, 1, 10, and 70 mM during five cycles of cleaning-in-place (CIP) of a XG fouled SWRO membrane. Cleaning performance is evaluated from water flux, fouling rate, salt flux, salt rejection, statistical analysis, and membrane surface chemistry perspectives. Over five fouling and CIP cycles, salt rejection reduction is less than 3%. H$_2$O$_2$ is found to enhance both initial and final water flux while causing less than 10% decline in salt rejection. 70 mM H$_2$O$_2$ is the most effective among the cleaning concentrations evaluated.

Chapter 6 summarizes findings of this thesis and recommendations for future work.
Chapter 1. Introduction

References


Jiang SX, Li YN, Ladewig BP. 2017. A review of reverse osmosis membrane fouling


CHAPTER 2

Review of literature
CHAPTER 2

2.1 Introduction

Due to climate change, extreme weather, and increasing population and demand, we are facing a century full of challenge caused by freshwater scarcity. Finding alternative water supplies, such as seawater, is now imperative. Production of freshwater by desalination is projected to increase to 97.5 million m$^3$/d by 2015 (Bennett 2013). The growth of desalination industry is blooming worldwide, especially in Saudi Arabia, United States, and Australia (Gude 2015). However, the application of desalination is limited due to its energy consumption and membrane fouling. To mitigate membrane fouling and develop control strategies, better understanding of causes of fouling, membrane fouling mechanisms, and membrane cleaning options is required. Membrane biofouling is considered as the most challenging and inevitable fouling type compared to inorganic, organic, and particulate /colloidal fouling, and is found extensively in RO systems even after pre-treatment and disinfection (Flemming et al. 1997; Matin et al. 2011). In order to elucidate materials that initiate biofouling, bacteria and extracellular polymer substances (EPS) have been studied extensively. Recently, a subset of EPS, namely transparent exopolymer particles (TEP), has drawn attention due to TEP’s abundance in ocean, gel-particle nature (i.e. self-assembling, aggregation, adhesion, and ability to be retained on membrane), and identification as food source for microbials (Bar-Zeev et al. 2015; Passow 2002a; Verdugo 2012). Their role in initiating biofilm formation and their occurrence in desalination systems have been investigated (Bar-Zeev et al. 2015; Berman et al. 2011; Le Lan et al. 2015). TEP is an operationally defined substance whose concentration and presence are based on size fractionation processing and a functional group staining method. TEP does not have an overall defined chemical structure or a unique composition, but simply exhibits a certain filterability (albeit one that varies significantly with water composition) and a
measurable concentration based on the concentration of stainable functional groups (albeit, only loosely and variably related to the total mass or reactivity of the substance present). Consequently, there is a need to get a better understanding of (1) what is measured (2) the fouling potential in terms of different size faction, and (3) the cleaning of TEP fouled membranes without undue membrane performance loss.

2.2 TEP

2.2.1 History and definition of TEP

Fig. 2.1. In situ observation of planktonic protobiofilm (a and c) and uncolonized TEP (b and d) in seawater passing through a flow cell (Bar-Zeev et al. 2012b).

TEP have long been ignored by scientists because of their transparency, making them hard to be observed under light microscope. Transparent marine particles were first detected by staining coupled electron microscopy method by Gordon in 1970 (Gordon 1970). Until 1993, their abundance, origin, and significance were noticed by scientist
due to the development of TEP quantification methods. It was first defined as gel-like acidic polysaccharide particles that retained on 0.4 μm polycarbonate filters which can be stained by AB at pH 2.5 (Fig. 2.1) (Alldredge et al. 1993). Their semi-quantification method was developed thereafter (Passow and Alldredge 1995). Accordingly, TEP is an operationally defined entity other than defined by their chemical composition or physical characteristics.

2.2.2 Role of TEP in marine systems

Fig. 2.2. TEP plays a critical role in the sequestration of CO$_2$ from the atmosphere, into organic carbon in the ocean. (a) Under present level of CO$_2$, and (b) Enhanced CO$_2$ concentrations due to uptake of CO$_2$ by phytoplankton (Arrigo 2007).

After discovering these carbon-rich gel-particles in marine and fresh water, the role of TEP in biogeochemical cycling processes and climate change has been studied extensively for the past two decades (Fig. 2.2) (Arrigo 2007, Buchan et al. 2014, Engel

In general, the significance of TEP are: (1) TEP provide a site that allows bacterial attachment and serve as food web structure for bacteria growth and biofilm formation; (2) TEP help cycling of metals, organic substances and nutrient elements by complex interaction and aggregation (Decho 1990; Passow 2002a); (3) TEP act as an abiotic pathway for converting dissolved organic carbon (DOC) to particulate organic carbon (POC) (Arrigo 2007; Verdugo et al. 2004); (4) TEP coagulate with other particles into marine snow aggregates; and (5) TEP increase the sinking rate of particulate matter by sedimentation (Engel et al. 2004; Mari and Kiorboe 1996).

2.2.3 TEP formation

TEP can be produced either biotically or abiotically (Fig. 2.3). In terms of the biotic pathway, TEP are formed as “chemical gel” via the release of particulate material by bacteria and phytoplankton (Passow 2002b) which are characterized by covalently crosslinked and irreversibly attached biopolymers (Mari et al. 2005; Verdugo 2012). Accordingly, TEP are considered as a subclass of extracellular polymeric substance (EPS) in marine and freshwater systems.

The TEP abiotic formation pathway (Fig. 2.3) is basically a spontaneous formation from dissolved precursor substances or aggregation, similar to self-assembled gels (Fig. 2.4) (Chin et al. 1998; Verdugo 2012). It is considered as the predominant pathway leading to “physical gel” formation (Verdugo 2012). The abiotic pathway starts with
release of dissolved fibrillar polysaccharides from various planktonic organisms (extracellular release) (Engel et al. 2004, Passow 2002a, Villacorte et al. 2013), and lysis or breakage of cells through bacterial or viral infections (intracellular release) (Passow 2000, 2002a, 2012; Verdugo 2012). These fibrils are 1 - 3 nm in diameter and 100 s of nanometres long (Leppard et al. 1977), which enables TEP precursors to pass through 8 KDa pore size membranes (Passow 2000). Then nano- and micro-gels are formed from free fibrillar polysaccharides by weak hydrophobic and ionic bridging interactions, resulting in a 3-dimensional (3D) tangled polymer network. The structure of such gels is further stabilized by cation bridging (especially Ca$^{2+}$ (Fig. 2.5)) and hydrogen bonding (Chin et al. 1998; Verdugo 2012).

![Fig. 2.3. TEP formation pathways (Bar-Zeev et al. 2015).](image-url)
Fig. 2.4. Kinetics of dissolved organic carbon (DOC) self-assembly (Verdugo 2012).

Fig. 2.5. Dissolved organic carbon (DOC) assembly and formation of self-assembled microgels (SAG) that are stabilized by entanglements and Ca\(^{2+}\) bonds (Verdugo 2012).

2.2.4 TEP properties

2.2.4.1 Chemical composition

The major components for EPS are polysaccharides and other organics such as amino acids, amino sugars, phosphate, pyruvate, and acyl groups, uronic acids, and glycoproteins, which are excreted by bacteria, phytoplankton, macroalgae and fish (Decho 1990; Hoagland et al. 1993). The diverse origins from marine systems lead to variations in chemical composition of EPS. Even among diatoms, the chemical composition of released substances varies both with species and physiological stage (Myklestad 1977). Being in the same category of EPS, the exact chemical composition
of TEP is also unclear. Hence TEP are accepted as a chemically diverse group of particles, which depends heavily on its origins (Engel and Passow 2001).

Acid polysaccharides are typically referenced as the components of TEP (Discart et al., 2015; Villacorte et al., 2015). For marine samples, the majority of the acidity of TEP comes from the presence of sulphate half-ester groups (R-OSO$_3$) (Zhou et al. 1998), while only traces of uronic acids (R-COO$^-$) are found in TEP (Mopper et al. 1995). In contrast, sulphated polysaccharide mucilage is rare in the exopolymers of fresh water organisms (Kloareg and Quatrano 1988). Other studies show that surface active polysaccharides and TEP formed by bubble coagulation of seawater are enriched in the deoxy sugars, fucose and rhamnose (Mopper et al. 1995; Zhou et al. 1998). These findings suggest that the formation mechanisms of TEP may differ, but the predominant sugars in TEP are the same (Passow 2002a).

### 2.2.4.2 Physical characteristics

TEP are distinguished from other EPS for their particulate properties rather than dissolved molecules or coatings (Alldredge et al. 1993). They can undergo reversible phase transitions between condensed and hydrated phases (Tanaka 1981). Consequently, depending on environmental factors (such as pH, temperature, pressure and ion density), they may change in size and volume without significant changes in mass (Passow 2002b, Verdugo 2012). This high flexibility allows them to pass through pores many times smaller than their apparent size, therefore posing a challenge for water pre-treatment technology. TEP also exhibit high stickiness, owing to metal ion bridges and hydrogen bonds that are formed by sulphate half-ester groups (-OSO$_3$).
2.3 TEP quantification methods

In general, TEP are quantified through three steps: (1) without filter or pre-filter water sample, (2) treat with a single stain or a double stain, and (3) detect by a microscope or a spectrophotometer. Different methods for TEP quantification mostly measure different fractions, in terms of their size and compositions (Discart et al. 2015). It is important to know (1) which TEP size fraction the method is measuring, and (2) at what pH the TEP are stained to compare TEP concentration results from different research groups. It is also important to keep in mind that TEP particle size is more of an operational concept than a hard-solid characterization, because TEP as well as surrogates are deformable chain organic molecules.

The following section focuses on the Alcian blue (AB) staining mechanism which is the most commonly used stain for TEP, and summarizes AB based TEP quantification methods according to TEP sizes: pTEP, cTEP and TEP precursors.

2.3.1 Alcian blue (AB)

AB is a family name for polyvalent basic dyes. Among the AB family (AB 2GX, AB 5GX, AB 7GX and AB 8GX), the most accepted and principally used by market is AB 8GX (Scott et al. 1964). Alcian is a trademark of the manufacturer ICI. It was first used in 1950 by Steedman for mucin staining (Steedman 1950). Since then it has been widely used in histology for biological cell and tissue structure study (Discart et al. 2015), namely mucin stain. Mucins are a family of high molecular weight glycosylated protein with multiple chains of carbohydrates (polysaccharide) that secrete from epithelial
tissue, such as gastrointestinal, respiratory and reproductive tract, which exhibit gel properties (Marin et al. 2000). Although the use of AB became very popular right after 1950, its structure was kept a top secret by the dye industry over the subsequent two decades. In 1972, Professor Scott revealed the structure of AB (Fig. 2.6) which was confirmed by ICI in 1973 (Scott 1973).

![Fig. 2.6. Molecular structure of Alcian blue 8GX (Scott 1973).](image)

AB is a bulky molecule composed of a copper phthalocyanine nucleus and four basic isothiouronium side chains (Discart et al. 2015; Scott 1973). The lower the number for the AB family member (AB 2GX, AB 5GX, AB 7GX), the fewer the isothiouronium groups. For example, AB 7GX carries fewer isothiouronium groups than AB 8GX. The copper in the center is responsible for its blue pigment and the side isothiouronium groups each carry a positive charge so that AB is able to bind with anions by electrostatic force (Scott et al. 1964). Also, the side chains in a positive charged state keep AB soluble in water (Scott et al. 1964). Isothiouronium chains are highly selective...
for binding with carboxylated (-COOH) and sulfated (-OSO$_3$) polyanions at proper pH. AB forms a stable and insoluble salt that allows permanent staining and cannot be destained by subsequent harsh treatment (Discart et al. 2015; Quintarelli et al. 1964; Scott et al. 1964). In other words, AB doesn’t stain neutral substances and its staining ability is restricted by pH and the pK$_a$ of target groups. For example, it stains both carboxyl and sulphate mucosubstances at pH 2.5, but it only stains sulphated compounds at pH 1.0 and 0.5 (Lev and Spicer 1964). In general, AB is used specifically for negatively charged polysaccharides (Decho 1990).

Because of the excellent contrast of blue in AB, other stains (i.e. Alcian yellow and basic red 18) could not substitute the use of AB in histochemical. However, the major disadvantage of AB staining is that it doesn’t stain reproducibly (Quintarelli et al. 1964). Moreover, the presence of salt (such as in marine samples), creates artifacts (Discart et al. 2015). Therefore, pre-treatment of marine samples is needed prior to AB staining.

2.3.2 TEP surrogates

Fig. 2.7. Molecular structure of (A) xanthum gum (XG) and (B) alginic acid (AA) (Discart et al. 2015).
AB staining reproducibility suffers due to batch variation (in purity and solubility) as well as aging of dye content. (In practice reaggregated dye particles are removed during prefiltration.) Therefore, surrogates are needed to calibrate the AB staining solution for each batch of TEP quantification (Passow and Alldredge 1995). Ideal standard surrogates must satisfy two criteria: (1) Dissolve in water, form gel-like particles, and be retained by filters; (2) Exhibit a high enough staining capacity by AB to be measurable by colorimetric method; and (3) The mass of particles must be high enough for measurement (i.e. dry weight (Fatibello et al. 2004; Passow and Alldredge 1995), TOC methods (Villacorte et al. 2009a, b; Villacorte et al. 2015), and phenol-sulfuric acid assay (Thornton et al. 2007)).

Among different carbohydrates and proteins, XG and AA have been used extensively as TEP surrogates. While XG is the most popular TEP surrogate due to its better replicability in TEP assays (Passow and Alldredge 1995; Thornton et al. 2007; Villacorte et al. 2015), AA was suggested to be more representative to APS produced by microorganisms in marine environments (Hung et al. 2003).

2.3.3 pTEP and cTEP quantification methods

2.3.3.1 Microscopic method

The first quantitative microscopic determination method of pTEP was developed by Alldredge et al. in 1993. Fresh seawater sample is first filtered through a 0.4 μm Nuclepore filter. The retained particles are then stained by 0.06% acetic acid and 0.02% alcian blue for less than 2 seconds. Following filter-transfer-freeze (FTF) technique (Hewes and Holmhansen 1983), the stained particles are transferred to a slide and then observed and counted under a standard light microscope at 200 × magnification. TEP
concentration is expressed as the number of particles or total surface area covered by TEP per milliliter of water sample. However, the transfer efficiency of FTF is low and unstable that causes many problems (Discart et al., 2015), including (1) possible artefacts, (2) low contrast of stained particles, and (3) laborious, slow and complex processing.

The FTF microscopic method was simplified by Logan et al. in 1994 so that the stained TEP particles (≥ 0.2 μm) can be observed directly on filters using clearing slides. Despite a low contrast disadvantage, subsequent studies using microscopic method proved to be successful in TEP quantification (Berman and Viner-Mozzini 2001; Mari and Kiorboe 1996).

2.2.3.4 Spectrophotometric method

The most widely used particulate TEP (pTEP, a.k.a. TEP_{0.4μm}) quantification method is the spectrophotometric method which was developed by Passow and Alldredge in 1995. Same as microscopic method, seawater samples are pre-filtered through a 0.4 μm polycarbonate filter at a constant vacuum of 0.2 bar. Salt moisture is removed by rinsing with DI water prior to AB staining. Then pre-filtered 0.02% AB solution in 0.06% acetic acid (pH 2.5) is added and reacted for 10s. Excess dye is rinsed off using DI water. The filter is then soaked in 80% sulfuric acid for 2 hrs. Finally, the absorbance of stained material in sulfuric acid is measured at 787 nm wavelength. In this method, surrogates (i.e., XG, AA) are used for TEP calibration, and TEP concentration is expressed as surrogate equivalents (mg/L surrogate_{eq}). The procedure for measuring pTEP (TEP_{0.4μm}) is shown in Fig. 2.8.
In 2009, Villacorte et al. proposed the term pTEP and cTEP based on the International Union of Pure and Applied Chemistry (IUPAC) definition, and modified the Passow and Alldredge method to quantify smaller TEP size fractions by serial filtration using 0.4, 0.2, 0.1 and 0.05 μm polycarbonate filters (Villacorte et al. 2009b). Such categorization is a significant improvement since cTEP (82 - 93%) was found to contribute more to total TEP than pTEP (7 - 18%) in seawater (Villacorte et al. 2009a).

Another difference to the Passow and Alldredge method is the calibration method. Instead of weighing the mass of XG on filter, TOC measurement was used to quantify XG mass.

![Fig. 2.8. Procedural diagram for measuring TEP0.4μm (Villacorte et al. 2015).](image)

Although spectrophotometric method is used extensively in TEP quantification, the following limitations should be noted: (1) still laborious, (2) some particles still difficult to see on the filter, (3) weighing method for calibration is very difficult because of the
very low weight of XG being used, and (4) absolute TEP concentration cannot be obtained from this method.

The above two methods that stain TEP on the filter. Another two methods stain TEP in solution using pre-filtered 0.02% AB solution and 0.06% acetic acid at pH 4 or pH 2.5 (Fatibello et al. 2004; Thornton et al. 2007). It’s worth noting that other than particle size, the composition of stained TEP may differ from the Passow and Alldredge method due to a different staining pH. Prior to staining, the water sample may be first filtered or left unfiltered. The stained solution is then centrifuged or filtered through a 0.2 μm surfactant free cellulose acetate (SFCA) membrane. The absorbance of the supernatant/filtrate is measured at 620 nm or 610 nm. In these methods, concentration of pTEP is inversely proportional to absorbance value and is expressed as XG equivalents (mg XGeq/L). Because of the direct staining of TEP in solutions, it is only applicable to quantify TEP in freshwater samples. Unless coupled with pre-treatment (i.e. dialysis) to remove salt components, this method is not suitable for seawater samples.

2.3.4 TEP precursors quantification methods

A modified colorimetric method was developed for quantification of TEP$_{10kDa}$ which include both pTEP and TEP precursors (Villacorte et al., 2015). First, the water sample is filtered through 10kDa regenerated cellulose filters using a syringe. This is followed by injection of air and DI water, which ensures all sample liquid is filtered as well as removes residual salt in water sample. The filter retained TEP material is then resuspended in DI water by soaking the filter in DI water, vortexing and sonicating for 2 hrs. Acetic acid is added to resuspended the solution to reach a final pH of 2.5. The
pre-filtered AB is then added. After staining, the solution is filtered through a 0.1 μm polycarbonate membrane filter using a syringe. The absorbance of the filtrate is measured at 610 nm wavelength. The concentration of TEP_{10kDa} is inversely proportional to absorbance value and is expressed as XG equivalents (mg XG\textsubscript{eq}/L). The procedure for measuring pTEP and TEP precursors is shown in Fig. 2.9.

![Procedural diagram for measuring TEP\textsubscript{10kDa} (Villacorte et al. 2015).](image)

**Fig. 2.9.** Procedural diagram for measuring TEP\textsubscript{10kDa} (Villacorte et al. 2015).

### 2.4 TEP and membrane fouling

#### 2.4.1 Desalination

To meet increasing demands for freshwater and ease water stress, desalination technology has been developed since 1950s. It has been widely used for municipal, industrial, irrigation and other purposes (Fig.2.10B). Desalination is a process using thermal energy and/or electricity power to remove dissolved salts from saline water (i.e. seawater and brackish water). It can be classified as either of two processes: thermal
process or membrane process (Fritzmann et al. 2007; Gude 2016; Khawaji et al. 2008). Thermal processes involve evaporation and condensation to produce freshwater from feedwater (i.e. solar distillation (SD), multi-effect distillation (MED), multi-stage flash distillation (MSF), thermal vapor compression (TVC) and mechanical vapor compression (MVC)). Membrane processes, including electrodialysis (ED) and reverse osmosis (RO), use membranes to remove dissolved salts and microbial as well as organics from feedwater. Other techniques combine both thermal and membrane processes to produce potable water (i.e. membrane distillation (MD), RO-MSF, and RO-MED). Alternatively, renewable energy such as solar, wind, geothermal and wave can be used as an energy source for desalination systems for long term sustainability (Gude 2015).

Fig. 2.10. Desalination industry by (A) Technology, (B) Users, and (C) Cost components (Gude 2016).
2.4.2 Reverse osmosis

Today, RO is the leading technology in desalination industry, representing 63% of the total (Fig.2.10A). It also shows competitiveness in cost compared to MSF and MED (Fig.2.10C). RO is a pressure driven process in which the applied external pressure must be higher than the osmotic pressure of feedwater to allow water to pass through the membrane. For seawater, where the osmotic pressure is around 2300 to 2600 KPa and can be as high as 3500 KPa, the applied feed pressure typically ranges from 6000 - 8000 KPa (Greenlee et al. 2009).

A semi-permeable membrane is used to reject dissolved salts (i.e. sodium, chloride), small organic molecules and bacteria. Over the last decades, salt rejection for seawater RO (SWRO) membranes has increased to more than 99% and can achieve as high as 99.8% under standard test conditions (32,000 mg/L NaCl, 5.5 MPa, 25 °C, pH 8, 8% recovery) (Fritzmann et al. 2007; Misdan et al. 2012; Reverberi and Gorenflo 2007).

Salt rejection is typically used in evaluation of RO membrane performance:

\[ R_s = \left(1 - \frac{C_p}{C_F}\right) \times 100\% \]

where \( R_s \) is percent salt rejection; \( C_p \) is permeate salt concentration; \( C_F \) is feed water salt concentration.

RO membrane performance can also be measured by salt flux through the membrane:

\[ J_s = B(C_F - C_p) \times 100\% \]

\[ B = \frac{D_s K_s}{l} \]

where \( J_s \) is salt flux across the membrane, \( B \) is a constant that depends on membrane characteristics, \( D_s \) is salt diffusivity through the membrane, \( K_s \) is the salt partition coefficient between the solution and membrane phases, and \( l \) is the membrane thickness.
Unlike porous filters, RO membranes do not have distinct pores (Greenlee et al. 2009). The structure of the material is web-like, layered polymer which is asymmetric containing one polymer layer or composite layer (Greenlee et al. 2009; Malaeb and Ayoub 2011). The most widely used materials in RO are cellulose acetate (CA) and polyamide (PA) membranes. PA membranes used in SWRO are typically thin-film composite membranes that consist of a PA active layer and a polysulfone supporting layer. While PA membranes have higher salt rejection and produce higher water flux, they are more hydrophobic and susceptible to fouling, and have very low tolerance to chlorine compared to CA membranes (Crittenden et al., 2012). Commercial RO membranes are typically available as spiral-wound and hollow fiber modules, which can be used in dead-end and crossflow filtration (Fig. 2.11) (Malaeb and Ayoub 2011; Ruiz-Garcia et al. 2017).

Two limiting factors to the RO process are membrane lifetime and permeate flux decline caused by concentration polarization and membrane fouling (Fig. 2.12). Under operation conditions, solute concentration near the membrane surface is higher than the concentration in the bulk solution. This phenomenon is called concentration polarization. It is caused by building up of salt solutes which results in increased osmotic pressure (decrease in net driving force), reduced permeate water flux, reduced salt rejection, or even precipitation/scaling when the concentration locally exceeds the solubility limit (Crittenden et al., 2012).
Fig. 2.11. Comparison of RO filtration modes: dead-end and cross-flow (Ruiz-Garcia et al. 2017).

Fig. 2.12. A schematic representation of concentration polarization and fouling at the membrane surface (Goosen et al. 2004).

### 2.4.3 Membrane Fouling

Membrane fouling is caused by accumulation of foulants on membrane surface and/or pore clogging within the membrane pores (Van der Bruggen et al. 2003). Surface fouling is more frequent for RO processes, because RO membranes do not have distinct pores and are considered as nonporous membranes (Greenlee et al. 2009). It includes...
(1) inorganic fouling/scaling, (2) organic fouling, (3) particulate/colloidal fouling, and (4) biofouling (microbial/biological fouling) (Flemming 2002; Nguyen et al. 2012).

Fig. 2.13. SEM of four fouling types on membrane surfaces. (A) Biofouling (B) Organic fouling (C) Inorganic scaling (D) Colloidal fouling (Jiang et al. 2017).

SEM images of four fouling types on membrane are shown in Fig. 2.13. Inorganic scaling occurs when solutes such as calcium, silica, phosphate and carbonate ions are supersaturated (saturation index greater than one) (Malaeb and Ayoub 2011). Organic fouling is caused by deposition of organic matter, such as humic substances, polysaccharides, proteins, lipids, nucleic acids and amino acids, organic acids, and cell components (Cho et al. 1999; Flemming 2002; Jeong et al. 2016). Particulate/colloidal fouling occurs when particulate matter accumulates on RO membranes, which forms a cake layer and adds resistance to flow (Crittenden et al., 2012). Particulate fouling is a concern in RO as a backwashing step is not included during operational cycle. However, coagulation and filtration as well as advanced pre-treatment (i.e. UF and MF) are
usually installed to reduce particulate fouling (Malaeb and Ayoub 2011). Biofouling is caused by organic, typically microbial, attachment to membrane surface, which forms biofilms containing microbial cells as well as EPS produced by the microbes (Greenlee et al. 2009; Ivnitsky et al. 2005).

In practice, membrane fouling is not restricted to one type of fouling, i.e. SWRO membranes are primarily fouled by organic, particulate, and biological fouling (Greenlee et al. 2009; Jiang et al. 2017).

2.4.4 TEP and biofilm formation

Understanding fouling types is essential for finding appropriate pre-treatment or post chemical cleaning reagents to mitigate membrane fouling. For the interest of this research, we focus on biofouling formation mechanism, particularly TEP caused biofouling.

The classic concept of biofilm formation on a membrane surface involves five steps (Fig. 2.14): (1) reversible attachment, (2) irreversible attachment, (3) cell proliferation, (4) biofilm maturation, and (5) cell death and biofilm dissolution. At first, bacteria cells reversibly attach to the membrane surface via van der Waals, hydrophobic, and hydrogen bonding interactions that overcome electrostatic repulsive forces (Hori and Matsumoto 2010; Redman et al. 2004). As cells excrete self-produced EPS that helps binding to the surface, irreversible attachment by hydrophobic interactions, strong dipole-dipole forces, and hydrogen bonding of polysaccharides to the surface evolves (Hori and Matsumoto 2010). Then cell proliferation begins in specific areas over subsequent hours and days, resulting in formation of a mature biofilm. Cell death and dispersal occurs after that, leading to dissolution of the center of microcolonies and
leaving behind remnant structures. This may happen over days and months.

Fig. 2.14. Stages in the formation of biofilms (Mansouri et al. 2010).

A revised paradigm (Fig. 2.15) was proposed involving TEP precursors, TEP, and protobiofilms (Bar-Zeev et al. 2012b). The first stage is the adsorption of TEP precursors to the surface very soon after membranes are exposed to TEP-containing water. This results in a conditioning layer that significantly enhances bacteria and “food” (i.e. polysaccharides, proteins, lipids, nucleic acids and etc) attachment by hydrophobic interactions and hydrogen bonding (de Kerchove and Elimelech 2007; Hwang et al. 2013). Meanwhile, additional TEP becomes part of the EPS layer, which in the early stage may originate from TEP itself (Bar-Zeev et al. 2009a; Bar-Zeev et al. 2015).
2.4.5 TEP pre-treatment

Pre-treatment is essential to a SWRO desalination process. It aims to improve RO feedwater quality and mitigate membrane fouling by removing undesirable large particles, organics, and bacteria. Techniques include chlorination, coagulation, acid addition, multi-media filtration, micron cartridge filtration (i.e. MF and UF), and de-chlorination. The choice of pre-treatment is highly dependent on source water quality, membrane type, and product water quality (Khawaji et al. 2008).

2.4.5.1 pTEP removal

Conventional TEP removal technologies are coagulation and rapid sand filtration (RSF), either used alone or in combination with ultrafiltration (UF) or microfiltration (MF). The TEP removal efficiency of coagulation-RSF pre-treatment varies in different
studies (with up to 80% TEP removal) mostly because of the variation of TEP size distributions and concentrations of TEP and other components in the feed water (Bar-Zeev et al. 2009a; Bar-Zeev et al. 2012a; Kennedy et al. 2009; Villacorte et al. 2009b). Compared to algae, TEP are not easily removed by filtration due to their elastic and gel properties (Bar-Zeev et al. 2009a; Villacorte et al. 2009a). Higher TEP removal efficiencies (70%) were obtained under a high coagulant dose (aluminium sulphate at a concentration of 10 mg Al\textsuperscript{3+}/L) compared to a low dose of 1.5 mg Al\textsuperscript{3+}/L (27% TEP removal) (Kennedy et al. 2009). Additionally, it is reported that a granular activated carbon (GAC) pilot system was able to remove 84% of TEP (Naidu et al. 2013).

UF membranes usually have a pore size of 0.01 - 0.1 \( \mu \)m and MF have a pore size of 0.1 - 1\( \mu \)m. In theory, pTEP are much bigger than the UF membrane pore size, so they couldn’t get through them and be able to reach the subsequent RO unit. But in fact, studies showed moderate TEP removal (35 - 66%) by using MF (Villacorte et al. 2009a, b; Villacorte et al. 2010), and complete (Kennedy et al. 2009; Van Nevel et al. 2012), or partial measurable TEP removal (Gasía-Bruch et al. 2011; Van Nevel et al. 2012; Villacorte et al. 2009b; Villacorte et al. 2010) by using UF. It is postulated under low transmembrane pressure (typically between 0.1 - 5 bar), TEP are fragmented by hydrodynamic shear forces, pass through the filter as nanoscale TEP, and are reassembled in the downstream before reaching the subsequent RO unit (Bar-Zeev et al. 2015). This makes them detectable in the RO feed water (Bar-Zeev et al. 2009a; Villacorte et al. 2009b; Villacorte et al. 2010).

Lately, several new approaches have been developed for reducing TEP concentrations in feed water. A self-cleaning, automatic microfiber filter (AMF), with a nominal cut
off ranging between 2 and 20 μm, was proven to remove TEP, chlorophyll (Chl), particles, turbidity and total suspended solids (TSS) from a lake water source (Eshel et al. 2013). After water filtration, biofilm formation was markedly inhibited for 30 days according to observation by confocal laser scanning microscopy. However, TEP removal efficiency is much lower (47 ± 21%) compared to other substances such as Chl (90 ± 3%). In a subsequent study, it is pointed out that the decrease of TEP concentration by using AMF may result from a change of microbial community composition in biofilm within different time frames (Lakretz et al. 2014). It is worth noting that in these two studies, cTEP is not included and only pTEP is measured. Therefore, the removed portion of TEP is unlikely to be TEP precursors, cTEP or smaller pTEP, but could be TEP colonized bacteria (“protobiofilm”) as proposed by Bar-Zeev (Bar-Zeev et al. 2009a; Bar-Zeev et al. 2015). Another recent technique used is a nanoalumina depth filter (Disruptor) (El-Azizi et al. 2011; Komlenic et al. 2013). It is an electropositive, submicron polishing medium that removes submicron contaminants such as TEP by adsorption. This nanofiber filter has a mean flow pore size of 0.7 μm. It is shown that its TEP removal efficiency for seawater is around 80% and substantially reduces biofilm formation in a SWRO system (El-Azizi et al. 2011). The removal particles are bacterial colonized TEP (Bar-Zeev et al. 2009b). However, a later study using a similar system showed varied TEP removal efficiencies ranging from 36% to 82%, indicating a high variability of TEP removal efficiency even for the same source water (Komlenic et al. 2013). A technology, namely rapid bioflocculation filter (RBF), based on the adhesive properties of TEP and EPS was tested as an alternative way to RSF as pre-treatment in SWRO systems (Bar-Zeev et al. 2013). The idea is using TEP and EPS as chemical free bioflocculants. While TEP removal efficiency was low (26 ± 25%), Chl, silt density index (SDI), and other parameters were comparable
to those of using chemical coagulants $[\text{Fe}_2(\text{SO}_4)_3]$ in conventional RSF during one-year operation.

### 2.4.5.2 TEP precursors removal

Due to their small size, TEP precursors pose a great challenge for pre-treatment technology and lead to fouling issues in a subsequent RO system (Villacorte et al. 2009a). Conventional pre-treatment, including a combination of coagulation and sand filtration, removed 67% of the total TEP (Van Nevel et al. 2012). By using UF, TEP precursors removal increased to 88 - 97% (Van Nevel et al. 2012; Villacorte et al. 2009a). MF showed a lower TEP precursors removal efficiency, ranging from 49 - 80% (Villacorte et al. 2009a, b). In general, TEP precursors are the fractions most difficult to be removed by pre-treatment process, although they haven’t been paid much attention compared to pTEP.

### 2.4.6 Significance of TEP to membrane systems

Direct or indirect evidence of roles and responsibilities of TEP for membrane biofouling are listed below:

1. Cake fouling potential. Autopsies of SWRO membranes from desalination facilities and pilot plants and bench-scale fouling studies revealed the cake fouling potential of TEP (Lee et al. 2015; Villacorte et al. 2009b). This cake layer contains biopolymers, bacterial clusters, algae, and fungal cells (Bar-Zeev et al. 2015; Gasia-Bruch et al. 2011; Khan et al. 2013). It is likely that these fouling components are partly mediated by TEP and TEP precursors from the RO feed (Wu et al. 2013).
(2) Enhanced bacterial deposition on RO membranes with increased cake fouling potential of TEP (Lee et al. 2015).

(3) TEP association with organic, biofouling, particulate/colloidal fouling in RO membrane systems. This association is of high importance for flux-decline (Berman et al. 2011; Discart et al. 2013).

(4) Effect of membrane microbial community composition, and in turn, low nutrient levels and a high N/P ratio stimulate a higher TEP production by microbes (Lakretz et al. 2014; Le Lan et al. 2015).

2.5 Membrane cleaning

Despite RO feedwater quality having greatly improved because of development of advanced desalination pre-treatment technologies (i.e. MF and UF), complete foulants removal is not guaranteed. Therefore, periodic cleaning is often routinely employed in SWRO plants with the aim to remove accumulated foulants through physical, chemical and biochemical (enzymatic) processes (Al-Juboori and Yusaf 2012; Jiang et al. 2017; Regula et al. 2014) and to recover or maintain water flux and permeability (Filloux et al. 2015; Porcelli and Judd 2010). MF and UF membrane cleaning are relatively low-cost activity compared to pre-treatment, and is applied as an in-situ cleaning process (i.e. chemically enhanced backflush (CEB), cleaning in place (CIP), and cleaning in air (CIA)) or an ex-situ cleaning process (Goh et al. 2018; Porcelli and Judd 2010; Wang et al. 2014).

2.5.1 Physical and chemical cleaning

Physical cleaning, which is applied predominantly only to MF and UF membranes, dislodges and removes loosely attached deposits on membrane surfaces (“reversible
fouling”) by utilizing hydraulic, mechanical and electrical forces (Regula et al. 2014; Shi et al. 2014). It includes backwashing, pulsing, forward flushing with air and sonication (Goh et al. 2018). In practice, rinsing with water is the most used physical cleaning method (Jiang et al. 2017). Chemical cleaning uses alkaline, acidic, chelating, and oxidant agents to remove fouling by changing electrostatic repulsion between the foulants and membrane or by decomposing foulants into solution (Porcelli and Judd 2010; Shi et al. 2014; Varin et al. 2013). Biochemical (enzymatic) agents are used to remove specific biopolymers (i.e. proteins and lipids) (Wang et al. 2014). To improve cleaning efficiency, a combination of different cleaning methods is typically used in the desalination industry. Cleaning protocols are often provided by the membrane manufacturer and the composition of commercially available cleaning agents is often not disclosed to consumers.

Chemical cleaning usually involves six steps (Porcelli and Judd 2010; Shi et al. 2014; Wang et al. 2014): (1) bulk reaction (hydrolysis, ionization and other reaction) of cleaning agents, (2) transport of cleaning agents to membrane surface, (3) transition through foulant layers to membrane surface, (4) reaction with foulants, (5) transport of modified foulants to interface, and (6) transport of modified foulants to bulk solution. Generally, alkaline agents (i.e. sodium hydroxide) are used to remove organics and biofilms; acidic agents (i.e. hydrochloric acid, nitric acid and sulfuric acid) are used to remove inorganic salts and metal oxides; chelating agents are used to remove metal; oxidizing agents (i.e. chlorine-based agents and hydrogen peroxide) are used to remove organics and biological materials through oxidation and disinfection; and enzymatic agents are used to remove proteins and lipids (Jiang et al. 2017; Porcelli and Judd 2010; Shi et al. 2014).
The factors that influence membrane cleaning efficiency include cleaning types, chemical agents and operation conditions (i.e. filtration fluxes, temperature, agent concentration, agent sequence and soaking time) (Wang et al. 2014). It has to be noted that frequent membrane cleaning, inappropriate cleaning types (i.e. in-situ or ex-situ), cleaning agents (i.e. harsh (hypochlorite) or moderate chemicals agents) and cleaning conditions (i.e. pH, temperature,) can shorten membrane lifespan. Therefore, correct identification of fouling types, careful selection of cleaning techniques/agents and optimization of cleaning conditions are crucial for successful membrane cleaning and maintenance of membrane integrity and operational lifespan.

2.5.2 Chlorine-based agents and hydrogen peroxide

Free chlorine-based agents (i.e. hypochlorous acid (HOCl), chloramines (i.e. monochloramine (NH₂Cl), and chlorine dioxide (ClO₂)) are the most commonly used cleaning and disinfection agents targeting organic and biological foulants on SWRO membrane surface (Matin et al. 2011). By adding oxidants, functional groups of organic foulants are oxidized to ketonic, aldehydic, and carboxylic groups, which are more hydrophilic and less adhesive to membrane surface. In addition, microorganisms are killed (Porcelli and Judd 2010; Wang et al. 2014). As a result, oxidized products as well as dead cells are detached from membrane surface. Although the majority of RO membrane cleaning/disinfection studies focus on chlorinated oxidants (Gohil and Suresh 2017; Gu et al. 2012; Verbeke et al. 2017), their drawbacks include (Richardson et al. 2007; Shi et al. 2014; Wang et al. 2014): (1) membrane swelling; (2) membrane damage caused by changes in membrane functional groups, mechanical properties, and physical structure, and (3) disinfection byproducts (i.e. absorbable organic halogen
(AOX), trihalomethanes (THMs), and N-nitrosodimethylamine (NDMA)) which cause adverse environmental and health effects.

The major advantage of hydrogen peroxide is that RO membranes have been observed to be more tolerant to hydrogen peroxide compared to chlorinated-based oxidants (Abejon et al. 2013). In addition, peroxide doesn’t produce documented toxic byproducts and is more environmentally friendly (Ling et al. 2017). However just as with other strong oxidants, there is a safety issue in that precautions are required for chemical storage. In some industrial applications, hydrogen peroxide combined with peracetic acid is used for membrane cleaning. Such combination poses a high risk of explosion, causing safety issues and increasing costs (Brepols et al. 2008). So far, far less studies have focused on hydrogen peroxide compared to sodium hypochlorite and chlorine containing cleaning agents. Few studies worked on hydrogen peroxide cleaning of polysulphone and polyvinylchloride membranes (Arnal et al. 2008; Hijnen et al. 2012; Li et al. 2005). Up to now, no study has been done about organic cleaning performance of hydrogen peroxide in UF (Regula et al. 2014). No peer reviewed references were found evaluating hydrogen peroxide cleaning of RO membranes.
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CHAPTER 3

Comparison of Alcian blue and total carbohydrate assays for quantitation of transparent exopolymer particles (TEP) in biofouling studies

The following chapter is a modified (primarily expanded to include supplementary information) version of the published paper:

Abstract

Transparent exopolymer particles (TEP) and their precursors are gel-like acidic polysaccharide particles. Both TEP precursors and TEP have been identified as causal factors in fouling of desalination and water treatment systems. For comparison between studies, it is important to accurately measure the amount and fouling capacity of both components. However, the accuracy and recovery of the currently used Alcian blue based TEP measurement of different surrogates and different size fractions are not well understood. In this study, we compared Alcian blue based TEP measurements with a total carbohydrate assay method. Three surrogates; xanthan gum, pectin and alginic acid; were evaluated at different salinities. Total carbohydrate concentrations of particulates (>0.4 μm) and their precursors (<0.4 μm, >10kDa) of all surrogates were dependent on water salinity and method of recovery. As xanthan gum is the most frequently used surrogate in fouling studies, TEP concentration is expressed as xanthan gum equivalents (mg XGeq/L) in this study. At a salinity of 35 mg/L sea salt, total carbohydrate assays showed a much higher particulate TEP fraction for alginic acid (38%) compared to xanthan gum (9%) and pectin (12%). The concentrations of particulate TEP therefore may only represent ~10% of the total mass; while precursor TEP ~90% of the total TEP. This highlights the importance of reporting both particulate and precursor TEP for membrane biofouling studies. The calculated concentrations of TEP and their precursors in seawater samples are also highly dependent on type of surrogate and resulting calibration factor. A linear correlation between TEP recovery and calibration factor was demonstrated in this study for all three surrogates. The relative importance and accuracy of measurement method, particulate size, surrogate type, and recovery are described in detail in this study.
Keywords: Transparent exopolymer particles (TEP), TEP precursors, total carbohydrate assay, membrane fouling, biofouling, Alcian blue

3.1 Introduction

Interest has steadily risen over the past decade in the role played by transparent exopolymer particles (TEP) in the biofouling of membranes (Bar-Zeev et al. 2009; Berman 2005; Berman et al. 2011; de la Torre et al. 2008). This interest quickly expanded from a focus only on particulate TEP to include smaller size fractions of these extracellular polymeric substances (EPS) now termed colloidal and precursor TEP (Bar-Zeev et al. 2015; Discart et al. 2015; Li et al. 2016a; Villacorte et al. 2009a, b; Villacorte et al. 2015). TEP was itself identified as an important player in marine ecosystems and biogeochemical processes about a decade prior to that when a tractable means of quantitatively identifying this gel-like, natural material was developed by Passow and Alldredge (1995). The likelihood that these biogenic particles were implicated in ocean desalination plant reverse osmosis membrane fouling seemed mechanistically plausible due to their ubiquitous presence, particulate nature, documented tendency to aggregate and adhere to surfaces, and identification as a food source for microbial growth (Chin et al. 1998; Passow 2000; Rochelle-Newall et al. 2010; Uthicke et al. 2009; Verdugo 2012). TEP are a subset of EPS, which are released from microbial aquatic organisms and so, despite their microbial origin, contribute to biofouling of membranes even when no viable microorganisms are present (e.g., after disinfection).

TEP is an operationally defined entity. It is the mass of material retained on a 0.4 µm filter which stains with Alcian blue (denoted herein as TEP\(_{0.4\mu m}\), ‘particulate’ TEP, or
pTEP) (Bar-Zeev et al. 2015; Passow and Alldredge 1995). Subsequently, extensions of this concept have been utilized. Colloidal TEP (cTEP) is defined as Alcian blue (AB) stainable material smaller than 0.4 μm but larger than 0.05 μm (Bar-Zeev et al. 2015; Villacorte et al. 2009a, b), although filtration bounds of 0.2 μm and 0.1 μm have also been studied (Passow 2000; Villacorte et al. 2009a, b). In addition, TEP and their precursors (denoted here as TEP\textsubscript{10kDa}) have been defined and studied as the mass of material which is retained by a 10 kDa filter and stains with Alcian blue (Villacorte et al. 2015). By this definition, precursor TEP includes cTEP plus any additional AB stainable material retained by a 10 kDa filter, which are smaller than 0.4 μm. Consequently, precursor TEP is a measure of any “non-dissolved” (i.e., filterable) constituent passing a 0.4 μm filter which stains with Alcian blue. It is worth noting that Alcian blue stain is not specific for TEP, that it stains all carboxylated (-COO\textsuperscript{-}) and sulfated (-OSO\textsubscript{3}\textsuperscript{-}) polyanions at pH 2.5. Other compounds, such as glycopolymers, proteins, and other acidic polysaccharides (i.e. bacterial cell walls) stain as well.

The difficulties and variabilities in collecting natural TEP samples motivate the use of more easily prepared TEP surrogates. Even more critically, controlled experiments such as quantitative and mechanistic biofouling studies require use of a reproducible, quantifiable material rather than an operationally defined material to which no standard value or mass balance can be applied. Thus, for controlled behaviour trials, TEP surrogates have been routinely used (Fatibello et al. 2004; Passow and Alldredge 1995; Thornton et al. 2007; Villacorte et al. 2009a, b; Villacorte et al. 2015). Relatively easily quantified surrogates allow normalization of measurements of natural samples to some known and reproducible standard (e.g., TEP expressed as mg equivalents xanthum gum/L (Passow and Alldredge 1995)). In addition, and particularly relevant to
controlled parametric trials on membrane fouling, it allows known quantities of a foulant (albeit a surrogate) to be utilized and the response observed under a variety of conditions (Le Lan et al. 2015; Li et al. 2016a; Villacorte et al. 2009a, b). This approach gives rise to several questions, both in evaluating the ‘real’ world impact of study results, as well as when comparing the results between different studies. These include how well do the surrogates mimic TEP including size variants, how well do different surrogates’ behaviour compare one to another, to what interferences or conditions are the measurements sensitive, and how well are the surrogates (and thus by implication the TEP) recovered and size fractionated in the various conditions and methods commonly utilized by researchers? However, few studies have attempted to answer these questions and none have done so comparatively among surrogates and over the range of salinities and TEP size fractions utilized in membrane biofouling trials.

In this study, we investigate TEP method recovery of different fractions of TEP using three surrogates (xanthan gum (XG), pectin (PN) and alginic acid (AA)); at three selected salinities representing close to fresh, brackish and seawater conditions; and for the size fractions of TEP implicated in biofouling studies. To understand the influence of recovery on TEP quantification, the relationship between TEP recovery and calibration factor was simulated. The surrogates are then compared to a natural seawater TEP material to evaluate the correlation between surrogate behaviour and behaviour of a real world TEP. The seawater TEP was also analysed to compare with previous TEP studies using different calibration factors.
3.2 Materials and methods

3.2.1 TEP quantification

To be most consistent with the previous TEP definitions, in this study TEP$_{0.4\mu m}$ is defined as particulate TEP and is the AB stainable mass retained by a 0.4 μm filter. TEP$_{0.1\mu m}$ is defined as colloidal TEP and is the AB stainable mass passing 0.4 μm filtration and retained by 0.1 μm filtration. TEP and their precursors (TEP$_{10kDa}$), on the other hand, are defined as all AB stainable mass retained by a 10 kDa filter, which includes particulate plus colloidal TEP plus any AB stainable material retained by a 10 kDa filter.

The procedure for measuring 0.4 μm fraction of TEP was based on Passow and Alldredge (Passow and Alldredge 1995), and 0.1μm fraction of TEP was based on Villacorte (Villacorte et al. 2009a, b). Fifty milliliters of sample was filtered through a 0.4 μm polycarbonate filter (Nuclepore Track-Etch membrane, Whatman, 47 mm diameter) and the filtrate was filtered through 0.1 μm polycarbonate filter (Nuclepore Track-Etch membrane, Whatman, 47 mm diameter) by applying a constant vacuum of 0.2 bar (Bettervac Pty Ltd, Australia). To remove residual salt, 2 mL of distilled MilliQ water was subsequently added and filtered. 1.0 mL of pre-filtered 0.02% AB solution in 0.06% acetic acid (pH 2.5) was added so that the filter was soaked in the stain. After 10 s, the excess dye was rinsed off using 2.0 mL of distilled MilliQ water and vacuum filtration. The filter was then transferred to a 50 mL glass beaker face down and 6.0 mL of 80% sulphuric acid added. The beaker was covered with parafilm and mixed on a shaker table for 2 hrs. Samples were taken from the beaker and absorbance measured at 787 nm wavelength (UVmini-1240, Shimadzu). Concentrations of TEP$_{0.4\mu m}$ and
TEP$_{0.1\mu m}$ were calculated as follows and expressed as surrogate equivalents (μg/L surrogate$_{eq}$):

\[ \text{TEP}_{0.4\mu m} = \frac{1}{m_{787}^{0.4\mu m}} \left( A_{\text{sample}} - A_{\text{filter blank}} - A_{\text{sample blank}} \right) \]

\[ \text{TEP}_{0.1\mu m} = \frac{1}{m_{787}^{0.1\mu m}} V \left( A_{\text{sample}} - A_{\text{filter blank}} - A_{\text{sample blank}} \right) \]

\[ f_{787} = \frac{1}{m_{787}} \]

Where TEP$_{0.4\mu m}$ is the concentration of particulate TEP, TEP$_{0.1\mu m}$ is the concentration of colloidal TEP (μg/L surrogate$_{eq}$); $m_{787}^{0.4\mu m}$ and $m_{787}^{0.1\mu m}$ are the slopes of the calibration curves for 0.4 μm and 0.1 μm fractions [(abs/cm)/μg surrogate$_{eq}$]; V is the volume of filtered sample (L); $A_{\text{sample}}$ is the absorbance of Alcian blue stained sample (abs/cm); $A_{\text{filter blank}}$ is the absorbance of Alcian blue on a negative control filter (abs/cm); $A_{\text{sample blank}}$ is the absorbance of unstained sample filter (abs/cm); and 1/$m_{787}$ is the calibration factor ($f_{787}$) [μg/(abs/cm)].

The procedure for measuring seawater TEP and their precursors was based on Villacorte (Villacorte et al. 2015). Fifty milliliters of sample was filtered through regenerated cellulose filters (Ultracel Ultrafiltration Discs, 24 mm diameter, 10 kDa, Millipore) using a disposable syringe. Injection of ten milliliter of air followed to ensure the excess sample liquid was filtered. 2.0 mL of distilled MilliQ water was then filtered to remove residual salt. Finally, 10 mL of air was injected to remove excess MilliQ water. The filter was then soaked in 10.0 mL of distilled deionized water with TEP side facing down in a 40 mL plastic container, vortexed for 10 s and sonicated for 2 hrs. 4.0 mL of the sonicated solution was transferred to a 7 mL glass vial, and acetic acid added to adjust the pH to 2.5. Then 0.50 mL of pre-filtered AB was added, vortexed for 10 s,
and 4.0 mL withdrawn and filtered through a 0.1 µm polycarbonate membrane filter (Nuclepore Track-Etch membrane, Whatman, 24 mm diameter,) manually using a syringe. The absorbance of the filtrate was measured at 610 nm wavelength. Concentration of TEP_{10kDa} was calculated from the following and expressed as surrogate equivalents (mg surrogate_{eq}/L):

\[
\text{TEP}_{10kDa} = \frac{V_F}{m_{610}} \left( \frac{A_{\text{sample}} - A_{\text{filter blank}} - A_{\text{sample blank}}}{V} \right)
\]

\[
f_{610} = \frac{1}{m_{610}}
\]

Where TEP_{10kDa} is the concentration of particulate TEP plus colloidal TEP plus any additional AB stainable mass retained by a 10 kDa filter (mg surrogate_{eq}/L); \(m_{610}\) is the slope of the calibration curve \([(\text{abs/cm})/(\text{mg surrogate}_{eq}/L)]\); \(V\) is the volume of filtered sample (L); \(V_F\) is the final re-suspended sample volume, which is 10 mL for this method (L); \(A_{\text{sample}}\) is the absorbance of Alcian blue stained sample (abs/cm); \(A_{\text{filter blank}}\) is the absorbance of Alcian blue on a negative control filter (abs/cm); and \(A_{\text{sample blank}}\) is the absorbance of unstained sample filter (abs/cm). Also, \(1/m_{610}\) is defined as the calibration factor \((f_{610})\) in \([(\text{mg/L})/(\text{abs/cm})]\).

### 3.2.2 Total carbohydrate analysis

To quantify recovery and comparatively evaluate the TEP assay, the phenol-sulfuric acid method was used. The phenol-sulfuric acid method is a widely used colorimetric method to determine the total carbohydrate concentration (Dubois et al. 1956). The predominant monomers found in biofilms and exocellular polysaccharides are glucose, mannose, galactose, glucuronic acid and galacturonic acid based on the composition analysis of both pure strain (Uhlinger and White 1983) and activated sludge (Horan and Eccles 1986). Therefore, these five sugars were selected for phenol-sulfuric acid, total
carbohydrate, and method validation. In addition, three TEP surrogates were investigated: XG, PN and AA. Each surrogate contains one or more of the corresponding sugar monomers: XG consists of glucose, mannose and glucuronic acid units; PN consists of galacturonic acid units; and AA consists of glucuronic acid. Among the three surrogates, XG is the most widely used standard in TEP measurement due to its reported better replicability (Passow and Alldredge 1995; Villacorte et al. 2009b; Villacorte et al. 2015). AA is less frequently used (Hung et al. 2003), while to our knowledge no studies have used PN as a surrogate for TEP. However, PN contains the AB stainable galacturonic acid groups absent from either XG or AA, and was therefore included in this study.

Stock sugar solutions were made by dissolving 20 mg of D-glucose (Sigma-Aldrich), D-Mannose (Sigma-Aldrich), D-Galactose (Sigma-Aldrich), D-Glucuronic acid (Sigma-Aldrich) or D-Galacturonic acid (Fluka) to 500 mg of distilled milliQ water. Mixed sugar solution was made from D-glucose, D-Mannose and D-Glucuronic acid with a molar ratio of 2.8: 2.0: 2.0 to mimic the composition of XG.

For calibration curves, 0.25 - 1.75 mL of above stock solutions containing 10-70 μg sugar was pipetted to a 10 mL glass tube. Distilled milliQ water was added to reach a volume of 2.00 mL, followed with 80% phenol solution (VWR) and 5.0 mL of sulfuric acid (95%, VWR). To obtain a good reaction, the sulfuric acid needs to be added rapidly and directly to the liquid surface. The vial was capped, vortexed (Crown scientific) for 10 s, and set aside for 20 min. Full scan of each standard was examined before reading the absorbance (UVmini-1240, Shimadzu). The maximum wavelength for each standard is listed in Table 3.1. All samples were measured in triplicate.
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Table 3.1. Parameters for phenol-sulfuric acid method validation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wavelength (nm)</th>
<th>Range</th>
<th>Precision</th>
<th>Linearity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mass (μg)</td>
<td>Concentration (mg/L)</td>
<td>RSD(^a) (%)</td>
</tr>
<tr>
<td>D-Glucose*</td>
<td>485</td>
<td>10-70</td>
<td>1.52-10.65</td>
<td>1.19-8.12</td>
</tr>
<tr>
<td>D-Mannose*</td>
<td>487</td>
<td>10-70</td>
<td>1.52-10.65</td>
<td>0.95-3.84</td>
</tr>
<tr>
<td>D-Galactose*</td>
<td>487</td>
<td>10-70</td>
<td>1.52-10.65</td>
<td>0.36-6.63</td>
</tr>
<tr>
<td>D-Glucuronic acid*</td>
<td>480</td>
<td>10-70</td>
<td>1.52-10.65</td>
<td>2.09-7.25</td>
</tr>
<tr>
<td>D-Galacturonic acid*</td>
<td>480</td>
<td>10-70</td>
<td>1.52-10.65</td>
<td>1.05-8.31</td>
</tr>
<tr>
<td>Mixed sugar standards†</td>
<td>485</td>
<td>10-70</td>
<td>1.52-10.65</td>
<td>1.04-4.94</td>
</tr>
<tr>
<td>Xanthan Gum**</td>
<td>485</td>
<td>7.5-75</td>
<td>1.14-11.42</td>
<td>7.03-15.20(^b)</td>
</tr>
<tr>
<td>Pectin**</td>
<td>480</td>
<td>7.5-75</td>
<td>1.14-11.42</td>
<td>1.05-6.14</td>
</tr>
<tr>
<td>Alginic acid**</td>
<td>480</td>
<td>7.5-75</td>
<td>1.14-11.42</td>
<td>1.32-6.37</td>
</tr>
<tr>
<td>Seawater</td>
<td>485</td>
<td>N/A(^c)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

a. RSD: Relative standard deviation. All samples were measured in triplicate.

b. RSD value was 1.1% for 37.5 μg (5.71 mg/L) same batch xanthan gum (n=10).

c. N/A: Not available.

*. Monomer solutions ranged from 10 μg (1.52 mg/L) to 70 μg (10.65 mg/L) per testing tube.

#. Mixed sugar standards include all the five sugar monomers with the same concentration (D-Glucose, D-Mannose and D-Glucuronic acid). Molar ratios of the three monomers were 2.8: 2.0: 2.0.

**. Surrogate standards ranged from 7.5 μg (1.14 mg/L) to 75 μg (11.42 mg/L) per testing tube.

3.2.3 Surrogates and Alcian blue

Stock surrogate solutions were freshly made by adding 15.00 mg of XG (Sigma-Aldrich), AA (Sigma-Aldrich) or PN (Sigma-Aldrich) to 200.0 mL of distilled
deionized water. The solution was mixed constantly with a magnetic stir bar, and then
ground with a tissue grinder 3 times before use.

Stock Alcian blue solution was prepared by dissolving 40.00 mg of Alcian blue (Sigma-Aldrich) to 200.0 mL of distilled deionized water. Acetic acid (ACS reagent, Sigma-Aldrich) was added dropwise to adjust pH to 2.5. The solution was stirred for 12-18 hrs and stored in the dark at 4°C. Stock AB solution was filtered drop by drop through a 0.05 μm polycarbonate membrane (Nuclepore Track-Etch membrane, 24 mm diameter, Whatman) using a syringe prior to staining. A new stock solution was prepared every 4 weeks. Calibration curves were generated for each batch of total carbohydrate analysis and TEP measurement.

3.2.4 TEP recovery method

Recoveries of the TEP surrogates; XG, PN and AA; were tested under three different salinities: close to fresh water (prepared in distilled deionized water), brackish water (4000 mg/L sea salt (Sigma-Aldrich)) and seawater (35000 mg/L sea salt). Surrogate processing followed the TEP methods discussed above including filtration, rinsing, staining with Alcian blue, and re-rinsing. Total carbohydrate concentrations of the initial surrogate solution and the filtrate were measured following total carbohydrate analysis method described above. The filter was transferred to a 10 mL vial, 2.0 mL of distilled deionized water was added, vortexed for 30 s and sonicated for 2 hrs. Finally, total carbohydrate concentration of the re-suspended filter solution was measured. Recoveries were calculated as:

\[ R (\%) = \frac{C_{\text{filter}}V_{\text{filter}} + C_{\text{filtrate}}V_{\text{filtrate}}}{C_{\text{initial}}V_{\text{initial}}} \times 100 \]

where \( C_{\text{filter}}, C_{\text{filtrate}} \) and \( C_{\text{initial}} \) are total carbohydrate concentrations of the re-suspended
filter solution, filtrate and initial surrogate solution, respectively (μg/mL); and $V_{\text{filter}}$, $V_{\text{filtrate}}$ and $V_{\text{initial}}$ are volume of the re-suspended filter solution, filtrate and initial surrogate solution, respectively.

The percentage of recovered carbohydrates in the surrogate retained on the membrane filters ($F_{\text{filter}}$) and the percentage in the filtrate ($F_{\text{filtrate}}$) were calculated as:

$$F_{\text{filter}}(\%) = \frac{C_{\text{filter}}V_{\text{filter}}}{C_{\text{filter}}V_{\text{filter}} + C_{\text{filtrate}}V_{\text{filtrate}}} \times 100$$

$$F_{\text{filtrate}}(\%) = \frac{C_{\text{filtrate}}V_{\text{filtrate}}}{C_{\text{filter}}V_{\text{filter}} + C_{\text{filtrate}}V_{\text{filtrate}}} \times 100$$

### 3.2.5 Seawater samples

Raw seawater samples were collected from the Trigg Beach in Perth, WA, Australia (Water Corporation) in February, 2017. TEP and total carbohydrate analyses were carried out within 24 hours after sample collection.

### 3.3 Results and discussion

#### 3.3.1 Monomers and surrogate quantification

To evaluate phenol-sulfuric acid method performance, three parameters were considered: method linearity, linearity range and method precision. Calibration results showed that the coefficient of determination for linear regression was higher than 0.98 for sugar standards within the range of 10 μg (1.52 mg/L) to 70 μg (10.65 mg/L) and higher than 0.99 for surrogates within the range of 7.5 μg (1.14 mg/L) to 75 μg (11.42 mg/L) (Table 3.1). The relative standard deviations (RSD %) of all sugars and surrogates were less than 9% and 7%, respectively. The standard deviation of 10 independent blank measurements was 0.02 abs/cm. The lower limits of detection (LOD)
of the phenol-sulfuric acid method were 1.39, 1.67, 1.67 mg/L for XG, PN, and AA, respectively.

3.3.2 Alcian blue effect on quantification by phenol-sulfuric acid

In this study, the phenol-sulfuric acid assay was used to quantify the total carbohydrate concentration of both surrogate and seawater samples processed using the TEP filtration method. TEP quantification is a two-step process; filtration and Alcian blue (AB) staining. Typically filtration occurs before staining, although the reverse order has also been utilized (Passow and Alldredge 1995; Thornton et al. 2007). Thus, for this study effects of the filtration process were separated from those of AB staining. AB is a blue-purple dye while phenol-sulfuric acid presents a yellow-orange color. Thus, the effect of AB on phenol-sulfuric acid analysis was subtracted in the measure of total carbohydrate of any TEP (filter or filtrate) sample.

Spectral scans of AB in XG, PN and AA were performed using the total carbohydrate analysis. The peak wavelength for the three surrogates remained unchanged with the presence of AB: 485 nm for XG and 480nm for both PN and AA. However, AB did increase the absorbance for all three surrogates. In subsequent analyses this was corrected by subtracting the AB control in the phenol-sulfuric acid assay. To avoid AB batch variations, a new correction was done for each batch of AB solution.

3.3.3 TEP method recovery

Although method recovery (mass balance) is an important parameter in method validation, it has not often been done in TEP analysis (Table 3.2). Recovery in a filtration method is expressed as the sum of the mass retained on the filter and the mass
in the filtrate divided by the initial mass subjected to filtration. The Passow and Alldredge, 1995 method (Passow and Alldredge 1995) measured the 0.4 μm retained fraction of surrogate XG on the filter by dry weight measurement, but didn’t measure the amount in the filtrate nor in the initial solution. This follows the logic used in measuring TEP in natural samples in which there is no independent means of measuring the “total TEP” in the sample subjected to filtration, since TEP is itself operationally defined by filtration. In contrast, Villacorte et al, 2009 method (Villacorte et al. 2009a) measured the total organic carbon (TOC) of the XG surrogate in the initial solution and in the filtrate, but didn’t analyse TOC of the filter, deducing that quantity by difference (and de facto assuming 100% recovery). Neither of these two methods directly tested method recovery. To determine TEP method recovery, phenol-sulfuric acid method was used in this study to analyse the initial surrogate solution, filter and filtrate.

In this study, TEP_{0.4μm} and TEP_{0.1μm} and TEP_{10kDa} represent the AB stainable fraction larger than 0.4 μm; fraction between 0.1 and 0.4 μm; and fraction larger than 10 kDa, respectively. Recoveries using four versions of the TEP method were investigated, namely: TEP_{0.4μm} by Passow and Alldredge (Passow and Alldredge 1995), TEP_{0.1μm} by Villacorte et al (Villacorte et al. 2009a), TEP_{10kDa} by Villacorte et al (Villacorte et al. 2015), and TEP_{pre-stain} by Thornton et al (Thornton et al. 2007) using AB staining prior to filtration by 0.4 μm PC filter. The first three methods were selected because the Passow and Alldredge, 1995 method (Passow and Alldredge 1995) is the most widely used method for TEP (> 0.4 μm) monitoring, Villacorte et al, 2009 method (Villacorte et al. 2009a) is developed based on the Passow and Alldredge’s method by using a series of filtration targeted at colloidal TEP (0.05 μm < TEP size < 0.4 μm) measurement, and Villacorte et al, 2015 method (Villacorte et al. 2015) is a recent
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developed method for TEP/TEP precursor (>10 kDa) analysis. TEP\textsubscript{pre-stain} of Thornton (Thornton et al. 2007) is also of interest since the method offers some operational advantages over the other methods (such as ability to measure total stainable mass of the initial sample) and since the fouling mechanisms as a function of particle size and solution composition for any kind of TEP are not fully understood.

Table 3.2. Comparison of surrogate calibration method used in transparent exopolymer particles (TEP) assays.

<table>
<thead>
<tr>
<th>Passow and Alldredge, 1995 (Passow and Alldredge 1995)</th>
<th>Villacorte et al. 2009 (Villacorte et al. 2009a)</th>
<th>This study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry weight method(^a)</td>
<td>TOC(^b) method</td>
<td>Phenol-sulfuric acid method</td>
</tr>
<tr>
<td>Initial feed</td>
<td>×(^*)</td>
<td>√(^**)</td>
</tr>
<tr>
<td>Filter</td>
<td>√(^)</td>
<td>×(^)</td>
</tr>
<tr>
<td>Filtrate</td>
<td>×(^)</td>
<td>√(^)</td>
</tr>
<tr>
<td>Mass balance</td>
<td>×(^)</td>
<td>×(^)</td>
</tr>
</tbody>
</table>

\(^a\) Dry weight method of measuring surrogates on preweighed filter (Sharp 1991).
\(^b\) TOC: Total organic carbon.

\(^*\) Symbol ×: not measured.

\(^**\) Symbol √: measured by correspond surrogate calibration method.

Mass balances (the sum of the filtered + filtrate masses divided by the initial mass) quantified using the phenol-sulfuric acid method show recoveries for all three surrogates at three different salinities (DDI: distilled deionized water, 4000 mg/L sea salt and 35000 mg/L sea salt) were in the range of 80 - 120% (Table 3.3). Across the variables of both salinity and size fraction, no surrogate showed noticeably better or worse recovery than the others. Likewise, the relative standard deviation among
replicates was not noticeably different for one surrogate or set of conditions than for the others.

3.3.4 Recovered carbohydrate of TEP\(_{0.4\mu m}\)

In DDI water, the XG TEP\(_{0.4\mu m}\) fraction was only 13% of the total XG mass and similarly the PN TEP\(_{0.4\mu m}\) fraction was only 12% of the total mass (Fig. 3.1). For XG, this was consistent with recent TOC results for seawater in which TEP\(_{0.4\mu m}\) was only 16.5% of the TOC of the initial feed seawater (Li et al. 2016a). In contrast, the particulate fraction for AA was 68%. However, the higher TEP\(_{0.4\mu m}\) fraction of AA is not consistent with its relative molecular weight (32000 - 400000 g/mol (Lee and Mooney 2012)) compared to XG (2 MDa - 20 MDa g/mol (Garcia-Ochoa et al. 2000)) and PN (60 - 130000 g/mol(Muzzarelli et al. 2012)). One possible explanation is that AA has a higher particulate fraction than XG and PN, however this explanation was not tested. Since TEP is operationally defined as the mass of material retained on a 0.4 \(\mu\)m filter and stained by AB, then use of XG or PN as surrogates is problematic in that only a small fraction of surrogate mass in a sample would meet the TEP criteria of filterability in fresh water. Choosing AA as a surrogate for TEP\(_{0.4\mu m}\), will capture much more of the AB stainable material than using XG or PN as TEP\(_{0.4\mu m}\) surrogates.
Table 3.3. Transparent exopolymer particles (TEP) recovery (R (%)) by phenol-sulfuric acid assay.

<table>
<thead>
<tr>
<th>Salinity</th>
<th>TEP&lt;sub&gt;pre-stain&lt;/sub&gt;&lt;sup&gt;*&lt;/sup&gt;</th>
<th>TEP&lt;sub&gt;10kDa&lt;/sub&gt;&lt;sup&gt;*&lt;/sup&gt;</th>
<th>TEP&lt;sub&gt;0.1μm&lt;/sub&gt;&lt;sup&gt;*&lt;/sup&gt;</th>
<th>TEP&lt;sub&gt;0.4μm&lt;/sub&gt;&lt;sup&gt;*&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>XG&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Pectin</td>
<td>AA&lt;sup&gt;d&lt;/sup&gt;</td>
<td>XG</td>
</tr>
<tr>
<td>DDI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.39±5.78**</td>
<td>87.33±2.94</td>
<td>84.83±9.79</td>
<td>85.44±7.64</td>
</tr>
<tr>
<td>4000 mg/L SS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.02±4.46</td>
<td>97.65±5.61</td>
<td>106.30±10.42</td>
<td>96.39±12.45</td>
</tr>
<tr>
<td>35000 mg/L SS</td>
<td>98.19±1.19</td>
<td>93.86±7.09</td>
<td>103.39±5.98</td>
<td>87.03±8.01</td>
</tr>
</tbody>
</table>

a. DDI: distilled deionized water; b. SS: sea salt; c. XG: xanthan gum; d. AA: alginic acid.

* TEP<sub>pre-stain</sub> , TEP<sub>10kDa</sub> , TEP<sub>0.1μm</sub> and TEP<sub>0.4μm</sub> stand for method that AB staining before to filtration by 0.4 μm PC filter (Thornton et al. 2007), Villacorte et al, 2015 method (Villacorte et al. 2015) Villacorte et al, 2009 method (Villacorte et al. 2009a, b) and Passow and Alldredge, 1995 method (Passow and Alldredge 1995) respectively.

**. ±: standard deviation of three repetitive filtration measurement.
Fig. 3.1. Percentage of recovered carbohydrate in xanthan gum, pectin and alginic acid retained by 0.4 μm membrane filter versus in the filtrate at different salinities. Error bars show standard deviation of three repetitive filtration measurements.

Two salinities, 4000 mg/L and 35000 mg/L of sea salt, were selected to simulate brackish water and seawater, respectively (Fig. 3.1). As the sample salinity increases the mass retained by the 0.4 μm filter is unchanged for PN (12%, 11% and 12%, respectively) and only modestly diminished for XG (13%, 13% and 9%, respectively). The greatest observed change caused by salinity is for AA; the retained TEP_{0.4μm} fraction gradually decreased from 68% to 38% as salinity increased. Therefore, the drawback of selecting AA as TEP surrogate is its sensitivity to salinity change, even though in fresh water it behaves much more like a true TEP_{0.4μm} surrogate (than XG or PN) in which the AB stainable material would be completely retained on a 0.4 μm filter. The issue of poor retention of TEP_{0.4μm} for the three surrogates is unmitigated or worsened in seawater salinity concentration samples compared to fresh or brackish...
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water salinity concentration samples.

3.3.5 Recovered carbohydrate of TEP$_{0.1\mu m}$

![Graph showing the percentage of recovered carbohydrate in xanthan gum, pectin, and alginic acid retained by a series of 0.4 and 0.1 μm membrane filter versus in the filtrate at different salinities. Error bars show standard deviation of three repetitive filtration measurements.](image)

Fig. 3.2. Percentage of recovered carbohydrate in xanthan gum, pectin, and alginic acid retained by a series of 0.4 and 0.1 μm membrane filter versus in the filtrate at different salinities. Error bars show standard deviation of three repetitive filtration measurements.

In terms of filter retention, TEP$_{0.1\mu m}$ of XG, PN, and AA in DDI water contrasted with TEP$_{0.4\mu m}$ in that the retention for AA was much less than XG and PN (Fig. 3.2). But the retention difference of TEP$_{0.1\mu m}$ between AA, XG, and PN shrunk. The same as with TEP$_{0.4\mu m}$ results, the retention of TEP$_{0.1\mu m}$ for PN was not affected by sample salinity, while that for AA declined with increased salinity (Fig. 3.2). However, TEP$_{0.1\mu m}$ for XG reduced from 43% to 26% at salinity of 4000 mg/L and to 10% at salinity of 35000 mg/L. This declining retention trend indicates the salt interference effect is more pronounced on the colloidal size particles (less than 0.4 μm) than on the larger particles.
for XG. Possible explanations are that higher salinity causes some disaggregation of particles and stabilization of colloids or that conformational change of the XG polymeric material is toward a less filterable (perhaps flexible) geometry due to increased salinity and compression of diffuse and fixed layer charge proximate to charged functional groups (Verdugo et al. 2004). However, this decrease in filterability with increasing size is seemingly at odds with the enhancement of dissolved organic carbon (DOC) self-assembly and annealing as salinity increases reported by Verdugo (2012). Passow and Alldredge (Passow and Alldredge 1995) did compare post sample rinse and no rinse of the 0.4µm filtered material, but their results showed no measureable difference for TEP_{0.4µm}. This is consistent with this study’s results, as a rinse of the material retained on the filter would remove only salts and increase the material’s filterability, whereas the additional fraction that would have been retained in less saline water has already been passed through to the filtrate prior to rinsing.

3.3.6 Recovered carbohydrate of TEP_{10kDa}

Unlike TEP_{0.4µm}, the retention of TEP_{10kDa} for XG (93%) in DDI water is much better than PN (77%) and AA (73%) (Fig. 3.3). Also, the XG and PN mass retained by the 10 kDa filter were minimally affected by salinity change. This suggests that XG may be a reasonable surrogate for precursor TEP across a range of salinities using the Villacorte et al., 2015 method (Villacorte et al. 2015). This is in stark contrast to its poor performance as a surrogate for pTEP and cTEP compared particularly to AA, but also PN (Fig. 3.4). While the TEP_{10kDa} retention of AA declined with increasing salinity, this trend is similar to the effect of salinity on TEP_{0.4µm} and TEP_{0.1µm} retention of AA.
Fig. 3.3. Percentage of recovered carbohydrate in xanthan gum, pectin and alginic acid retained by 10 kDa membrane filter versus in the filtrate at different salinities. Error bars show standard deviation of three repetitive filtration measurements.

3.3.7 Recovered carbohydrate of different TEP size fractions

The surrogates XG and PN exhibit a broad size distribution across the spectrum from 0.4 µm to 10 kDa retention with $\text{TEP}_{10\text{kDa}} > \text{TEP}_{0.1\text{µm}} > \text{TEP}_{0.4\text{µm}}$ (Fig. 3.4). This is expected when a sample contains a broad range of particle sizes or in terms of gels, a wide range of tendencies toward self-assembly. In general, the $\text{TEP}_{0.1\text{µm}}$ fractions of XG were higher than $\text{TEP}_{0.4\text{µm}}$, but their mass ratio varied depending on salinity. The $\text{TEP}_{0.1\text{µm}}$ fractions of PN were about three times the $\text{TEP}_{0.4\text{µm}}$ fraction, and $\text{TEP}_{10\text{kDa}}$ fractions were about seven times the $\text{TEP}_{0.4\text{µm}}$ fraction. However, the $\text{TEP}_{0.1\text{µm}}$ fractions of AA were far less than $\text{TEP}_{0.4\text{µm}}$, and even less than the 10 kDa filterable fraction (Fig. 3.4). This suggests that most of the filterable AA is in particles of effective diameter
greater than 400 nm with the balance in a dissolved state of <10 kDa. Despite its molecular weight range being much less than that of XG, its polymeric units appear to much more readily assembled or aggregated to large microgels, than those of XG. Interestingly, as the salinity of the AA sample decreases, the fraction of the total AA mass in the particulate state increases without a significant fraction appearing in the intermediate size fractions of 0.1 µm and 10 kDa.

Fig. 3.4. Percentage of recovered TEP_{0.4µm}, TEP_{0.1µm} and TEP_{10kDa} fractions of carbohydrate in xanthan gum, pectin and alginic acid. Error bars show standard deviation of three repetitive filtration measurements.

It is recognized and important to keep in mind that all of the surrogates studied are deformable chain organic molecules, so particle size is more of an operational concept.
than a hard-solid characterization. This is particularly noticeable in the trials at the various salinities described above. Xanthan gum is the most widely used TEP surrogate (Fatibello et al. 2004; Passow and All dredge 1995; Thornton et al. 2007; Villacorte et al. 2009a). However, the reported XG TEP\(_{0.4\mu m}\) concentration is only a small portion of the total XG TEP (particulate, colloidal and dissolved). On the other hand, a much greater fraction of XG is observed in the 0.1 \(\mu\)m to 10 kDa size range than for either AA or PN. Thus, to the degree that the colloidal and precursor TEP fractions are implicated in membrane biofouling, as has been suggested by several studies (Bar-Zeev et al. 2015; Li et al. 2016a; Li et al. 2016b), XG may be a better surrogate than PN or AA. Consequently, in studies on the mechanisms of biofouling, which use XG as a surrogate, the possible effects of these smaller size fractions shouldn’t be ignored.

### 3.3.8 Recovered carbohydrate of TEP\(_{pre-stain}\)

The TEP method of Thornton (Thornton et al. 2007) adds Alcian blue to the sample prior to filtration. In marked contrast to the results of TEP\(_{0.4\mu m}\) fractional retention, TEP\(_{pre-stain}\) of XG in DDI water retains over 90% of the sample mass and much more than compared to PN and AA (Fig. 3.5). Particulate XG (\(> 0.4 \mu m\)) retained in TEP\(_{pre-stain}\) method showed a much greater fraction (93%) of total carbohydrate compared to TEP\(_{0.4\mu m}\) fraction (Fig. 3.1) in DDI water. The likely explanation is that the AB dye forms insoluble substances and promotes stable XG assembly into microgels from otherwise sub 0.1\(\mu\)m fractions (Horobin and Flemming 1990).

For XG, the retained TEP\(_{pre-stain}\) at salinity of 4000 mg/L (91%) was very close to that in DDI water (93%), but dropped significantly to 13% at seawater salinity (35000 mg/L) (Fig. 3.5). Presumably the seawater stabilized the small size XG fractions and largely
prevented their aggregation into particulate size TEP. AA showed the same trend as XG, albeit with a lesser fraction no longer retained in seawater. This illustrates that the effect of AB on the size distribution of XG and AA was essentially nullified at seawater salinity as the fractions retained were nearly the same for the pre-stain and post-stain cases. Like XG and AA, pre-staining of PN increased the 0.4μm retained fraction, but the effect was more evenly distributed across the three size fractions investigated. From the perspective of biofouling potential and based on the surrogates’ results, pre-staining of samples with brackish or freshwater salinities significantly increases the apparent particle size and therefore overstates the predicted fouling potential. This effect is not pronounced for seawater samples.

Fig. 3.5. Percentage of recovered carbohydrate in pre-stained xanthan gum, pectin and alginate acid retained by 0.4 μm membrane filter versus in the filtrate at different salinities. Error bars show standard deviation of three repetitive filtration measurements.
### 3.3.9 Seawater analysis

When using XG as TEP surrogate, seawater TEP$_{0.4\mu m}$ and TEP$_{0.1\mu m}$ concentrations were 0.49 mg XG$_{eq}$ per liter and 0.44 mg XG$_{eq}$ per liter, respectively (Fig. 3.6). The calibration factor was 213 [μg XG$_{eq}$/abs/cm)] for seawater TEP$_{0.4\mu m}$ and TEP$_{0.1\mu m}$, and -42 [(mg XG$_{eq}$/L)/(abs/cm)] for TEP$_{10kDa}$. Total carbohydrate concentrations of seawater 0.4 μm and 0.1μm fractions were lower than TEP concentrations. The calibration factor used for seawater total carbohydrate concentration was 164 [μg XG$_{eq}$/abs/cm)]. In order to compare the seawater TEP results to other studies, we also took calibration factors from two widely used TEP method studies to calculate our seawater TEP concentrations. Two of the calibration factors were from Passow and Alldredge (Passow and Alldredge 1995): 88 [μg XG$_{eq}$/abs/cm] and 139 [μg XG$_{eq}$/abs/cm)]; another two were from Villacorte: 476 [μg XG$_{eq}$/abs/cm]) (Villacorte et al. 2009b) and -30 [(mg XG$_{eq}$/L)/(abs/cm)] (Villacorte et al. 2015). As shown in Fig. 6, TEP concentrations were proportional to calibration factors. That is to say, the higher the calibration factor, higher the TEP concentration results, and a greater difference in calibration factor results in a greater difference in TEP concentration. Villacorte attributed their big calibration factor difference compared to Passow and Alldredge’s to a lower concentration of Alcian blue stain. In this study, we noticed the apparent calibration factor difference could be due to projected TEP recovery (R$_{projected}$) difference, which is shown in Fig. 3.7. By manipulating the projected TEP recovery, it changes the mass of surrogates on the filter, and the slope of calibration curve (the reciprocal of calibration factor) changes thereafter. Its impact is especially important when using TOC or total carbohydrate method to calculate the mass of surrogates on the filter. Unlike direct mass measurement including method recovery, indirect methods have a default assumption that TEP method recovery is 100%. This is problematic,
since TEP method recovery changes from different batches of experiment, and varies between different surrogates at different salinities (Table 3.3). Linear regression between calibration factor and projected TEP recovery showed a coefficient of determination (R²) of 0.9992 for XG, 0.9981 for PN and 0.9486 for AA. Therefore, a higher calibration factor could be due to a higher TEP recovery.

Fig. 3.6. Comparison of seawater TEP₀.₄μm, TEP₀.₁μm and TEP₁₀kDa concentrations by using different calibration factors from references versus this study. (expressed as xanthan gum equivalent) Error bars show standard deviation of three repetitive filtration measurements.
Fig. 3.7. Projected effect of transparent exopolymer particles (TEP) method recovery (\(R_{\text{projected}}\) (%)) on calibration factor of surrogate xanthan gum, pectin and alginic acid.

\[ R_{\text{projected}}(\%) \times C_{\text{initial}}V_{\text{initial}} - C_{\text{filtrate}}V_{\text{filtrate}} = m_{\text{filter}} \]

where \(R_{\text{projected}}\) is projected TEP recovery in percentage; \(C_{\text{filtrate}}\) and \(C_{\text{initial}}\) are total carbohydrate concentrations of the filtrate and initial surrogate solution, respectively (\(\mu g/mL\)); \(V_{\text{filtrate}}\) and \(V_{\text{initial}}\) are volume of filtrate and initial surrogate solution, respectively; and \(m_{\text{filter}}\) is the mass of retained surrogates on the filter (\(\mu g\)).

Since the calibration factor also changed when using different surrogates for TEP analysis, Figure 3.8 illustrates how the calculated seawater TEP concentrations changes when expressed as different surrogate equivalents. The calibration factor for surrogate XG, PN and AA were 213, 909 and 833 [\(\mu g\) XG_{eq}/(abs/cm)], respectively. The TEP recoveries of above surrogates for the same batch seawater analysis were 84%, 85% and 85%, respectively.
Fig. 3.8. Seawater TEP$_{0.4\mu m}$, TEP$_{0.1\mu m}$ and TEP$_{10kDa}$ concentrations expressed as different surrogate equivalent. Error bars show standard deviation of three repetitive filtration measurements.

### 3.4 Summary and conclusions

- TEP measurement by definition means quantification using the Alcian blue method. The use of surrogates to simulate TEP (TEP$_{0.4\mu m}$) and its smaller size fraction variants (TEP$_{0.1\mu m}$ and TEP$_{10kDa}$) is commonly practiced in membrane biofouling research in an effort to undertake more controlled and quantifiable parametric studies than is possible using natural water TEP. However, there is a high degree of variability in quantification as TEP of a single surrogate with variation of the water’s salinity and the filter size applied, as well as between surrogates for the same water salinity and filter size applied. This makes comparison between studies using different surrogates and/or different salinities and size fractions problematic. Furthermore, to the degree that surrogate behaviour is indicative of the behaviour of TEP in general, there is little basis on which to confidently compare results from
different studies whose results are based on measuring natural TEP and between which either the water salinity or size fraction vary.

- TEP$_{0.4\mu m}$ only represents a small fraction (~10%) of the total mass for the xanthan gum surrogate. The major component of TEP is in the form of precursor TEP. Therefore, measuring the concentration of TEP$_{0.4\mu m}$ alone will likely underestimate the fouling potential for a membrane system. Thus, it is recommended to report both concentrations of particulate TEP and TEP precursors.

- Measured TEP concentrations were dependent on surrogate, salinity, method of recovery, and calibration factor. Thus, it is recommended to determine and report each of these for each study. It is especially useful when a comparison needs to be done between different research groups.

- Alcian blue based TEP staining method is also limited by batch variability of the standard. It is also laborious. Although semi-quantification of TEP levels in feed water is possible, the variability of standards makes evaluation and comparison of biofouling potential between studies problematic. Another drawback of Alcian blue method is that it also stains other polysaccharides (i.e. bacterial cell walls) that are not classified as TEP. Therefore, further techniques (i.e. cell fixation) may be used to identify cell wall polysaccharides. The colorimetric total carbohydrate method was more reproducible and simpler than the AB stain method. If one could simulate and correlate total carbohydrate concentration to TEP concentration, then total carbohydrate analysis could be used as a more comparatively accurate measurement tool for TEP.
Acknowledgements

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CHAPTER 4

Evaluation of transparent exopolymer particles (TEP) and their precursors fouling potential on seawater reverse osmosis (SWRO) membranes

Li, X., Bowden R.S., Clarke B.R., Li, D. and Ela, W.P. Evaluation of transparent exopolymer particles (TEP) and their precursors fouling potential on seawater reverse osmosis (SWRO) membranes. Under revision.
Abstract

Membrane fouling is a major challenge for seawater reverse osmosis (SWRO) desalination. In the category of extracellular polymeric substances (EPS) which were biological foulants released from microorganism, transparent exopolymer particles (TEP) were considered playing a key role in initiating biofilm formation on reverse osmosis (RO) membrane surface. However, fouling potential of TEP and TEP precursors were not fully understood. In this study, we investigated fouling behaviours of total TEP using surrogate xanthan gum (XG), pectin (PN), and alginic acid (AA). To further identify fouling potential difference between total TEP, TEP, and TEP precursors, we studied fouling behaviours of total XG, < 0.4 µm XG, and ≥ 0.4 µm XG. A reliable t-test was developed to differentiate fouling behaviour difference between different surrogates and TEP fractions. AA showed no significance (p > 0.05) compared to PN and sea salt baseline but was significantly from XG (p < 0.05). No significant difference was detected among total XG, ≥ 0.4 µm XG, and < 0.4 µm XG. It suggested similar fouling potential of total TEP, TEP, and TEP precursors. Intensity and density of XG on fouled SWRO membranes from confocal laser scanning microscopy (CLSM) results showed no significance between XG of different sizes. This study strongly suggested TEP concentrations rather than TEP sizes correlated more to membrane fouling.

Keywords: Transparent exopolymer particles, TEP, TEP precursors, membrane fouling, statistical analysis, T-test
4.1 Introduction

Transparent exopolymer particles (TEP) are gel-like particles that can be stained by Alcian blue (AB) and retained on 0.4 µm polycarbonate filters (Alldredge et al. 1993; Passow and Alldredge 1995). Due to their abundance in ocean, gel-particle nature (i.e. self-assembling, aggregation, adhesion, and ability to be retained on membrane), and identification as food source for microbials, TEP and their precursors were implicated as active players in biofilm formation and membrane fouling (Bar-Zeev et al. 2015; Passow 2002; Verdugo 2012). Over the past decades, TEP have been widely studied in desalination (Le Lan et al. 2015; Villacorte et al. 2009a, b; Villacorte et al. 2010) and other water/membrane systems (Berman et al. 2011; de la Torre et al. 2008; Linares et al. 2012). Until recently, the fact that TEP might only represent 10% of total TEP, and the majority (80%) is in the form of TEP precursors have gained attention (Li et al. 2018). In addition, TEP precursors are more difficult to be removed from seawater reverse osmosis (SWRO) feed water by either conventional pre-treatment systems (i.e. sand filtration and coagulation) or advanced technologies (i.e microfiltration (MF) and ultrafiltration (UF)) (Bar-Zeev et al. 2015). Even if MF/UF could completely remove TEP, it’s unlikely to remove all TEP precursors from RO influent which leads to aggregation of TEP precursors and formation of TEP due to their self-assemble gel-particle nature (Bar-Zeev et al. 2015; Verdugo 2012). Therefore, it is important to get in-depth understanding of fouling behaviours of both TEP and TEP precursors in SWRO systems to mitigate SWRO membrane fouling and improve pre-treatment design.

Today, great efforts have been made in developing quantification methods of TEP (Passow and Alldredge 1995; Fatibello et al. 2004; Thornton et al. 2007) and TEP
precursors (Villacorte et al. 2009a, b; Villacorte et al. 2015), and monitoring their occurrence in desalination source water and along the treatment scheme (Bar-Zeev et al. 2009; Li et al. 2016a; Miyoshi et al. 2016). Little knowledge is available about their fouling behaviour difference. In fact, size-dependent TEP fouling studies have been done either by seawater pre-filtration (Kumar et al. 2006; Ladner et al. 2010), multiple membrane array system (MMAS) using TEP surrogates (Alginic acid (AA)) (Lee et al. 2015), or correlating concentrations of TEP and colloidal TEP (cTEP) (0.1 - 0.4 μm) to fouling rate (Miyoshi et al. 2016). However, filters used in seawater pre-filtration studies were not selected based on TEP size categorization, and fouling studies were not controlled at the same TEP concentrations. In other words, observed fouling behaviour differences between different TEP fractions, in terms of flux decline or fouling index, could be due to their differences in total TEP composition (Li et al. 2018). In addition, previous studies only compared fouling potential between TEP and cTEP and found that TEP were more correlated to membrane fouling than cTEP (Lee et al. 2015; Miyoshi et al. 2016). These studies evaluated fouling potential in an indirect way and fouling potential of TEP precursors were ignored. Compared to TEP fouling studies, TEP precursors have been studied far less. A recent study pointed out that TEP precursors caused obvious fouling on RO membranes (Li et al. 2016b) but their fouling potential relative to TEP is still unclear. This gives rises to several questions: (1) Is there a fouling difference between total TEP, TEP, and precursor TEP? Which fraction causes more flux decline? (2) Since TEP composition is dependent on selected surrogates (Li et al. 2018). How different is it between TEP surrogates (i.e. xanthan gum (XG), pectin (PN), and AA) in terms of membrane fouling potential? (3) How to quantify fouling difference between two foulants?
In this study, we developed a reliable hour-by-hour T-test method to quantify TEP fouling potential difference on SWRO membrane using TEP surrogates XG, PN and AA. XG were further fractionated to different sizes simulating total TEP, TEP and TEP precursors for identification of their fouling potential difference.

4.2 Materials and methods

4.2.1 Lab-scale seawater reverse osmosis (SWRO) unit

A lab-scale crossflow reverse osmosis (RO) unit was used for fouling experiment (Fig. 4.1). The unit was comprised of a feed tank (Sterlitech Corp.), a crossflow cell (CF042, Sterlitech Corp.), a high-pressure pump (Hydra-Cell, Wanner Engineering Inc.), a chiller (PolyScience), a flow meter (Blue-White Industries, Ltd.), a digital balance (A & D Australasia Pty. Ltd.), a digital pressure sensor (Techsis), two digital thermocouples (Omega Engineering), a Labjack (Labjack Corp.), and a data acquisition system (Labview). No spacer was used in the RO cell. The RO cell is 9.207 cm in length, 3.9 cm in width, and 0.228 cm in depth with an effective membrane area of 42 cm². Feed water temperature was controlled by circulating cooling water through coiled stainless steel which was submerged in the feed tank. Temperature of both feed and RO inlets was monitored by thermocouples and the signal was sent to a computer through USB data acquisition module. Transmembrane pressure can be manipulated by a pressure valve. The voltage signal of a pressure sensor was monitored and sent to a computer through Labjack and converted to pressure signal in LabVIEW. Permeate was collected in a beaker on a digital balance and the weight was recorded by computer. The concentrate was recirculated back to the feed tank.
4.2.2 Xanthan gum fractionation

Stock solution was freshly made by adding 0.2 g of XG (Sigma-Aldrich) to 200.0 mL of deionized water. The solution was mixed constantly with a magnetic stir bar, and then ground with a tissue grinder 3 times before vacuum filtration. Every six milliliters of grounded stock solution were filtered through a 0.4 μm polycarbonate filter (Nuclepore Track-Etch membrane, Whatman, 47mm diameter) by applying a constant vacuum of 0.2 bar (Bettervac Pty Ltd, Australia). XG greater than 0.4 μm were collected on the filter and a total mass of 80 mg of ≥ 0.4 μm XG were obtained for fouling experiment. Filtrate was also collected in a glass bottle and stored in fridge at 4 °C. The filters were transferred to a 500 mL glass beaker face down and 200 mL of deionized water was added. The beaker was covered with parafilm and mixed on a shaker table for 5 hrs. Concentrations of both re-suspended (≥ 0.4 μm) and filtrate XG (< 0.4 μm) were measured following the phenol-sulfuric acid method (Li et al. 2018). The absorbance of sample was measured at 485 nm wavelength (UVmini-1240, Shimadzu).
4.2.3 Fouling protocol

A thin film composite polyamide RO membrane (SWHR, Dow Chemical Company) was used for fouling experiments which was provided by Perth desalination plant, WA, Australia. Membrane was received as spiral-wound and was cut into small coupons to fit previous described RO cell. Pressure and temperature were set at 900 psi and 20 ± 1°C. Cross-flow velocity was approximately 2.2 cm/s.

The RO membrane was firstly soaked in deionized (DI) water overnight, and then compacted with 4 L of DI water for 20 hrs. After compaction, 140 g of sea salt (Olsson’s, WA, Australia) was added into feed tank to reach a concentration of 35 g/L and an overhead stirrer was introduced. The composition of this commercial sea salt was listed in Table 4.1. Surrogates of XG, XG filtrate (< 0.4 μm), re-suspended XG (≥ 0.4 μm), pectin (Sigma-Aldrich) and alginic acid (Sigma-Aldrich) were tested in fouling experiment. After 24 hrs of sea salt baseline, 80 mg of surrogate was dissolved in permeate for 5 hrs and then added back to feed tank. All fouling experiments were operated in triplicate for each surrogate and lasted for 24 hrs.

4.2.4 Flux calculation

The raw permeate flux \( J_r \) is defined as permeate flowrate per unit membrane area:

\[
J_r = \frac{Q_w}{A_m} = \frac{\Delta m_w}{\rho \Delta t A_m}
\]

where \( Q_w \) is permeate flowrate (L/h), \( A_m \) is the effective membrane area (m\(^2\)), \( \Delta m_w \) is the mass of permeate (g) collected during time \( \Delta t \) (h) and \( \rho \) is solution density (g/L).
Table 4.1. Commercial sea salt composition.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Olsson’s (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride</td>
<td>16310</td>
</tr>
<tr>
<td>Sodium</td>
<td>11025</td>
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<tr>
<td>Potassium</td>
<td>770</td>
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<tr>
<td>Calcium</td>
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<td>Sulfate</td>
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<tr>
<td>Magnesium</td>
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<tr>
<td>Manganese</td>
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<tr>
<td>Iodine</td>
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<td>Selenium</td>
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</tr>
<tr>
<td>Copper</td>
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<tr>
<td>Iron</td>
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</tr>
<tr>
<td>Tin</td>
<td>1.6</td>
</tr>
<tr>
<td>Silver</td>
<td>1.1</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Since osmotic pressure changes over time as a result of removing permeate from feed solution, raw permeate flux was corrected to net driving force as well as temperature:

\[
J = \frac{J_r(TCF)}{P} = \frac{J_r(TCF)}{P - \Delta \pi_m} = \frac{J_r(TCF)}{P - \pi_b - \pi_p} = \frac{J_r(TCF)}{P - \pi_b}
\]

TCF = \(\text{EXP}\left[3020\left(\frac{1}{298} - \frac{1}{273 + T}\right)\right]\)

where \(J\) is corrected flux (LMH/Pa), TCF is temperature correction factor and the equation are for temperature below 25°C provided by Dow Chemical Company, \(P\) is applied pressure (Pa), \(\Delta \pi_m\) is transmembrane osmotic pressure (Pa), \(\pi_b\) is osmotic pressure at the bulk (Pa), \(\pi_p\) is osmotic pressure on the permeate side (Pa). For high resistant membrane, permeate osmotic pressure is significantly lower than feed osmotic pressure and therefore can be neglected.
Osmotic pressure was calculated from following equation:

\[ \pi_b \text{ (bar)} = \varphi C_b RT \]

where \( \varphi \) is osmotic coefficient and is calculated using Phreeqc (United States Geological Survey (USGS), USA), \( C_b \) is bulk concentration of solutes (mol/L), \( R \) is universal gas constant (0.083145 L·bar/mol·K), \( T \) is thermodynamic (absolute) temperature (K).

To evaluate fouling potential, normalized corrected flux (Norm \( J \)) were calculated:

\[ \text{Norm } J = \frac{J_t}{J_0} \]

where \( J_t \) is corrected flux at time \( t \) and \( J_0 \) is corrected initial flux.

### 4.2.5 Statistical test

In this study, a statistical method was developed for testing significant difference for time series analysis using MINITAB 2018 for windows (National Institute of Standards and Technology, USA). Normalized flux results were analysed to determine: (1). Whether there was a difference in flux decline with respect to the first reading by the finish of the experiment between: (a) XG of different sizes, and (b) different types of surrogates as well as the sea salt baseline and the DI compaction; (2). Beyond which time point further testing is not required.

Three replicates were analysed for each solution to ensure reliable statistical results. It was a result of compromise between cost of experimentation and statistical efficiency.

An estimate using a sample of size three has a standard error that is \( \frac{1}{\sqrt{3}} = 0.58 \) times the standard deviation of the original data whereas a sample of size four has a \( \frac{1}{\sqrt{4}} = \)
0.50 multiplier. So, there is a decrease in “error” of 0.08 for a 0.33 increase in cost. Hence a sample of size four was deemed financially unsustainable. Conversely a sample of size two presents substantial issues with the stability of the T-test outcome.

To focus on testing the significant difference between solutions for the latest time points, only most recent data were used in statistical test. A simple T-test was performed by comparing the normalized flux of each solution’s data at each one-hour interval for the 3 v 3 data points at that interval only. The statistically significant p-value was set to 0.05. A three-hour interval T-test was also analysed. One drawback of three-hour interval T-test is autocorrelation caused by contamination of irrelevant less recent data. But it showed similar statistical power compared to one-hour T-test, that is the chance of rejecting the null hypothesis of no difference in normalized flux. The results of three-hour interval T-test for normalized flux of different sizes of XG were shown in Appendix (Fig. S1.).

The second prospective test is Hotelling’s $T^2$ test which is a multivariate test where the data points for the three-time intervals represent three separate random variables. This test is likely to have low power due to the need to estimate three means, three variances and three cross-correlations. This was born out by the results and again this test wasn’t further employed.

In statistical analysis section, normalized flux of total XG, PN, AA, sea salt baseline, DI compaction, $< 0.4 \mu m$ XG filtrate, and $\geq 0.4 \mu m$ XG were also denoted as solution 1, 2, 3, 4, 5, 6 and 7, respectively. For statistical view, normalized flux plots were replotted in a different x-axis scale and were shown in Appendix (Fig. S2.).
4.2.6 Membrane characterization

Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) (Perkin Elmer, US) and Confocal Laser Scanning Microscope (CLSM) (Nikon C2, Japan) were used for membrane characterization.

All membrane samples were first cut into coupons with a size of approximately 1 cm$^2$. Three replicates were analysed for each membrane sample. All fouled membranes were then thoroughly air dried before ATR-FTIR analysis. Each spectrum was averaged from 80 scans over the range of 600 - 1900 cm$^{-1}$.

Before CLSM analysis, XG fouled membranes were stained with a FITC conjugated Conacavalin A lectin (Life Technologies GmbH) to visualize polysaccharides. The details of labelling procedure was described elsewhere (Nagaraja et al. 2017). Firstly, the 1 cm$^2$ membrane coupon was placed on a clean glass slides in a petri dish, and then 100 μl of 100 μg/mL ConA FITC was added on top of membrane. The staining process was carried out in dark by covering the petri dish with aluminium foil for 10 mins. The stain was then disposed and excessive stain was removed by phosphate-buffered saline (Sigma-Aldrich). After staining, the membranes were kept wet on clean glass slides, secured with coverslips and observed on a CLSM at 20×magnification using associated software (NIS-Elements) to collect z-stacks from at least 3 sections per membrane. All images were acquired and processed using the same scales and parameters. To quantify mass and density of polysaccharides on fouled membrane, voxels and mean intensity was calculated using ImageJ (National Institutes of Health, USA) using following equations:
\[ I = \frac{\sum v_n \times I_n}{\sum v_n} \]
\[ D = \frac{I}{d} \]

where \( I \) is mean intensity for entire 3-D stacks; \( v_n \) is voxel for layer \( n \); \( I_n \) is mean intensity for layer \( n \), \( D \) is density; \( d \) is polysaccharide depth of each image. For the first layer of each image, the background was subtracted by setting a threshold intensity which was obtained from the maximum intensity of a clean RO membrane.

4.3 Results and discussion

4.3.1 Normalized flux of surrogates and baseline

(a) Surrogates and baseline

- DI compaction
- Sea salt baseline
- Alginic acid
- Pectin
- Xanthan gum
Fig. 4.2. Normalized flux of different (a) surrogates and baseline and (b) xanthan gum in different size fractions. Test conditions: initial feed water containing 35g/L sea salt and 20 mg/L foulants, pressure of 900 psi, temperature of 20 ± 1 °C, cross-flow velocity of 2.2 cm/s. Error bars show one standard deviation measured from triplicate samples.

Normalized flux of surrogates XG, PN, and AA as well as sea salt baseline and DI compaction were shown in Fig. 4.2 (a). Initial raw flux of sea salt baseline ranged from 11.63 to 15.07 LMH due to inconsistencies of different sections of membrane. It was relatively low compared to other studies: 23.4 LMH for filtered seawater (Kumar et al. 2006) and 30 LMH for 32 g/L NaCl under constant pressure of 6.9 MPa (Ladner et al. 2010). This can be caused by relatively low cross-flow velocity of 0.022 m/s in this study compared to others: 0.3 m/s (Kumar et al. 2006) and 0.47 m/s (Ladner et al. 2010). Salt rejection in this study was between 98.2 to 99.2%, which was expected for SWRO membranes. After compaction for 20 hrs, a constant flux was reached (34.2 - 45.9 LMH). A decline in flux of 13% was observed for sea salt baseline after 24-hr operation.
CHAPTER 4

This may be caused by concentration polarization and the normalized flux can get recovered by dividing by a concentration polarization factor (Ladner et al. 2010).

In 35 g/L sea salt solutions (calcium concentration around 0.01 M), AA (20 mg/L) showed less flux decline (~13%) after 24-hr operation compared to PN (~19%) and XG (~33%). Its flux decline, however, was comparable to sea salt baseline, indicating low fouling potential on SWRO membrane. This can be explained by “egg-box” model that calcium ions bind preferentially to carboxylate groups of alginates in a highly organized manner and form bridges between adjacent alginate molecules leading to the egg-box-shaped gel network (Davis et al., 2003). In fact, AA is a small molecular weight polysaccharide (12 - 80 kDa (Lee and Elimelech 2007)) compared to XG (2 MDa - 20 MDa g/mol (Garcia-Ochoa et al. 2000)). Calcium is needed for AA to form larger size macromolecules, and such cross-linked gel complex could increase the deposition of AA on membrane surface causing more fouling with the presence of calcium as a result (Ang et al. 2011; Jin et al. 2009; Lee and Elimelech 2006). It’s also worth noting that AA (100 mg/L) fouled less in synthetic seawater (28% flux decline after 27hrs) than in sodium chloride solution (41% flux decline after 27hrs) at the same calcium concentration of 0.009 M (Jin et al. 2009) which implied a hindered calcium-alginate interaction in seawater compared to in sodium chloride solution.

4.3.2 Normalized flux of xanthan gum

Normalized flux of surrogate XG in size fractions of total, ≥ 0.4 μm, and < 0.4 μm were shown in Fig.4.2 (b). At concentration of 20 mg/L, the three size fractions of XG showed similar flux decline, indicating similar fouling potential among total TEP, TEP and TEP precursors. This is consistent with findings from seawater fractionation studies.
(Kumar et al. 2006; Ladner et al. 2010). Noted that filter sizes were different in these studies and were not selected based on TEP size categorization. Results from sequential filtration of natural seawater suggested comparable flux decline contribution between larger size of TEP (0.1 - 1 μm) and smaller size of TEP precursors (20kDa - 100kDa) fractions (Kumar et al. 2006), and no significance between different fractions of seawater (≥ 0.7 μm, 100kDa - 0.7 μm, 30kDa - 100kDa, 20kDa - 30kDa, and < 20 kDa) in flux decline rate (Ladner et al. 2010).

4.3.3 Statistical analysis

After replication for each solution undertaken for this paper it is apparent that there is substantial variation in the trend of the normalized flux between replications for the same solution (Fig. 4.2). A suitable statistical test based on well understood scientific and probabilistic properties is the only way to reliably conclude whether the change in flux readings for two solutions are statistically significantly different. The statistical analysis results were discussed below.

4.3.3.1 Surrogates and baseline

Figure. 4.3. presents the main effects of normalized flux of surrogates XG, PN, and AA as well as sea salt baseline and DI compaction, being time and solution type. The statistical significance of these variations is explored below.
Fig. 4.3. Main effects plot for normalized flux of (1) xanthan gum (2) pectin (3) alginic acid (4) sea salt baseline and (5) deionized water compaction. Dashed line represents the overall mean.

Fig. 4.4. Comparison at one-hour intervals of p-values for pairs of independent parameters: (1) xanthan gum, (2) pectin, (3) alginic acid, (4) sea salt baseline, and (5)
deionized water compaction. Dashed line indicates a p-value = 0.05. P-value below significance level of 0.05 indicates a significant difference between two independent parameters at the particular time point.

The hour-by-hour p-values were plotted in Fig. 4.4. The p-values for the comparison between (1) XG (2) PN (3) AA (4) sea salt baseline and (5) DI compaction showed no significant differences in fouling potential between PN and AA, AA and sea salt baseline during 24 hours operation. After 5 hours operation, no significant differences was observed between PN and sea salt baseline. Significant difference in fouling potential was observed between surrogates XG and PN, XG and sea salt baseline, XG and DI compaction, and between PN and DI compaction during 24 hours operation. After 1, 10, and 10 hours operation, significant difference was measured between surrogates XG and AA, AA and DI compaction, and between sea salt baseline and DI compaction.

### 4.3.3.2 Xanthan gum

Main effects of normalized flux of XG (1) total (6) < 0.4 μm and (7) ≥ 0.4 μm were plotted in Fig. 4.5. The slope of solutions effect was much less negative compared to time effect, suggesting time is the main effect factor for different XG size solutions. During 24 hrs fouling operation, p-values of two-sample t-test between XG (1) total (6) < 0.4 μm and (7) ≥ 0.4 μm were all above the statistically significance line of 0.05 (Fig. 6.), indicating no significant difference in fouling potential between different sizes of XG in sea salt solutions.
Fig. 4.5. Main effects plot for normalized flux of xanthan gum (1) total, (6) $< 0.4 \, \mu m$ and (7) $\geq 0.4 \, \mu m$. Dashed line represents the overall mean.

Fig. 4.6. Comparison of p-values for paired xanthan gum size fractions of (1) total, (6) $< 0.4 \, \mu m$, and (7) $\geq 0.4 \, \mu m$ at one-hour intervals. Dashed line indicated p-value =
A p-value below significance level of 0.05 would indicate a significant difference between two size fractions at that time point.

### 4.3.4 ATR-FTIR

Figure 4.7 showed ATR-FTIR spectrum of (a) AA powder (b) PN powder (c) XG powder, (d) AA fouled RO membrane (e) PN fouled RO membrane (f) XG fouled RO membrane (g) RO membrane after sea salt baseline and (h) virgin RO membrane. Assigned peaks were compared to other studies and were shown in Table 4.2. For polysaccharides XG, PN and AA, one of their signature bands was near 1750 cm\(^{-1}\) which associated to C=O axial deformation from esters, carboxylic acid, aldehydes and ketones (Hazirah et al. 2016; Urias-Orona et al. 2010). Another carbohydrate fingerprint band was in the range of 1200 to 950 cm\(^{-1}\) (Leal et al. 2008). For XG, the peaks at 1604 cm\(^{-1}\), 1400 cm\(^{-1}\) and 1020 cm\(^{-1}\) might associated to C=O axial deformation from enols (–diketones), and deflection angle of C=H, respectively (Hazirah et al. 2016).

![ATR-FTIR spectra](image)

Fig. 4.7. ATR-FTIR spectra of (a) alginic acid powder (b) pectin powder (c) xanthan
gum powder, (d) alginic acid fouled RO membrane (e) pectin fouled RO membrane (f) xanthan gum fouled RO membrane (g) RO membrane after sea salt baseline and (h) virgin RO membrane.

As expected, the polyamide fingerprint bands were observed in virgin RO membranes (1671, 1608 and 1542 cm\(^{-1}\)). According to Tang et al., 2009, the amide I band at 1671 cm\(^{-1}\) was associated to the C=O stretching, C–N stretching, and C–C–N deformation vibration in a secondary amide group. The aromatic amide band at 1608 cm\(^{-1}\) was due to N–H deformation vibration and C=C ring stretching vibration. The amide II band at 1542 cm\(^{-1}\) was assigned to N–H in-plane bending and N–C stretching vibration of a – CO–NH– group. For polysulfone supporting layer, the peaks at 1584, 1504, and 1486 cm\(^{-1}\) were due to aromatic in-plane ring bend stretching vibration. The weak peak at 1364 cm\(^{-1}\) were assigned to C–H symmetric deformation vibration of >C(CH\(_3\))\(_2\). The peaks at 1322 and 1293 cm\(^{-1}\) were assigned to asymmetric SO\(_2\) stretching vibration and those at 1169 and 1148 cm\(^{-1}\) were assigned to symmetric stretching vibration. The prominent peak at 1236 cm\(^{-1}\) was associated with the C–O–C asymmetric stretching vibration of the aryl–O–aryl group in polysulfone. The peak at 832 cm\(^{-1}\) was due to the in-phase out-of-plane hydrogen deformation of para substituted phenyl groups. All fouled membranes showed similar peak intensity over the wavelength range of 1800 to 600 cm\(^{-1}\).
Fig. 4.8. ATR-FTIR spectra (a) xanthan gum powder (b) total xanthan gum fouled RO membrane (c) < 0.4 μm xanthan gum fouled RO membrane (d) ≥ 0.4 μm xanthan gum fouled RO membrane and (e) virgin RO membrane.

ATR-FTIR spectra of (a) XG powder (b) total XG fouled RO membrane (c) < 0.4 μm XG fouled RO membrane (d) ≥ 0.4 μm XG fouled RO membrane and (e) virgin RO membrane were shown in Fig. 4.8. All sizes of XG fouled membranes showed identical peaks, except at 1010 cm⁻¹ the peak for particulate XG (≥ 0.4 μm) fouled membrane weakened compared to the other two XG sizes.
Table 4.2. Peak assignment for ATR-FTIR spectra of fouled polyamide RO membrane.

<table>
<thead>
<tr>
<th>Peak (cm⁻¹)</th>
<th>Assignment</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other study</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td><strong>Active layer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1663ᵃ</td>
<td>1671</td>
<td>C=O stretch, C–N stretch, C–C–N deformation vibration</td>
</tr>
<tr>
<td>1609ᵃ</td>
<td>1608</td>
<td>N–H deformation vibration or C=C ring stretching vibration</td>
</tr>
<tr>
<td>1541ᵃ</td>
<td>1542</td>
<td>N–H bend, N–C stretch</td>
</tr>
<tr>
<td><strong>Supporting layer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1587, 1504, 1488ᵇ</td>
<td>1584, 1504, 1486</td>
<td>C–C stretch</td>
</tr>
<tr>
<td>1385-1365ᵃ</td>
<td>1364</td>
<td>C–H symmetric deformation vibration</td>
</tr>
<tr>
<td>1350-1280ᵃ</td>
<td>1322, 1293</td>
<td>Asymmetric SO₂ stretching vibration</td>
</tr>
<tr>
<td>~1245ᵇ</td>
<td>1236</td>
<td>C–O–C asymmetric stretching vibration</td>
</tr>
<tr>
<td>1180-1145ᵃ</td>
<td>1169, 1148</td>
<td>Symmetric SO₂ stretching vibration</td>
</tr>
<tr>
<td>~830ᵃ</td>
<td>832</td>
<td>Hydrogen deformation</td>
</tr>
<tr>
<td><strong>Xanthan gum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1800-1700ᵇ</td>
<td>1722</td>
<td>C=O axial deformation</td>
</tr>
<tr>
<td>1604.41ᵇ</td>
<td>1604</td>
<td>C=O axial deformation</td>
</tr>
<tr>
<td>1405.19ᵇ</td>
<td>1400</td>
<td>C=H deflection</td>
</tr>
<tr>
<td>1021.28ᵇ</td>
<td>1020</td>
<td>C–O axial deformation</td>
</tr>
<tr>
<td><strong>Pectin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1750ᶜ</td>
<td>1734</td>
<td>C=O stretch</td>
</tr>
<tr>
<td>1200-950ᶜ</td>
<td>1012</td>
<td>C–O axial deformation</td>
</tr>
<tr>
<td><strong>Alginic acid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1750ᶜ</td>
<td>1725</td>
<td>C=O stretch</td>
</tr>
<tr>
<td>1035.6ᵈ</td>
<td>1029</td>
<td>C–O axial deformation</td>
</tr>
</tbody>
</table>

ᵃ: (Tang et al. 2009); ᵇ: (Hazirah et al. 2016)
ᶜ: (Urias-Orona et al. 2010)
ᵈ: (Leal et al. 2008); ᵇ: Not available.
4.3.5 CLSM

To specifically characterize fouling differences between different sizes of XG, CSLM images were taken for XG (a) total (b) < 0.4 μm (c) ≥ 0.4 μm fouled membranes and (d) RO membrane after sea salt baseline (Fig. 4.9). Compared to sea salt treated RO membrane, all XG fouled membranes were covered by XG that TEP fraction (≥ 0.4 μm XG) had the most coverage. To further quantify relative XG mass and density on membrane surface, overall mean intensity of all fouling layers and density were calculated and plotted in Fig. 4.10. The mass (overall mean intensity = total intensity/voxel) in ≥ 0.4 XG μm was highest, followed by < 0.4 XG μm and total XG. The density (mean intensity/depth), however, was highest in < 0.4 XG μm and followed by total XG and ≥ 0.4 μm XG. This was further confirmed by plotting overall mean intensity of all layers versus depth (Fig. 4.11). It also suggested development of different sizes of XG over time on membrane surface. The ≥ 0.4 μm of XG developed the most in depth, followed by total XG and < 0.4 μm XG. This indicated that TEP fraction formed the thickest layer and the highest total mass on membrane surface but least compact, while TEP precursors was least in thickness and mass but the most compact, and total TEP was in between.
Fig. 4.9. Confocal laser scanning microscope (CLSM) 3D images (aerial views): (a) total xanthan gum fouled RO membrane, (b) < 0.4 \( \mu \)m xanthan gum fouled RO membrane, (c) \( \geq 0.4 \mu \)m xanthan gum fouled RO membrane and (d) RO membrane after sea salt baseline.
Fig. 4.10. Relative comparison of (a) overall mean intensity of all layers and (b) density in xanthan gum (XG) fouled and sea salt treated RO membranes. Error bars show one standard deviation measured from triplicate samples.

Fig. 4.11. Relative comparison of overall mean intensity of all layers versus depth in xanthan gum (XG) fouled RO membranes. Noted that the last 1 - 2 points didn’t show error bars due to variation between replicates in depth. Error bars show one standard
deviation measured from triplicate samples.

4.4 Summary and conclusions

- An hour-by-hour T-test was developed to quantify and develop a criterium for differentiating fouling behaviour of different surrogates and TEP fractions. Several statistical testing procedures were evaluated for determining if there was difference in normalized flux based on different treatment. These included comparisons of regression line slopes after polynomial fitting (data not shown), Hotelling’s $T^2$ test, and the two-sample T-test. For the latter two tests, it was evident in the face of small p-values ($<0.05$) that biofouling could be statistically identified at around the same time and continuing for all times after that. In essence the authors opted for the simplest test, being the two-sample T-test based on a difference of two means and using 3 replications in each sample. This test showed similar power, to the other more complicated tests canvassed and because of its simplicity and universality we report statistical testing using this test. Researchers may want to perform T-tests beyond the time when the p-value first falls below 0.05. Our experience has been that the T-test once it becomes significant, continues to do so after that, subsequently confirming that there is a significant difference in treatment. In the event of no significant difference in treatment, p-values of the preferred test tend to be as for a test under the null hypothesis.

- Alginic acid showed no significance ($p > 0.05$) compared to pectin and sea salt baseline, whereas xanthan gum showed significant fouling ($p < 0.05$). Therefore, AA and PN might not be the best surrogate for TEP fouling potential study in seawater, whereas XG was good for studying TEP fouling behaviour.
• No significant difference was detected between total xanthan gum, $\geq 0.4 \mu m$ xanthan gum, and $< 0.4 \mu m$ xanthan gum. Similar fouling potential was measured with total TEP, TEP, and TEP precursors at same concentrations. Therefore, TEP concentrations rather than TEP sizes were more likely correlated to membrane fouling. For predicting fouling more accurately, it is important to monitor both TEP and precursor TEP in RO feed water. It highlighted the importance of developing new pre-treatment technique for TEP precursors removal, since these contribute the greatest mass to total TEP.

• ATR-FTIR provided chemical bonding information of both virgin and fouled membrane. CLSM provided semi-quantitative information of polysaccharides on membrane surface. It’s useful to combine multiple membrane characterization techniques to identify membrane fouling.

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Garcia-Ochoa F, Santos VE, Casas JA, Gomez E. 2000. Xanthan gum: Production,


CHAPTER 5

Evaluation of cleaning performance of hydrogen peroxide for xanthan gum fouled seawater reverse osmosis (SWRO) membrane

Abstract

Hydrogen peroxide (H$_2$O$_2$) is a membrane disinfection/cleaning agent which is considered as less destructive to reverse osmosis (RO) membrane and more environmentally friendly compared to the most commonly used chlorine-based chemicals (i.e. hypochlorous acid (HOCl)). Effect of H$_2$O$_2$ to RO membranes, however, have been far less studied than chlorine-based disinfectants. Up to now, its cleaning performance is still unclear. To understand H$_2$O$_2$ cleaning performance on potential biofilm initiator transparent exopolymer particles (TEP) fouled RO membrane. H$_2$O$_2$ at concentrations of 0, 1, 10, and 70 mM were used in cleaning-in-place (CIP) for five cycles TEP surrogate xanthan gum (XG) fouled RO membrane cleaning. Cleaning performance was evaluated from water flux, fouling rate, salt flux, salt rejection, and membrane surface chemistry. H$_2$O$_2$ was found to enhance water flux. This is hypothesized as possibly due to re-compaction of XG layer on membrane surface, although it also could be because of the breakdown of XG into smaller fragments. H$_2$O$_2$ at 70 mM showed greater flux rejuvenation and similar salt rejection deterioration compared to 1 and 10 mM. Membrane degradation due to exposure to H$_2$O$_2$ during five cycles of CIP was not supported by attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), salt flux, and salt rejection results. This study shed a light into potential usage of H$_2$O$_2$ for RO membrane cleaning.

Keywords: Hydrogen peroxide, xanthan gum, reverse osmosis, cleaning performance, statistical analysis
5.1 Introduction

Membrane biofouling is a major limitation in reverse osmosis (RO) desalination industry. Since RO membranes do not have distinct pores, they are particularly susceptible to surface fouling which is caused by accumulation of inorganics, organics, particulates/colloidal, and bacteria on membrane surface (Greenlee et al. 2009; Jiang et al. 2017; Nguyen et al. 2012; Van der Bruggen et al. 2003). Although pretreatment technologies (i.e. coagulation and sedimentation, microfiltration (MF), and ultrafiltration (UF) are usually installed for membrane fouling control, foulants such as transparent exopolymer particles (TEP), TEP precursors, and bacteria cannot be completely removed from RO feed water are likely to form biofilm on RO membrane surface (Bar-Zeev et al. 2015). As biofouling occurs, permeate flux decreases which shortens membrane lifespan and increases cost for membrane replacement. Other mitigation strategies including membrane cleaning/disinfection and developing anti-fouling membranes are considered.

To recover water flux and remove foulants from membrane surface, periodic cleaning in place (CIP) is routinely operated in full-scale plants (Porcelli and Judd 2010). Free chlorine (i.e. hypochlorous acid (HOCl)) and chloramines (i.e. monochloramine (NH₂Cl) and chlorine dioxide (ClO₂)) are often used for removing organic and biological foulants from seawater reverse osmosis (SWRO) membranes surface (Matin et al. 2011). The attack mechanism of chlorine, their cleaning efficiency, and influence on RO membrane performance have been studied extensively (Gohil and Suresh 2017; Gu et al. 2012; Verbeke et al. 2017). Their drawbacks, however, are that they cause membrane swelling/peeling, degrade membrane and cause damage, form disinfection by-products (i.e. absorbable organic halogen (AOX), trihalomethanes (THMs), and N-
nitrosodimethylamine (NDMA)), and cause environmental and health issues (Richardson et al. 2007; Shi et al. 2014; Wang et al. 2014).

Hydrogen peroxide (H$_2$O$_2$) is considered as an alternative disinfection agent but has been studied far less for RO membranes. The advantages of H$_2$O$_2$ over chlorine-based agents are: (1) polyamide RO membranes are relatively tolerant to H$_2$O$_2$ (Abejon et al. 2013) (2) no toxic by-product (3) H$_2$O$_2$ in RO effluent can be used in ultra violet (UV)/H$_2$O$_2$ advanced oxidation processes (Ling et al. 2017). The challenging part of using H$_2$O$_2$ in membrane cleaning is to find the proper concentration not too low to remove organics and kill bacteria, and not high enough to damage membrane (Regula et al. 2014). Although the cleaning agent components (H$_2$O$_2$ and peracetic acid) are available commercially from Dow Chemical Company, their paired cleaning efficiency and how frequently it can be applied as CIP are still unknown. Understanding the effect of H$_2$O$_2$ as a cleaning (and disinfection) agent will potentially provide insight into a better means of biofouling control in some RO systems.

The objective of this study was to understand H$_2$O$_2$ cleaning performance on potential biofilm initiator TEP, and tolerant cleaning cycle of polyamide SWRO membrane to H$_2$O$_2$. In this study, we investigated H$_2$O$_2$ cleaning performance on TEP surrogate xanthan gum (XG) fouled polyamide SWRO membrane. Cleaning performance of different concentrations of H$_2$O$_2$ during five cycles of fouling-cleaning were evaluated from water flux, flux decline rate, salt flux, and salt rejection.
5.2 Materials and methods

5.2.1 Chemicals

Sea salt was purchased from a local company (Olsson’s, WA, Australia) in Australia. The salt was originated from the Great Barrier Reef and the Great Australian Bight. Fouling agent xanthan gum (XG) was obtained from Sigma-Aldrich. Xanthan gum stock solution was prepared in advance and dissolved in deionized (DI) water and stirred overnight for complete dissolution. Analytical grade hydrogen peroxide (30% w/w) (Chem-Supply, Australia) and L-ascorbic acid (Chem-Supply, Australia) were used as cleaning agents. The cleaning solution was freshly made prior to cleaning to achieve concentrations of H$_2$O$_2$ at 0, 1, 10 and 70 mM in 0.24 mg/L ascorbic acid.

5.2.2 RO membrane

A thin-film composite polyamide reverse osmosis membrane was selected as model RO membrane. Membrane samples were collected following autopsy of a newly installed spiral-wound membrane unit (SWHR, Dow, USA) provided by a full-scale desalination plant in Perth, WA, Australia. In order to minimize variations due to conservation condition and membrane aging. Membrane samples used in this study were cut into coupon size, sealed in zip bag, and stored in fridge at 4°C simultaneously. Under operation conditions (35 g/L sea salt solution, 900psi and 20°C), salt rejection was observed between 98.2 to 99.2%.

5.2.3 Lab-scale seawater reverse osmosis (SWRO) unit

Fouling and cleaning experiment were conducted using a lab-scale crossflow reverse osmosis unit. It consists of a feed tank (Sterlitech Corp.), a crossflow cell (CF042, Sterlitech Corp.), a high-pressure pump (Hydra-Cell, Wanner Engineering Inc.), a
chiller (PolyScience), a flow meter (Blue-White Industries, Ltd.), a digital balance (A & D Australasia Pty. Ltd.), a digital pressure sensor (Techsis), two digital thermocouples (Omega Engineering), a labjack (Labjack Corp.) and a data acquisition system (Labview). The RO cell is 9.207 cm in length, 3.9 cm in width, 0.228 cm in depth with an effective membrane area of 42 cm². Concentrate was recycled back to the feed tank and permeate was collected in a beaker on a digital balance. Temperature and transmembrane pressure were monitored using thermocouples and pressure sensor, respectively. All signals were sent to a computer and recorded. Fouling experiments were operated at pressure of 900 ± 20 psi, temperature of 20 ± 1°C, and cross-flow velocity of approximately 2.2 cm/s. No pressure was applied to cleaning experiments. Conductivity was recorded at the end of each fouling cycle.

5.2.4 Fouling and cleaning protocol

The procedure of five cycles of xanthan gum fouling and hydrogen peroxide/ascorbic acid cleaning was listed Table 5.1. Firstly, RO membrane was cut into coupon size and soaked in DI water overnight prior to compaction. After 20 hrs of compaction, sea salt was loaded to feed tank to reach a concentration of 35 g/L and was operated for 5 hrs. Following sea salt baseline, prepared xanthan gum stock solution was added to achieve a final concentration of 20 mg/L. Fouling experiment was then carried out for 24 hrs. After fouling runs, feed solution was disposed and DI water was introduced to flush off any salt residues left along the high-pressure hose for 15 min. Then H₂O₂/ascorbic acid solutions at desired concentration were added to feed tank and recycled at a flux of 0.5 LPM for 15 min at 25 °C. Temperature for H₂O₂ cleaning should be controlled not higher than 25 °C to avoid severe membrane degradation. At the end of cleaning cycle, the cleaning solution was emptied and DI water was introduced to remove remaining
cleaning chemicals for another 30 min. The fouling-cleaning procedure was repeated five times simulating five-year CIP in desalination plant where a CIP was conducted annually. A final sea salt baseline was operated for 5hrs after five cycles of CIP.

Table 5.1. Fouling and cleaning procedure.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Procedure</th>
<th>Duration (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RO membrane conditioning</td>
<td>overnight</td>
</tr>
<tr>
<td>2</td>
<td>DI compaction</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Initial sea salt baseline</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Xanthan gum fouling</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>DI flush</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>H$_2$O$_2$/ascorbic acid cleaning</td>
<td>0.25</td>
</tr>
<tr>
<td>7</td>
<td>DI flush</td>
<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>Repeat steps 4-7 four times</td>
<td>101</td>
</tr>
<tr>
<td>9</td>
<td>Final sea salt baseline</td>
<td>5</td>
</tr>
</tbody>
</table>

5.2.5 Cleaning performance calculation

The most common way to evaluate cleaning performance is to calculate water recovery by comparing pure water flux before and after cleaning. However, a good water recovery does not ensure a good operational flux and therefore was not reliable (Shi et al. 2014). In this study, cleaning performance of hydrogen peroxide was evaluated from fouling potential differences before and after cleaning, and from membrane damage potential using parameters: (1) water flux (2) fouling rate (3) salt flux and (4) salt rejection. A better cleaning performance resulted in less decrease in initial water flux,
water flux at the end of each fouling cycle, fouling rate, and salt rejection, and less increase in salt flux compared to first XG fouling.

5.2.5.1 Water flux

Water flux was determined by normalizing water flux of fouling runs to the initial sea salt baseline. Raw water flux ($J_r$) was corrected for temperature (25°C) and net driving force because of change in osmotic pressure by withdrawing permeate from feed tank. Equations used for corrected water flux ($J$) calculation were:

\[
J = \frac{J_r(TCF)}{P - \pi_b}
\]

\[
J_r = \frac{\Delta m_w}{\rho \Delta t A_m}
\]

\[
TCF = EXP\left[3020 \left(\frac{1}{298} - \frac{1}{273 + T}\right)\right]
\]

\[
\pi_b (bar) = \phi C_b RT
\]

where TCF is temperature correction factor and the equation are for temperature below 25°C provided by Dow Chemical Company, P is applied pressure (Pa), $\pi_b$ is osmotic pressure at the bulk (Pa), $\Delta m_w$ is the mass of permeate (g) collected during time $\Delta t$ (h), $\rho$ is solution density (g/L), $A_m$ is the effective membrane area (m²), $\phi$ is osmotic coefficient which was calculated using Phreeqc (United States Geological Survey (USGS), USA), $C_b$ is bulk concentration of solutes (mol/L), R is universal gas constant (0.083145 L·bar/mol·K), and T is thermodynamic (absolute) temperature (K).

Corrected water flux was then normalized to initial water flux of sea salt baseline using following equation.
Norm \( J = \frac{J_t}{J_{ss,0}} \)

where Norm \( J \) is normalized water flux to initial sea salt baseline, \( J_t \) is water flux at time \( t \), and \( J_{ss,0} \) is initial water flux of sea salt baseline.

### 5.2.5.2 Fouling rate

As membrane fouling gets worse or cleaning efficiency becomes weaker, water permeability will decrease more rapidly over time/cleaning cycle. Consequently, the slope of normalized water flux is more negative. Fouling rate of different XG fouling cycles at different \( \text{H}_2\text{O}_2 \) concentrations, therefore, can be evaluated from the slope of normalized flux. To understand \( \text{H}_2\text{O}_2 \) cleaning effect on XG, fouling rate were calculated for each fouling cycle. In this study, fouling rate values were presented as an absolute value of the slope of normalized water flux. Noted that first 1 to 3 data points of fouling cycle 2 to 5 were not counted in slope calculation due to unstable initial state of re-compaction.

### 5.2.5.3 Salt flux

Salt flux (\( J_s \)) is normally expressed as a mass flux with units of mg/m\(^2\)/h and can be determined by equation:

\[
J_s = \frac{\Delta m_s}{\Delta t A_m}
\]

where \( \Delta m_s \) (g) is the mass of salt passes through membrane during time \( \Delta t \) (h). In this study, an overall salt flux was calculated for 24-hour fouling operation (\( \Delta t = 24 \)). As a result, the mass of salt passed through membrane during 24hrs is:

\[
\Delta m_s = C_{p,24} V_{p,24}
\]
where \( C_{p,24} \) (mg/L) is permeate concentrations at 24 hrs, \( V_{p,24} \) (L) is permeate volume at 24 hrs. Permeate concentration correlated to permeate conductivity \( (\sigma_p, \text{mS/cm}) \) which can be obtained by conducting a calibration curve of conductivity versus sea salt mass.

To evaluate membrane damage caused by \( \text{H}_2\text{O}_2 \), normalized salt flux \( (\text{Norm } J_s) \) was calculated using overall salt flux \( (J_{s,nM}, n = 0, 1, 10, \text{ and } 70) \) divided by that of 0 mM \( \text{H}_2\text{O}_2 \) cleaning condition \( (J_{s,0mM}) \) at each cleaning cycles.

\[
\text{Norm } J_s = \frac{J_{s,nM}}{J_{s,0mM}}
\]

### 5.2.5.4 Salt rejection

Due to inconsistencies of different sections of membrane, initial salt rejection \( (R) \) of each membrane used in cleaning study varied. To avoid the intrinsic difference and compare salt rejection between different membranes after cleaning, salt rejection \( (R) \) ratio between fouling cycle \( n \) \( (R_n) \) and first XG fouling cycle \( (R_1) \) was calculated for each membrane. As a rule of thumb, when salt rejection ratio drops about 10\%, it indicates membrane damage (Gohil and Suresh 2017). Salt rejection can be determined simply from conductivity of permeate \( (\sigma_p) \) and feed solution \( (\sigma_f) \).

\[
R \ (\%) = \left(1 - \frac{\sigma_p}{\sigma_f}\right) \times 100\%
\]

### 5.2.6 ATR-FTIR

Fouled membranes were cut into 1×1 cm\(^2\) and air dried thoroughly before attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) analysis (Perkin Elmer, US). Each spectrum was averaged from 80 scans over the range of 600 -1900
Three replicates were done for each membrane sample. ATR-FTIR spectrum showed in this study were the mean results of the three replicates.

5.3 Results and discussion

5.3.1 Normalized water flux

Cleaning performance of H$_2$O$_2$ at concentrations of 0, 1, 10, and 70 mM were studied during five cycles of XG fouling and H$_2$O$_2$/Ascorbic acid cleaning. Normalized water flux of initial sea salt baseline, five cycles of XG fouling, and final sea salt baseline at each cleaning conditions were shown in Fig. 5.1 (a) to (d). Two reference lines 1.0 and 0.6 indicated initial flux of sea salt baseline and flux of first XG fouling at the end of 24-h fouling operation, respectively.

Compared to first XG fouling, an increasing trend in initial flux was observed with increased cleaning cycle for all cleaning conditions. This can be caused by decompaction-compaction procedure. At cleaning conditions, no pressure was applied to RO membrane, and hydrophilic colloid XG on membrane surface was less compacted and loosely attached. Once under pressure, a re-attachment process to membrane surface resulted in increase in initial water flux and rapid initial flux decline; as it reached equilibrium condition, a slow flux decline phase was observed (Matin et al. 2011). When long enough DI compaction (20 hrs) was done after cleaning but before refouling, such increase in initial water flux was much less noticeable (Fig. 5.2).
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(a) 0 mM H$_2$O$_2$

(b) 1 mM H$_2$O$_2$

(c) 10 mM H$_2$O$_2$
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Fig. 5.1. Normalized water flux during five cycles of xanthan gum (XG) fouling and 
H₂O₂ cleaning at concentrations of (a) 0 mM (b) 1 mM (c) 10 mM and (d) 70 mM. 
Upper and lower reference line represented normalized flux of 1.0 and 0.6, respectively.

Fig. 5.2. Normalized water flux during five cycles of xanthan gum (XG) fouling and 70 
mM H₂O₂ cleaning where 20-hr deionized water compaction was done between each 
cleaning and fouling cycle.
Normalized initial water flux of fouling cycle 1 to 5 at different H₂O₂ cleaning concentrations was plotted in Fig. 5.3. A good correlation between initial water flux and fouling cycle was observed with coefficient of determination (R²) ranged from 0.8684 to 0.9933. Initial water flux increasing rate (slope) at 0, 1, 10 and 70 mM H₂O₂ cleaning concentrations were 0.0212, 0.0368, 0.0513, and 0.0447 per cycle, respectively. Compared to DI water cleaning (0 mM H₂O₂), H₂O₂ showed stronger effect in increasing initial water flux possibly due to breakdown of XG molecules into smaller filaments. Alternatively, it could be because of H₂O₂ degradation effect to RO membrane, which might not be the case here because the highest H₂O₂ concentration (70 mM) didn’t show the greatest effect. Also, membrane degradation was unlikely during such short H₂O₂ exposure time (15 - 60 min) since polyamide membrane was reported stable in 50 mM H₂O₂ for at least 10 days (Ling et al. 2017). The hypothesis was further discussed in section 5.3.4.

![Fig. 5.3. Normalized initial water flux of different xanthan gum fouling cycle n (n = 1 - 5) at H₂O₂ cleaning concentrations of 0, 1, 10, and 70 mM.](image-url)
Fig. 5.4. Normalized final water flux ratio between xanthan gum fouling cycle n (n = 1 - 5) and first fouling cycle at H\textsubscript{2}O\textsubscript{2} cleaning concentrations of 0, 1, 10, and 70 mM.

Since initial flux increased with increased fouling cycle, flux decline ratio (Hu et al. 2016) might not be appropriate for evaluating flux decline difference between fouling cycles, normalized water flux at the end of 24-h fouling operation at cycle n (Norm J\textsubscript{n,24}) was compared to that of first XG fouling (Norm J\textsubscript{1,24}) (Fig. 5.4). Among all cleaning conditions, 0 mM H\textsubscript{2}O\textsubscript{2} showed a decline trend in last flux data which indicated the worst cleaning performance; while 1, 10, and 70 mM H\textsubscript{2}O\textsubscript{2} showed an increasing trend in the last fouling flux indicating a comparable cleaning performance.

### 5.3.2 Fouling rate

To further identify cleaning performance, fouling rate for all fouling cycles at H\textsubscript{2}O\textsubscript{2} concentrations of 0, 1, 10 and 70 mM were plotted in Fig. 5.5. Fouling rate of 1 and 10 mM increased rapidly for first three or four cycles and then flattened suggesting similar cleaning performance. Fouling rate of 70 mM slightly increased during first three cycles,
then increased at 4th fouling cycle suggesting good cleaning performance before cycle 3 but was not effective afterwards. Fouling rate of 0 mM didn’t follow an increasing trend, instead, it increased for first three fouling cycles but decreased for last two cycles.

Fouling rate of initial sea salt baseline at all cleaning conditions were between 0.008 to 0.009; fouling rate of final sea salt baseline at H2O2 concentrations of 0, 1, 10 and 70 mM were 0.016, 0.030, 0.029, and 0.027, respectively. Fouling rate of final sea salt baseline at higher H2O2 concentrations were greater than 0 mM H2O2, which was consistent with findings from initial water flux increasing rate (Fig. 5.3). The hypothesize was that a higher increase in initial water flux due to re-compaction (re-attachment of XG to membrane surface) resulted in a more rapid initial flux decline before equilibrium state (compacted). This can be confirmed from conducting DI compaction experiment between cleaning and fouling cycles (Fig. 5.2), where fouling rate of final sea salt baseline (0.004) was comparable to initial sea salt baseline (0.003).

![Fouling rate of xanthan gum during five cycles of fouling and H2O2 cleaning at concentrations of 0, 1, 10, and 70 mM.](Fig. 5.5. Fouling rate of xanthan gum during five cycles of fouling and H2O2 cleaning at concentrations of 0, 1, 10, and 70 mM.)
To compare the acceleration rate of fouling rate for 1, 10, and 70 mM, the slope of fouling rate was plotted against H₂O₂ concentration in Fig. 5.6. A good correlation between them observed with a R² value of 0.9552. The acceleration rate of fouling rate of 70 mM H₂O₂ was smaller than that of 1 and 10 mM, indicating that RO membrane after 70 mM H₂O₂ fouled less quickly than 1 and 10 mM H₂O₂ cleaned membrane. So, 70 mM H₂O₂ was considered to show better cleaning performance compared to 1 and 10 mM H₂O₂.

![Graph showing acceleration rate of fouling rate](image.png)

Fig. 5.6. Acceleration rate of fouling rate of five-cycle xanthan gum fouling at different H₂O₂ cleaning concentrations.

5.3.4 Salt flux and salt rejection

Salt flux and salt rejection are two indicative parameters for evaluating membrane performance. Permeate conductivity increased with increased fouling cycles from ~1500 to up to 4000 µs/cm for all cleaning conditions. To identify whether such an increase was due to increased fouling cycles or membrane degradation caused by H₂O₂, salt flux at 1, 10, and 70 mM were normalized to that of 0 mM H₂O₂ for each fouling
cycle and shown in Fig. 5.7. Normalized salt flux was between ~0.8 to 1.1, suggesting that salt flux did not increase due to exposure to \( \text{H}_2\text{O}_2 \).

![Normalized salt flux during five cycles of xanthan gum fouling and \( \text{H}_2\text{O}_2 \) cleaning at concentrations of 0, 1, 10, and 70 mM.]

Fig. 5.7. Normalized salt flux during five cycles of xanthan gum fouling and \( \text{H}_2\text{O}_2 \) cleaning at concentrations of 0, 1, 10, and 70 mM.

![Salt rejection ratio between xanthan gum fouling cycle \( n (n = 1 - 5) \) and first fouling cycle \( R_1 \) at \( \text{H}_2\text{O}_2 \) cleaning concentrations of 0, 1, 10, and 70 mM.]

Fig. 5.8. Salt rejection ratio between xanthan gum fouling cycle \( n (n = 1 - 5) \) and first fouling cycle \( R_1 \) at \( \text{H}_2\text{O}_2 \) cleaning concentrations of 0, 1, 10, and 70 mM.
Salt rejection ratio between fouling cycle n and 1st XG fouling at H$_2$O$_2$ concentrations of 0, 1, 10, and 70 mM were shown in Fig. 5.8. Compared to initial salt rejection, it decreased with increased fouling cycles for all cleaning conditions. At most cases, if rejections were not affected, the rise of permeate production rate would be a positive result of RO membrane degradation (Abejon et al. 2013). This implied that salt rejection decline in this study was not caused by H$_2$O$_2$. In fact, it was found that H$_2$O$_2$ enhanced water flux without diminishing salt rejection before reaching breakthrough point where it was 18 and 10 days for exposure to 25 and 50 mM H$_2$O$_2$, respectively (Ling et al. 2017). Although the mechanism is unclear, it was suggested that water flux and salt rejection were controlled by different structural features of the polyamide layer (Fujioka et al. 2015), that the outer polyamide layer contributes to flux resistance and the “inner-nodular back layer” is responsible for the solute-water separation (Pacheco et al. 2016).

Another criterion for evaluating membrane damage is the percentage of salt rejection decline. As the rule of thumb, if salt rejection drops by 10%, membrane damage such as loss of material and formation of cracks should be considered (Gohil and Suresh 2017). This was not the case here because salt rejection decline was less than 3% in this study. Although low levels of degradation might occur affecting membrane properties such as permeability (Gohil and Suresh 2017).

5.3.5 ATR-FTIR

To investigate how the chemical structure of membrane changed due to exposure of H$_2$O$_2$, surface chemistry of H$_2$O$_2$ treated RO membranes were compared to virgin and sea salt treated RO membranes by ATR-FTIR (Fig. 5.9). Membrane degradation can be evaluated from shift in peak and decline in polyamide bands intensity. A polyamide RO
membrane was often considered damaged by chlorination with signs of amide I band (1663 cm\(^{-1}\)) shift to a higher frequency, amide II band (1541 cm\(^{-1}\)) shift to lower frequency, and decrease of signature bands (1663, 1609, and 1541 cm\(^{-1}\)) peak intensities (Kwon and Leckie 2006). Compared to reference polyamide signature bands, the observed bands in virgin RO membrane in this study were 1671, 1608, and 1542 cm\(^{-1}\) which can be assigned to C=O stretching/C-N stretching/C-C-N deformation vibration, N-H deformation/C=C ring stretching vibration motion, and N-H bending/N-C stretching, respectively (Tang et al. 2009). For \(\text{H}_2\text{O}_2\)/sea salt treated membranes, those peaks shifted to 1647, 1613, and 1546 cm\(^{-1}\), respectively. On the contrary of RO membrane chlorination/damage, amide I band (1671 cm\(^{-1}\)) shifted to a lower frequency and amide II band (1542 cm\(^{-1}\)) shifted to a higher frequency due to stronger hydrogen bonding between C=O double bond and N-H bond (Kwon and Leckie 2006), the intensity of amide I and II band increased implying the number of -NH- bonds and hydrogen bonding sites increased in membranes (Kwon et al. 2008). In fact, there was a variation in peaks of polyamide signature bands due to different manufacturers, that amid I band ranged from 1670 - 1640 cm\(^{-1}\), amide II band ranged from 1550 - 1530 cm\(^{-1}\), and aromatic ring band ranged from 1609 - 1610 cm\(^{-1}\) (Ettori et al. 2011; Kang et al. 2007; Oh and Jang 2016; Tang et al. 2009). In contrast to exposure with \(\text{H}_2\text{O}_2\), no changes of these signature bands were reported for polyamide RO membrane exposed to 50 mM \(\text{H}_2\text{O}_2\) for 24 days (Ling et al. 2017).

A difference in carbohydrate fingerprint band in the range of 1200 to 950 cm\(^{-1}\) (Hazirah et al. 2016; Leal et al. 2008) was also noted for DI water and \(\text{H}_2\text{O}_2\) cleaned membranes. Carbohydrate peak intensity/width was in the order of 0 = 1 > 10 > 70 > sea salt > clean RO membrane. This indicated that 70 mM \(\text{H}_2\text{O}_2\) cleaned RO membrane was cleaner.
than 0, 1, and 10 mM \( \text{H}_2\text{O}_2 \) cleaned RO membranes. This was consistent with findings from supporting layer. For polysulfone bands, peak at 1584, 1486, 1293, 1236, 1169, 1148, and 832 cm\(^{-1}\) (Tang et al. 2009) of 70 mM \( \text{H}_2\text{O}_2 \) cleaned RO membrane showed similar intensity to sea salt treated and clean RO membrane; whereas for 0, 1, and 10 mM \( \text{H}_2\text{O}_2 \) treated RO membranes those peaks were less intense. Such difference in polysulfone peak intensity suggested that XG layer on 70 mM \( \text{H}_2\text{O}_2 \) cleaned RO membrane was thinner compared to 0, 1, and 10 mM \( \text{H}_2\text{O}_2 \).

![ATR-FTIR spectra of RO membranes after five cycles of xanthan gum fouling and \( \text{H}_2\text{O}_2 \) cleaning at concentrations of 0, 1, 10, and 70 mM, RO membrane after sea salt baseline, and virgin RO membrane.](image)

**Fig. 5.9.** ATR-FTIR spectra of RO membranes after five cycles of xanthan gum fouling and \( \text{H}_2\text{O}_2 \) cleaning at concentrations of 0, 1, 10, and 70 mM, RO membrane after sea salt baseline, and virgin RO membrane.

### 5.4 Summary and conclusions

- A laboratory test was designed simulating five cycles of CIP using \( \text{H}_2\text{O}_2 \) at different concentrations. Initial flux after each cleaning cycle was found increased at all
cleaning conditions. It’s possibly due to re-compaction of loosely attached XG layer or H$_2$O$_2$ caused breakdown of XG into smaller fragment. Cleaning with DI water alone lead to more water flux decline after each cleaning cycle. Therefore, DI water flush is not recommended to use alone for membrane cleaning, and combination of DI with other chemical cleaning is needed. Water flux results provided rough estimation of cleaning performance in terms of water flux enhancement or diminution.

- The acceleration rate of fouling rate of 70 mM was smaller than that of 0 and 1 mM. This indicated that XG fouled RO membrane after 70 mM H$_2$O$_2$ cleaning fouled less quickly than 1 and 10 mM H$_2$O$_2$ cleaned membrane.

- Normalized salt flux after each H$_2$O$_2$ cleaning cycle was similar to those after DI water cleaning. Salt rejection decline after five cycles of H$_2$O$_2$ CIP were less than 3%. These suggested that exposure to H$_2$O$_2$ did not enhance salt flux nor deteriorate salt rejection.

- ATR-FTIR showed XG fouled RO membrane after 70 mM H$_2$O$_2$ cleaning was cleaner compared to 0, 1, and 10 mM cleaned RO membrane. Polyamide (PA) signature band of RO membrane after H$_2$O$_2$ cleaning showed no difference from sea salt baseline control. Membrane degradation was not detected by ATR-FTIR.

- In general, H$_2$O$_2$ at 70 mM showed greater flux rejuvenation and similar salt rejection deterioration compared to 1 and 10 mM. Membrane exposure to H$_2$O$_2$ cleaning didn’t show significance in salt flux increase nor salt rejection decline. The
loss of salt rejection during five cycles of fouling and cleaning-in-place (CIP) was not detected by ATR-FTIR. The work strongly suggests peroxide cleaning be further evaluated with field trials as an alternative to commonly used, but more destructive, cleaning techniques.

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CHAPTER 5

References


CHAPTER 6

Conclusions and recommendations
6.1 Conclusions

The objectives of this study were to get better understanding of potential biofilm initiator transparent exopolymer particles (TEP) and TEP precursors in reverse osmosis (RO) systems. In this study, we evaluated TEP and TEP precursors from three perspectives (a) quantification methods, (b) fouling potential, and (c) membrane cleaning through the studies of:

- Comparing Alcian blue based TEP and TEP precursors quantification method with a total carbohydrate essay in fresh, brackish, and seawater.
- Quantifying fouling potential difference between TEP surrogates (xanthan gum (XG), pectin (PN), and alginic acid (AA)) and TEP size fractions (total TEP, TEP, and TEP precursors) by developing statistical analysis and using membrane characterization techniques.
- Investigating cleaning performance of H$_2$O$_2$ on TEP surrogate fouled RO membranes.

As a result, methods accuracy and recovery, fouling potential in terms of flux decline and significance, and cleaning performance in terms of water flux, fouling rate, salt flux, salt rejection, and significance were determined and evaluated.

It was found that:

- Alcian blue based TEP staining method is limited by batch variability which makes evaluation and comparison of biofouling potential between studies problematic whereas total carbohydrate assay is less laborious and better in reproducibility.
- Measured TEP concentrations were dependent on surrogates, salinity, method of recovery, and calibration factor. TEP only represents a small fraction (~10%) of the
total mass for the XG. The major component of total TEP is TEP precursors. Therefore, measuring the concentration of TEP alone will likely underestimate the fouling potential for a membrane system.

- An hour-by-hour T-test was developed to quantify and differentiate fouling behaviour of different surrogates and TEP fractions. The T-test performed at one-hour interval or three-hour interval showed similar power.

- AA and PN exhibited significant fouling potential difference compared to XG, whereas similar fouling potential was measured with total XG, < 0.4 µm XG, and ≥ 0.4 µm XG. Therefore, TEP concentrations rather than TEP sizes were more likely correlated to membrane fouling.

- H$_2$O$_2$ enhanced water flux possibly due to re-compaction/re-attachment of XG on membrane surface and following breakdown of XG into smaller fragments.

- Taken attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), salt flux, and salt rejection results together, there was no supportive evidence showing membrane degradation due to exposure to H$_2$O$_2$ during cycles of fouling and cleaning-in-place (CIP).

Based on these findings, it is useful to:

- Report both concentrations of TEP and TEP precursors as well as surrogate, salinity, method of recovery, and calibration factor for each study for comparison purpose.

- Develop new pre-treatment technology for TEP precursors removal.

- Use both water flux, fouling rate, salt flux, salt rejection, statistical analysis, and multiple membrane characterization techniques (i.e. ATR-FTIR and Confocal Laser Scanning Microscopy (CLSM)) to compare/quantify membrane fouling and degradation.
The desalination industry can benefit from the following perspectives:

- To comparatively analyse new membrane product and/or cleaning approaches, it provides a relatively short and feasible testing period of time.
- To mitigate membrane biofouling by developing new pretreatment methods for effective TEP removal or new membrane cleaning methods targeting less TEP attachment.
- To improve membrane cleaning methods.
- To reduce operation, maintenance and repair (OM&R) cost.
6.2 Recommendations

The valuable findings in this study regarding TEP and their precursors in RO systems have led to solid recommendations to future studies:

- Development of direct TEP quantification method is recommended since it doesn’t seem practicable to apply Alcian-blue based TEP method for field in-situ TEP concentration monitoring due to its poor reproducibility and batch variability. If one could simulate and correlate a “internal standard” to TEP concentration, then it can be used as a comparatively accurate measurement tool for TEP concentration analysis and can be used in pilot-scale SWRO unit or even full-scale SWRO desalination plant for TEP monitoring and biofouling control.

- On the one hand, the exact chemical composition of TEP is still unknown. For better understanding of biofouling mechanism as well as making progress in biofouling prevention (i.e. pre-treatment) and membrane fouling mitigation (i.e. membrane cleaning), it is recommended for future study to analyze TEP composition.

- Up to now, TEP surrogates were commonly used in membrane fouling studies which does not necessarily reflect fouling conditions in full-scale desalination plant. Therefore, there is a need for future studies to conduct fouling experiment using natural TEP materials such as seawater TEP and aquatic organism (i.e. bacteria and phytoplankton) released TEP.

- To get fully understanding of cleaning performance of H₂O₂ in full scale desalination plant, it is recommended for future studies to study cleaning performance of H₂O₂ on different TEP fractions derived from TEP surrogates, natural seawater, and bacteria, and on different membrane types.
Fig. S1. Comparison of p-values (two-sample t-test at three-hour intervals) between xanthan gum (1) total (6) < 0.4 μm and (7) ≥ 0.4 μm. Dashed line indicated p-value = 0.05.
Fig. S2. Normalized flux of (a) surrogates and baseline and (b) xanthan gum of different sizes. An adjusted x-axis scale for statistical view. Error bars show one standard deviation measured from triplicate samples.