Evaluation of Biofouling and Its Control by Hypochlorite on Polyamide Reverse Osmosis Membranes

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Evaluation of Biofouling and Its Control by Hypochlorite on Polyamide Reverse Osmosis Membranes

（ポリアミド RO 膜でのバイオファウリングの評価と次亜塩素酸塩によるバイオファウリングの制御）

Cervinia V. Manalo

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Abstract

Biofouling is a prevailing problem associated with the performance of reverse osmosis (RO) membrane technology, which has emerged as a promising technology directed towards solving the world's problem in water scarcity and increasing global water demand, through its application in seawater and brackish water desalination and wastewater reuse and reclamation. Since biofouling potential is a key ingredient in biofouling studies, a part of this research aims to develop a biofouling potential test that is novel, simple, fast, and suitable for determination of biofouling on RO membranes. In this research, the method developed evaluated biofouling potential by direct analysis of RO membrane through fluorescence intensity analysis of biofilm formed on the membrane surface, thereby incorporating fouling tendencies of both feedwater and membrane. Evaluation of the biofouling potential on the RO membrane was done by accelerated biofilm formation through soaking of membranes in high biofouling potential waters obtained by adding microorganisms and glucose in test waters. The biofilm formed on the soaked membrane was quantified by fluorescence intensity microplate analysis. The soaking method's capability in detecting biofilm formation was confirmed when percentage coverage obtained through fluorescence microscopy and intensity values exhibited a linear correlation (R^2 = 0.96). Continuous cross-flow experiments confirmed the ability and reliability of the soaking method in giving biofouling potential on RO membranes when a good correlation (R^2 = 0.87) between intensity values of biofilms formed on the membrane during soaking and filtration conditions was obtained. Applicability of the test developed was shown when 3 commercially available polyamide RO membranes are assessed for biofouling potential. This new method can also be applied for the determination of biofouling potential in water with at least 3.6 mg L^{-1} easily degradable organic carbon, thus is more applicable for high fouling waters. The applicability of this method would be most prominent in current times since water sustainability technologies are geared towards the re-use of polluted waters so as not to further deplete traditional water sources.

Particles and colloids in feedwater for RO processes are typically removed by pretreatment to silt density index (SDI) allowable levels to prevent accumulation on membranes. However, the accumulation is mostly caused due to combined biofouling-particulate accumulation and it is important to quantitatively understand particle accumulation as affected by biofilm. Since biofilm formation cannot be avoided on membrane surfaces, its influence on the accumulation of inorganic suspended solids (SS) on

RO membranes needs further understanding. Thus, a part of this research aims to examine qualitative and quantitative information regarding the influence of biofilm on suspended solid (SS) accumulation. Continuous flow (without filtration) experiments of 1 μm filtered secondary effluent water and pure water (as control) with kaolin as representative SS particles were conducted and results indicate that organic matter (mainly coming from biofilm) deposited does not correlate with the amount of initial kaolin in the feedwater. However, inorganic matter amount deposited showed a correlated increase with initial kaolin amount in the feedwater. The rate of inorganic matter deposition is twice as fast when secondary effluent water was used as feedwater and showed a higher linear correlation (R^2 = 0.997) in contrast to pure water. With the same kaolin concentration contained on the feedwater, the amount of inorganic material deposited is greater by 0.16 mg/cm^2 when secondary effluent water was used in contrast to pure water, signifying quantitative enhancement of accumulated SS on the membrane. Amount of glucose in feedwater did not result in a related increase in inorganic material since deposition seemed to be influenced by biofilm coverage on a preformed biofilm, as indicated by similar biofilm percentage coverage with and without glucose in feedwater. Micrographs indicated the preferential deposition of SS on the spacer filaments and membrane areas that were covered with biofilm. The initial site for biofilm formation seemed to be a result of the continuous flow under no filtration conditions, thereby an almost negligible concentration polarization, which makes the biofilm in greater contact with the spacer rather than on the membrane surface. The SS preferentially deposited on the biofilm formed on the membrane surface again due to the greater contact of the biofilm to the SS flowing in the liquid. This effect of biofilm on inorganic SS accumulation will be highly useful in designing pretreatment strategies by addressing biofilm control to prevent both biofilm formation and SS accumulation.

One of the critical issues in membrane processes is the low tolerance of the RO membranes to oxidants like chlorine. But with the current trend of developing chlorine-resistant membranes direct chlorine washing will be a viable option to avoid biofouling on these chlorine-resistant membranes. Since it has been shown that particle accumulation is exacerbated by the presence of biofilm, particle accumulation has the potential of being prevented by biofilm control, specifically chlorine washing. Thus, expensive pretreatment for particle removal can either be removed or simplified, which could lessen associated cost in pretreatment operations. A part of this research, aims to determine the effective hypochlorite washing condition required for controlling biofilm formation as well as inorganic particle

accumulation on a RO membrane in a continuous flow channel with RO membranes and spacer. Results showed that comparable biofilm formation control can be achieved with continuous and intermittent washing (10 mg/L chlorine), but with lesser exposure to chlorine during intermittent washing. For 48 h of soaking tests, the fluorescence intensity, a measure of biofilm on the membrane surface, was 470 and decreased to 0 by hypochlorite washing with 10 mg/L chlorine concentration, 2 times/day washing interval, and 30 min washing time. Results showed that the chlorine concentration required to control biofilm formation decreased as the chlorine concentration (0.5–10 mg/L), the washing interval (1–4 times/d), or the washing time (1–30 min) increased. For the sample solutions used in the study, 10 mg/L chlorine concentration with 2 times/day interval, and 5 min washing time was required for biofilm control. The optimum hypochlorite washing condition obtained from soaking experiments proved to be applicable also in controlling biofilm formation in continuous flow experiments. In addition, based on calculation of CT (Concentration of free chlorine-time of exposure) values, the intermittent chlorine washing conditions are within the limits of allowed exposure conditions for commercial RO membranes within expected membrane lifetimes.

Particle accumulation control by chlorine washing experiments were done and results showed that for the sample water with kaolin and hypochlorite, the accumulation amounts were 0.03 mg/cm^2 for organic and 0.14 mg/cm^2 for inorganic, respectively, which were lower than that for sample water without hypochlorite (0.14 mg/cm^2 and 0.33 mg/cm^2, respectively). The amount of biofilm formed was 79% controlled by continuous washing with 10 mg/L of free chlorine concentration, and the inorganic accumulation amount was decreased by 58% to levels similar to that of pure water with kaolin (0.17 mg/cm^2). These results confirmed the acceleration of particle accumulation due to biofilm formation, and that the inhibition of biofilm growth can almost completely reduce further particle accumulation. In this research, it was shown that effective hypochlorite washing condition which can control both biofilm formation and particle accumulation could be achieved.

In the current state of membrane development, RO membranes cannot have the perfect resistance to free chlorine as long as polyamide (PA) is used as a membrane material due to its sensitivity to chlorine. Therefore, studies that deal on PA membrane degradation by chlorine is very important. Water sources and their quality are vital in the RO membrane processes, and determining the effects of the quality of the source on the PA membrane degradation will be helpful in designing and developing chlorine-resistant membranes. Since source waters have a wide array of metal ions and of variable concentrations, and because

metal ions have been reported to accelerate membrane degradation, part of this research aims to determine the enhancing effect of the coexistence of metal ions on PA membrane degradation by hypochlorite using a commercial PA membrane. The mechanism of the PA membrane degradation by hypochlorite was also examined. Results showed that the acceleration of membrane degradation by hypochlorite was caused by all monovalent (Na^+, K^+) and divalent metal ions (Ca^{2+}, Mg^{2+}, and Ba^{2+}) used in this study, as shown by the decrease in salt rejection and an increase in flux. The acceleration of PA membrane degradation was caused by divalent ions in much lower concentrations than the monovalent metal ions, indicating the potency of divalent ions in membrane degradation. The Na^+ did not accelerate the degradation of the PA membrane in less than 100 mM concentration, whereas $Mg^{2+}$and Ca^{2+} did not have a threshold limit. The membrane degradation in the presence of both monovalent and divalent metal ions seemed to be influenced by the threshold limit of the monovalent ion within the range of concentration of each of the ions present. Below that threshold limit, the divalent ion gives greater effect in the membrane degradation while higher than the threshold limit, the monovalent ion has a greater effect. The effect of chlorination was shown for both size exclusion and electric charge repulsion performance of the RO membrane. For the membrane degradation mechanism, as indicated by FTIR results and zeta potential analysis of the membranes, the degradation process of PA membranes does not change even if metal ions are present in the reaction. However, acceleration of amide hydrolysis was possibly catalyzed by the divalent ion leading to PA membrane degradation.

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Chapter 1: Preface

1.1 Brief introduction

1.1.1 The global water demand and the role of RO membrane technology

Water is a very essential part of human life: from consumption and daily living use, up to non-potable use for recreation, agriculture, and industrial applications. Freshwater distribution and availability through the natural water cycle like precipitation and run-off are very irregular worldwide due to the differences in seasons and location, resulting in significant variations in per capita water availability between countries and with some countries experiencing absolute scarcity in freshwater (Figure 1.1 (left), WWAP, 2015). The availability, demand, and use of water have also suffered huge impacts due to climate change, socio-economic development, and population growth (Arnell, 2004; Alcamo et al., 2007; Vörösmarty et al., 2010). Aside from population growth and urbanization, food and energy security policies, and macro-economic processes (trade globalization and changing consumption patterns) have influenced the global water demand, and due to the expected growing demand from major sectors such as domestic use, manufacturing, and thermal electricity, the demand is projected to increase by 55% by the year 2050 (WWAP, 2015).

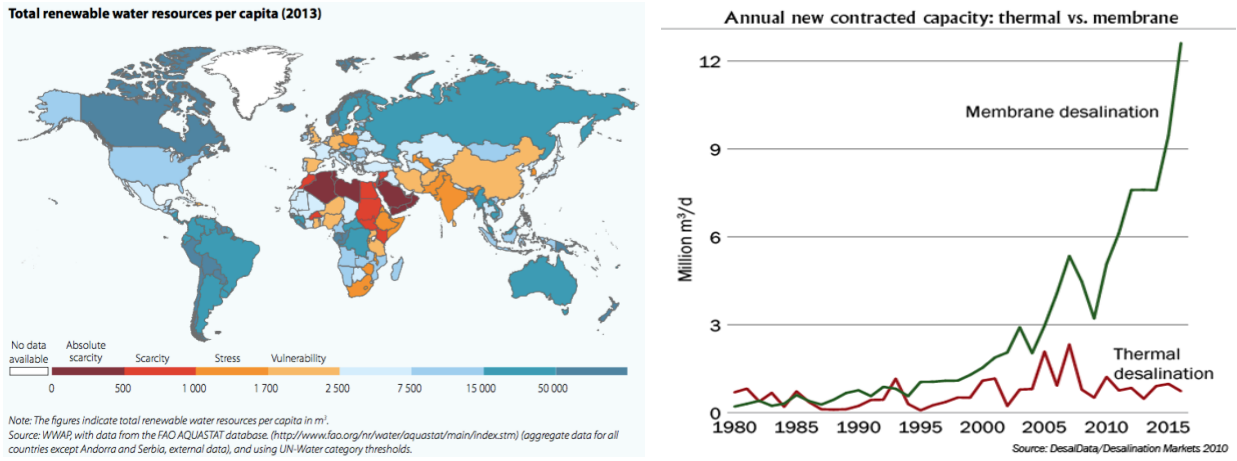

Figure 1. 1 (left) Total renewable water resources per capita in the world (2013) (adapated from WWAP, 2015). (right) Comparison between membrane-based and thermal-based desalination capacity (observed and projected from 2010) (adapted from Misdan et al., 2012; Source: Desaldata/Desalination Markets 2010).

Research studies and recent technologies are geared towards the management of existing water resources as well as development from alternative ones such as water reclamation, recycling, water reuse, desalination of seawater and inland saline aquifers, and others (Miller, 2006; Shannon et al., 2008). On a global scale, desalination, despite the limits posed due to energy concerns associated with the technology, has great development potential due to the following reasons (Ghaffour et al., 2013): firstly, large cities lacking fresh water sources are located along coastal regions, thus have ready access to sea water and secondly, desalination has a secure and unlimited source of water supply since feed water supply is unaffected by climatic conditions. Desalination can be categorized based on the mechanism of separation: 1) thermal processes (multi-stage flash, MSF; multiple effect distillation MSD; vapor compression distillation, VCD) and 2) membrane-based processes (electrodialysis, ED; nanofiltration (NF), reverse osmosis (RO) (Greenlee et al., 2009). Compared to the thermal-based processes, membrane desalination is increasing and is projected to increase exponentially in terms of capacity (Figure 1.1 (right) adapted from Misdan et al., 2012, Source:Desaldata/Desalination Markets 2010). This is due to substantial improvements and innovations towards reducing the costs of desalinated water compared to other water resources, particularly in the RO process (Ghaffour et al., 2013), with a reported 55% growth rate per year in desalination capacity (GWI/IDA DesalData, 2013). RO membrane desalination technology dominates up to 44% of the total world desalination capacity (Greenlee et al., 2009). The four major membrane suppliers, DOW, Hydranautics, Toray, and Toyobo are responsible for some of the state-of-the-art seawater reverse osmosis (SWRO) large scale desalination plants which are found in Perth in Australia, Llobregat in Spain, Tuas in Singapore, and Fukuoka in Japan, respectively (Lee et al., 2011). As of 2013, there are about 17,000 desalination plants worldwide (IDA, 2014) and the RO technology

accounts for about 60% of installed capacities (IDA, 2013). Compared to conventional thermal desalination, RO membrane systems do not suffer from corrosion, has the ability to use low grade heat, and has been shown to reduce energy consumption owing to efficient energy recover systems, and the development of more robust membranes (Shannon et al., 2008). The applicability of RO process has extended far and wide, originally for the desalination of seawater and brackish water (Fritzmann et al., 2007; Greenlee et al., 2009; Afonso et al., 2004) to treatment of different water types with varying inorganic and organic contaminants present in the feed water (Malaeb and Ayoub, 2011; Ang et al., 2011), more specifically in the treatment of municipal and industrial wastewater (Ridgway et al., 1983; Wilf and Alt, 2000; Bódalo-Santoyo et al., 2003; Ang et al., 2011). In the past, wastewater treatment is focused on pollution abatement but in the last two decades, there is an increased amount of municipal wastewater recovered for reuse (Levine and Asano, 2004). Global interests and efforts are also made in utilizing reclaimed wastewater for both potable and non-potable purposes (Levine and Asano, 2004; Toze, 2006). Although conventional treatment can produce water to meet existing regulations, information of its effectiveness to control harmful trace contaminants are limited such that advance treatment technologies such as membrane bioreactors, microfiltration (MF), ultrafiltration (UF), NF, and RO proved to be more effective for wastewater reuse (Levine and Asano, 2004). The RO membrane process plays a vital part in this goal due to its higher rejection of impurities, essentially producing clean water only compared to other membrane-based technology (Figure 1.3, adapted from Van der Bruggen et al., 2003), and its production of higher quality water at a lower cost (Pandey et al., 2012). RO technology's capability and its acceptance for wastewater reclamation purposes had been validated due to the presence of large-scale commercial RO membrane plants. These 6 large-scale plants are found in West Basin and Orange County in California, Kranji, Bedok, and Ulu in Singapore, and in Sulabaiya, Kuwait (Bartels, 2006).

The range and scope of studies in RO membrane technology encompass the whole process; from applicability of the RO process to different types of water until post treatment of clean water, despite the challenges posed during operation (Greenlee et al., 2009; Malaeb and Ayoub, 2011). For both desalination and water reclamation and reuse, the application of RO membranes faces a significant challenge because of the decline in membrane performance due to membrane fouling.

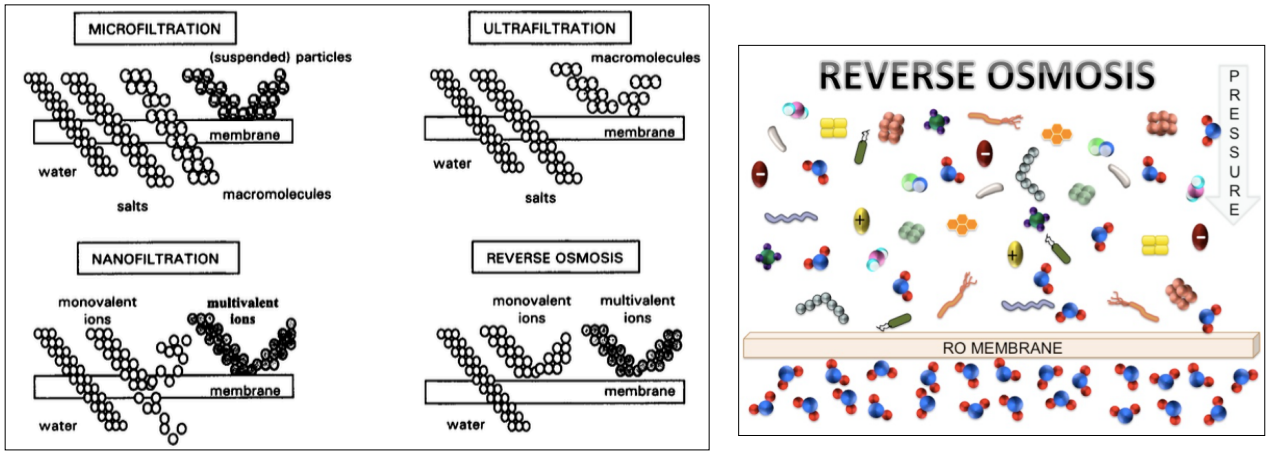

Figure 1. 2 (left) Comparison of membrane-based processes based on rejection of water components (adapted from Van der Bruggen et al., 2003). (right) Clean water production of RO process

1.1.2 Biofouling

1.1.2.1 Biofouling: definition and mechanism

Major challenges that RO desalination face are relatively low recovery for sea water desalination (less than 55%), which results in large volumes of concentrated brine, relatively low removal of low-molecular-weight contaminants, particularly boron in sea water, and membrane fouling (Shannon et al., 2008). Membrane fouling is the accumulation of unwanted substances on the membrane surface and remains a critical issue to the desalination industry worldwide since it decreases performance efficiency of the RO process. Figure 1.3 shows the main types of membrane fouling which are crystalline fouling/scaling, organic fouling, particulate and colloidal fouling, and microbiological fouling or biofouling, which

are categorized based on materials responsible for the fouling (Flemming, 1997), such as sparingly soluble inorganic compounds, dissolved and macromolecular organic substances, suspended and colloidal particles, and microorganisms, respectively (Pandey et al., 2012).

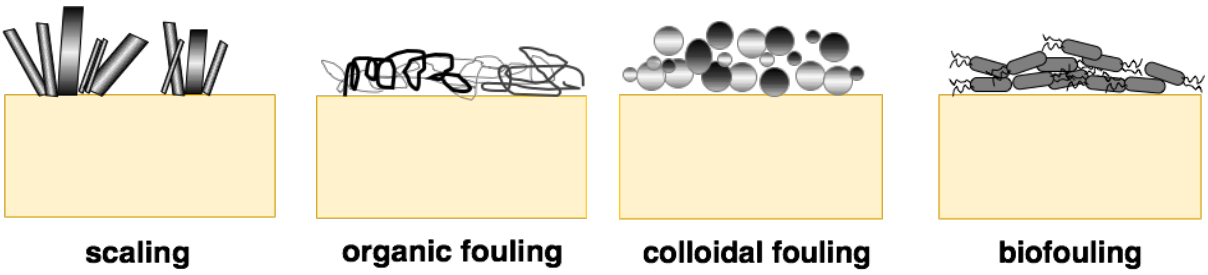

Figure 1. 3 Membrane fouling types.

Membrane fouling is a complex and dynamic process involving many steps in the development of the fouling layer. During organic, inorganic, and colloidal fouling, a rapid initial step involves foulant-membrane interactions and then a gradual long term-step, which involves foulant-foulant interactions (Tang et al., 2009) while biofouling involves conditioning, cell attachment, cell growth, and then cell dispersion (Khan et al., 2013). Biofouling refers to the deposition and growth of biofilms to the point that their presence on the membrane systems lead to performance decline (Flemming, 1997). A biofilm is a complex assembly of sessile microbial communities permanently attached to the membrane due to the presence of self-produced extracellular polymeric substances (EPS), which are mainly polysaccharides and proteins (Kwan et al., 2015). Steps in biofilm formation along with the deposition of organic substances as shown in a diagram by Khan et al., 2013 (Figure 1.4) include 1) the surface conditioning film formation (initial phase), which is the rapid accumulation of humic substances and polysaccharides (predominantly α-linked) on the membrane surface, with accompanying cell adhesion and adsorption of cells on the membrane surface serving as the starting point for growth, 2) the microbial growth (intermediate) phase, which involves production of more polysaccharides and formation of

microcolonies, and then the 3) biofilm phase, wherein microcolonies grow into macrocolonies while embedded in released EPS. The last phase is the plateau phase, whereby biofilm growth is limited either by effective shear forces (So et al., 2015) or by nutrient concentration (Hunt et al., 2004), the resultant growth rate, and the mechanical stability of the biofilm (Matin et al., 2011).

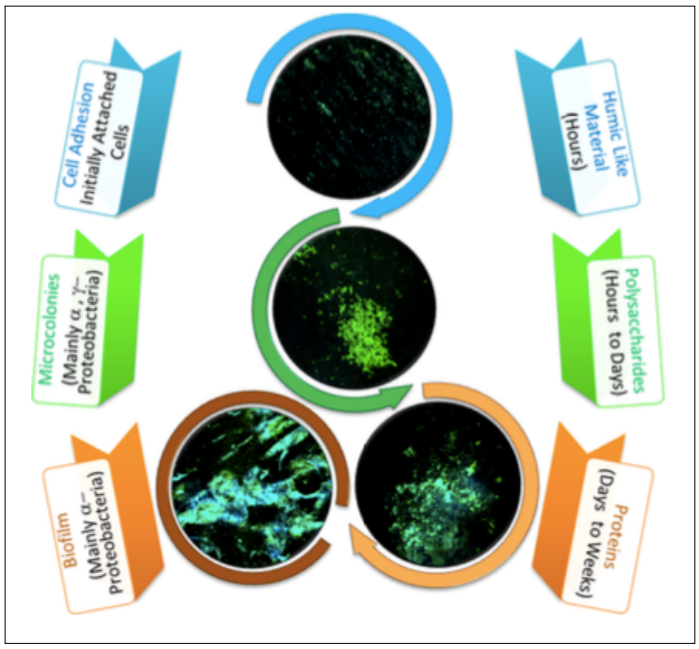

Figure 1. 4 Changes in abundance of organic and microbial components of fouling layer occurring during biofilm formation on SWRO membrane (adapted from Khan et al., 2013).

It is generally accepted that initial bacterial adhesion is a key part in the biofilm development process, which eventually leads to biofouling. Factors that influence bacterial adhesion include bacterial characteristics (hydrophobicity, surface charge, bacterial surface structure), membrane characteristics (surface hydrophobicity, membrane surface charge, membrane chemical composition, roughness, and surface morphology and microtopography), and operational/environmental conditions (conditioning layers, permeate flux, and hydrodynamics and mass transport) (Habimana et al., 2014). A hypothesized mechanism of the factors affecting initial adhesion between cells and membrane during NF/RO filtration processes was proposed by Habimana et al., 2014 (Figure 1.5) and involves the following: 1)

During the filtration process involving permeate flux and high pressures, divalent cations, organic matter, and microorganisms are concentrated onto the membrane surface as the feed water passes, a phenomenon referred to as concentration polarization (CP), which leads to an increase in the osmotic pressure of the feed, resulting in a reduced water flux. As filtration progresses, a gradual flux decline is observed due to the accumulation of foulant on the membrane surface; 2) Relevant to the initial interaction between bacterial cell and the surface of the membrane are the membrane material properties. Membrane roughness enhances bacterial adhesion and low electronegative surface charge and high surface hydrophobicity have been shown to be correlated to high bacterial adhesion; 3) The bacterial cell wall properties can influence bacterial adhesion by the presence of substances that enhance irreversible adhesion; 4) Environmental factors (temperature, pH, salt concentration, and the presence of signal molecules) are known to induce a number of different mechanisms at the cell level that might induce adhesion.

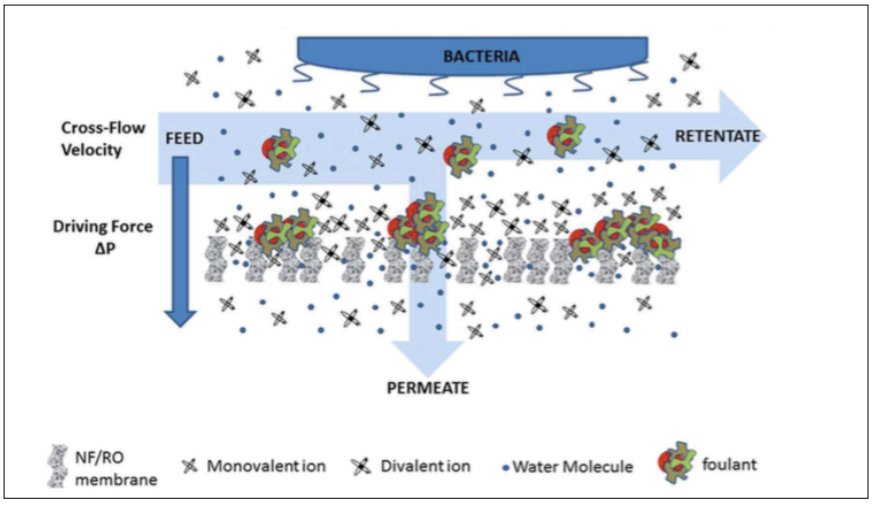

Figure 1. 5 Schematic outline of nanofiltration and reverse osmosis process operation, including fouling components and salts, the direction of cross-flow and permeate flow, the concentration polarization effect and the presence of microbes (adapted from Habimana et al., 2014)

Although, initial bacterial adhesion onto the membrane surface is a critical step in membrane biofouling, the conditioning stage which involves adsorption of effluent organic

matter onto the membrane surface may precede bacterial deposition, and change the virgin membrane surface properties, such that an initial decrease in membrane productivity is more likely due to direct organic fouling (i.e., gel formation) and biofouling predominates at a later time when mature biofilms have developed (Subramani et al., 2009). This is why pretreatment of RO feed water is an important part in the RO operation process (Figure 1.6), which is mainly designed to reduce microorganisms and particulate matter.

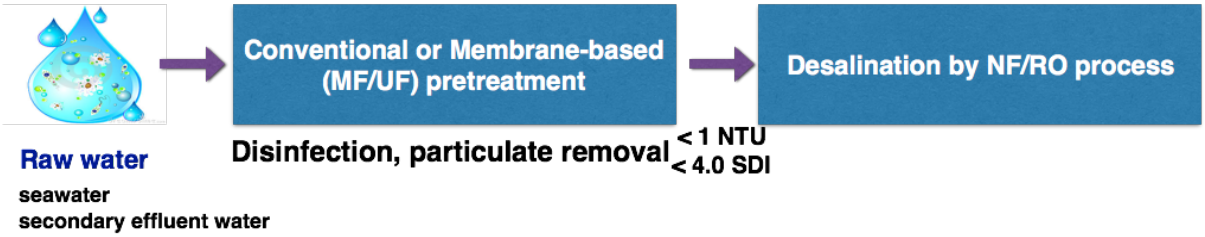

Figure 1. 6 Schematic diagram of the RO process.

In order to prevent particulate fouling by large and visible particles, RO feed water needs to have turbidities of less than 1 NTU (= 1 mg/L using kaolin standard), and silt density index (SDI) of less than 4.0 (Jamaly et al., 2014). However, deposition of nano- or colloidal particles as carryover from pretreatment (Ning and Troyer, 2007) can lead to colloidal fouling in NF or RO membrane units (Ning and Troyer, 2007; Kim et al., 2008; Xu et al., 2010). Colloidal particles, in particular, have high tendency to aggregate and its removal from membrane surface becomes more problematic when they are coated with organic foulants or embedded in biofilms (Xu et al., 2010). During continuous pressure-driven membrane filtration, the membrane inherently retains dissolved substances, which then accumulates (phenomenon of CP) (Matin et al, 2011). It has been reported that biofilm enhanced crystal growth and nucleation rates, suggesting the synergistic influence of biofilm on mineral scaling (Thompson et al., 2012). This presence of different types of foulants and the accompanying occurrence of multiple fouling mechanisms result in multiple fouling layers on NF and RO membranes used for the treatment of produced water from the

petroleum industry for reuse as a potential water resource, although biofilm formation dominated over organic fouling (Alzahrani, et al., 2013). Since the presence of biofilm has been suggested to enhance other types of fouling, biofilm formed on the RO membrane surface can eventually lead to membrane fouling, which has a significant correlation to RO membrane performance decline.

1.1.2.2 Effects of biofouling on membrane performance

Biofouling is a consequence when the biofilm on the membrane surface interferes with the membrane performance. For membrane systems like RO, the biofilms interfere in the separation process and gives rise to enhanced concentration polarization, increased hydraulic resistance, decreased membrane permeability, and decreased salt rejection but in terms of process efficiency, the decline in permeate flux and decrease in salt rejection are considered to be the main concerns (Matin et al., 2011).

The kinetics of flux decline usually shows an initial rapid decline typically correlated with the early attachment and growth of microorganisms on the membrane surface and a slow decline (plateau) phase whereby an equilibrium condition between biofilm growth and EPS production, and biofilm loss is taking place (Flemming and Geesey, 1991). Rather than a result of changes in the inherent properties of the membrane, the flux decline is most likely a consequence of the biofilm acting as a transport barrier leading to increased hydraulic resistance to water transport (Matin et al., 2011). Studies related to fouling mechanism reported that bacterial cells and EPS play major roles in the flux decline (Herzberg and Elimelech, 2007; Chong et al., 2008; Huertas et al., 2008). Increase in transmembrane pressure (TMP) is a result of a hindered back diffusion of salt, elevating the osmotic pressure on the membrane surface due to the presence of biofilm, resulting in permeate flux decline (Herzberg and Elimelech, 2007; Chong et al., 2008; Huertas et al., 2008). The associated EPS

surrounding the biofilm, on the other hand, increases the hydraulic resistance to permeate flow (Herzberg and Elimelech, 2007; Huertas et al., 2008), contributing to the decline in flux. A similar mechanism has been reported to be responsible for the decrease in salt rejection, wherein EPS and biofilm have significant contributions (Herzberg and Elimelech, 2007; Matin et al., 2011). Because the biofilm consists of a viscous EPS matrix that can suppress turbulent mixing at the membrane surface, a boundary layer can form and stabilize between the membrane and the solution, which favors the accumulation of dissolved salts (Matin et al., 2011). The presence of bacterial cells in the EPS matrix hinder back diffusion of salts thereby increasing the salt concentration near the membrane surface (Herzberg and Elimelech, 2007). These phenomenon result in enhanced solute transport through the membrane due to the increased ionic activity in the boundary layer (Matin et al., 2011), leading to a decrease in salt rejection.

In addition, adverse effects of biofilm growth on boron rejection were attributed to both an increase in hydraulic resistance to permeate flow due to bacterial extracellular polymeric substances (EPS) and a BEOP near the membrane surface (Huertas et al., 2008). The decrease in salt rejection can also be attributed to biodegradation or biodeterioration of the RO membrane due to the presence of bacteria, fungi, and other microorganisms in the biofilm which may directly (via enzyme) or indirectly (via localized pH or redox potential changes) degrade the membrane polymer, which were specifically reported for cellulose acetate (CA) membranes, but nonetheless could occur on the noncellulosic aromatic or aliphatic cross-linked polyamide or other thin film composite (TFC) membranes due to the presence of aromatic and amide groups that could serve as nitrogen and reduced carbon sources for microbial growth (Matin et al., 2011).

1.1.2.3 Biofouling potential tests

Knowing the characteristics of the RO membranes that could affect biofilm formation is a huge advantage in choosing the best RO membrane to use in membrane filtration treatment for desalination and wastewater reclamation applications. However due to the inevitability of fouling events on RO membrane surfaces, a variety of fouling potential tests are used to be able to diagnose the propensity for RO membranes to foul. Biofouling potential tests usually involve the use of chemical and biological quality parameters of the water as indicators of biofouling. SDI (Schneider et al., 2005; Sauvet-Goichon, 2007; Teng et al., 2003; Huang et al., 2013; Jamaly et al., 2014), total organic carbon (TOC) (Pandey et al., 2012; Teng et al., 2003; Huang et al., 2013), and turbidity (Teng et a.l, 2003; Berman et al., 2011; Huang et al., 2013; Jamaly et al., 2014) are used to determine the quality of raw feed water or of pretreated water before the RO unit. Biological parameters of the feed water quantifying substances that can promote bacterial adhesion and growth such as chlorophyll (Berman et al., 2011), transparent exopolymer substances (TEP) (Berman et al., 2011), assimilable organic carbon (AOC) (Schneider et al., 2005; Vrouwenvelder et al., 2008) and parameters quantifying microorganisms in the feed water such as total direct cell counts (Vrouwenvelder et al., 2008), heterotrophic cell counts (HPC) (Vrouwenvelder et al., 2008), adenosine triphosphate (ATP) for active biomass (Veza et al., 2008; Vrouwenvelder et al., 2008) are more related to biofilm formation but the presence and/or concentration of microorganisms and the precursors in the feed water does not fully indicate biofouling occurrence (Flemming, 1997) nor low values signify absence of biofouling (Vrouwenvelder et al., 2008). Similar to chemical water parameters, the biological parameters from the feed water and its relation to biofouling or fouling in general are also location-dependent (Schneider et al., 2005; Huang et al., 2013; Berman et al., 2011).

Biofouling occurrence is not only determined by characteristics of the feed water but

also by the properties of the membrane, characteristics of feed spacers, and hydrodynamic conditions of the membrane (Schneider et al., 2005; Herzberg and Elimelech, 2007; Vrouwenvelder et al., 2008; Khan et al., 2010; Khan et al., 2011; Chen et al., 2013; Suwarno et al., 2014). For about 30 years since after the 1980's, many studies have been conducted in the field of fouling reduction and improvement of membrane performance through development and modification of RO membranes (Lee et al., 2011). Therefore, biofouling indicators for assessment of developed, modified, and commercially available membranes are expected, which could be obtained through analysis of fouled membranes.

Microscopic techniques after staining or labeling of cells are typically used for the analysis of fouled membranes, particularly for bacterial enumeration and biofilm characterization on the membranes (Ridgway et al., 1983; Vrouwenvelder et al., 2008; Berman et al., 2011; Huang et al., 2013; Nguyen et al., 2012; Xu et al., 2013). Fluorescence microscopy and the use of fluorescent stains have gained popularity in favor of the traditional plate counting methods owing to the deficiency of these culture methods to examine physiological activity and species diversity of many microbial communities especially on complex matrices like biofilms (McFeters et al., 1995) and the enumeration of only culturable active, bacteria which are able to initiate cell division at a sufficient rate to form colonies (Boulos et al., 1999). Fluorescent dyes commonly used in biofilm analysis are acrydine orange (AO), 4',6-diamidino-2-phenylindole (DAPI), both used for total bacterial count, 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), for determination of active bacteria, and SYTO 9 and Propidium Iodide (PI) pair for live and dead cells determination, respectively (Schneider et al., 2005; Huang et al., 2013; Berman et al 2011; McFeters et al., 1995; Boulos et al., 1999; Seo et al., 2010; Berney et al., 2007; Biggerstaff et al., 2006; Khan et al., 2011). Estimations by AO and Baclight of total bacterial numbers (TBN) were relatively accurate and interchangeable for quantitative interpretation, while DAPI staining showed

underestimation of TBN (Seo et al., 2010). Boulos, et. al. (1999) cited that reliability, rapidness, ease-of-use, and determination of viable and total counts in one step are clear advantages of SYTO 9 and PI to the other fluorescent dyes in the analysis of drinking water samples. Despite certain disadvantages found in using Baclight for complex environmental samples (Berney et al., 2007; Biggerstaff et al., 2006), it has found its use for analysis of biofouling on fouled membrane analysis in conjunction with microscopic techniques, due to its one step capability in differentiating between live and dead cells. Biofouling was confirmed by confocal laser scanning microscopy (CLSM) investigation of membrane biofilm thickness and live and dead bacterial cell counts (Huang et al., 2013) while characteristics of bacterial components such as biovolume, percent surface coverage, surface to biovolume ratio, thickness and roughness were determined by CLSM after SYTO 9 staining (Berman et al., 2011). Epifluorescence microscopic analysis of fouled RO and nanofiltration (NF) membranes stained with SYTO 9 and PI were characterized and the effects of biofouling on the membrane surfaces were also differentiated (Khan et al., 2011). All of these show that fluorescence microscopic analysis has become a powerful detection tool for biofouling propensity.

1.1.3 Biofouling control by hypochlorite on polyamide reverse osmosis membranes

Since biofilm formation is inevitable once the water comes in contact with the membrane surface, Flemming (2002) suggested that the key to prevent biofouling is to “keep biofilm development under control” and he described this as “biofilm management”. In this way, biofilm is controlled in such a way that its growth is limited to the point where its presence will not interfere with the performance of the membrane.

1.1.3.1 Biofouling control strategies

Prevention and control of biofouling has been achieved by one or a combination of the following methods (Greenlee et al., 2009; Flemming, 1997; Pandey et al., 2012; Flemming, 2002; Kim et al., 2009), i) conventional and advanced/membrane treatment, ii) biocide application, iii) membrane cleaning, and iv) surface modification. The main goal of any pretreatment process is to reduce the tendency of fouling by the feed water, may it be complete removal of the unwanted material or its reduction to acceptable levels (Greenlee et al., 2009; Prihasto, et al., 2009). For biofouling, pretreatment means controlling the factors that support biofilm growth, such as limiting the access of microorganisms to the membrane surfaces, and though shear forces limit excessive biofilm development, nutrients should be considered as potential biomass, and that nutrient limitation is the key to biofilm growth (Flemming 2002). Conventional pretreatment methods include acid addition, coagulation/flocculation, media filtration, cartridge filtration, and disinfection (Ridgway et al., 1983; Sauvet-Goichon, 2007). Although conventional pre-treatment systems applied in various seawater reverse osmosis (SWRO) plants in several parts of the world can still meet the feed water quality standards for the RO process, problems arising during the RO process and related operational costs (Prihasto et al., 2009) and its inability to reduce fouling in the RO unit (Wilf and Alt, 2000; Schneider et al., 2005) has furthered research in this area into advanced pretreatment methods using microfiltration and ultrafiltration or hybrids of these treatments (Afonso et al., 2004; Wilf and Alt, 2000; Prihasto, et al., 2009; Teng et al., 2003; Kim et al., 2009). Dosing feed water continuously with biocides or antimicrobial substances is another method used for biofouling control (Schneider et al., 2005; Kim et al., 2009; Berman et al., 2011). Several review articles give detailed description and modes of action of existing disinfecting agents (chlorine, ozone, UV) (Flemming, 1997; Pandey et al., 2012; Flemming, 2002; Kim et al., 2009; Nguyen et al., 2012). There are two main applications of

biocides in membrane systems. The biocide may be added continuously or intermittently (e.g., via a metering pump) to the feed water to suppress or control biofilm growth on the membrane surfaces and during extended periods of membrane storage or plant shutdown, the biocide is used to preserve the polymer membranes and related module components (e.g., glues, plastic spacers, other materials of construction) (Matin et al., 2011). A recent study reported the development of a new disinfectant (Yu et al., 2013) that is more effective in disinfection with less adverse effects to the environment or to the membrane.

For long-term water treatment, fouling of the membrane cannot be avoided such that membrane cleaning is part of the RO operation. Its main aim is to restore the membrane performance to an acceptable level either in terms of permeate flow and/or salt rejection at a reasonable cost (Prihasto, et al., 2009). Cleaning, whether by mechanical or chemical/biochemical specifically ensures to overcome the adhesion of the biofilm to the surface and the cohesion forces which keep the biofilm together, so that biofilm can be dispersed (Flemming 2002; Flemming 2011). Two general strategies include addition of chemical agents to weaken the biofilm matrix or by employing physical processes to remove the biofilm (Ang et al., 2011; Flemming, 1997; Nguyen et al., 2012).

Aside from developing cleaning strategies and pretreatment methods, RO studies delved into developing membranes with enhanced desalination performance (Lee et al., 2011), as well as improved antifouling properties (Kang and Cao, 2012; Saeki et al., 2013; Xu et al., 2013). This focus on membrane surface modification aims to prevent or slow down bacterial adhesion, microcolony formation, and biofilm maturation, which are important factors that lead to biofouling (Matin et al., 2011).

As a summary, Flemming (2011) has perfectly described an integrated anti-fouling strategy (Figure 1.7), which involves the following: 1) Feed water before coming into the RO unit should have very low bacterial count that could potentially start biofilm formation. 2)

Since 100 % removal of bacteria is very ideal, membrane surface should be improved or modified so that microorganisms that are present in the feed water will be prevented in attaching to membrane surface, thus avoiding biofilm formation. 3) In the course of biofilm formation on the membrane surface, biofilm growth should then be avoided by limiting the nutrients in the feed water that could support microbial activity and growth. 4) Surface membrane cleaning strategies should be done, both to ensure that biofilm which has formed are removed and that its dispersal will not serve as nutrients for viable microorganisms that remain in the feed water. 5) Monitoring of membrane surface should be done to provide early warning signs of biofilm growth at a point wherein biofilm that has formed can be easily removed by membrane surface cleaning.

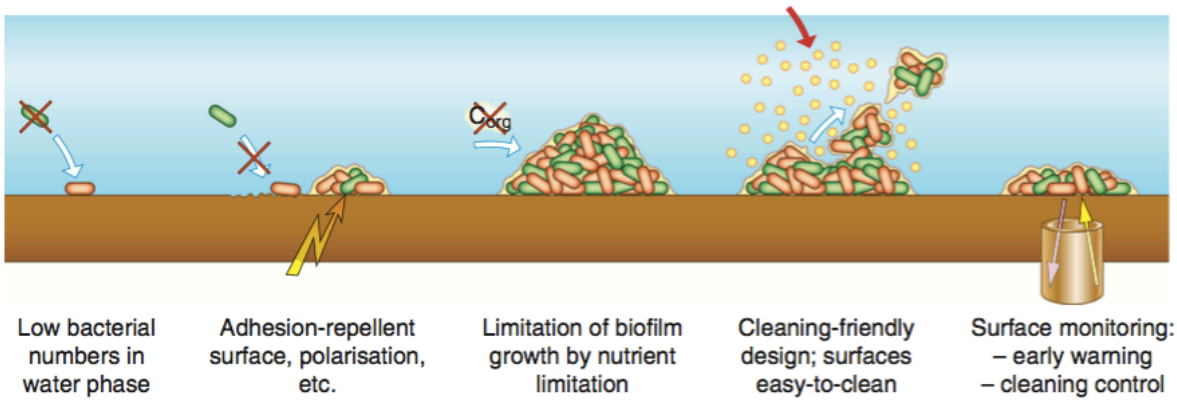

Figure 1. 7 An integrated fouling control strategy (adapted from Flemming 2011).

1.1.3.2 Reverse osmosis membranes

Membrane materials are the key determinants for the RO separation performance efficiency and water productivity. Cellulose acetate (CA) and polyamide (PA) are the leading membrane materials used for this purpose. However, future RO desalination membranes will ideally have high water flux per unit of pressure applied, near-complete rejection of dissolved species, low fouling propensity, and high tolerance to oxidants used in pretreatment for biofouling control (Shannon et al., 2008). From the discovery of CA in the late 1950s, to its

popularity as the best membrane material for RO until 1969, highlights in the major development of asymmetric CA membranes up to the 1980s was focused on the improvement of CA membrane transport properties and its capability for industrial applications, such as its stability in a wider range of temperatures and pH, higher resistance to chemical and biological attack, controlling severe flux decline, higher permeability and selectivity, and greater resistance to compaction (Lee et al., 2011). However, since acetate group in these membranes are chemically susceptible to hydrolysis under acidic and alkaline conditions, and the membranes are prone to microbial contamination, the CA membranes' applicability and durability is restricted (Edgar et al., 2001). Polyamide thin-film composite (PA-TFC) membranes, discovered in the late 1970's, have emerged as the premier technology for the production of pure water through desalination (Cadotte, et al., 1980). However, despite its very high salt rejection (99.4 – 99.8%) at standard test conditions of 32,000 ppm NaCl solution at 5.5 MPa and 25 °C and greater water permeability compared to other asymmetric membranes, TFC membranes have low boron rejection efficiencies, are easily fouled, and are sensitive to chlorine (Misdan et al., 2012).

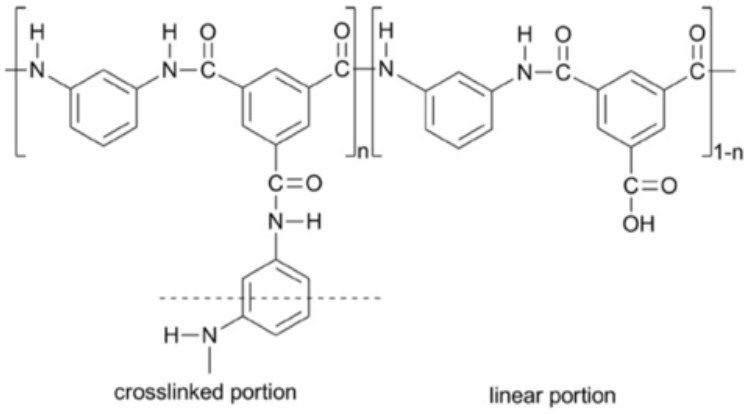

Figure 1. 8 Structural formula of crosslinked aromatic polyamide (adapted from Xu et al., 2013).

Due to the adverse reaction of PA RO membrane with chlorine, research studies focusing on the development and preparation of the membrane that are more resistant to

chlorine without sacrificing its flux and salt rejection capabilities has spanned for about 20 years. Since the nitrogen functional groups and the aromatic rings in the membrane are sensitive to chlorine attacks (Arthur, 1989; Glater, et al., 1994), improvements and modifications are geared towards the use of suitable starting materials for the synthesis of the chlorine-resistant polyamide membrane (Shintani, et al., 2007). After the 1980s, instead of finding the optimized polymeric membrane material, RO membrane performance has been improved through modification of chemical reactions for membrane formation and through the utilization of poly-condensation catalysts and additives, until the present wherein the emergence of nano-technology, including inorganic membranes (such as zeolite membranes), thin film nano-composite membranes, carbon nano-tube membranes, biomimetic membranes have been considered as new materials for enhanced RO desalination performance, despite problems associated to their practical implementation (Lee et al., 2011). For example, RO membranes are either developed through novel fabrications (Kim et al., 2003; Saeki et al., 2013; Gong et al., 2015) or improved through surface modification (Xu et al., 2013; Blok et al., 2014; Karkhanechi et al., 2014; Matin et al., 2014).

1.1.3.3 Polyamide membrane degradation by hypochlorite

When biofilms have settled on the membrane surface, removal by treatment with antibiotics, oxidizing agents, or biocides are extremely difficult due to the resistance of biofilm and detached biofilm clusters provided by EPS (Flemming and Wingender, 2010; Xue et al., 2012). Thus, free chlorine injected at the head of a pretreatment process in the form of chlorine gas (Cl_2) or sodium hypochlorite (NaOCl) (Kim, et al., 2009) is a standard pretreatment to control biofouling in the RO membrane processes. However, the amide linkage in PA RO membranes is susceptible to chlorine attack. The free chlorine is believed to initially attack the amide nitrogen, replacing the hydrogen with chlorine, forming a N-

chlorinated amide in a reversible and instantaneous reaction followed by an irreversible, ring chlorination reaction via Orton rearrangement (Glater, et al., 1994; Kawaguchi and Tamura, 1984). Glater, et al., (1994) cited another mechanism, which is a direct ring chlorination reaction governed by electrophilic aromatic substitution. More recent studies suggest the possibility of a competing mechanism between N-chlorination and hydrolysis of the amide C-N bonds in the chlorination of fully aromatic polyamide membranes, with the dominant mechanism prevailing depending on the chlorination conditions (Do et al., 2012; Donose, et al., 2013). Figure 1.8 shows the proposed membrane mechanism by hypochlorite (left, adapted from Kang et al., 2007) and the competing mechanism between N-chlorination and hydrolysis (right, adapted from Do et al., 2012).

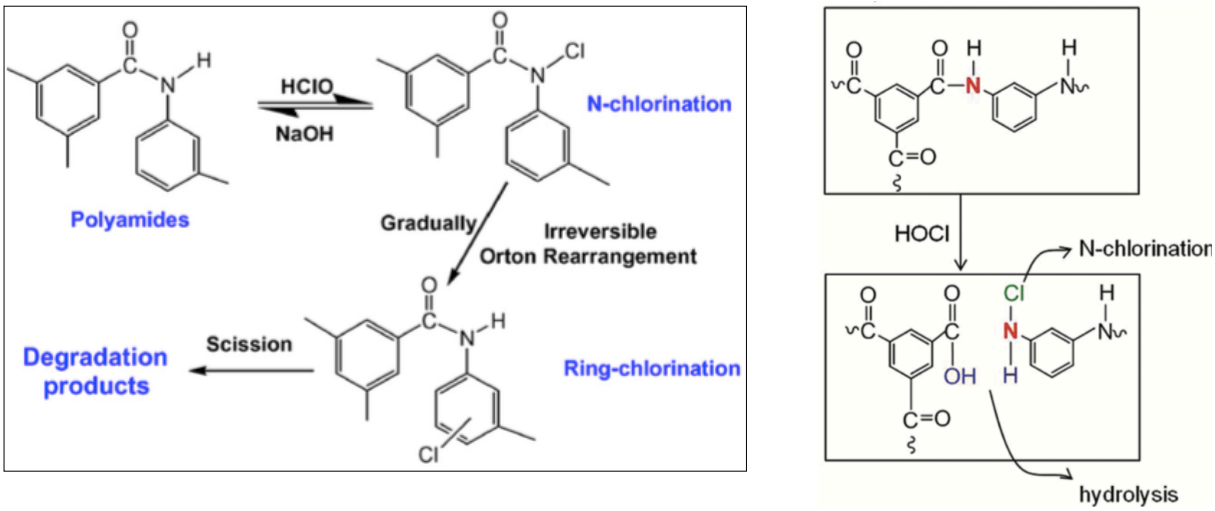

Figure 1. 9 (left) Proposed mechanism of hypochlorite degradation of polyamide membrane (adapted from Kang et al., 2007); (right) Proposed competing mechanism between N-chlorination and amide hydrolysis (adapted from Do et al., 2012).

Numerous studies document the decline in PA RO membrane performance due to various factors of chlorinating conditions such as chlorine concentration, pH, exposure time, as well as means of exposure. Higher permeability and increased salt passage were attributed to the effects of chlorination whereby intermolecular hydrogen bonds were disrupted and the crystalline regions of the polyamide were transformed to an amorphous state (Avlonitis, et al., 1992; Kang, et al., 2007; Antony, et al., 2010). Studies have shown the dependency of the aromatic chlorination on the pH during chlorination and that aggravated effects on membrane

permeability, were observed in low pH where the main oxidizing chlorine species are hypochlorous acid (HOCl) and aqueous chlorine (Soice, et al., 2003; Kwon, et al., 2006; Ettori, et al., 2011; Donose, et al., 2013). Upon exposure of membranes to a dose of ca. 400 ppm-h of HOCl, Ettori, et al. (2011) found that a permanent RO membrane performance loss in terms of water permeability and salt rejection is expected. Oxidative degradation conditions on which the membranes are exposed showed differences in membrane performance depending on the degree of exposure (active (pressurized and stirred) versus passive (unpressurized and unstirred) exposure). A significant change in permeability and salt rejection was observed under pressurized conditions and only permeability was affected under passive conditions (Antony, et. al., 2010). Gu et al., (2012) found that chlorination done in a pressurized mode showed decreased water flux, regardless of pH conditions. Thus, depending on the chlorinating conditions, the degree of damage on the PA RO membrane varies.

Studies also indicate that the presence of metal ions, such as ferrous iron enhance the oxidation of the PA RO membrane (Gabelich, et al., 2005), and that despite the higher tolerance of the membrane to chloramine solutions, another disinfecting agent used in water treatment, the presence of other metal ions (copper, aluminum, and iron) seem to catalyze the formation of chloramine radicals, which eventually attacks the polyamide structure of the membrane (Gabelich, et al., 2005; Cran, et al., 2011).

1.2 Significance of the study

RO membrane technology is originally used as the desalination process for seawater and brackish water desalination for pure water production. However, with the steady increase in the global demand for potable and non-potable water, its application has extended to the treatment of highly polluted water for the purpose of water reuse and wastewater reclamation. Thus, recent trends in studies and research regarding the RO process target the effective and

optimized performance of the RO membrane technology to suit the goals of the RO treatment, whether by desalination or wastewater reclamation. Membrane performance decline has been associated with membrane fouling wherein biofouling is considered to be the most problematic since it is difficult to prevent and control. Because microorganisms are ubiquitous in the environment and biofilm formation cannot be avoided on membrane surfaces, they play a big role in fouling of RO membranes. Thus a diverse area of research in RO membrane technology is directed towards a better understanding, prevention, and control of biofouling. A fast, easy, and suitable method of analysis that detect the propensity for biofouling is a key ingredient in biofouling studies. Existing tests involve the use of chemical and biological quality parameters of the feed water, biological determinations of substances that promote bacterial adhesion or growth, and microbial concentrations in the feed water. However, even when feed water quality has passed the standards required by membrane manufacturers, biofouling has still been observed suggesting that feed water quality alone is inadequate in giving biofouling potential. Evaluation of the changes in membrane and feed spacer properties and hydrodynamic conditions of the membrane system usually involve *in situ* determinations and thus the RO process is ongoing and diagnosis for biofouling might come in too late. In addition, autopsy analysis of fouled membranes entails stopping the operation since the membranes have to be analyzed. Thus, a biofouling potential test that can be done quickly with sample pieces of the RO membrane and the sample feed water to be used would make the biofouling potential determination much less complicated. Membrane and bacterial interaction has been shown to play a major role in biofouling occurrence in membrane surfaces, and thus direct evaluation of biofouling potential based on the formed biofilm on the RO membrane has more bearing on biofouling propensity since the evaluation incorporates the biofouling potential of the feed water type used and the biofouling potential of the RO membrane. Thus, a part of this research is the development of a method/test that

evaluates biofouling potential easily and rapidly. The novelty of this biofouling potential test lies on the direct analysis of the biofilm formed on the membrane used, which to our knowledge does not currently exist.

The problem of biofouling is significantly challenging when the purpose of RO treatment is wastewater reclamation. Secondary effluent wastewater has very high fouling potential due to the presence of suspended solids (SS), colloidal and organic matter, and high level of biological activity. And despite the effectiveness of existing pretreatment technologies, presence of biofilm formed on the membrane surface can have an enhancing effect on other foulant materials, resulting in composite fouling that can lead to performance decline. In this regard, it is essential to gain qualitative and quantitative information on how biofilm affect other impurities to understand composite fouling in RO processes. Knowledge of this interaction would have an impact on the modification/improvement or creation/design of pretreatment strategies, such that the focus of pretreatment could be on biofilm control. In this way, control of biofilm can result in controlling or preventing the deposition or accumulation of other foulant materials. Thus, a part of this research deals with the study of the effect of biofilm on inorganic SS accumulation on reverse osmosis membranes and spacer under non-filtration conditions. Another part of the study is biofilm control and the related effects of biofilm control on inhibition of SS accumulation.

The options for biofouling control vary from conventional and advanced/membrane pretreatment, membrane cleaning, biocide application, and membrane surface modification. Numerous studies have been done to control biofouling through conventional and advanced/membrane pretreatments but a 100% bacterial removal from feed water is yet to be found. This is especially difficult since biofouling itself is a complex and complicated phenomenon. Since biofilm formation cannot be avoided particularly in long-term water treatment, membrane cleaning is part of the RO operation to restore the membrane

performance to acceptable levels. Frequent membrane cleaning however incurs greater operational costs and if the membrane cannot produce the required amount or quality of the treated water, membrane replacement has to be done. Biocide application, in the form of free chlorine, is a standard practice to disinfect feed water, whereby the levels of bacteria in the feed water are reduced. However, the use of chlorine in treatment, especially when PA RO membrane is used, brings about problems due to the degradation effect of chlorine to the PA. Thus, investigations are being done either in the development of new disinfectants which are less harmful to the environment or to the membrane, development of new robust membrane that is more tolerant to chlorine and other biocides, and development or modification of the PA RO membrane surface to lessen the adverse effect of chlorine. Part of this research deals with the study of the effect of metal ions in the degradation of PA RO membranes treated with hypochlorite. This study is important to the understanding of the damage that could be done on the PA membrane during biofouling control of seawater for RO desalination applications, especially in the coexistence of metal ions that are naturally found in seawater. Since chlorine is the standard reagent used for disinfection, part of this study also deals with the optimization of various washing (disinfection) conditions using hypochlorite as a source of free chlorine to control biofouling within limits of allowed exposure of the PA membrane to free chlorine. Results of this study will be valuable in designing effective hypochlorite washing conditions for biofouling control in PA RO membrane applications for both seawater desalination and wastewater reclamation without sacrificing membrane lifetime and RO performance efficiency.

1.3 Objectives of the research

This research aims to:

1. Develop a novel, rapid, and acceptable biofouling test to evaluate the biofouling potential of reverse osmosis membranes
2. Determine the effect of biofilm on inorganic suspended solids accumulation on reverse osmosis membranes under no filtration conditions
3. Determine the effective washing conditions of the polyamide reverse osmosis membrane using hypochlorite to control biofilm formation and inorganic particle accumulation
4. Determine the effect of metal ions in water on the degradation of polyamide reverse osmosis membrane by hypochlorite

1.4 Research flow

Chapter 1 is the preface, which includes a brief introduction, significance of the research study, objectives of the research, and the research flow.

Chapter 2 deals with the development of the biofouling potential test, which was used for the determination of biofouling potential or biofilm formation throughout the study.

Chapter 3 investigates the effect of biofilm on SS accumulation on RO membranes and spacer under no filtration conditions.

Chapter 4 investigates the hypochlorite washing conditions to control biofilm formation and inorganic particle accumulation on RO membranes and spacers.

Chapter 5 focuses on the polyamide membrane degradation as influenced by the presence of metal ions in source waters.

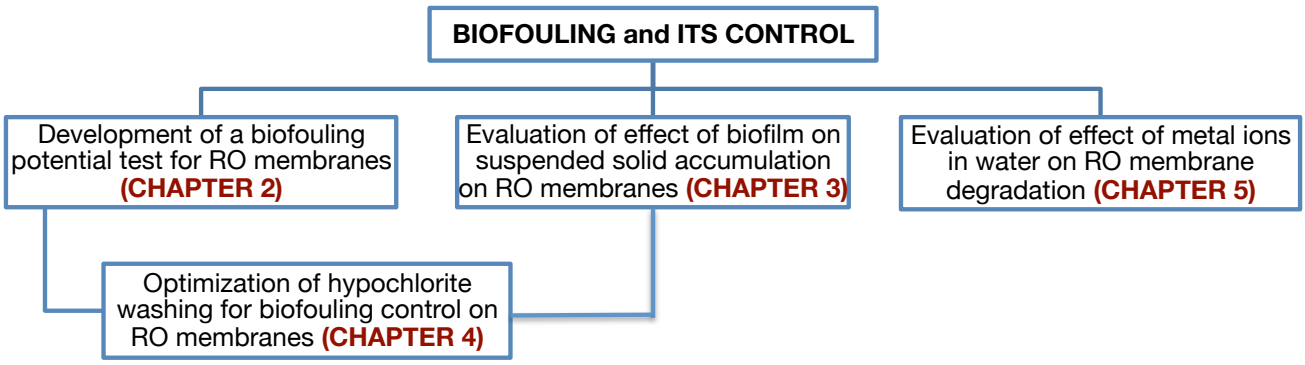

Figure 1. 10 Schematic diagram of the research flow in this dissertation.

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Chapter 2: Development of a novel biofouling potential test for polyamide (PA) reverse osmosis (RO) membranes

2.1 Introduction

Microorganisms are ubiquitous in the environment and every operational plant possesses biofilm from the start of operation and the extent to which it grows and proliferates determines its adverse effect on the reverse osmosis (RO) process (Flemming, 2002). Biofouling, unlike the other types of membrane fouling, can only be prevented and controlled with the proper selection of anti-fouling strategies (Pandey et al., 2012). Because of the diverse areas of research in RO membrane technology directed towards a better understanding, prevention, and control of biofouling, suitable methods of analysis that detect the propensity for biofouling is a key ingredient. Determining biofouling potential would specifically help in designing cost-effective RO processes, especially in the treatment of waters with high fouling potential, such as secondary treated effluent municipal waters (Pandey et al., 2012;) and industrial wastewaters (Ridgway et al., 1983).

Existing biofouling potential tests involves the use of chemical and biological quality parameters of the water as indicators of biofouling. However, even when SDI and/or turbidity values are within or below the allowable limits (Schneider et al., 2005; Huang et al., 2013), fouling still occurred. Since the presence of major bacterial groups in the raw source water or the pretreated RO feed does not necessarily indicate the formation of such bacterial groups on the RO membrane as biofilm (Khan et al., 2013), determination of biofouling potential based on the formed biofilm on the RO membrane is of greater merit in depicting actual biofouling tendencies. A microbiology-based assay which is recently developed assessed the early stage bacterial attachment of *Klebsiella oxytoca* to RO and nanofiltration membranes and showed the importance of membrane-bacterial interaction as a basis for evaluating the susceptibility

of membranes to bacterial attachment, subsequently to biofilm growth, (Lutskiy et al., 2015) and in the case of filtration processes, biofouling. Removing the attached biofilm from the membrane and ensuring its quantitative transfer for bacterial analysis is an added step that needs to be optimized (Lutskiy et al., 2015), thus direct evaluation of biofilm formed on the membrane with easy and rapid fluorescence analysis is an adequate way to determine the biofouling potential of the membrane. The novelty of this biofouling potential indicator method lies on the direct analysis of the biofilm formed on the membrane used, which to our knowledge, does not currently exist. In this study, accelerated biofilm formation on the membrane was achieved by using high biofouling potential water through addition of microorganisms and glucose. This study aims to develop a rapid and reliable biofouling potential test that will be highly useful in determining biofouling potential of membranes being developed or improved as well as commercial RO membranes used for treatment. Moreover, the method developed in this study can also be applied in evaluating biofouling potential of waters with relatively high biofouling potential.

This method involved soaking a small piece of the RO membrane in the test water and the amount of biofilm formed after soaking, considered to be a measure of biofouling potential, is determined by staining the soaked membranes with the green fluorescent dye, SYTO 9. The amount of biofilm formed on the membrane surface is quantified in terms of fluorescence intensity measured by the fluorescence microplate reader. Epifluorescence microscopy is an established technology for viewing biofilms on surfaces but is limited to the analysis of very thin biofilms (McFeters et al., 1995) and as such is employed in this study to confirm the applicability of the soaking method for biofilm formation by comparing percentage coverage obtained by fluorescence microscopy with fluorescence intensity from microplate analysis. Continuous cross-flow experiments were done employing similar conditions used in the soaking method to evaluate the reliability of the soaking method and

the quantification through fluorescence microplate analysis in determining biofouling potential on RO membranes used in real filtration conditions.

2.2 Materials and methods

2.2.1 Sample preparation

For all experiments, deionized (MilliQ) autoclaved water was used as control sample (blank). Secondary effluent water from Higashi Hiroshima Wastewater Treatment Plant was used as sample water. Average (±SD) raw water quality for the secondary effluent water was 4.028 (0.752) mg/L dissolved organic carbon (DOC), 4.700 (1.273) mg/L total SS, 1.104 (0.023) mS conductivity, and pH 6.22 (0.04). To accelerate biofilm formation on the membrane surface, secondary effluent water samples for soaking and continuous cross-flow experiments were added with either glucose (Wako Pure Chemical Industries, Ltd, Japan) and/or *Bacillus subtilis* (JCM 2499, Riken, Japan) to enhance nutrient concentration and/or microorganism growth, respectively.

2.2.2 Bacteria stock preparation

B. subtilis has emerged as an alternative model organism for studying the molecular basis of biofilm formation (Vlamakis et al., 2013). It has also been found to be one of the many bacterial species that participate in biofouling on RO membranes (Matin et al., 2011; Ridgway et al., 1983), and thus has been used in studies that involve seawater RO membrane biofouling (Lee et al., 2010) and in the development of antibacterial polyamide RO membranes (Saeki et al., 2013), and thus the use of *B. subtilis* is acceptable for enhancing biofilm development on the membrane surface**.** The bacterial stock was prepared by growing overnight cultures in 3 % Tryptic Soy Broth (TSB, Merck, Germany) with shaking at 45 rpm at 37 °C for 24 hours. The bacterial cells were harvested by centrifugation at 2,000 rpm for 5 minutes and washed for at least three times with 0.85% NaCl (Nacalai Tesque, Inc., Japan). The pellet was resuspended in 0.85% NaCl to achieve an optical density of 0.4 – 0.5 (OD

550nm, UV-1800, SHIMADZU), resulting to a bacterial concentration in the range of 3 – 6 x 10^8 cfu mL^{-1} in the stock mixture, indicated by the McFarland Turbidity Standard (bioMérieux, Inc., USA). This stock of *B. subtilis* suspension was then added to the sample waters using the necessary dilutions required by the experiment.

2.2.3 Optimum conditions for biofilm formation

In order to confirm enhancement of biofilm formation by using secondary effluent water and addition of glucose and to determine the optimum time that is required for biofilm formation, unfiltered secondary effluent water without glucose, unfiltered secondary effluent water with 1 mM glucose as well as blank water were used as soaking solutions. The optimum soaking time was determined from 6 until 48 hours. Since all secondary effluent feed waters contain microorganisms, which can be regarded as colloidal particles and are removed during filtration pretreatment to limit biological growth (Pandey et al., 2012), the pretreatment, if needed, for the secondary effluent water is then evaluated. Secondary effluent water samples were filtered with GF 75 (0.3 μm pore size, Advantec), GF/F (0.7μm pore size, Whatman), and GF/B glass filters to determine the effect of filtration as pretreatment for the removal of particulates in the sample water. To determine the extent of increase in biofilm growth, the amount of dissolved organic carbon is increased by adding 1 mM glucose to the secondary effluent water. To further achieve an enhanced biofouling behavior, a suspension of *B. subtilis* was added to the nutrient-enhanced samples (with 1.00 mM glucose) and the dilution necessary to produce such enhancement was determined.

2.2.4 Soaking experimental procedure

A commercial thin-film composite polyamide RO membrane (NTR 759HR, Nitto Denko, Japan) was used for the soaking experiments. The membranes were received as a flat sheet and were cut using sterile scissors into 2 cm x 2 cm pieces. Three membranes for each sample waters were analyzed by placing the cut membranes in separate wells of a 6 well-

plate container containing 10 mL of the test water. The well-plate containers were wrapped in aluminum foil and shaken at 45 rpm and at 37 °C to allow biofilm formation. The conditions used for biofilm formation (soaking time, pretreatment, and amount of *B. subtilis*) were based on optimization studies.

2.2.5 Filtration experiments

In order to simulate the conditions that happen during water treatment and to evaluate the reliability of the soaking method and subsequent fluorescence intensity quantification as a biofouling potential test, 24-hour filtration experiments were conducted. The secondary effluent water was first filtered through GF/B glass filter (1 μm, Whatman). The secondary effluent water samples contained 3 – 6 x 10^6 cfu mL^{-1} of *B. subtilis*. Three (3.0) L of the sample water was fed in a laboratory scale continuous cross-flow unit equipped with a pump, feed water reservoir, and a conductivity meter (Multi-Function Water Quality Meter, MM-60R, DKK-TOA Corporation, Japan) (Figure 2.1). Permeate and retentate were recirculated to the feed water reservoir and the filtration was run at a constant applied pressure of 1.5 MPa, feed temperature of 25 °C, initial flux of ~ 0.8 $m^3/m^2/d$, and a cross-flow velocity of 50 mL min^{-1}. NTR 759HR membranes used for these filtration experiments were received as circular sheets with 75 mm diameter size. After filtration, the membrane was retrieved and was very carefully cut with sterile scissors into six 2 cm x 2 cm pieces for the determination of biofilm amount.

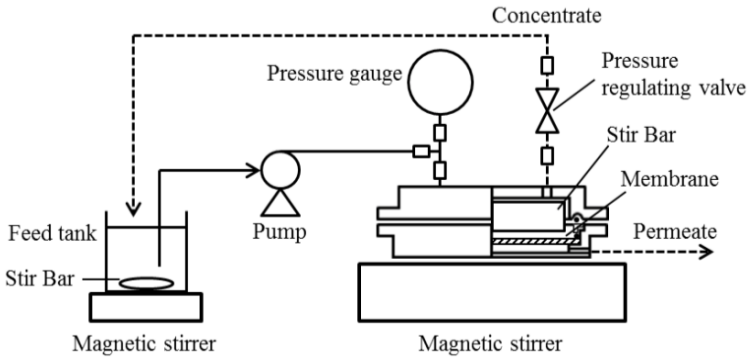

Figure 2. 1 Schematic diagram of the laboratory cross-flow RO set-up

2.2.6 SYTO 9 staining procedure

After soaking or filtration experiments, the membranes were retrieved, and then subjected for staining. The green dye, SYTO 9 from the BacLightTM Bacterial Viability Kit L13152 (Invitrogen/Molecular Probes, USA) stains both live and dead cells with a fluorescent green color. The SYTO 9 solution was prepared according to manufacturer specifications and the solutions were kept in the dark and inside the refrigerator until analysis. A 2-cm piece of the membrane was stained with 100 μL of SYTO 9 and then allowed to stand for 30 minutes at the minimum. After 30 minutes, for the soaked membranes, the excess dye was washed off with 100 μL of deionized filtered (0.22 μm, Millipore) autoclaved water while cut filtered membranes were not washed. Repeated experiments showed that for the soaked membranes, this washing procedure did not remove the biofilm on the membrane surface while the foulants on the filtered membranes were easily detached. All measurements reported herein are within the precision errors described below. All stained membranes were then subjected to fluorescence microscopic analysis and fluorescence microplate analysis.

2.2.7 Fluorescence analysis

Biofilm formed on the dyed membranes is quantified by coverage (expressed as percentage) and fluorescence intensity. The green fluorescence from the microorganisms present was viewed with a fluorescence microscope (Olympus CX-RFL-2). Sixteen-microscopic field shots were obtained from each membrane using Canon EOS Kiss X-50. The images obtained were then analyzed for coverage using the free software ImageJ (Abramoff et al., 2004). Fluorescence intensity was analyzed using the Gemini EM Microplate Reader with SoftMax®Pro Microplate Microplate Data Acquisition & Analysis Software with Excitation scan set at 485 nm and Emission scan set at 545 nm. SoftMax®Pro Microplate Data Acquisition & Analysis Software gave fluorescence intensity values for 144

points per membrane. The percentage coverage and fluorescence intensity values are then reported as averages of 3 pieces of soaked membranes, and averages of 6 cut pieces from the membrane used in filtration. Consequently, precision is reported as standard deviations for n = 3 and n = 6 membranes, for soaking and filtration experiments, respectively.

2.2.8 Biofouling potential test on 3 PA RO membranes

The soaking method was used to evaluate the biofouling potential of three commercially available PA RO membranes: ES20 (Nitto Denko, Japan), NTR 759HR, and SU 700 (Toray Industries, Japan). Fluorescence intensity of biofilm formed on the membrane surface after 24 hours of soaking in secondary effluent water with 1 mM glucose and *B. subtilis* concentration range of 3 – 6 x 10^7 cfu mL^{-1} was determined. Contact angle, as a measure of the surface hydrophobicity of the virgin membranes, was determined to characterize the surface of the membranes using Drop Master DM-300 (Kyowa Co., Japan). Ten water contact angle measurements at different locations on one membrane sample were carried out to get average and standard deviation values.

2.3 Results and Discussion

2.3.1 Optimum conditions for biofilm formation

Fluorescence intensities from the biofilm formed on the membrane were determined after 6, 24, and 48 and results are shown on Figure 2.2. These results indicate that optimum growth of microorganisms is expected to occur around 24 hours of soaking the RO membranes in secondary effluent water for both with and without glucose, and thus 24 hours is employed for biofilm formation time. The decrease in intensity at 48 hours signify that the amount of biofilm on the membrane has lessened and can be explained by possible detachment of microorganisms from the membrane surface and dispersal due to nutrient resource limitation (Vlamakis et al., 2013).

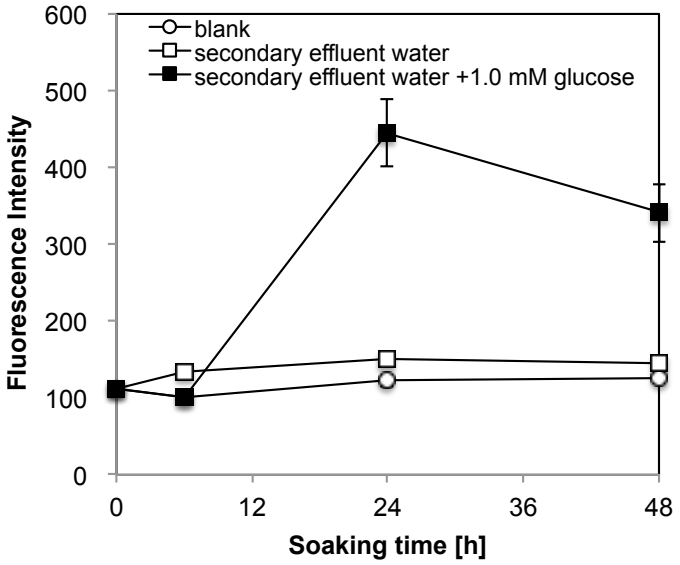

Figure 2. 2 Fluorescence intensity of biofilm formed on membranes soaked in different test waters. Error bars show standard deviation (n=3).

Secondary effluent waters can have variable loads of suspended solids, colloidal materials, organics, and bacteria and to reduce cell deposition, and bacterial growth in actual RO operations, appropriate pre-treatment methods are employed (Pandey et al., 2012). After establishing the amount of time needed for biofilm formation (Figure 2.2), the effect of filtration in removing particles from the secondary effluent water on the fluorescence intensity of the biofilm formed was determined (Figure 2.3). The extent of increase of biofilm growth by adding dissolved organic carbon in the form of glucose was also determined. To assess biofilm formation on all samples after 24 hours, difference of fluorescence intensity (ΔFluorescence intensity) values from fluorescence intensity of membrane samples soaked in test waters after 24 hours and the fluorescence intensity of membranes at zero hour (i.e. virgin membranes) were determined. Membranes soaked in blank water samples showed the lowest ΔF of 11, while filtered samples without added glucose using 0.3 μm, 0.7 μm, and 1.0 μm filter have lower intensity values, 20, 19, and 28, respectively. In contrast, membranes soaked in unfiltered samples without glucose have ΔF of 47. As expected, addition of the 1.0 mM glucose greatly enhanced the fluorescence intensity of the biofilm formed on the

membrane surface, with ΔF of 99, 98, 85, and 353 for 0.3 μm-, 0.7 μm-, 1.0 μm- filtered, and unfiltered sample waters, respectively, signifying increased biofilm formation due to the added dissolved organic carbon source for the microorganisms. These results also indicate that the sensitivity of the soaking method and detection of fluorescence intensity is low without the added carbon, and thus conditions to increase sensitivity should be done. As expected also, filtration through 0.3 μm to 1 μm filters reduced the biofilm formation. Bacteria in the secondary effluent water may not exist in completely dispersed form but are in floc form. Since filtration would significantly reduce the bacterial number that could form biofilm in 24 hours, for the establishment of conditions necessary for the soaking method, secondary effluent water would not be filtered.

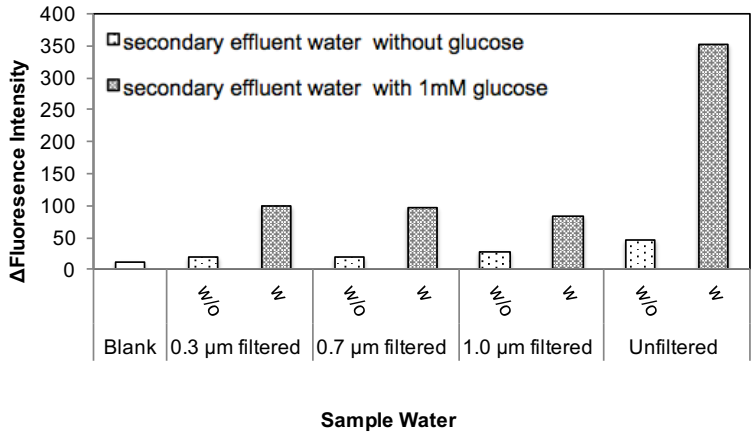

Figure 2. 3 Effect of filtration as pretreatment of secondary effluent water on the fluorescence intensity of biofilm formed after 24 hours of membrane soaking. ΔFluorescence intensity = fluorescence intensity at 24 h – fluorescence intensity at 0 h. Error bars show standard deviation (n=3).

Moreover, since concentration of particulate matters in the secondary effluent water was very variable, addition of microorganisms in the soaking method was evaluated next in order to have a stable biofouling potential (Figure 2. 4). Results of average intensity values (± SD) indicate that unfiltered secondary effluent water samples with 1.00 mM glucose with no *B. subtilis*, and samples with 3 – 6 x 10^4 cfu mL^{-1}, 3 – 6 x 10^5 cfu mL^{-1}, and 3 – 6 x 10^6 cfu mL^{-1} of *B. subtilis* suspension had relatively the same fluorescence intensity: 194 ± 43,

191 ± 26, 187 ± 30, and 220 ± 34, respectively. There was marked increased in fluorescence intensity in samples containing 3 – 6 x 10^7 cfu mL^{-1} of *B. subtilis* (451 ± 95), indicating more bacterial growth and thus this concentration was considered for all soaking experiments.

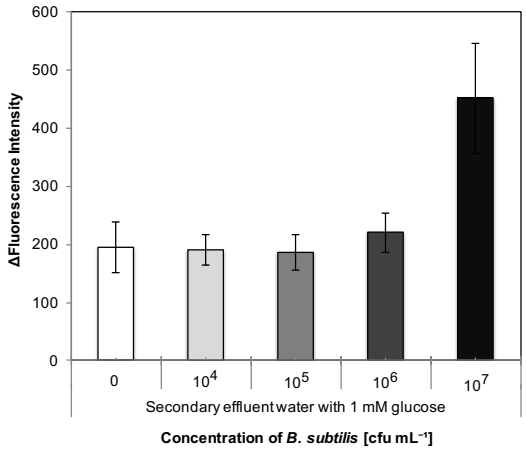

Figure 2. 4 Effect of amount *Bacillus subtilis* on fluorescence intensity of biofilm formed after 24 hours of membrane soaking. ΔFluorescence intensity = fluorescence intensity at 24 h – fluorescence intensity at 0 h. Error bars show standard deviation (n=3).

2.3.2 Biofilm formation determination during soaking method

In order to evaluate the new method, the relationship between amount of glucose concentration in the sample waters and the percentage coverage and fluorescence intensity from the biofilm formed on the membrane surface using the optimum soaking conditions were determined. An increase in percentage coverage (Figure 2.5a) and fluorescence intensity (Figure 2.5b) was not observed from 0 to 0.05 mM glucose concentration. A continuous increase in coverage and intensity was observed from 0.05 to 0.80 mM glucose concentration due to biofilm growth and development, and a linear correlation (R^2 = 0.96, Figure 2.6) exists between percentage coverage and fluorescence intensity in this range. From 0.80 mM glucose concentrations, the coverage and fluorescence intensity leveled off, suggesting that addition of 0.80 or 1.00 mM final concentration of glucose into the secondary effluent water is enough to accelerate biofilm formation for the evaluation of biofouling potential on the membrane.

The increase in percentage coverage and fluorescence intensity with glucose concentration between 0.05 to 0.80 mM, corresponding to 3.6 to 58 mg L^{-1} carbon, shows that this new method can also be applied to evaluate biofouling potential of water within this range. Glucose is a typical biodegradable organic matter and, therefore, this new method will be applicable to relatively organically polluted water with 3.6 to 58 mg L^{-1} of biodegradable organic carbon.

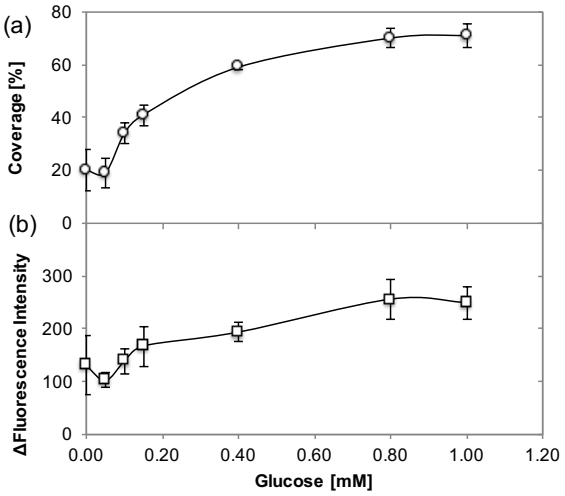

Figure 2. 5 (a) Percentage coverage (○) and (b) fluorescence intensity (□) of biofilm formed after 24 hours of membrane soaking in secondary effluent water with *B. subtilis* concentration range of 3 – 6 x 10^7 cfu mL^{-1}. ΔFluorescence intensity = fluorescence intensity at 24 h – fluorescence intensity at 0 h. Error bars show standard deviation (n=3).

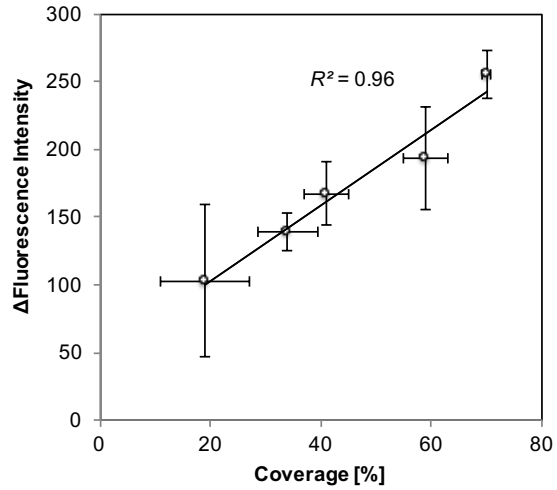

Figure 2. 6 Correlation between fluorescence intensity and percentage coverage during 24 hours of biofilm formation under soaking conditions. ΔFluorescence intensity = fluorescence intensity at 24 h – fluorescence intensity at 0 h. Error bars show standard deviation (n=3).

The soaking test was applied in the determination of biofouling potential of river water having less than 2 mg L^{-1}, in comparison to the secondary effluent water. Results shown in Figure 2.7 indicate the ability of the test to determine the biofouling potential of less than 3.6 mg L^{-1} of dissolved organic carbon, provided that conditions to enhance the sensitivity of detection are done, such as increasing the soaking time (from 24 h to 72 h) with the soaking solution being replaced every 24 h to avoid nutrient depletion and increasing the soaking solution volume (from 10 mL to 50 mL). However, an extended time to 3 days for biofouling potential determination even using accelerated biofilm formation conditions means a lot of time is spent waiting for results, which could be obtained using other quicker biofouling potential tests. Despite this disadvantage, considering that freshwater resources are already scarce and would be the least choice for RO feed, the biofouling potential test developed is most applicable for the current trend in wastewater reuse and reclamation, which is the use of non-traditional water resources.

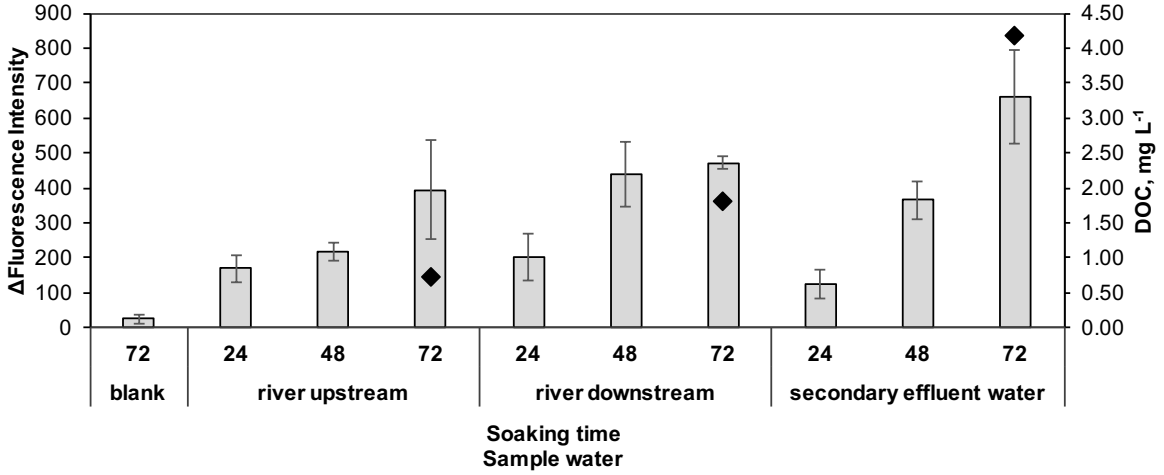

Figure 2. 7 Fluorescence intensity of biofilm formed after 72 hours of membrane soaking in sample waters with 1.00 mM glucose and *B. subtilis* concentration range of 3 – 6 x 10^7 cfu mL^{-1}. Soaking solution replacement done every 24 h. ΔFluorescence intensity = fluorescence intensity at time, h – fluorescence intensity at 0 h. Error bars show standard deviation (n=3).

2.3.3 Biofouling determination in real filtration conditions

Previous studies show that biofilm formation occurs within 24 hours of contact between membrane surface and feed water (Bar-Zeev et al., 2012; Schneider et al., 2005), although the extent of biofilm growth and accumulation varies depending on the conditions employed. To assess the reliability of the soaking method and fluorescence intensity quantification as a biofouling potential test, cross-flow filtration experiments were performed. Results showed that percentage coverage ranged from ~75 to 95% (Figure 2.8), suggesting an almost completely covered fouled membrane. Fluorescence intensity increased from 0 to 1.00 mM glucose concentration as shown in Figure 2.8b, signifying a steady biofilm growth and accumulation on the membrane surface. The results indicate that in conditions of greater chances of biofouling, such as filtration during water treatment, the biofilm steadily covers the whole surface area of the membrane (thus an almost constant coverage observed), and when the membrane is fully covered, biofilm growth and accumulation in the vertical direction takes place.

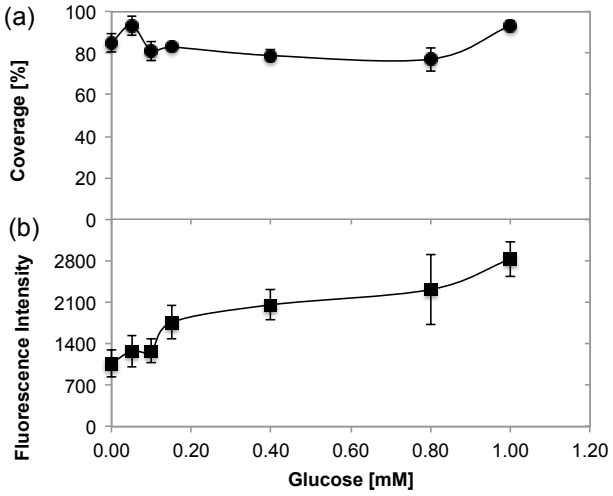

Figure 2. 8 (a) Percentage coverage (●) and (b) fluorescence intensity (■) of biofilm formed during 24-hour continuous cross-flow experiments. Run conditions: 1.5 MPa, 25 °C, secondary effluent water with 3 – 6 x 10^6 cfu mL^{-1} *B. subtilis*. Error bars show standard deviation (n=6).

Figure 2.9 shows that fluorescence intensity values from the biofilm formed during filtration conditions, even at no additional glucose, are at least 8 times higher than fluorescence intensity values obtained from biofilms that were formed during the soaking method. This can be explained by the difference in the amount of liquid or amount of glucose in contact with the surface area as expressed in terms of liquid volume- or glucose-membrane surface ratio, that is, 2.50 and 68.0 mL cm^{-2}, and 0.0225 and 0.611 mg cm^{-2} in soaking and filtration experiments, respectively. As also shown in Figure 2.9, fluorescence intensity values from biofilm formed during filtration and soaking experiments have a slightly linear correlation (R^2 = 0.87), indicating the capability of the soaking method to depict biofouling potential in real filtration conditions. All conditions considered, except for the amount of glucose at the start of filtration, thus different biofouling potentials, continuous filtration would still lead to a rise in fluorescence intensity and reach a maximum because the feed water continuously delivers nutrient, which the microorganisms in the feed water can use for biofilm growth and development (Chen et al., 2013).

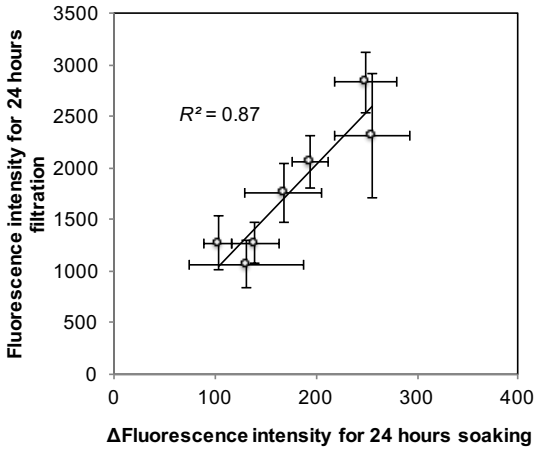

Figure 2. 9 Correlation of fluorescence intensity of biofilm formed on membranes during filtration and soaking conditions for the 24-hour biofilm formation at increasing glucose concentrations (0.00, 0.05, 0.10, 0.15, 0.40, 0.80, 1.00 mM). ΔFluorescence intensity = fluorescence intensity at 24 h – fluorescence intensity at 0 h. Error bars show standard deviation (n=3 for soaking and n=6 for filtration conditions).

Studies have reported the contribution of biofilm and the associated extracellular polymeric substances (EPS) in permeate flux decline due to a hindered back diffusion of salt and resulting elevation of osmotic pressure on the membrane surface due to the presence of biofilm and an increased hydraulic resistance to permeate flow due to the EPS surrounding the biofilm (Herzberg and Elimelech, 2007; Chong et al., 2008; Huertas et al., 2008). A plot of fluorescence intensity with change in flux revealed this decrease in flux corresponded to an increase in the amount of biofilm formed, except for the filtration condition using 1.00 mM glucose, which showed an unexpected increase in permeate flux (Figure 2.10). This discrepancy needs further investigation in order to verify if the observed increase is due to an error or due to a change in the characteristic of the biofilm. Biofilm structural parameters (porosity, bio-volume, and thickness) and biofilm components (cells, polysaccharides, and proteins) has been reported to have a relationship with membrane permeability as well as operating mode (flux) (Sun et al., 2011). Analysis of the biofilm components and structure and its relation to the changes in flux could explain this particular increase in flux at higher fluorescence value, whereby porosity of the biofilm could result in increase in flux. However, such differentiation of biofilm structure and components is beyond the scope of this part of the research, but could be addressed in future studies.

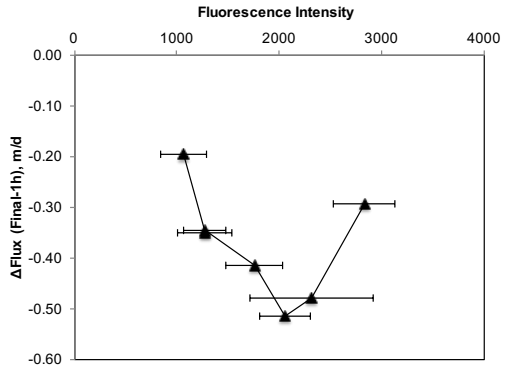

Figure 2. 10 Change in flux (ΔFlux) and fluorescence intensity after 24 hours of continuous cross-flow experiments. Run conditions: 1.5 MPa, 25 °C, secondary effluent water with 3 – 6 x 10^6 cfu mL^{-1} *B. subtilis* and increasing glucose concentrations (0.00, 0.05, 0.10, 0.15, 0.40, 0.80, 1.00 mM). ΔFlux = Final flux (flux at 24 h) – flux at 1 h. Error bars show standard deviation (n=6).

2.3.4 Biofouling potential test on 3 PA RO membranes

Membrane characteristics (surface hydrophobicity, surface charge, chemical composition, roughness, and surface morphology and microtopography) are known to influence biofouling, specifically on the initial adhesion of bacteria on the membrane surface (Habimana et al., 2014). It has been reported that membranes with neutral or highly negative surface charge, with smooth surfaces, and that are less hydrophobic tend to minimize membrane fouling (Norberg et al., 2007). Thus, the biofouling potential test developed in this study was applied to 3 commercially available PA RO membranes, and the fluorescence intensities obtained were compared to virgin membranes' contact angles, as a measure of surface hydrophobicity. Figure 2.11 shows fluorescence intensity of ES 20 is lowest (120 ± 30), while NTR 759HR (300 ± 85) and SU 700 (317 ± 105) have comparable fluorescence intensity values. Contact angle measurements of virgin membranes revealed a similar trend with ES 20 having the lowest contact angle, 15.6 ± 5.3°, while NTR 759HR and SU 700 have similar values, 30.8 ± 5.0° and 31.5 ± 2.2°, respectively. Contact angle results indicate that ES 20 will have the least biofouling tendency out of all the three membranes studied. This is supported by the fluorescence intensity values obtained where ES 20 showed the lowest value, suggesting least biofilm formation. A previous study showed that greater accumulated cells were observed on RO and NF membranes with higher initial hydrophobicity, indicated by higher contact angle values (Khan et al., 2011), which could explain these results observed. These results further showed the applicability of the method developed in assessing biofouling potential on PA RO membranes.

Since current technology is also geared towards improvement and development of RO membranes, the test was applied for the determination of biofouling potential of a membrane material made of organosilica with bridging organic groups, which was developed and applied to various membrane separation processes due to their amazing hydrothermal

stability, adjustable pore sizes, and excellent molecular sieving abilities (Gong et al., 2015). Results shown in Figure 2.12 indicate the ability of the test to determine the biofouling potential of membranes being developed using the accelerated biofilm formation conditions used, specifically for the membrane material, 1,2-bis(triethoxysilyl)ethylene (BTESEthy), which was synthesized on a glass cover slip (provided by Dr. Tsuru's research group).

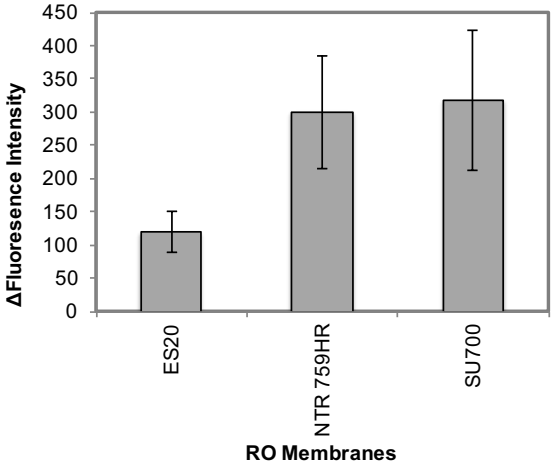

Figure 2. 11 Fluorescence intensity of biofilm formed after 24 hours of membrane soaking in secondary effluent water with 1.00 mM glucose and *B. subtilis* concentration range of 3 – 6 x 10^7 cfu mL^{-1}. ΔFluorescence intensity = fluorescence intensity at 24 h – fluorescence intensity at 0 h. Error bars show standard deviation (n=3).

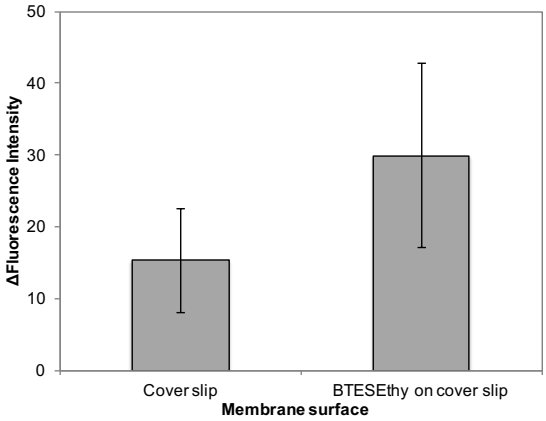

Figure 2. 12 Fluorescence intensity of biofilm formed after 24 hours of membrane soaking in secondary effluent water with 1.00 mM glucose and *B. subtilis* concentration range of 3 – 6 x 10^7 cfu mL^{-1}. ΔFluorescence intensity = fluorescence intensity at 24 h – fluorescence intensity at 0 h. Error bars show standard deviation (n=3).

2.3.5 Comparison of fluorescence microscopic analysis and fluorescence microplate analysis

Under soaking conditions, both methods of detection were able to portray the biofilm growth and accumulation on the surface of the membrane, indicated by the linear correlation in Figure 2.6. In most biofilm development, during the first 24 hours, the microorganisms tend to cover the surface of the membrane, rather than grow in thickness (Bar-Zeev et al., 2012). When the membrane surface is completely covered, microorganisms tend to grow and accumulate thus increasing in thickness or volume. Although epifluorescence microscopy can provide 2-dimensional distribution of microorganisms (Al-Juboori and Yusaf, 2012) as well as bacterial activity and viability, examination of the depth of the biofilm is not possible (Wolf et al., 2002), as shown in Figure 2.8a. Thus, fluorescence microplate analysis holds an advantage in determining the propensity for biofouling of highly polluted waters since it could depict growth and accumulation of a fully-biofilm-covered membrane.

In addition, despite ImageJ software having macros to automate often-repeated tasks (Abramoff et al., 2004), in this study, repeated trials indicate more reliable results when individual pictures/shots are assessed for coverage by manually adjusting the brightness slider based on the microorganisms stained with the fluorescent green color, while hue and saturation ranges are fixed. Therefore, increasing the number of membrane samples as well as the microscopic field shots correspond to added amount of time allotted for data processing. In contrast, microplate assay boasts of its simple high-throughput method (Merritt et al., 2005). Combining these points, it is more advantageous to use the fluorescence microplate analysis for detecting biofouling potential on RO membranes.

Furthermore, the capability of the soaking method and fluorescence microplate analysis in simulating biofouling potential tendencies using a laboratory cross-flow filtration set-up has important implications in the ability of the method in analyzing fouled spiral

wound RO commercial membranes and spacers. Fluorescence microscopy with fluorescence staining have been used in detecting and quantifying biofoulants (Khan et al., 2011). However, with the simplicity offered by just cutting portions of the fouled membrane, the quantitative analysis of biofouling given through fluorescence intensity measurements particularly for highly fouled membranes, and the quick output rendered by the microplate analysis, biofouling occurrence in fouled commercial membranes can now be easily analyzed using the method developed.

2.5 Summary and conclusion

A new, capable, and rapid method was developed by analyzing fouled RO membranes for the determination of biofouling potential. The method involved soaking small pieces of the RO membranes in test waters added with microorganisms and glucose for accelerated biofilm formation and the biofilm formed on the membrane surface was measured by fluorescence intensity through fluorescence microplate analysis. A correlation between fluorescence intensity values obtained from biofilms formed on the membrane during soaking (no pressure) and filtration (under constant pressure) conditions indicated the reliability of the membrane soaking method as a biofouling potential indicator. This method can be applied for determination of biofouling potential in water with more than 3.6 mg L^{-1} easily degradable organic carbon and showed capability of assessing biofouling potential of 3 commercially available PARO membranes. On top of being a novel method that gives biofouling potential based on direct analysis of biofilm formed on the membrane, capability of showing biofouling potential tendencies in real filtration conditions, and the fast output of data analysis are clear benefits of using the soaking method and fluorescence microplate analysis for the determination of biofouling potential on RO membranes.

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Chapter 3: Effect of biofilm on inorganic suspended solid accumulation on RO membranes under no filtration conditions

3.1 Introduction

Population growth and intense urbanization have increased the global water demand. And as countries seek supplements to natural water resources, growth in desalination has increased significantly over the past 20 years (WWAP, 2014). Out of all desalination technologies, the most internationally widespread is the seawater reverse osmosis (SWRO) technology (). Some of the state-of-the-art SWRO large scale desalination plants are found in Perth in Australia, Llobregat in Spain, Tuas in Singapore, and Fukuoka in Japan, whose RO membranes are supplied by the four major membrane suppliers, DOW, Hydranautics, Toray, and Toyobo, respectively (Lee et al., 2010). In the US, the highest installed capacity as of 2005 are found in the coastal states of California, Florida, and Texas, and the arid state of Arizona (Cooley et al., 2006) but in the last decade, northeastern states have existing facilities in New Jersey, Massachusetts, and a proposed desalination plant will be in New York (Vedachalam and Riha, 2012). The increase in desalination capacity is caused by the significant reduction in desalination cost as a result of substantial technological advances, particularly in the RO process, which made water produced by desalination of comparable cost with other resources (Greenlee et al., 2009; Ghaffour et al., 2013). Such advances and innovations are geared to reduce energy consumption during the process, to obtain higher water flux membranes, and to decrease the harmful effects of fouling, including scaling, on RO membranes (Peñate and García-Rodríguez, 2012). Another promising technology to augment water supply is through wastewater reuse and reclamation. In the past, wastewater treatment is focused on pollution abatement but in the last two decades, there is an increased amount of municipal wastewater recovered for reuse (Levine and Asano, 2004). Global

interests and efforts are also made in utilizing reclaimed wastewater for both potable and non-potable purposes (Levine and Asano, 2004; Toze, 2006). The RO membrane process plays a vital part in this goal due to its higher rejection of impurities and its production of higher quality water at a lower cost (Pandey et al., 2012). For both desalination and water reclamation and reuse, the application of RO membranes faces a significant challenge because of the decline in membrane performance due to membrane fouling.

Membrane fouling is categorized into crystalline fouling or mineral scaling, organic fouling, particulate and colloidal fouling, and microbiological fouling or biofouling, which is based on the materials (foulants) deposited on the membrane (Flemming, 1997). Numerous investigations have been done on the effects of fouling in membrane treatment processes. For example, source waters with significant amount of colloids, colloidal fouling is inevitable especially for composite polyamide RO membranes due to their distinct surface roughness (Zhu and Elimelech, 1997). The feedwater source and its composition and quality largely influence the fouling behavior on RO membranes (Khan et al., 2013). For any type of application, raw water undergoes conventional pretreatment or non-conventional/membrane pretreatment (microfiltration (MF) or ultrafiltration (UF) or nanofiltration (NF)) before being fed into the RO filtration unit (Speth et al., 2000; Schneider et al., 2005; Ning et al., 2007; Kim et al., 2008; Pearce et al., 2008; Thompson et al., 2012; Jamaly et al., 2014). To prevent fouling by large and visible particles, RO feed water needs to have turbidities of less than 1 NTU (= 1 mg/L using kaolin standard), and silt density index (SDI) of less than 4.0 (Jamaly et al., 2014). Operational cost of conventional pretreatment is lower than non-conventional pretreatment technologies but the latter is the preferred pretreatment for RO processes due to a better quality of produced water (Pearce et al., 2008; Jamaly et al., 2014). However, both pretreatment types have been shown inadequate in mitigating fouling on NF or RO units (Schneider et al., 2005; Ning et al., 2007; Kim et al., 2008; Speth et al., 2000). For example,

due to biological growth on the membrane and impurities passing through the pretreatment (Schneider et al., 2005; Kim et al., 2008; Speth et al., 2000), one study reported that severe fouling caused the feed spacers, which are spiral wound membrane elements used to separate several RO membranes (Greenlee et al., 2009), to be forced out of the membrane element (Schneider et al., 2005). Another study suggested that mineral scaling, which has been assumed to occur solely on tail elements in a RO system, may also occur in lead elements or any elements in the RO train where biofilm has formed (Thompson et al., 2012). Several pilot scale and plant studies reported that colloidal fouling observed was attributed to inorganic particles, most likely in nano- and colloidal form, that were able to pass through the MF or UF unit (Ning et al., 2007; Kim et al., 2008; Xu et al., 2010).

It is an established fact that more than a single type of foulant is responsible for fouling in RO systems, which largely depends on the source water quality (Khan et al., 2013). And combined biological-mineral scaling (Thompson et al., 2012) organic-organic (Li et al., 2007), and organic-inorganic (Higgin et al., 2010) fouling have been studied in RO processes to further understand the effects of composite fouling on membrane performance decline. It was found that biofilm-gypsum (Thompson et al, 2012) and bovine serum albumin (BSA)-alginate (Li et al., 2007) could have synergistic effects on the fouling of RO membranes, while specific alginic-silica interactions could mitigate fouling (Higgin et al, 2010).

Analysis of available experimental data have shown that in the case of particulate fouling, the later stages of fouling are greatly influenced by synergistic particle-fluid, particle-particle, and particle-surface interactions (Henry et al., 2012). It has been observed in other environments that inorganic materials interact with biofilms (Lowe et al., 1984; Sheikholeslami, 1999; Leon-Morales et al., 2004; Yang et al., 2015). A study on a porous media (sand columns) showed that introduction of laponite at high and low ionic conditions caused cell detachment, with the cell detachment attributed to interactions between the

inorganic materials and biofilm or to hydrodynamic changes within the column (Leon-Morales et al., 2004). In another study, the presence of kaolin showed initial inhibition of biofilm growth, but with the accompanying increase in biofilm weight it was suggested that presence of SS may have also enhanced cell attachment or have increased entrapment of SS within the attached cells or both (Lowe et al., 2004). A study on the interactions between suspended matter and biofouling formed in a treated sewage heat exchanger showed that biofilm formed are added surface area to which particles can attach, but smaller particles are more likely to attach and increase the fouling weight than the larger particles (Yang et al., 2015). These various effects when inorganic materials and biofilm are present need further understanding, especially on RO membrane systems.

Because microorganisms are ubiquitous in the environment and biofilm formation cannot be avoided on membrane surfaces (Flemming, 1997), biofilm plays a big role in fouling of RO membranes. For wastewater reclamation of secondary effluent water where there are high levels of organic matter and bacteria, and high suspended solids (SS) loads and colloidal matter (Pandey et al., 2012), biofilm can pose a significant impact on the behavior of particles that passed through a failed pretreatment scheme. Colloidal particles, in particular, have high tendency to aggregate and its removal from membrane surface becomes more problematic when they are coated with organic foulants or embedded in biofilms (Xu et al., 2010). Thus it is advantageous to gain qualitative and quantitative information on how biofilm affect the behavior of SS to be able to understand composite fouling in RO processes (Sheikholeslami, 1999).

This study aims to determine the effect of biofilm on the inorganic SS accumulation on RO membranes with spacer under continuous cross flow with no filtration. The findings of this study could help in designing control of composite fouling due to biofilm and SS deposition through creation/design or modification of pretreatment schemes that is directed

towards biofilm control and thus could lessen costs associated with pretreatment due to particulate removal. When applied to continuous cross flow filtration conditions the findings would have significant implications on RO membrane performance as influenced by composite fouling due to biofilm and SS deposition and the cleaning strategies related to such fouling events.

3.2 Materials and methods

3.2.1 Materials

Glucose and kaolin (5-10 μm) were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Red dyed monodisperse polystyrene microsphere particles (3 μm, no surface functionalization) purchased from Polybead, Polysciences Inc., USA, were added into pure water (Milli-Q Reference Ultrapure Water, Merck Millipore Corp., Darmstadt, Germany) to prepare a resulting concentration of 100 mg/L in the suspension, determined by turbidity measurements (Turbidimeter ANA-148, Tokyo Photoelectic Co., Ltd., Japan). These red dyed particles were used instead of kaolin to visually represent SS on the membrane and spacer, specifically for fluorescence analysis due to the contrast in color between the red dyed particles and the fluorescent green biofilm, stained by SYTO 9 (Baclight™ Bacterial Viability Kit L13152, Invitrogen/Molecular Probes, Oregon, USA). Kaolin turbidity standard was purchased from Chameleon reagent (Osaka, Japan). Secondary effluent water, discharged after activated sludge treatment and sedimentation was taken from Higashi-Hiroshima Wastewater Treatment Plant. Average(±SD) raw water quality during the duration of this study was 4.028(0.752) mg/L dissolved organic carbon (DOC), 4.700(1.273) mg/L total SS, 1.104(0.023) mS conductivity, and pH 6.220(0.044). The secondary effluent water was filtered through GF/B glass fiber filter (1 μm pore size, Whatman, UK) and the filtered secondary effluent water was used for all experiments in this study. Pure water was used to prepare SS suspensions of kaolin or red dyed particles.

3.2.2 RO cross flow set-up

A schematic diagram of the laboratory cross flow cell unit (without permeation) used for the experiments is shown on Figure 3.1. The cross flow cell set-up is equipped with the custom made cross flow cell, a digital tubing pump (DSP-100SA, AS ONE, Japan) to control flow rate of solutions at 30 mL/min and a feed container placed on top of a magnetic stirrer (HS-1A, AS ONE, Japan) to keep all 1-L test water solutions continuously stirred during experiments. The flow cell consists of two pieces of acrylic plates. One plate has an internal space of 28.9 x 4.6 x 1.4 cm, where a plastic support and cut pieces of membrane with spacer on top are placed. The inside of the plate was surrounded by an O-ring to prevent any leakage. The cover plate has one hole on top for inlet and another hole at the end for outlet. Both plates are held together by screws to hold the entire cell. The flow cell was then operated at 25 °C without pressure with a calculated standard error of the mean of about 2% for runs conducted three times. Commercially available polyamide RO membrane, NTR 759HR, used in this study was provided by Nitto Denko Co. (Osaka, Japan). A diamond type polypropylene spacer (3 x 3 mm mesh size), commercially used in RO membrane elements, was used in this study. Membranes and spacers were received as flat sheets, and then were cut into 4.5 x 7.5/8.0 cm sizes with sterile scissors while being rinsed with copious amount of pure water before placing them inside the flow cell. Top view of the cut spacer and membrane inside the cell is shown on Figure 3.2.

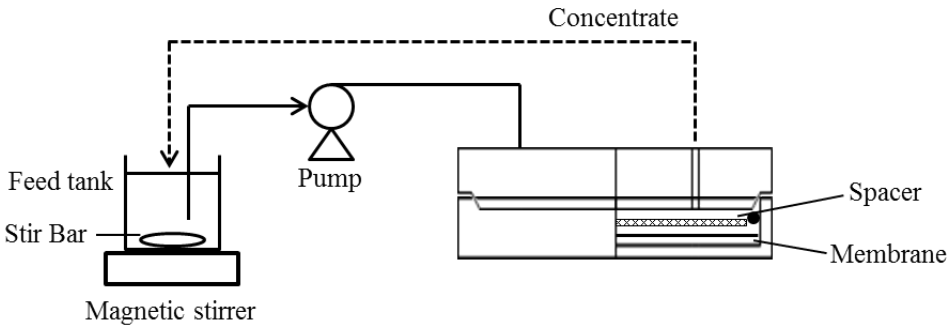

Figure 3. 1 Schematic Diagram of the Laboratory Cross-flow RO Test Cell (without permeation).

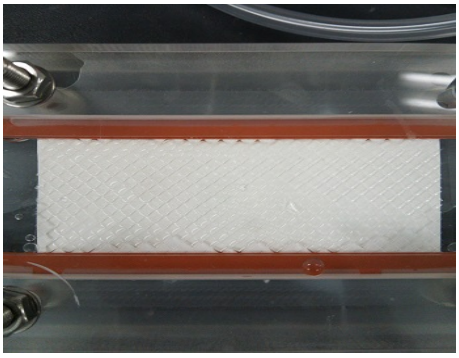

Figure 3. 2 Top View of Polypropylene Spacer and RO Membrane inside the Flow Cell.

3.2.2 Experimental protocol

A summary of this protocol is presented in Table 3.1. In order to determine the correlation of initial kaolin concentration in feedwater to the amount of organic and inorganic matter that is formed on the membrane (Run A), filtered secondary effluent water was added with 0-300 mg/L of kaolin and then continuously flowed through the flow cell for 24h (one step). Pure water added with 0-300 mg/L of kaolin was also flowed for 24h as basis for a feedwater sample with lower biofouling potential compared to secondary effluent water. Each of these runs were performed using one cut membrane and spacer (n = 1). After obtaining the initial kaolin concentration that could significantly deposit on the membrane, a two-step run condition was done to determine the effect of preformed biofilm on SS accumulation (Run B). Biofilm formation occurs within 24 hours of contact between membrane surface and feedwater (Schneider et al., 2005), although the biological growth and accumulation depends on the conditions employed. Since the flow experiments were done under no filtration conditions, in order to enhance the biofilm amount that could form on the membrane and spacer, secondary effluent water was run through the flow cell for 48h. After which, 300 mg/L of kaolin suspension was run for 1h to deposit the SS on the preformed biofilm. To increase the biofilm formation potential of secondary effluent water and to determine the effect of increasing glucose, secondary effluent water was prepared to have a total of 0.0-1.0 mM glucose. Glucose is a typical biodegradable organic matter and is an

applicable nutrient to mimic relatively organically polluted water. Each of these runs were performed using one piece of membrane and spacer, and subsequently cut into three smaller pieces for loss on ignition tests (n = 3). Lastly, in order to obtain qualitative information on biofilm formation and SS deposition, a longer two-step run was performed for biofilm formation to obtain more evident biofilm development through optical micrographs and longer SS deposition, with red dyed particles substituting for kaolin to obtain clearer results under fluorescence microscopic analysis (Run C). Secondary effluent water with 1.0 mM glucose was fed through the flow cell for 72h and biofilm formation observed through light microscopic analysis. 100 mg/L red dyed particle suspension was then run through the flow cell for 24h. To avoid nutrient depletion on the secondary effluent water used for more than 24h, the feedwater was replaced with freshly prepared feedwater every 24h until the end of the experiment. Each of these runs were performed using one cut membrane and spacer (n = 1).

Table 3. 1 Experimental design and corresponding analytical objectives for biofilm formation and SS deposition run of secondary.

Type of run	Sample feedwaters	Run time	Analytical objective
A. Simultaneous run of secondary effluent water/pure water with kaolin	1) Secondary effluent water with kaolin (0-300 mg/L) 2) Pure water with kaolin (0-300 mg/L)	24h 24h	Correlation of amount of inorganic matter and organic matter deposited with initial SS concentration in feed water; amount of kaolin for significant accumulation
B. Two-step (secondary effluent water first, then SS suspension)	Secondary effluent water with glucose (0-1 mM) 300 mg/L kaolin suspension	48h 1h	Effect of amount of pre-formed biofilm on amount of inorganic matter and inorganic matter deposited; percentage biofilm coverage
C. Two-step (secondary effluent water first, then SS suspension)	Secondary effluent water with glucose (0-1 mM) 100 mg/L red dyed polystyrene microsphere particles	72h 24h	Qualitative description of biofilm formation and SS deposition; percentage biofilm coverage

3.2.2 Analytical methods

Accumulated particle deposits on the membrane were determined through LOI tests. Pure water was filtered through GF/B filter as rinsing and then dried for 1 hour at 110°C. The dried filter is then burned to 550°C for 30 minutes to get the mass of dried and ignited filter (m_1). Membrane and spacer were carefully removed from the flow cell after each experiment and the spacer was also carefully removed from the membrane top. The accumulated mass on the membrane surface was thoroughly removed from the membrane surface by rinsing and brushing using nylon toothbrush (Lion Co., Japan) while dipping in pure water. The resulting water was then filtered in the pre-weighed GF/B filter. The collected mass and filter was then dried for 1 hour at 110°C and then weighed (m_2). Total dried mass was calculated based on equation (1). Filter and dried mass was further ignited to 550°C and then weighed (m_3). Inorganic mass, which is the residue after ignition, was obtained based on equation (2). Organic mass was obtained by the difference in total mass and organic mass as shown in equation (3).

$$\textit{Total mass} = m_2 - m_1 \quad (1)$$

$$\textit{Inorganic mass} = m_3 - m_1 \quad (2)$$

$$\textit{Organic mass} = \text{Total mass} - \text{Inorganic mass} \quad (3)$$

Light and fluorescent microscopic images were taken to qualitatively depict the formation of biofilm and SS accumulation on the membrane and spacers. Before taking out the membrane and spacer from the flow-cell unit for LOI tests, images of the membranes and spacers were taken using a digital microscope (AM 2001 Dino-Lite Digital Microscope, Taiwan) to analyze the site for biofilm formation and SS deposition. Light microscopic images are taken at the lowest magnification (20x) at 0h, and every 24 hours of biofilm formation or SS accumulation. For biofilm formation detection, membranes and spacers were

taken out of the flow-cell, were cut into 2 x 2 cm pieces, and then stained with the fluorescent green dye, SYTO 9, which stains both live and dead cells with a fluorescent green color. 100 µL of SYTO 9 was placed on the membrane with the spacer on top and then allowed to stand in the dark for 30 minutes at the minimum. After 30 minutes the excess dye was washed off with 100 µL of deionized filtered (0.22 µm, Millipore) water. The green fluorescence from the microorganisms that were present was viewed with a fluorescence microscope (Olympus CX-RFL-2) and images were obtained using Canon EOS Kiss X-50. For additional information, biofilm formed on the dyed membrane was quantified in terms of percentage coverage of the fluorescent green color, which was obtained by analyzing the fluorescent micrographs using the free imaging software ImageJ (Abramoff et al., 2004).

3.3 Results and discussion

3.3.1 Deposition of inorganic particles with and without biofilm

The raw secondary effluent water was filtered through 1µm glass fiber filter and thus suspended particles were already removed from the secondary effluent water feedwater. Since microorganisms are considered to be a major organic contributor to fouling (Henry et al., 2012), organic matter in this study is due mainly to biofilm formed and extracellular materials generated through biological growth on the membrane after flow experiments. Since kaolin was added in the feedwater (pure water or filtered secondary effluent water), inorganic matter, in this study refers primarily to SS from kaolin (in pure water) or kaolin and other inorganic colloidal particles that have remained in the filtered secondary effluent water. All reference to secondary effluent water from here on would pertain to the filtered secondary effluent water.

Results for Run A (simultaneous run) are shown on Figure 3.3 and Figure 3.4, where the amounts of organic matter and inorganic matter, respectively, that have accumulated by flowing pure water (without biofilm) and secondary effluent water (with biofilm) flowing

were determined as the initial kaolin concentration in the feedwater was increased (0-300 mg/L). Figure 3.3 shows that the amount of organic matter deposited using secondary effluent water was higher compared to that which was deposited using pure water owing to a high biofilm formation potential of the secondary effluent water, due to the presence of dissolved organic matter (DOM), which could support microbial activity. The presence of organic matter on both sets of fouled membranes indicates the nature of bacteria, which despite being eliminated by pretreatment, a few bacteria can still enter the filtration system, adhere to surfaces, and multiply in the presence of biodegradable substances (Flemming, 2002). The lack of correlation between organic matter deposited and increasing initial kaolin concentration for both types of sample water indicates that under these conditions, deposition of organic matter was not influenced by the amount of SS in the feedwater.

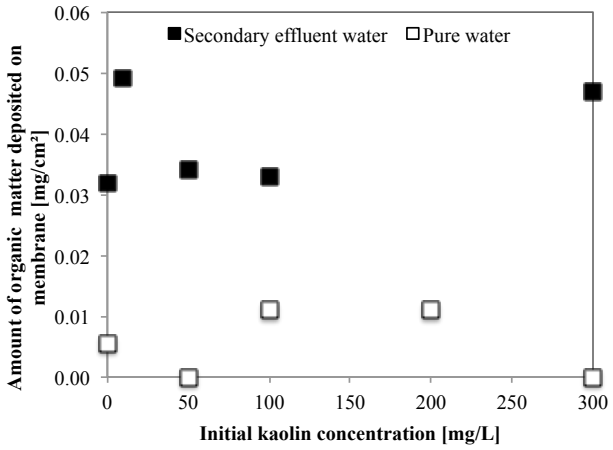

Figure 3. 3 Correlation of Amount Organic Matter Deposits with Increasing Initial Kaolin Concentration during Simultaneous 24-hour run of Feedwater with Kaolin.

As shown in Figure 3.4, the inorganic matter deposited on the membrane when both types of feedwater was used increased in proportion to the initial kaolin concentration in the feedwater, but with a higher linearity observed for the secondary effluent water. With an initial amount of 300 mg/L kaolin in both feedwater, the amount of inorganic matter deposited on the membrane using secondary effluent water as feedwater was greater by 0.16

mg/cm^2, indicating quantitative enhancement of inorganic matter due to biofilm, probably attributed to the inherent sticky, gel-like property of extracellular polymeric substances (EPS) surrounding the biofilm. However, deposited inorganic matter in the secondary effluent water is only 4% of the maximum amount that could be deposited on the membrane based on the initial 300 mg/L kaolin in the feedwater. This small percentage of deposition could be due to the absence of filtration during these continuous flow experiments. However, under these no filtration conditions, results showed that inorganic matter deposition, with and without biofilm, is influenced by SS in the feedwater, and that biofilm formation enhance the deposition. This particular observation implies the need for new pretreatment schemes that address both biofouling and SS fouling, focusing on control of biofilm, since presence of biofilm influence the SS accumulation on the membrane.

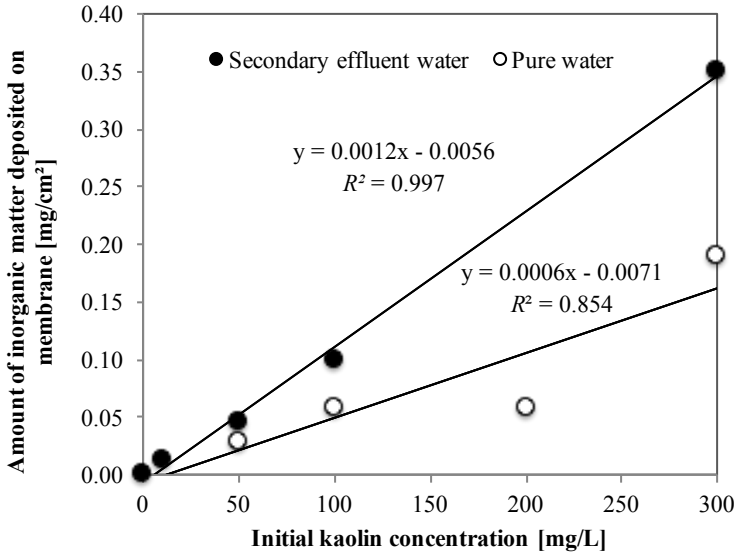

Figure 3. 4 Correlation of Amount of Inorganic Matter Deposits with Increasing Initial Kaolin Concentration during Simultaneous 24-hour run of Feedwater with Kaolin.

3.3.2 Interaction between biofilm and suspended solids

Results for Run B (Two-step) are shown on Figure 3.5 indicating a related increase in organic matter deposited (R^2 = 0.938) at increasing glucose concentrations, signifying increasing amount of biofilm formed on the membrane, attributed to the added nutrients that

could support such biofilm growth. The amount of inorganic matter deposited, on the other hand, was almost the same for all glucose concentrations, which is unexpected since there was increased amount of biofilm formed, as indicated by the increasing amount of organic matter deposited. The increase in organic matter at increasing glucose concentrations suggests that after covering the membrane surface, biofilm growth and accumulation happened in the vertical direction, i.e., in thickness, which in this case cannot be verified since epifluorescence microscopy, although an established technology for viewing biofilms on surfaces, is limited in its applicability for analyzing thickness (McFeters et al., 1995). However, considering the conditions set for Run A and Run B were different, the SS deposition mechanism might be different in the two runs. In Run A, the biofilm and kaolin are flowing simultaneously while in Run B, the kaolin suspension was flown over a pre-formed biofilm. After 24h of continuous flow of secondary effluent water, percentage biofilm coverage was found to be 80%, indicating that the membrane surface was almost completely covered with biofilm. Therefore, during Run B (48h), the SS suspension was already flowing on a biofilm-covered membrane and spacer. Thus, in these conditions, SS deposition was not influenced by the amount of biofilm but by the surface area of biofilm cover.

At the same kaolin concentration used (300 mg/L) and without added glucose, the amount of inorganic matter deposited for three runs are: 0.16 mg/cm^2 (pure water, Run A), 0.35 mg/cm^2 (secondary effluent water, Run A), and 0.62 mg/cm^2 (secondary effluent water, Run B). These quantitative results show enhancement when secondary effluent water was used as feedwater in contrast to pure water, and despite the difference in biofilm formation conditions (Run A, simultaneous; Run B, two-step). These results confirm that the biofilm on the membrane and spacer influence the SS deposition.

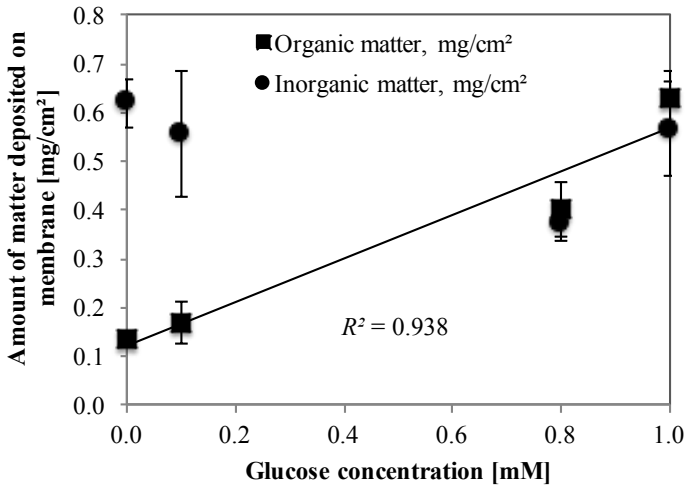

Figure 3. 5 Correlation of Amounts of (a) Organic and (b) Inorganic Matter Deposits at Increasing Amount of Glucose in 48-hour run of Secondary Effluent Water Followed by 1-hour Passing of 300 mg/L Kaolin Suspension.

3.3.3 Qualitative description of biofilm formation and SS deposition

Figure 3.6 and Figure 3.7 show qualitative information regarding the biofilm formation and SS deposition. Flow was from left to right (white arrow, Figure 3.6) indicating the entrance region/upstream from the feed side of the flow cell towards the exit region/downstream. Results show that biofilm formation was enhanced with increasing run time (24h to 72h), and with preferential formation of biofilm on the spacer filaments or at membrane areas near the spacer filaments, indicated by the dark portions in the optical micrographs (Figure 3.6) and the darker green portions on the fluorescent micrographs (white diamond line (spacer filament), Figure 3.7). This preferential formation of biofilm on spacer filaments or membrane regions near the spacer filaments had been observed in other studies (Vrouwenvelder et al., 2009; Suwarno et al., 2012; Radu et al., 2014) as well as on polyethersulfone (PES) microstructured membranes, where the spacer filaments or in the latter's case, the microstructures serve as points of attachment by the microorganisms (Ngene et al., 2010). One study reported that spacer fouling, more than membrane fouling, was considered to be a greater problem in NF/RO membranes operated with and without

permeation (Vrouwenvelder et al., 2009). Spacers on top of the membrane may provide additional surface area for bacterial deposition and would most likely be in greater contact with the flowing fluid rather than the membrane, thus biofilm formed on the spacer filaments rather than on the membrane surface. Suwarno *et al*., (2012) discussed conditions in which membrane fouling or spacer fouling dominates and postulated that in a fixed flux and increasing cross flow conditions, spacer fouling increases and membrane fouling decreases due to reduced concentration polarization. Relating this in the context of our study, the flow experiments were done without permeation (no flux), and thus there is negligible concentration polarization on the membrane surface that could enhance deposition of foulant materials on the surface of the membrane. Therefore, the microorganisms are in greater contact with the spacer filaments rather than on the membrane surface, favoring spacer fouling.

The biofilm formation on the spacers and membrane surfaces during 72 hours of biofilm formation at no glucose (Figure 3.7, upper left) and with 1 mM glucose (Figure 3.7, upper right) in secondary effluent water resulted to similar percentage biofilm coverage as measured by ImageJ from the area covered by the green fluorescent color on the micrographs (78% and 87%, respectively). The red dyed particles are shown to have deposited on the biofilm that has preformed on the membrane and spacer filaments (Figure 3.6 and Figure 3.7), which supports the observations in the previous section, that the biofilm area controls SS deposition under the conditions in these experiments, and not biofilm amount.

Biofilm and the red dyed particles qualitatively mirror initial formation and deposition, respectively, which is based on the material that they have greater contact. Instead of forming on the membrane surface, the microorganisms have greater contact with the spacer filaments, thus initially formed on the spacer. Then, the red dyed particles have greater contact with the biofilm that had formed on the spacer, thus deposited on the biofilm. This

similar position of initial particle deposition and biofilm formation has also been observed in PES microstructures membranes (Ngene et al., 2010). Kang *et al*., (2004) reported the similarity of initial deposition of biofilm and SS as governed by hydrodynamic properties and chemical interactions. In our study, the initial biofilm deposition is governed by the fixed cross flow velocity under no flux conditions and a greater contact between the spacer and biofilm during flow of secondary effluent water. The red dyed particles in the suspension during fixed flow velocity comes in contact with the biofilm which has formed on the spacer filaments, and thus are deposited on these areas.

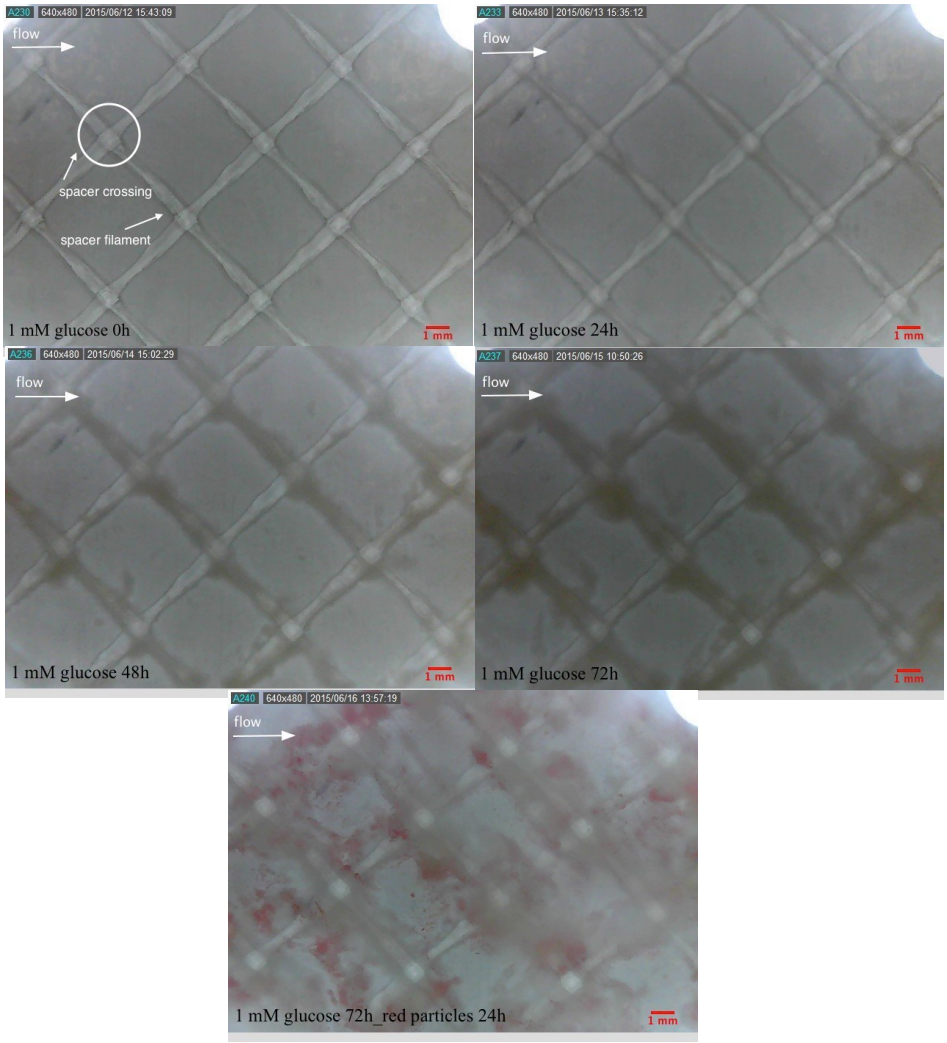

Figure 3. 6 Optical Micrographs of Biofilm Formation during 72-hour Flow of Secondary Effluent Water with 1.0 mM Glucose Followed by 24-hour Flow of Red Dyed Microsphere Particles.

Figure 3.7 (bottom right) is a magnified micrograph of the area where the red dyed particles are situated on the biofilm. Darker red portions appeared to have been deposited on top of the biofilm while lighter red portions seemed to have been deposited within the biofilm. The presence of these red dyed particles on/in the biofilm-filled spacer confirmed the inorganic SS accumulation as influenced by biofilm. For future studies involving real filtration conditions, the results in this study could imply synergistic effect, which had been observed between colloidal materials and natural organic matter in NF membranes (without spacers) that lead to a significant flux decline (Li and Elimelech, 2006).

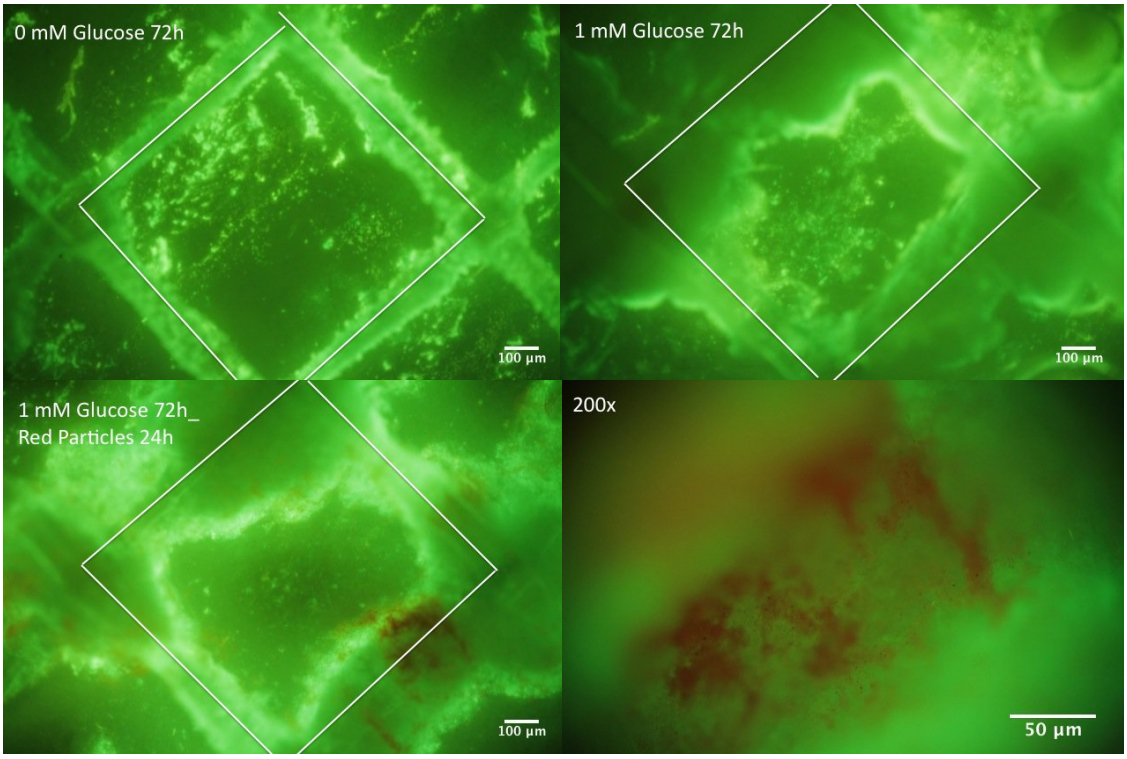

Figure 3. 7 Fluorescence Micrographs of Biofilm Formed after 72-hour Flow of Secondary Effluent Water with 0 mM Glucose (upper left), 1 mM Glucose (upper right) and 1 mM Glucose Followed by 24-hour Flow of Red Dyed Microsphere Particles (bottom left) (magnification 40x; white lines represent spacer filaments); Red Dyed Microsphere Particles Magnified 200x (bottom right).

3.5 Summary and Conclusion

Continuous cross flow experiments under no filtration conditions revealed the enhancement of inorganic matter deposited on the RO membrane due to biofilm. Enhancement occurred whether the SS deposition and biofilm formation are happening simultaneously or SS suspensions are flowed over a preformed biofilm. Under the conditions set in this study, biofilm formation and SS deposition seemed to preferentially occur on the spacer filaments. Fluorescent micrographs suggest deposition of SS on and within the biofilm formed. The findings in this study, particularly the influence of biofilm on SS deposition and accumulation, is beneficial regarding pretreatment schemes that could be improved since a composite fouling due to biofilm-SS interaction can be addressed by focusing on biofilm control such that SS deposition could also be prevented. In addition, the findings on this study have significant implications when continuous cross flow experiments are done under filtration conditions, since other hydrodynamic properties such as permeate flux can influence the fouling events.

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Chapter 4: Hypochlorite washing studies for biofilm formation control and inorganic particle accumulation on RO membranes

4.1 Introduction

Water demand has increased due to growth caused by increased population, industrial expansion, tourism, and agriculture development in many water-stressed or arid regions or countries (Ghaffour et al., 2013). Moreover, the global needs for water are expected to reach 6900 billion m^3 by the year 2030 which is about 150% increase from the demand in 2009 (4500 billion m^3) (Misdan et al., 2012, Douglas, 2009). Desalination is an increasingly common solution to supply fresh water in many regions of the world where this resource is scarce (Peñate and García-Rodríguez, 2012). Reverse osmosis (RO) is the most widely used desalination technology globally for desalination to purify water for drinking and other purposes (Lee et al., 2011, Peñate and García-Rodríguez, 2012), with RO technology accounting for 59.9% of the desalination amount in the world (GWI/IDA DesalData, 2013). In 2012, the sources for desalination are split to about 58.9% from seawater, 21.2% from brackish groundwater sources, and the remaining percentage from surface water and saline wastewater (GWI/IDA DesalData, 2013). Recently, secondary effluent water has been also used as feed water for RO membrane technology because of worldwide water shortage (Li et al., 2007, López-Ramírez et al., 2003, Glueckstern et al., 2008).

In membrane technology, membrane fouling is categorized into crystalline fouling including mineral scaling, organic fouling, particle and colloid fouling, and microbiological fouling or biofouling (Flemming, 1997). The first three types of fouling can generally be controlled by pretreatment for foulant removal from feed water (Flemming, 1997); however, despite silt density index (SDI), biological, and chemical parameters of feed water being

within limits prescribed by RO membrane suppliers, severe membrane fouling can still occur, with fouling layers mostly organic and of biological origin with minor inorganic amounts (Schneider et al., 2005). An inevitable problem of RO membrane technology is biofouling, which is caused by adhesion and accumulation of microorganisms, followed by growth and formation of biofilms among all the types of fouling (Flemming, 1997, Mansouri et al., 2010). Biofouling is characterized by the presence of a biofilm on the membrane leading to increase in resistance and decline in membrane performance, such as water permeation and rejection of solutes (Suwarno et al., 2014). Microorganisms are present in nearly all water systems and are capable of colonizing almost any surface (Matin et al., 2011). Moreover, spiral wound elements, which contain feed spacer to keep membrane sheets apart and create the flow channel, were used for RO in current industrial practice (Schwinge et al., 2004). The deposition of biofilms or particles was analyzed for different feed spacer orientations such as diamond and ladder (Neal et al, 2003, Ngene et al, 2010) and it has been reported that the position of initial particle deposition seems qualitatively similar to biofouling (Ngene et al, 2010). In membrane elements, biofilms may form around the spacer along with inorganic particles, if some particles contaminate the feed water or are carried over from pretreatment of the raw water (Speth et al., 2000).

Use of free chlorine has been considered as a promising and effective method for control of biofilm formation. However, most of polyamide RO membranes, which have been primarily used for water recycling and desalination applications (Antony et al., 2010, Xu et al., 2013), have no resistance to chlorine at present and, therefore, it has been pointed out that residual free chlorine in the feed solution can cause the degradation of polyamide if free chlorine is not completely removed before RO membrane filtration (Kwon et al., 2006, Kang et al., 2007, Shintani et al., 2007, Do et al., 2012). The pretreatment of feed solution with free chlorine has been widely used as a standard practice in current RO systems (Matin et al.,

2011, Colquhoun et al., 2010). Moreover, continuous chlorine treatment has been the industrial standard for years (Fritzmann et al., 2007, Kim et al., 2009), but intermittent chlorine treatment is also considered in recent years. In a study by Fujiwara et al., 2008, when the required minimum chlorine concentration and injecting time of intermittent chlorine treatment based on the experiments of the growth and sterilization rates of microorganisms in seawater of Middle East were simulated, the operational results proved that intermittent chlorine treatment was the most effective chlorine injection mode to achieve best RO performance for desalination. The reduction of biofilm by continuous or intermittent shock chlorination of seawater for the pretreatment of RO system was evaluated in other studies (Dionisio-Ruiz et al., 2014, Xu et al., 2010). However, the intermittent shock chlorination every 15 days at 1 mg/L of residual free chlorine for 2 h of exposure time could not reduce the biofilm formation, and improvement of the application method (higher frequency or different dosage) was suggested to prevent the development of the biofilms on the RO membranes (Dionisio-Ruiz et al., 2014). The microorganisms, which could not be reduced completely during pretreatment most likely form biofilms on the RO membrane. On the other hand, it was reported that the biofilm formed by *Pseudomonas aeruginosa* PAO1 GFP on the RO membrane surface was inactivated by the continuous chlorine treatment with 10 mg/L of initial chlorine concentration for 30 minutes (Yu et al., 2013). Direct chlorine treatment will be able to reduce biofilm formation thoroughly and will allow simplification of the conventional pretreatment similar to disinfection of microorganisms in feed water. The intermittent chlorine treatment, on the other hand, which may be able to reduce biofilms with an exposure amount smaller than the continuous chlorine treatment, is also effective from the point of view of membrane degradation by chlorine since it will be able to reduce biofilm formation with minimum membrane damage. However, despite many reports about the degradation of polyamide RO membrane by hypochlorite treatment, the reduction of biofilm

on the RO membrane has not been examined in various conditions of chlorine treatment, and have not also been quantitatively evaluated.

Chlorine-resistant polyamide membranes are being developed to allow direct treatment with free chlorine and chloramines to avoid biofilm formation on the membrane (Shintani et al., 2007, Xu et al., 2013, Buch et al., 2008, La et al., 2010, Yu et al., 2009, Kwon et al., 2012, Shin et al., 2011), and are expected to be used in the near future. Therefore, chlorine washing will be an option to avoid biofouling on the chlorine-resistant membrane. Furthermore, chlorine washing has potential to avoid the accumulation of particles on membrane. If particle accumulation control is possible by using chlorine washing, expensive pretreatment for particle removal will be removed or simplified. Thus, it will be very important in designing and developing chlorine-resistant membranes to quantitatively understand the control of biofilm formed and inorganic particle accumulated on the membrane surface by chlorine treatment.

In this study, the reduction of the biofilm formed on the RO membrane by hypochlorite was examined by optimization of various chlorine treatment/washing conditions: free chlorine concentration, washing frequency, and washing time, using a conventional polyamide RO membrane. Moreover, the reduction of inorganic particle accumulated with biofilm formation in a continuous flow channel with membrane and spacer was also examined.

4.2 Materials and methods

4.2.1 Materials

Commercially available polyamide RO membrane NTR-759HR, provided by Nitto Denko Co. (Osaka, Japan) was used in the study. All virgin membranes were thoroughly washed with pure water first before use. A diamond type polypropylene spacer (3 × 3 mm

mesh size) was used in the study. *Bacillus subtilis* was used JCM 2499 (Riken, Japan). Tryptic Soy Broth (TSB) was purchased from Merck (Darmstadt**,** Germany). McFarland Turbidity Standard was purchased from bioMérieux, Inc. (Marcy l'Etoile, France). Glucose ($C_6H_{12}O_6$), kaolin (5-10 μm), and sodium chloride (NaCl) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Sodium hypochlorite (NaOCl) (approx. 10% available chlorine) and Chicago Sky Blue 6B were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Other reagents were purchased as analytical grade from Kanto Chemical Co. Inc. (Tokyo, Japan). DPD (N,N-diethyl-p-phenylenediamine) was purchased for free chlorine analysis from Hanna Instruments Japan, Inc. (Chiba, Japan). Pure water was prepared using a Milli-Q Reference Ultrapure Water Purification System (Merck Millipore Corp., Darmstadt, Germany). A 0.85% NaCl was prepared from NaCl. Secondary effluent water discharged after activated sludge treatment and settling but before disinfection was taken from Higashi-Hiroshima Wastewater Treatment Plant, and then was filtered by a glass fiber filter (1.0 μm, GF/B, Whatman, UK). The filtered secondary effluent water was used for all experiments for biofilm formation.

5.2.2 Bacteria stock preparation

Bacillus subtilis has emerged as an alternative model organism for studying the molecular basis of biofilm formation (Vlamakis et al., 2013). It has also been found to be one of the many bacterial species that participate in biofouling on RO membranes (Matin et al., 2011, Ridgway et al., 1983), and thus has been used in studies that involve seawater RO membrane biofouling (Lee et al., 2010) and in the development of antibacterial polyamide RO membranes (Saeki et al., 2013), and thus the use of *B. subtilis* is acceptable for enhancing biofilm development on the membrane surface**.** The bacterial stock was prepared by growing overnight cultures in 3 % Tryptic Soy Broth with shaking at 45 rpm at 37°C for 24 h. The bacterial cells were recovered by centrifugation at 2,000 rpm for 5 min and washed for at

least three times with 0.85% NaCl. The recovered bacterial suspension was resuspended in 0.85% NaCl to achieve an optical density of 0.4–0.5 at 550 nm using spectrophotometer (UV-1800, Shimadzu Co., Kyoto, Japan), resulting to a bacterial concentration in the range of 3–6 × 10^8 cfu/mL in the stock mixture, indicated by the McFarland Turbidity Standard. This stock of *B. subtilis* suspension was then added to the sample waters with the necessary dilutions required by the experiment.

4.2.3 Biofilm formation reduction by soaking test

Reduction of biofilm formation on the RO membrane surface was conducted by soaking test with continuous or intermittent chlorine washing of the membrane. The membrane was cut using sterile scissors into 20 × 20 mm pieces, and was washed with pure water. To enhance biofilm formation potential on the membrane surface, the filtered secondary effluent water was added with 1 mM of glucose and/or 3–6 × 10^7 cfu/mL of *Bacillus subtilis*, and was used as soaking solution for all experiment. The membranes were soaked in the soaking solution, and then the biofilm was allowed to form at 37°C in the dark with shaking at 45 rpm for 48 h. The chlorine washing for reduction of biofilm formation was continuously or intermittently conducted using aqueous sodium hypochlorite solution. In continuous washing, the soaking solution used was added with hypochlorite with free chlorine concentration maintained at 1.1 ± 0.2 mg/L. The soaking solution was replaced with fresh solution every 12 h. In intermittent washing, different conditions for chlorine washing were devised: the residual free chlorine concentration in the soaking solution, the washing frequency, and the washing time. The soaking solution was replaced with secondary effluent water added with hypochlorite every 6, 12, and 24 h, and then the membranes were exposed to the water with free chlorine for 1–30 minutes. For the secondary effluent water added with hypochlorite, the residual free chlorine concentrations of the secondary effluent water with free chlorine were within 0.5–10 mg/L, and the free chlorine concentration under study was

maintained during the washing. After the membrane was washed, the secondary effluent water with free chlorine was replaced with fresh soaking solution. As control washing experiment, the membrane was exposed to the secondary effluent water without free chlorine, and then was replaced with fresh soaking solution. Biofilm formed on the membrane, before and after the chlorine washing, was determined by fluorescence intensity analysis.

4.2.4 Biofilm formation reduction by continuous flow test

To confirm the validity of the chlorine washing condition suggested by the soaking test, the reduction of biofilm formation on the RO membrane surface was conducted by continuous flow test with intermittent chlorine washing. A schematic diagram of a cross-flow test cell with permeation is presented in Figure 4.1.a. The stainless steel continuous cross-flow RO test cell (spin flow cell; Tritec Co., Ltd., Tokyo, Japan) with internal diameter of 75 mm and equipped with a pump (KP-22, Flom, Tokyo, Japan) was used. The filtered secondary effluent water was used as feed water, and was adjusted to pH 6.0 using HCl and NaOH during the operation. The membrane was then placed inside the RO test cell, and the test cell was operated by running feed water with stirring at a flow rate of 50 mL/min (initial flux 19.3 $m^3/m/^2d$), operating pressure of 1.5 MPa, and at 25°C for 72 h. The effective filtration area of the operating membrane was 37.4 cm^2. Concentrate and permeate were disposed during the initial 4 minutes (200 mL), and then were circulated into the feed tank. The secondary effluent water with free chlorine was intermittently flowed for 30 minutes of washing time every 12 h to reduce the biofilm formation on the membrane. The residual free chlorine concentrations in the secondary effluent water were within 3–10 mg/L, and the free chlorine concentration under study was maintained by adding hypochlorite solution during the washing.

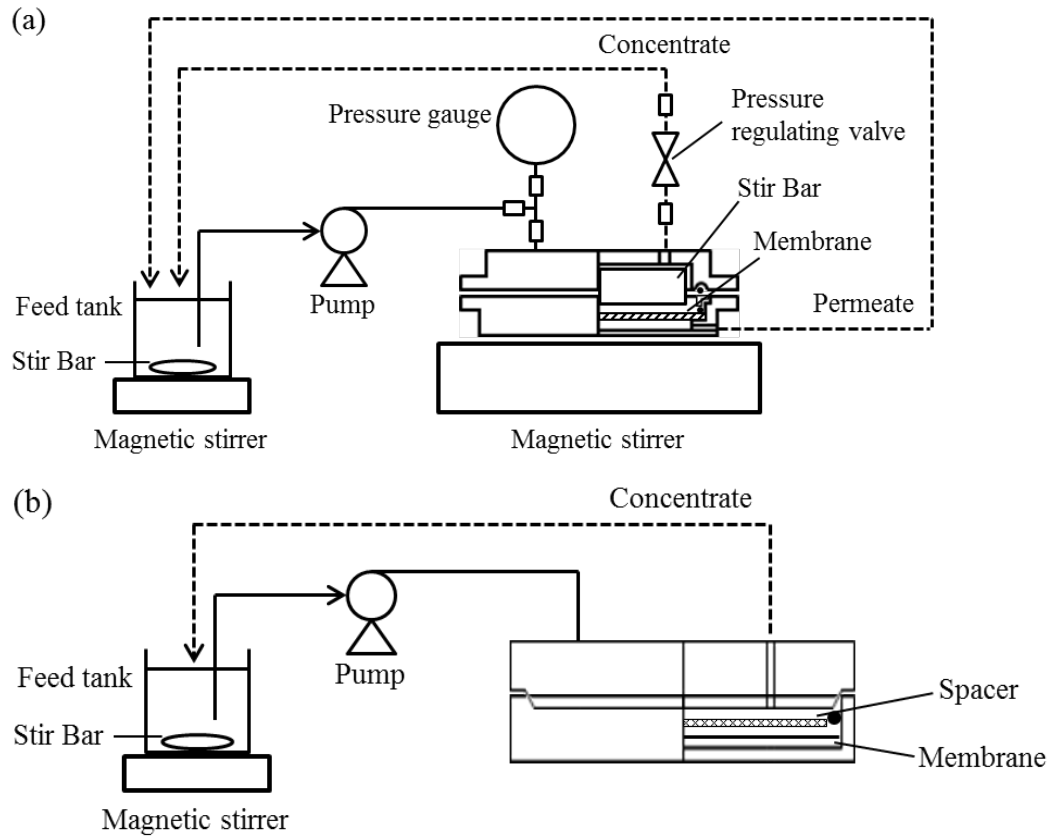

Figure 4. 1 Schematics of test cells for continuous test: (a) Cross-flow test cell with permeate, (b) Cross-flow test cell without permeate.

4.2.5 Reduction of inorganic particle accumulation with biofilm formation

To evaluate the reduction of inorganic particle accumulation attributed by the biofilm formation on the membrane surface and spacer, continuous chlorine washing was also employed to a membrane with biofilm and particle deposited during continuous flow experiments. A schematic diagram of a cross-flow test cell without permeation is presented in Figure 4.1.b. The acrylic test flow cell with internal space to put the polyamide RO membrane (45 × 90 mm) and the spacer and equipped with a pump (DSP-100SA, AS ONE Co., Osaka, Japan) was used. The biofilm and the inorganic particle were allowed to form and deposit on the membrane with a spacer placed on top of the membrane. To determine the accumulation of an inorganic particle quantitatively with and without hypochlorite, filtered secondary effluent water added with 1 mM of glucose and 3–6 × 10^7 cfu/mL *B. subtilis* to

enhance biofilm formation and added with 300 mg/L of kaolin, was used as feed water. The membrane and spacer were then placed inside the test flow cell, and the test flow cell was operated by running feed water at a flow rate of 30 mL/min and at 25°C for 24 h without pressure. The effective filtration area of the operating membrane was 40.5 cm^2. The chlorine washing was continuously conducted, and the residual chlorine concentration in the secondary effluent water was maintained at 10.1 ± 1.6 mg/L during the washing.

To detect the presence of the kaolin particles, the membrane and spacer with biofilm and inorganic particles were stained by running Chicago Sky Blue 6B solution through the test flow cell, and then images of the membrane and spacer surface were taken using a digital microscope (Dino-Lite Basic DINOAM2001, Thanko Co., Tokyo, Japan) to analyze the site for biofilm formation and inorganic particles deposition. Light microscopic images were taken at 20× magnifications at 0 h and 24 h. To detect biofilm formation, the membrane and spacer with biofilm and inorganic particles were taken out of the test flow cell, carefully cut into 20 × 20 mm pieces, and then were stained with SYTO 9 using the method described in Section 2.6. The green fluorescence from the microorganisms present on the stained membrane and spacer was viewed using a fluorescence microscope which is an upright microscope (CX-41, Olympus Co., Tokyo, Japan) equipped with a power supply unit (U-RFLT50, Olympus Co., Tokyo, Japan). Images were obtained to detect the biofilm formation using a camera (EOS Kiss X50, Canon Inc., Tokyo, Japan) which is attached to the fluorescence microscope. Accumulated particle deposits on the membrane were determined through loss on ignition (LOI) tests. Pure water was filtered through a glass fiber filter (1.0 μm, GF/B) as rinsing, and then the filter was dried for 1 h at 110°C. The dried filter is then burned to 550°C for 30 minutes to get the mass of dried and ignited filter (m_1). Membrane and spacer were carefully removed from the test flow cell after each experiment, and the spacer was also carefully removed from the membrane top. The accumulated mass on the

membrane surface was thoroughly removed from the membrane surface by rinsing and brushing using nylon toothbrush (Lion Co., Japan) in pure water. The resulting water was then filtered in a pre-weighed GF/B filter. The collected mass and filter was then dried for 1 h at 110°C, and then weighed (m_2). Total mass was calculated as m_1 mass subtracted from m_2 mass. The dried mass and filter was further ignited to 550°C and then weighed (m_3) to obtain inorganic mass. Inorganic mass was calculated as m_1 mass subtracted from m_3 mass, and organic mass was calculated as inorganic mass subtracted from total mass.

4.2.6 SYTO 9 staining procedure and fluorescence analysis

After soaking or filtration experiments, the membranes were retrieved, and then subjected for staining. The green dye, SYTO 9 from the BacLightTM Bacterial Viability Kit L13152 (Invitrogen/Molecular Probes, USA) stains both live and dead cells with a fluorescent green color. The SYTO 9 solution was prepared according to manufacturer specifications, and was kept in the dark and inside the refrigerator until analysis. A 20 × 20 mm piece of the membrane was stained with 100 μL of SYTO 9 for 30 minutes in the dark, and then the excess dye was carefully removed from the membrane by pipetting.

Amount of biofilm formed on the dyed membranes is quantified by fluorescence intensity (FI). The FI was analyzed using a microplate reader (Gemini EM, Molocular Devices Japan Co., Tokyo, Japan) with SoftMax®Pro Microplate Microplate Data Acquisition & Analysis Software with Excitation scan set at 485 nm and Emission scan set at 545 nm. The FI values for 144 points per membrane were analyzed. Averages of FI values for each membrane were then calculated. The FI values are then reported as the averages of 3 replicate membranes for soaking tests and averages of 6 cut pieces from the membrane used in continuous filtration tests, and the precision reported as standard deviations for n = 3 and 6 membranes, respectively. The ΔFI, which indicates the amount of biofilm remaining, was calculated as the FI of the virgin membrane subtracted from the FI of the membrane with biofilm formed.

4.3 Results and discussion

4.3.1 Biofilm formation control by continuous washing and intermittent washing

Behavior of biofilm formation (ΔFI) by continuous washing with 1 mg/L of residual free chlorine and intermittent washing every 12 h with 1 and 10 mg/L of residual free chlorine are shown in Figure 4.2. The biofilm formation on the RO membrane without chlorine washing increased with soaking time, and ΔFI after 48 h was 440. The ΔFI by continuous washing with 1.1 ± 0.2 mg/L of free chlorine decreased every sampling, and was 12 (97% reduction) after 48 h. According to Redondo et al. (2001), a residual free chlorine concentration of 0.5–1.0 mg/L should be maintained throughout the whole pretreatment for disinfection of microorganisms. This was also the same on the RO membrane. On the other hand, the ΔFI by intermittent washing every 12 h was 454, and was the same for the control experiment. It has been reported that continuous chlorination enables better reduction of total aerobic bacteria than intermittent chlorination (Dionisio-Ruiz et al., 2014). ΔFI before chlorine washing after 48 h was 864. The intermittent chlorine washing reduces the biofilm by removing the biofilm that has formed on the membrane. The microorganisms and biofilm fragments, which detached from the biofilm during intermittent chlorine washing create an increased risk of biofilm formation on RO membrane (Dionisio-Ruiz et al., 2014). The intermittent washing with 1 mg/L of the free chlorine, in this case, seemed to be an extremely insufficient condition which aids instead of reduce biofilm formation. In the intermittent washing with 10 mg/L of the free chlorine, the ΔFI was 0 (100% controlled), and reduced biofilm formation completely. The CT value in the continuous washing with 1 mg/L of the free chlorine for 1 day, which is calculated as the product of free chlorine concentration and the washing time, was 24 mg·h/L. On the other hand, CT values in the intermittent washing for 1 day were 1 mg·h/L for 1 mg/L of free chlorine and 10 mg·h/L for 10 mg/L of free chlorine, respectively. These results suggest that the intermittent washing, which can reduce

the biofilm formation with small CT value is more beneficial than the continuous washing, since it can also be effective in preventing membrane degradation by hypochlorite due to the low CT value.

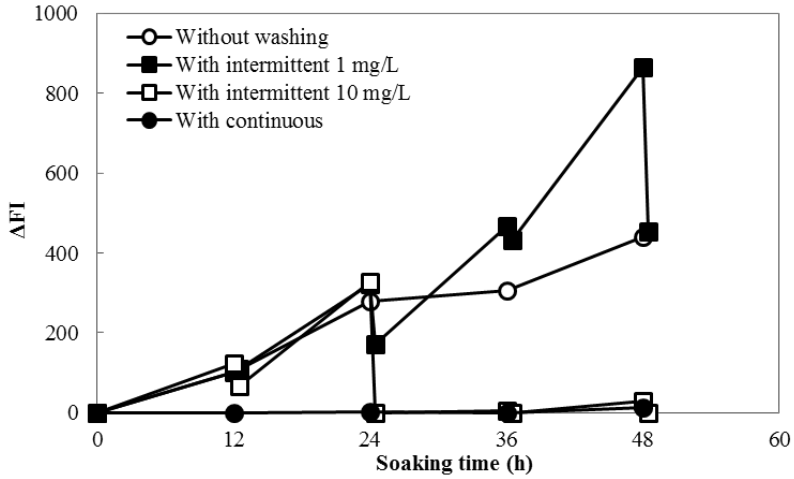

Figure 4. 2 Behaviors of biofilm formation by continuous washing and intermittent washing every 12 h with 1 mg/L of residual free chlorine concentration.

4.3.2 Optimization of washing condition for biofilm on the membrane

The optimization of washing condition for the intermittent chlorine washing was evaluated to reduce the biofilm formation completely and efficiently. The ΔFI after 48 h was evaluated to determine the effect of the different conditions during intermittent washing. The amounts of biofilm remaining using intermittent chlorine washing under different conditions are shown in Figure 4.3. The washing conditions constitute 0.5–10 mg/L of free chlorine concentration, every 6, 12, and 24 h of washing frequency, and 1–30 min of washing time. ΔFI after 48 h for the control experiments (without free chlorine) with replacement of solutions at 6, 12, and 24 h were 338, 440, and 701, respectively. Figure 4.3.a shows the effect of free chlorine concentration and washing frequency to the reduction of biofilm formation. Results show that the ΔFI with 30 min of washing time decreased as the free chlorine concentration increased in all washing frequencies employed, indicating that there is concentration dependence existing between reduction of biofilm formed on the membrane

and the free chlorine amount. In particular, for every 12 h of washing frequency, ΔFI for 3 mg/L of free chlorine remarkably decreased to 146 from that for 2 mg/L of free chlorine (396). The intermittent chlorine washing using 1 and 2 mg/L of free chlorine might have been insufficient resulting to an increase of biofilm formation after 48 h since at chlorine concentration of 2 mg/L, the ΔFI at 6, 12, and 24 h was 46.8, 396, and 423, respectively. The biofilm formation decreased as the washing frequency increased, and every 6 h of washing frequency indicated the highest reduction effect, which was 93%. After 6 and 12 h, ΔFI values of control experiment for every 6 h of washing were 18 and 129, respectively (data not shown). The every 6 h of washing frequency could reduce the biofilm most effectively, because the free chlorine could attack before or during the early stages of biofilm formation on the membrane. To reduce biofilm formation to more than 90% during intermittent washing, the necessary residual chorine concentrations were less than 2 mg/L for every 6 h of washing, and more than 10 mg/L for every 12 and 24 h washing. The minimum washing conditions, which could reduce the biofilm by 100% on the RO membrane were 10 mg/L of free chlorine concentration and every 12 h of washing frequency.

Every 12 h of washing frequency was selected and then CT value was used to evaluate the optimum washing condition of residual free chlorine concentration and washing time for reduction of biofilm formation (Figure 4.3.b). ΔFI decreased as the CT value increased. The 90% reduction of biofilm formed on the membrane could be achieved by 5 mg/L of free chlorine and 5 min of washing time, with the CT values required to reduce the biofilm by 90% were 1.7–20 mg·h/L for 48 h, which was calculated at 5–20 mg/L of free chlorine and 3–30 min of washing time. Based on this, it can be suggested that the optimum condition for intermittent chlorine washing as 10 mg/L of free chlorine concentration, washing frequency of every 12 h, and 5 min of washing time to sufficiently reduce the biofilm formed on the RO membrane (CT = 0.07 mg/L for 1 h).

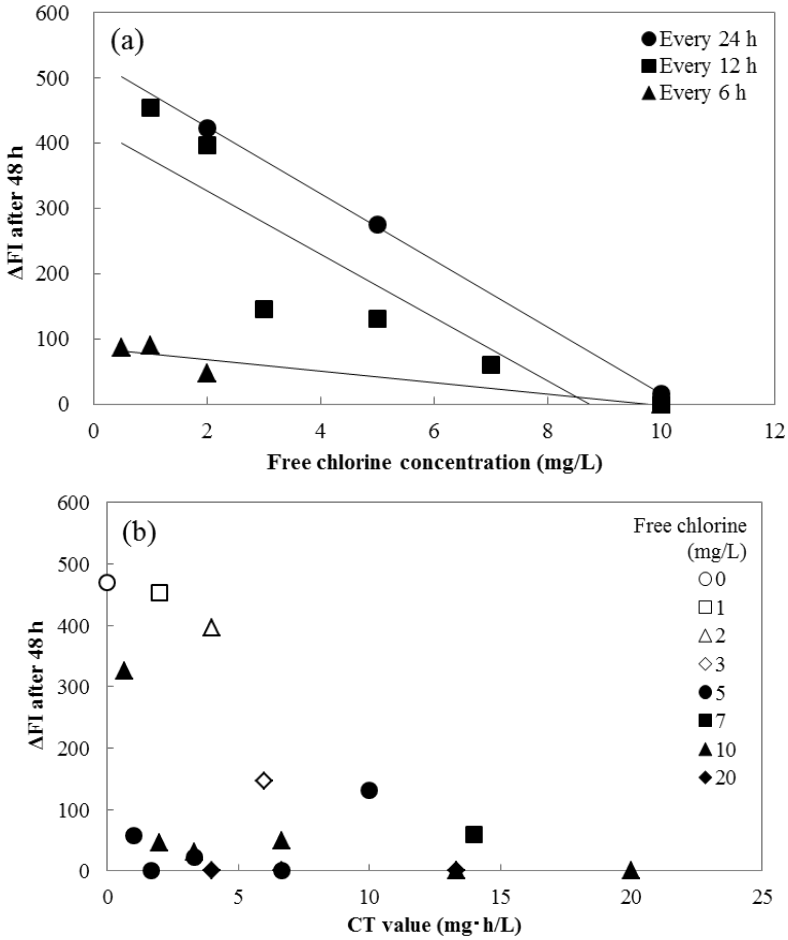

Figure 4. 3 Amount of biofilm remained after 48 h by intermittent chlorine washing in different washing conditions; Washing conditions are 0.5–10 mg/L of free chlorine concentration, every 6 h, 12 h, and 24 h of washing frequency, and 1–30 minutes of washing time: (a) examination of free chlorine concentration and washing frequency with 30 minutes of constant washing time, (b) examination of free chlorine concentration and washing time with every 12 h of constant washing frequency.

For general use, the polyamide membranes are designed to be exposed to maximum chlorine concentration of below 0.1 mg/L (Sajjad and Rasul 2015; Bódalo-Santoyo et al., 2004). Our results of free chlorine concentration required for intermittent washing for reduction of biofilm formation was within the application conditions for the polyamide membrane, and thus the intermittent washing condition can be applied for conventional membranes. On the other hand, membrane lifetime has been estimated as 3-5 years for continuous operation, depending on the feed stream characteristics and the operating conditions (Baker, 2004; Madaeni and Samieirad, 2010; Shemer and Semiat, 2011), and the

general maximum chlorine resistance of the polyamide RO membrane is calculated to be 2,628 mg·h/L during 3 years of membrane lifetime. The membrane lifetime allowed for direct chlorine washing was estimated to be 1,546 days using the suggested intermittent washing condition (CT = 1.7 mg·h/L for 1 day). Furthermore, in order to achieve the general membrane lifetime of 3-5 years, CT values based on the conditions suggested by this study were 1,825–3,042 mg · h/L, which are the chlorine resistance values for the polyamide RO membrane.

4.3.3 Confirmation of validity of optimized washing condition

To confirm the validity of the condition of intermittent chlorine washing based from the soaking test, the amount of biofilm remaining was evaluated by continuous filtration test with intermittent chlorine washing. The variations of amount of biofilm remaining after 72 h are shown in Figure 4.4. Continuous filtration was conducted with 3–10 mg/L of chlorine concentration, 30 min of washing time, and every 12 h of washing frequency for 72h. ΔFI of control (without the free chlorine) in the continuous filtration test was 1,791, which was 3.8 times higher than that in the soaking test (470). Biofilm formation was easier in the continuous filtration test because the membrane surface was always subjected to pressure and velocity, enhancing contact between microorganisms in the feed water and the membrane surface. The ΔFI did not change until 3 mg/L of free chlorine concentration, which is hardly reduced until 4 mg/L of free chlorine concentration, unlike the results in the soaking test. However, ΔFI at 4 mg/L of free chlorine was 142.8 after 72 h, and is expected to increase upon continued filtration. The biofilm formed on the membrane was completely reduced at greater than 5 mg/L of free chlorine concentration. The reduction of biofilm formation in the continuous filtration test required a free chlorine concentration that is lower than that in the soaking test, because the membrane surface is continuously exposed to the chlorine washing

due to the applied pressure and flow of the washing solution. However, biofilm that is remaining due to insufficient washing can still accumulate with the extension of operating time. Based on these results, for the intermittent washing, the sufficient free chlorine concentration, 5 mg/L, which was lower than the soaking test, could reduce the biofilm formed on the RO membrane during continuous filtration operations. The CT value in intermittent chlorine washing for 30 min is 5 mg·h/L for 1 day, which was higher than the optimum washing condition during the soaking test (1.7 mg·h/L), and thus, shorter washing time should be examined in future continuous filtration tests.

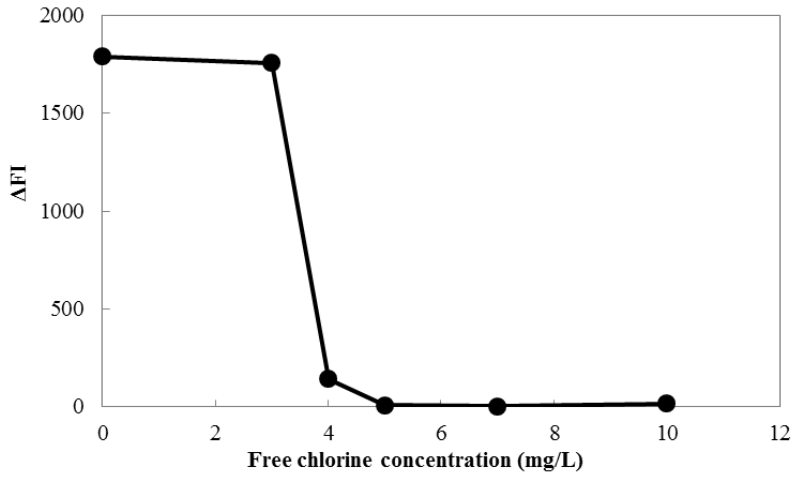

Figure 4. 4 The variation of the amount of the biofilm remained by the intermittent chlorine washing in the continuous filtration test for 72 h.

Direct chlorine washing is a non-conventional method of washing because of the known membrane degradation of polyamide due to chlorine. However, in the conditions performed in this study, comparison of salt rejection performance for 72 hours of cross-flow filtration of untreated secondary effluent water and treated secondary effluent water (10 mg/L free chlorine, 30 min washing for every 12 h), with a calculated CT value of 30 mg·h/L showed no change in rejection (99 % rejection all throught) for both samples, indicating that the chlorine washing conducted did not damage the membrane. This is particularly significant

because direct chlorine washing will be a simplified but effective pretreatment for developing and future chlorine-tolerant membranes.

4.3.4 Reduction of inorganic particle accumulation with biofilm formation

The continuous chlorine washing was conducted to evaluate the reduction of the accumulation of inorganic particles with biofilm formation on the membrane and spacer. The light images and fluorescence images of the membrane and spacer after 24 h with biofilm formed and accumulated kaolin with or without the chlorine washing are depicted in Figure 4.5. The micrographs show that without free chlorine conditions, the accumulated kaolin, which was stained by a blue dye was observed mainly around the spacer, and the fluorescent green color from the biofilm formed was also observed mainly around the spacer. On the other hand, the presence of biofilm and kaolin on the membrane and spacer was not observed in the condition with 10.1 ± 1.6 mg/L of free chlorine. This suggests that continuous chlorine washing reduced the accumulation of inorganic particles on the membrane and spacer along with the biofilm formed.

The reduction of the inorganic particles accumulation with the biofilm formation was quantitatively evaluated. After 24 h, the amounts of organic and inorganic materials deposited on the membrane and spacer in control experiment (pure water and 300 mg/L kaolin, without free chlorine) were 0 and 0.17 mg/cm^2, respectively. For secondary effluent water added with glucose, *B. subtilis*, and 300 mg/L kaolin, the amounts of organic and inorganic materials deposited were 0.14 and 0.33 mg/cm^2, respectively; indicating an amount of 0.16 mg/cm^2 increase in inorganic material deposited due to biofilm formation on the membrane and spacer. It can be concluded that biofilm formation aided the accumulation of inorganic particle from the feed water on the membrane and spacer. The continuous chlorine washing with free chlorine concentration ranging from 8.9–11.2 mg/L, resulted to 0.11 mg/cm^2 (79%) and 0.19 mg/cm^2 (58%) reduction in the amount of organic and inorganic

material deposited (0.03 and 0.14 mg/cm^2), respectively. The deposited amounts after 24 h with chlorine washing were reduced to the same levels as those of the control experiment (without the free chlorine). These results confirmed the role of biofilm on the accumulation of inorganic particles. The results also indicate that biofilm growth can be reduced and that essentially can also reduce the accumulation of not only the biofilm but also the inorganic suspended particles.

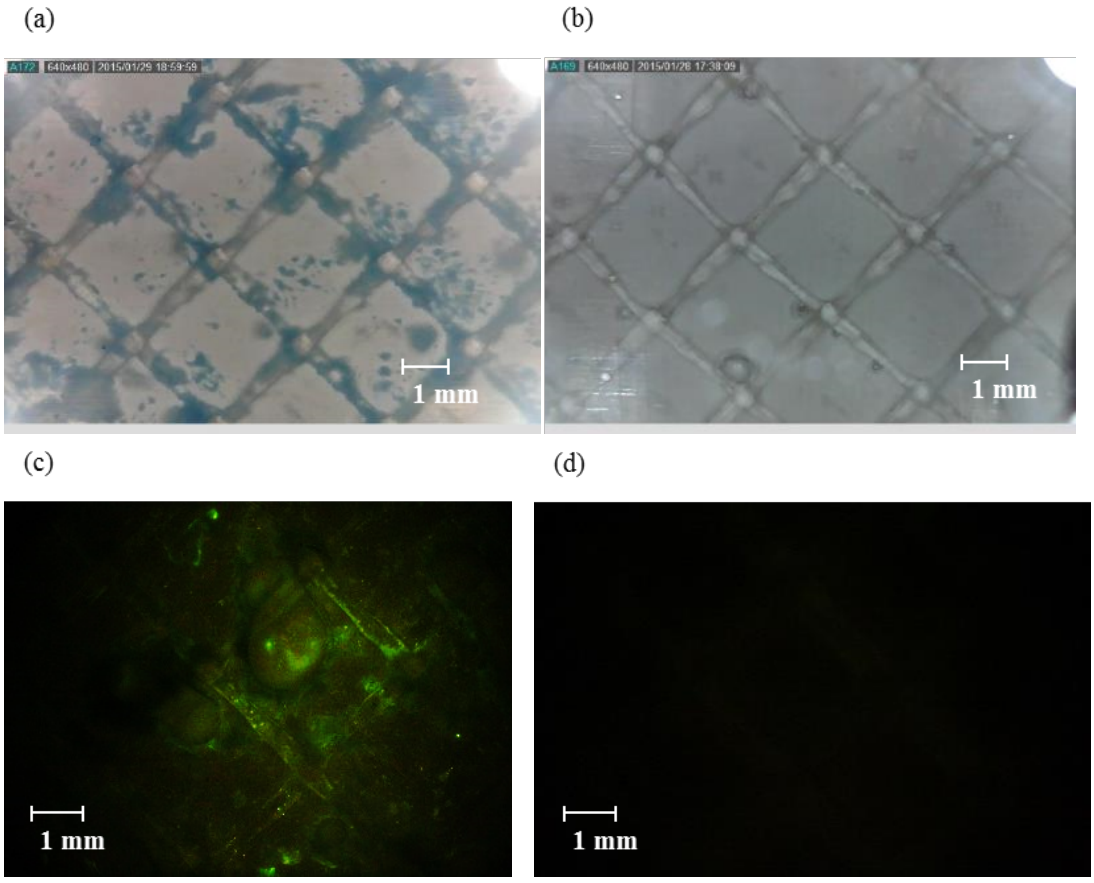

Figure 4. 5 Light images and fluorescence images of the membrane and spacer after 24 h which was formed the biofilm and accumulated the kaolin with or without the chlorine washing: (a) and (b) light images of the membrane and spacer, (c) and (d) fluorescence images of the membrane and spacer, (a) and (c) the membrane and spacer without the chlorine washing, (b) and (d) the membrane and spacer with the chlorine washing.

4.5 Summary and Conclusion

The reduction of biofilm formed on the RO membrane using hypochlorite was examined by optimization of the various washing condition using a conventional polyamide

membrane. Intermittent washing which can reduce biofilm formation with a small CT value is more useful than continuous washing. The suggested optimum condition for the intermittent chlorine washing is 10 mg/L of free chlorine concentration, washing frequency of every 12 h, and 5 min of washing time for sufficient reduction of the biofilm formed on the RO membrane (CT = 0.07 mg/L for 1 h). The free chlorine concentration required for intermittent washing was found to be within the allowable application condition for conventional polyamide membrane. Moreover, based from the conditions suggested by this study, CT values that can be applied to achieve the usual membrane lifetime expected for RO membranes were also estimated, and were also indicated as the chlorine resistance values allowed for polyamide RO membrane. Reduction of biofilm formation in the continuous filtration test required a free chlorine concentration lower than that from the soaking test. Furthermore, the reduction of inorganic particles, which accumulated with biofilm formation on the membrane and spacer in a continuous flow channel, was also examined. The inorganic particles accumulated and biofilm formed, which were observed mainly around the spacer were reduced by continuous chlorine washing. The results indicate hypochlorite washing can essentially reduce the accumulation of not only the biofilm but also the inorganic suspended particles.

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Chapter 5: Effect of the presence of metal ions on PA membrane degradation by hypochlorite

5.1 Introduction

Increased population, industrial expansion, tourism, and agriculture development have triggered an increase in water demand such that many water-stressed or arid regions or countries are supplementing their water supply with desalinated water (Ghaffour et al., 2013). The total global desalination capacity is around 66.4 million m^3 d^{-1} in 2012, and is expected to reach about 100 million m^3 d^{-1} by 2015 (GWI/IDA DesalData). Moreover, the global needs for water are expected to reach 6900 billion m^3 by the year 2030 which is about 150% increase from the demand in 2009 (4500 billion m^3) (Misdan et al., 2012; Douglas, 2009). In 2012, the source water for desalination is split to about 58.9% from seawater, 21.2% from brackish groundwater sources, and the remaining percentage from surface water and saline wastewater (GWI/IDA DesalData, 2013). These percentages are constantly changing because the desalination market is growing very rapidly (Ghaffour et al., 2013). Desalination is an increasingly common solution to supply fresh water in many regions of the world where this resource is scarce (Peñate and García-Rodríguez, 2012). Recently, reverse osmosis (RO) is the most widely used desalination technology globally (Peñate and García-Rodríguez, 2012; Lee et al., 2011), with RO technology accounting for 59.9% of the desalination amount in the world (GWI/IDA DesalData, 2013). Desalination is no longer a marginal or supplemental water resource in some countries (Ghaffour, 2009); the desalting supply ratio reached 100% in Qatar and Kuwait. Natural water such as surface water and groundwater can be used as raw water for RO systems in the future, because of worldwide water shortage. As the most successful commercial RO membrane, thin-film composite (TFC) aromatic polyamide (PA) RO membranes have been primarily used in water recycling and desalination applications

from saline water and other wastewater sources due to their ability to withstand wide range of pH values and high recoveries (Antony et al., 2010; Li and Wang, 2010; Elimelech and Phillip, 2011; Xu et al., 2013).

However, among all the types of fouling, an inevitable problem of RO membrane technology is biofouling, which is caused by adhesion and accumulation of microorganisms, followed by growth and formation of biofilms (Flemming, 1997). With a sterilization or disinfection that is less than 100% effective, complete reduction or removal of biofilm is very difficult to achieve by feed treatment because of the ability of biofouling organisms to self-replicate since some cells remain alive and use the dead cells as nutrient source (Mansouri et al., 2010). Microorganisms are present in nearly all water systems and are capable of colonizing almost any surface forming biofilms (Matin et al., 2011). Biofouling is characterized by the presence of a biofilm on the membrane leading to increase in resistance and decline in membrane performance, such as water permeation and rejection of solutes (Suwarno et al., 2014) and results to increase in RO operational costs significantly since it is irreversible (Kim et al., 2009). Among the different types of biocides summarized in a review by Kim et al. (2009), free chlorine (i.e., hypochlorous acid, HOCl or hypochlorite, OCl^-) injected at the head of a pretreatment process has been the standard practice to control biofilm formation in the RO process (Matin et al., 2011; Kim et al., 2009; Colquhoun et al., 2010). However, it has been pointed out that PA is very sensitive to free chlorine and, therefore, residual free chlorine in the feed solution can cause the degradation of PA if free chlorine is not completely removed before RO membrane filtration (Glater et al., 1994; Kwon and Leckie, 2006; Kang et al., 2007; Shintani et al., 2007; Ettori et al., 2011; Do et al., 2012). It has been considered that the degradation process of PA membranes mainly involves an initial N-chlorination by substituting the hydrogen on the amide nitrogen with chlorine, followed by ring-chlorination via an intermolecular rearrangement called Orton

Rearrangement (Glater et al., 1994). Chlorination of the PA has been hypothesized to facilitate C–N bond hydrolysis which lead to additional carboxylic acid groups as a result from the decrease in the number of C–N bonds (Do et al., 2012). It has been also reported that the presence of metal ions with chloramines or free chlorine resulted in the accelerated degradation of PA membranes (Gabelich et al., 2005; Cran et al., 2011). Fe(III) and Fe(II) ions accelerated the membrane degradation with monochloramine and free chlorine (Gabelich et al., 2005). The effect of chloroamine in the presence of several metal ions to the degradation of polyamide membranes was tested and the presence of Cu^{2+} was found to accelerate the reduction of the polyamide (II) peak (Cran et al., 2011). Shintani et al. (2007) used purified water with 500 mg L^{-1} of Ca^{2+} to evaluate chlorine resistance of PA membrane developed because it was empirically known that Ca ion accelerates PA degradation. However, evidence of acceleration of PA degradation by the addition of Ca ion was not shown.

Different types of water sources are used as raw water for RO processes and the metal ions and their concentrations in the water sources vary depending on the type of water. Understanding the effect to membrane degradation of the presence of main ions in the raw water for RO process is necessary. Feed water for RO processes are usually seawater or brackish water, which contain several metal salts (alkali metal and alkaline earth metal such as Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+}), with water qualities in varying degrees: pH values from 6.0–8.2, 7.4–472 mM Na^{+}, 0.2–56 mM Mg^{2+}, 0.005–10 mM K^{+}, and 1.9–11 mM Ca^{2+} (Leparc et al., 2007; Sachit and Veenstra, 2014). In seawater, concentrations of trace ions were reported to be 0.11 μM for Ba^{2+} and 74.2 μM for Sr^{2+} (Leparc et al., 2007). Other various feed water such as secondary effluent water and Colorado River water were also supplied for RO processes (Gabelich et al., 2003; Franks et al., 2007; Xiao et al., 2014), and the concentrations of the metal ions ranged from 13.5–24 mM Na^{+}, 0.27–2.1 mM Mg^{2+}, 0.05–0.3

mM K^+, and 3.6–7.3 mM Ca^{2+}. The wastewater from a semiconductor industry also contain trace ions of 5.5 μM Cu, 6.1 μM Zn, 77.6 μM Sr, 139.4 μM Al, and 17.7 μM Fe (Xiao et al., 2014). Despite the presence of these metal ions in these various feed water sources for RO processes, the effects of these ions or their concentration in the degradation of the PA membrane have not been reported.

Due to increased demand in water supply, RO membrane technology is expected to make drinking water and reclaimed water from various water resources, even with resource waters containing different types and concentrations of coexisting metal ions and with high biofouling potential waters such as secondary effluent water. Chlorine-resistant PA membranes are being developed to allow direct washing of water with free chlorine and chloramines to avoid biofilm formation on the membrane (Shintani et al., 2007; Buch et al., 2008; Yu et al., 2009; La et al., 2010; Shin et al., 2011; Kwon et al., 2012; Xu et al., 2013), and are expected to be used in the near future. In the current state of membrane development research however, the membrane cannot have the perfect resistance to free chlorine as long as polyamide is used as a membrane material, such that research focusing on understanding PA membrane degradation by chlorine is very important. Since water sources and their quality are vital in RO membrane processes, determining the effects of water quality on the PA membrane degradation will be very important in designing and developing chlorine-resistant membranes.

In this study, the enhancing effect of the coexistence of metal ions on PA membrane degradation by hypochlorite was examined using a commercial PA membrane. The mechanism of the PA membrane degradation by hypochlorite was also examined.

5.2 Materials and methods

5.2.1 RO membrane and reagents

Commercially available PA RO membrane NTR-759HR, provided by Nitto Denko Co. (Osaka, Japan) was used in the study. Sodium chloride (NaCl), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) and potassium chloride (KCl) were purchased as analytical grade reagent from Kanto Chemical Co. Inc. (Tokyo, Japan). Sodium dihydrogenphosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), sodium sulfate (Na_2SO_4), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), and calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$) were purchased as analytical grade from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Sodium hypochlorite (NaOCl) (approx. 10% available chlorine) was purchased from Sigma-Aldrich Corp. (St. Louis, MO). Other reagents were purchased as analytical grade from Kishida Chemical Co., Ltd. (Osaka, Japan) and Kanto Chemical Co. Inc. (Tokyo, Japan). DPD (N,N-diethyl-p-phenylenediamine) was purchased for free chlorine analysis from Hanna Instruments Japan, Inc. (Chiba, Japan). Pure water was prepared using a Milli-Q Reference Ultrapure Water Purification System (Merck Millipore Corp., Darmstadt, Germany). Phosphate buffer solution of pH8 was prepared from $NaH_2PO_4 \cdot 2H_2O$ and NaOH.

5.2.2 Hypochlorite treatment of RO membrane

The membranes were soaked in 300 mL of 2.70 mM (200 mg L^{-1}) NaOCl solution with each metal ion under study for the measurement of the membrane degradation. As control experiment, the membrane was soaked in 2.70 mM NaOCl solution without any metal ion. The soaking of the membrane was conducted at 25°C in the dark, and was shaken at 45 rpm for 48 h. The pH values of the soaking solutions were initially adjusted to 8.0 using HCl and NaOH, which is within the pH range usually found for seawater desalination plants (Ettori et al., 2011). Effective free chlorine species of hypochlorous acid (pKa=7.5, 25°C) (Morris, 1966) are undissociated (HOCl), dissociated (OCl^-), and dissolved chlorine (Cl_2),

although the effect of Cl_2 which has unstable characteristics such as volatilization can be excluded (Pellegrin et al., 2013). The soaking solutions used were either a metal chloride salt or a metal sulfate salt, with metal ion concentrations as follows: 130–469 mM for Na^+, 9.7–300 mM for K^+, 0.5–7.0 mM for Mg^{2+}, 1.0–10.0 mM for Ca^{2+}, and 5.0 mM for Ba^{2+}. Experimental systems were constructed by a combination of these conditions, each metal salt added and the concentrations in the soaking solutions are summarized in Table 5.1. To maintain greater than 80% residual free chlorine concentration in the solution the soaking solution for the membrane degradation was replaced with fresh solution at 2, 12, 24, and 36 h soaking time for the 48 h duration of soaking. The free chlorine concentration was measured by the DPD absorption photometry using the residual chlorine meter (HI96711; Hanna Instruments Japan, Inc., Chiba, Japan). The soaked membrane was taken out of the soaking solution after 48 h, and then washed with pure water. After soaking, the membranes are subjected to cross-flow filtration to measure membrane performance by determining salt rejection and flux before and after the filtration using an RO test cell apparatus.

For mechanism analysis, isopropyl alcohol (IPA) rejections of the virgin membrane and membranes soaked in 2.70 mM NaOCl solution with and without 5 mM Mg^{2+} were also measured. To evaluate the effect of a counter anion of monovalent ions and divalent ions in the degradation, the membranes were similarly soaked for 48 h in 300 mL of 2.70 mM NaOCl solution with 187 mM Na^+ or 4 mM Ca^{2+} with chloride or sulfate as the counter anions.

Table 5. 1 Soaking solution conditions and membrane performance.

	Metal ion concentration (mmol L^{-1})	Added salt	Salt rejection (%)	Permeate flux (m^3 m^{-2} d^{-1})
Control	0	-	96.9 ± 1.8	0.8 ± 0.2
Na^+	130	NaCl	92.1 ± 4.8	1.8 ± 0.7
	186	NaCl	82.8 ± 8.5	1.6 ± 0.5
	327	NaCl	56.1 ± 7.0	1.2 ± 0.1
	469	NaCl	34.9 ± 5.7	1.7 ± 0.2
	186	Na_2SO_4	73.1 ± 12.4	1.5 ± 0.7
K^+	200	KCl	52.4 ± 5.9	1.3 ± 0.1
Mg^{2+}	0.5	$MgCl_2$	86.9 ± 2.4	1.0 ± 0.0
	1.0	$MgCl_2$	85.6 ± 6.0	1.2 ± 0.3
	2.0	$MgCl_2$	65.5 ± 7.4	1.4 ± 0.2
	4.0	$MgCl_2$	54.5 ± 3.8	1.3 ± 0.0
	5.0	$MgCl_2$	46.3 ± 1.0	1.5 ± 0.1
	7.0	$MgCl_2$	38.6 ± 11.4	2.1 ± 0.8
Ca^{2+}	1.0	$CaCl_2$	72.9 ± 3.6	1.1 ± 0.0
	2.0	$CaCl_2$	53.9 ± 10.1	1.2 ± 0.1
	3.7	$CaCl_2$	47.8 ± 2.7	1.3 ± 0.1
	4.4	$CaCl_2$	29.3 ± 5.4	1.5 ± 0.2
	5.0	$CaCl_2$	28.0 ± 3.1	1.6 ± 0.1
	7.0	$CaCl_2$	11.1 ± 5.7	2.5 ± 0.1
	10.0	$CaCl_2$	1.8 ± 1.1	5.4 ± 0.5
	4.0	$CaSO_4$	42.0 ± 4.4	1.4 ± 0.1
Ba^{2+}	5.0	$BaCl_2$	1.0 ± 0.5	2.7 ± 0.2

5.2.3 Measurement of membrane performance

A schematic diagram of a cross-flow RO test cell is presented in Figure 5.1. The stainless steel continuous cross-flow RO test cell (spin flow cell; Tritec Co., Ltd., Tokyo, Japan) with internal diameter of 75 mm and equipped with a pump (KP-22, Flom, Tokyo, Japan) was used for the measurement of salt rejection and permeate flux of the membrane. The effective filtration area of the operating membrane was 37.4 cm^2. Feed water (electric conductivity 327 ± 3 mS cm^{-1}) was made by dissolving NaCl in phosphate buffer solution (pH 8) to exclude any effects of pH change. The concentration of Na^+ of the feed water prepared was always adjusted to have the same concentration as a 2000 mg L^{-1} NaCl solution. All virgin membranes were washed with pure water first before use. The membrane was then placed inside the RO test cell, and the test cell was operated by running feed water with stirring at a flow rate of 50 mL min^{-1} (initial flux 19.3 m^3 m^{-2} d^{-1}), operating pressure of

1.5 MPa, and at 25°C. Concentrate and permeate were drained during the initial 4 minutes, and then were circulated into the feed tank. Electric conductivity of the feed water and permeate as well as permeate flow rate were measured after 30 minutes of filtration. The salt rejections of the membrane, *R(S)* (%), were calculated in percentage from the conductivities using Eq. (1).

$$R(S) = \left(1 - \frac{Permeate\ conductivity}{Feed\ conductivity}\right) \times 100 \qquad (1)$$

The permeate flux, *F* ($m^3\ m^{-2}\ d^{-1}$), were calculated using Eq. (2) as (Zhai et al., 2011).

$$F = \frac{V}{S \cdot t} \qquad (2)$$

where v denotes the volume of the solution permeated during the test time (m^3), S denotes the effective membrane area (m^2), t denotes the operation time (d).

To evaluate size exclusion effect associated with hypochlorite treatment, rejection of a 2000 mg L^{-1} IPA solution was determined. IPA was used as filtration target, in this case since it is a neutral organic molecule, which does not render effects due to electric charge repulsion. IPA rejection test as well as salt rejection test of the membrane were determined, and the IPA concentration of the feed water and permeate were measured after 30 minutes of filtration. The IPA concentration was measured as organic carbon concentration using a TOC-V CSN (Shimazu Co., Tokyo, Japan). The limit of detection of the organic carbon concentration was 0.1 mg L^{-1}. The IPA rejections of the membrane, *R(I)* (%), were calculated in percentage from the IPA concentrations of the feed water and permeate using Eq. (3) as (Zhai et al., 2011).

$$R(I) = \left(1 - \frac{C_p}{C_f}\right) \times 100 \qquad (3)$$

where C_p and C_f denotes the IPA concentrations of permeate and feed water (mg L^{-1}), respectively.

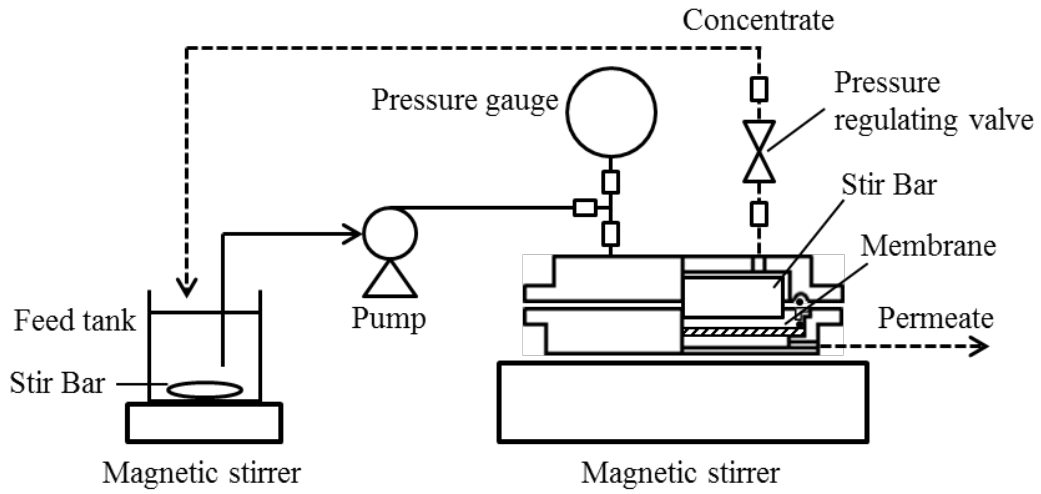


Figure 5. 1 Schematic diagram of the Cross-flow RO test cell.

5.2.4. Examination of degradation mechanism

To clarify the degradation mechanism of the PA membrane, virgin and soaked membranes were evaluated on the change of surface properties and the effect of the metal ions on the degradation mechanism. The membranes soaked in 2.70 mM NaOCl solution with and without 10 mM Ca^{2+} were observed using field emission scanning electron microscopy (FE–SEM, S-5200; Hitachi High–Tech Manufacturing & Service Corp., Ibaraki, Japan) equipped with an energy dispersive x-ray spectroscope (EDX, Genesis XM2; Ametek Co., Ltd., Tokyo, Japan) (FE–SEM/EDX). All membranes were coated with a carbon layer (purity 99.995%) using a carbon coater (CADE; Meiwafosis Co., Ltd., Tokyo, Japan).

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscope (ALPHA-G; Bruker Optics K. K., Tokyo, Japan) equipped with diamond crystal was used to determine the chemical changes in the membranes before and after hypochlorite exposure. The virgin membrane and the membrane soaked in 2.70 mM NaOCl solution with and without 5 mM Mg^{2+} or Ca^{2+} were dried, and then were used for the analysis. FTIR spectra of the membranes were recorded in wavenumber of 550–5000 cm^{-1} at 25°C. Typical amide bands at 1446 cm^{-1} and 1608 cm^{-1} (C=C) and 1540 cm^{-1} (N–H) (Gabelich et al., 2005), and

carboxylic acid at 1700 cm^{-1} (C=O) were identified to track the changes of the surface structure of the membrane.

Zeta potential of the membrane surface was measured using the zeta electrometer (Zetasizer Nano ZS; Malvern Instruments Ltd., UK) to determine the chemical change of the surface by the degradation. The membranes soaked in 2.70 mM NaOCl solution with and without 5 mM Mg^{2+} or Ca^{2+} were measured.

5.3 Results and discussion

5.3.1 Effect of metal ions on the membrane degradation

The salt rejections of the membranes after 48 hours of soaking in the hypochlorite solution coexisting with ca. 200 mM of monovalent metal ions or 5 mM of divalent metal ions are shown in Figure 5.2. The salt rejections of the membranes soaked in hypochlorite solution with 200 mM of the monovalent metal ions, Na^+ and K^+, were 82.8 ± 8.5% and 52.4 ± 5.9%, respectively were lower than that of the control (96.9 ± 1.8%) soaked in hypochlorite solution without metal ion. On the other hand, the salt rejections of membranes soaked in hypochlorite solution with 5 mM of the divalent metal ions, Mg^{2+}, Ca^{2+}, and Ba^{2+} were 46.3 ± 1.0%, 28.0 ± 3.1%, and 1.0 ± 0.5%, respectively. The membrane degradation in the hypochlorite solution was more significantly accelerated in the presence of the divalent metal ions rather than the monovalent ions even if the concentration of the divalent metal ions used were much smaller than that of the monovalent metal ions, indicating that valency of metal ions is a determining factor in the degradation. The membrane degradation was also confirmed by the change in flux observed. The fluxes of the membrane soaked in the hypochlorite solution with coexisting metal ions were 1.6 ± 0.5 $m^3 m^{-2} d^{-1}$, 1.3 ± 0.1 $m^3 m^{-2} d^{-1}$, 1.5 ± 0.1 $m^3 m^{-2} d^{-1}$, 1.6 ± 0.1 $m^3 m^{-2} d^{-1}$, and 2.7 ± 0.2 $m^3 m^{-2} d^{-1}$ in Na^+, K^+, Mg^{2+}, Ca^{2+}, and Ba^{2+}, respectively, and were higher than that of control (0.8±0.2 $m^3 m^{-2} d^{-1}$). The membrane degradation in the presence of the three divalent metal ions was remarkably

increasing in accordance with their atomic number. It has been previously suggested that membrane degradation in the hypochlorite solution is accelerated by the coexistence of Ca ion (data is not shown) (Shintani et al., 2007). However, this study clearly clarifies that acceleration of membrane degradation by hypochlorite was caused by all monovalent and divalent metal ions used in this study. Moreover, the acceleration of membrane degradation was caused by divalent metal ions in lower concentration than the monovalent metal ions.

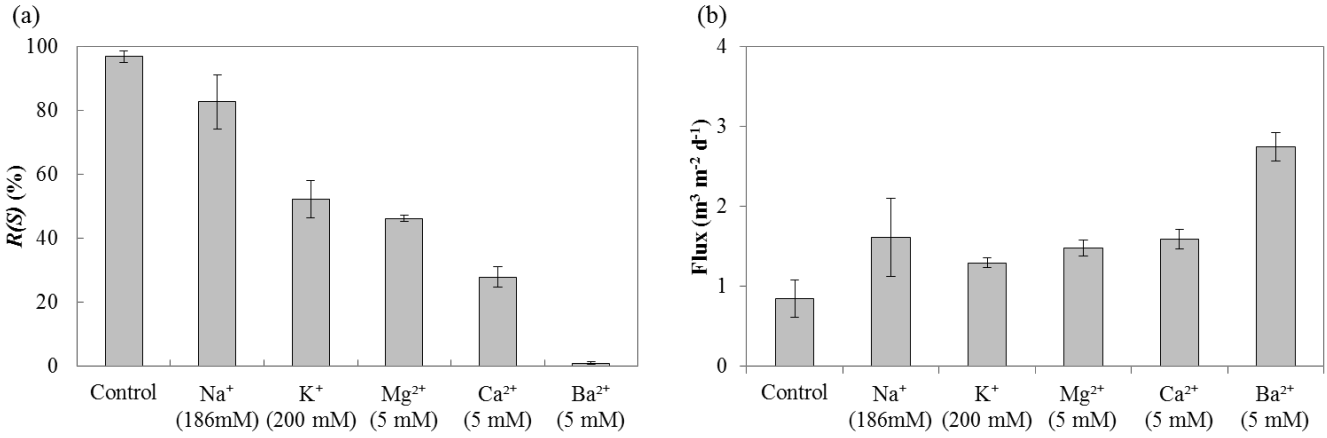


Figure 5. 2 Salt rejection and permeate flux of the membrane soaked in 2.70 mM of hypochlorite solution with and without ca. 200 mM of monovalent metal ions or 5mM of divalent metal ions (n=3): (a) salt rejection, (b) permeate flux.

5.3.2 Metal ion concentration dependence of membrane degradation

Concentration dependence of the acceleration of membrane degradation by hypochlorite was evaluated for the ions except for Ba^{2+}, which has extremely low concentrations in typical water sources. The effect of the metal ion concentration in the soaking solution on membrane degradation was evaluated by calculating the ratio of the salt rejection of the membrane soaked with coexisting the metal ions, R, to that of the salt rejection of the membrane for the control, R_C (Figure 5.3). Na^+ was selected as a typical monovalent ion for this evaluation because of its much higher concentration than K^+ in various target water sources for RO membrane treatment such as seawater, fresh water, and

secondary wastewater. The R/R_C decreased in solutions with concentrations greater than 100 mM Na^+ (Figure 5.3a), suggesting a threshold limit for Na^+ in terms of accelerating membrane degradation. In Japan, Na^+ concentrations found in surface water and ground water for drinking water sources were 0–4.4 mM and 0–8.7 mM (average 0.71 mM), respectively (Database of water quality of aqueducts, 2006), while those in Europe with high water hardness had 0.004–16 mM (average 0.85 mM) (Banks et al., 2015). Larger concentration of Na^+, 6.5–24.4 mM (average 10.2 mM) was reported in the US (VanLandeghem et al., 2012). The Na^+ concentrations found in drinking water sources are much lower than the threshold limit of Na^+ found in this study, suggesting that Na^+ in drinking water sources will have no effect on PA membrane degradation by hypochlorite solution.

As shown in Figure 5.3b, the membrane degradation was also accelerated by the divalent metal ion in a range of much lower concentration than the monovalent ion. The R/R_C in the solution with Mg^{2+} and Ca^{2+} were significantly correlated ($p < 0.01$) with the metal ion concentration (R^2 = 0.939 and 0.937 for Mg^{2+} and Ca^{2+}, respectively). The Ca^{2+} accelerated the membrane degradation more strongly than Mg^{2+} in the same concentration. In addition, the coefficient of membrane degradation in Ca^{2+} (-0.102 L $mmol^{-1}$), which was evaluated by the decrease in salt rejection, was about 1.3 times larger than that in Mg^{2+} (-0.080 L $mmol^{-1}$). Moreover, the coefficient of membrane degradation was different by two orders of magnitude between the divalent ions (Mg^{2+} and Ca^{2+}) and the monovalent ion Na^+ (-0.0018 L $mmol^{-1}$). Our results also indicate that the acceleration of PA membrane degradation by Mg^{2+} and Ca^{2+} seemed to have no threshold limit, in contrast to what was shown earlier for Na^+, which has a threshold limit of 100 mM. In Japan, Mg^{2+} and Ca^{2+} concentrations of drinking water sources calculated from water hardness (Database of water quality of aqueducts, 2006) ranged from 0 to about 3 mM in total, while in the tap water in Europe, Mg^{2+} and Ca^{2+} concentrations were

0.006–2.5 mM (average 0.54 mM) and 0.03–3.9 mM (average 1.5 mM) (Banks et al., 2015) and in river water in the US, 0.3–7.7 mM Mg^{2+} (average 3.4 mM) and 0.6–11.2 mM Ca^{2+} (average 3.0 mM) (VanLandeghem et al., 2012). This lack of threshold limit for Mg^{2+} and Ca^{2+} is crucial since our results suggest that the levels of Mg^{2+} and Ca^{2+} concentration in drinking water sources are enough to accelerate degradation of PA membrane by hypochlorite.

The concentrations of metal ions in treated wastewater from domestic and industrial uses, which are also target water sources of reclaimed water by RO membrane treatment, will be higher than those in found drinking water sources. Thus, the acceleration of PA membrane by divalent ions is to be expected. Moreover, in seawater desalination, accelerated degradation of PA membrane by hypochlorite is unavoidable since both monovalent and divalent metal ions are present in abundance in seawater.

Although monovalent ions concentrations in natural water and treated wastewater are too small to accelerate degradation of PA membrane by hypochlorite, synergistic acceleration of membrane degradation by divalent and monovalent ions may be expected. Therefore, the effect of coexisting monovalent ion (Na^+) and divalent ion (Mg^{2+}) on the acceleration of membrane degradation was evaluated (Figure 5.3c). The membrane degradation was compared for membranes soaked in solutions of hypochlorite with constant 5 mM Mg^{2+} concentration mixed with different concentrations of Na^+ (130 mM – 469 mM) and solutions with Na^+ only. The membrane degradation in the hypochlorite solution with 5 mM Mg^{2+} did not change until 300 mM of Na^+ and then was accelerated with an increase in Na^+ concentration. The R/R_C in the hypochlorite solution with 300 mM of Na^+ was almost the same as that with 5 mM of Mg^{2+}. These results suggest that even in coexistence with other metal ions, Na^+ seemed to have a threshold limit. Within the concentration range studied, the threshold of Na^+ in the presence of Mg^{2+} is higher (300 mM) compared to when it was

present as a single ion (100 mM). Since below 300 mM of Na^+, the R/R_C is the same as that for a solution with 5 mM Mg^{2+} only, this suggests that degradation in this range is dominated by Mg^{2+}. Considering that the slope of the R/R_C for 5 mM of Mg^{2+} with greater than 300 mM of Na^+ (-0.00185) is almost the same as the slope of the R/R_C for Na^+ without Mg^{2+} (-0.00176) and that membrane degradation is accelerated with increasing Na^+ concentration, at concentrations greater than 300 mM of Na^+, the degradation of the membrane is dominated by the Na^+. Thus, in the presence of the both the monovalent and divalent ion, threshold limit of the monovalent ion is the determining factor, whereby below that limit, the membrane degradation is accelerated due to the concentration of the divalent ion, while beyond the threshold limit, the membrane degradation due to the monovalent ion is dominant compared with that of the divalent ion.

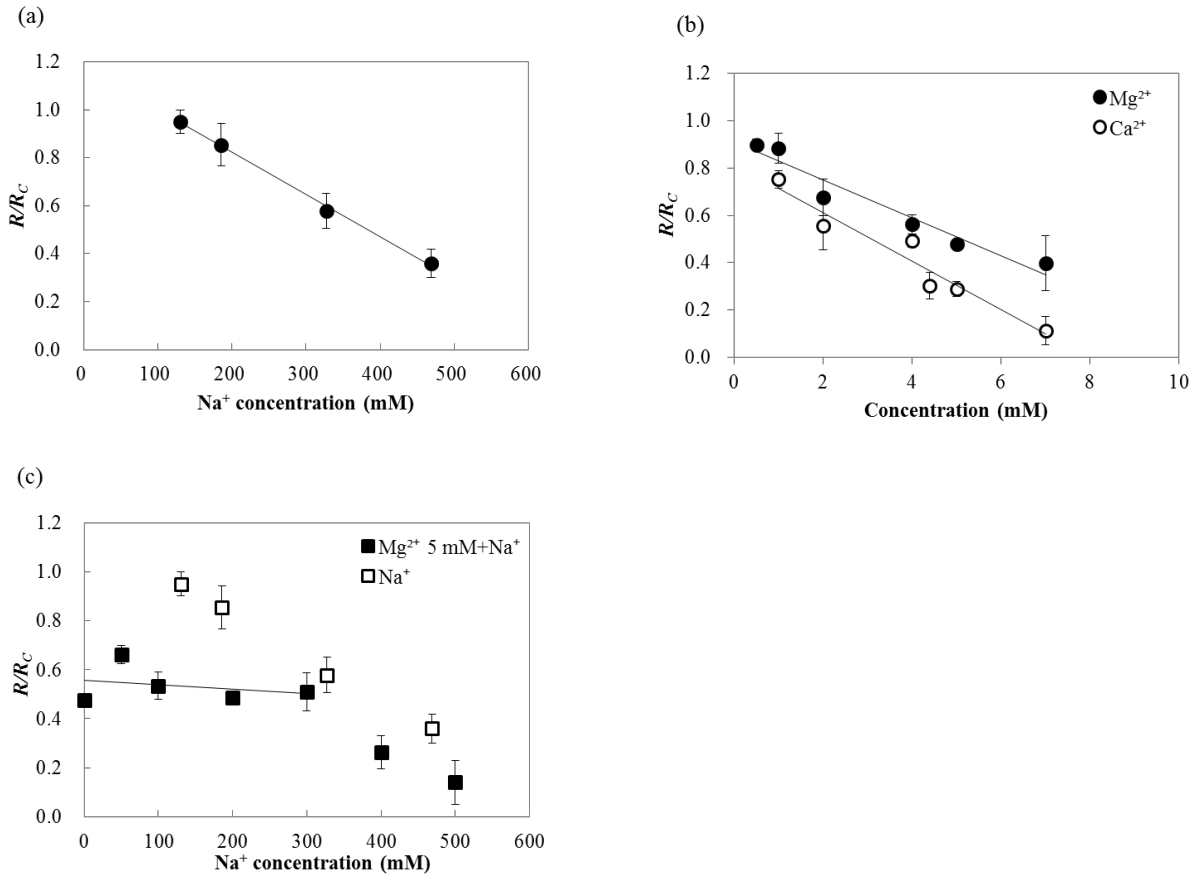


Figure 5. 3 Effect of metal ion concentrations in soaking solution on the R/R_C of the membrane: (a) concentration variation by monovalent metal ions, (b) concentration variation by divalent metal ions, and (c) concentration variation by Na^+ with 5 mM of Mg^{2+}.

5.3.3 Effect of counter anion to membrane degradation

The effects of counter anions on the acceleration of the membrane degradation by Na^+ and Ca^{2+} were examined using Cl^- and SO_4^{2-} (Table 5.1). In 186 mM of Na^+, the salt rejections in the presence of Cl^- and SO_4^{2-} were 82.8 ± 8.5% and 73.1 ± 12.4%, respectively. In 4 mM of Ca^{2+}, the salt rejections in the presence of Cl^- and SO_4^{2-} were 47.8 ± 2.7% and 42.0 ± 4.4%, respectively. In the concentrations used in this study for both Na^+ and Ca^{2+}, there was no significant difference in membrane degradation in the presence of Cl^- and SO_4^{2-} using student t-test (significance level= 0.05).

5.3.4 Change in membrane property

The changes in the physicochemical property and performance of the membrane due to degradation were evaluated. The SEM surface images in the virgin membrane and the membranes soaked in 200 mg L^{-1} of hypochlorite solution with and without 10 mM of Ca^{2+} are shown in Figure 5.4. A ridgy structure was observed in all membrane surfaces (Figure 5.4a and 5.4b), whereas parts of the surface changed in structure on the membranes treated by the hypochlorite solution with and without Ca^{2+} (Figure 5.4c–4f).

The zeta potentials of the membrane decreased with degradation of membrane in the hypochlorite solution with and without metal ions. The decrease in the zeta potential corresponded with the decrease in the salt rejection (Figure 5.5), suggesting that membrane degradation by hypochlorite regardless of the presence of metals can be explained by the increase of carboxyl group formed by the hydrolysis in the amide C–N (Do et al., 2012).

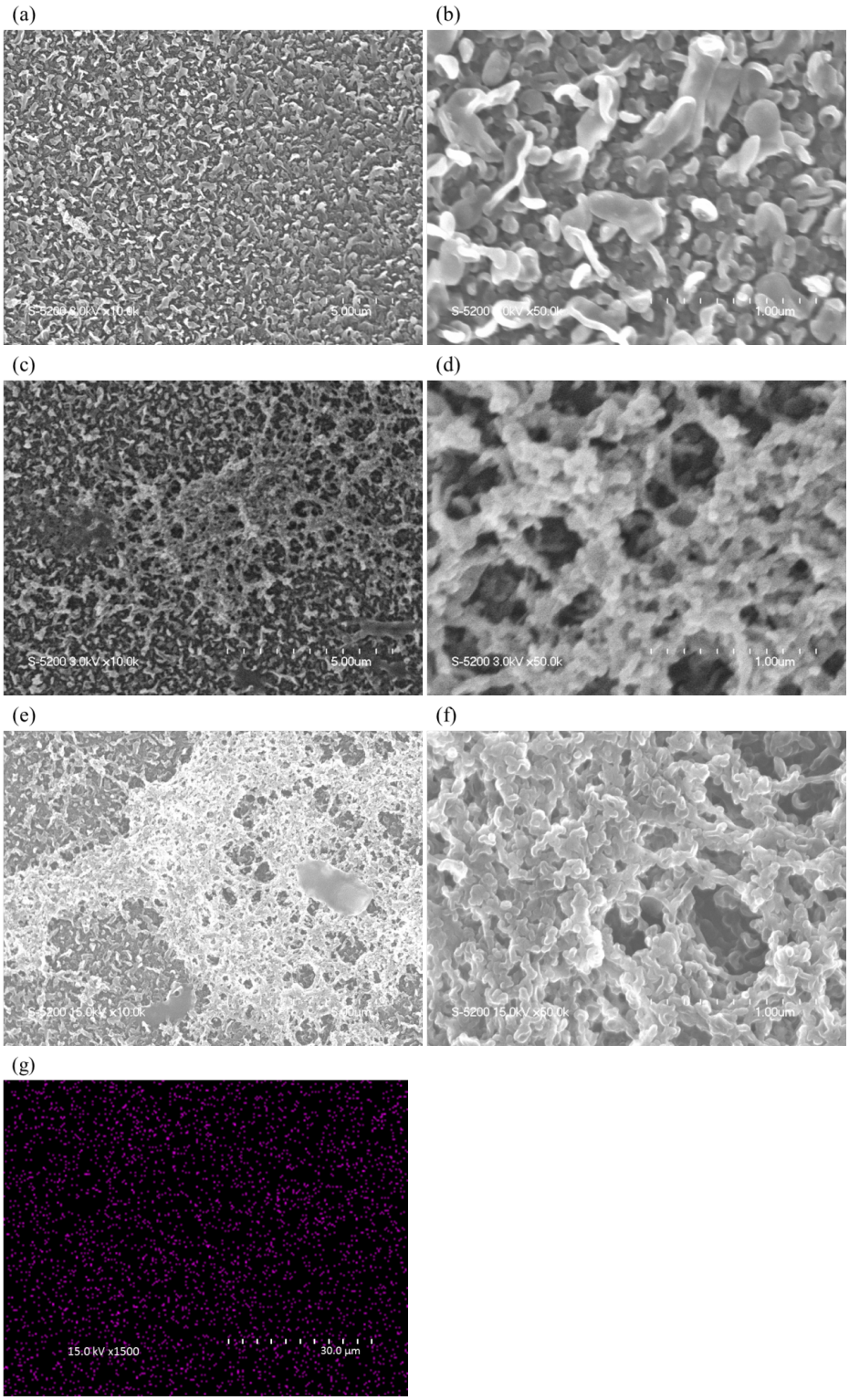


Figure 5. 4 SEM surface images and map of cross-sectional chemical composition of the virgin membrane and the membranes soaked in 2.70 mM of hypochlorite solution with and without 5 mM of Ca^{2+}: (a) and (b) SEM image of the virgin membrane, (c) and (d) SEM image of the membrane soaked by hypochlorite (control), (e) and (f) SEM image of the membrane soaked in 2.70 mM of hypochlorite solution with 10 mM of Ca^{2+}, and (g) map of Cl of (c). (a), (c), and (e) are images in 10000 magnifications, (b), (d), and (f) are images in 50000 magnifications, (g) is an image in 1500 magnification.

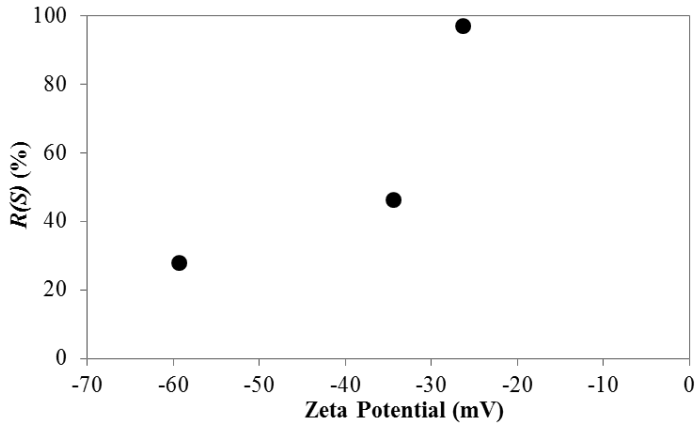


Figure 5. 5 Relation between salt rejection and zeta potential of the surface of the membrane soaked in 2.70 mM of hypochlorite solution with and without 5 mM of Mg^{2+} or Ca^{2+}.

The permeation performances such as the salt rejection and IPA rejection in the virgin membrane and the membranes soaked in 200 mg L^{-1} of hypochlorite solution with and without 5 mM of Mg^{2+} are shown in Figure 5.6. The IPA rejection of the virgin membrane and the membranes soaked in hypochlorite solution without and with 5 mM of Mg^{2+} were 84.6 ± 2.1%, 69.3 ± 1.2%, and 5.7 ± 5.8%, respectively. This observed decrease in IPA rejection of the membrane during soaking in hypochlorite solution with and without Mg^{2+} confirmed that hypochlorite deteriorates both size exclusion performance as well as the electric charge repulsion performance of the PA membrane.

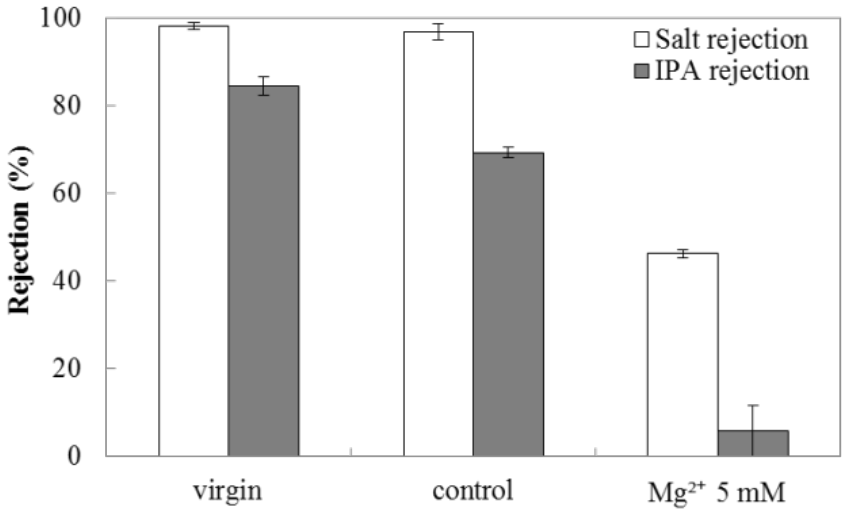


Figure 5. 6 Salt rejection and IPA rejection of the virgin membrane and the membranes soaked in 2.70 mM of hypochlorite solution with and without 5 mM of Mg^{2+}.

5.3.5 Mechanism of membrane degradation by hypochlorite with divalent metal ion

The ATR-FTIR spectra for the virgin membrane and the membranes soaked in 2.70 mM hypochlorite solution with and without 5 mM of Mg^{2+} or Ca^{2+} were obtained (Figure 5.7). The oxidation of the membrane was observed in all membranes soaked in hypochlorite solution. The intensities of the N–H bond near 1540 cm^{-1} and the C=C ring vibrations near 1610 cm^{-1} and 1446 cm^{-1} in amide bond decreased. The changes observed in the characteristic peaks for PA membranes in this study agreed with reports presented in other studies (Gabelich et al., 2005; Kwon and Leckie, 2006; Kang et al., 2007; Ettori et al., 2011; Do et al., 2012). Moreover, an increase in the intensity in the carboxyl C=O bond near 1700 cm^{-1} was observed in the membranes soaked in the hypochlorite solution with and without metal ions, which can support the changes observed in zeta potential (Figure 5.5), as a consequence of hydrolysis on amide C-N (Do et al., 2012). Consequently, the different changes of chemical structure in the treatment of hypochlorite between with and without metal ions were not clarified by FTIR spectra.

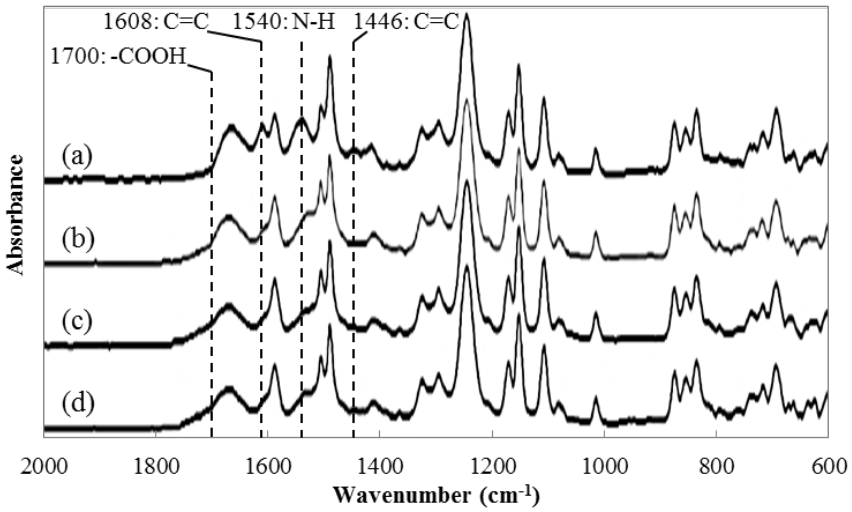


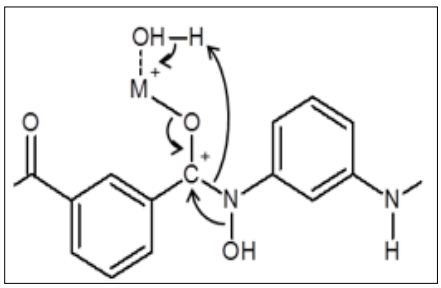
Figure 5. 7 ATR-FTIR spectra of the virgin membrane and the membranes soaked in 2.70 mM of hypochlorite solution with and without the metal ions: (a) virgin, (b) membrane soaked in hypochlorite (control), (C) membrane soaked in hypochlorite with 5 mM of Ca^{2+}, (d) membrane soaked in hypochlorite with 5 mM of Mg^{2+}.

The maps of cross sectional chemical composition in the virgin membrane and membranes soaked in 2.70 mM hypochlorite solution with 10 mM of Ca^{2+} are shown in Figure 5.4. The Cl and Ca species were mapped for the membranes soaked in the hypochlorite solution with and without Ca^{2+} (Figure 5.4g), and Cl was detected in all membranes, indicating chlorination has occurred upon treatment. On the other hand, the Ca^{2+} ion coexisting was not detected on the membrane surface (data is not shown) suggesting that the metal ion was not part of the reaction but possibly acted as a catalyst on the membrane degradation.

The assumed mechanism of PA membrane degradation soaked in hypochlorite solution with metal ions is illustrated in Figure 5.8. The degradation process of PA membranes will not change even if metal ions are involved in the reaction. The degradation will start from N–chlorination by substituting the hydrogen on amide nitrogen with chlorine (Kwon and Leckie, 2006), and then hydrolysis in the amide bond will occur. The rate-limiting step in amide hydrolysis in neutral pH conditions is the breakdown of the C-N cleavage. The metal ion can accelerate amide hydrolysis in neutral pH and the acceleration by metal ion can be explained by the ability of metal-bound water to serve as a general acid catalyst in protonating the leaving nitrogen and/or the ability of the metal to facilitate the breakdown of the C-N cleavage directly (Sayre, 1986). Therefore, the metal ion possibly acted as a catalyst in the degradation of the PA membrane, thereby accelerating amide hydrolysis, which completely breaks down the polyamide chains.

Provisions for metal ion-catalyzed amide hydrolysis indicate that 1) metal ion should remain stoichiometrically coordinated forming the tetrahedral intermediate (inset on Figure 5.8), throughout the hydrolysis reaction, and that 2) stereolectronic requirements for the amide hydrolysis is not hindered by geometrical constraints of the amide structure, which can arise when the metal ion interacts with the leaving nitrogen in the intermediate stage,

preventing the required protonation of the N-leaving group (Sayre, 1986). The observed threshold limit by which Na^+ can enhance membrane degradation might be due to the inability of the Na^+ ion to satisfy one or both of these requirements, especially during low concentrations of the Na^+. Divalent ions can form chelate compounds (complex of a metal ion with 2 or more groups), and most probably, even in lower concentrations are able to form coordinates with the O in the C-N group, without interacting with N. However, monovalent ions do not have this capability, and thus at lower concentrations even upon coordination with the O in the C-N group, there are still free amide groups wherein the monovalent metal ion can interact with the N.



Tetrahedral intermediate

Figure 5. 8 Degradation mechanism of PA membrane soaked in hypochlorite solution (a) with and (b) without divalent metal ion. M^+ denotes divalent metal ions. Inset: Tetrahedral intermediate.

5.5 Summary and Conclusion

RO membranes cannot have the perfect resistance to free chlorine as long as PA is used as a membrane material since PA is known to degrade with chlorine. Thus, studies geared towards the development of chorine-resistant PA membrane have been increasing to allow chlorine washing which is very essential to avoid biofilm formation on the membrane. However, the degradation of PA membranes by free chlorine needs further understanding in order to develop these chlorine-resistant PA membranes. In this regard, it is very important to know the effect of water quality on the degradation of PA membrane by free chlorine, particularly the presence of metal ions and their concentration since metal ions are typical components of source water. In this study, the accelerating effect of coexisting metal ions on the PA membrane degradation by hypochlorite was evaluated, and the acceleration mechanism by the metal ions was also examined.

The acceleration of membrane degradation by hypochlorite was caused by all monovalent (Na^+, K^+) and divalent metal ions (Ca^{2+}, Mg^{2+}, and Ba^{2+}) used in this study. The acceleration of PA membrane degradation was caused by divalent ions in much lower concentrations than the monovalent metal ions. The Na^+ did not accelerate the degradation of the PA membrane in less than 100 mM concentration, whereas Mg^{2+} and Ca^{2+} did not have a threshold limit. The acceleration of PA membrane degradation by metal ions showed concentration dependence. The membrane degradation in the presence of monovalent and divalent metal ions seemed to be influenced by the threshold limit of the monovalent ion within the range of concentration of each of the ions present. Below that threshold limit, the divalent ion gives greater effect in the membrane degradation while higher than the threshold limit, the monovalent has a greater effect. The degradation process of PA membranes does not change even if metal ions are present in the reaction, although the divalent ion can possibly catalyze and accelerate amide hydrolysis leading to PA membrane degradation.

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Chapter 6: Summary and Conclusions

Chapter 1 revealed the need to investigate biofouling and biofouling control by hypochlorite on polyamide (PA) reverse osmosis (RO) membranes. RO membrane technology is a promising technology geared to augment the increasing need for potable and non-potable water through desalination and water reuse and wastewater reclamation. The prevailing problems associated with biofouling on RO membranes specifically 1) the effect of biofilm and biofouling on other foulant materials, and 2) the effect of hypochlorite for biofouling control on PA RO membranes bear further investigation since findings will help in the improvement of the RO process through the design of pretreatment strategies, which could lessen costs of operations associated with the decline of membrane performance due to biofouling or costs associated with membrane replacement or membrane cleaning.

Chapter 2 described the development of a novel method for determining biofouling potential by direct analysis of the RO membrane through fluorescence intensity analysis of biofilm formed on the membrane surface, thereby incorporating fouling tendencies of both feedwater and membrane. Evaluation of the biofouling potential on the RO membrane was done by accelerated biofilm formation through soaking of membranes in high biofouling potential waters obtained by adding microorganisms and glucose in test waters. The soaking method's capability in detecting biofilm formation was confirmed when percentage coverage obtained through fluorescence microscopy and intensity values exhibited a linear correlation (R^2 = 0.96). Continuous cross-flow experiments confirmed the ability and reliability of the soaking method in giving biofouling potential on RO membranes when a good correlation (R^2 = 0.87) between intensity values of biofilms formed on the membrane during soaking and filtration conditions was obtained. Applicability of the test developed was shown when 3 commercially available polyamide RO membranes are assessed for biofouling potential. This

new method can also be applied for the determination of biofouling potential in water with more than 3.6 mg L^{-1} easily degradable organic carbon.

Chapter 3 revealed the effect of biofilm on suspended solid (SS) accumulation on RO membranes under no filtration conditions. Results showed that even with the same initial kaolin concentration contained on the feedwater, the amount of inorganic material deposited is greater by 0.16 mg/cm^2 when secondary effluent water was used in contrast to pure water, signifying quantitative enhancement of accumulated SS on the membrane. The amount of glucose (taken as biomass) in feedwater did not result in a related increase in inorganic material since deposition seemed to be influence by biofilm coverage on a preformed biofilm, as indicated by similar biofilm percentage coverage with and without glucose in feedwater. Micrographs indicated the preferential deposition of SS on the spacer filaments and membrane areas that were covered with biofilm. This effect of biofilm on inorganic SS accumulation has significant impact in designing pretreatment strategies membrane filtration processes by addressing biofilm control to prevent both biofilm formation and SS accumulation.

Chapter 4 deals with optimization studies using hypochlorite treatment on PA RO membranes. Results showed that the chlorine concentration required to control biofilm formation decreased as the chlorine concentration (0.5–10 mg/L), the washing interval (1–4 times/d), or the washing time (1–30 min) increased. The optimum hypochlorite washing condition obtained from soaking experiments proved to be applicable also in controlling biofilm formation in continuous flow experiments, as shown by the 79% and 58% removal of amount of biofilm and inorganic particle accumulated, respectively, by continuous washing with 10 mg/L of free chlorine concentration. The results also confirmed the enhancement of particle accumulation due to biofilm formation and that effective hypochlorite washing condition that controls both biofilm formation and particle accumulation could be achieved.

Chapter 5 revealed the effect of metal ions in water sources on the PA membrane degradation. Results showed that the acceleration of membrane degradation by hypochlorite was caused by all monovalent and divalent metal ions used in this study: Na^+, K^+, Ca^{2+}, Mg^{2+}, and Ba^{2+}. The study also revealed the potency of divalent metal ions in PA membrane degradation since divalent ions in much lower concentration than the monovalent metal ions caused membrane degradation. Na^+ did not accelerate the degradation of the PA membrane in concentrations less than 100 mM while Mg^{2+} and Ca^{2+} showed no threshold limits. Accelerated membrane degradation in the presence of both monovalent and divalent metal ions seemed to be influenced by the threshold limit of the monovalent ion within the concentration range of each of the ions present. Membrane degradation below the threshold limit is dominated by the divalent ion while above the threshold limit the monovalent ion dominated the degradation. The study showed that the degradation mechanism of PA membranes with or without metal ions is the same. However, in the presence of the divalent ion, it possibly acts as a catalyst in the amide hydrolysis, leading to accelerated membrane degradation.

Recommendations

The following are future areas for research which could further the understanding of biofouling and its control by hypochlorite washing:

1. The accumulation of suspended solids (SS) due to the presence of biofilm has been confirmed under continuous cross-flow conditions without filtration. In real life water treatment processes, these observations would have a greater impact due to the presence of permeate flux accompanying filtration. Thus, accumulation of particulate matter as influenced by biofilm should be further studied under cross-flow filtration conditions in order to simulate real water treatment processes.

2. The influence of biofilm on the enhancement of organic fouling also needs further investigation because non-traditional sources for RO feed water can have ample amounts of organic matter, which could lead to the deposition of multiple layers of foulants that could lead to a decrease in membrane performance.

3. The effectiveness of direct chlorine washing has been shown. However, studies have shown that any biofilm fragments could serve as nutrients for remaining viable microorganisms in the feed water. Therefore, investigations on new or improved cleaning approaches (chemical or physical) for the removal of biofilm fragments are important to have a complete biofouling control strategy.

List of Achievements

Original Papers

1. **Cervinia V. Manalo**, Masaki Ohno, Tetsuji Okuda, Satoshi Nakai, Wataru Nishijima. Rapid novel test for the determination of biofouling potential on reverse osmosis membranes. (Water Science and Technology, selected for publication, 2016; related to Chapter 2)
2. **Cervinia V. Manalo**, Masaki Ohno, Tetsuji Okuda, Satoshi Nakai, Wataru Nishijima. Effect of Biofilm on Inorganic Suspended Solids Accumulation on Reverse Osmosis Membranes (Journal of Water and Environment Technology, accepted for publication, 2016: related to Chapter 3)
3. Masaki Ohno, **Cervinia Manalo**, Tetsuji Okuda, Satoshi Nakai, Wataru Nishijima. 2016. Control of Biofilm Formation and Inorganic Particle Accumulation on Reverse Osmosis Membrane by Hypochlorite Washing. 18th International Conference on Water and Wastewater Treatment Plants Proceedings. 18(1), Part 11, 233-241. (related to Chapter 4)
4. Masaki Ohno, **Cervinia Manalo**, Laura Rossetto, Tetsuji Okuda, Satoshi Nakai, Wataru Nishijima. 2016. Effect of coexisting metal ions on the degradation of polyamide reverse osmosis membrane by hypochlorite treatment. *Desalination* 381:126-134. (related to Chapter 5)

International Conferences

5. **Cervinia V. Manalo**, Masaki Ohno, Tetsuji Okuda, Satoshi Nakai, Wataru Nishijima. 2014. Rapid novel test for the determination of biofouling potential on reverse osmosis membranes. *4th IWA Regional Conference on Membrane Technology 2014*. Rex Hotel, Ho Chi Minh City, Vietnam, 3 – 6 December 2014. Oral Presentation (related to Chapter 2)
6. Masaki Ohno, **Cervinia Manalo**, Tetsuji Okuda, Satoshi Nakai, Wataru Nishijima. 2016. Control of Biofilm Formation and Inorganic Particle Accumulation on Reverse Osmosis Membrane by Hypochlorite Washing. *18th International Conference on Water and Wastewater Treatment Plants*. Singapore SG, 7 – 8 January 2016 (related to Chapter 4)

Domestic Conferences

7. **Cervinia V. Manalo**, Masaki Ohno, Tetsuji Okuda, Satoshi Nakai, Wataru Nishijima. 2014. Evaluation of Biofouling Potential on Reverse Osmosis Membranes. *48th Annual Meeting of the Japanese Society on Water Environment.* Kawauchi Kita Campus, Tohoku University, Sendai, Miyagi, Japan, 17 – 19 March 2014. Oral Presentation (related to Chapter 2)
8. **Cervinia V. Manalo**, Masaki Ohno, Tetsuji Okuda, Satoshi Nakai, Wataru Nishijima. 2015. Effect of Biofilm on Suspended Solid Accumulation on Reverse Osmosis Membranes. *Water and Environment Technology Conference 2015 (WET2015)***.** Kawauchi Kita Campus, Tohoku University, Sendai, Miyagi, Japan, 17 – 19 March 2014. Oral and Poster Presentation (related to Chapter 3)