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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**DEVELOPMENT OF MONITORING TOOLS BASED ON
ELECTRICAL IMPEDANCE SPECTROSCOPY FOR WATER
TREATMENT AND INTEGRATED PROCESS FOR WASTEWATER
RECOVERY**

HO JIA SHIN

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

2017

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RECOVERY**

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Abstract

In this thesis, a real time and non-invasive electrical impedance spectroscopy (EIS) fouling monitor was employed for determination of threshold flux, to monitor biofouling and fouling behavior in a real water reclamation plant. EIS is able to provide reliable information on fouling events in reverse osmosis (RO) and to give insights into the phenomenological events happening in the diffusion polarization (DP) layer during colloidal as well as biofouling.

When RO was operated near the threshold flux for silica fouling, the nature of the flowing colloidal suspension of silica on the membrane surface changed. This transition was well-defined and was reflected in the changes of the slope of transmembrane pressure (TMP) with flux and the conductance of the diffusion polarization (DP) layer determined by EIS. The threshold flux increased with increasing crossflow velocity. The effect of the presence of a spacer in the feed channel was also investigated and resulted in a higher threshold flux. The conductance of the diffusion polarization layer (G_{DP}) derived from the low frequency region in the EIS was identified as the most importance parameter for signaling the onset of fouling in this thesis.

EIS was also used to monitor the biofilm formation on the membrane surface. In this case, the G_{DP} showed two stages of biofilm formation, first increasing then decreasing. The first stage was related to the accumulation of bacterial cells and the formation of the respiration products from the bacteria. The second stage referred to the accumulation of extracellular polymeric substances (EPS) which was the main component for the formation of the biofilm matrix. The effect of sodium azide which acts as the biostat was also evaluated and its presence slowed down the growth of bacterial on the membrane surface. Sodium azide's effect was reflected in the normalized G_{DP} plot. The sustainable flux, at

which the fouling was minimal, could be estimated from the rate of change of the initial increase of normalized G_{DP} with respect to flux.

In order to investigate the applicability of EIS fouling monitor in real plant, it was used to monitor the performance of a RO treatment train in one of the wastewater reclamation plants in Singapore. The EIS measurement system coupled with the canary cell was installed in a side stream of the RO train. The canary cell was operated at the same average hydrodynamic and flux conditions of the spiral wound modules of the RO train. The response of the EIS fouling monitor was found to be correlated well with the plant performance. A maxima was observed from the normalized real impedance of the diffusion polarization layer ($Z_{real-DP}$) versus time plot well before several cycles of chemical cleaning. The occurrence of $Z_{real-DP}$ can be used as an indicator to initiate mitigation measures to control fouling.

List of Publications

Journal Publications:

1. **Ho, J.S.**, Toh, C.-S., 2013. A Rapid Low Power Ultra-Violet Light-Assisted Bacterial Sensor for Coliform Determination. American Journal of Analytical Chemistry 4, 10, 8.
2. Cheng, M.S., **Ho, J.S.**, Lau, S.H., Chow, V.T.K., Toh, C.-S., 2013. Impedimetric microbial sensor for real-time monitoring of phage infection of *Escherichia coli*. Biosensors and Bioelectronics 47, 340-344.
3. **Ho, J.S.**, Ma, Z., Qin, J., Sim, S.H., Toh, C.-S., 2015. Inline coagulation-ultrafiltration as the pretreatment for reverse osmosis brine treatment and recovery. Desalination 365, 242-249.
4. **Ho, J.S.**, Sim, L.N., Gu, J., Webster, R.D., Fane, A.G., Coster, H.G.L., 2016a. A threshold flux phenomenon for colloidal fouling in reverse osmosis characterized by transmembrane pressure and electrical impedance spectroscopy. Journal of Membrane Science 500, 55-65.
5. **Ho, J.S.**, Low, J.H., Sim, L.N., Webster, R.D., Rice, S.A., Fane, A.G., Coster, H.G.L., 2016b. *In-situ* monitoring of biofouling on reverse osmosis membranes: Detection and mechanistic study using electrical impedance spectroscopy. Journal of Membrane Science 518, 229-242.
6. **Ho, J.S.**, Sim, L.N., Webster, R.D., Viswanath, B., Coster, H.G.L., Fane, A.G., 2017. Monitoring fouling behaviour of reverse osmosis membranes using electrical impedance spectroscopy: A field trial study. Desalination 407, 75-84.

Patents:

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2. **Ho, J. S.**; Toh, C. –S. “A Rapid Low Power Ultra-Violet Light-assisted Membrane-based Bacterial Sensor for Coliform Determination”, 2nd Regional Symposium on Biosensors, Biodiagnostics & Biochips 2013, 11-13 December, Chiang Rai, Thailand.
3. **Ho, J. S.**; Qin, J.; Sim, S.; Ma, Z; Toh, C. –S. “Inline Coagulation-Ultrafiltration as the Pretreatment for Reverse Osmosis Brine Recovery”, International Congress on Natural Sciences and Engineering 2014, 7 – 9 May 2014, Kyoto, Japan.
4. **Ho, J. S.**; Sim, L. N.; Gu, J.; Toh, C.-S.; Coster, H. G. L.; Fane, A. G. “Critical flux determination for silica fouling in reverse osmosis: Detection by electrical impedance spectroscopy”, The 10th International Congress on Membranes and Membrane Processes (ICOM 2014), 20-25 July 2014, Suzhou, China.
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List of Symbols

Symbol	Description (units)
a_p	Radius of particle (m)
A	Surface area of the electrode (m ²)
$[c]$	Concentration of <i>E. coli</i> (cfu/mL)
C	(I) Capacitance (F) (II) Concentration of redox probe (M)
C_b	Concentration of solute in bulk solution (ppm or kg/m ³)
C_p	Concentration of solute in permeate (ppm or kg/m ³)
C_w	Concentration of solute at the membrane wall (ppm or kg/m ³)
D	Diffusion coefficient of solute in bulk solution (m ² /s)
D_{Br}	Diffusion coefficient of particle based on Brownian diffusion model (m ² /s)
D_{SID}	Diffusion coefficient of particle based on shear-induced diffusion model (m/s)
f	Frequency (Hz)
F	Faraday constant (C/mol)
G	Conductance (S)
G_{feed}	Conductance of feed (S)

Symbol	Description (units)
G_{measured}	Measured conductance (S)
$G_{\text{normalized}}$	Normalized conductance (S)
G_{DP}	Conductance of diffusion polarization layer (S/m^2)
$G_{\text{DP-0}}$	G_{DP} at time 0 (S/m^2)
$G_{\text{DP-Normalized}}$	Normalized G_{DP} ($G_{\text{DP}}/ G_{\text{DP-0}}$, dimensionless)
G_R	Real part of conductance (S)
jG'	Imaginary part of conductance (S)
i	Current (A)
i_c	Capacitive current (A)
i_f	Faradaic current (A)
i_o	Amplitude of current (A)
I_p	Oxidative current in the presence of <i>E. coli</i> (A)
$I_{p,0}$	Oxidative current in the absence of <i>E. coli</i> (A)
J_v	Permeate flux ($\text{L}/\text{m}^2 \text{ h}$)
J_{Br}	Flux based on Brownian diffusion model ($\text{L}/\text{m}^2 \text{ h}$)
J_{SID}	Flux based on shear-induced diffusion model ($\text{L}/\text{m}^2 \text{ h}$)
J_{IL}	Flux based on inertial lift model ($\text{L}/\text{m}^2 \text{ h}$)
J_{th}	Threshold flux ($\text{L}/\text{m}^2 \text{ h}$)
k	Electron transfer rate constant (m/s)

Symbol	Description (unit)
k^0	Electron transfer rate constant at the non-hindered electrode surface (m/s)
k_B	Boltzmann's constant
k_m	Mass transfer coefficient (m/s)
L	Membrane channel length (m)
n	Frequency independent exponent (dimensionless)
Q	Frequency independent admittance ($\Omega^{-1} \text{ s}^n$)
R_{et}	Electron transfer resistance (Ω)
R_{et}^0	Electron transfer resistance prior to infection (Ω)
R_m	Membrane resistance (m^{-1})
R_s	Ohmic resistance of bulk solution (Ω)
R_{obs}	Observed retention (dimensionless)
R_{real}	Real retention coefficient (dimensionless)
TMP	Transmembrane pressure (bar)
TMP_0	Transmembrane pressure at time 0 (bar)
T	Temperature (K)
t	Time (h)
$v_{L,o}$	Inertial lift velocity (m/s)
v	Electrical potential (V)

Symbol	Description (unit)
v_o	Amplitude of electrical potential (V)
Y	Frequency dependent admittance (Ω^{-1})
$ Z $	Magnitude of impedance (Ω)
Z_{Re}	Real part of impedance (Ω)
$Z_{\text{real-DP}}$	Real impedance of diffusion polarization layer (Ω)
Z_{Im}	Imaginary part of impedance (Ω)
$Z_{\text{normalized}}$	Normalized impedance (dimensionless)
Z_{measured}	Measured impedance (Ω)
Z_w	Warburg impedance ($\Omega/\text{s}^{1/2}$)
Greek letters	
β	Magnitude of the capacitance (dimensionless)
γ_o	Shear rate (s^{-1})
δ	Boundary layer thickness (m)
δ_{channel}	Channel height (m)
$\Delta\Pi_b$	Osmotic pressure difference between feed and permeate solution (Pa or bar)
η	Viscosity (Pa.s or kg/s.m)
θ	(I) Phase difference – Chapter 2 (deg)
	(II) Surface coverage - Chapter 6 (dimensionless)

Symbol	Description (unit)
μ	Viscosity of solution (Pa.s)
Π_w	Osmotic pressure at the membrane wall (Pa or bar)
Π_p	Osmotic pressure of permeate solution (Pa or bar)
ρ_o	Fluid density (kg/m ³)
σ_{feed}	Conductivity of feed (S/cm)
ϕ_w	Particle volume fraction at the edge of the cake layer
ϕ_w	Particle volume fraction at the membrane wall (dimensionless)
ϕ_b	Particle volume fraction in the bulk solution (dimensionless)
ω	Angular frequency (rad/s)

List of Abbreviations

Abbreviations	Descriptions
ACH	Aluminum chlorohydrate
AOC	Assimilable organic carbon
AOPs	Advanced oxidation processes
ATP	Adenosine triphosphate
AU	Arbitrary units
BAC	Biological activated carbon
BB	Building blocks
BEOP	Biofilm enhanced osmotic pressure
BSA	Bovine serum albumin
BP	Biopolymers
CDI	Capacitive deionization
CECP	Cake-enhanced concentration polarization
CEOP	Cake enhanced osmotic pressure
cfu	Colony forming unit
CIP	Cleaning-in-place
CLSM	Confocal laser scanning microscopy
CP	Concentration polarization

Abbreviations	Descriptions
CV	Cyclic voltammetry
DLVO	Derjaguin-landau-verwey-overbeek
DOC	Dissolved organic carbon
DOTM	Direct observation through membrane
DP	Diffusion polarization
<i>E. coli</i>	<i>Escherichia coli</i>
EEM	Excitation-emission matrix
EIS	Electrical impedance spectroscopy
EISFM	Electrical impedance spectroscopy fouling monitor
ELISA	Enzyme-linked immunosorbent assay
EPS	Extracellular polymeric substances
FESEM-EDX	Field-emission scanning electron microscopy with energy dispersive X-ray
FO	Forward osmosis
HS	Humic substances
IC	Ion chromatography
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
LC-OCD	Liquid chromatography-organic carbon detection
LMW	Low molecular weight
LPS	Lipopolysaccharide

Abbreviations	Descriptions
MF	Microfiltration
MF/UF	Microfiltration/ultrafiltration
MLD	Megalitres per day
MOI	Multiplicity of infection
NB	Nutrient broth
NF	Nanofiltration
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NOM	Natural organic matter
O&M	Operation and maintenance
PACl	Polyaluminium chloride
PA01	<i>Pseudomonas aeruginosa</i> 01
PB	Prussian blue
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
RNI	Reactive nitrogen intermediates
RO	Reverse osmosis
ROI	Reactive oxygen intermediates
SAM	Self-assembled monolayer

Abbreviations	Descriptions
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
SPCE	Screen printed carbon electrode
SWRO	Seawater reverse osmosis
TFC	Thin film composite
TOC	Total organic carbon
UF	Ultrafiltration
UTDR	Ultrasonic time domain reflectometry
UV	Ultra-violet

1 Chapter 1 – Introduction

1.1 Background

A myriad of problems in the world have been associated with the shortage of clean and fresh water supplies. There are more than 1.1 billion people who do not have access to safe drinking water and an estimated 2.6 billion individuals who have no sanitation services (Montgomery and Elimelech, 2007). In addition to this, the global water demand is projected to increase by 55% by 2050 and this is mainly attributed to the growing demand from manufacturing, domestic use and thermal electricity generation (WWAP United Nations World Water Assessment Programme, 2015).

The main sources of fresh drinking water supplies around the globe come from surface water or groundwater. However, the availability of the freshwater resources, through precipitation can be erratic because of different areas in the world receiving different quantities of water throughout the year (WWAP United Nations World Water Assessment Programme, 2015). This is the reason why some parts of the world, for example, in Africa, the Middle East and Asia are facing water scarcity or are highly vulnerable to water stress as shown in **Figure 1.1**.

Since the natural water resources are diminishing (Gleeson et al., 2012), it is thus of utmost importance to explore new technologies to produce sustainable water resources. Desalination and recovery of reclaimed wastewater using reverse osmosis (RO) technology serve to provide solutions to these water shortage problems. This technology is extremely attractive due to the numerous advantages it offers, including but not limited to its small footprint with modular construction, process stability and its ability to produce permeate with excellent water quality (Bartels et al., 2005b; Pérez-González et al., 2012; Wintgens

et al., 2005). Furthermore, over the years of research and development in RO membranes and its processes, the average production cost of desalinated seawater using RO has been steadily reduced and is estimated to be around US\$ 0.50/m³ - US\$ 0.70/m³ (WaterReuse Association Desalination Committee, 2011).

Figure 1.1 Total renewable water resources per capita for 2013. (Copyright: www.fao.org/nr/water/art/2007/scarcity)

Typically, for a 190 megalitres per day (MLD) brackish groundwater RO desalting plant, a significant contribution of approximately 53% of the total operation and maintenance (O&M) cost was spent on power consumption (Bartels et al., 2005a). **Figure 1.2** features the breakdown costs of a desalination plant with energy demand accounting for about 40% of the total cost (Lenntech BV, 2016). Thus, potential savings can be made from the reduction of energy usage. Energy usage in RO processes is often related to membrane fouling issues.

Figure 1.2 Desalination plant costs breakdown. (Adapted from www.lenntech.com)

1.2 Problem statement

Membrane fouling for RO refers to the accumulation of unwanted materials on the membrane surface which is likely to result in undesirable consequences, such as a decline in the permeate flux in constant pressure filtration or increase in transmembrane pressure (TMP) for constant flux operation, increases in energy and operation costs, reduced membrane lifetimes and downtime for chemical cleaning as well as costs associated with chemicals and their disposal. Besides these, there might also be loss in water quality (Xu et al., 2006). There are several types of membrane fouling in RO systems, including particulate/colloidal fouling, inorganic fouling, organic fouling and biological fouling (Goosen et al., 2005).

Membrane fouling can be minimized by (1) pre-empting the fouling event using sensitive monitoring tools so that appropriate countermeasures can be performed on time and (2) operation below threshold flux. A study has shown that with an early warning system, the potential annual savings for seawater reverse osmosis (SWRO) with a capacity

of 10,000 m³/h was close to US\$ 2 million (Vrouwenvelder et al., 2011). This clearly indicates that pre-empting the fouling event might bring about potential savings for the O&M costs. Besides these, with proper monitoring techniques in place, membrane lifetimes could be extended and hence, reducing the membrane replacement cost.

Typically, the performance of the RO system in the plant is monitored through the pressure drop along the channel, transmembrane pressure (TMP) and rejection of the salt. The increase in TMP or feed pressure as well as the deterioration in permeate quality are used as the indications of membrane fouling (Tay and Song, 2005) in most of the water treatment plants. As a rule of thumb, most plants take 10 to 15% increase in pressure as the benchmark to trigger the application of cleaning countermeasures. However, the raised pressure approach is insensitive to initial fouling of the membrane. By the time the trigger pressure is reached, the system could be extensively fouled. The conventional pressure approach is unable to pre-empt the fouling events and gives no information of the type of fouling. Besides proper monitoring tools, current RO industries still lack a sensitive sensor that can determine the threshold flux. Threshold flux is “a flux at or below which a low and near constant rate of fouling occurs but above which the rate of fouling increases markedly” (Bacchin et al., 2006; Field and Pearce, 2011). Most RO plants tend to operate in a conservative manner where operational flux is typically low. Such practices are able to minimize fouling, but may underutilize the plant’s full capacity. Therefore, it is of pressing need to develop a sensitive tool that can identify the threshold flux so that the plant can always be operated at its optimum level. Hence, significant effort has to be put into developing a more reliable and sensitive monitoring tool to provide (1) early warning of incipient fouling, (2) characterize the type of fouling and (3) identification of a suitable operation flux.

In this dissertation, a real time and non-invasive method using electrical impedance spectroscopy (EIS) has been employed to monitor fouling in RO and to understand the phenomenological events during colloidal fouling and biofouling. In this study, EIS was also employed to determine threshold flux for colloidal fouling in RO operations. The impedance measurements were conducted by injecting a small sinusoidal alternating current at a series of known frequencies and after appropriate signal processing the properties of the diffusion polarization (DP) layer were examined (Ho et al., 2016a; Sim et al., 2016; Sim et al., 2013b). The method was shown to be capable of detecting changes in the nature of the foulant layer when it undergoes transition (Ho et al., 2016a; Ho et al., 2016b; Sim et al., 2013b). This sensor was also suited to be integrated into a side stream ‘canary’ cell which simulated the flow and flux of a bank of spiral wound modules in a water treatment plant and it was demonstrated to provide early warning of fouling events in the main plant (Chapter 5). In addition to fouling monitoring using EIS, other monitoring techniques for the determination of coliform and real-time phage monitoring in the feed water using electrochemistry methods were also evaluated in this thesis (Appendix).

1.3 Objectives

The main objective of this study was to investigate the capability of EIS to monitor colloidal fouling, including the determination of threshold flux and biofouling of the RO processes. The EIS fouling monitor has been integrated into a side stream ‘canary’ cell simulating the hydrodynamic conditions of the spiral wound modules for early warning of fouling events so that the most appropriate counter measures can be carried out.

1.4 Structure of the dissertation

According to the objectives outlined in Section 1.3, the structure of the thesis is as follows:

- Chapter 2 – A critical review of RO technology, non-invasive methods of fouling detection, EIS and techniques to determine coliform in water.
- Chapter 3 – Characterization of threshold flux phenomenon for colloidal fouling in RO by using transmembrane pressure and EIS.
- Chapter 4 – Adaptation of EIS for *in-situ* monitoring and a mechanistic study of biofouling on RO membranes.
- Chapter 5 – Demonstration of an EIS fouling monitor for early warning of fouling events in a real wastewater treatment plant.
- Chapter 6 – Conclusions and recommendations based on the findings in this dissertation.

In summary, Chapter 2 provides a detailed review of the different topics included in this dissertation. Chapter 3 presents the unique features of EIS for the determination of threshold flux and correlation of the results with conventional TMP methods. The effects of crossflow velocity and spacer on threshold flux are also evaluated in this chapter. Chapter 4 illustrates the ability of EIS for biofouling monitoring and the mechanisms for biofilm formation based on EIS analysis. The effects of a biostat, sodium azide are also investigated in Chapter 4. Biofilm dispersal using nitric oxide and the monitoring process using EIS are also evaluated in Chapter 4. Chapter 5 features the adaptation of an EIS fouling monitor installed as part of the canary cells in a real wastewater treatment plant in Singapore. The last chapter, Chapter 6 concludes by elaborating future outlook of the EIS fouling monitor.

Additionally, a complementary study was conducted related to RO brine treatment and recovery and is presented outside the main body of the dissertation in the Appendix section. This section describes the pretreatment of RO brine treatment and recovery using an inline coagulation-ultrafiltration method. The optimization of coagulants and the characterization of dissolved organic carbon (DOC) are described in this section.

Chapter 2 - Literature Review

Membrane processes have been widely employed in various water treatment processes. This chapter provides a critical review of RO technology, non-invasive methods of fouling detection, EIS and techniques to determine coliform in water.

2.1 Osmosis and reverse osmosis

Osmosis (**Figure 2.1**) is a phenomenon that occurs naturally where water passes from the solution with lower solute concentrations (higher chemical potential of water) to the solution with higher solute concentrations (lower chemical potential of water) through a semi-permeable membrane until both solutions become equal in concentration or the pressure of the more concentrated solution increases to match the difference in osmotic pressure ($\Delta\Pi$).

Figure 2.1 Schematic diagram of osmosis process.

In reverse osmosis (**Figure 2.2**), a pressure higher than the osmotic pressure is applied on the more concentrated (higher solute concentration) side to drive the water (but not solutes) through the semi-permeable membrane into the solution at the other side (lower

solute concentration). The RO membrane used in the process acts as a barrier to all the dissolved salts and inorganic molecules as well as most of the organic molecules. On the other hand, water can pass freely through the membrane, separating the feed into two streams. One of the streams is the purified product water (permeate) and the other portion contains a high concentration of ions and organic compounds (brine/retentate/reject).

Figure 2.2 Schematic diagram of reverse osmosis process.

2.2 Reverse osmosis membranes and membrane modules

The current market of RO membranes is dominated by thin film composite (TFC) polyamide membranes (Lee et al., 2011). The TFC membrane consists of three layers: structural support layer made up of the polyester web, a microporous polysulfone interlayer and an ultrathin barrier polyamide layer on the upper surface (Lee et al., 2011; Porter, 1990). Due to the highly porous and irregular nature of the polyester web layer, it is unable to provide direct support to the barrier layer. Hence, the microporous polysulfone layer is added in between these two layers so that the ultrathin barrier polyamide layer is able to withstand the compression by high pressure. Both the bottom substrate and top barrier layer could be tailored and optimized individually to obtain desired flux and rejection rate by using the interfacial polymerization technique (Lau et al., 2012).

Generally, there are four types of popularly known membrane modules for the RO processes, namely; tubular, plate and frame, hollow fiber and spiral wound. However, the most extensively used design in RO desalination is the spiral wound membrane module (Lee et al., 2011). The attractive features of the spiral wound configuration include its highly specific membrane surface area and packing density, the ease of scale up operation, inter-changeability, relatively low replacement costs and it is the least expensive way to produce TFC membranes from its flat sheet form (Lee et al., 2011; Pearce, 2007). Spiral wound modules utilize flat sheet membranes, feed spacers and permeate spacers wrapped around a hollow tube, called the permeate tube. It is essentially a crossflow device and due to its configuration it can be constructed into different modules with varying length, diameter and membrane material. Spiral wound elements consist of membranes, feed spacers, permeate spacers and a permeate tube (**Figure 2.3**). The feed water passes through the flow channels tangentially along the length of the module while permeate moves in the radial direction towards the permeate collection tube, passing through the permeate spacer. The feed spacers which form part of the spiral wound module provide an open channel for the flowing feed water by maintaining separation between the membrane sheets. On the other hand, they also function at promoting mixing within the feed channel, bringing away salts and other rejected substances from the membrane surface and reducing concentration polarization (discussed in Section 2.4).

Figure 2.3 Spiral wound reverse osmosis module. (Reprinted with permission from McGraw Hill, “Standard Handbook of Environmental Engineering”)

2.3 Performance of membrane

Membrane performance is commonly defined in terms of flux and selectivity. The rate of permeate transported per unit membrane area is known as permeate flux, J_v ,

$$J_v = \frac{\text{TMP} - \Delta\Pi_b}{\mu R_m} \quad (2.1)$$

where TMP refers to the transmembrane pressure, $\Delta\Pi_b$ is the osmotic pressure difference of the solution between the wall and permeate ($\Delta\Pi_b = \Pi_w - \Pi_p$), taking into account the effect of concentration polarization (detailed explanation in Section 2.4), μ is the viscosity of the permeate while R_m refers to the membrane resistance. Equation 2.1 is used for a clean membrane.

Another parameter to describe the performance of the membrane is its selectivity, represented by the fraction of solute rejected or retained by the membrane. The observed solute retention, R_{obs} refers to the relative change in the concentration of solute from the feed side, C_b to the permeate side, C_p and it is defined as

$$R_{\text{obs}} = \frac{C_b - C_p}{C_b} \quad (2.2)$$

If concentration polarization is taken into account, the real retention coefficient, R_{real} becomes

$$R_{\text{real}} = \frac{C_w - C_b}{C_w} \quad (2.3)$$

where C_w is the concentration of solute at the membrane wall.

2.4 Concentration polarization (CP)

As discussed previously, the major concern in RO process for system design and process optimization is the permeate flux. The permeate flux of the RO process is initially controlled by the properties of the membrane. In other words, the initial permeate flux of the RO process depends upon the membrane permeability/resistance to water. A layer of high concentration solutes which are being rejected by the membrane forms on the membrane surface as filtration proceeds overtime. As a result of the high concentration of the rejected compounds, there is also a back diffusion from this thin layer on the membrane surface into the bulk. This phenomenon is known as concentration polarization (CP) (Song and Yu, 1999). **Figure 2.4** shows a schematic diagram of the concentration polarization in reverse osmosis.

Figure 2.4 Schematic diagram of concentration polarization in reverse osmosis.

In steady state, the flux of solute to the membrane, $J_v C$, the flux of solute through the membrane, $J_v C_p$ and the solute back diffusion, $-D(\partial C/\partial x)$ are in equilibrium and this

phenomenon can be expressed mathematically based on the convective-diffusion equation (Field, 2010).

$$J_v C_p = J_v C - D \frac{\partial C}{\partial x} \quad (2.4)$$

Integration of Equation 2.4 taking the following boundary conditions into account,

$$x = 0, C = C_w$$

$$x = \delta, C = C_b$$

gives:

$$J_v = \frac{D}{\delta} \cdot \ln \left(\frac{C_w - C_p}{C_b - C_p} \right) \quad (2.5)$$

Where C_w refers to the concentration of solutes at the membrane wall (feed), C_p defines the concentration of solutes at the permeate side and C_b is the concentration of solutes in the bulk, D refers to the diffusion coefficient of solute in bulk solution and δ is the boundary layer thickness.

Rewriting Equation 2.5 in exponential terms gives,

$$\exp \left(\frac{J_v}{D/\delta} \right) = \frac{C_w - C_p}{C_b - C_p} = CP \quad (2.6)$$

As $D/\delta = k_m$, where k_m is the boundary layer mass transfer coefficient,

$$CP = \exp \left(\frac{J_v}{k_m} \right) \quad (2.7)$$

This is the Film Model for CP in membrane processes, CP is undesirable due to the following reasons. Firstly, the osmotic pressure on the membrane surface is raised due to the accumulation of high concentration of solutes on the membrane surface. This implies

that the osmotic pressure ($\Pi_w - \Pi_p$) is increased, hence higher pressure needs to be applied on the system to compensate for this increase. Secondly, due to high concentration of solutes on the membrane wall, more solutes can pass through the membrane since the concentration gradient across the membrane is the driving force for solute transport. Thirdly, the high concentration of solute at the membrane interface could cause the composition of membrane material to change due to chemical attack (Matthiasson and Sivik, 1980). Lastly, when the concentration of solutes on the wall increases to the saturation point, precipitation or formation of a gel layer or inorganic scale occurs on the membrane surface and results in membrane fouling (refer to Section 2.5) (Matthiasson and Sivik, 1980; Sablani et al., 2001).

2.4.1 Cake enhanced concentration polarization (CECP)

The formation of foulant layer does not just result in higher osmotic pressure but also lead to cake enhanced concentration polarization (CECP) effect (Chong et al., 2008a; Hoek and Elimelech, 2003). When there is a cake layer formed on the membrane surface, the back diffusion of solutes to the bulk solution will be hindered and the solutes need to diffuse through tortuous pathway within the cake layer. Furthermore, solutes at/near the cake layer do not experience the same tangential force as the free flow channel, hence, mass transfer is impacted. When the effective back diffusion drops, the concentration and the osmotic pressure near the membrane wall will be enhanced greatly. In practical, the increase in hydraulic resistance together with the CECP contribute to the overall loss in RO performance.

Despite the undesirable impacts resulting from CECP effect, there are only few methods available for its characterization (Gutman et al., 2013). The direct estimation method developed by Hoek and Elimelech can be applied on idealized systems. The models were characterized by monodispersed-size particles and fouling layer with constant

porosity (Hoek and Elimelech, 2003). Another method, the sodium chloride tracer pulse technique derived by Chong et al., is suitable for any type of fouling and systems operating with constant permeate flux (Chong et al., 2008a).

2.5 Types of reverse osmosis membrane fouling

Membrane fouling refers to the deposition of non-dissolved or unwanted materials in the membrane pore or on the surface or a mixture of both (Epstein, 1983; Field, 2010). For non-porous RO membrane, the fouling is a surface phenomenon. There are several types of fouling in the RO process. They are categorized as scaling, organic fouling, particulate/colloidal fouling and biofouling (Matthiasson and Sivik, 1980). Scaling is the formation of insoluble precipitates from dissolved ions on the membrane while organic fouling refers to the adsorption of organic compounds onto the membrane. Particulate/colloidal fouling defines the deposition of particulate matter or colloids on the membrane and biofouling is the adhesion of microorganisms, such as bacteria and subsequent biofilm formation on the membrane. Fouling causes unwanted consequences such as a decline in flux for constant pressure operation and an increase in transmembrane pressure (TMP) for constant flux operation. Fouling is commonly observed when the operating flux exceeds the so-called critical flux (refer to Section 2.5.1) (Field et al., 1995). The fouling problem is further complicated in commercial plants because it evolves differently from that in the laboratory scale. The membrane modules in the plant are more susceptible to fouling maldistribution along the module length and width of a single module and along several modules in a pressure vessel. Among the different types of fouling, biofouling is recognized as the most serious problem in RO plant operation (Uemura and Henmi, 2008). Colloidal fouling is also considered as a persistent problem in RO processes. It is thus of interest to highlight colloidal fouling and biofouling in the following sections (Sections 2.5.1 and 2.5.2 respectively).

2.5.1 Colloidal fouling

Colloids are ubiquitous in natural waters. The size of colloids ranges from nanometers to a few micrometers. Aquatic colloids include clay minerals, iron, manganese oxides, organic colloids, suspended matter, calcium carbonate precipitates and colloidal silica (Zhu and Elimelech, 1997). Most of the colloids carry a negative charge at the pH of natural water.

Membrane fouling refers to the accumulation of colloids on the membrane surface or within the membrane pores. This affects both the permeate flux and the quality of the product water (Zhu and Elimelech, 1997). It is worthwhile to note the difference when defining fouling of pressure-driven membranes. Colloidal fouling is caused by the accumulation of particles on the membrane surface, forming a cake layer in RO, nanofiltration (NF) and tight ultrafiltration (UF) membranes. The cake layer creates an additional hydraulic resistance to water that permeates through the membrane, reducing the product water flux (Zhu and Elimelech, 1995). For microfiltration (MF) membranes, the more common case is pore plugging by colloidal particles together with the accumulation of particles on the membrane surface. Due to the non-porous nature of RO membrane, the only mechanism for RO colloidal fouling is cake formation.

1. Particle transport

Colloidal fouling occurs due to the transport of the foulant from the bulk solution to the membrane surface then followed by the attachment of particles. In a crossflow RO system, permeation flux (perpendicular to the membrane surface) induces particles to move towards the membrane surface whilst crossflow (tangential to the surface) causes particles to move away from the membrane surface and leads to the back transport into the bulk.

Several transport mechanisms of back diffusion which carries materials away from the membrane surface include Brownian diffusion, shear-induced diffusion and inertial lift (Lawler and Kweon, 2004).

i) Brownian diffusion

One of the major transport mechanisms is Brownian back diffusion. Sub-micron sized particles ($< 0.1 \mu\text{m}$) suspended in flowing fluid are subjected to Brownian diffusion (Ounis and Ahmadi, 1990). Brownian diffusivity, D_{Br} , can be estimated from the Stokes-Einstein equation (Einstein, 1956)

$$D_{Br} = \frac{k_B T}{6\pi\eta a_p} \quad (2.8)$$

where k_B refers to the Boltzmann's constant, T is the temperature, η is the viscosity and a_p is the particle radius.

The Film Model equation (Equation 2.7) can be rewritten with ϕ (particle volume fraction) instead of C (concentration) to give,

$$J_v = k_m \ln \left(\frac{\phi_w}{\phi_b} \right) \quad (2.9)$$

where J_v is the permeate flux, ϕ_w and ϕ_b are the particle volume fraction at the edge of the membrane wall and in the bulk respectively and k_m is the mass transfer coefficient. The length-averaged mass transfer coefficient, $\langle K \rangle$ is determined by the Leveque solution for thin boundary layers in laminar flow (Belfort et al., 1994),

$$\langle K \rangle = 0.81 \left(\frac{\gamma_o D^2}{L} \right)^{1/3} \quad (2.10)$$

where L is the tube or channel length, γ_o is the shear rate and D is the particle diffusivity.

Combining Equation 2.8, 2.9 and 2.10, yields the following expression for the length averaged permeate flux, $\langle J \rangle$ based on Brownian diffusion,

$$\langle J_{Br} \rangle = 0.81 \left(\frac{\gamma_o D_{Br}^2}{L} \right)^{1/3} \ln \left(\frac{\phi_w}{\phi_b} \right) \quad (2.11)$$

The predicted flux for the Brownian diffusion mechanism increases with shear rate to the power of one third but decreases with particle radius to the two-third power (D_{Br} from Equation 2.8) (Belfort et al., 1994).

ii) Shear-induced diffusion

Shear-induced hydrodynamic diffusion occurs due to the random displacements of individual particles from the streamlines in a shear flow as they interact and tumble over other particles (Belfort et al., 1994; Zydney and Colton, 1986).

Eckstein *et al.* (Eckstein et al., 1977) measured the shear-induced diffusion and obtained

$$D_{SID} = 0.03 \gamma_o a_p^2 \quad (2.12)$$

The shear-induced hydrodynamic diffusion is proportional to the square of the particle size whilst Brownian diffusion as detailed in the above section is independent of shear rate and inversely proportional to particle radius. Shear-induced diffusion is important for micron-sized and large particles. If Equation 2.12 is incorporated into the Leveque expression for the mass transfer coefficient, Equation 2.10, the flux is given as,

$$\langle J_{SID} \rangle = 0.078 \gamma_o \left(\frac{a_p^4}{L} \right)^{1/3} \ln \left(\frac{\phi_w}{\phi_b} \right) \quad (2.13)$$

It is worthwhile to note that the permeate flux becomes proportional to the shear rate when D_{SID} replaces D_{Br} in Equation 2.11. Additionally, it also increases with particle size.

iii) Inertial lift

Nonlinear interaction of a particle with the surrounding flow field occurs when the Reynolds number based on the particle size is not negligible (Belfort et al., 1994). In the case where the inertial lift velocity is sufficient to offset the opposing permeate velocity, deposition of particles on the membrane are not expected to occur. Most of the operations for the crossflow filtration occur under fast laminar flow conditions. For the condition of fast laminar flow with thin fouling layers, the flux predicted by inertial lift theory at the steady state is expressed as (Drew et al., 1991):

$$J_{IL} = v_{L,o} = \frac{0.036\rho_0 a_p^3 \gamma_o^2}{\eta_o} \quad (2.14)$$

where $v_{L,o}$ refers to the inertial lift velocity, ρ_o is the fluid density. The inertial lift velocity for fast laminar flow is proportional to the cube of the particle radius as well as the square of the tangential shear rate. Thus, the inertial lift is significant for high flow rates and large particles. In addition, the permeate flux is inversely proportional to the solution viscosity and independent of the length of the filter and the concentration of bulk in the suspension. When the permeate flux of a clean membrane exceeds the inertial lift velocity, deposition of particles occur and a concentrated layer of particles forms on the membrane surface. Fouling layers with high resistance reduce the permeate flux up to a point when the inertial lift velocity is balanced (Belfort et al., 1994).

2. Particle attachment

Interaction of colloid and membrane is one of the determining factors for the attachment of particles onto the membrane surface. The interactions include hydrophobic or van der Waals forces (Van der Meeren et al., 2004). The nature of the interactions

depends largely on the surface properties of the membrane as well as particles and the solution chemistry (Bacchin et al., 2011; Hoek et al., 2003; Van der Meeren et al., 2004).

i. DLVO interaction

Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Derjaguin and Landau, 1941; Verwey, 1947) for colloid stability is used widely to describe the attachment of colloidal particles. According to this theory, balance between the electrostatic interaction and the van der Waals interaction between particles determines the stability of a suspension of colloidal particles (Ohshima, 2012). The characteristic of the van der Waals interaction is that it is always attractive whilst the electrostatic interaction (i.e. electrical double layer interaction force) is attractive only if the particle and the wall possess opposite sign but repulsive if these charges are of opposite signs (Ohshima, 2012). The total interaction energies involved leads to large energy barriers which must be overcome before particles attach on the wall (Chong, 2008). The interaction energy is dependent on several physicochemical parameters, for example the size of the particle and its zeta potential, the solution ionic strength and surface roughness (Elimelech et al., 1995; Hoek et al., 2003).

3. Critical/threshold flux

Field *et al.* introduced the empirical concept of critical flux for microfiltration (MF) (Field et al., 1995). Critical flux for MF refers to the observation that “on start-up there exists a flux below which a decline of flux with time does not occur; above it fouling is observed”. The critical flux depends on the hydrodynamics as well as other variables (Field et al., 1995). A theoretical model was proposed by Bacchin *et al.* which described the deposition of colloids on a membrane surface accounting for surface interactions (Bacchin et al., 1995). The existence of critical/threshold flux refers to the edge between pure filtration (where convection overcomes surface repulsion) and non-fouling (where the

dominant phenomena is surface repulsion) (Bacchin et al., 1995). Howell then pointed out the concept of ‘sub-critical flux operation’ which refers to “a flux below which there is no fouling by colloidal particles” (Howell, 1995).

Since the concept of critical flux alone does not provide sufficient guidelines for plant operators in the water industry, attention is given to the threshold flux instead (Field and Pearce, 2011). The threshold flux defines “the flux at or below which a low and near constant rate of fouling increases markedly” (Field and Pearce, 2011). The threshold flux can also be referred to as the transition between low or negligible fouling to significant fouling (Ho et al., 2016a).

i.) Methods of measurement for critical flux

The critical flux has often been obtained by the flux or pressure stepping method and the transmembrane pressure (TMP) or the corresponding flux is measured. Other than that, measurements have also been carried out using Direct Observation Through Membrane (DOTM), the mass balance methods and the salt pulse tracer response technique. These methods are described in the following section.

a) Flux stepping

Either the constant pressure or constant flux method can be used to determine the critical flux. However, the flux step method is preferred over the pressure step because of the ease in controlling the flux and the build-up of the foulant occurs more easily due to the constant flow towards the membrane (Defrance and Jaffrin, 1999a, b). The TMP should remain constant at each flux for the flux step method as any increase in the TMP indicates that the critical point has been exceeded. Before the measurement is carried out, it is important to ensure that the filtration is initially at sub-critical flux then increased to the critical flux (Bacchin et al., 2006). This stepwise filtration procedure by adjusting the flux

incrementally has been widely used to determine the “critical point” for fouling, for example by Chen *et al.* and Wu *et al.* (Chen et al., 1997; Wu et al., 1999). It is worth noting that the plot of raw data flux, J and the TMP alone does not give the critical flux. Thus, $dTMP/dt$ needs to be calculated for a series of fluxes for the determination of critical flux (Bacchin et al., 2006). Le Clech *et al.* showed a zero rate of TMP increase might never be obtained for their study using real and synthetic sewage (Le Clech et al., 2003). The rate of fouling, $dTMP/dt$ was identified as a useful parameter for the identification of critical/sustainable flux as distinct differences have been found below and above the critical point.

b) Direct Observation Through Membrane (DOTM)

Some studies have observed a critical flux for cake formation (Li et al., 1998, 2000). However, this cannot be observed from the flux or pressure data as those changes may not be related to cake formation. Direct Observation Through Membrane (DOTM) was used to identify the flux at which the deposition started at a given crossflow and feed suspension (Li et al., 1998). A membrane module made of Perspex with a transparent membrane was used to allow light transmission from the feed side of the module. Li *et al.* (Li et al., 1998) showed that pressure rise was barely perceptible even after particle deposition had occurred as observed from DOTM. This clearly shows that DOTM is a sensitive tool to detect an incipient fouling events. However, its application is limited to the use of transparent membranes and modules with a transparent permeate side (Bacchin et al., 2006).

c) Mass balance

Kwon *et al.* monitored the change of the particle concentration at the outlet stream so that the amount and the rate of the particle deposition on the membrane surface can be determined at different permeate fluxes (Kwon et al., 2000). Passive adhesion of particles was first determined at zero flux before starting each experiment. The concentration of

particles at the outlet stream was then calculated at different permeate fluxes to obtain the amount of deposited particle on the membrane. A graph of deposition rate versus permeate flux was then plotted to obtain the critical flux for particle deposition. The TMP method was also used to obtain the critical flux. However, the critical flux based on the TMP method was found to be two times higher than the mass balance method, illustrating the relative insensitivity of the TMP method. Gésan-Guizou *et al.* also studied the critical flux of latex particles by mass balance and pressure stepping method (Gésan-Guizou et al., 2002). However, they did not specify whether the results correlated to each other or the averaged value of the two was presented in the work. The use of mass balance method is recommended when it is used in conjunction with another technique. Its application has not been shown to be useful for molecules which might accumulate in the concentration polarization layer of the membrane (Bacchin et al., 2006).

d) Salt tracer response technique

Chong *et al.* determined the critical flux and the cake enhanced osmotic pressure (CEOP) effect of silica fouling in RO using the sodium chloride tracer response technique (Chong et al., 2008a). The method involved the injection of a raised concentration of salt into the system during the filtration with silica particles in the feed for a fixed duration at each flux. The critical flux was obtained from the salt pulse data by estimating the highest flux at which the deposition rate (dm_f/dt) was zero. However, this method requires several steps of calculation before the mass of deposit, m_f could be obtained.

ii) Effect of crossflow velocity on critical flux

Several studies have shown that critical flux increases with increased crossflow velocity (Chong et al., 2008a; Li et al., 1998, 2000). At the critical flux, the particles are balanced by the convective force towards the membrane and back transport away from the membrane (Cui et al., 2013). The increase of the crossflow velocity results in the increase

of back transport rate of particles and higher wall shear stress and this delays the deposition of particles to the membrane surface. Hence, the critical flux increases with increasing crossflow velocity. It should be noted that the original concept of critical flux applied to particulate MF but has been shown relevant to RO (Chong et al., 2008a) where back transport is by Brownian diffusion.

iii) Effect of spacer on critical flux

Neal *et al.* studied the effect of filament orientation in a spacer-filled channel on critical flux (Neal et al., 2003). The degree of critical flux enhancement was found to be depended on the orientation of the spacers. The presence of the spacer in the filtration channel introduces eddies and mixing effects, leading to higher critical flux to induce particle deposition. Other than that, Chong *et al.* have also investigated the effect of spacer-filled channels on critical flux with nano-sized colloidal particles using the salt-pulse tracer technique (Chong et al., 2008a). Enhancement of critical flux was also observed in their study but at a lower extent probably due to the different transport mechanisms (Brownian and shear-induced diffusions) that governed in both the studies.

2.5.2 Biofouling

Biofouling refers to the formation of a biofilm that has several stages. The first stage involves the attachment of organic matter onto the membrane surface, leading to the formation of a conditioning layer followed by bacterial attachment. In the second stage, the bacteria cells grow and proliferate, forming micro-colonies and embed themselves in a self-produced matrix of extracellular polymeric substances (EPS), resulting in the formation of a mature biofilm (O'Toole et al., 2000). For the last stage, detachment of the bacteria cells may occur and this stage depends largely on the concentration of the nutrients, growth rates of bacteria, the accumulation of dispersal signals, the mechanical stability of the biofilm

and also the effective shear force of the feed water (Flemming, 1997). Biofouling is regarded as the most severe form of fouling in RO processes due to its almost inevitable nature and costly problems incurred by the plant operators despite various cleaning protocols that might be in place. There was estimated to be \$730,000 spent on biofouling control per year and biofouling accounted for approximately 30% of the operating cost for the RO plant at Water Factory 21 in Orange County, California (Flemming et al., 1994). The global overall cost for biofilms caused by downtime due to cleaning and pretreatment is exceptionally high (Flemming, 2011).

1. Events in biofouling

The biofilm formation can be divided into several stages. The process has been reviewed extensively by some of the authors (Flemming, 1997; P. Stoodley et al., 2002). The detailed stages of biofouling will be described in the following section.

i) Surface conditioning and attachment of bacteria cells to the surface

When the membrane is in contact with water, macromolecules, such as nutrients and organic matters present in the water are adsorbed onto the membrane surface. This forms a conditioning layer on the membrane surface (Flemming, 1997). The attachment is affected by various physical, biological and chemical factors (Donlan, 2002). In the RO process, the concentration of nutrients on the membrane surface is higher due to the application of flux which leads to the concentration polarization effect (Herzberg and Elimelech, 2007b; Suwarno et al., 2014). The adsorption of nutrients and organic matter on the membrane surface changes the hydrophilicity and roughness, enhancing bacterial adhesion (Subramani et al., 2009).

ii) EPS production for irreversible attachment of bacteria cells

Soon after bacteria cells attach on the membrane surface, they excrete EPS, which is the primary component that is responsible for the structural integrity of the biofilm (Donlan, 2002). Different bacteria may produce different amount of EPS and the amount of EPS increases with the age of biofilm.

iii) Early development of biofilm

There are at least three mechanisms for biofilm formation. First, it is by the redistribution of the attached cells using surface motility (P. Stoodley et al., 2002). The second mechanism is by the binary division of the attached cells (Heydorn et al., 2000). In this case, the daughter cells spread outward and upward from the attachment surface for the formation of cell clusters during the cell division stage. The third mechanism describes the aggregation of the cells from the recruitment of bacteria in the bulk liquid to the developing biofilm (Tolker-Nielsen et al., 2000). The dominant mechanism for biofilm formation depends on the organisms involved, nature of the surface for colonization, chemical and physical conditions of the environment.

iv) Maturation of biofilm

Following the early development of a biofilm, the synthesis of EPS is increased, making a three-dimensional structure within the bacteria community (Branda et al., 2005; Herzberg and Elimelech, 2007b). Mature biofilms were found to have different gene expression to those grown in the chemostat (P. Stoodley et al., 2002). The growth pattern of a biofilm is also affected by the hydrodynamic conditions. It is postulated that biofilms grown in higher shear stress are more rigid and stronger compared to those grown at lower shear (Stoodley et al., 1998). EPS is responsible for the both mechanical stability and integrity maintenance of the biofilms (Flemming et al., 2000).

v) Detachment/dispersal of cells from biofilm

Detachment refers to the release of cells, individually or in a group from a biofilm. One of the most likely signals for detachment might be starvation (O'Toole et al., 2000) which allows bacteria cells to search for a more nutrient-rich habitat (P. Stoodley et al., 2002). Sauer *et al.* showed that bacteria cells in the dispersion stage resembled more like planktonic bacteria than that of bacteria in the mature stage (Sauer et al., 2002). The dispersal of bacteria cells has been associated with the depletion or lack of nutrient level (Sauer et al., 2004) and generation of nitrosative stress by reactive nitrogen intermediates, including nitric oxide (NO) (Barraud et al., 2006; Barraud et al., 2009a). Dispersal induced by NO will be discussed in detail in the following section.

2. Biofouling in RO process

Biofouling in RO membranes often results in permeate flux decline as well as decrease in salt rejection (Herzberg and Elimelech, 2007a). Biofouling is a major problem because it cannot be eliminated by pretreatment alone (Matin et al., 2011). A few surviving cells can adhere to the surface, grow and proliferate to form biofilm. Hence, biofouling has found to occur in RO system even after pretreatment of the feed and with the addition of disinfectant (Flemming et al., 1997).

The conditions of liquid mass transport affect the microbial adhesion as these influence the rate of the accumulation of bacteria/microorganisms on the membrane surface (Al-Juboori and Yusaf, 2012). This suggests that having a higher shear force (higher flow) on the membrane surface might hinder the growth of bacteria but it could also result in thinner yet mechanically stable biofilm (Al-Juboori and Yusaf, 2012). The concentration of nutrient in the bulk solution is also an important factor for biofilm growth on the membrane surface. From the study by Liu *et al.*, the initial growth period of biofilm could

be shortened and there was a higher mass of biofilm when the carbon content was increased (Liu et al., 2011). Thus, biofilm growth could be negatively affected when there is a lower concentration of available nutrients.

Herzberg and Elimelech (Herzberg and Elimelech, 2007a), Chong *et al.* (Chong et al., 2008b) and Suwarno *et al.* (Suwarno et al., 2012) pointed out biofilm enhanced osmotic pressure (BEOP) which contributed strongly to the flux decline. There were two mechanisms identified for the flux decline, which included the increase of hydraulic resistance on the membrane and the hindered back diffusion of salt. On the other hand, Vrouwenvelder *et al.* studied biofouling in the presence of spacer and concluded that biofouling is a feed spacer problem in spiral wound module (Vrouwenvelder et al., 2009). Most of these studies employ *ex-situ* and destructive method for the monitoring of biofouling, it is thus of significance to study the potential of *in-situ* method to understand biofilm formation on membrane.

2.5.3 Biofouling control

Control of biofouling is needed during plant operation as well as during the extended periods of plant inactivity (Matin et al., 2011). Biofouling control can be carried out by the pretreatment of feed water, application of biocide, surface modification of the membrane, membrane cleaning (Matin et al., 2011) and biofilm dispersal (Barraud et al., 2006).

Pretreatment is commonly carried out by the conventional physico-chemical processes or by microfiltration/ultrafiltration (MF/UF) processes. For successful operation of RO, pretreatment is considered as one of the most critical steps as it reduces bacteria and substances that cause membrane biofouling (Kumar et al., 2006). In this section, the application of biocide and control using NO will be discussed in detail.

1. Control using biocide/biostat

Biocides refer to disinfecting agents which are used for inactivating microorganisms (Matin et al., 2011). Free chlorine, for example, HOCl and OCl⁻ are commonly used for the inactivation of microbial growth in the seawater reverse osmosis (SWRO) process (Kim et al., 2009). However, the strong oxidation potential and possible chemical reaction with amide functional groups on RO membranes limit the application of chlorine as a biocide (Kang et al., 2007; Kwon and Leckie, 2006).

Chloramine which causes lesser impact on the RO membrane was also found to be effective in controlling microbial growth (Applegate et al., 1989; Bartels et al., 2005b). By reacting aqueous ammonia with free chlorine, combined chlorines, such as NH₂Cl, NHCl₂ and NCl₃ are produced *in-situ*. Monochloramine (NH₂Cl) is most often used among these three due to its higher stability (Kim et al., 2009). Chloramination is more commonly used in water reclamation plant with RO. Despite its wide applications in SWRO process, some studies have shown that disinfection of system feed water using chlorine does not necessarily guarantee that membrane biofouling does not occur (Hassan et al., 1989; Ridgway et al., 1984).

Sodium azide acts as a biostat and is known to decrease the biological activity (Peter-Varbanets et al., 2010) and to inhibit the growth of bacteria by the inhibition of catalase activity (Kay and Gronlund, 1969; Lichstein, 1944). However, the use of sodium azide has not been evaluated in large scale applications although it is used in lab studies. Thus, it is of interest to evaluate the use of sodium azide in biofouling control in the following chapter (Chapter 4).

Ozone has also been employed widely as a disinfectant in water treatment due to its strong oxidation potential and the production of fewer disinfection by-products (Kim et al.,

2009; Matin et al., 2011). Kaur *et al.* showed that ozone weakens the biofilm matrix and thus, the removal of biomass was facilitated by shear force (Kaur et al., 1992). However, the main disadvantage of ozone is the fourfold-higher generation cost as compared to the use of chlorine (Matin et al., 2011).

2. Biofilm dispersal

There is a surge of interest in biofilm dispersal due to its potential application in biofouling mitigation (Barraud et al., 2006; Barraud et al., 2009a; Webb, 2006). Several processes are known to cause biofilm dispersal. These include 1) breakdown of the biofilm matrix by enzymes, 2) loosening of cells from the biofilm by the production of surfactants, 3) the control of quorum sensing, for example, variation in the intracellular di-cyclic GMP levels, changes in nutrient availability and the production of free radical species (Webb, 2006). For the bacterial strain that is most commonly used in the laboratory, *Pseudomonas aeruginosa* (*P. aeruginosa*), cell dispersal is commonly related to both the activation of prophage and the generation of oxidative and nitrosative stress in the microcolonies (Barraud et al., 2006). Oxidative stress refers to either exogenous exposure or endogenous production of reactive oxygen intermediates (ROI), for example, superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical ($HO\cdot$). The other type of stress is the nitrosative stress which involves exposure to and production of reactive nitrogen intermediates (RNI). These include nitric oxide (NO), peroxynitrite ($ONOO^-$), nitrous acid (HNO_2), nitrogen trioxide (N_2O_3) etc. (Barraud et al., 2006). In the following sections, only biofilm dispersal using NO will be discussed in further detail, as it will be assessed in Chapter 4.

i) Properties and sources of NO

Nitric oxide is a colorless diatomic gas. It is a free radical with a half-life of several seconds and due to the availability of an unpaired electron on N and it can cause nitration, lipid peroxidation and S-nitrosylation of protein (Cutruzzola and Frankenberg-Dinkel, 2015). In bacterial systems, NO acts as an intermediate or by-product of specific metabolic pathways, for example denitrification, which is a form of anaerobic respiration. Studies using different NO-releasing compounds have been carried out to characterize the difference in response of biofilms to exogenous NO (Barraud et al., 2009b; Barraud et al., 2012; Yepuri et al., 2013). Several NO donors were used in this studies, such as sodium nitroprusside (SNP) (Barraud et al., 2006), cephalosporin-3'-diazoniumdiolate NO-donor prodrug which selectively releases NO upon contact with bacterial enzyme β -lactamase (Yepuri et al., 2013) and PROLI-NONOate (Barnes et al., 2014; Barnes et al., 2015; Saavedra et al., 1996). In the following section, only the reaction mechanism of PROLI-NONOate will be discussed as it was used as the NO donor in the control and dispersal studies of biofilms in Chapter 4.

Figure 2.5 shows the synthesis of PROLI-NO and its dissociation in the aqueous media to proline and NO. PROLI-NO was found to dissociate rapidly at pH 7.4 with a half-life of ~ 1.8 s (rate constant 0.39 s^{-1}) (Saavedra et al., 1996). The dissociation of NO donor is sufficiently slow in alkaline solutions but sufficiently rapid at physiological pH to be effectively complete within the system of interest (Saavedra et al., 1996).

Figure 2.5 Synthesis of PROLI-NO and its dissociation in aqueous media to proline and NO (shown in left arrow).

ii) Dispersal of biofilm induced by exogenous NO source

The entire molecular events which cause biofilm dispersal in *P. aeruginosa* are still far from completely understood (Cutruzzola and Frankenberg-Dinkel, 2015). Barraud *et al.* has pointed out that NO induces biofilm dispersal by stimulating the phosphodiesterase activity, leading to the degradation of cyclic di-guanylate monophosphate (c-di-GMP), culminating in changes in the gene expression which favors the planktonic mode of growth (Barnes et al., 2015; Barraud et al., 2009b). However, the response of a biofilm towards NO depends on aerobic or anaerobic conditions where anaerobic conditions favor the accumulation of NO, eventually leading to the formation of biofilm as a stress defense response (Cutruzzola and Frankenberg-Dinkel, 2015).

2.6 Non-invasive methods of fouling monitoring

The study of membrane processes involves observation of events in three regions, namely the membrane, the boundary layer of fluid, including the membrane-fluid interface and the possible presence of cake or fouling layer as well as the bulk fluid in the membrane module (Chen et al., 2004c). Most of the monitoring techniques available rely on the overall flows, concentrations, pressures and by autopsies of the used membranes. There are very

few studies which focus on the microscopic processes occurring *in-situ* during the filtration process. Thus, novel *in-situ*, non-invasive and rigorous methods are required for breakthroughs in the understanding of fouling phenomena (Chen et al., 2004a).

There are several characteristics that need to be considered for *in-situ* real-time monitoring of phenomena in membrane modules and membrane filtration processes (Chen et al., 2004c). These include non-invasiveness which has either external signal generation and detection or minimal invasion to the membrane, specificity, real time observation which implies a rapid response to signal change and complexity. The various monitoring methods available can be grouped into two categories, namely the optical and the non-optical methods. High magnification cameras or microscopic techniques are used to observe fouling or particle deposition near the membrane surface (Chen et al., 2004a; Chen et al., 2004c). Optical techniques provide truly real time observations and are very sensitive. However, this method requires membrane cells that are specially designed for the application while some need the use of specific transparent membranes (Chen et al., 2004c). In contrast, the non-optical methods do not rely on refractive index, fluorescence dyes or optical densities. Their resolution can reach down to the angstrom level for the small angle neutron scattering (SANS) method and the hydrodynamic conditions within the membrane module can be probed by using nuclear magnetic resonance (NMR) imaging or an electrochemical method such as electrical impedance spectroscopy (EIS).

EIS has been successfully employed to characterize various types of membranes, including synthetic (Antony et al., 2013; Chilcott et al., 1995; Chilcott et al., 2002; Coster et al., 1992; Fortunato et al., 2006; Gaedt et al., 2002; Gao et al., 2013; Xu et al., 2011) biological membranes (Chilcott et al., 1983; Ogata et al., 1983; Smith et al., 1985) and recently, hollow fiber membranes (Bannwarth et al., 2015; Bannwarth et al., 2016). The porosity of the membrane can be estimated from the capacitance determined from EIS when

the dielectric constant and the thickness of the membrane polymer are known (Coster et al., 1996; Coster et al., 1992). This technique is also capable of detecting fouling or scaling in membrane processes in real time and in a non-invasive manner (Bannwarth et al., 2016; Hu et al., 2014; Kavanagh et al., 2009; Park et al., 2006; Park et al., 2005; Sim et al., 2013a). The various EIS applications to membrane technology are listed in **Table 2.1**. EIS has also been proven to be applicable beyond laboratory scale studies such as to detect real foulants on the membrane surface in the treatment of wastewater from a fermentation process (Cen et al., 2012). *In-situ* monitoring techniques for membrane filtration processes are of paramount importance for facilitating our understanding in the fundamental processes governing membrane fouling (Chen et al., 2004b). To date, EIS has not been correlated directly with flux nor used to detect changes at the membrane surface below, at and above the threshold flux and for the monitoring of biofouling in RO processes.

EIS stands out as an outstanding monitoring tool due to its ability in detecting the phenomenon occurring at the membrane-solution interface, the flowing cake layer and the stagnant cake layer formed on the membrane surface. The working principles of EIS will be discussed in the following sections as it is the main fouling monitoring tool used in the following chapters.

Table 2.1 Summary of various EIS applications to membrane technology.

Applications	Observations	References
Reverse osmosis (RO) membrane		
1. Fouling monitoring		
a. Colloidal/organic fouling	i.) Shift of Nyquist plot as the indication of silica and organic fouling	(Sim et al., 2016; Sim et al., 2013b)
	ii.) Identification of threshold flux for silica fouling using the change in conductance of diffusion polarization layer (G_{DP})	(Ho et al., 2016a)
	iii.) Decrease in capacitance was observed for bovine serum albumin (BSA) and molasses wastewater and the increase of capacitance was observed for silica fouling.	(Cen et al., 2015)
b. Inorganic scaling	i.) Changes in both the conductance and impedance values during calcium carbonate (CaCO_3) precipitation	(Kavanagh et al., 2009)
	ii.) Changes of conductance value for the molecular polyamide/coating layer for CaCO_3 scaling	(Antony et al., 2013)
	iii.) Changes of conductance around 38 Hz, corresponding to the coating layer on the membrane surface for calcium sulfate (CaSO_4) scaling	(Hu et al., 2014)

	iv.)	Decreases in the electrical potential across the depletion layer that were correlated with the onset of rapid flux decline	(Chilcott et al.)
c. Industrial effluent	i.)	Decrease in capacitance at low frequency for fouling of molasses process water	(Cen et al., 2012; Cen et al., 2015)
2. Characterization	i.)	Inclusion of the hydrodynamic circuit in the modelling facilitated Maxwell-Wagner characterizations of the filtrate, substrate, support and active layers of thin-film composite RO membranes	(Chilcott et al., 2015)
Hollow fiber membrane			
1. Fouling monitoring			
a. Colloidal fouling	i.)	Changes in the impedance value for silica fouling	(Bannwarth et al., 2016)
2. Characterization	i.)	Use of 2-terminal method and equivalent circuit system to model the experimental impedance data	(Bannwarth et al., 2015)
Nanofiltration (NF) membrane			
1. Characterization	i.)	The resistance of the active layer was influenced by the concentration and ionic species of electrolytes	(Xu et al., 2011)

Applications	Observations	References
Forward osmosis (FO) membrane		
1. Characterization	i.) The effects of internal concentration polarization (ICP) was clearly observed from the impedance spectra	(Gao et al., 2013)
	ii.) Maxwell Wagner theory was used to reveal the distinct structures including the active separation layer and porous support of cellulose triacetate (CTA-ES) FO membrane	(Yeo et al., 2014)
Ultrafiltration (UF) membrane		
1. Characterization	i.) EIS characterization of platinum coated polysulfone UF membrane in solution, giving estimations of membrane porosity and surface roughness	(Chilcott et al., 2002; Coster et al., 1992; Gaedt et al., 2002)
Ion-exchange membrane		
1. Organic fouling	i.) Decrease of conductance and capacitance dispersions with the accumulation of bovine serum albumin (BSA) fouling layer on the membrane	(Park et al., 2006; Park et al., 2005)
2. Interfacial properties	i.) An equivalent circuit was selected for quantitative analysis of components in the system and the diffusion boundary layer thickness was estimated	(Zhang et al., 2016)

2.6.1 Fundamentals of EIS

Impedance measurements are conducted by the injection of small sinusoidal alternating currents, $i = i_o \sin(\omega t)$ at a series of known frequencies ω and amplitude i_o into the system and measuring the voltage $v = v_o \sin(\omega t - \theta)$ across the sample. The voltage amplitude v_o and phase difference θ between the voltage and current are measured. The magnitude of the impedance is given by,

$$|Z| = \frac{v_o}{i_o} \quad (2.15)$$

Impedance is a complex quantity, with real and imaginary components. The impedance can be decomposed into these components using the following expression:

$$Z = |Z| e^{j\angle Z} = \frac{v_o}{i_o} e^{-j\theta} = \frac{v_o}{i_o} (\cos \theta - j \sin \theta) \quad (2.16)$$

where $|Z| (= \frac{v_o}{i_o})$ and $\angle Z (= -\theta)$ define the impedance magnitude and phase respectively.

This gives the impedance in terms of the measureable parameters, such as i_o , v_o , θ and the imaginary unit vector, j where $j^2 = -1$.

The admittance is derived from the reciprocal of impedance and is represented by

$$Y = \frac{1}{Z} = \frac{i_o}{v_o} e^{j\theta} = \frac{i_o}{v_o} (\cos \theta + j \sin \theta) \equiv G + j\omega C \quad (2.17)$$

Here, the admittance is expressed in terms of a conductance element, G in parallel with a capacitance element, C describing its ability to conduct and store charge respectively. This single conductance in parallel with a capacitance is known as a Maxwell-Wagner element (see inset of **Figure 2.7**), where the impedance measurement provides a measure of,

$$G = \frac{1}{|Z|} \cos \theta \text{ and } C = -\frac{1}{\omega |Z|} \sin \theta \quad (2.18)$$

where $\omega = (2\pi f)$ is the angular frequency. The variation or dispersion of G and C with frequency can be used to determine the number of circuit elements with different time constants present in the system. In the context of membranes, such elements arise from layers within the system and diffusion polarization layers or processes. This provides a means of real time and *in-situ* monitoring of the accumulation of particulates that could potentially foul the membrane (Gaedt et al., 2002). Rearrangement of Equation (2.17), leads to

$$Z(\omega) = \frac{1}{G + j\omega C} \quad (2.19)$$

Equation (2.19) can also be expressed as

$$Z(\omega) = \frac{G}{G^2 + C^2\omega^2} - j \frac{C\omega}{G^2 + C^2\omega^2} \quad (2.20)$$

Since Z is a complex variable, $Z = Z_{\text{Re}} - jZ_{\text{Im}}$ where Z_{Re} represents the real part of Z and Z_{Im} refers to the imaginary component of Z . Therefore,

$$Z_{\text{Re}} = \frac{G}{G^2 + C^2\omega^2} \text{ and } Z_{\text{Im}} = \frac{C\omega}{G^2 + C^2\omega^2} \quad (2.21)$$

A bode plot of the magnitude of the impedance as a function of frequency is shown in **Figure 2.6**. Note that the impedance is highest in the very low frequency region. This plot can be translated into a Nyquist plot using the phase measurements at each frequency, to split the impedance into its real and imaginary components, where $-Z_{\text{Im}}$ is plotted against Z_{Re} . The Nyquist plots consist of a number of overlapping semicircles where a single Maxwell-Wagner element produces a semicircular plot. The number of semicircles and the degree of overlapping are determined by the number of elements with different time

constants and how close those time constants are to each other. In most cases, the Nyquist plot provides direct insights into the layers as well as the electrochemical transport processes occurring.

Figure 2.6 Representative bode plot (impedance vs frequency plot) for a fouled RO system.

The frequency dependent complex impedance of the membrane can be modeled using a number of circuit elements in series representing internal and external layers in the membrane. This is the so-called Maxwell-Wagner model. Detailed explanations on the equivalent Maxwell-Wagner circuit model have been described in the literature (Antony et al., 2013; Coster et al., 1992). Briefly, at low frequencies (<10 kHz), there exists an element representing the diffusion polarization of ions at the membrane-solution interface. At mid frequencies, the dispersion in capacitance can be ascribed to elements representing membrane substrate, membrane skin layer and partial diffusion polarization effect. At higher frequencies (>10 kHz), there is an element representing the electrolyte connected in series with the membrane (Antony et al., 2013; Coster et al., 1992; Fortunato et al., 2006).

Figure 2.7 Representative Nyquist plot obtained from RO system. *Inset:* Maxwell Wagner model used to obtain the fitted results using the dedicated software. Four Maxwell-Wagner elements are shown.

1. Low frequency element - Diffusion polarization (DP) layer

As the element at low frequencies represents the phenomena occurring at the membrane-solution interface, the electrical properties of this element are of special interest. This diffusion polarization (DP) layer originates from a phenomenological event caused by the alternative accumulation and depletion of ions at the membrane-solution interface during the measurement of impedance using AC currents. During one half cycle (**Figure 2.8a**), when the injected AC current is towards the feed side of the membrane, there is a transport of Na^+ towards the membrane at the feed side and Cl^- away from the membrane. In the membrane the electric current carried by the cations and anions may not be equal; that is, they have different transport numbers. This can arise, for instance if the membrane has a net residual charge as is the case for the RO membranes used which are slightly

negatively charged. Thus, the fraction of the electric current in the membrane carried by Na^+ is larger than that carried by Cl^- , whilst in the feed solution these fractions are equal. As a result, there will be a depletion of Na^+ ions at the surface. Similarly, fewer Cl^- ions arrive through the membrane at the membrane surface than are transported away in the feed solution, thus also lowering the Cl^- ion concentration at the membrane surface. The opposite effect occurs at the permeate side. Further, the entire profiles reverse during the next half cycle of the AC current when the electric current is reversed, as illustrated in **Figure 2.8b**. The occurrence of electrical diffusion polarization only occurs for low frequencies of the AC current injected when the half cycle is long enough for substantial changes in the ion concentrations to build up near the surface of the membrane. At high frequencies there is insufficient time in each half cycle for concentration profiles to build up. Thus, the AC ion concentration perturbations are frequency dependent. If the conductance G has a real (G_R) and an imaginary component (jG'), that is, if

$$G = G_R + jG'(\omega) \quad (2.22)$$

This imaginary component appears as a frequency dependent capacitance. The capacitance can be written in terms of the imaginary part of the admittance in Equation (2.17) as:

$$C = \frac{I_m\{Y(\omega)\}}{j\omega} \quad (2.23)$$

Substitution of Equation (2.22) into Equation (2.17) with Equation (2.21) gives the total capacitance:

$$C^*(\omega) = C + \frac{G'(\omega)}{\omega} \quad (2.24)$$

This can be written as:

$$C^*(\omega) = C + C'(\omega)$$

$$\text{where } C'(\omega) = \frac{G'}{\omega} = \frac{\beta}{\omega} \quad (2.25)$$

where β is a constant describing the magnitude of this frequency dependent capacitance arising from diffusion polarization.

For a salt rejecting membrane, like RO, ions such as Na^+ and Cl^- also tend to accumulate on the RO membrane surface during filtration as a result of the pressure driven flux as illustrated in **Figure 2.8**. This is the so called concentration polarization effect. It should be noted that the DP layer defined here is not identical with the pressure driven concentration polarization layer but is very sensitive to the concentration profile of the Na^+ and Cl^- near the membrane surface, both at the feed side as well as at the permeate side (**Figure 2.8**). The DP profile will be asymmetrical due to the differences in NaCl concentration on both sides of the membrane.

Modeling using a series combination of Maxwell-Wagner elements (Inset **Figure 2.7**) allows determination of the conductance of the AC DP layer, designated here as G_{DP} . This parameter allows one to probe the changes at the membrane-solution interface in order to provide clearer understanding of the events occurring below, around and above threshold flux and during the biofouling process. The parameter G_{DP} is used in this thesis in Chapters 3 and 4.

Figure 2.8 Schematic illustration of electrical driven diffusion polarisation (DP) process at low frequencies. The profiles of concentration shown in (a) are for that part of the AC cycle when the electric current is towards the membrane from the feed side to the permeate side. It is assumed in this illustration that in the membrane the transport number (fraction of current carried) for Na^+ is greater than that for Cl^- . In the next half cycle of the AC current, the concentration profiles are reversed as shown in (b). Note that a DP exists at both the feed and permeate sides of the membrane and the impedance elements associated with these are in series.

2.7 Research gaps and opportunities

This chapter provides a brief overview of the reverse osmosis process, fouling problems, including colloidal fouling and biofouling, non-invasive methods for fouling monitoring and finally, water quality monitoring tools.

A major concern for fouling control in RO process is an appropriate early warning system which is able to give plant operators enough information in order to conduct the most strategic countermeasures with economic considerations. This can be achieved by integrating the fouling monitors (such as EIS) into the system and this allows the monitoring process to be carried out in real time, *in-situ* and a non-destructive manner.

It is a common practice for plant operators to determine the most sustainable flux for water production during the start-up stage prior to its normal operation. Currently, the flux that plants use is based on the recommendations from membrane manufacturing companies and they are mostly run at a very conservative throughput. In order to optimize the entire process (maximize water production with minimal fouling), the operating flux can be determined based on the ‘threshold’ value. In this thesis, EIS is combined with flux step method to determine threshold flux, and this is discussed in Chapter 3.

While biofouling remains the most problematic fouling problem, most of the detection methods for biofouling remain cumbersome, time consuming and tedious. The EIS fouling monitor is capable of providing a non-invasive and online method for the determination of biofouling occurrence and this will be detailed in Chapter 4. Commonly, biocides or biostats are used to minimize biofouling but this is not based on any measure of the fouling. The control of biofouling using a dispersal agent (e.g. PROLI/NO) and monitoring using EIS is also discussed in Chapter 4.

In most of the RO water reclamation plants, the problems of membrane fouling are probed through the changes in delta P along the channel, TMP and the salt rejection. Most plant operators rely on arbitrary values; say 15% increase in delta P in order to initiate the cleaning-in-place (CIP). However, membranes in the system might already be well-fouled when such observations are made. Hence, the use of an EIS fouling monitor installed in a side-stream ‘canary cell’ was evaluated to monitor the fouling behavior of one train of the RO modules in a real wastewater reclamation plant in Singapore and this is discussed in Chapter 5.

3 Chapter 3 - A Threshold Flux Phenomenon for Colloidal Fouling in Reverse Osmosis Characterized by Transmembrane Pressure and Electrical Impedance Spectroscopy^{†1}

3.1 Introduction

Membrane fouling refers to the deposition of unwanted materials on the membrane surface which is likely to cause undesirable consequences, including permeate flux decline in constant pressure filtration or increase in transmembrane pressure (TMP) in constant flux filtration, an increase in energy and operational costs, reduced membrane lifetimes, down-time for chemical cleaning as well as costs associated with chemicals and their disposal. There may also be a loss in water quality (Xu et al., 2006). There are several types of particulate fouling, including colloids, suspended solids and microbial cells. However, colloidal fouling remains a persistent problem in the applications of water treatment by membranes (Boerlage et al., 1998).

Fouling tends to be flux-driven and one of the measures to mitigate fouling and improve the filtration performance is to operate the system below a certain flux. For example, the concept of critical flux was first introduced in 1995 by Field et al. (Field et al., 1995) for microfiltration (MF) which asserts that “on start-up there exists a flux below which a decline of flux with time does not occur whilst above this “critical flux”, fouling

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^{†1} Findings in this chapter have been published in: Ho, J.S., Sim, L.N., Gu, J., Webster, R.D., Fane, A.G., Coster, H.G.L., 2016. A threshold flux phenomenon for colloidal fouling in reverse osmosis characterized by transmembrane pressure and electrical impedance spectroscopy. *Journal of Membrane Science* 500, 55-65 and PCT has been filed as: Coster, H. G. L.; Sim, L. N.; Ho, J. S.; Fane, A. G. “Critical flux phenomena in reverse osmosis characterized by electrical impedance spectroscopy” Singapore, Filed 24 April 2015.

will occur. This critical flux level would depend on the hydrodynamics and probably other variables. Others discussed a 'transition' between concentration polarization and cake formation for colloids (Chen et al., 1997). Howell (Howell, 1995) pointed to "a flux below which there is no fouling by colloidal particles" which then leads to the concept of "sub-critical flux operations of microfiltration" which provides for a sustainable flux (Bacchin et al., 2006).

Operation above the critical flux would result in the deposition of a colloidal layer on the membrane surface and lead to an additional resistance to the permeate flow, which is known as the cake resistance and this increases the overall hydraulic resistance (Chong et al., 2008a). For RO membranes, the deposited layer on the membrane surface could hinder back-diffusion of solutes, such as salt, leading to the cake-enhanced concentration polarization (CECP) phenomena as identified by Hoek et al. (Hoek and Elimelech, 2003). Further, the solutes in this 'unstirred' cake layer are not exposed to the shear forces of the crossflow, causing the concentration and the osmotic pressure of solutes at the membrane surface to be enhanced further (Chong et al., 2008a; Hoek and Elimelech, 2003). Thus, the loss of overall RO performance results both from the increase in hydraulic resistance and the CECP effect.

The concept of critical flux is useful but alone does not give sufficient guidance for plant operators to optimize the performance in real world situations (Field and Pearce, 2011). A more practical concept is that of a 'threshold flux', introduced by Bacchin et al. (Bacchin et al., 2006; Field and Pearce, 2011), and defined as the "flux at or below which a low and near constant rate of fouling occurs but above which the rate of fouling increases markedly." Thus, threshold flux is a transition between low (negligible) fouling and significant fouling. Studies of transitional fluxes in RO have been relatively limited. However in previous work (Chong et al., 2008a) using a model colloidal silica foulant, operated over a range of fixed fluxes, it was observed that the deposition rate ($d(\text{mass})/dt$),

estimated from a tracer technique, was flux dependent. A flux for transition from negligible to detectable net deposition was identified and termed ‘critical flux’ although it may more correctly satisfy the definition of threshold flux. In this chapter, the term threshold flux is used to identify the fouling transition.

In previous work in the Singapore Membrane Technology Centre (SMTTC) which involves EIS, it was shown that there is a threshold phenomenon during RO crossflow filtration when the nature of the foulant layer undergoes a transition that is reflected in changes in the electrical conductance of the concentration polarization layer (Sim et al., 2013b). In the current work, the dependence of the nature of the polarized or deposited foulant layer on flux has been investigated. This was done by measuring the TMP as a function of flux and crossflow velocity using the flux-step method (discussed in Section 2.5.1). EIS was simultaneously used to characterize the electrical properties of the diffusion polarization (DP) layer as a function of the flux. The effect of crossflow velocity and the presence of a spacer on the feed side were also investigated to compare TMP data and EIS measurements around the transition.

3.2 Materials and methods

3.2.1 EIS equipment

The main components of the EIS equipment are a crossflow impedance cell fitted with a pair of current injecting electrodes, a pair of electrodes for measuring the voltage across the membrane, the EIS spectrometer and an amplifier unit. The current injecting plates (plates 3 and 6 in **Figure 3.1**) serve as the current electrodes and the RO membrane is placed between these two plates, insulated by a plastic gasket. The voltage electrodes are mounted in the top and bottom of these two plates (but insulated from them) to measure the voltage developed across the membrane. Plates 1 and 8 are present to allow operations at high pressures. All of the stainless steel plates are insulated by the plastic gaskets (plate

2, 4 and 7) and cylindrical insulating tubes around clamping bolts to prevent the filtration cell from being electrically short circuited. Both the current and voltage electrodes are made of stainless steel. The phase difference between the current and voltage is measured by the spectrometer in the specified range of frequencies and recorded through dedicated software. The measured parameters then yield the capacitance and conductance (as detailed in Section 3.2) of the sample at each frequency. The amplifier unit contains a reference circuit (Randall circuit) so that the accuracy for the measurements over a wide range of frequencies can be optimized. This system employs the four-terminal method where four electrodes are used for the measurement of impedance to eliminate the complicating effects of the frequency dependent impedance at the voltage electrode-solution interface (Kavanagh et al., 2009).

The dimensions of the cell are $302\text{ mm} \times 60\text{ mm} \times 0.95\text{ mm}$ with an effective membrane area of 0.01812 m^2 . The cell was connected to an INPHAZE^{TM} Electrical Impedance Spectrometer (Sydney, Australia) and the electrical properties of the membrane were measured at frequency ranges from 10^{-1} to 10^5 Hz. This spectrometer has a phase resolution of 0.001° and is therefore capable of measuring the capacitance even at low frequencies where the admittance is dominated by the (high) conductance. Three replicates of impedance spectra were obtained per scan and the time required per scan over the frequency range (10^{-1} to 10^5 Hz) was approximately 30 minutes.

Figure 3.1 Cross section (bottom) and explode view (top right) of the stainless steel RO-EIS crossflow cell for impedance measurement. (1 and 8 – metal plates, 2, 4 and 7 – insulating plastic gaskets, 3 and 6 – metal plates that form the chamber and also act as the current electrodes. The voltage electrodes protrude through the plates that act as the current injecting electrodes and are insulated from it. The tip of the voltage electrodes make contact direct with the solutions on either side of the membrane (feed or permeate). The bolts that clamp the components of the flow chamber together are insulated from the current injecting electrodes by plastic sleeves. Note: Prof Hans G. L. Coster is the primary designer of the RO-EIS crossflow cell.

3.2.2 Model foulant, background electrolyte and membrane

Colloidal silica (Sigma-Aldrich, Ludox TMA) was used as the model foulant in this study, as used in previous studies in the SMTC (Sim et al., 2013b). It was supplied in the form of a 34 wt% suspension in deionized water at pH 7.0. The nominal size of the silica particles was 20 nm based on the datasheet provided by the manufacturer. Sodium chloride (Merck) was used as the background electrolyte. Milli-Q water was used to prepare the silica and sodium chloride solution. A 0.45 μm filter (Millipore, model HAWP04700) was used to filter the sodium chloride solution to remove unwanted particulate impurities before use.

Commercial RO membranes (DOW FilmTec, TW30) with a membrane hydraulic resistance of $1.06 \times 10^{14} \text{ m}^{-1}$ were used. The observed salt rejection of the membranes was about 97%, measured at a constant flux of $25 \text{ L/m}^2 \text{ h}$ with 2000 ppm NaCl aqueous solution. RO membranes were wetted in ethanol for 2 hours and rinsed thoroughly before storing them in Milli-Q water for at least 24 hours before use. Most of the studies used a spacer-free feed channel but one set of experiments used a conventional diamond-shaped spacer.

3.2.3 RO setup

A schematic diagram of the RO setup is shown in **Figure 3.2**. A high pressure pump (Hydra-Cell Pump, model D03) was used to deliver the solution from a 10 L feed tank to the RO-EIS crossflow cell. An overhead stirrer (Panasonics, model MX8G5B) was installed in the feed tank to ensure that the solution was mixed thoroughly throughout the experiment. The temperature in the feed tank was maintained with cooling water from a chiller (PolyScience, model 9106) at $23 \pm 1 \text{ }^\circ\text{C}$. The reject and permeate were recycled back to the feed tank in the course of filtration where the permeate flux was controlled by a mass flow controller (Brooks Instrument, model 5882). The system pressure was controlled by a backpressure regulator (Swagelok, model KBP series) while the pressures of the feed and permeate were monitored using pressure transducers (Ashcroft, model 302174). The flow of the feed stream was measured with two flow meters (Cole-Palmer, model PMR1-010640 and model PMR1-010-210) while conductivity meters (Eutech Instruments, model Alpha COND 500) were installed to measure the conductivity of the feed and permeate. Pressure, flux and conductivity readings were recorded with a data acquisition system (National Instrument, model PCI-6014 and LabView 10 software).

Figure 3.2 Schematic diagram of the RO-EIS crossflow filtration system.

3.3 Experimental procedure

3.3.1 Membrane compaction

The membrane was compacted at a flux of $40 \text{ L/m}^2 \text{ h}$ for at least 48 hours prior to the fouling experiments. This was to ensure that there were no significant changes in the membrane properties as these changes are detectable by EIS. The final concentration of NaCl solution in the feed tank was adjusted to 2000 ppm to simulate brackish water conditions.

3.3.2 Silica fouling

1. Flux step method

The system was adjusted to the desired (lowest) flux and crossflow velocity before silica was added into the feed tank. After the system was stabilized, concentrated silica was added to the feed tank to achieve the desired silica concentration of 200 ppm. The fouling stage at a particular flux was maintained for 2 hours and EIS measurements were taken at

1 and 2 hours intervals. The flux was then increased incrementally after a fixed duration and the sequence continued until the highest desired flux was reached.

Some prolonged fouling experiments were conducted and lasted for about 24 hours and EIS measurements were recorded regularly to monitor the membrane responses throughout the experiment.

3.4 Results and discussion

3.4.1 Rationale for results presentation

Nyquist plots (**Figure 3.3**) are one of the most useful ways to represent EIS data, where each data point is obtained from a particular frequency as detailed in Section 2.6.1. This uses Equation (2.21) and is a plot of $-Z_{\text{Im}}$ with respect to Z_{Re} whereby the shape of the plots and shift in the plots provide real time information of the onset of fouling processes as well as the type of foulant (Sim et al., 2013b). When the experimental data were fitted to the Maxwell-Wagner model, it revealed the presence of various elements and processes occurring in the system. The fitting was done using the INPHAZE^{TM} Structure Refinement Software (Sydney, Australia). As explained in Section 3.2, elements at low frequencies (~1-10 Hz) correspond to the DP layer and its conductance, G_{DP} values were derived from the fitted values as explained in Section 2.6.1. The detailed procedure for fitting has been described elsewhere (Antony et al., 2013; Kavanagh et al., 2009). **Figure 3.3** shows a typical Nyquist plot for a RO system, where the low frequencies element corresponds to the DP layer, mid frequencies corresponds to the membrane layers and solution is the dominant feature in the high frequencies range. The remaining discussions in this chapter are in terms of the Nyquist plot and the parameter, G_{DP} which provides insights into the interfacial layer at the membrane surface when the operating conditions approach the threshold flux and the onset of the CECF effect.

Figure 3.3 Representative Nyquist plot of a silica-fouled RO system. *Conditions:* RO feed = 200 ppm silica with 2000 ppm NaCl; Flux = 30 L/m² h; crossflow velocity = 0.15 m/s. The full line represents the fitted results obtained from the Maxwell Wagner model using the INPHAZE™ Structure Refinement Software.

3.4.2 Threshold flux determined by the flux step method

The critical flux as originally defined (Field et al., 1995) is usually determined by the flux step method. This is preferred over the pressure step due to the more manageable control of flux and a constant flow of foulants towards the membrane can be established more easily (Defrance and Jaffrin, 1999a, b). Therefore, a similar flux step procedure was used here, coupled simultaneously with EIS measurements to investigate the effect of flux on the nature of the concentration polarization layer.

1. TMP and EIS measurements using the flux step method

The TMP with foulant as well as the salt rejection profiles for the flux step method are presented in **Figure 3.4a**. The TMP values remained almost constant from 15 to 25 L/m² h throughout the course of filtration. A slope change occurred at about 30 L/m² h, suggesting the onset of detectable foulant deposition and membrane fouling. As shown in **Figure 3.4b**, the threshold flux (J_{th}) was found to be ~ 28 L/m² h from the intersection of the dTMP/dt slopes.

Figure 3.5 presents the Nyquist plots of the flux step method for silica fouling. The Nyquist plot shifted to the right as the flux increased from 15 to 30 L/m² h. When the system was operated beyond J_{th} , the movement of the Nyquist plot changed direction and shifted to the left. The shift to the left of the Nyquist plot was due to a decrease in the overall Z (hence increase in G). The trend was similar to observations in a previous study in the SMTC (Sim et al., 2013b).

The G_{DP} values derived from the theoretical fitting result as detailed in Section 3.2 are shown in **Figure 3.6a**. The G_{DP} decreased as flux increased from 15 to 30 L/m² h which means the polarized layer became less conducting as flux increased. This is probably due to the presence of the flowing colloidal suspension of non-conducting particles near the membrane surface which would reduce the overall conductance (per unit volume) of that layer. As flux increased, the concentration of the non-conducting particles in the dynamic polarization layer would increase and thereby, decrease overall conductance in that layer. The nature of this colloidal suspension is likely to be flowing as observed which a similar feed using nuclear magnetic resonance (NMR) flow imaging (Airey et al., 1998). As a result, the mass transfer coefficient for the salt that controls its concentration polarization at the surface would not be affected substantially as the layer remains hydro-dynamically active. When the flux exceeded the threshold level of 30 L/m² h, G_{DP} started to rise. It is proposed

that this was because the concentration of salt in the DP layer increased as a result of concentration polarization. This would be expected if the salt mass transfer coefficient was decreasing due to hindered diffusion in a cake layer. That would imply that the nature of the foulant layer has undergone a transition from a flowing colloidal suspension or loose cake structure to a stagnant cake (Chen et al., 1997).

Figure 3.4 (a) TMP, permeate flux and salt rejection versus time plot; (b) $d\text{TMP}/dt$ versus permeate flux for the flux step experiment. *Conditions:* RO feed = 200 ppm silica with 2000 ppm NaCl; crossflow velocity = 0.15 m/s.

Figure 3.5 Nyquist plots obtained from EIS measurement with the flux step method. The points in the plot represent the measured data whereas the solid lines are obtained from the fitted data using the Maxwell-Wagner model. Error bars (generally smaller than the size of the symbols) were obtained from three scans for a particular imposed flux. Note that the x and y -axes of the plot are presented in different scale in order to better illustrate the shifts in the Nyquist plots. *Conditions:* RO feed = 200 ppm silica with 2000 ppm NaCl; crossflow velocity = 0.15 m/s.

In order to test these ideas, membranes operated below and above the threshold flux was examined for silica deposition by SEM. **Figure 3.7a** shows the membrane after 2 hours of filtration at an imposed flux of 25 L/m² h (below the apparent threshold flux). Interestingly, a modest amount of silica particles was observed on the membrane surface even below the threshold flux. This further affirmed that the decrease of G_{DP} prior to the threshold flux is due to the accumulation of silica particles close to and on the membrane surface. Above the threshold flux (35 L/m² h), **Figure 3.7b** shows that there was a greater accumulation of silica on the surface and there were fewer voids and patches as compared to that at 25 L/m² h (**Figure 3.7a**). However, denser silica layers did not result in lower G_{DP} values which are consistent with the notion that the dense cake structure resulted in a significantly hindered back transport of NaCl, which produces the CECF effect.

Figure 3.6 (a) G_{DP} (b) G_{skin} and (c) G_{base} of membrane obtained from the fitted model for silica fouling in the flux step method. The time in (a) indicates the duration after the flux has been adjusted to the desired one. *Conditions:* RO feed = 200 ppm silica with 2000 ppm NaCl; crossflow velocity = 0.15 m/s.

Figure 3.7 SEM images of silica particles on membrane surface at x20,000 magnification after 2 hours of fouling at (a) 25 L/m² h; (b) 35 L/m² h and (c) clean membrane. *Conditions:* RO feed = 200 ppm silica with 2000 ppm NaCl; crossflow velocity = 0.15 m/s.

Whilst the properties of these layers cannot be determined from the SEM images, their impact on TMP and the G_{DP} would indicate substantial differences. As observed in **Figure 3.6a**, there was a distinct minima around J_{th} where G_{DP} increased sharply with increasing flux and this is interpreted to coincide with the formation of a more structured cake layer from the incipient silica fouling layer on the membrane surface. It is assumed that above J_{th} , as the cake layer grew thicker and denser it enhanced the CECF resulting in a higher salt concentration at the membrane surface which gave rise to the higher G_{DP} as the fouling proceeded further (1 and 2 hr data after flux adjusted in **Figure 3.6a**. The higher G_{DP} values at fluxes beyond J_{th} for fluxes maintained for 1 and 2 hours lead to slightly different estimates of J_{th} from ~ 31 to 29 L/m² h. The latter is closer to the value of J_{th} of ~ 28 L/m² h derived from the TMP data. It is worthwhile to note that the threshold flux

defined here is dependent on the time scale and in this case the threshold flux from the G_{DP} plot was $\sim 29 \text{ L/m}^2 \text{ h}$ for a flux step of 2 hours each.

In order to study the fouling behavior of colloidal silica below and above the threshold flux, prolonged silica fouling experiments were conducted below and above the threshold flux (imposed flux of $25 \text{ L/m}^2 \text{ h}$ and $35 \text{ L/m}^2 \text{ h}$) to investigate the change of G_{DP} as opposed to TMP with time. Below the threshold flux, the G_{DP} decreased for the first 3.5 hours of filtration and then increased as shown in **Figure 3.8a**. This phenomenon was previously observed by Sim et al. where the Nyquist plot was originally shifted to the right direction and then to the left (Sim et al., 2013b). This could further affirm that there is a gradual transition over time for the foulant layer from loose cake to stagnant cake where CECF becomes evident. However, the formation of this layer above the threshold is probably different as discussed above. Above J_{th} , the cake appeared to form much more rapidly which reduced the mass transfer coefficient of NaCl, leading to a significant CECF effect at the initial stage of the fouling. This can also be observed from **Figure 3.8b** where the G_{DP} increased immediately as fouling proceeded. This scenario is supported by other studies in SMTC using ultrasonic time domain reflectometry (UTDR) which detected the formation of a consolidated silica layer adjacent to the membrane. It has been proposed that, at a fixed flux, there is a transition over time from a less dense colloidal suspension in the polarization layer to a more dense gel-like phase (Sim et al., 2014). The rate of this transition was flux-dependent and showed ‘threshold’ behavior.

The TMP profile (**Figure 3.8c**) shows insignificant changes throughout the course of filtration of 20 hours below the threshold flux. It would appear that the *in-situ* EIS method is more sensitive than the TMP method in determining the incipient silica fouling event.

Figure 3.8 (a) G_{DP} of membrane obtained from the fitted model for silica fouling as a function of time in a prolonged fouling experiment at a flux of 25 $L/m^2 h$. (b) G_{DP} of membrane obtained from the fitted model for silica fouling as a function of time in a prolonged experiment at a flux of 35 $L/m^2 h$ and (c) TMP at an imposed flux of 25 and 35 $L/m^2 h$ in the prolonged fouling experiment. *Conditions:* RO feed = 200 ppm silica with 2000 ppm NaCl; crossflow velocity = 0.15 m/s.

The conductance of the skin layer (G_{skin}) and base layer (G_{base}) of the membrane were also deduced using the INPHAZE^{TM} Structure Refinement Software. **Figure 3.6b** shows the changes in G_{skin} as the flux was increased. The change in G_{skin} was not as distinct as G_{DP} around the J_{th} although it does show a minor minima around 30 L/m² h. G_{base} , as shown in **Figure 3.6c** does not differ much before and around the J_{th} . These results confirm that the diffusion polarization layer was the dominant layer where fouling behavior can be observed.

2. Effect of crossflow velocity

The critical flux is expected to be dependent on the crossflow velocity (Chong et al., 2008a; Field et al., 1995; Li et al., 1998, 2000). Likewise, we would expect the threshold flux to change with crossflow velocity. This was examined in a separate series of experiments. The dTMP/dt data versus flux in **Figure 3.9** shows J_{th} at two crossflow velocities (0.15 and 0.30 m/s). The trend of J_{th} with crossflow velocity is similar to that observed in the case of critical fluxes (Chong et al., 2008a; Li et al., 1998, 2000). In the present study, the J_{th} with the increased crossflow velocity (from 0.15 to 0.30 m/s) resulted in a higher J_{th} of ~ 37 L/m²h (up from 30 L/m²h). The turning point for G_{DP} also shifted to a higher flux of around 40 L/m² h that is relatively close to the TMP-derived J_{th} (**Figure 3.10**). It is suggested that the increase crossflow provides a greater shear rate, causing increased back transport of particles from the membrane-solution interface and a thinner layer of silica below the J_{th} . Beyond the threshold flux of about 40 L/m² h, however, the cake would start to form at the surface leading to CECP and a rise in the G_{DP} as before.

Figure 3.9 Comparison of $dTMP/dt$ versus flux plot for flux step experiments with different crossflow velocities. *Conditions:* RO feed = 200 ppm silica with 2000 ppm NaCl; crossflow velocity = 0.15 m/s and 0.30 m/s.

Figure 3.10 Comparison of G_{DP} for flux step experiments with different crossflow velocities. *Conditions:* RO feed = 200 ppm silica with 2000 ppm NaCl; crossflow velocity = 0.15 and 0.30 m/s.

3. Effect of spacer

Commercial RO systems and side-stream (canary) cells use spacer-filled channels. In the case of critical fluxes, several studies have shown that J_{crit} can be enhanced in spacer-filled channels (Chong et al., 2008a; Neal et al., 2003). As a result, it is of interest to use EIS to investigate the effect of the spacer on the threshold flux. This was done by the inclusion of a diamond shaped spacer in the feed channel. The $d\text{TMP}/dt$ in the spacer-filled channel is presented in **Figure 3.11** and the estimated threshold flux, J_{th} , was $\sim 33 \text{ L/m}^2 \text{ h}$ at the crossflow rate of 0.15 m/s . The spacer gave an enhancement of the J_{th} by $\sim 18\%$. The turning point of the G_{DP} plot also shifted to a higher flux as shown in **Figure 3.12** with a J_{th} as determined by EIS of $\sim 38 \text{ L/m}^2 \text{ h}$. This value is higher, but relatively close to the value obtained by $d\text{TMP}/dt$ analysis. A longer flux step period could bring the two methods closer, as suggested by **Figure 3.6a**. It has been suggested by Neal et al. (Neal et al., 2003) that the presence of the spacer introduces complex mixing behavior as the stream flows over and under the attached filaments, leading to a patchy and heterogeneous particle deposition and in the present study it led to a higher value of J_{th} . The fact that the EIS can determine J_{th} , which is dependence on the presence of spacers further supports the use of a side-stream ‘canary cell’ incorporating EIS to characterize plant performance (Sim et al., 2013b; Sim et al., 2015). To simulate the plant, the canary cell requires spacers in the flow channel and the non-invasive detection method needs to provide a ‘membrane-averaged’ signal; EIS delivers this information.

Figure 3.11 dTMP/dt versus permeate flux for flux step experiment with and without a spacer in the feed channel. *Conditions:* RO feed = 200 ppm silica with 2000 ppm NaCl; crossflow velocity = 0.15 m/s.

Figure 3.12 G_{DP} of membrane obtained from the fitted model for silica fouling in the flux step experiments with no spacer in the feed channel and with a spacer in the channel. *Conditions:* RO feed = 200 ppm silica with 2000 ppm NaCl; crossflow velocity = 0.15 m/s.

3.5 Concluding remarks

EIS was employed to characterize the electrical properties associated with diffusion polarization as a function of the flux. The G_{DP} decreased with increasing flux due to presence of a flowing colloidal suspension with enhanced concentrations of silica that result from the pressure driven flux. As the flux was increased beyond a threshold flux value, the G_{DP} was found to increase, which is attributed to the formation of a stagnant/cake layer and the accumulation of salt in this layer; the so-called cake enhanced concentration polarization (CECP) effect. This hypothesis was confirmed when a higher G_{DP} was obtained beyond J_{th} at longer filtration times when a thicker stagnant layer forms with a higher concentration of salt.

The threshold flux increased with increasing crossflow velocity. The minima of G_{DP} shifted to a higher flux, indicating the initial formation of a cake layer at a higher flux, which is attributed to the increased shear force at higher crossflow velocities that tend to drag the particles along on the membrane surface. The efficacy of the EIS in characterizing the threshold flux was also confirmed in a spacer-filled channel. The enhanced J_{th} obtained by the $dTMP/dt$ measurement was also detected by EIS which revealed a shift of the turning point in the G_{DP} plots to higher flux values.

EIS is able to provide quantitative data for the onset of cake formation and the CECP effect while the TMP measurements on their own provide no direct information for these events. While the other *in-situ* fouling detection method, such as ultrasonic time domain reflectometry (UTDR) (Sim et al., 2012) and salt pulse tracer test (Chong et al., 2008a) depends largely on the foulant thickness, the size of the particle and the injection of high salt concentration into the system, EIS could detect the incipient fouling event (happening below the threshold flux) in brackish water condition. EIS is therefore a promising tool that could be incorporated into a side stream canary cell for fouling and cake formation

monitoring in high pressure membrane systems to optimize plant performance. The flux that RO plants are operated at is often conservative and based on *in-situ* EIS measurements, the plant could be tuned to operate more efficiently at or just below the threshold flux.

4 Chapter 4 - *In-situ* Monitoring of Biofouling on Reverse Osmosis Membranes: Detection and Mechanistic Study using Electrical Impedance Spectroscopy^{†2}

4.1 Introduction

Microorganisms found in seawater or wastewater feed poses a great challenge to the use of membrane technologies due to membrane biofouling (Bereschenko et al., 2008; Flemming, 2002; Mayer et al., 1999). Biofouling is a biofilm phenomenon that involves several stages starting with the attachment of organic matter onto the membrane surface forming a conditioning layer followed by bacteria. In the second stage, the bacteria cells grow and proliferate forming micro-colonies and embed themselves in a self-produced matrix of extracellular polymeric substances (EPS), forming a mature biofilm (O'Toole et al., 2000). Lastly, the bacteria cells may detach from the membrane surface and this stage is determined by the concentration of nutrients, growth rates, the accumulation of dispersal signals, the mechanical stability of the biofilm and also the effective shear force of the feed water (Flemming, 1997). Biofouling is considered to be the most severe form of fouling in processes using reverse osmosis (RO) membranes. It is an inevitable and costly problem which occurs on the membrane surface despite cleaning protocols that may be in place. For example, biofouling accounted for approximately 30% of the operating cost for RO plant at Water Factory 21 in Orange County, California and the amount spent on biofouling control there was estimated to be \$730,000 per year (Flemming et al., 1994). In another

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² [†]Part of the findings in this chapter have been published in: Ho, J.S., Low, J.H., Sim, L.N., Webster, R.D., Rice, S. A., Fane, A.G., Coster, H.G.L., 2016. *In-situ* monitoring of biofouling on reverse osmosis membranes: detection and mechanistic study using electrical impedance spectroscopy. Journal of Membrane Science 518, 229-242.

study, the potential annual savings that could be achieved by early warning of biofouling for large-scale seawater reverse osmosis (SWRO) plants was estimated to be \$2 million (Vrouwenvelder et al., 2011).

Biofilm formation on membrane surfaces results in a severe decline in flux, or an increase in transmembrane pressure (TMP) and feed pressure required to maintain flux (Hoek et al., 2008). This leads to higher energy consumption, and a deterioration of system performance and water production. Typically, these changes in performance are monitored by inlet pressure increases and/or a drop in permeate quality. Rules of thumb, such as 10 to 15% increase in pressure are used to trigger the application of cleaning counter-measures, however, the raised pressure approach is insensitive to initial fouling. In a multi-module pressure vessel, pressure drop is a global parameter caused by fouling along the pressure vessel. Overtime, the fouling shifts from inlet to outlet due to the fouling creep (or 'flux levelling') (Hoek et al., 2008). By the time the trigger pressure is reached; the whole system could be well-fouled. Identified as the most crucial problem in the operation of seawater reverse osmosis (SWRO) plants, a common method to prevent biofouling in these plants is to employ the use of continuous chlorine dosing of the raw seawater feed upstream and a de-chlorination process just prior to the RO membrane stage (Uemura and Henmi, 2008). This approach is slowly being replaced by low pressure membrane pretreatment. However, even with a microfiltration (MF) pretreatment step, biofouling in RO cannot be eliminated completely as it only requires a few residual microorganisms to slip through the MF membranes and deposit on the RO membrane to eventually form a mature biofilm (Flemming, 2002). The best available strategy currently is to accept the presence of the biofilm and minimize its impacts on the operation.

Studies have shown that the feed water quality plays a crucial role in membrane biofouling, thus, the use of biological parameters to assess the biofouling potential of the

feed water is of great importance (Vrouwenvelder et al., 2011). Adenosine triphosphate (ATP) quantification (Holm-Hansen and Booth, 1966) and direct cell count using fluorescence microscopy (Hobbie et al., 1977) are related to the concentration of microorganisms and can be used as indicators for biofouling. The amount of assimilable organic carbon (AOC) (van der Kooij, 1992), that is a growth promoting nutrient and a proxy to biofilm formation rate (van der Kooij et al., 1995), has been considered as a biofouling indicator (Vrouwenvelder et al., 2011). These parameters may be applicable for screening of the biofouling potential in the feed water (Vrouwenvelder et al., 2008) but are not suited to *in situ*, real-time, monitoring. Furthermore, most industrial plants do not have systems installed to assess the biological activities or the initiation of biofilm development, despite the enormous amount of investment spent on tackling biofouling. Without a definitive means to determine the onset of biofilm formation, most of the treatment plants carry out cleaning based on a preset schedule or when there is apparent evidence of growth (such as pressure reaching a threshold). This can lead to the possibility of improper dosage of biocides or suboptimal cleaning schedules. Hence there is a need for a novel, non-invasive and *in-situ* monitoring tool for monitoring the biofouling processes and also to identify the minimum flux levels for mitigating biofouling.

Common methods for fouling control include membrane cleaning and pretreatment of feed water. The application of a biocide is one of the most common methods used against biofouling problems (Flemming et al., 1997). However, this approach might result in severe drawbacks as the biocide might not remove the biofouling layer completely and the remnants of this layer might invite new biofouling (Flemming et al., 1997). Even after 99.9-99.99% removal of bacteria by the pretreatment step using microfiltration (MF), biofouling cannot be completely eliminated as only the presence of a few initial colonies on the membrane is required to form a biofilm (Chong et al., 2008b; Flemming, 2002; Flemming

et al., 1997). Therefore, it is of urgent need to formulate new strategies for biofouling control and it would be extremely useful to couple this with an effective monitoring system.

Recent studies have demonstrated that one of the biological messengers, nitric oxide (NO) acts as a signal for biofilm dispersal, inducing the transition from the biofilm growth mode to the free swimming planktonic state (Barraud et al., 2006; Barraud et al., 2009b). The dispersal NO occurs due to the stimulation of phosphodiesterase activity, leading to the degradation of cyclic-di-guanylate monophosphate (c-di-GMP), resulting in the change of gene expression which favors the planktonic mode (Barraud et al., 2009b). Apart from the destructive methods such as autopsy or confocal laser scanning microscopy (CLSM), a non-destructive and online monitoring tools for the dispersal event have not previously been investigated.

Electrical impedance spectroscopy (EIS) has been employed to detect various types of RO membrane fouling and scaling as well as to characterize the threshold flux phenomena in RO processes (Chapter 3) (Hu et al., 2014; Kavanagh et al., 2009; Park et al., 2006; Park et al., 2005; Sim et al., 2013b); see also **Table 2.1**. EIS has also been used to characterize different types of membranes, such as biological (Chilcott et al., 1983; Ogata et al., 1983; Smith et al., 1985) and synthetic membranes (Antony et al., 2013; Chilcott et al., 1995; Chilcott et al., 2002; Coster et al., 1992; Fortunato et al., 2006; Gaedt et al., 2002; Gao et al., 2013; Xu et al., 2011). Hence, EIS has the potential to characterize the growth of a biofilm non-invasively and in real time on a membrane surface. However, to date, EIS has not been applied for membrane biofouling detection and monitoring. Therefore, this chapter investigates the ability of EIS to elucidate the mechanisms of biofilm formation on RO membranes. This includes a study of the effect of extracellular polymeric substances (EPS) on the EIS signals and the potential of EIS in monitoring biofouling control using nitric oxide (NO) dispersal.

This fouling monitor is potentially useful when installed in a side stream as a ‘canary cell’ in situations where the biofouling problems are prevalent so that control and cleaning strategies can be well formulated.

4.2 Previous studies of fouling using EIS

Briefly, based on results reported in Chapter 3 and results from our group (Sim et al., 2013b), the key signals which could be obtained from the EIS measurements include (1) the Nyquist plot and its temporal shift and (2) the conductivity of the diffusion polarization layer (G_{DP}). The general trends of G_{DP} and its implications are summarized in **Table 4.1**. The trends evident in **Table 4.1** can be used to help interpretation of the observed EIS parameters for membrane biofouling in this study.

Table 4.1 Summary of G_{DP} plot and its implications.

GDP plot	Implications
	A drop in G_{DP} signifies a build-up in a less conductive layer adjacent to the membrane. For example: colloidal cake and organics (Sim et al., 2013b).

G _{DP} plot	Implications (cont.)
	An increase in G_{DP} signifies an increase in conductivity of the layer adjacent to the membrane. For example, an increase in ionic species, e.g. salts (Sim et al., 2013b).
	A decline then increase in G_{DP} with flux signifies a transition in polarization and fouling. The increase of slope of G_{DP} with time is typical of a developing cake with cake enhanced concentration polarization of ionic species (Chapter 3).

4.3 Materials and method

4.3.1 Bacteria stock preparation

Pseudomonas aeruginosa PA01 (ATCC, BAA-47) was used as the model bacterium in this study. A single colony on agar plates, freshly cultured from frozen glycerol stocks was sub-cultured on Nutrient Broth (NB) (Difco NB-BD diagnostics) agar plates (NB, 8 g/L supplemented with 14 g/L agar – Difco agar, BD diagnostics). The bacterial stock solution was prepared by growing the culture in nutrient broth (NB) (5g/L NB, 2 g/L NaCl) with shaking at 150 rpm (Stuart, model SSL1) at room temperature for 24 hours. The bacteria cells were then harvested by centrifugation at $4000 \times g$ at 4 °C for 30

minutes. The pellets were subsequently washed and suspended in NaCl solution (2 g/L, the same concentration as the experimental condition) to achieve an optical density (OD₆₀₀, Shimadzu, model UV1800) of 0.1.

A dead bacteria stock solution was also prepared using the above procedure followed by heating in 80°C for 2 hours to ensure that the bacteria were killed. Viable bacterial counts were carried out for the dead bacterial stock and no colonies were found to be formed on the plates even after 36 hours of incubation at 37°C.

4.3.2 RO biofouling setup

The schematic diagram of the RO setup is illustrated in **Figure 4.1**. The setup is similar to the one described in Section 3.3.3. Modifications of the setup include the installation of the injection pump as well as micro-filters in the bypass and retentate streams. A high pressure pump (Hydra-Cell Pump, model D03) was used to pump the feed solution from a 10 L feed tank to the RO-EIS crossflow cell. To ensure that the solution in the feed tank could be mixed thoroughly, an overhead stirrer (Panasonics, model MX8G5B) was installed in the feed tank. The temperature in the feed tank was maintained with cooling water from a chiller (PolyScience, model 9106) at 23 ± 1 °C. The system pressure was controlled by a backpressure regulator (Swagelok, model KBP series) and the pressures of the feed as well as permeate were monitored using pressure transducers (Ashcroft, model 302174). A mass flow controller (Brooks Instrument, model 5882) was installed at the permeate line to maintain constant flux throughout the experiment. Two rotameters (Cole-Palmer, model PMR1-010640 and model PMR1-010-210) were installed in the feed stream to indicate the flow to the RO-EIS cell. The conductivity of feed and permeate were measured using two conductivity meters (Eutech Instruments, model Alpha COND 500). All the readings, including pressure, permeate flux, feed and permeate conductivities were

recorded through a data acquisition system (National Instrument, model PCI-6014 and LabView 10). During the biofouling studies, the bacteria stock solution was injected at a predetermined rate into the system before entering the RO-EIS cell using an injection pump (ELDEX, model 5979-OptosPump 2HM). The system was operated in a full recirculation mode where the bypass, retentate and permeate streams were returned to the feed tank. A set of micro-filters were installed in the bypass and retentate streams (KAREI, 5 μm and 0.2 μm for concentrate, 0.2 μm for bypass) before they flowed into the feed tank to collect suspended bacteria and prevent the feed tank from turning into an “active bioreactor”.

4.4 Experimental procedure

4.4.1 Biofouling experimental protocol and EIS measurements

Before each experiment, the RO flat sheet membranes (DOW FilmTec, TW30) were cut and sterilized in absolute ethanol (Merck) for 2 hours. The membranes were then rinsed thoroughly and stored in Milli-Q water (Millipore) for at least 24 hours before using.

Figure 4.1 Schematic diagram of the RO-EIS crossflow filtration system for biofouling.

The experimental procedures included: (i) overnight membrane compaction at 50 L/m² h with background NaCl (2000 ppm), (ii) addition of nutrient broth (NB) (to obtain a final concentration of 24 mg/L), (iii) continuous injection of the bacteria stock solution, (iv) biofouling of the RO membrane and (v) cleaning of the RO setup upon completion of the experiment. The repeatability of the biofouling experiments was calculated using the standard deviation of the initial increase of normalized G_{DP} plot and it was estimated to be $\pm 10\%$.

The RO membrane was compacted at a flux of 50 L/m² h for 24 hours prior to the biofouling experiment. This was to ensure that there were no significant changes in the membrane properties as any such changes would be detected by EIS. After compaction, the flux was adjusted to the desired value and fresh NaCl solution was added into the feed tank. The system was then allowed to stabilize for 1 hour. NB was added into the feed tank to achieve 24 mg/L or 7.8 mg/L of total organic carbon (TOC) and the system was allowed to stabilize for another hour.

After stabilization, the biofouling experiment was commenced with continuous injection of the bacterial stock solution into the feed line at a dilution rate of 1:1000 based on the RO crossflow velocity. This was equivalent to a bacterial load of 10⁵ cfu/mL into the system. The bacterial stock solution was replaced every 48 hours. The biofilm was allowed to grow for durations of 1 – 5 days. The experiments were conducted in a constant flux mode (ranging from 8 to 40 L/m² h) and the increases in TMP were monitored. EIS measurements were conducted every 2.5 hours (over a frequency range: 10⁻¹ to 10⁵ Hz) and according to the specifications described in Section 3.2.1. The solution in the feed tank was replaced twice daily with the same concentration of NaCl and NB in order to maintain the freshness level of the feed.

Upon completion of each experiment, the RO system was cleaned with HNO₃ solution (pH 2, 1.5 hours, Merck) followed by NaOH (pH 11, 1.5 hours, Merck) and then rinsed thoroughly with Milli-Q water.

4.4.2 Salt pulse tracer test

A salt pulse tracer test (Chong et al., 2008a) was used to estimate fouling resistance and the cake-enhanced CP due to fouling. Briefly, the protocol involved a sodium chloride solution, 200 g/L was injected into the high pressure feed line of the RO-EIS crossflow using an injection pump (ELDEX, model 5979-OptosPump 2HM). The flowrate of the high concentration salt solution was maintained at 0.6 mL/min and each pulse length lasted for 10 minutes. During the tracer test, the retentate and permeate were drained to maintain the conductivity of the feed solution. The feed solution was topped up to 20 L prior to the test. Changes to TMP and conductivity were monitored during the test; a detailed procedure of the salt pulse tracer test has been described elsewhere (Chong et al., 2008b).

4.4.3 Biofilm control using NO dispersal

The NO dispersal was carried out by dosing 1 mg/mL of PROLI NONOate dissolved in 10 mM NaOH into the system for 30 min at the beginning of day 2 and 3 respectively in separate tests using an injection pump (ELDEX, model 5979-OptosPump 2HM) to give a final concentration of 40 µM. The bacteria injection was stopped during the NO dispersal. Immediately after NO treatment, the bacteria injection into the system resumed.

4.4.4 Membrane autopsy

The fouled membrane was removed from the RO-EIS cell for autopsy studies when the experiment was completed. The membrane was first cut into four segments. Three segments (3 cm × 4 cm) covering the inlet, middle and outlet of the RO-EIS cell were used

for the viable bacterial counts and quantifications of protein and polysaccharides which made up the EPS. The results presented are based on the averaged value of the three samples. The other segment (1 cm × 3 cm) was cut from the middle of the membrane for the confocal laser scanning microscopy (CLSM) observation.

The cut membrane segments (3 cm × 4 cm) were soaked in 25 mL NaCl (2000 ppm, the same as the experimental conditions) in separate centrifuge tubes. The tubes were sonicated using a probe sonicator (Fisher Scientific, model FB15068) for 3 minutes to detach the biofilm from the membrane surface. The tubes were then vortexed (Velp Scientifica, model RX3) for 10 seconds and 10 µL of the solution was withdrawn from the tube for viable bacterial counts. A 1.0 M of solution of NaOH was added into the remaining solution in the tubes, mixed and stored at 4 °C for 24 hours before the analysis of EPS was carried out.

1. EPS extraction

The polysaccharide content of the EPS was measured by the colorimetric method (DuBois et al., 1956; Suwarno et al., 2012). One mL of 5% (v/v) phenol solution and 5 mL of concentrated H₂SO₄ were added into a 2 mL of sample solutions. The solutions were mixed and left to cool down to room temperature for 15 minutes before the UV absorbance at 490 nm (A₄₉₀) was measured using a UV spectrometer. Glucose (Merck) was used as the polysaccharide standard for calibration.

The protein content of the EPS was quantified using the Bicinchoninic acid (BCA) Assay Kit (Pierce, #23227). 2 mL of the working solution was mixed with 1 mL of sample solution and incubated in darkness at room temperature for 2 hours. The UV absorbance was then measured at 562 nm (A₅₆₂). Bovine serum albumin (BSA, Pierce) was used to construct the standard calibration curve.

2. Viable bacterial counts

Viable bacterial counts were conducted using the modified Miles and Misra method (Barnes et al., 2014; Miles et al., 1938). Briefly, 10 μL of 10^{-1} to 10^{-5} dilutions were pipetted onto the NB agar plates. The plates were incubated at 37 °C for 24 hours before colony counting. Viable cells were expressed as colony forming units (cfus) per cm^2 of membrane.

3. Confocal Laser Scanning Microscopy (CLSM)

Membrane samples used for CLSM analysis were obtained from the same site for all experiments. Biofilms were stained with LIVE/DEAD BacLight Bacterial Viability Kit (Molecular Probes, L7012) according to the manufacturer's specifications. In brief, 3 μL of SYTO 9 and PI each were mixed in 1 mL of buffer solution (0.85% NaCl) to obtain the working solution for staining. The membrane samples were then soaked in the working solution for 45 minutes in darkness at room temperature. After the incubation, the membrane samples were rinsed three times with the buffer solution before placing them on a glass slide under a cover slip. The microscopic observation and image acquisition were obtained using a CLSM (Zeiss, model LSM710). The biovolume was calculated using the IMARIS software (Bitplane, version 7.1.3).

4.5 Results

4.5.1 Rationale for results presentation

1. Normalized G_{DP} (G_{DP}/G_{DP-0})

The experimental data obtained from the EIS measurements were fitted with the Maxwell-Wagner model, which reveals the number of elements and/or processes occurring in the system. The fitting was done using the INPHAZE^{TM} Structure Refinement Software (Sydney, Australia). The elements at the low frequencies (~ 1 -10 Hz), corresponding to the DP layer can be derived from the fitting as detailed in Section 2.6.1. The detailed procedures for fitting have been described by Antony et al. (Antony et al., 2013) and Kavanagh et al. (Kavanagh et al., 2009). G_{DP} has been identified as the main EIS parameter associated with membrane fouling as it reveals the conditions at the interfacial layer on the membrane surface (Chapter 3). Each membrane sample could possibly have different initial values of G_{DP} as it is affected by the membrane surface properties and ion transport on the membrane surface (see also Section 2.6.1). To allow for this, the G_{DP} results represented here have been normalized with the value of G_{DP} at the start of the experiment (designated as G_{DP-0})

$$G_{DP\text{-Normalized}} = \frac{G_{DP}}{G_{DP-0}} \quad (4.1)$$

4.5.2 TMP and EIS measurement

Figure 4.2 shows the normalized TMP profile when bacteria were injected continuously into the system. Two stages of TMP profile were observed. The first stage shows only insignificant changes from day 0 to day 1.7 and then TMP jump was observed, at ~ 1.8 days after which the normalized TMP increased rapidly (47%) up to day 5. These

trends were reproducible and match those reported using similar protocols (Suwarno et al., 2012).

Figure 4.2 Normalized TMP profile as a function of time in the presence of bacteria (concentration $\sim 10^9$ cfu/mL). *Conditions:* permeate flux = 30 L/m² h, crossflow velocity = 0.15 m/s, RO feed = 24 mg/L NB with 2000 mg/L NaCl.

Figure 4.3 displays the normalized G_{DP} obtained from the EIS measurement for the 5-day long biofouling experiment. The normalized G_{DP} increased initially from day 1 to day 1.5 where a maximum point could be observed. From day 1.5 onwards, the plot shows an opposite trend in which the normalized G_{DP} decreased for the remaining course of biofouling.

To confirm the trend obtained for the EIS measurements, different durations of biofouling were carried out with the EIS scans recorded *in-situ* and in real time. **Figure 4.4** features the normalized G_{DP} as a function of time for 3-day biofouling. Similar to the results obtained for 5-day biofouling, there was a maximum point at around 1.5 days followed by a gradual decrease of normalized G_{DP} . For the 1-day biofouling experiment, the normalized

G_{DP} (**Figure 4.5**) shows a slight increase, which is consistent with the results shown for 5-day and 3-day biofouling experiments.

Figure 4.3 Normalized G_{DP} as a function of time for a 5-day biofouling experiment. *Inset*: CLSM images for live/dead staining of biofilm on RO membrane at end of the 5-day experiment. Note that the live cells are shown in green whilst dead cells are in shown red. *Conditions*: permeate flux = 30 L/m² h, crossflow velocity = 0.15 m/s, RO feed = 24 mg/L NB with 2000 mg/L NaCl.

In order to investigate whether concentration polarization of the components in the medium, rather than the initial biofilm, was responsible for the results obtained, experiments were performed using only the medium without the injection of bacteria. **Figure 4.6a** shows the normalized TMP plot for nutrient fouling and there was no TMP jump observed. The normalized G_{DP} for nutrient fouling (without bacteria) shows an increasing trend for 5-day operation (**Figure 4.6b**). In contrast to the normalized G_{DP} plot for biofouling, no maximum point was observed in this case. The increasing trend in the normalized G_{DP} is likely to be due to gradual accumulation and concentration polarization (CP) of nutrient when no bacteria are present. Enhanced CP of background salinity could also contribute as shown in **Figure 4.7**. This result implies some foulant-enhanced CP.

Figure 4.4 Normalized G_{DP} as a function of time for a 3-day biofouling experiment. *Inset*: CLSM images for live/dead staining of biofilm on RO membrane at the end of the 3-day experiment. Note that the live cells are shown in green whilst dead cells are in shown red. *Conditions*: permeate flux = 30 L/m² h, crossflow velocity = 0.15 m/s, RO feed = 24 mg/L NB with 2000 mg/L NaCl.

Figure 4.5 Normalized G_{DP} as a function of time for a 1-day biofouling experiment. *Inset*: CLSM images for live/dead staining of biofilm on RO membrane at the end of the for 1-day experiment. *Conditions*: permeate flux = 30 L/m² h, crossflow velocity = 0.15 m/s, RO feed = 24 mg/L NB with 2000 mg/L NaCl.

Figure 4.6 (a) Normalized TMP and (b) normalized G_{DP} as a function of time for nutrient fouling in the absence of bacteria. *Conditions:* permeate flux = 30 L/m² h, crossflow velocity = 0.15 m/s, RO feed = 24 mg/L NB with 2000 mg/L NaCl.

Figure 4.7 CP profile for nutrient fouling. *Conditions without salt pulse:* permeate flux = 30 L/m² h, crossflow velocity = 0.15 m/s, RO feed = 24 mg/L NB with 2000 mg/L NaCl. *Conditions for salt pulse:* additional salt concentration: 200 mg/L NaCl, pulse length = 10 min.

4.5.3 Fouling with dead bacteria

To investigate whether the maximum in the normalized G_{DP} trend are related to the presence and colonization by live bacteria on the membrane surface, we performed experiments in which dead bacteria were used in the fouling experiments. **Figure 4.8** shows a plot of the normalized G_{DP} as a function of time for fouling with dead bacteria. Similar to fouling with nutrient medium only (**Figure 4.6**), the normalized G_{DP} for fouling using dead bacteria increased monotonically as the fouling proceeded and no maximum could be seen.

Figure 4.8 Normalized G_{DP} as a function of time during fouling with dead bacteria (dead bacteria = stock solution heated in 80°C water bath for 2 hours). *Inset*: CLSM images for live/dead staining of biofilm on RO membrane at the end dead cell biofouling. *Conditions*: permeate flux = 30 L/m² h, crossflow velocity = 0.15 m/s, RO feed = 24 mg/L NB with 2000 mg/L NaCl.

To investigate whether or not EPS was associated with the G_{DP} trends when live bacteria were used, the effects of EPS-like materials on normalized G_{DP} were studied using mixtures of dead bacteria with two different concentrations of alginate. The concentrations of alginate used matched the TOC equivalence for 1 and 5 day biofouling.

When the alginate concentration (100 ppm) was as high as the EPS produced for the 5-day old biofilm, the normalized G_{DP} decreased (**Figure 4.9a**). However, when the alginate concentration (16 ppm) was low, at an equivalence to the TOC in the 1-day old biofilm, the normalized G_{DP} increased slightly (**Figure 4.9b**), similar to the trend of fouling with dead bacteria alone. The results obtained thus indicate that the maxima observed in time with fouling with live bacteria can be simulated by fouling with dead bacteria when a build-up of alginate is included.

Figure 4.9 Normalized G_{DP} as a function of time for (a) fouling with dead bacteria mixed with 100 ppm alginate and (b) fouling with dead bacteria mixed with 16 ppm alginate. *Conditions:* permeate flux = 30 L/m² h, crossflow velocity = 0.15 m/s, RO feed = 24 mg/L NB, 100 mg/L or 16 mg/L alginate with 2000 mg/L NaCl.

4.5.4 Characterization of biofilms on the membrane surface

At the end of the 1, 3 and 5-day biofouling experiments, images were made of the membrane surface. From the CLSM images, biofilms developed from individual planktonic cells (Inset **Figure 4.5**) to a slightly dispersed biofilm (Inset **Figure 4.4**) and then to a more complete coverage at day 5 (Inset **Figure 4.3**). The concentrations of proteins and polysaccharides which made up the main components of the EPS were determined using the extraction method and the results are shown in **Figure 4.10a**. The total EPS increased by 13% from day 1 to day 3 whilst there was a 46% increase from day 3 to day 5. This clearly shows that the amount of EPS produced by day 5 was significantly higher as compared to the early stage of the biofouling process.

The bio-volume for live and dead cells was determined and the live cell biovolume for day 3 was more than four-fold compared to that of day 1 (**Figure 4.10b**). However, the amount of dead cells for day 5 was twice the amount observed on day 1. The increase in biovolume was also validated by the viable bacterial count (**Figure 4.10c**) which increased from 2.08×10^7 (day 1) to 2.78×10^7 (day 3) and then to 6.25×10^7 cfu/cm² (day 5).

Figure 4.10 Characterization of biofilms on the RO membranes. (a) Concentrations of EPS (proteins and polysaccharides) determined by the extraction method for different durations of biofouling. (b) Average biovolume which consists of live and dead bacteria cells calculated using the IMARIS software. (c) Viable bacteria count for different durations of biofouling. Bars represent standard error, $n = 3$.

4.5.5 Effect of sodium azide

In order to evaluate the EIS signal response when the biofilm growth was disrupted, the biofouling studies were performed with NaN_3 dosing during the experiment. **Figure 4.11** (a) Normalized TMP profile, (b) Normalized G_{DP} as a function of time for biofouling where sodium azide was dosed into the system for 2 hours (concentration = 0.05wt%). After the dosing of sodium azide was stopped, the system was allowed to run continuously without introducing further bacteria. *Inset of (a)*: CLSM images for live/dead staining of biofilm on RO membrane at the end of biofouling (sodium azide dosing at day 1.5). (c) Normalized G_{DP} as a function of time for biofouling with and without the dosing of sodium azide. Sodium azide was dosed into the systems for one hour each. Concentration of sodium azide for first and second dosing = 0.03wt% and 0.05wt%. After the sodium azide dosing was stopped, the system was left to run continuously with bacteria injection. **Figure 4.11a** displays the normalized TMP profile when NaN_3 was dosed into the system at day 1.5. **Figure 4.11b** shows the normalized G_{DP} when NaN_3 was dosed into the system at day 1.5, where the maximum of the peak was normally observed. In contrast to the normalized G_{DP} plots shown in **Figure 4.3** and **Figure 4.4**, the normalized G_{DP} dropped to the initial value when NaN_3 was introduced and was then followed by an increase of the normalized G_{DP} when the system was allowed to run continuously. To further examine the effect of sodium azide, another experiment was carried out with two cycles of dosing and with a bacterial supply after the introduction of the biostat. In this case (**Figure 4.11b**), the first dosing with lower concentration (0.03 wt%) showed an almost unchanged normalized G_{DP} . However, the second dosing with higher concentration of NaN_3 (0.05 wt%) showed a larger drop of normalized G_{DP} as compared to the first dosing. Interestingly, after two cycles of NaN_3 dosing, and with the continuous injection of bacteria into the system, the trend of normalized G_{DP} developed after the disturbance was similar to that of biofouling without dosing with NaN_3 (**Figure 4.3**).

Figure 4.11 (a) Normalized TMP profile, (b) Normalized G_{DP} as a function of time for biofouling where sodium azide was dosed into the system for 2 hours (concentration = 0.05wt%). After the

dosing of sodium azide was stopped, the system was allowed to run continuously without introducing further bacteria. *Inset of (a)*: CLSM images for live/dead staining of biofilm on RO membrane at the end of biofouling (sodium azide dosing at day 1.5). (c) Normalized G_{DP} as a function of time for biofouling with and without the dosing of sodium azide. Sodium azide was dosed into the systems for one hour each. Concentration of sodium azide for first and second dosing = 0.03wt% and 0.05wt%. After the sodium azide dosing was stopped, the system was left to run continuously with bacteria injection.

4.5.6 Effect of flux

Since the rate of biofilm growth has been reported to increase with imposed flux (Chong et al., 2008b; Suwarno et al., 2012), it was of interest to compare the initial increase of the normalized G_{DP} at different fluxes (**Figure 4.12**). The slopes of the normalized G_{DP} were similar for operating fluxes of 8 L/m² h (**Figure 4.12a**) and 15 L/m² h (**Figure 4.12b**) whilst it increased three-fold when the flux was increased from 15 L/m² h to 20 L/m² h (**Figure 4.12c**). The increase in slope was smaller for 20 L/m² h and 30 L/m² h (**Figure 4.12d**) as compared to the significant increase (~ 1.4 times) when the flux was changed from 30 L/m² h to 40 L/m² h (**Figure 4.12e**). It should be noted that at 8 and 15 L/m² h the normalized G_{DP} profile did not go through a maximum during 5 or 3 days operation respectively, unlike the behavior at higher flux (**Figure 4.3** and **Figure 4.4**),

The magnitude of the initial increase of the normalized G_{DP} might give an indication of the flux below which the mature biofilm is less likely to form. If that was the case, the process might be more sustainable at these lower fluxes. **Figure 4.12f** plots the $dG_{DP-Norm}/dt$ and $dTMP/dt$ versus imposed flux. The data suggest a sustainable flux for this feed is about 20 LMH and show that the EIS trends ($dG_{DP-Norm}/dt$) mirror the TMP trends ($dTMP/dt$).

Figure 4.12 Normalized G_{DP} as a function of time for biofouling at (a) 8 $L/m^2 h$, (b) 15 $L/m^2 h$, (c) 20 $L/m^2 h$, (d) 30 $L/m^2 h$ (e) 40 $L/m^2 h$ and (f) $dG_{DP-Norm}/dt$ and $dTMP/dt$ vs flux plot for biofouling. Conditions: crossflow velocity = 0.15 m/s, RO feed = 24 mg/L NB with 2000 mg/L NaCl.

4.5.7 Biofilm control using nitric oxide

1. TMP and EIS measurements

Since NO is known to induce dispersal of biofilms (Barnes et al., 2015; Barraud et al., 2006), PROLI NONOate was dosed into the system at different durations of biofouling to investigate the most appropriate day to conduct such a control measures. The TMP profiles of the RO system inoculated with PA01, treated with and without NO at day 2 are shown in **Figure 4.13**. The RO membrane without NO treatment fouled in 5 days with TMP increase of ~50%. The TMP showed a slow initial increase over the first two days of biofouling, followed by an abrupt rise over the next three days. Over the same 5 day period, the TMP for the RO system injected with NO increased by only ~20%, this accounts for a 30% difference for NO-treated and untreated systems.

Figure 4.13 TMP profiles of the RO system inoculated with PA01, untreated versus treated with 40 μM PROLI NONOate at day 2. *Conditions:* permeate flux = 30 $\text{L}/\text{m}^2 \text{ h}$, crossflow velocity = 0.15 m/s , RO feed = 24 mg/L NB with 2000 mg/L NaCl.

In order to evaluate the potential of EIS in monitoring the biofilm dispersal induced by NO, EIS measurements were carried out before and after the introduction of NO into the RO system. **Figure 4.14** shows the normalized G_{DP} plot of RO system with treatment of NO at day 2. There were two turning points observed as compared to just one when NO was not present in the system (**Figure 4.3**). The normalized G_{DP} increased from the start of biofouling and reached a maximum around day 1 before it showed a decreasing trend. Upon introduction of NO into the system, the normalized G_{DP} increased for another 0.5 day and then decreased again for the remaining course of biofouling.

Figure 4.14 Normalized G_{DP} as a function of time for the RO system inoculated with PA01 and treated with 40 μ M PROLI NONOate at day 2. Red arrow shows the day when PROLI NONOate was injected into the system. *Inset*: CLSM images for live/dead staining of biofilm on RO membrane at end of the 5-day experiment. Note that the live cells are shown in green whilst dead cells are in shown red. *Conditions*: permeate flux = 30 L/m² h, crossflow velocity = 0.15 m/s, RO feed = 24 mg/L NB with 2000 mg/L NaCl.

Due to the decreased biofouling rate after PROLI NONOate was introduced into the system, the experiment was repeated but NO was injected at day 3 rather than at day 2.

Figure 4.15 presents the TMP profiles of NO treated RO system inoculated with PA01 and non-NO treated system. Similar to **Figure 4.13**, the TMP profile increased by only ~18% when NO was injected into the system, compared with ~50% without NO treatment.

Figure 4.16 depicts the normalized G_{DP} plot for the RO system with the treatment of NO at day 3. The results are comparable to **Figure 4.14** where there were two turning points observed. The normalized G_{DP} showed an initial increase for ~ 1 day and then decreased. Upon introduction of NO, the normalized G_{DP} increased again, followed by a decreasing trend. The transient behaviour of the G_{DP} profiles during fouling and fouling control is discussed in Section 4.6.

Figure 4.15 TMP profiles of the RO system inoculated with PA01, untreated versus treated with 40 μ M PROLI NONOate at day 3. *Conditions:* permeate flux = 30 L/m² h, crossflow velocity = 0.15 m/s, RO feed = 24 mg/L NB with 2000 mg/L NaCl.

2. Characterizations of biofilm on membrane surface

At the end of every experiment, the concentrations of proteins and polysaccharides which made up the main component of EPS were determined with the chemical extraction method and the results are shown in **Figure 4.17**.

The total EPS was reduced by 61% and 80% respectively for NO treatment at day 2 and 3. For NO treatment at day 2 and 3, the viable bacterial count was decreased by 97% and 94% respectively. As for the live cell biovolume calculated from the confocal images (**Figure 4.17c**), NO treatment at day 2 and 3 demonstrated reduction of 97% and 92% respectively.

Figure 4.16 Normalized G_{DP} as a function of time for the RO system inoculated with PA01 and treated with 40 μ M PROLI NONOate at day 3. Red arrow shows the day when PROLI NONOate was injected into the system. *Inset*: CLSM images for live/dead staining of biofilm on RO membrane at end of the 5-day experiment. Note that the live cells are shown in green whilst dead cells are in shown red. *Conditions*: permeate flux = 30 L/m² h, crossflow velocity = 0.15 m/s, RO feed = 24 mg/L NB with 2000 mg/L NaCl.

Figure 4.17 Characterization of biofilms on RO membranes. (a) Concentration of EPS (proteins and polysaccharides) determined by the extraction method for different durations of biofouling. (b) Viable bacterial count for different durations of biofouling. (c) Average live bacterial cell biovolume calculated using the IMARIS software. Bars represent standard error, $n = 3$.

4.6 Discussion

4.6.1 Mechanisms of biofilm formation

These studies clearly indicate that EIS is able to detect the formation of a mature biofilm on the RO membrane surface and provide insights into its development. The normalized G_{DP} plots (**Figure 4.3** and **Figure 4.4**) with an initial rise followed by a decrease show a maximum point at around 1.5 days which correlates well with the abrupt rise of TMP/TMP_0 (**Figure 4.2**). Such a TMP jump was also observed in other biofouling studies (Herzberg and Elimelech, 2007a; Suwarno et al., 2012).

The normalized G_{DP} trends in **Figure 4.3** and **Figure 4.4** can be explained as follows. The accumulation of live bacteria on the membrane surface led to an initial increase of the normalized G_{DP} as the bacterial cells and their respiration products are very conductive (Krishnamurti and Kate, 1951; Lu et al., 2008). The normalized G_{DP} is also enhanced by the accumulation of material by concentration polarization. This period also coincides with the induction phase of biofilm development where bacteria start to attach on the membrane surface (Flemming, 1997) before forming micro-colonies (Matin et al., 2011). This is consistent with the confocal images taken for a 1 day biofouling experiment where the biofilm had not yet formed on the membrane surface (Inset **Figure 4.5**). With the longer biofouling experiments, upon reaching a maximum point, the normalized G_{DP} started to drop due to the substantial formation of EPS. Once bacteria attached on the membrane surface, the cells began to grow and multiply. During this stage, EPS was produced continuously in order to provide the biofilm with greater structural integrity (Matin et al., 2011) and this was evident in the confocal image for 5-day biofouling (Inset **Figure 4.3**). The accumulation of a tighter EPS matrix between cells reduced the electrically conductivity in the fouling layer, despite concentration polarization effects, resulting in a lower normalized G_{DP} after ~ 1.5 days. The increase of the EPS content (proteins and

polysaccharides) with the longer biofouling duration was confirmed by the extraction method and viable bacterial cell counts. However, the slight increase of the dead cell biovolume from 3 day to 5 day (**Figure 4.10b**) suggests that the deposition of EPS within the matrix has a greater impact on the EIS than the accumulation of dead cells itself.

To validate the proposed mechanisms deduced from the EIS signal response, several control studies were conducted, namely (1) nutrient fouling, (2) dead bacteria fouling and (3) mixtures of alginate and dead bacteria fouling.

As nutrient broth (NB) was also added into the system, it was relevant to investigate its effect on the EIS signal response without the presence of bacteria. The normalized G_{DP} (**Figure 4.6**) was found to increase throughout the course of filtration, due to the CP effect as NB accumulated on the membrane surface. The increasing normalized G_{DP} could also be due to modest levels of enhanced salt polarization. The slow background fouling of a membrane by NB was also observed by Suwarno et al. (Suwarno et al., 2012) in the absence of bacteria. The CP effect (**Figure 4.7**) was confirmed by the salt pulse tracer test and it clearly indicated the presence of the CP effect during the nutrient fouling. The data in **Figure 4.7** shows a CP rise of about 7% per day over the 5 day period.

The bacteria stock solution heated in a 80°C water bath was used in the study to elucidate its effect on the EIS signal. When dead bacteria were injected, no maximum point was observed in the normalized G_{DP} plot (**Figure 4.8**). With live bacteria, the NB, background salinity and the cell content in the bacteria cells could all contribute to the early conductance increases as explained above, hence, causing the normalized G_{DP} to increase.

A surrogate for EPS, alginate (Wloka et al., 2004), mixed with dead bacteria was also used in the study to further validate whether the decreasing trend in the normalized G_{DP} profiles could be attributed to the accumulation of EPS in the matrix. The changes of

$dG_{DP-Norm}/dt$ for dead bacteria and its mixtures with alginate are summarized in **Table 4.2**. The decrease in normalized G_{DP} with an alginate concentration equivalent to the TOC for a 5-day biofouling experiment (**Figure 4.9a**) supports the view that the effect of EPS build-up outweighed the accumulation of dead cells. However, when a lower concentration of alginate (TOC equivalent to 1-day old biofilm) was used with dead bacteria, the accumulation of the bacteria cells on the membrane surface caused a slight increase of normalized G_{DP} (**Figure 4.9b**).

Table 4.2 Comparison of $dG_{DP-Norm}/dt$ for fouling with dead bacteria and mixture of alginate with dead bacteria.

Conditions	$dG_{DP-Norm}/dt$ (1/day)
Dead bacteria	0.5635
16 ppm alginate & dead bacteria	0.0215
100 ppm alginate & dead bacteria	-0.2528

4.6.2 Effect of sodium azide on biofilm growth

Sodium azide is known to inhibit the catalytic activity and growth of bacteria (Kay and Gronlund, 1969; Lichstein, 1944). In the present study, PA01 biofilms were allowed to grow and sodium azide was dosed into the system at day 1.5. Its effect could be observed clearly from the normalized TMP and G_{DP} plot (**Figure 4.11a** and **b**). The normalized TMP increased slightly and there was no TMP jump observed (**Figure 4.11a**). Dosing of sodium azide into the system was believed to 1) inhibit the growth of *P. aeruginosa*, leading to reduced production of respiration products and 2) cause the detachment of some bacteria from the membrane surface, resulting in lower normalized G_{DP} after injection (**Figure 4.11b**). When the system was allowed to continue (after sodium azide dosing) even without the supply of new bacteria, the normalized G_{DP} followed the trend of a 5 day biofouling

experiment. This implies that the residual cells subsequently attached onto the membrane surface continued to proliferate and grow, eventually forming a matrix of biofilm material.

When a lower concentration of sodium azide was used (**Figure 4.11b**), the normalized G_{DP} remained almost unchanged immediately after the sodium azide dosing. Thereafter, the normalized G_{DP} increased again due to the accumulation of the new bacterial cell supply. However, for the second dosing with a higher concentration of sodium azide, the normalized G_{DP} dropped to a lower value, similar to the case in **Figure 4.11a**. When fresh bacteria solution was reintroduced into the system the normalized G_{DP} profile showed a similar trend to the 5 day biofouling without NaN_3 dosing. Thus, a maximum point in normalized G_{DP} could be observed after ~1 day of biofouling after which the normalized G_{DP} decreases due to the accumulation of EPS. This result demonstrates that EIS could potentially be employed to assess cleaning efficiency or the effectiveness of control strategies for biofouling in water treatment plants.

4.6.3 Sustainable flux derived from EIS parameters

Since the initial increase of the normalized G_{DP} was hypothesized to result from the deposition of bacterial cells and their respiration products, it was worthwhile to investigate the rate of the initial increase with respect to flux (**Figure 4.12**). The slope of the normalized G_{DP} plot increased with flux (as did the TMP rise), implying that the rate of biofilm growth also varies with flux; this was established in our earlier studies (Suwarno et al., 2012). This allows for an estimation of sustainable flux, referred to as “a flux in which fouling is minimized to avoid frequent cleaning” (Field and Pearce, 2011), using EIS measurement. This could provide opportunities to tune operations to provide more economically sustainable performance with minimum fouling. This type of monitoring tool for assessing the sustainable flux could be useful in the wastewater industry for plant process optimization at the start-up stage.

4.6.4 Monitoring of biofilm dispersal using EIS

There is an increasing need for improvements in water production for developed and developing nations around the world and attention has been focused on membrane purification methods. Despite the widespread use of membrane filtration method, biofouling and biofilm formation remain a significant issue for this technology. Hence, there is an urgent need for the development of novel approaches for biofouling control. Several recent discoveries have shown that bacterial cells produce and respond to NO which can also act as a natural dispersal signal (Barraud et al., 2006; Barraud et al., 2009a; Barraud et al., 2009b) and thereby help in controlling biofouling in RO processes (Barnes et al., 2014; Barnes et al., 2015). In this chapter, the potential of EIS to monitoring the dispersal of RO biofilms by PROLI NONOate was investigated.

It has been demonstrated in this chapter that EIS is capable of indicating when the biofilm was dispersed by NO. This is shown in the normalized G_{DP} plot (**Figure 4.14** and **Figure 4.16**) where the normalized G_{DP} demonstrates an increasing trend after NO treatment. This suggests that part of the biofilm formed on the membrane surface had been dispersed after NO treatment, resulting in a new biofouling cycle with the re-attachment of bacterial cells on the membrane surface and the production of respiration products which could lead to the increase of normalized G_{DP} . The dispersal of biofilm was confirmed when a significant amount of bacterial were obtained in a sample collected from the retentate during the NO treatment. The plate count for retentate collected during NO treatment (without bacteria injection) was only ~ 10 order of magnitude lower than that before and after NO treatment (with bacteria injection). After 0.5 – 1 day after the injection of NO, the effect of EPS started to outweigh that of bacterial attachment and respiration products (as described in Section 4.6.1), leading to the decrease of normalized G_{DP} (**Figure 4.14** and **Figure 4.16**).

Studies have shown that the accumulation of bacterial cells on the membrane surface could reduce salt rejection and permeate flux by the biofilm enhanced osmotic pressure (BEOP) effect and the EPS layer could impact permeate flux adversely by increasing the hydraulic resistance to permeate flow (Herzberg and Elimelech, 2007a; Herzberg et al., 2009). The study conducted in this chapter also demonstrated a reduction in EPS constituents (proteins and polysaccharides) and a decrease in cell count as well as biovolume (for NO treatment at day 2 and 3) which led to significantly lower biofouling rate as shown in the slower TMP rise from the plots (**Figure 4.13** and **Figure 4.15**). The confocal images also showed lesser coverage and more hollow voids (Barraud et al., 2006) when there was NO treatment (insets of **Figure 4.14** and **Figure 4.16**) as compared to the one without NO treatment (inset of **Figure 4.14**). These results suggest that biologically-derived strategies, such as by NO dispersal, might represent another approach for the control of biofouling and together with EIS measurements, the implementation could be monitored.

4.7 Concluding remarks

This study demonstrates that biofouling can be detected and its development mechanisms elucidated using the non-invasive and real time monitoring tool of EIS. There were two distinct stages in the time profile of the normalized G_{DP} where the first stage, an initial increase, can be related to the accumulation of live bacteria on the membrane surface and the production of the respiration products. The second stage, with declining normalized G_{DP} , corresponded to the formation of EPS and a biofilm matrix.

The introduction of a biostat (sodium azide) during cleaning operations would slow down the growth of bacteria and would ultimately lead to a detachment of the bacteria from the membrane. The EIS changes observed, mirrored these effects and might be of assistance

to the plant operators as it establishes the ability of EIS to assess the cleaning efficiency and effectiveness of control measures for biofouling.

The initial increase of the normalized G_{DP} plot that occurs during biofilm development could also provide indications of the sustainable flux at which filtration could be carried out in a more economically efficient way with minimal fouling.

This chapter also demonstrates the potential of EIS to be used as biofouling control monitoring tool. The normalized G_{DP} shows two turning points when the NO treatment was present. This indicates that the dispersal event could be observed using EIS where the re-attachment of bacterial cells led to the second maximum point of normalized G_{DP} . Following the re-attachment, EPS was produced and the normalized G_{DP} showed a decreasing trend due to its non-conductive nature and its effect outweighed that of the bacterial cells. The EPS concentration and the cell counts on the membrane coupons were reduced following the NO treatment. The TMP results together with the EIS signals suggest that NO was useful in delaying biofouling and its application could be monitored using EIS. The combined exposure of NO and antimicrobial agents was shown to be effective in the removal of an established biofilm (Barraud et al., 2006), and this novel strategy may offer an alternative for the control of biofouling in various water treatment plants.

EIS in combination with TMP and CLSM autopsy provides considerable insights into the mechanisms of biofilm formation on the membrane surface while TMP or CLSM measurements alone give no direct information on this process. EIS is therefore a potential tool to be incorporated *in-situ* into a side stream ‘canary cell’ in high pressure membrane systems to assess their biofouling conditions or the cleaning efficiency in real time, non-invasively and online.

5 Chapter 5 - Monitoring of Fouling Behavior of Reverse Osmosis Membranes using Electrical Impedance Spectroscopy – a Field Trial Study^{†3}

5.1 Introduction

Most of RO water treatment plants rely on three parameters, namely the delta P along the channel, TMP and salt rejection to determine whether membranes in the modules have been fouled. However, these parameters represent the averaged values over the whole system and therefore provide little information on incipient fouling, that can be initially a localized phenomenon. As a result, plant operators can only rely on arbitrary values of these parameters to initiate a chemical clean-in-place (CIP) cycle. As a rule of thumb, CIP is conducted when a 15% increase in delta P or TMP or 15% drop in salt rejection is observed. In most cases, delta P is more sensitive than TMP in signaling fouling due to channeling (Vrouwenvelder et al., 2009) where debris, foulants and scale could form within the flow channels (feed spacers) of the element. High delta P could bring problems to operations as this indicates that the flux profile is affected in such a way that the lead elements are operated at an excessively higher flux but the tail elements run at a very low flux. However, when such observations are made, the membrane may already be significantly fouled. This may lead to more frequent or more difficult chemical cleaning events to restore the membrane performance especially during the later part of the lifespan of the membrane. Therefore, there is a pressing need to have sensitive tools to monitor the membrane fouling

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³ [†]Findings in this chapter have been published in: Ho, J.S., Sim, L.N., Webster, R.D., Viswanath, B., Coster, H.G.L., Fane, A.G., 2017. Monitoring fouling behaviour of reverse osmosis membranes using electrical impedance spectroscopy: A field trial study. Desalination 407, 75-84.

process so that specific countermeasures can be taken before significant fouling is observed. Countermeasures such as physical cleaning could be carried out instead of CIP to control fouling so as to reduce the number of CIP events and to minimize costs incurred in chemical usage.

One way to provide sensitive observation of the spiral wound RO elements in the plant is to install appropriate sensors on a side-stream cell (Sim et al., 2015). The role of this side-stream cell (also known as a “canary cell”) is to mimic the fouling conditions in the RO elements, where the sensors integrated into the cell provide information on the incipient fouling. Sim et al. (2015) have successfully shown the capability of the canary cell to simulate the spiral wound module under controlled hydrodynamic and flux conditions, provided that the canary cell consists of the same material in terms of membrane and spacers, has the same spatial dimensions such as channel height and most importantly has similar hydrodynamics conditions (Sim et al., 2015).

In this chapter, an electrical impedance spectroscopy fouling monitor (EISFM) was integrated into a canary cell to monitor the fouling behavior of a bank or train of RO modules in a water reclamation plant in Singapore. The main objective of this chapter is to identify which EIS signal parameter best correlated with reported plant performance data and CIP events. Most importantly, the ability of the EIS signal to provide early indications of performance changes in the modules caused by incipient fouling was evaluated. As such, this study focused only on monitoring the changes of EIS parameter over time as there was no provision to exercise control over when a CIP was to be performed. At the end of the study, based on tracking the operation at the plant, we proposed several criteria that could be used to initiate fouling mitigation measures based on EIS parameter. Furthermore, a membrane autopsy was also performed to confirm fouling mechanisms suggested by the EISFM.

5.2 Materials and methods

5.2.1 Installation of EISFM in NEWater plant

An EISFM was installed at the Bedok NEWater factory in Singapore to monitor the performance of one of the RO trains in the plant. The feed to the RO is secondary treated municipal wastewater that has been pretreated by MF with a nominal pore size of 0.1 μm . The RO trains consist of two stages, operating at an overall recovery of 75%. In this study, the EISFM canary cell was located on side stream of stage 1 of the RO system. The layout of the RO process and the side stream EISFM canary cell is shown in **Figure 5.1**

Figure 5.1 Layout of the RO process in Bedok NEWater Factor and the location of the EISFM canary cell. Note that the schematic diagram is not drawn to scale.

As the EISFM canary cell was installed on a side stream of RO stage 1, it was subjected to a similar pressure as the lead part of RO stage 1. In order to mimic the fouling conditions in the RO spiral wound module element, the canary cell had similar membranes and spacers, the same spatial dimensions in terms of channel height and product spacer channels, and the same hydrodynamics and flux (Sim et al., 2015). The flow to the canary cell was regulated by a flow control valve and was maintained at a similar Reynold number as RO stage 1 (based on the average flux of the RO train) and at the same production flux

using mass flow controller (Brooks Instrument, Model 5882), located in the permeate stream. The membrane used in this study was a RO flat sheet membrane (Hydranautics Nitto Group Company, ESPA 2). Furthermore, in the setup as shown in **Figure 5.1**, the canary cell would undergo the same cleaning regime as the main RO train.

The operational permeate flux of the canary cell was set at $17.6 \text{ L/m}^2 \text{ h}$ and its crossflow velocity was maintained at 0.18 m/s , equivalent to $Re = 184$ (the method to obtain Reynolds number of a spacer-filled channel is described by Schock and Miquel (Schock and Miquel, 1987)). Pressure and flux readings were recorded with a data acquisition system (National Instrument, Model PCI 6014 and Labview software). It should be noted that the membrane coupon in the EISFM cell was new but the RO trains in the NEWater plant had been operated for several years. Therefore, it was assumed that the values of parameters deduced from the impedance measurement provide relative trends rather than absolute values directly equivalent to those for the membranes in the plant.

5.2.2 Description of the EISFM cells

The EISFM cells were similar to the EIS-RO crossflow cell described in Section 3.2.1 but with a smaller dimension of $210 \text{ mm} \times 6 \text{ mm} \times 0.73 \text{ mm}$, with an effective membrane area of 0.0126 m^2 .

5.2.3 EISFM signal analysis

The theory underlying the use of EIS for signal analysis has been discussed in Section 2.6.1. **Figure 5.2** shows a typical Nyquist plot obtained for the RO filtration system in this study. Each data point in the plot corresponds to a different frequency from a low frequency (right side of the plot) to high frequency (left side of the plot).

Figure 5.2 Nyquist representation of signals from of an EISFM canary cell installed at the side stream of a RO train. Operating conditions: Feed = RO stage 1 feed; Permeate flux= 17.6 L/m² h; Crossflow velocity = 0.18 m/s. The full curves are theoretical fits using a Maxwell-Wagner model representing the various layers detected. The frequencies indicated are the characteristic frequencies of each of the impedance elements (in series) identified by fitting the EIS measurements.

Typically, the conductivity of the RO feed fluctuated as much as 100 $\mu\text{S}/\text{cm}$ throughout the period of study. As the fluctuation of conductivity can contribute to changes to the measured frequency dependent conductance of the membrane, it was therefore necessary to normalize the measured conductance (G_{measured}) against the conductance of the feed (G_{feed}), as follows:

$$G_{\text{normalised}} = \frac{G_{\text{measured}}}{G_{\text{feed}}} \quad (5.1)$$

where

$$G_{\text{feed}} = \frac{\sigma_{\text{feed}}}{\delta_{\text{channel}}} \quad (5.2)$$

and σ_{feed} refers to the conductivity of the feed and δ_{channel} is channel height (=0.73mm).

Impedance, Z is the inverse of conductance, G , therefore,

$$Z_{\text{normalised}} = \frac{1}{G_{\text{normalised}}}, \quad Z_{\text{measured}} = \frac{1}{G_{\text{measured}}} \quad (5.3)$$

Substituting Equation 5.2 into Equation 5.4 gives

$$Z_{\text{normalised}} = \frac{G_{\text{feed}}}{G_{\text{measured}}} \quad (5.4)$$

Substituting Equation 5.3 into Equation 5.5 gives,

$$Z_{\text{normalised}} = Z_{\text{measured}} \frac{\sigma_{\text{feed}}}{\delta_{\text{channel}}} \quad (5.5)$$

5.2.4 Membrane autopsy

After 8 months of operation, the RO membrane was removed from the canary cell for autopsy. This allowed further identification of potential foulants in the plant. The techniques used for surface characterization of the fouled membrane included visual observation, field-emission scanning electron microscopy with energy dispersive X-ray (FESEM-EDX), total organic carbon (TOC) analyzer, Liquid chromatography-organic carbon detection (LC-OCD) and Fluorescence Excitation-Emission Matrix (EEM). Analysis of extracellular polymeric substances (EPS) was also carried out (see below).

The membrane was cut into four segments. Two segments of the membrane with area of 4×4 cm were cut about 5 cm from the inlet and the outlet of the fouled membrane canary coupon. They were then soaked in 50 mL sterilized NaCl solution (300 ppm; same salinity as plant) and sonicated at the sonicator (Fisher Scientific, model FB15068) for 5 minutes and subsequently vortexed (Velp Scientifica, model RX3) for 10 seconds to detach the foulants from the membrane surface. Fluorescence EEM, TOC, EPS contents were then performed using the dissolved foulants. Another membrane segment of 2×1 cm was cut from the center of the fouled membrane coupon for FESEM-EDX analysis.

1. EPS extraction

The EPS extraction was carried out according to the protocol described in Section 4.4.3.

5.2.5 Analytical methods

1. FESEM-EDX

A field-emission scanning electron microscopy (FESEM, JSM-7600F, JEOL, Japan) was used to characterize the fouled membrane. The fouled membrane was dried in an oven at 50 °C before SEM analysis for about 24 hour. The sample was then sputter coated with a thin layer of platinum using JFC-1600 auto fine coater.

2. TOC

Organic fouling intensity on the membrane surface was evaluated by measuring the TOC using a TOC analyzer (Shimadzu Corporation). The solutions were filtered through a 0.45 µm syringe filter before the measurement was carried out.

3. LC-OCD analysis

Organic matter in the RO feed and dissolved foulant from the membrane coupon were characterized by size exclusion chromatography (SEC). The SEC system (DOC-LABOR Dr. Huber, Germany) is comprised of an LC-OCD-UVD-OND (liquid chromatography-organic carbon detector-UV detector-organic nitrogen detector). It is equipped with a size exclusion column HW-50S (Toyopearl, Tosoh Bioscience, USA) of particle size 30 µm, length 250 mm and internal diameter 20 mm for the separation of organic molecules according to their molecular weight. The mobile phase employed was phosphate buffer at pH 6.8 (2.5 g/L KH_2PO_4 and 1.5 g/L $\text{Na}_2(\text{HPO})_4 \cdot 2\text{H}_2\text{O}$) at a flow rate of 1.1 mL/min. An injection volume of 1000 µL was utilized for measurement. This method facilitates the

distribution of DOC into five fractions, namely the biopolymers (BP), humic substances (HS), building blocks (BB), low molecular weight (LMW) neutrals and LMW acids.

4. Fluorescence EEM

Fluorescence EEM measurements were conducted using a PerkinElmer LS-50B luminescence spectrometer. The maximum emission intensity of the spectrometer is 1000 arbitrary units (AU). The spectrometer is equipped with a xenon excitation source. The excitation and emission slits were set to a 10 nm band-pass. In order to obtain fluorescence EEMs, the excitation wavelengths were increased from 200 to 500 nm in 10 nm band-pass. In order to obtain fluorescence EEMs, the excitation wavelengths were increased from 200 to 500 nm in 10 nm steps while the emission was detected at longer wavelengths from 230 to 550 nm in 10 nm steps. The scanning speed was maintained at 500 nm/min.

5.2.6 Determination of threshold flux using lab scale RO system

In order to assess the fouling propensity of the feed under the imposed flux and hydrodynamic conditions of the plant, the ‘threshold flux’ of the feed water collected from the NEWater plant was estimated in the EISFM using a combination of EIS and TMP methods which have been developed and discussed in Chapter 3. Briefly, the membrane was compacted at 50 L/m² h for 24 hours with salt solution (similar conductivity to the plant water) prior to the feed being replaced with RO feed water collected from the NEWater plant. The system was adjusted to the lowest flux (10 L/m² h) and the fouling signals (EIS and TMP) collected for at least 20 hours. The flux was then increased incrementally after a fixed duration and the sequence continued until the highest flux (35 L/m² h) was reached. EIS measurements were carried out for a fixed time interval.

5.3 Results and discussion

5.3.1 Feed water characteristics

Feed water analysis is crucial for understanding the fouling phenomenon occurring on the membrane surfaces. The elemental analysis was carried out by inductively coupled plasma-optical emission spectroscopy (ICP-OES) while the anion analysis was conducted using the ion chromatography (IC). **Table 5.1** shows the properties of the RO feed averaged through the study period.

5.3.2 Hydraulic performance of RO train

The performance of the RO train was provided in terms of three parameters: (1) Pressure drop along the train (ΔP); (2) hydraulic resistance (TMP/permeate flowrate for first stage) and (3) salt rejection. **Figure 5.3** shows the hydraulic performances of the stage 1 of the RO train. It should be noted that the shaded bars indicate the train was not in operation and the red bar indicates when the clean-in-place (CIPs) were carried out.

Table 5.1 RO stage 1 feed water analysis

Parameter	Average values
pH	6.1 ± 0.2
Turbidity (NTU)	0.3 ± 0.2
Conductivity (mS/cm)	0.6 ± 0.1
TOC (mg/L)	8.3 ± 1.3
Fe (mg/L)	0.1 ± 0.01
Ca (mg/L)	26.2 ± 0.3
Si (mg/L)	5.3 ± 0.1

Parameter	Average values (cont.)
F ⁻ (mg/L)	1.4±0.1
NO ₃ ⁻ (mg/L)	12.1±0.2
SO ₄ ²⁻ (mg/L)	76.2±2.8
PO ₄ ³⁻ (mg/L)	9.6±0.1

a

b

c

Figure 5.3 Performance of stage 1 RO train in a NEWater plant (a) average hydraulic resistance, (b) average deltaP (pressure drop) and (c) average rejection across stage 1.

As can be seen from **Figure 5.3**, the performance of the stage 1 was stable for the first three months of operation (up to day 100). No obvious increase in deltaP (~2%) or hydraulic resistance (~4%) was observed throughout this period. Also rejection remained stable throughout the first three months of operation. A routine clean in place (CIP) was performed after 3 months of operation. After the CIP, from day 100 to day 150, the hydraulic resistance remained relatively constant, however, about a 6% increase in ΔP was observed, which initiated another cycle of CIP. In between day 160 to day 220, about 10% and 6% increase in deltaP and hydraulic resistance were observed, but rejection remained relatively rather constant. On day 244, a CIP was performed because of the obvious drop in the rejection and increase in deltaP. The increase in deltaP and hydraulic resistance throughout the monitoring period are summarized in **Table 5.2**.

5.3.3 Correlating plant performance with EISFM responses

1. Hydraulic resistance of EISFM canary cell

The hydraulic resistance of the EISFM canary cell was also monitored throughout the study and is shown in **Figure 5.4**. The hydraulic resistance increased slightly from day 60 to day 80, before the first cycle of CIP was carried out. However, the hydraulic resistance remained almost unchanged before the second and third cycles of CIP. There was again a slight increase in the hydraulic resistance prior to the fourth cycle of CIP. These data indicate that the hydraulic resistance alone provides little information on the incipient fouling conditions of the membrane in the canary cell.

Figure 5.4 Hydraulic resistance of EISFM canary cell.

2. EIS analysis and trend

Figure 5.5 shows the trend of normalized Z_{real} of the DP layer (normalized $Z_{\text{real-DP}}$) over time. When fitted with polynomial models, maxima in the normalized $Z_{\text{real-DP}}$ were observed well before the CIPs were carried out. The initial increase in the $Z_{\text{real-DP}}$ could be due to the accumulation of less conductive material close to or on the membrane surface, leading to the decrease of conductivity at the membrane-solution interface. When the foulant layer became consolidated sufficiently to hinder the back diffusion of salt from the membrane surface, the conductance increased and impedance decreased. Similar phenomenon were discussed in Section 3.4.2 and observed in a previous laboratory study by our group (Sim et al., 2013a) where colloidal silica was used as a model foulant and a transition was observed from accumulating colloid in the boundary layer (impedance increase) to cake effects (impedance decrease). On the basis of this, it is hypothesized that colloidal/particulate deposition could be a major form of fouling in this RO plant. To test this, membrane autopsy studies of the canary cell were performed after 260 days of operation (Section 5.3.4).

Figure 5.5 The normalized $Z_{\text{real-DP}}$ over time

Based on the observed trends, the occurrence of $Z_{\text{real-DP}}$ (norm) peaks could have been used to initiate mitigation measures to control fouling other than performing a CIP. For instance, a temporary increase in crossflow velocity of the system, short term flushing or by reduction in recovery of the system. Several studies have shown that an increase in crossflow velocity tends to decrease the load of particles deposited on the membrane surface (Chong et al., 2008a; Ho et al., 2016a; Suwarno et al., 2012; Tang et al., 2011). Through the creation of eddies or turbulence, the accumulated layer of foulant may be disturbed and the concentration polarization (CP) of solute on the membrane surface would also be reduced. In addition, physical cleaning methods could be applied to remove the reversible fouling on the membrane surface and these methods include forward and reverse flushing (Ebrahim, 1994), backwashing (Sagiv and Semiat, 2005; Sagiv et al., 2008) and air flushing (Ducom et al., 2002; Zamani et al., 2015). Forward flushing is a commonly applied method in current RO systems. This is carried out by pumping permeate water at high cross-flow velocity through the feed side in order to dislodge foulants from the membrane surface. Due to the use of cleaner feed as well as more rapid flow, the reversible foulant layer absorbed on the membrane surface can be partially released as a result of the shear forces applied. It should be noted that in one of the studies carried out by our group have previously shown that EIS can also monitor cleaning events and characterize the efficiency of cleaning of membranes (Sim et al., 2016).

Table 5.2 Correlation of EIS data with the observed plant performance for four CIP cycles.

Period	Day when CIP was performed	Delta P channel	Averaged Hydraulic resistance	EIS Peak detected	Recommended “day” to initiate fouling control based on criteria 1	Recommended “day” to initiate fouling control based on criteria 2
1st CIP cycle (0-100 days)	98*	~2% increase	~4% increase	33	50	43
2nd CIP cycle (100-150 days)	148	~6% increase	Relatively constant	130	137	140
3rd CIP cycle (160-220 days)	216	~10% increase	~6% increase	180	190	190
4th CIP cycle (221-259 days)	244	~10% increase	~4% increase†	224	230	234

*Train no operation from day 52 to 65.

† Comparison between day 232 and 240

Criteria 1: The minima after a peak

Criteria 2: 10 days after a peak

Based on the EIS trend, there are several alternative criteria that could be used to initiate fouling control measures: (i) when the $Z_{\text{real-DP}}$ (norm) reaches a maximum followed by a clear downward trend, or (ii) 10 days after the peak (this would allow 5 to 7 days to monitor the slow decline in $Z_{\text{real-DP}}$ (norm) after a peak). Either of these criteria could occur, and in this study, were earlier than the plant-scheduled CIP dates. Initiation of fouling control measures could thereby avoid potential irreversible fouling resulting from delayed action. **Table 5.2** shows the correlation of EIS data with the observed plant performance for four cycles of CIP and the EIS recommended “day” to initiate fouling control strategies.

The advantages of performing fouling control strategies based on EIS trends include effective removal of incipient deposition on the membrane surface and the potential to delay CIP events. As such, the frequency of CIP and overall operating cost could be reduced.

5.3.4 Membrane autopsy studies

1. Visual inspection

When the membrane coupon was taken out from the crossflow chamber, brownish debris was found scattered over the membrane. **Figure 5.6** shows the digital camera and scanning electron microscopy (SEM) images of the fouled membrane. At low resolution, no thick foulant could be observed on the membrane surface. However, from the SEM images, a thick foulant cake layer can be seen on some of the membrane surface (**Figure 5.6b**), but in other locations for example **Figure 5.6c**, the foulant layer was rather scattered. **Figure 5.6c** shows that particles attached on the membrane surface were around 1 μm . The use of UF membrane as pre-treatment for RO membranes should have prevented these large particles from reaching the membranes. However, nano-sized particles or colloids could still have remained in the feed after the pretreatment stages and subsequently coagulated and formed part of the foulant layer. In order to identify the composition of the foulant layer on the membrane surface, different types of foulant analysis were performed using the various methodologies described in the following sections.

Figure 5.6 Visual inspection of the fouled membrane by (a) digital image, (b) and (c) SEM images obtained from different locations from the fouled membrane at $\times 5,000$ magnification.

2. Foulant analysis

i) SEM/EDX analysis

SEM/EDX study was performed to identify the dominant compositions of foulant on the membrane surface (**Figure 5.7**).

Figure 5.7 SEM-EDX mapping of foulant from different locations of the membrane surface.

Table 5.3 SEM-EDX analysis of the foulant deposited on the membrane surface (relative weight % excluding carbon, oxygen and sulfur)

Element	Relative weight %
Phosphorous	32
Calcium	30
Fluoride	13
Aluminium	13
Chloride	7
Iron	5

The SEM-EDX mapping data indicates the dominant presence of calcium and phosphorous (**Table 5.3**) in the foulant and other elements such as F and Al were also found

in the foulant analysis. The results suggest that the major type of foulant could be calcium phosphate. The results coincide with the findings by Bartels et al. (Bartels et al., 2005b) who identified calcium phosphate as the key foulant that limits the RO system recovery rate based on their experience in the Bedok demonstration plant operation. Typically, calcium phosphate has been identified as a common type of inorganic fouling problem encountered on RO membranes (Antony et al., 2011; Bartels et al., 2005b; Greenberg et al., 2005; Qin et al., 2009; Raffin et al., 2012). However, from the SEM images (**Figure 5.6**), the morphology of the foulant layer is more like that of a cake-like structure rather than a crystallized structure. The latter would be expected if the calcium phosphate arrives at the surface dissolved in the feed and deposits out to form a growing crystalline phase. The more open cake like structure suggests that calcium phosphate already in particle form when it arrives at the RO surface (Bartels et al., 2005b; Ozcan et al., 2011). This is consistent with the findings of Ning and Troyer (Ning and Troyer, 2007) who demonstrated that nano-colloids of calcium phosphate could pass through MF/UF pretreatment, leading to RO fouling in a wastewater reclamation plant. Our observations from the EIS data support this colloidal fouling mechanism where a less conductive material (in this case colloidal calcium phosphate) close to and on the membrane surface, causes an increase in impedance over time. The impedance would then start to decrease as the foulant layer becomes thick enough to hinder the back diffusion of salt causing a cake enhanced concentration polarization effect (Chong et al., 2007; Sim et al., 2013a).

It is important to understand the nature or the state of the calcium phosphate before it arrives at the RO system. If the calcium phosphate is in its colloidal form, the deposits are more likely to form a porous cake on the membrane surface, instead of scale crystallization. In this case, antiscalant might not be as effective at their typical dosage levels and anticoagulant or dispersants might be more suitable (Antony et al., 2011).

ii) Organic analyses

The determination of the total organic carbon (TOC) is a measure of organic matter present in the feed water or on the fouled membrane. From the TOC analysis of the deposit, the amount of organic content attached on membrane surface of $3.67 \mu\text{g}/\text{cm}^2$ was insignificant considering the average TOC of the RO intake was around $8.3 \text{ mg}/\text{L}$ ($<1\%$ of organic deposition over the 10 days filtration after the last cycle of chemical cleaning). Fluorescence EEM analysis provides additional characterization of the dissolved organic carbon (DOC) in the water or on the membrane samples. The EEM spectra for the RO feed water collected from stage 1 and the membrane samples are shown in **Figure 5.8**. The spectra can be divided into five regions using consistent boundaries for excitation and emission wavelengths while the peaks are detailed below as peaks 1 – 5.

- 1) 230/303 nm – protein-like fluorescence, such as tyrosine
- 2) 230/355 nm – protein-like fluorescence, such as tryptophan
- 3) 230/407 nm – fulvic acid-like fluorescence
- 4) 310/353 nm – soluble microbial byproduct-like fluorescence
- 5) 330/410 nm – humic acid-like fluorescence

Figure 5.8 EEM spectra of a) stage 1 RO feed and b) extraction from fouled membrane sample.

As shown in **Figure 5.8a**, there was a relatively high content of humic and fulvic acids and protein (Peaks 2, 3 and 5) in the RO feed water. Characterization of the extraction from the fouled membrane samples is shown in **Figure 5.8b**. Only modest amounts of a

protein-like material could be observed. This further confirms that there was minor deposition of organic matter on the membrane surface.

Figure 5.9 LC-OCD chromatograms of foulants extracted from fouled membrane surface.

The DOC value of the wash solution from the fouled membrane was 1056 ppb, equivalent to a loading of $3.3 \mu\text{g}/\text{cm}^2$. This value is similar to that obtained from TOC analysis. LC-OCD (**Figure 5.9**) shows a biopolymer peak and from the analysis it indicates that it mainly consists of protein. This is supported by the fluorescence EEM analysis which shows the presence of a protein-like material. Another interesting piece of information from the chromatogram is that there is a significant peak coinciding with the presence of inorganic colloids as compared to the organic carbon peaks (Huber, 2008).

The extent of biofouling on the membrane was evaluated by the presence of EPS, which consists mainly of proteins and polysaccharides. However, the fouled membrane extracted from the side stream canary cell contained negligible amounts of proteins and polysaccharides as shown in **Table 5.4**. No evidence of biofouling was observed in this RO train probably because most of the microorganisms had been removed by the MF/UF

pretreatments and the biocide, monochloroamine that was dosed in-line upstream of the MF/UF pre-filter to control biofouling. Our autopsy results confirm that biofouling is not a major foulant type in this RO process as evidenced by the small amount of biopolymers from LC-OCD and the negligible amount of EPS from the extraction method.

Table 5.4 Polysaccharide, protein and TOC concentrations of fouled membrane.

Polysaccharide ($\mu\text{g}/\text{cm}^2$)	Protein ($\mu\text{g}/\text{cm}^2$)	TOC ($\mu\text{g}/\text{cm}^2$)
3.67	3.42	3.67

5.3.5 Determination of threshold flux using EIS

Since EIS has been successfully used to determine the threshold flux in our previous laboratory study (Ho et al., 2016a) as described in Chapter 3, it was considered useful to evaluate the threshold flux of RO stage 1 operation using EIS and TMP characterization. The idea of a threshold flux evolved from that of a ‘critical flux’, which was first described by Field et al. (Field et al., 1995) for microporous membranes. The notion of threshold flux is more applicable for plant operations and it refers to the transition from low or negligible fouling to significant fouling (Bacchin et al., 2006; Field and Pearce, 2011; Ho et al., 2016a) and is associated with a transition of a colloidal foulant layer to a more cake like structure (Ho et al., 2016a).

The dTMP/dt vs permeate flux plot for the flux step experiment using the NEWater feed is shown in **Figure 5.10a**. The threshold flux was estimated to be $\geq 18.5 \text{ L}/\text{m}^2 \text{ h}$ from the intersection of the dTMP/dt plot. The TMP remained almost constant for fluxes of 10 and $15 \text{ L}/\text{m}^2 \text{ h}$ throughout the course of filtration, whereas, there was a drastic change of slope from $20 \text{ L}/\text{m}^2 \text{ h}$ and above.

The conductance of the diffusion polarization layer (G_{DP}) can provide information on the properties of the fouling layer near the membrane surface (Ho et al., 2016a) and a detailed explanations of G_{DP} trends were described in Sections 2.6.1 and 4.2 as well as from the literatures (Ho et al., 2016a; Sim et al., 2016; Sim et al., 2013a). G_{DP} is the inverse of the $Z_{real-DP}$ and its variation with flux provide another method for the determination of the threshold flux for the real plant feed (Ho et al., 2016a). Changes of G_{DP} with respect to permeate flux for RO are presented in **Figure 5.10b**. The G_{DP} decreased when the flux was increased from 10 to 20 L/m² h. This is attributed to the accumulation of the non-conducting substances, such as nano-colloids which cause reduction of the overall conductance per unit volume in the polarization layer. Once the permeate flux reaches the threshold value, G_{DP} starts to increase and a turning point is observed; in this study a value of 20 L/m² h is indicated (similar to the dTMP/dt-determined value of > 18.5 L/m² h). As explained earlier, this is due to the increased concentration of conducting species (salt) in the diffusion polarization (DP) layer resulting from the cake enhanced concentration polarization (CECP) effect.

In summary, the threshold flux determined from the EIS method correlates well with that obtained from the TMP measurement. The operational flux of the plant was quoted as 17.6 L/m² h which is just below the threshold flux characterized by the TMP and EIS methods (18.5 – 20.0 L/m² h). Operation below the threshold flux should greatly reduce the fouling of membranes. However, local values in the plant could be significantly higher than the plant average due to variations of local driving force across the spiral wound module leaf and the gradients in the pressure vessel (Fane, 2004). EISFM canary cells, strategically placed in the cascade could help to avoid localized fouling.

Figure 5.10 (a) $dTMP/dt$ versus permeate flux and (b) GDP of membrane obtained from the fitted model in the flux step experiment for RO feed collected from Bedok NEWater Factory.

5.4 Concluding remarks

This chapter describes, for the first time, an electrical impedance spectroscopy fouling monitor (EISFM) integrated into a side-stream RO “canary cell” to monitor the performance and fouling behavior of a large-scale water reclamation plant. The canary cell is designed to experience the same hydrodynamic conditions and to undergo the same cleaning patterns as the RO train. By doing so, the canary cell is able to mimic the fouling conditions in the RO train and the fouling phenomena are detectable by the EISFM.

In this study, EIS measurements were taken periodically. As the diffusion polarization layer is the dominant layer where fouling behavior can be detected, the changes of the impedance of this element ($Z_{\text{real-DP}}$) were monitored during the course of the fouling process. Throughout the monitoring period, maxima in the normalized $Z_{\text{real-DP}}$ were observed well before each chemical cleaning process was performed. Between cleaning events, the trend of $Z_{\text{real-DP}}$ in time always showed an increase initially followed by a decrease. It is hypothesized that the initial increase was due to the accumulation of non-conductive material in the boundary layer and on the membrane; when the foulant layer became more consolidated so as to hinder the back diffusion of the dissolved salt, $Z_{\text{real-DP}}$ would start to decrease. Based on previous studies with model feed (Ho et al., 2016a; Sim et al., 2016; Sim et al., 2013a), it was anticipated that colloidal/particulate material could be the major form of fouling in this RO train. The autopsy data supported this hypothesis. The results showed that the major foulant type was calcium phosphate which can be present in the feed as nano-colloidal particle form. The threshold flux for the RO feed from the wastewater reclamation plant was also validated using TMP and EIS measurements in the laboratory.

The occurrence of $Z_{\text{real-DP}}$ peaks in the signal from the EISFM can be used as an indicator to initiate mitigation measures to control fouling such as physical cleanings. As

such, the frequency of chemical CIPs and the cost associated with the cleaning can be reduced. Based on the trend of $Z_{\text{real-DP}}$, appropriate cleaning agents and methods can be applied for more effective mitigation measures. Importantly, the EISFM provides early indications of fouling not evident from pressures and flows. Moreover, by observing the trend of pressure and flow, it is impossible to identify the possible foulant type. This field trial study provides a good foundation for developing fouling control protocols and also confirms the value of EIS as a promising fouling monitoring technique.

6 Chapter 6 - Conclusions and Recommendations

6.1 Summary and conclusions

RO technologies serve to provide solutions for problems associated with water shortages around the world. Despite the widespread use of RO in wastewater treatment and reclamation, membrane fouling remains as the main hurdle which limits its application. Membrane fouling can be minimized by pre-empting the fouling event using sensitive monitoring tools for the implementation of appropriate control measures and by operation that is well below the threshold flux. Hence, the development of a real time and non-invasive monitoring tool is critical so that signaling of early warning of incipient fouling and identification of suitable operation flux can be achieved. In this thesis, a real time and non-invasive method using EIS was used to monitor fouling events in RO and to understand the phenomenological events occurring in the DP layer during colloidal fouling and biofouling.

Chapter 2 includes the fundamentals of RO and an overview of various types of fouling. This is followed by the fundamentals of EIS, including the description of the low frequency element, the DP layer and the main parameters extracted from the EIS measurement for comparison. This chapter also comprises a brief overview of the water quality monitoring tools. Lastly, research gaps and opportunities were outlined here.

In Chapter 3, the electrical properties of the DP layer as a function of flux were characterized using EIS. The nature of a flowing colloidal suspension of silica on the membrane surface changes when a transition or threshold flux was achieved. The transition was well-defined and it was reflected in the changes of the slope in the plot of the transmembrane pressure (TMP) with flux and the conductance of the diffusion polarization

(G_{DP}) determined from EIS measurement. The G_{DP} derived from the low frequency region in EIS was identified as the most important parameter for the signaling of the onset of cake formation and the cake enhanced concentration polarization (CECP) effect. TMP measurements on their own provide limited information of these phenomena. The threshold flux was affected strongly by the crossflow velocity and the presence of a spacer in the feed channel. These were illustrated by the higher threshold flux from the $dTMP/dt$ plot and also the change of the minimum of G_{DP} with increasing flux.

Chapter 4 illustrates the *in-situ* and non-invasive monitoring of biofilm formation on a RO membrane surface. The changes of the G_{DP} were also monitored in this chapter and showed two stages of biofilm formation. The first stage was related to the accumulation of the bacterial cells and the formation of respiration products from the bacteria. The second stage referred to the accumulation of the extracellular polymeric substances (EPS) which was the main component for the formation of the biofilm matrix. The effect of a biostat, sodium azide was also investigated and its presence slowed down the growth of bacterial. The effects of sodium azide were reflected in the normalized G_{DP} plot. The sustainable flux at which the fouling was minimal, could be estimated from the rate of change of the initial increase of the normalized G_{DP} with respect to flux. Conventional monitoring methods such as TMP and Confocal Laser Scanning Microscopy (CLSM) on their own gave little insights into the mechanisms of biofilm formation. The change of normalized G_{DP} was also monitored when NO treatment was introduced as it has been identified as a biofilm dispersal agent. The TMP results together with the normalized G_{DP} suggest that NO has potential to be used to delay the biofouling.

The applicability of EIS in real settings was evaluated in Chapter 5. The EIS fouling monitor (EISFM) was employed to monitor the performance of a RO treatment train in one of the NEWater plant in Singapore. A side stream ‘canary cell’ equipped with EIS was installed in the plant. The canary cell was operated under the same hydrodynamic and flux

conditions as the RO train in order to simulate the fouling conditions in the spiral wound modules. The correlation between the response of EISFM and the plant performance was assessed. When trending the real part of the impedance of the low frequency element, the DP layer ($Z_{\text{real-DP}}$) over time, there was a maximum observed well before several cycles of the chemical cleanings. It is worth noting that the $Z_{\text{real-DP}}$ is the inverse of G_{DP} described in the previous chapters. The occurrence of the $Z_{\text{real-DP}}$ peaks can therefore be used as an indicator to initiate mitigation measures, for example, by tuning the flux/crossflow velocities or implementing physical cleanings to control fouling. As such, the frequency of chemical cleaning-in-place (CIP) could be reduced. Furthermore, the fouling mechanism could be deduced to be a build-up of a particulate layer, such as the inorganic colloid, from the observation of the trend of $Z_{\text{real-DP}}$. The mechanism was validated by membrane autopsy studies. The threshold flux of the RO feed water collected from the NEWater plant was estimated using the EIS characterization method described previously and it was found to be marginally greater than the operating flux in the plant.

6.2 Recommendations

Several areas of future research directions are outlined below.

- i. Further studies on the estimation of foulant layer thickness before, during and after the threshold flux using EIS or EIS coupled with ultrasonic time domain reflectometry (UTDR) would be of great interest. The onset of metastability (Sim et al., 2012) and nature of the cake layer could then be determined using combined EIS-UTDR.
- ii. It is useful to investigate the effect of NO dispersal combined with antimicrobial agents and EIS signal response towards these control measures. Since biofouling was difficult to be eliminated completely, control measures

combined with an online non-destructive monitoring tool is of importance for water treatment plant operations.

- iii. The applicability of the EIS fouling monitor has been evaluated using synthetic feed of organic, inorganic and biofouling as well as real wastewater. Its application can be expanded into seawater reverse osmosis (SWRO) where biofouling is the major challenge.
- iv. Currently, a side stream 'canary cell' installed with electrodes for EIS measurement is used for the characterization of the fouling conditions in real water treatment plant. Incorporation of voltage and current electrodes into the spiral wound modules coupled with EIS fouling monitor would allow detection of membrane fouling in a more direct way. Such 'smart' modules would be located strategically within the RO plant.
- v. Other parameters obtained from the EIS measurement (e.g. G_i – the imaginary part of the conductance) could be investigated and to correlate the changes of EIS parameters with fouling conditions quantitatively.

A1 Appendix 1 - Other Approaches of Sensing for Water

Quality Monitoring^{†4}

A1.1 Background

Apart from monitoring the state of the process during the water treatment, it is also important to observe the state of the feed to ensure that it is of the best quality when being directed into the system. To achieve a sustainable water supply, it is crucial to ensure the safety of water sources by monitoring its chemical and microbiological parameters. (Dufour and World Health Organization, 2003; Scandura and Sobsey, 1997). Pathogenic contamination of water sources is of great concern as it can cause serious health risks, for example diarrhea, nausea and various symptoms to the consumer, especially infants and people with severely compromised immune system. The discharge of raw domestic wastes which contains a wide variety of human enteric viruses could lead to water pollution as well (Rao et al., 1981). It is worth noting that most of the waterborne diseases originate from human and animal fecal pollution and they remain as serious public health issues. Thus, various methods have been investigated and developed to detect and identify fecal pollution (Fiksdal et al., 1994; Shanks et al., 2007; Shanks et al., 2009).

In order to ensure that water sources are not contaminated or polluted, fecal indicator bacteria or viruses, for example coliphages and *Bacteriodes fragilis* phages are being monitored when the analysis of water samples of public health concern is carried out

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⁴ [†]Findings in this chapter have been published in: Ho, J.S., Toh, C.-S., 2013. A Rapid Low Power Ultra-Violet Light-Assisted Bacterial Sensor for Coliform Determination. American Journal of Analytical Chemistry 4, 1-8 and Cheng, M.S., Ho, J.S., Lau, S.H., Chow, V.T.K., Toh, C.-S., 2013. Impedimetric microbial sensor for real-time monitoring of phage infection of Escherichia coli. Biosensors and Bioelectronics 47, 340-344.

(Rodríguez et al., 2012). Point source contamination is revealed when there is presence of fecal indicator bacteria or viruses. The presence of coliphages has been used for the correlation of fecal contamination and this is particularly advantageous because it serves as a complementary method to bacterial indicators. In particular, the enumeration of coliphages appears to be much simpler (Leclerc et al., 2000; Rodríguez et al., 2012). Currently, total coliform and fecal coliform and/or *Escherichia coli* (*E. coli*) are employed as the indicator organisms in the monitoring of drinking water in developed countries. *E. coli* has been identified as the most suitable indicator organisms for bacterial contamination in drinking water due to its prevalence in human and animal feces as compared to other coliform and also the availability of rapid and affordable detection methods (Grabow and Coubrough, 1986; Rompré et al., 2002; Tallon et al., 2005).

However, there may be cases where *E. coli* is difficult to detect but where coliphages are present. Traditional plaque assays have been extensively used in the detection and quantification of coliphages in water (Grabow and Coubrough, 1986). Despite their usage widely, their application is limited by the time-consuming procedures, tediousness in real time operation and it is not feasible for the detection of non-lytic phages (Cheng et al., 2013). While for the detection of fecal indicators, detection methods include the multiple tube fermentation (method of most probable number) or membrane filtration (Rompré et al., 2002). These methods require long incubation times and are dependent on the generation of visible presumptive reactions. Thus, they are not desirable as the methods are time consuming, not specific and susceptible to non-target interferences. In spite of this, the low level of training required and low cost nature make these methods widely acceptable in the routine testing of drinking water and wastewater quality (Ashbolt et al., 2001).

An immunological method has also been developed for the detection of fecal indicator and the detection is conducted using an enzyme-linked immunosorbent assay

(ELISA). It is a simple, rapid and sensitive method which makes the detection of low antigenic protein content possible. However, the detection of specific cells from the naturally contaminated samples becomes limited when there is a high concentration of non-specific organisms associated with the sample and this may interfere with the specificity of ELISA (Hanai et al., 1997).

Molecular methods such as polymerase chain reaction (PCR) is another technique that is capable of rapid and specific detection of bacteria (Calvo et al., 2013; Horáková et al., 2006; Kong et al., 2002; Tani et al., 1998; Yang et al., 2003). Traditional amplification method techniques such as nested (Calvo et al., 2013) and multiplex PCR (Kang et al., 2007) are being replaced by fully automated quantitative real time PCR assays which have higher sensitivity and specificity (Rompré et al., 2002; Shanks et al., 2009). Although PCR has been widely recognized and accepted as a highly specific detection tool, the instrument is very costly and requires specially trained personnel for operation. Another inherent limitation for the analysis of environmental samples using PCR is the possibility of enzymatic reaction inhibition as humic substances available in the water are known as polymerization enzyme (e.g. *Taq*-polymerase) inhibitors. Colloid particles present in the water have high affinity to DNA as well (Way et al., 1993). Hence, the development of a rapid, low cost and simple bacterial sensor with early warning capability and of low power is of significant for the detection and sensing of coliform and coliphage in water and this will be discussed in this chapter.

To ensure that water sources are free from microbial pollutants or contamination, fecal indicator bacteria or phages (e.g. coliphages and *Bacteriodes fragilis* phages) are typically monitored when analyzing water samples for microorganisms of public health concern (Rodríguez et al., 2012). The presence of fecal indicator bacteria or viruses reflects the presence of point-source contamination or fecal contamination. Currently, the total coliform and fecal coliform and/or *Escherichia coli* (*E. coli*) are used as the indicator

organisms for monitoring the quality of drinking water in developed countries. Amongst these, *E. coli* has been identified as the most suitable indicator for bacterial contamination in drinking water due to its prevalence in the intestinal flora of warm-blooded animals (Rompré et al., 2002).

Phages are used as models for the indication of the likely presence of pathogenic enteric bacteria in a water body (Ashbolt et al., 2001). The traditional plaque assay has been used extensively in the detection and quantification of phage. However, this method is time-consuming, difficult to operate in real-time and not feasible for the detection of non-lytic phages such as filamentous phages M13 and f1. Another commonly used method for coliform enumeration is the multiple-tube fermentation technique which has been employed as a water quality monitoring tool for at least 80 years (Rompré et al., 2002). This method involves the inoculation of a series of tubes with appropriate dilutions of the water sample. Despite its easy implementation and the need for unsophisticated equipment, this technique is extremely time-consuming as it requires 48 hours for presumptive results and another 48 hours for a confirmative outcome.

Hence, there is an urgent need to develop monitoring techniques which are portable, easy to use and of low cost for the determination of coliform and real-time phage monitoring based on electrochemical methods. There are two main sections in this chapter, part 1: a rapid low power ultra-violet light-assisted bacterial sensor for coliform determination (Section A1.2) and part 2: an impedimetric microbial sensor for real-time monitoring of phage infection of *Escherichia coli* (Section A1.3).

A1.2 Part 1 – A rapid low power ultra-violet light-assisted bacterial sensor for coliform determination

A1.2.1 Background

Water contamination is one of the most problematic issues where millions of people die from water borne diseases due to the consumption of unsafe or contaminated water. *Escherichia coli* (*E. coli*) have been identified as one of the agents for waterborne diseases and its presence in water represents water quality deterioration and contamination by human or animal wastes (Paul et al., 2001). This poses serious health risks, such as diarrhea, nausea and other symptoms to consumers, especially infants and those with a severely compromised immune system. The contamination of water sources by bacteria and pathogen also leads to limited sustainable water supply where one-fifth of world's population has no clean access to water and this scenario is more prominent in developing countries (Haller, 2008). Hence, there is an urgent need to develop a rapid, low cost and simple bacterial sensor with early warning capability and of low power (Battistel et al., 2012; Neumann et al., 2000) in order to address these problems. Electrochemical sensing which is portable and inexpensive has shown its superior performances in the detection of cells, viruses and biological samples (Cheng et al., 2011; Cheng et al., 2012; Diculescu et al., 2006; Yadav et al., 2013). However, real-time detection and regeneration of sensing materials have always been the limitations for biosensing (Brett and Oliveira-Brett, 2011). An electrochemical sensor which exhibits immediate response upon detection and the ability to be reused is expected to be highly useful due to the potential enhanced performance for on-site analysis.

TiO₂ photocatalyst has been used extensively for the photocatalytic degradation of organic compounds for water purification (Herrmann, 1999; Matsunaga et al., 1985). However, the development of photocatalytic reactors remains a challenge due to the high

recombination rates of holes and electrons (Sun and Bolton, 1996) and the lack of ability to regenerate and reuse the material. On the contrary, a polymer incorporated with TiO_2 would be expected to partly resolve the problem of recombination because the polymer serves as the electron acceptor in the conduction band. To develop a low power bacterial sensor, we chose iron hexacyanoferrate polymer which is also known as Prussian blue (PB), incorporated with titanium dioxide (TiO_2) particles as the sensing material. Two electrode configurations, namely a porous alumina membrane and a screen-printed carbon electrode (SPCE) have been employed in this study to investigate the performance of sensor. The polymer incorporated TiO_2 was deposited onto the platinum-coated porous alumina membrane template and SPCE, which acted as the working electrode in this study. The setup of polymer-coated membrane sensor operated like a conventional two-electrode electrochemical system but required only a very low power ultra-violet (UV) lamp to function while SPCE functioned as a conventional three-electrode system.

An ultrathin Prussian blue (PB) film was employed in this work because it can significantly improve the signal response and improve the sensitivity owing to the fast mass transfer of analytes (Henstridge and Compton, 2012; Nguyen et al., 2009). In this study, the sensing of bacteria was carried out without the labeling of antibody as well as with small volume which significantly simplified the procedures for operation. To further enhance the sensing performance of sensor, large TiO_2 particle size was chosen owing to its good mechanical adhesion (Baram et al., 2009) and to maximize interaction between particles and bacteria. The two-electrode system used in this work is simple and potentially useful in the application of field measurement conditions. Herein, we utilized a TiO_2 particle incorporated PB film to construct a novel membrane sensor with bacterial sensing capability with small volume of analytes with the aid of low power UV light (Fig. 6.1). *E. coli* sensing and detection of total coliform in river water were performed by the sensor using cyclic voltammetry. To further evaluate the application of the TiO_2 -incorporated PB

sensor on *E. coli* detection, the performance of SPCE coated with PB and TiO₂ was also investigated in this study.

A1.2.2 Experimental

1. Materials and instruments

Nanoporous alumina membranes (Anodisc^{TM}, 13 mm diameter, 0.02 μ m pore size) were purchased from Whatman (Maidstone, Kent, UK). SPCE was obtained from Dropsens. The working electrode (4mm diameter) of SPCE was carbon (C) while the counter and reference electrodes were platinum (Pt) and silver (Ag) respectively. Hydrochloric acid (HCl, 37%) was obtained from P. P. Chemical. Potassium chloride (KCl) was purchased from Sinopharm Chemical Reagent Co. Ltd. Potassium hexacyanoferrate (III) (K₃Fe(CN)₆) and Nafion perfluorinated ion-exchange resin were obtained from Sigma-Aldrich. Anhydrous iron (III) chloride (FeCl₃) was purchased from Merck. Titanium (IV) oxide (TiO₂, -325 mesh powder, anatase, 99.6%) was obtained from Alfa Aesar. Methanol (MeOH, $\geq$ 99.9%) was purchased from Tedia Company, Inc, toluene (C₆H₅OH, 99.5%) was obtained from RCI Labscan Limited and 1,4-dioxane (C₄H₈O₂, $\geq$ 99.9%) was purchased from Merck. *E. coli* K12 was obtained from ATCC and it was prepared in 0.01 M phosphate buffered saline (PBS, pH 7.4). All chemicals and solvents were used as received. Ultrapure water (Sartorius Ultrapure Water System) was used for all solution preparation, unless otherwise stated. Sputter coating of Pt onto the nanoporous alumina membrane was performed by JEOL Auto Fine Coater (JFC-1600). Coating of TiO₂ incorporated PB onto the electrodes and electrochemical measurements were carried out by e-corder 401 (eDaQ) and potentiostat (eDaQ EA161) controlled by a PC.

2. *E. coli* culturing

E. coli K12 was purchased from ATCC. Luria Broth (LB) containing 10.0 g tryptone, 5.0 g yeast extract and 10.0 g sodium chloride was used to grow the pure culture of *E. coli*. The culture was grown on an orbital shaker at 37 °C for 18 h and it was subsequently diluted to 10 cfu/mL with 0.01 M PBS, pH 7.4. *E. coli* cell number was enumerated by the spread plate method where 0.1 mL of diluted solution was spread evenly on LB agar plate and incubated at 37 °C for 24 h. *E. coli* colonies on the plates were counted for determination of the number of viable cells in colony-forming units per milliliter (cfu/mL).

3. River water spiking

One-liter of water sample was collected from Kallang river, Singapore on 16 Jan 2013. The water sample was divided into three equal volumes and two of the solutions were spiked with different volumes of 30 cfu/mL *E. coli* solution to make up the desired spiked concentration. All solutions were finally made up to equal volumes using PBS. The electrochemical measurement was then carried out according to the procedures stated below.

4. Sensor fabrication

Fabrication of TiO₂ incorporated PB membrane sensor was based on the procedure described elsewhere (Nishizawa et al., 1996). Both sides of the nanoporous alumina membrane were sputtered with conductive Pt layers and it was subsequently electrodeposited with PB (**Figure A1.1a**). The schematic diagram of the fabrication of the membrane-based bacterial sensor is shown in **Figure A1.1b**. The active side of the sputtered membrane served as the working electrode while the passive side of the membrane as counter electrode and silver/silver chloride (Ag/AgCl) in 1M KCl was used

as the reference electrode during the coating process. The membrane was coated using a galvanostat for 1 h in an aqueous solution of 20 mM $\text{K}_3\text{Fe}(\text{CN})_6$ and 20 mM FeCl_3 with pH adjusted to be around 2.0 using HCl. TiO_2 particles were suspended in the same solution with a concentration of 15 g/dm³. The solution was stirred and dropped onto the surface of the membrane every 10 min to ensure that TiO_2 was coated together with PB. The current density was 20 $\mu\text{A}/\text{cm}^2$ and total electrolysis charge passed was 72.2 mC/cm². The TiO_2 incorporated PB membrane was then rinsed with ultrapure water and dried overnight at room temperature. The passive side of the membrane was coated with Nafion before it was used for sensing. The coating of SPCE was accomplished by the same procedures except Pt and Ag were used as the counter and reference electrodes respectively.

5. Sensing and disinfection of *E. coli*

E. coli sensing was carried out by spreading 20 μL of *E. coli* solution on both sides of the membrane and the working electrode of SPCE prior to irradiation and the signal response was obtained from cyclic voltammogram (CV). The sensor was then subjected to UV light irradiation where the source was a 4 W mercury detector (MD401, Khind) before the determination of electrochemical response. After the first measurement, the sensor was rinsed with copious amounts of PBS to remove bacteria from the previous scan. Higher concentration of *E. coli* solution was applied onto the sensor and the signal response was determined again. The electrochemical measurement system comprised an integrated two-electrode setup or SPCE as well as e-corder 401 (eDaQ) and potentiostat (eDaQ EA161) controlled by a PC.

a **b**

Figure A1.1 (a) Fabrication of the membrane-based bacterial sensor by sputtering ~ 50 nm thick platinum layer on both sides of $60\ \mu\text{m}$ thick nanoporous alumina membrane, followed by coating of TiO_2 incorporated PB film galvanostatically onto the Pt electrode and (b) schematic diagram of the procedures for the preparation of the membrane-based bacterial sensor.

A1.2.3 Results and discussions

1. Model for bacterial sensor signal response

The TiO₂ incorporated PB membrane sensor presents a large signal response towards *E. coli* solution upon irradiation in contrast to the signal response before exposure of light (**Figure A1.2a**). This remarkably high electroactivity of the bacterial sensor cannot be observed on the sensor in the absence of PB films (**Figure A1.3a**) because electron transfer is harder to occur in TiO₂ due to its larger band gap of 3.2 eV (Jin et al., 2011). The change of peak current is insignificant when PB sensor without TiO₂ was used (**Figure A1.3b**). This clearly indicates that bacteria sensing happens only when there are photogenerated electrons and highly reactive radical species from TiO₂ particles. Further evaluation of the sensor in differentiating its signal response from an organic compound, i.e. methanol, reveals insignificant change of signal response (**Figure A1.2b**). The difference in electrochemical behaviors can be explained by the chemical reaction of PB and methanol (Goel et al., 2012) causing the PB film to dissolve away thereby, reducing electrochemical activity.

a **b**

Figure A1.2 Cyclic voltammogram of TiO_2 incorporated PB membrane sensor with (a) 20 μL 10 cfu/mL of *E. coli* before (.....) and after (- . -) UV light irradiation (b) 20 μL of methanol before (- . -) and after (—) UV light irradiation. Conditions: scan rate = 20 mV/s, potential range = -0.4 to 0.4 V, *E. coli* concentration = 10 cfu/mL, methanol concentration = 20 % (v/v).

a **b**

Figure A1.3 Cyclic voltammogram of (a) TiO_2 sensor without PB before (—) and after (- -) irradiation; (b) PB sensor without TiO_2 before (—) and after (- -) irradiation. Conditions: scan rate = 20 mV/s, potential range = -0.4 to 0.4 V, *E. coli* concentration = 10 cfu/mL.

The significantly high oxidative current of the bacterial sensor upon irradiation is owing to the formation of Prussian white (PW) as a result of injection of photo-excited electrons into PB as shown in **Figure A1.4**. A hole (h^+) and electron (e^-) pair is generated in the valence and conduction bands respectively when TiO_2 is illuminated with light of appropriate wavelength (300-400 nm). The valence band h^+ reacts with a water molecule to produce a hydroxyl radical ($\text{OH}^\bullet$) and the recombination of $\text{OH}^\bullet$ produces hydrogen peroxide (H_2O_2). To prevent the extremely deleterious $e^- - h^+$ recombination reaction, PB with compatible band gap energy (Yu et al., 2011) was chosen to incorporate with TiO_2 particles as the bacteria sensing material. In this study, the bacteria sensing is proposed to be coupled with disinfection of bacteria, where highly reactive $\text{OH}^\bullet$ and H_2O_2 are responsible for cell wall decomposition, subsequently changing the cell membrane permeability (Foster et al., 2011; Lu et al., 2003; Maness et al., 1999; Sunada et al., 2003; Sunada et al., 1998). Hence, the disinfection of *E. coli* is mainly due to the destruction of its cell wall leading to cell membrane which permeability change that allows the penetration of highly reactive species into the cytoplasmic membrane. Direct oxidation of cells by h^+ has also been proposed previously but the detailed mechanism has not been discussed extensively (Foster et al., 2011; Wang et al., 2012).

Figure A1.4 Schematic diagram of principle of operation of bacterial sensor and photocatalytic inactivation of *E. coli* by TiO_2 under UV light irradiation.

2. Analytical performance of bacterial sensor

Figure A1.5 illustrates the correlation between oxidative current with increasing concentration of *E. coli* upon irradiation. The bacterial sensor shows significant change of response upon irradiation and the short analysis time outperforms the conventional methods for detection of *E. coli* in water, including multiple-tube fermentation and membrane filter techniques which require time-consuming and labor-intensive procedures (Rompré et al., 2002). The correlation between logarithm concentration of *E. coli* and oxidative peak current is somewhat linear between 10 to 10⁵ cfu/mL (inset of **Figure A1.5**) and it can be correlated using the equation:

Figure A1.5 Cyclic voltammogram of TiO₂ incorporated PB membrane sensor before irradiation (.....) and after irradiation in the presence of 10 cfu/mL (- - -), 10² cfu/mL (———), 10³ cfu/mL (- · - ·), 10⁴ cfu/mL (- · · ·), 10⁵ cfu/mL (— · —). I_p represent the peak current signals in the presence of *E. coli* cells after UV irradiation and $I_{p,0}$ represents the peak current signal before irradiation. All potential sweeps were carried out in the presence of UV irradiation with increasing concentration of *E. coli*. Inset: Linear correlation between oxidative peak current with logarithm concentration of *E. coli* derived from three sets of experiments. Conditions: scan rate = 20 mV/s, potential range = -0.4 to 0.4 V.

$$\frac{I_p - I_{p,0}}{k_1} = \log \frac{[c]}{k_2} \quad (6.1)$$

where I_p and $I_{p,0}$ are the oxidative peak currents in the presence and absence of *E. coli*, k_1 and k_2 are constants and $[c]$ is the concentration of *E. coli*.

The calibration plot is obtained from three independent sets of experiments with three different sensors. The increase contact between TiO₂ particles and higher concentration of *E. coli* explains the relationship of signal response and *E. coli* concentration (Foster et al., 2011). The h^+ generated can react with cells (Wang et al., 2012) more rapidly and it can also be filled more readily by electrons generated from the decomposition of cells which have close contact with TiO₂ particles (Foster et al., 2011). Whereas for lower concentration of cells, less cells can be oxidized by the h^+ thus, resulting in lower oxidative current. This suggests direct oxidation of cells as one of the mechanisms occurring in our study of cell disinfection.

3. Interference study

The effect of organic contaminants on the sensor output is evaluated with three organic compounds, toluene, methanol and 1,4-dioxane (**Figure A1.6**) which are commonly found in industrial effluent and ground water contaminated by point emissions (Bedding et al., 1982; Coleman et al., 2007; World Health Organization, 2011). All sensor signal responses are derived from the peak currents of the potential scans and offset against the potential scan obtained in the absence of *E. coli* cells. **Figure A1.6** reveals the sensor signal response toward *E. coli*, mixture of *E. coli* with organic contaminants and 1 h after suspension of *E. coli* in the organic mixtures. The sensor was first applied with 10 cfu/mL *E. coli* solution to obtain the signal response (lower than the required USEPA recreational water quality criteria) (United States Environmental Protection Agency, 2012). A mixture

of three organic compounds, 0.05 ppm 1,4-dioxane, 0.002 ppm methanol and 0.002 ppm toluene were then added into the *E. coli* solution and applied onto the sensor to obtain the second signal response. These concentrations were chosen because these are the possible levels of contaminants present in polluted water (Bedding et al., 1982; Coleman et al., 2007; World Health Organization, 2011). There is insignificant change of signal response even after the sensor was exposed to organic contaminants, demonstrating that the presence of organic contaminants does not passivate nor interfere with the sensor performance on bacteria sensing. After 1 h suspension of *E. coli* in organic mixtures, the solution was applied onto the sensor to acquire the next signal response. The difference in signal observed might be attributed to the mineralization of organic compounds by *E. coli* after prolonged mixing, contributing to the increase in signal response (Díaz et al., 2001). This sensor shows its superior performance in *E. coli* detection (10 cfu/mL which is lower than the recreational water bacteria limit) (United States Environmental Protection Agency, 2012) even in the presence of organic contaminants, which are known to react with TiO₂ (Herrmann, 1999; Matsunaga et al., 1985), leading to its potential application in contaminated water analysis.

4. Reproducibility and regeneration of the bacterial sensor

The reproducibility of the fabrication procedure was determined by coating PB incorporated TiO₂ onto platinum sputtered porous alumina membrane under identical conditions and measured the signal response toward 10 cfu/mL *E. coli*. The coefficient of variation of fabrication reproducibility for three different sensors was found to be within 8%.

To investigate the reusability of the bacterial sensor, change of signal response before and after irradiation was monitored at 10 cfu/mL *E. coli* in three independent experiments. After each analysis, the sensor was immersed in PBS for 10 min to ensure that

bacteria cells had detached from the sensor prior to the next experiment. Single factor ANOVA test shows that there is no significant difference of the sensor signal response at 95% confidence level. As shown in **Figure A1.7**, the sensors can be readily regenerated after rinsing with PBS solution and this presents an outstanding feature of the sensor since reusability has always been regarded as one of the most important features of bacterial biosensors for practical use (Yagi, 2007).

Figure A1.6 Change of oxidative peak current of TiO_2 incorporated PB membrane sensor ($I_p - I_{p,0}$) toward *E. coli*, *E. coli* + organics 1 (*E. coli* in a mixture of methanol, toluene and 1,4-dioxane) and *E. coli* + organics 2 (*E. coli* incubated in a mixture of methanol, toluene and 1,4-dioxane for 1 h). I_p represents the peak current signal in the presence of *E. coli* and *E. coli* in the mixture of organics upon irradiation while $I_{p,0}$ represents peak current signal before irradiation. Conditions: scan rate = 20 mV/s, potential range = -0.4 to 0.4 V, *E. coli* concentration = 10 cfu/mL, methanol concentration = 0.002 ppm, toluene concentration = 0.002 ppm, 1,4-dioxane concentration = 0.05 ppm.

5. Applications of the bacterial sensor

To demonstrate useful applications of the bacterial sensor for the analysis of total coliform in freshwater samples, analysis was conducted using untreated river water samples collected in clean sterile containers and subsequently spiked with 30 cfu/mL *E. coli*. The *E. coli* concentration in the water sample was analyzed by comparing the TiO₂ incorporated PB membrane sensor signal response to the standard calibration curve constructed in Figure A1.5.

Figure A1.7 Change of oxidative peak current of TiO₂ incorporated PB membrane sensor ($I_p - I_{p,0}$) toward *E. coli* for three different sets of experiments. I_p represents signal response after *E. coli* was applied onto the sensor followed by irradiation while $I_{p,0}$ represents the signal response before irradiation. Conditions: scan rate = 20 mV/s, potential range = -0.4 to 0.4 V, *E. coli* concentration = 10 cfu/mL.

Real sample analysis of total coliform in river water samples shows excellent correlation of spiked concentrations and experimentally determined values at 95% confidence level. The sensor developed therefore can be applied for sensing and

deactivation of bacteria in river water and it can also be widely used in the analysis of water samples from other sources.

Performance of the sensor was then evaluated on a non-porous electrode, SPCE to compare with that of porous alumina membrane electrode. **Figure A1.8** gives the signal response of PB-TiO₂ coated SPCE with increasing concentration of *E. coli*. The correlation between logarithm concentration of *E. coli* and peak current follows Equation 6.5 and a linear range from 10 to 10⁵ cfu/mL is established. However, the current range obtained from PB-TiO₂ coated SPCE is lower than that of TiO₂ incorporated PB membrane sensor due to its non-porous structure and smaller surface area (Yuan et al., 2005). This suggests the highly regular, rigid and dense porous material with high pore density favors high PB loading and it can also act as reservoir of PB that restricts PB leaking, resulting in a higher current range (Curulli et al., 2004).

Figure A1.8 Cyclic voltammogram of PB-TiO₂ coated SPCE before irradiation (.....) and after irradiation in the presence of 10 cfu/mL (- - -), 10² cfu/mL (———), 10³ cfu/mL (- . -), 10⁴ cfu/mL (— . .), 10⁵ cfu/mL (— · -). I_p represent the peak current signals in the presence of *E. coli* cells after UV irradiation and $I_{p,0}$ represents the peak current signal before irradiation. All potential sweeps were carried out in the presence of UV irradiation with increasing concentration of *E. coli*. Inset: Linear correlation between oxidative peak current with logarithm concentration of *E. coli* derived from three sets of experiments. Conditions: scan rate = 20 mV/s, potential range = -0.4 to 0.4 V.

A1.2.4 Concluding remarks

In conclusion, we describe a unique bacterial sensor comprising PB coated porous membrane or SPCE electrode incorporated with TiO_2 particles which demonstrate high sensitivity bacterial sensing utility and simultaneously, deactivate the bacteria. Unlike other detection methods which require lengthy incubation time, this sensor exhibits change of signal response immediately after the application of *E. coli* solution onto the sensor and at the start of the detection procedure. The sensor also shows its specificity towards *E. coli* when tested against organic contaminants which are commonly present in industrial effluents and polluted groundwater and this feature is extremely useful in the monitoring of water quality where bacteria acts as the contamination indicator. The simple integrated two electrode setup of TiO_2 incorporated PB membrane sensor shows its superior analytical performance in detection of total coliforms in a water sample derived from river water. In addition, the higher current output of the membrane sensor at low analyte concentrations as compared to PB- TiO_2 coated SPCE suggests its potential development into a low cost and portable water disinfection system which can be highly applicable in developing countries where UV irradiation is readily available. Due to the potential ease of miniaturizing of the two electrode setup, this sensor could be developed into a powerful contamination monitoring tool for on-site analysis of water from various sources.

A1.3 Part 2 – Impedimetric microbial sensor for real-time monitoring of phage infection *Escherichia coli*

A1.3.1 Background

This section mainly discusses the development of a rapid phage detection tool based on electrochemical impedance spectroscopy. Phages are used as the common models for indicating the possible presence of pathogenic enteric in a water source (Ashbolt et al., 2001). Filamentous bacteriophage (phage) M13, which is ~0.8 µm in length and 6 nm in diameter is a phage/virus that cause infection specifically on male *Escherichia coli* (*E. coli*) with F-pili (Loeb, 1960; Salivar et al., 1964). Infection caused by phage M13 particles does not cause lysis to the infected *E. coli* cells (Hofschneider and Preuss, 1963) but they continue to grow and proliferate at a lower rate (Brown and Dowell, 1968).

To date, physical and morphological changes of the infected phage M13 by *E. coli* are hardly differentiated with the uninfected one despite the vast contributions made in microscopy techniques. Infection starts with the adsorption of phage M13 to the F-pili of *E. coli* cells. Subsequently, the phages are brought closer to the bacterial co-receptor TolA. Coat proteins and DNA are then integrated into the bacterium (Bradley and Dewar, 1967; Shu et al., 2008; Valentine and Strand, 1965). This is then followed by the *E. coli* secretion of the progeny phages which assembled on the bacterial membrane together with the release of lipopolysaccharide (LPS) found on the outer surface of cells (Falaschi and Kornberg, 1965). Studies have shown that the *E. coli* cells lose surface rigidity, become fragile and softer because of the damage of the outer surface of the cell and the release of LPS (Chen et al., 2009; Roy and Mitra, 1970).

Impedimetric methods which are portable and *in-situ* have been used in the real-time and non-invasive study viral infections, including cytopathic effect induced by herpes simplex virus in Vero cells (Cho et al., 2007) and cytopathic effects of West Nile virus in

Vero cell cultures (Fang et al., 2011). In comparison to these studies which require additional incubation time of one day to grow the cell layers, surface immobilized monolayers of covalently-bound antibodies would be anticipated to achieve shorter preparation time of less than 30 min for antibody-cell binding at the near equilibrium condition. The monolayer of the cells employed in this study was able to provide rapid responses to the changes that happened in the cells and their environment. This approach can also be applied to other virus-host systems as specific antibodies could be attached on the sensor for different cell types.

Herein, we report a method to investigate the interaction of M13 phage with *E. coli* by constructing an impedimetric tool using a mercaptoacetic acid (MACA) gold electrode for the self-assembled monolayer (SAM) of amine functionalized antibody molecules. Its outstanding performance is evident from the rapid detection time for phage infection and the simple analysis procedures which might be potentially useful in water quality monitoring.

A1.3.2 Experimental

1. Reagents and materials

Rabbit anti-*E. coli* antibody (immunoglobulin G, IgG, 4.0 mg/mL) was obtained from AbD Serotec. M13 phage was purchased from New England Biolabs, Inc and *E. coli* (ATCC 11775) was obtained from ATCC. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC, $\geq 97\%$), thioglycolic acid (MACA, $\geq 98\%$), N-hydroxysuccinimide (NHS) and potassium hexacyanoferrate (III) ($K_3Fe(CN)_6$) were purchased from Sigma Aldrich. Potassium chloride (KCl) was obtained from Scharlau. 0.3 and 1.0 μm alumina powders were purchased from Allied High Tech Products, Inc. *E. coli*, phage and antibody solutions were prepared in 0.01 M phosphate buffered saline (PBS, pH

7.4). All chemicals and solvents of analytical grade were used as received. All solutions were prepared in ultrapure water (Sartorius Ultrapure Water System), unless otherwise stated.

2. *E. coli* culturing

The pure culture of *E. coli* (O1:K1:H7, ATC 11775) was grown in Luria Broth, LB (10.0 g tryptone, 5.0 g yeast extract and 10.0 g sodium chloride) on an orbital shaker at 37 °C for 18 h. The culture was serially diluted to 10 cfu/mL with 0.01 M PBS, pH 7.4. *E. coli* cell number was enumerated by the spread plate method. 0.1 mL of diluted solution was spread evenly on a LB agar plate and incubated at 37 °C for 24 h. *E. coli* colonies on the plates were counted to determine the number of viable cells in colony-forming units per milliliter (cfu/mL).

3. Pretreatment of gold electrode

A gold electrode (diameter 2 mm, CH instruments Inc., Austin, TX) was polished with alumina slurries (1.0 followed by 0.3 and 0.05 µm grain size) for 20 min respectively and sonicated in water for 5 min. After polishing, the electrode was pretreated with 1 M sodium hydroxide for 20 min, 1 M hydrochloric acid for 5 min and Piranha solution (1:3 mixtures of 30% hydrogen peroxide/concentrated sulfuric acid) for 5 min. The pretreated electrode was then rinsed with ethanol and ultrapure water. Lastly, the electrode was cleaned in 50 mM sulfuric acid using cyclic voltammetry (conditions: 12 cycles, scan rate = 100 mV/s, potential range = -0.4 – 1.4 V) and then dried under nitrogen.

4. Construction of microbial sensor and monitoring of infection process

Self-assembled monolayer (SAM) modified gold electrode was fabricated by immersing a pretreated electrode in 1.0% MACA in ethanol solution for 24 h. Terminal

carboxylic acid groups were transformed into NHS ester group by the subsequent treatment of the electrode with 0.4 M EDC and 0.1 M NHS for 1 h. 10 μ L of 1.0 mg/mL of rabbit anti-*E. coli* antibody, IgG was then applied onto electrode surface and stored at 4 $^{\circ}$ C for overnight. Lipopolysaccharide (LPS) can be found on the outer surface of the cells (Falaschi and Kornberg, 1965). It contains a negatively-charged repetitive polysaccharide polymer, known as O-antigen (Raetz and Whitfield, 2002) which can be specifically recognized by the rabbit anti-*E. coli* antibody selected in this study (specific towards O- and K-antigenic serotypes of *E. coli*). Unbound antibodies on the electrode were removed by rinsing with copious amounts of 0.01 M PBS, pH 7.4. The construction of a microbial sensor was carried out by adding 10 μ L of 10^4 cfu/mL *E. coli* onto the surface of the electrode and incubating for 1 h and then followed by rinsing with 0.01 M PBS, pH 7.4 to remove non-specifically bound antibodies and cells. M13 phage infection was monitored using the prepared microbial sensor immersed in 10 mL of 2.5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ (1:1) in 0.01 M PBS at room temperature. 100 μ L of stock solution of 5×10^6 pfu/mL M13 phage was added into the analysis solution and the infection was monitored using EIS for up to 20 h. EIS experiments were carried out using an electrochemical analyzer Autolab PGSTAT128N and FRA2 module with a NOVA 1.6 software in a one-compartment three-electrode cell with the microbial sensor as the working electrode, platinum mesh as the auxiliary electrode and silver/silver chloride (1 M KCl) as the reference electrode. Electrochemical impedance spectra were recorded at frequency ranges from 10^{-1} to 10^6 Hz with an excitation amplitude of 10 mV and sine-modulated AC potential of 200 mV.

A1.3.3 Results and discussions

Figure A1.9a shows the schematic diagram for the construction of SAM-based microbial sensor and monitoring of the phage infection. The typical Nyquist plot obtained after each step of sensor construction (in 2.5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$) is featured in **Figure A1.10a**.

Electron transfer resistance (R_{et}) increases following the formation of highly-ordered and defect free MACA-SAM due to its insulating nature which hindered the penetration of redox species at the electrode surface (Brett et al., 2003). The schematic diagram for the preparation of microbial sensor is shown in **Figure A1.9b**.

As can be observed from **Figure A1.9**, the negatively-charged carboxylic tail groups of MACA was replaced by the positively-charged NHS ester intermediate after activation by NHS/EDC. The electrostatic interaction between the ester group and negatively-charged redox species promotes the transport of $\text{Fe}(\text{CN})_6^{3-/4-}$ to the underlying gold electrode, leading to the smaller radius of semi-circle for the Nyquist plot (**Figure A1.10a**) and lower R_{et} value (**Table A1.1**). In the subsequent step, amine covalent binding of the antibody stabilizes the biorecognition layer. This prevented solvent-mediated desorption and antibody attached in the right orientation for the bindings of antigens (Disley et al., 1998). The immobilization of antibody to the SAM and the subsequent specific binding of *E. coli* cells hinders the electron-transfer process at the electrode surface to a larger extent. This results in the significant increase of R_{et} values which is attributed to the hydrophobic and insulating nature of proteins and cells.

a

b

Figure A1.9 (a) Schematic diagram of the microbial sensor preparation: grafting of antibody monolayer, followed by capture of *E. coli* host cells and subsequent *in-situ* monitoring of live phage-cell interaction using Faradaic impedance spectroscopy. “FC” represents $\text{Fe}(\text{CN})_6^{3-/4-}$ and (b) Schematic diagram of the three-terminal electrode system for the preparation of microbial sensor.

In this work, *E. coli* cells were infected with M13 phage at a multiplicity of infection (MOI) of 5. MOI refers to the ratio of number of phage to number of bacteria (*E. coli* in this case). A high level of MOI was chosen due to the weak adsorption of M13 phage to the host cell (Pratt and Erdahl, 1968). **Figure A1.10b** shows the decrease of R_{et} (smaller semi-circle of Nyquist plot) after 5 h and longer infection time. These reductions could be attributed to several possibilities. Firstly, the damage on the LPS or O-antigen could shatter the antigen-antibody binding and minimized the blockage to the penetration of $\text{Fe}(\text{CN})_6^{3-/4-}$ to the underlying electrode. Secondly, infection caused the release of negatively-charged LPS which reduced cell adhesion (Abu-Lail and Camesano, 2003), hence, this facilitated the transport of the negatively-charged $\text{Fe}(\text{CN})_6^{3-/4-}$ toward the gold electrode. Thirdly, the

release of LPS, which is responsible for the structural rigidity of cells, could possibly induce slight changes to the surface morphology of the infected cells (Chen et al., 2009; Schwartz and Zinder, 1968). This might result in the aggregation of $\text{Fe}(\text{CN})_6^{3-/4-}$ in the space between *E. coli* and the gold electrode. These statements were then further verified by fitting the EIS data with the Randles circuit (**Figure A1.11**) using the EIS Spectrum Analyzer software (version 0.1b).

Figure A1.10 (a) Nyquist plot of sensor preparation after each step. Bare Au electrode ($\blacklozenge$), coating with MACA ($\blacktriangle$), activation with NHS/EDC ($\blacklozenge$), immobilization with antibody ($\bullet$) and binding of *E. coli* cells ($\blacksquare$). (b) Nyquist plot of *in-situ* monitoring of M13 phage infection in the presence of 2.5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ in 0.01 M PBS, pH 7.4. Binding of *E. coli* cells ($\blacksquare$), phage infection at 1 h ($\blacktriangle$), 3 h ($\bullet$), 5 h ($\blacklozenge$), 7 h ($\blacklozenge$), 10 h ($\blacklozenge$), 14 h ($\blacklozenge$) and 20 h ($\blacklozenge$). Lines are fitted using Randles

equivalent circuit. Inset: Reciprocal plot of R_{et} values at different infection time t normalized to control R_{et}^0 value obtained prior to infection, in the presence of phage (◆) and absence of phage (■). Conditions: frequency range = 10^{-1} - 10^6 Hz, excitation amplitude = 10 mV, sine-modulated AC potential = 200 mV, phage concentration = 5×10^4 pfu/mL.

Figure A1.11 Randles equivalent circuit of the microbial sensor.

The Randles circuit describes an electrode which undergoes a heterogeneous electron-transfer process. It consists of ohmic resistance of the bulk solution (R_s), electron-transfer resistance (R_{et}) and Warburg impedance (Z_w), resulting from the diffusion of the redox species to the electrode surface. Furthermore, a constant phase element (CPE) which comprises of admittance (Q) and frequency independent exponent (n) was employed to model the non-ideal double layer capacitance at the antibody-grafted electrode surface. In this work, the use of CPE was more appropriate than the capacitor as the surface of the antibody-grafted electrode was not homogeneously smooth and the non-uniform coating of antibody molecules led to the differently-charged localized regions on the electrode surface. In addition, the wide deviation in protein surface charges and charge densities could

contribute significantly to the variation in the overall electrode surface capacitance under the influence of frequency.

Current which passed through the uncompensated resistance of the bulk analysis solution was made up of Faradaic current (i_f) and capacitive current (i_c). In the branch circuit, the Z_w was connected in series with R_{et} and parallel with CPE. Simulated values of all elements in the equivalent circuit at different phage infection times are shown in **Table A1.1**. CPE and R_{et} vary according to the dielectric and insulating features at the solution/electrode interface. As shown in **Table A1.1**, the somewhat constant CPE values imply minimal changes on the electrode surface charges during the course of phage infection. The decreasing simulated R_{et} values after 5 h of infection correlated well with the reduced radius of semi-circle in the Nyquist plot (**Figure A1.10b**). This also justifies the hypothesis stated above that the electron-transfer rate gradually increased with longer infection time. The rate constant, k depends on the surface coverage, θ of the blocking film of bacteria cells on the electrode following the equation below (Koh et al., 2007):

$$k = k^0(1 - \theta) \quad (6.2)$$

where k^0 refers to the heterogeneous electron transfer of redox probe at the non-hindered electrode surface. The electron transfer rate constant, k could be estimated from the R_{et} values in Table 6.1 using the dependence of exchange current i_0 on R_{et} , where

$$R_{et} = RT / Fi_0 \quad (6.3)$$

and the relation between i_0 , k and C , concentration of the redox probe,

$$i_0 = FAcC \quad (6.4)$$

i.e. R_{et} is inversely proportional to k , where A refers to the surface area of the electrode. Due to the blocking property of the surface-bound *E. coli* cells, the electron transfer rate constant, k for $\text{Fe}(\text{CN})_6^{3-/4-}$ at the electrode was estimated to be $\sim 2.2 \times 10^{-4}$ cm/s (with 2 mm diameter

of electrode and 2.5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ by using Equation 6.4. The k for bare Au electrode and IgG-coated electrode was estimated to be 0.016 and 5.6×10^{-4} cm/s respectively. According to Equation 6.2, the coverage of antibody IgG on the electrode was approximately 96% while ~ 61% of the IgG-coated electrode surface was covered with *E. coli* cells before phage infection. Upon phage infection, the k increases further due to the release of the LPS molecules from the cell surface and the cell adhesion was reduced because of the disruption in the surface-bound antibody-LPS interaction. If the surface coverage changes linearly with time during phage infection, then Equation 6.2 could be expressed in terms of infection time, t as in Equation 6.5. Since k is inversely proportional to R_{et} , hence,

$$R_{et}^0 / R_{et} = 1 + At \quad (6.5)$$

where A is a proportionality constant.

The reciprocal plot of R_{et} values at different infection times, t were obtained from three separate phage infection experiments. The R_{et} values were normalized to control R_{et}^0 value obtained before phage infection, i.e. R_{et}^0/R_{et} versus t gives a linear relation ($R^2=0.99$) which is shown in the inset of **Figure A1.10b** (with 1-10% relative standard errors). This clearly indicates that there was a direct relationship between the phage infection time and how well the cell adhered to the electrode via the antibody-LPS binding interaction. This suggests that the impedance analysis serves as a very useful indicator tool of *E. coli* cell damage resulted from phage infection. In contrast, the resistance of the analysis solution, R_s remained relatively constant (**Table A1.1**) since it represents the properties of the bulk solution. The initial increase of the Warburg impedance (Z_w) with infection time suggests the enhanced resistive blockage to the mass transfer diffusion of $\text{Fe}(\text{CN})_6^{3-/4-}$ towards the underlying gold electrode. The diffusion path of $\text{Fe}(\text{CN})_6^{3-/4-}$ was blocked by the negatively-charged progeny phages or non-specifically adsorbed phages near the electrode surface.

In comparison, there were no apparent differences observed from the images obtained from optical microscopy for bacterial cells infected with phage for different durations of infection (**Figure A1.12**).

Figure A1.12 Confocal Raman microscopic images of (a) uninfected *E. coli* cells, (b) phage-infected *E. coli* cells for 3 h, (c) overnight phage-infected *E. coli* cells.

Table A1.1 Simulated values of elements in the equivalent Randles circuit.

	$R_s (\Omega)$	$R_{et} (k\Omega)$	$Z_w (k\Omega s^{-1/2})$	$Q(\mu \Omega^{-1} s^n)$	n
Bare Au	114.16 ± 0.59	0.21 ± 0.01	1.65 ± 0.01	21.72 ± 1.05	0.64 ± 0.00
Coated with MACA	128.50 ± 0.35	12.49 ± 0.04	1.73 ± 0.01	1.27 ± 0.03	0.94 ± 0.00
NHS/EDC activation	126.07 ± 0.27	0.94 ± 0.07	1.66 ± 0.01	1.01 ± 0.04	0.92 ± 0.01
Immobilized with IgG	124.05 ± 0.39	6.01 ± 0.07	2.35 ± 0.01	1.57 ± 0.06	0.86 ± 0.00
Binding of E. coli	105.77 ± 1.59	15.28 ± 0.09	4.28 ± 0.04	1.88 ± 0.06	0.83 ± 0.00
Phage infection at 1 h	111.20 ± 1.51	15.51 ± 0.08	5.33 ± 0.06	1.94 ± 0.07	0.83 ± 0.00
Phage infection at 3 h	112.96 ± 0.84	15.17 ± 0.05	6.27 ± 0.07	2.00 ± 0.06	0.83 ± 0.00
Phage infection at 5 h	112.77 ± 0.96	14.25 ± 0.03	6.69 ± 0.08	2.08 ± 0.06	0.83 ± 0.00
Phage infection at 7 h	111.91 ± 1.05	13.54 ± 0.04	7.05 ± 0.09	2.25 ± 0.05	0.83 ± 0.00
Phage infection at 10 h	106.12 ± 1.22	13.16 ± 0.01	6.70 ± 0.04	1.79 ± 0.03	0.83 ± 0.00
Phage infection at 14 h	101.15 ± 0.04	12.35 ± 0.01	6.14 ± 0.17	1.66 ± 0.01	0.84 ± 0.00
Phage infection at 20 h	98.82 ± 0.12	1.09 ± 0.02	5.56 ± 0.01	1.66 ± 0.03	0.84 ± 0.00

A separate control experiment was carried out using the same procedure for the preparation of the surface bound *E. coli* cells on an antibody-grafted gold electrode surface but in the absence of phages. As shown in the inset of **Figure A1.10b** (represented by red square), the downward trend of R_{et}^0/R_{et} further confirms that phage infection was necessary to bring about the decrease in R_{et} overtime. The opposite trend of the control experiment was attributed to the continuous self-proliferation of *E. coli* cells on the electrode surface, resulting in the increasing R_{et} values with time. In order to confirm that the phage infection experiments and control study in the absence of phage were significantly different from each other, a student *t*-test with a *p*-value cutoff of 0.05 was carried out. The *p*-values of < 0.05 were observed for infection times beyond 5 h and the results are summarized in **Table A1.2**. Whereas for phage infection time < 5 h (for 1 and 3 h), there was no statistical difference observed (**Table A1.2**).

Equation Section (Next)

Table A1.2 *p*-values at different phage infection time.

Phage infection time (h)	<i>p</i> -value
1	0.795
3	0.234
5	0.049
7	0.019
10	0.015
14	0.008
20	0.004

Figure A1.13a shows the cyclic voltammograms (CVs) obtained during sensor construction indicating decreasing peak currents due to surface passivation by the insulating surface activation reagents, antibodies and cells. This is consistent with the

impedance results (**Figure A1.10a**). When *E. coli* cells were captured on the electrode surface, plateau-shaped CVs were observed, indicating typical hemispherical diffusion of the redox probe $\text{Fe}(\text{CN})_6^{3-/4-}$ to and from the electrode. This was likely due to the micrometer-sized spaces between the surface-attached *E. coli* cells. Decrease of the CV peak current upon phage infection as shown in **Figure A1.13b**, indicates that the mass transfer diffusion rate of redox probe was impeded by the *E. coli* cells and phages, consistent with the changes of Z_w in **Table A1.2** p -values at different phage infection time.. Thus, EIS is potentially a sensitive tool to detect and monitor phage infection of *E. coli* in real time by analyzing the impedimetric processes which occur at the solution/electrode interface.

a **b**

Figure A1.13 Cyclic voltammograms for (a) microbial sensor preparation and (b) *in-situ* phage infection in the presence of 2.5 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ and 0.01 M PBS, pH 7.4. Conditions: scan rate = 50 mV s^{-1} , potential range = -0.2-0.7 V.

Another control experiment was carried out using non-viable *E. coli* cells captured on the electrode surface and the impedance data are shown in **Figure A1.14a**. Even in the presence of M13 phage, the R_{et} values remained almost constant with the non-viable *E. coli* cells. The preparation of non-viable *E. coli* cells was carried out by autoclaving at 121 °C in a pressurized system of 15 psi. This caused the denaturation and damage of macromolecular and sub-cellular structures such as proteins, cytoplasmic membrane and nucleic acid (Perkins et al., 2004), thus preventing phages from attaching to the surface of the host cell and replicating. A control study was also conducted using a sensor prepared without *E. coli* cells. As shown in **Figure A1.14** EIS Nyquist plot of control studies. (a) non-viable *E. coli* cells captured onto sensor, (b) an antibody immobilized sensor without captured *E. coli* cells, (c) monitoring of phage-cell interaction using heat-inactivated phage and (d) phage solution of low MOI value of 1 instead of MOI value of 5 used in (a)-(c). Lines are fitted using the Randles equivalent circuit shown in Figure 2. Conditions: Background electrolyte: 2.5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$, 0.01 M PBS, pH 7.4. Impedance frequency range = 10^{-1} - 10^6 Hz, excitation amplitude = 10 mV, sine-modulated AC potential = 200 mV.b, the R_{et} value increased upon phage addition due to the impeded electron transfer process resulting from the non-specific adsorption of phage on the electrode surface. **Figure A1.14c** shows the control study using viable *E. coli* cells but infected with heat-inactivated phages and it displays a higher R_{et} value overtime. The heat-inactivated phages had lost their infectious activity and the increased R_{et} value is attributed to the self-proliferation of *E. coli* cells, similar to the result shown in **Figure A1.10b** inset in the absence of phages. When the phage concentration was significantly low (MOI = 1), the effect of phage infection was also not apparent where the R_{et} value remained higher than the initial value at time = 0 h and even after longer incubation time of 7 h (**Figure A1.14d**). This is caused by the insufficient amount of phage to effectively infect the cells.

In summary, the R_{et} values do not drop below the initial R_{et} values measured at time, $t = 0$ h throughout the course of experiment for these control experiments. In all experiments, there was always an initial increase of R_{et} values which could be attributed to surface passivation (or fouling) by the insulating phage particles, non-specific adsorption of other reagents in the phage samples or due to the cell proliferation. On the other hand, in the experiments using live *E. coli* cells and sufficiently high concentrations of active phage, R_{et} value increased initially followed by drastic decrease so that after 5 h, the R_{et} value was significantly less than the initial R_{et} value at $t = 0$ h.

b

c

d

Figure A1.14 EIS Nyquist plot of control studies. (a) non-viable *E. coli* cells captured onto sensor, (b) an antibody immobilized sensor without captured *E. coli* cells, (c) monitoring of phage-cell interaction using heat-inactivated phage and (d) phage solution of low MOI value of 1 instead of MOI value of 5 used in (a)-(c). Lines are fitted using the Randles equivalent circuit shown in Figure 2. Conditions: Background electrolyte: 2.5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$, 0.01 M PBS, pH 7.4. Impedance frequency range = 10^{-1} - 10^6 Hz, excitation amplitude = 10 mV, sine-modulated AC potential = 200 mV.

A1.3.4 Concluding remarks

In conclusion, this section describes a rapid and sensitive impedimetric antibody-captured microbial sensor for the monitoring of M13 phage infection of *E. coli* cells. The short detection time of phage infection time (5 h) outperforms the traditional microscopic methods which require monitoring of changes in cellular structures resulting from cytopathic effects such as cell death, cell lysis or morphological changes which become apparent only after several days of infection. The impedimetric method may plausibly overcome the challenging obstacle of monitoring of small morphological and physical changes of host cells *in-situ* during pathogen-host cell interactions; hence, it is potentially relevant for monitoring of water quality in the future.

A1.4 Overall conclusions

In summary, rapid and sensitive sensors for bacteria detection and monitoring of phage infection of *E. coli* cells have been described. Both sensors exhibit rapid analysis time and sensitive response when compared to the traditional methods. Both of these sensors are potentially useful to be developed into practical contamination monitoring tools for water quality analysis due to their ease of miniaturization and simple procedure

A2 Appendix 2 - Inline Coagulation-Ultrafiltration as the Pretreatment for Reverse Osmosis Brine Treatment and Recovery^{†5}

A2.1 Background

A short internship was carried out in the Public Utilities Board (PUB) of Singapore in 2013. The project was about the evaluation of the inline coagulation ultrafiltration (UF) as a pretreatment method for reverse osmosis (RO) treatment and recovery. This is a low cost, less footprint and low energy consumption pretreatment method performed in the laboratory to help resolve issues associated with the RO brine handling for inland water reuse plants. Polyaluminium chloride (PACl), aluminum chlorohydrate (ACH) and ferric chloride (FeCl_3) were chosen as the coagulants in this work. Liquid chromatography-organic carbon detector (LC-OCD) and fluorescence excitation-emission matrix (EEM) were used to characterize the DOC fractions removed by inline coagulation-UF.

This section is organized into the following parts. Section A2.2 consists of the introduction of inline coagulation-UF as the pretreatment of RO brine treatment and recovery. This is followed by Section A2.3 which comprised of the materials and experimental procedure. Section A2.4 gives the results and discussion whilst conclusions are outlined in Section A2.5.

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⁵ [†]Findings in this chapter have been published in: Ho, J.S., Ma, Z., Qin, J., Sim, S.H., Toh, C.-S., 2015. Inline coagulation–ultrafiltration as the pretreatment for reverse osmosis brine treatment and recovery. Desalination 365, 242-249.

A2.2 Introduction

The RO technology utilizes semi-permeable membranes which function to separate the feed into two streams, namely the permeate, a portion containing purified water that passes through the membrane and brine, a stream consisting high concentration of ions and organic compounds. The characteristics of the RO brine depend largely on the feed water quality, RO system recovery, membrane cleaning procedures employed and the pretreatment method (Squire, 2000; Squire et al., 1997; Watson, 1990). The concentration of constituents in the brine is approximately 150 to 350% of the inflow, depending on the RO recovery ratio and it normally contains a higher concentration of effluent organic matter (EfOM) (Al-Rifai et al., 2007; Bagastyo et al., 2011b; Dialynas et al., 2008).

The generation of increasing brine stream from water reclamation plant around the world, lack of cost effective disposal methods and direct brine discharge have become critical issues in the management of water and environmental resources (Roberts et al., 2010; Samer et al., 2006). For inland areas, measures for brine disposal include surface water discharge, deep well injection or concentrating brines from evaporation ponds (Afrasiabi and Shahbazali, 2011; Ahmed et al., 2001; Greenlee et al., 2009; Muniz and Skehan, 1990). However, these disposal methods are known to cause adverse impacts on the environment and marine life (Lattemann and Höpner, 2008; Sadhwani et al., 2005). Thus, proper management and handling of brine is necessary in order to meet the regulations set out for local discharge. To provide a more sustainable solution for brine disposal, recovery of RO brine by various treatment methods has gained increasing attention in the study of water reclamation (Lee et al., 2009a; Lee et al., 2009b; Tao et al., 2011). Feasible low cost treatment and recovery methods for RO brine provide a two-way solution for water reclamation where there is a reduction of RO reject volume while untapped water sources can be recovered to generate more useful water stream.

The key criterion for RO brine treatment system is low energy consumption for the removal of inorganic ions and low fouling potential where pretreatment method might be necessary to remove the potential foulants, especially organic matter which are extremely harmful to the downstream treatment process (Lee et al., 2009a). Capacitive deionization (CDI) has been demonstrated to remove more than 90% of salt from RO brine but electrodes used in CDI cell are highly susceptible to organic fouling and silica (SiO_2) scaling, resulting in increased pressure and higher energy consumption (Lee et al., 2009b; Oren, 2008; Tao et al., 2011). Biological pretreatment process using biological activated carbon (BAC) has been reported to remove only 20% of total organic carbon (TOC) and this shows that the organic matter in RO brine is highly resistant to biodegradation, indicating that biological pretreatment method is relatively ineffective in RO brine recovery unless being integrated with other process (Lee et al., 2009a; Lee et al., 2009b; Ng et al., 2008). Advanced oxidation processes (AOPs) such as photocatalysis using TiO_2 , sonolysis and electrolytic oxidation have been proved to be effective in organic matter removal but the performance deteriorates over short operation time, rendering the process cost ineffective and energy-consuming (Dialynas et al., 2008). However, the process efficiency on the removal of silica which remains as the most problematic foulant in the RO process has not been well-studied (Rakruam et al., 2010).

Inline coagulation-ultrafiltration (UF) is another alternative low cost pretreatment method for water reuse due to its usefulness in removing nutrients (e.g. phosphate) and foulants simultaneously with low chemical consumption while producing effluent with improved water quality (Qin et al., 2012; Zheng et al., 2012). With respect to dissolved organic carbon (DOC) removal in RO brine, previous studies investigated this issue mainly using conventional coagulation method with sedimentation (Dialynas et al., 2008). In contrast to the conventional process, inline coagulation requires shorter coagulation time and lower coagulant dosages which might result in different mechanisms of foulant removal

that still remain unclear to date (Lehman and Liu, 2009; Zheng et al., 2012). To the best of our understanding, the performance of different metal coagulants in inline coagulation-UF as the pretreatment method for inland RO brine recovery has rarely been reported. In this study, a series of coagulants including commonly used pre-polymerized aluminium coagulants, polyaluminium chloride (PACl), aluminium chlorohydrate (ACH) and monomeric ferric coagulant, ferric chloride (FeCl_3) has been investigated on the treatment of RO brine, which contains relatively high concentration of DOC, phosphate and silica. The effects of pH adjustment have been studied to control the metal concentration in the UF filtrate as well as to optimize the removal of DOC, phosphate and silica. The impact of treatment method on the removal of different DOC fractions using different coagulants have also been characterized by size exclusion LC-OCD and fluorescence EEM, the established methods used for separation and characterization of organic matter (Chen et al., 2003; Henderson et al., 2010; Huber et al., 2011). This study has focused on the optimized coagulation conditions as a significant consideration for multiple foulants (e.g. DOC, phosphate and silica) removal and the selection of suitable coagulant in the inline coagulation-UF process for RO brine recovery.

A2.3 Materials and methods

A2.3.1 RO brine

The RO brine used in this study was collected from the second stage RO process from the Kranji NEWater Factory, Singapore. It is a water reclamation plant that receives secondary treated domestic effluent, being produced from a domestic wastewater treatment facility consisting of primary settling and activated sludge process. The secondary treated effluent is then directed into the reclamation plant where it is further processed using the

MF and RO membrane processes. The operating conditions of the NEWater Factory are shown in **Table A2.1**. The water reclamation system recovers 75% of the feed water as NEWater (RO product water) while the balance remains as RO brine. The average water quality of RO brine during the experimental period is presented in **Table A2.2**.

Table A2.1 Operating conditions of NEWater Factory

Parameter	Unit	Value
MF membrane	-	Hollow fiber
MF operating mode	m ³ /h	Out-to-in
Pore size of MF membrane	μm	0.1
NaOCl dosage to the MF feed	mg/L	2
MF process recovery	%	95
TMP of MF	kPa	30 - 70
RO membrane	-	Polyamide thin film
Antiscalant dosage	mg/L	2
RO feed flow	m ³ /h	444
RO permeate flow	m ³ /h	333
RO process recovery	%	75
RO membrane flux	l/m ² . h	17
TMP of RO	kPa	700-950
Operating temperature	°C	28-32

Table A2.2 Characteristics of RO brine

Parameter	Unit	Value
pH	-	6.75 ± 0.6
Conductivity	μS/cm	1574 ± 49
TDS	mg/L	1046 ± 28
TOC	mg /L	24.3 ± 3.4
PO ₄ ³⁻	mg/ L	8.7 ± 4.0
SiO ₂	mg/L	38.9 ± 1.4
Al ³⁺	mg/L	0.08 ± 0.03
Fe ³⁺	mg/L	0.20 ± 0.02
Turbidity	NTU	0.28 ± 0.01
UV ₂₅₄	cm ⁻¹	0.52 ± 0.05
SUVA	l/mg. m	2.01 ± 0.003

A2.3.2 Coagulants

Three inorganic coagulants, FeCl_3 , ACH and PACl were used in this study. **Table A2.3** presents their basic characteristics and the dosage concentration range used.

A2.3.3 Lab scale inline coagulation-UF

To simulate an inline coagulation-UF process in laboratory as depicted in **Figure A2.1**, the coagulant (FeCl_3 , ACH or PACl) was added into a jar with 1 L of RO brine under rapid mixing at 200 rpm. After 1 min, the pre-coagulated liquor was drawn from the jar tester and filtered through 0.05 μm nitrocellulose membrane (MF^{TM}, Merck Millipore, USA) under constant pressure using a peristaltic pump. In the experiments which require pH adjustment (to pH 4.5, 5.0, 6.0, 6.5, 7.0), 0.1 M NaOH or H_2SO_4 was added before the coagulant. The filtrate was collected for further analyses. Temperature was monitored continuously and found to be at 26 ± 1 °C.

Figure A2.1 Schematic diagram of inline coagulation-UF pretreatment.

Table A2.3 Characteristics of the coagulants used

Coagulant	Type	Strength [†] (% w/w)	Concentration of stock solution (g/L as Fe or Al)	Supplier
FeCl ₃	Inorganic monomer	97 (FeCl ₃)	5.27	Sigma Aldrich
ACH	Inorganic polymer	23 (Al ₂ O ₃)	1.21	Chemkimia Sdn. Bhd.
PACl	Inorganic polymer	30 (Al ₂ O ₃)	1.65	Sinopharm Chemical Reagent Co. Ltd.

[†]Concentration of the coagulants in % weight of FeCl₃ or Al₂O₃ as provided by the manufacturers.

A2.3.4 Analysis

1. Water quality analysis

The water quality of samples before and after treatment, namely RO brine and inline coagulation-UF filtrate were analyzed for pH, conductivity, total dissolved solid (TDS), total organic carbon (TOC), phosphate and silica. pH was measured using a pH meter (Orion Star™ A211, Thermo Scientific, USA) while conductivity was quantified using a conductivity meter (Ultrameter II™, Myron L Company, USA). Turbidity was tested using a turbidimeter (2100Q, Hach, USA). Quantification of TDS was carried out in accordance to the Standard Methods (APHA-AWWA-WEF, 2012) and TOC was analyzed according to APHA : Pt 5310B. Phosphate was measured using APHA : Pt 4500-P (G) while filtrate silica were quantified according to APHA : Pt 3120B. Ultraviolet adsorption measurement was recorded at 254 nm (UV₂₅₄) following APHA : Pt 5910B.

2. LC-OCD analysis

Organic matters in the RO brine (feed) and filtrate were characterized by size exclusion chromatography (SEC). The SEC system (DOC-LABOR Dr. Huber, Germany) is composed of an LC-OCD-UVD-OND (liquid chromatography-organic carbon detector-UV detector-organic nitrogen detector. It is equipped with a size exclusion column HW-50S (Toyopearl, Tosoh Bioscience, USA) of particle size 30 µm, length 250 mm and internal diameter 20 mm for the separation of organic molecules according to their molecular weight. The mobile phase employed was phosphate buffer at pH 6.8 (2.5 g/L KH₂PO₄ and 1.5 g/L Na₂(HPO₄).2H₂O) at a flow rate of 1.1 mL/min. An injection volume of 1000 µL was utilized for measurement. Prior to the measurement with LC-OCD system, the samples were filtered through a 0.45 µm nylon syringe filter (Tisch Scientific, US) and then diluted to TOC level below 5 mg/L before analysis. This method facilitates the

distribution of DOC into five fractions, namely biopolymers (BP), humic substances (HS), building blocks (BB), low molecular weight (LMW) neutrals and LMW acids.

3. Fluorescence EEM analysis

Fluorescence EEM measurements were conducted using a Perkin Elmer LS-50B luminescence spectrometer. The maximum emission intensity of the spectrometer is 1000 arbitrary units (AU). The spectrometer is equipped with xenon excitation source. The excitation and emission slits were set to a 10 nm band-pass. In order to obtain fluorescence EEMs, the excitation wavelengths were increased from 200 to 500 nm at 10 nm steps while the emission was detected at longer wavelengths from 230 to 550 nm at 10 nm steps. The scanning speed was maintained at 500 nm/min. Origin 7.0 (OriginLab Inc., USA) was used for fluorescence EEM data processing. The plots were presented as elliptical shape of contours. The *x*-axis of the plots represents the emission wavelength from 230 to 550 nm whereas the *y*-axis refers to the excitation wavelength from 200 to 500 nm and the contour lines are shown to express the fluorescence intensity.

A2.4 Results and discussions

A2.4.1 Optimization of coagulants

1. TOC removal

In order to identify the optimum dose for coagulation, experiments were conducted with a range of dosages for three inorganic coagulants as depicted in **Figure A2.2a**. The TOC removal efficiency generally increased with higher coagulant dosages, with the maximum removal efficiency obtained at 0.556 mM as Fe/Al. Upon this dosage, the removal efficiency decreased again for all the coagulants used in this study. When coagulant dose was increased (below 0.556 mM), there were sufficient metal hydroxide or

polymerized materials present to be adsorbed and thereby, destabilized the suspension. As the concentration of the coagulant dosage increased beyond a critical point, restabilisation may occur due to an excess of counter-ion adsorption, giving rise to charge reversal. The removal of TOC with FeCl_3 appeared to be more efficient, achieved up to 60% at the optimal dosage while aluminium-based coagulants demonstrated lower removal efficiency of 31% and 27% for PACl and ACH respectively. The difference between the mechanisms of hydrolysis and precipitation behaviors of ferric and aluminium coagulants could give rise to this phenomena (Bratby, 2006). In this case, the hydrolysis of Fe^{3+} reached equilibrium in a shorter time than that of Al^{3+} and larger flocs were formed and removed by the membrane due to the rapid formation of $\text{Fe}(\text{OH})_3$ precipitate which settles down in a faster rate (Yu et al., 2007). Besides that, formation of ACH flocs is slow with the low turbidity feed and they are small, causing the poorest removal of TOC (Duan and Gregory, 2003). This clearly shows that pre-polymerized coagulants (PACl and ACH) exhibited insignificant improvements over monomeric coagulant (FeCl_3) as the polynuclear Al species formed, such as Al_{13} , could possibly be depolymerized in the presence of organic matter in the water medium (Duan and Gregory, 2003; Masion et al., 2000).

To further evaluate the effect of pH on the performance of metal coagulants on TOC removal, the removal efficiency was examined at the optimal dosage of 0.556 mM as Fe/Al and the results are shown in **Figure A2.2b**. This dosage was chosen as the optimal dosage because it gave the highest TOC removal efficiency for all the three coagulants as illustrated in Fig. A.2a. FeCl_3 again, showed its outstanding performance compared to the other two Al-based coagulants, presenting the highest TOC removal efficiency of 60% at pH 4.5 to 5 while PACl and ACH achieved a lower efficiency of 35% at pH 4.5 and 32% at pH 5. The optimum pH for TOC removal for monomeric coagulant, FeCl_3 was in the weakly acidic and neutral pH regions, therefore, TOC removal did not vary much for the range tested in this study. The high TOC removal of FeCl_3 could be attributed to further hydrolysis and

polymerization of the monomeric species (Fe^{3+}) to transform into the medium polymer species (i.e. $\text{Fe}_2(\text{OH})_2^{4+}$ or $\text{Fe}_3(\text{OH})_4^{5+}$) which was easily filtered out by the 0.05 μm UF membrane (Jiang and Graham, 1996; Yu et al., 2007). For the polymeric coagulants, PACl and ACH, the optimal pH range for TOC removal was ~ 4.5 to 5.0. Aluminium coagulants performed better in the acidic region due to the lower negative charge on the particles as well as on natural organic matter (NOM) (Hu et al., 2005). This reveals that the mechanism for TOC removal in this pH range might be charge neutralization which involved the interaction of negatively charged dissolved organic matter and positively charged hydrolysis species to form precipitate complexes, eventually be removed through the membrane. On the other hand, at pH above 6.5, there were lower amount of positively charged ions on the inorganic coagulants and adsorption of DOM on metal hydroxides precipitate decreased, leading to a lower TOC removal near the neutral pH region (Slavik et al., 2012). The optimum pH was chosen as pH 6.5 for Al and 7.0 for Fe based on the consideration of high TOC removal efficiency as well as low concentration of dissolved metal ions after filtration. Thus, at optimum pH, FeCl_3 obtained the highest TOC removal efficiency of 55% while it is 30% and 18% for PACl and ACH respectively.

a **b**

Figure A2.2 Effect of (a) coagulant dose and (b) pH on TOC removal with different coagulants. Dosage for (b) = 0.556 mM as Fe/Al.

2. Phosphate and silica removal

The removal of phosphate by inline coagulation-UF was also investigated and the results are shown in **Figure A2.3**. Increasing dosage of PACl and ACH improved the removal efficiency while FeCl_3 presented almost 100% removal at dosage level ranging from 0.407 to 0.889 mM (as Fe) as reported in **Figure A2.3a**. Lower removal efficiency of phosphate for the pre-polymerized coagulants as compared to monomeric one could be attributed to the lower charge density of metal-hydrolyzed species (Jiang and Graham, 1998). The partially hydrolyzed metal species, such as in PACl and ACH presented a lower efficiency in phosphate removal at the lower dosage region. This is consistent with the results of TOC removal where FeCl_3 presents the higher removal efficiency. The lower removal efficiency for Al coagulants at the lower dosage region may be attributed to the process of $\text{Al}(\text{OH})_3$ formation competing with the formation of aluminium-hydroxophosphate complexes, resulting in a reduced total cationic charge of Al^{3+} in contrast to Fe^{3+} originated from FeCl_3 (Ratnaweera et al., 1992).

Figure A2.3b illustrates the removal of phosphate at the optimal dosage of 0.556 mM as Fe or Al of coagulant at different pH. The removal efficiency of phosphate did not vary much in the pH range tested (pH 4.5 to 7). This could be attributed to the phosphate removal mechanism that the metal phosphate precipitation is dominant while the pH range for the minimum solubility of FePO_4 and AlPO_4 is coincided with the pH range tested. The observation is also in agreement with that from Jiang and Graham (Jiang and Graham, 1998). In the pH range of 5 to 7, the stable solid phases of metal phosphate were precipitated out and eventually, removed by the membrane, resulting in the lower concentration of phosphate in the filtrate. Again, the phosphate removal efficiency was the highest for FeCl_3 , followed by PACl and then ACH in the pH range tested. It should be emphasized that near 100% of phosphate removal could be achieved with FeCl_3 at its

optimal dosage, contributing to the production of better water quality ($\text{PO}_4 < 0.025 \text{ mg/L}$) for further reuse and reclamation.

a

b

Figure A2.3 Effect of (a) increasing dosage and (b) pH variation on phosphate removal. Dosage for (b) = 0.556 mM as Fe/Al.

Since silica has been known as one of the predominant foulants in water reuse and study on silica removal using inline coagulation has rarely been reported, the performance of different coagulants in silica removal at the optimum dosage was evaluated and it is shown in **Figure A2.4**. At the optimum pH for each coagulant, silica removal follows $\text{ACH} > \text{FeCl}_3 > \text{PACl}$ with 15%, 14% and 7% respectively. This suggests that the precipitation of metal hydroxides (i.e. $\text{Al}(\text{OH})_3$ and $\text{Fe}(\text{OH})_3$) with amorphous silica was likely the mechanism for removal since the sequence does not follow coagulant charge density; otherwise the removal efficiency for silica would be similar to that for TOC and phosphate where FeCl_3 , the fully hydrolyzed metal species, would be the most effective in this case (Chuang et al., 2007). This result further confirmed that FeCl_3 would be the most suitable coagulant for RO brine pretreatment, aiming at foulants removal at its optimum dosage and pH operating condition.

Figure A2.4 Effect of pH variation on silica removal for different coagulants. Dosage = 0.556 mM as Fe/Al.

A2.4.2 DOC characterization

1. LC-OCD

In order to assess the impact of treatment on the removal of different DOC fractions, LC-OCD was employed in this study to characterize the DOC in the feed RO brine. LC-OCD is capable of fractionating the DOC in the feed into five different fractions based on their different molecular weight. **Figure A2.5** illustrates the LC-OCD chromatograms of the RO brine, overlapping with PACl, ACH and FeCl₃ treated inline coagulation-UF filtrate at their optimal pH for all three detectors OCD, UVD and OND. Organic fractions identified were then quantified in terms of organic carbon concentration. The quantitative results of each DOC fraction characterized using LC-OCD for the feed and inline coagulation-UF filtrate is presented in **Table A2.4**. HS originated from effluent organic matter (EfOM) contributed to the largest fraction of DOC in the feed and this suggests that physical or chemical processes were favored compared to biological treatment in considering the treatment methods and this correlates with the Specific UV absorbance (SUVA) value presented in **Table A2.2** (Wei and Amy, 2012). SUVA 2 - 4 indicates that the NOM consists of mixture of molecular weight and reveals that coagulation could be a suitable method for DOC removal (Pemitsy and Edzwald, 2006). The feed in this study was also rich in BB and LMW neutrals because RO acted as a complete barrier for these compounds, resulting in their existence in the brine, which is the feed water in our case (Simon et al., 2012). However, LMW acids in the filtrate were mostly non-quantifiable, therefore, the values were not reported in **Table A2.4**.

The DOC in the feed removed by the inline coagulation-UF process using inorganic coagulants at their optimal operating conditions, ranges from 25% for PACl, 22% and 46% for ACH and FeCl₃ respectively, as reported in **Figure A2.6**. The trend of removal efficiency is in the order of FeCl₃>PACl>ACH. The higher removal efficiency of PACl

compared to ACH enhances the significance of the result shown in **Figure A2.2** and this again, clearly indicates that PACl is more efficient in removing hydrophilic organic matter in contrast to ACH. For the three coagulants tested, 73% to 83% of the DOC removed comprised of BP, reported to be made up of proteins (32%), as shown in the OND signal as well as polysaccharides (Huber et al., 2011). This is consistent with previous findings where coagulation mainly reduced the content of large molecular weight materials and the removal of biopolymers, which have been regarded as the main foulants, is very crucial in fouling control because this could potentially improve the filterability of membrane process (Bagastyo et al., 2011a; Bagastyo et al., 2011b; Zheng et al., 2012). PACl presented slightly higher biopolymer removal efficiency as compared to FeCl_3 and this could possibly be due to the formation of denser cake layer on the UF membrane that aids in the separation of organic fraction with the highest molecular weight (Zheng et al., 2012).

Figure A2.5 LC-OCD chromatogram of RO brine with inline-coagulation filtrate with PACl, ACH and FeCl₃, with responses of organic carbon detector (OCD), UV detection at 254 nm (UVD) and organic nitrogen detector (OND). Dosage = 0.556 mM as Fe/Al, pH = 6.5 for PACl and ACH, pH = 7.0 for FeCl₃.

Table A2.4 DOC and associated organic matter fractions in feed and coagulation-UF filtrate.

Coagulant	Dosage (mM as Fe/Al)	pH	DOC (mg/L)	Hydrophobics (mg/L)	BP (mg/L)	HS (mg/L)	BB (mg/L)	LMW neutrals (mg/L)	LMW acids (mg/L)
Raw	0	6.6	20.0	0.5	1.4	8.0	4.4	5.4	0.3
FeCl ₃	0.556	7.0	15.1	4.7	0.3	3.7	3.2	3.2	n.q.
PACl	0.556	6.5	15.1	0.4	0.2	7.3	3.6	3.6	n.q.
ACH	0.556	6.5	19.2	6.0	0.4	5.4	3.6	3.8	n.q.

n.q. = non-quantifiable

The removal of HS and BB was observed to be lower than that of BP, ranging from 10 to 49% and 17 to 27% respectively (**Figure A2.6**). This was expected due to the lower molecular weight of HS, which were not able to be adsorbed on flocs and likely to pass through the UF membrane. LMW neutrals removal was found to be ranging from 26 to 38%, which was higher than that of BB removal despite their lower molecular weight. This could be the result of adsorption of LMW neutrals on UF membrane, as shown in some of the previous studies (Henderson et al., 2011; Simon et al., 2012). Interestingly, FeCl_3 showed the highest removal efficiency in nearly all the DOC fractions characterized by LC-OCD. The removal of low molecular weight DOC compounds in the pretreatment method is highly recommended because this could significantly reduce the fouling potential of the downstream RO process (Gur-Reznik et al., 2008). The reduction of the UVD signal **Figure A2.5**) implies that FeCl_3 works effectively in the removal of aromatic organic carbon while the other two Al-based coagulants exhibit poorer performance.

Figure A2.6 Removal of DOC and associated organic matter fractions at optimal conditions using different coagulants where DOC = dissolved organic carbon; HS = humic substances; BB = building block; and LMW = low molecular weight. Dosage = 0.556 mM as Fe/Al, pH = 6.5 for PACl and ACH, pH = 7.0 for FeCl_3 .

2. Fluorescence EEM spectroscopy

Recently, fluorescence EEM has received much attention because it is extremely useful in characterizing dissolved organic matter and it is highly potential to be employed as an online monitoring tool. The EEM spectra for RO brine and inline coagulation-UF filtrate at optimized dosage for each coagulant are displayed in **Figure A2.7**. The intensity of fluorescence within the sample against the excitation and emission wavelength at which the excited fluorophores emit light is illustrated in the EEM spectra. The spectra were divided into five regions using consistent boundaries for excitation and emission wavelengths while the peaks are detailed below as peak 1-5 (Chen et al., 2003; Henderson et al., 2011). Subsequent fluorescence analysis as depicted in **Figure A2.7** was based on these wavelength pairs.

- 1) 230/303 nm – protein-like fluorescence, such as tyrosine
- 2) 230/355 nm – protein-like fluorescence, such as tryptophan
- 3) 230/407 nm – fulvic acid-like fluorescence
- 4) 310/353 nm – soluble microbial byproduct-like fluorescence
- 5) 330/410 nm – humic acid-like fluorescence

The fluorescence intensity for the wavelength pairs could provide quantitative information for the corresponding organic matter. According to **Figure A2.8**, FeCl_3 targets the removal of almost all the DOC fractions with the highest efficiency among the three coagulants, except for soluble microbial byproducts. FeCl_3 exhibited the highest removal efficiency of protein-like fluorescence (Peak 1 and 2), which had been identified as the tracer in wastewater effluent and this is consistent with the OND signal displayed in **Figure A2.5** (Haman et al., 2015). The reduction of proteinaceous content in treated water is highly

recommended because it accounts for 19-25% of soluble organics in secondary wastewater effluent (Rakruam et al., 2010). The trend of removal efficiency for EEM correlates well with that of LC-OCD and the robustness of this trend is very significant especially in extending the application of these tools in monitoring the performance of inline process (Henderson et al., 2011).

Figure A2.7 EEM spectra of a) RO brine and inline coagulation-UF filtrate with treatment of b) PACl, c) ACH and d) FeCl_3 at optimal conditions. Dosage = 0.556 mM as Fe/Al, pH = 6.5 for PACl and ACH, pH = 7.0 for FeCl_3

Figure A2.8 Fluorescence intensity of RO brine before and after treatment, where the numbers represent the wavelength pairs, peak 1 = protein-like fluorescence, such as tyrosine, 2 = protein-like fluorescence, such as tryptophan, 3 = fulvic acid-like fluorescence, 4 = soluble microbial byproduct-like fluorescence and 5 = humic acid-like fluorescence. Dosage = 0.556 mM as Fe/Al, pH = 6.5 for PACl and ACH, pH = 7.0 for FeCl₃.

A2.5 Concluding remarks

We describe the first study of inland RO brine treatment and recovery using inline coagulation-UF as the pretreatment method which potentially requires shorter coagulation time of one minute, smaller footprint and lower operating cost for the treatment system. The following conclusions can be drawn from the present study investigating the performance of three different coagulants for removal of RO foulants.

- Inline coagulation-UF with PACl and ACH attained TOC removals of up to 30% and 18% at the optimal pH 6.5. FeCl_3 presented the best performance among the three coagulants, achieving up to 55% of TOC removal at optimal pH of 7. Optimal molar dose for the three inorganic coagulants was 0.556 mM as Fe or Al. FeCl_3 demonstrated the highest phosphate removal efficiency of > 99% while PACl and ACH could only achieve up to 72% and 50% respectively at 0.556 mM as Fe or Al. FeCl_3 also elucidated relatively high silica removal efficiency of 14%, after ACH with 15%, and PACl displayed the lowest removal efficiency of 7% at the optimum conditions.
- FeCl_3 showed highest removals of nearly all the DOC fractions, namely humic substances, building blocks and LMW neutrals in contrast to the other two coagulants as well as proteins as illustrated in the EEM spectra. This suggests that the use of FeCl_3 in the inline coagulation-UF process is highly recommended because the removal of low molecular weight DOC and proteinaceous material could potentially reduce the fouling tendency in the downstream RO process.
- These results provide a basis for the selection of coagulants for the inline coagulation-UF pretreatment of RO brine which could potentially be useful in the mitigation measures of brine handling for inland water reuse plants. This study also provides information on the optimal operating conditions for each particular coagulant in order to obtain the required water quality suitable for the downstream membrane process and a database for the pilot study in the next step.

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