Synthesis and characterization of silver nanocomposite nanofiltration and forward osmosis membranes based on layer-by-layer assembly

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NANYANG
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UNIVERSITY

**SYNTHESIS AND CHARACTERIZATION OF SILVER
NANOCOMPOSITE NANOFILTRATION AND FORWARD
OSMOSIS MEMBRANES BASED ON LAYER-BY-LAYER
ASSEMBLY**

LIU XIN
SCHOOL OF CIVIL AND ENVIRONMENTAL ENGINEERING
2015

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ASSEMBLY**

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ABSTRACT

Membrane separation processes, such as nanofiltration (NF) and forward osmosis (FO), have attained great attention and interests due to their various environmental applications including water and wastewater treatment, power generation, seawater desalination, food processing and pharmaceutical industry. However, membrane fouling, especially the irreversible membrane biofouling, remains a major drawback in prolonged membrane operation. The incorporation of bactericidal nanoparticles (NPs), for example silver nanoparticles (AgNPs), in membrane is considered as an effective approach to overcome above obstacle. Layer-by-layer (LbL) assembly method is a promising technique for fabricating different kinds of membranes with versatile structures. This study first investigated the stability and bactericidal activity of AgNPs in environmentally relevant freshwater matrices, and then focused on the development of antimicrobial silver nanocomposite NF and FO membranes based on LbL assembly. Effects of incorporation of AgNPs in membrane substrate on formation of LbL selective layer were also systematically studied. Performances of silver nanocomposite membranes were evaluated and compared with the AgNPs-free controls.

The influences of various natural organic matter (NOM) and chelating agent, ethylenediaminetetraacetic acid (EDTA), on AgNPs stability (aggregation, dissolution) and bactericidal activity were investigated by comparing the particle size, surface zeta-potential, and bacterial viability, in terms of half maximal inhibitory concentration (IC_{50}) value, in environmentally relevant freshwater matrices. Bactericidal activity of AgNPs against *Bacillus subtilis* and *Escherichia coli* was not substantially affected with the presence of three model NOM, humic acid, bovine serum albumin and alginic acid, at concentration comparable to that of natural freshwaters. Nevertheless, the combined addition of Ca^{2+} and humic acid induced severe aggregation of AgNPs, resulting in a reduced nanotoxicity. The chelating agent, EDTA, exhibited a concentration-dependent effect on AgNPs bactericidal activity: at low concentration (1 mg/L), EDTA decreased toxicity of AgNPs likely by converting the relatively high toxic dissolved Ag species into a

series of low toxic Ag-EDTA complex; at high concentrations (5 and 20 mg/L), EDTA mainly acted as a synergist and increased the toxicity of AgNPs probably by reacting with divalent cations (such as Ca^{2+} and Mg^{2+}) on bacterial cell membranes. The results not only illustrate the influencing factors of AgNPs toxicity under natural freshwater conditions, but also benefit the understanding of nanotoxicity mechanisms as well as environmental impact assessments of nanomaterials.

Novel silver nanocomposite NF and FO membranes were first synthesized by adding the AgNPs in membrane selective layers based on LbL assembly. The incorporation of AgNPs in the membranes did not adversely affect the membrane separation performance in NF and FO processes at low AgNPs incorporation levels. The FO performance of the silver nanocomposite membranes was better than or comparable to most NF-like FO membranes reported in the literature. In addition, the silver nanocomposite NF and FO membranes exhibited excellent antibacterial properties against both Gram-positive *B. subtilis* and Gram-negative *E. coli*. The results showed that the performances of the silver nanocomposite LbL membranes with AgNPs in membrane selective layers are highly dependent on silver incorporation in the membranes, which could be controlled by using different membrane synthesis routines and doping of AgNPs.

Novel silver nanocomposite NF and FO membranes with enhanced water permeabilities and excellent antimicrobial properties were then fabricated by embedding the AgNPs in membrane substrates. The polyacrylonitrile silver nanocomposite membranes (PAN-Ag) were prepared using one-step phase inversion process and could be used as ultrafiltration (UF) membranes. With additional crosslinked layer-by-layer (xLbL) assembly on the UF substrates, xLbL-Ag nanocomposite membranes were obtained, which were applicable for both NF and FO operations. At the optimal concentration of AgNPs incorporation (0.02 wt.%), water permeability of PAN-Ag(0.02) UF membrane increased by 14.3 % compared with the AgNPs-free control; under the NF and FO testing conditions, corresponding water fluxes of NF/FO membrane increased by 10.1 % and 24.4 % respectively. In addition, the silver nanocomposite membranes exhibited excellent antibacterial performances. At the highest silver incorporation concentration of 0.10

wt.%, xLbL3.0-Ag(0.10) nanocomposite NF/FO membrane led to ~6 Log colony-forming units (CFU) reduction against Gram-positive *B. subtilis* and ~5 Log CFU reduction against Gram-negative *E. coli*.

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LIST OF SYMBOLS

<i>Symbol</i>	<i>Description</i>
A	Pure water permeability
B	Salt permeability coefficient
C_f	Concentrations of MgCl_2 in feed solution
C_p	Concentrations of MgCl_2 in permeate solution
J_s	Solute flux
J_s/J_v	Solute flux over water flux
J_v	Pure water flux
M_w	Weight-averaged molecular weight
R	Salt rejection
m_{dry}	Dry mass of membrane samples
m_{wet}	Wet mass of membrane samples
α	The percentage of silver
ε	Membrane porosity
ρ_w	Density of water
ρ_m	Density of PAN
ΔP	Trans-membrane pressure
$\Delta\pi$	Osmotic pressure difference across the membrane

LIST OF ABBREVIATIONS

<i>Abbreviation</i>	<i>Description</i>
AA	Alginic acid
AASS	Alginic acid sodium salt
AFM	Atomic force microscopy
AgNPs	Silver nanoparticles
AL-DS	Active layer facing draw solution
AL-FS	Active layer facing feed solution
BSA	Bovine serum albumin
CFU	Colony-forming units
CTA	Celluloses triacetate
DI	Deionized (water)
DIZ	Diffusion inhibition zone
DMF	N,N-dimethylformamide
DS	Draw solution
EDTA	Ethylenediamine-tetraacetic acid
EDX	Energy-dispersive X-ray
EPS	Extracellular polymeric substances
ESA	Electrostatic self-assembly
FA	Fulvic acid
FE-SEM	Field emission scanning electron microscope
FO	Forward osmosis
FOMBR	Forward osmosis membrane bioreactor
FS	Feed solution

<i>Abbreviation</i>	<i>Description</i>
GA	Glutaraldehyde
GPC	Gel permeation chromatography
HA	Humic acid
IC ₅₀	Half maximal inhibitory concentration
ICP	Internal concentration polarization
ICP-MS	Inductively coupled plasma mass spectrometer
IHSS	International Humic Substances Society
LB	Luria-Bertani
LbL	Layer-by-layer
LPS	Lipopolysaccharide
MBR	Membrane bioreactor
MD	Membrane distillation
MF	Microfiltration
MWCO	Molecular weight cut off
MWNTs	Multiwalled carbon nanotubes
NF	Nanofiltration
NOM	Natural organic matter
NPs	Nanoparticles
NZVI	Nanometer-sized zerovalent iron
OMBR	Osmotic membrane bioreactor
PAH	Poly(allylamine hydrochloride)
PAN	Polyacrylonitrile
PBS	Phosphate-buffered saline
PEMs	Polyelectrolyte multilayers

<i>Abbreviation</i>	<i>Description</i>
PES	Polyethersulfone
PSf	Polysulfone
PSS	Poly(sodium 4-styrene-sulfonate)
PRO	Pressure retarded osmosis
RO	Reverse osmosis
ROS	Reactive oxygen species
SEM	Scanning electron microscope
SRFA	Suwannee River fulvic acid
SRHA	Suwannee River humic acid
SRNOM	Standard Suwannee River natural organic matter
SWNTs	Single-walled nanotubes
TA	Tannic acid
TFC	Thin film composite
TFN	Thin film nanocomposite
UF	Ultrafiltration
xLbL	Crosslinked layer-by-layer

LIST OF PUBLICATIONS

Journal articles

1. **X. Liu**, S. Qi, Y. Li, L. Yang, B. Cao, C.Y. Tang, "Synthesis and characterization of novel antibacterial silver nanocomposite nanofiltration and forward osmosis membranes based on layer-by-layer assembly", *Water Research*, 47 (2013) 3081-3092.
2. **X. Liu**, X. Jin, B. Cao, C.Y. Tang, "Bactericidal activity of silver nanoparticles in environmentally relevant freshwater matrices: Influences of organic matter and chelating agent", *Journal of Environmental Chemical Engineering*, 2 (2014) 525-531.
3. **X. Liu**, L.-X. Foo, Y. Li, J.-Y. Lee, B. Cao, C.Y. Tang, "Synthesis and Characterization of Antibacterial Silver Nanocomposite Membranes with Enhanced Water Permeabilities and Versatile Environmental Applications". *In preparation*.
4. **X. Liu**, P. Yang, M.J. Gallagher, D.H. Fairbrother, K.L. Chen, "Evaluating the Antimicrobial and Anti-Adhesive Properties of Graphene Oxide Nanocomposite Membranes: Implications for Biofouling Control". *In preparation*.
5. J. Wei, **X. Liu**, C. Qiu, R. Wang, C.Y. Tang, "Influence of monomer concentrations on the performance of polyamide-based thin film composite forward osmosis membranes", *Journal of Membrane Science*, 381 (2011) 110-117.
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Conference presentations

1. **X. Liu**, S. Qi, Y. Li, L. Yang, B. Cao, C.Y. Tang (2013), “Synthesis and characterization of novel antibacterial silver nanocomposite nanofiltration and forward osmosis membranes based on layer-by-layer assembly”, *1st International Conference on Desalination using Membrane Technology*, Sitges, Spain.
2. **X. Liu**, L.-X. Foo, S. Qi, Y. Li, J.-Y. Lee, B. Cao, C.Y. Tang (2013), “Synthesis and Characterization of Antibacterial Silver Nanocomposite Membranes with Enhanced Water Permeabilities and Versatile Environmental Applications”, *NAMS 2013*, Boise, USA.
3. **X. Liu**, P. Yang, M.J. Gallagher, D.H. Fairbrother, K.L. Chen (2014), “Evaluating the Antimicrobial and Anti-Adhesive Properties of Graphene Oxide Nanocomposite Membranes: Implications for Biofouling Control”, *NAMS 2014*, Houston, USA
4. **X. Liu**, P. Yang, M.J. Gallagher, D.H. Fairbrother, K.L. Chen (2014), “Antimicrobial Properties of Low-pressure Graphene Oxide Nanocomposite Membranes for Water Purification”, *ACS 2014*, San Francisco, USA.

CHAPTER 1

Introduction

1.1. Background

Membrane separation processes, including nanofiltration (NF) and forward osmosis (FO) processes, have been widely used in a variety of applications, such as water and wastewater treatment (Nicolaisen, 2003; Sonune and Ghate, 2004; Alkudhiri et al., 2012), seawater desalination (Kessler and Moody, 1976), power generation (Kim and Logan, 2011; Logan and Elimelech, 2012), food and beverage processing (Garcia-Castello et al., 2009; Wei et al., 2011b), biochemical and pharmaceutical industries (Chang et al., 2008; Yang and Yen, 2013), gas separation (Baker, 2002; Sanders et al., 2013), and etc. Compared with the conventional separation techniques, membrane separation processes are more convenient, efficient and economical beneficial (Strathmann, 1981). However, membrane fouling, especially the irreversible membrane biofouling, remains a major obstacle for membrane operation (Elimelech and Phillip, 2011). Membrane biofouling refers to the growth of microorganisms and the formation of biofilms on membrane surfaces (Perreault et al., 2013). Membrane biofouling may result in the decrease in permeate water flux and membrane selectivity, the increase in energy consumption and overall operation costs, and a shorten membrane lifetime (Goosen et al., 2004; Le-Clech et al., 2006a; Matin et al., 2011). Several approaches have been adopted to prevent and control membrane biofouling, including the pretreatment of feed water, redesign of module and system, optimization of operation conditions (such as operating below the critical flux, applying ultrasound, shaking and microbubbles, and membrane cleaning), usage of biocides, and fabrication of antimicrobial membranes (including membrane surface modifications) (Nguyen et al., 2012). Among them, the fabrication of antimicrobial membranes has been considered as an effectively way to prevent membrane biofouling (Hilal et al., 2003). The antimicrobial membranes may prevent membrane biofouling in two aspects: 1) reduce bacterial affinity between microorganisms and membranes; 2) inhibit the growth of microorganisms on membrane surface.

Nanoparticles (NPs) can be used to fabricate the antimicrobial nanocomposite membranes mainly due to their relatively strong bactericidal effects against a wide range of aquatic organisms and microbial communities (Borm and Berube, 2008). Compared with the conventional membrane separation processes and those hybrid processes containing NPs, the nanocomposite membranes have several significant advantages: First, nanocomposite membrane is an effective strategy for membrane biofouling control, which may also prevent the internal scaling and over-growth of biofilm (Zhang et al., 2012b); Second, intrinsic membrane transport properties, for example, water permeability of nanocomposite membranes, can be enhanced due to the incorporation of NPs (Ma et al., 2013; Lee et al., 2014); Third, the leaching and recycle issues of the usage of slurry form NPs can be solved. Among all kinds of NPs, silver nanoparticles (AgNPs) is one of the most extensively studied biocides (Sondi and Salopek-Sondi, 2004; Li et al., 2008b; Choi et al., 2010), which can effectively control the growth and activity of various microorganisms and inactivate the planktonic microorganisms in aqueous suspensions (Lok et al., 2006; Nguyen et al., 2012).

Therefore, the investigation of synthesis and characterization of novel antimicrobial silver nanocomposite NF and FO membranes by incorporating AgNPs in membrane structures using a flexible fabrication method is highly desirable for membrane biofouling control.

1.2. Problem statement

Although the antimicrobial properties of AgNPs have been investigated and utilized in a wide range of environmental applications (Nguyen et al., 2012), little is known about the transport, fate, and toxicity of AgNPs in natural environments, especially in natural freshwaters (Jin et al., 2010; Liu et al., 2014). Previous studies suggested that toxicity of AgNPs in different matrices may differ greatly (Jin et al., 2010; Li et al., 2011a). While most currently documented ones were conducted in bio-relevant medium that is rich of various nutrients and with a high concentration of salts. Only a few studies have investigated the influence of specific ions on AgNPs stability and

bacterial inactivation in environmental relevant freshwaters (Jin et al., 2010; Chambers et al., 2013). Effects of natural organic matter (NOM) and chelating agent on the stability and bactericidal activity of AgNPs in environmentally relevant freshwater matrices are still unclear.

AgNPs have been successfully incorporated into nanocomposite microfiltration (MF) (Gunawan et al., 2011; Diagne et al., 2012), ultrafiltration (UF) (Taurozzi et al., 2008; Zodrow et al., 2009; Huang et al., 2012a), NF (Lee et al., 2007) and reverse osmosis (RO) (Yang et al., 2009a) membranes via various methods including membrane surface modification (Diagne et al., 2012), phase-inversion (Lee et al., 2007; Taurozzi et al., 2008; Zodrow et al., 2009; Huang et al., 2012a) and interfacial polymerization (Yang et al., 2009a) due to their antimicrobial properties (Liu et al., 2013b). Compared to above existing methods for synthesizing silver nanocomposite membranes, layer-by-layer (LbL) assembly is a highly versatile membrane fabrication method (Liu et al., 2014). LbL membranes can be used in a variety of environmental applications (Liu et al., 2013a). The layered structure can potentially allow the incorporation of AgNPs with great flexibility in controlling the loading conditions thus the membrane performance. Besides the drawbacks of membrane biofouling, current available NF and FO membranes are still limited in terms of water permeability and solute rejection. The incorporation of AgNPs in membrane based on LbL assembly may not only prevent membrane biofouling, but also enhance membrane water permeability. Nevertheless, LbL-Ag nanocomposite membranes with antibacterial properties have not been reported for NF and FO membrane fabrication.

1.3. Objectives

The overall objectives of this study are to explore a novel method (LbL assembly) for synthesizing the antibacterial silver nanocomposite membranes with potential versatile environmental applications, and to systematically investigate the different fabrication routines and influencing factors on membrane performances (including

the intrinsic membrane transport properties and the antibacterial performance). In particular, the specific tasks are listed as follows:

- (1) to investigate the stability and bactericidal activity of AgNPs in environmentally relevant freshwater matrices, especially the influences of NOM and chelating agent on AgNPs;
- (2) to synthesize novel antibacterial nanocomposite NF and FO membranes via LbL assembly method by incorporating the AgNPs in membrane selective layer and membrane substrate respectively;
- (3) to characterize membrane surface properties and performance of membranes under NF and FO testing conditions;
- (4) to investigate the effects of silver incorporation in membrane substrate on the formation of membrane selective layer using LbL assembly method;
- (5) to evaluate the antibacterial activities of the silver nanocomposite membranes against aquatic microbes (such as the Gram-positive *B. subtilis* and Gram-negative *E. coli*);
- (6) to study the concentration effect of AgNPs incorporation on membrane performances.

1.4. Scope and organization of the thesis

This thesis includes seven chapters and one appendix. Chapters 1, 2, 3, and 7 present the introduction, literature review, materials and methodology, and conclusions and recommendations, respectively. The experimental results and discussions are presented in Chapters 4–6. The contents of Chapters 4–6 and appendix are briefly summarized as follows:

In Chapter 4, the influences of natural organic matter and chelating agent, ethylenediaminetetraacetic acid (EDTA), on bactericidal activity of AgNPs in

environmental relevant freshwater matrices are systematically investigated. Under the experimental conditions, NOM show little effects on the bactericidal activity and stability of AgNPs in the absence of Ca^{2+} . While the introduction of additional Ca^{2+} may significantly decrease the bactericidal activity of AgNPs by inducing the Ca^{2+} -NOM-AgNPs aggregation. Chelating agent, EDTA, exhibit a concentration-dependent effect on AgNPs bactericidal activity. This part of work has been published in Journal of Environmental Chemical Engineering (Liu et al., 2014). Copyright permission for using this work is attached at the end of this thesis.

Chapter 5 presents the fabrication and characterization of antibacterial silver nanocomposite membranes based on layer-by-layer(LbL) assembly, which provides a novel method to fabricate the nanocomposite membranes. Different membrane synthesis routines and the effects of doping times of AgNPs are investigated. The performances of silver nanocomposite membranes are evaluated and compared to the AgNPs-free controls in both NF and FO processes. Different antibacterial tests are adopted to investigate the antibacterial properties of silver nanocomposite membranes against bacteria. This part of work has been published in Water Research (Liu et al., 2013b). Copyright permission for using this work is attached at the end of this thesis.

Chapter 6 investigates another routine of the silver nanocomposite membrane fabrication. Instead of incorporating the AgNPs in membrane selective layer via LbL assembly (Chapter 5), different amounts of AgNPs are first loaded in the support substrates, which could be individually used as silver nanocomposite UF membranes. Based on them, membrane selective layers are obtained via LbL assembly. Systematic membrane characterization are performed to investigate the effects of AgNPs incorporation on LbL assembly and membrane performances in UF, NF and FO processes. Antibacterial effects of the silver nanocomposite membranes are also evaluated. This part of work has been submitted to Desalination for potential publication.

Appendix presents A) Effects of NOM on live bacterial percentages; B) Dispersion and aggregation of AgNPs in PSS solution, Milli-Q water and PAH solution; C) Comparison of the NF and FO performances of the silver nanocomposite

membranes with membranes in literature; D) Silver leaching test of PAN-Ag nanocomposite UF membranes, and E) Evaluation of the increased costs of PAN-Ag nanocomposite UF membrane fabrication.

CHAPTER 2

Literature Review

2.1. Membrane separation process

Membrane technology refers to all engineering approaches for the transport and separation of the substances between two fractions by using membranes. Membrane separation processes are widely used in a variety of environmental applications (Strathmann, 1981; 2001; Cath et al., 2006; Bernardo et al., 2009; Takht Ravanchi et al., 2009; Zhao et al., 2012b). The widely used membrane processes include microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO), forward osmosis (FO), pressure retarded osmosis (PRO), electrodialysis, gas separation, membrane distillation (MD), pervaporation, and etc.

Membrane technology has been considered as an important separation method since 50 years ago (Mulder, 1996). In 1960, Loeb and Sourirajan (Loeb and Sourirajan, 1963) first developed and reported the technique for fabricating anisotropic pressure-driven RO membranes via phase inversion method using cellulose acetate. Since then, membrane separation technology has been attracting great attention as one of the most innovative fields and has been investigated intensively and used widely.

2.1.1. *NF membrane*

NF is a pressure driven membrane separation process that employs a semi-permeable membrane with the pore size in the range of nanometers. Although the NF membranes and their properties are quite diverse, however, they can be generally defined as the membranes having rejection characteristics ranging from “loose” RO to “tight” UF (Craig Bartels, 2007). Compared with RO membranes, NF membranes usually have much lower rejections of certain dissolved components, such as the monovalent ions, but higher water permeability and lower energy consumption (Van der Bruggen and Vandecasteele, 2003). Therefore, NF can be a great improvement for membrane separation technology.

2.1.2. Applications of NF

NF membranes were first invented for softening of surface water and groundwater in the drinking water industry (Van der Bruggen and Vandecasteele, 2003). As the development of NF technology, they can also be used for the removal of hardness (Sombekke et al., 1997), natural organic matter (NOM) (Cho et al., 1999), micropollutants such as pesticides (Kiso et al., 2000) and volatile organic compounds (Ducom and Cabassud, 1999), viruses and bacteria (Urase et al., 1996), salinity (Hassan et al., 1998), nitrates (Van der Bruggen et al., 2001), arsenic (Kartinen Jr and Martin, 1995), and etc.

In recent years, NF has been extended into other industries such as food processing (van der Horst et al., 1995), heavy metals removal (Muthukrishnan and Guha, 2006), and so on. Figure 2-1 shows the classification of membrane filtration processes as a function of molecular weight cut off (MWCO) and pore size. The typical substances that can be separated by NF membranes are highlighted in the figure.

Figure 2-1. Classification of membrane filtration processes as a function of molecular weight cut off and pore size (reproduced from www.epa.gov).

2.2. Forward osmosis

Membrane separation processes are dominated by the pressure-driven membranes which require the applied hydraulic pressure is higher than the osmotic pressure, such as NF and RO. These processes may cause high-energy consumption and severe membrane fouling. However, FO can be operated under low or even no applied hydraulic pressure with less energy consumption and low fouling propensity in various applications (Mi and Elimelech, 2008). Due to the global water scarcity and energy shortage, FO separation process has gained lots of attention and been investigated intensively with great interest in recent years. The working principles of FO process and its potential applications are introduced in this section.

2.2.1. Working principles of FO

Instead of using applied hydraulic pressure, osmotic pressure gradient ($\Delta\pi$) is the driving force in FO process, which automatically extracts water from a low concentrate feed solution (FS, i.e. freshwater) to a high concentrate draw solution (DS, i.e. brine) across a semi-permeable FO membrane (shown in Figure 2-2(a)). As a result, draw solution will be diluted and feed solution will be concentrated in this process. By contraries, the driving force in RO process is the pressure difference of applied hydraulic pressure exceeding osmotic pressure. Through this process, purified water is produced while solutes in the concentrated side are maintained. In other words, purified water can only be produced when the applied pressure is larger than the instinctive osmosis pressure of the solution. PRO is an intermediate process between FO and RO, where hydraulic pressure is also applied in the opposite direction of osmotic pressure gradient (similar to RO) (Cath et al., 2006). However, net water flux is still flow towards the concentrated draw solution (similar to FO) (S.Loeb, 1962).

The general equation that used to describe water transport in FO, RO, and PRO is:

$$J_w = A(\Delta P - \Delta\pi) \quad (2-1)$$

where J_w is the water flux, A is the membrane water permeability coefficient, ΔP is the applied hydraulic pressure, and $\Delta\pi$ is the osmotic pressure gradient. For FO,

hydraulic pressure difference (ΔP) is approximately zero, and water diffuses to the more saline side of the membrane due to osmotic pressure difference ($\Delta\pi$). For PRO, since pressure applied is between zero and the flux reversal point (i.e. $\Delta P < \Delta\pi$), less water diffuses to the brine side. For RO, water diffuses to the less saline side due to hydraulic pressure difference greater than the osmotic pressure difference (i.e. $\Delta P > \Delta\pi$). The principles of FO, RO and PRO are illustrated in Figure 2-2(a). The FO point, PRO zone, and RO zone, along with the flux reversal point, are illustrated in Figure 2-2(b) (Lee et al., 1981; Cath et al., 2006).

(a)

(b)

Figure 2-2. Working principles of FO, PRO and RO. (a) Water flows in FO, PRO and RO. (b) Direction and magnitude of water flux as a function of applied pressure in FO, PRO, and RO. Reproduced from the Ref. (Cath et al., 2006) with copyright permission.

FO offers a number of potential advantages including high rejection of various contaminants, reduction of energy consumption and brine discharge, and low membrane fouling propensity and mild operation conditions (Cath et al., 2006; Ng et al., 2006; Cornelissen et al., 2008; Achilli et al., 2009b).

2.2.2. Potential applications of FO process

Due to utilization of osmotic pressure gradient across the membrane as the driving force, FO separation process offers several significant advantages including less energy consumption, high rejection of a wide range of contaminants, low membrane fouling propensities (compared with the conventional pressure-driven membrane processes), and operation without requiring high temperature or pressure (Cath et al., 2006). Thanks to these, FO process has drawn great attention with potential applications in various fields (Mulder, 1996; Cath et al., 2006; Zhao et al., 2012b), such as water and wastewater treatment (Nicolaisen, 2003; Sonune and Ghate, 2004; Alkhudhiri et al., 2012), seawater desalination (Kessler and Moody, 1976), power generation (Kim and Logan, 2011; Logan and Elimelech, 2012), food and beverage processing (Garcia-Castello et al., 2009; Wei et al., 2011b), biochemical and pharmaceutical industries (Chang et al., 2008; Yang and Yen, 2013), and etc. The number of such applications is still growing. Major applications of FO technology were briefly introduced in the following parts to shed light on future studies of FO membrane fabrication and applications.

2.2.2.1. Water and wastewater treatment

FO membranes can be used in water and wastewater treatment in several aspects. Those applications of FO process include: 1) concentration of dilute industrial wastewater (F. Votta, 1974), 2) treatment of domestic sewage or landfill leachate (Innovations, 2011; Zhang et al., 2014), 3) direct potable reclamation of wastewater by using impaired water and saline water (or concentrated glucose solution in certain cases) as feed and draw solution respectively in FO process (Cath et al., 2005a; Cath et al., 2005b), and etc. Another promising application of FO membrane in wastewater treatment is FO membrane bioreactor (FOMBR), which is also known as the osmotic membrane bioreactor (OMBR). Conventional membrane bioreactor (MBR) is a prevalent and well-developed water reclamation technology that combines the biological degradation with membrane separation process (Judd, 2008). The innovative OMBR technology has been developed by adopting FO membranes in MBR system, which has gained great attention (Cornelissen et al., 2008; Achilli et al., 2009b; Lay et al., 2011; Lay et al., 2012; Zhang et al., 2012b).

The schematic of a submerged OMBR system is illustrated and shown in Figure 2-3. In the system, the FO membrane module is submerged in and contacted with the mixed liquor containing microbes. Water can permeate through the FO membrane into draw solution. After osmotic dilution, the diluted draw solution is reconcentrated by a post-treatment process (i.e. RO or MD unit) to produce high quality potable water; the by-product is a reconcentrated DS which can be reused in FO process (Achilli et al., 2009b; Zhao et al., 2012b). Compared with the conventional MBR process, OMBR can offer a much higher rejection at a lower hydraulic pressure (Achilli et al., 2009b; Xie et al., 2012), which minimized membrane fouling and thus reduced costs of the overall process (Cornelissen et al., 2008). The commercialization of OMBR may be realized in a near future (Zhao et al., 2012b).

Figure 2-3. Schematic of an OMBR system for wastewater treatment (figure adopted from Ref. (Achilli et al., 2009b) with copyright permission).

2.2.2.2. Seawater desalination

Due to water scarcity and the increasing global water demand, potable water obtained from seawater desalination has been considered as an important water source. FO membranes have been proved to be feasible in seawater desalination

process since 1970s (Kessler and Moody, 1976; McCutcheon et al., 2006; Choi et al., 2009b). In FO desalination process, seawater is used as the feed solution while a high concentration solution with higher osmotic pressure (i.e. ammonia-carbon dioxide solution and glucose solution) is chosen as the draw solution (McCutcheon et al., 2006). The diluted draw solution after FO process will be regenerated into draw solution. For example, the ammonia-carbon dioxide draw solution can be heated and decomposed into ammonia and carbon dioxide gas and then released. As a result, the freshwater can be obtained. Theoretically, the collected ammonia and carbon dioxide can be reused to regenerate new draw solution. Other hybrid FO desalination processes are also reported, including FO/RO (Choi et al., 2009b; Bamaga et al., 2011), FO/MD (Martinetti et al., 2009), FO/NF (Zhao et al., 2012a) and FO/UF (Ling and Chung, 2011) processes. In recent years, numerous large-scale seawater desalination plants have been built in water-stressed countries to augment available water resources. There are more to be constructed in near future (Elimelech and Phillip, 2011). Nevertheless, the lack of 1) FO membrane with high water flux and good rejection, and 2) proper draw solution with low cost and high recovery remain the obstacles for scale-up of FO desalination plants. The schematic of FO desalination process is shown in Figure 2-4.

Figure 2-4. Schematic of FO desalination process (figure adopted from Ref. (Achilli et al., 2009b) with copyright permission).

2.2.2.3. Power generation - PRO

Energy is an important issue for both economy and society. As a viable source of renewable energy, PRO has been investigated since 1970s with great interest (Norman, 1974; Loeb, 1975; Seppälä and Lampinen, 1999; Gerstandt et al., 2008). A typical design of a PRO plant and its working principle is exhibited in Figure 2-5. Similar principle is also reported by Achilli et al. (Achilli et al., 2009a). In the PRO plant, filtered freshwater is pumped into a PRO module containing membranes. In the module, freshwater flows on one side of the PRO membrane and diffuses through the membrane into the pressurized seawater side. The diluted and pressurized seawater is then split into two streams: one is depressurized in a turbine to generate power, and the other passes through a pressure exchanger to assist in pressurizing the incoming seawater. As observed in Figure 2-5, the plant has a high pressure loop for the salt and brackish water and a low pressure side for the freshwater (Aaberg, 2003).

The power generated by PRO from seawater and freshwater has certain features which make it attractive. For example, it is a large and unexploited resource, which is renewable, high-density (compared to other potential sources of energy from the ocean), and has minimal environmental impact (Loeb, 1976). Some estimations also suggested the global power production potential of salinity gradients is on the order of 2000 TWh per year (Loeb et al., 1976). If the PRO process can be developed to make use of this huge amount and environmental friendly energy source efficiently, it is likely to bring another energy revolution. Currently, Statkraft, a company working on renewable energy, has built the first prototype osmotic power plant at Tofte, Norway and lead the development of osmotic power in the world (Zhao et al., 2012b).

Figure 2-5. Schematic of a pressure retarded osmosis (PRO) power plant (figure adopted from www.statkraft.com).

2.2.2.4. Food and beverage industry

Compared with the conventional evaporative concentration techniques used in food and beverage processing, FO can provide several advantages in maintaining the physical properties of the liquid food (such as color, taste, aroma and nutrition) without deteriorating its quality (Petrotos and Lazarides, 2001). In addition, FO membrane can be operated under a mild environment (low temperature and pressure) while maintaining high rejection and low fouling tendency. Therefore, FO has been widely used to concentrate various water-containing foods (Zhao et al.). Two detailed summaries were published on the potential use of FO in the processing of liquid foods (Cath et al., 2006). Petrotos and Lazarides (Petrotos and Lazarides, 2001) reviewed several low pressure membrane processes (including FO) for concentration of liquid foods. Jiao et al. (Jiao et al., 2004) provided a more comprehensive review on membrane processes for concentration of fruit juices.

2.2.2.5. Pharmaceutical applications

In pharmaceutical industry, FO is mainly used in osmotic drug delivery and the enrichment of pharmaceutical products (Santus and Baker, 1995; Thombre et al., 1999a; Thombre et al., 1999b; Lin and Ho, 2003). Osmotic drug delivery systems are based on the principle of osmosis and designed for oral administration. It has

been widely used in medical fields, such as tablets/capsules coated with semipermeable membranes containing micro-pores, polymer drug matrix systems, and self-formulating in-line systems for parenteral drug delivery which is also known as osmotic pumps (Thombre et al., 1999a; Thombre et al., 1999b). The enrichment of pharmaceutical products using FO is similar to its application in food industry. Since most pharmaceutical products are large molecules and heat sensitive, FO process operated under low temperature has significant advantages compared with those conventional chemical or thermal concentration methods in pharmaceutical manufacture. Different from seawater desalination and wastewater treatment, the concentrates of FO are the target products in food and pharmaceutical industry. Since there is no need to further separate water from the diluted draw solution, FO has great potential in food and pharmaceutical product concentration (Cath et al., 2006; Zhao et al., 2012b).

2.2.2.6. Other applications

FO has also been proposed for many other applications. For example, hydration bags based on FO membranes (shown in Figure 2-6) have been commercially used for military, recreational and emergency relief situations where reliable drinking water is scarce (Cath et al., 2006); FO can play an important role in the production and separation of bio-energy as well as the protection of the environment (Hoover et al., 2011; Zhang et al., 2011a; Zou et al., 2011); FO has also been proposed for osmotic diluting of desalination brine before it is discharged into the sea (Hoover et al., 2011). NASA has also investigated an innovative algae bioreactor by using FO membranes and offshore ocean space as a sustainable energy source. The system consists of algae grown in flexible plastic bags with inserts of FO membranes floating offshore, where cities typically dump their wastewater. Oil-producing freshwater algae would naturally clean the wastewater by feeding on nutrients in the sewage. Biomass and oxygen will be produced by algae with the energy of sunlight. The cleansed freshwater is released into the surrounding ocean through FO membranes in the sides of the plastic bags (Marlaire, 2009). The schematic of algae bioreactor is presented in Figure 2-7.

Figure 2-6. Schematic of hydration bags based on FO membranes (figure adopted from Ref. (Cath et al., 2006) with copyright permission).

Figure 2-7. Schematic of algae bioreactor (figure adopted from www.nasa.gov).

2.3. Membrane fouling

2.3.1. Membrane fouling, influencing factors and controls

Membrane fouling is a process that solute or particles deposit onto membrane surfaces or into membrane pores which degrades the membrane performance. As applications of membrane technology growing rapidly worldwide, membrane fouling, especially the irreversible membrane biofouling, remains the greatest barrier to the efficient usage of membranes (Li et al., 2008b). Membrane fouling can significantly affect membrane performance thus the membrane life span. The energy consumption and overall operation costs will also be increased. The past decade has seen the extensive study on fouling issues in pressure driven membrane processes, variety of fouling behavior and control methods have been investigated (Elimelech et al., 1997; Hong and Elimelech, 1997; Yuan and Zydney, 1999; Vrijenhoek et al., 2001; Le-Clech et al., 2006a; Nguyen et al., 2012).

According to the types of foulants, membrane fouling can be divided into four categories: 1) organic fouling; 2) inorganic fouling; 3) colloidal fouling and 4) biofouling (Pan et al., 2010; Nguyen et al., 2012). Among all of them, biofouling has been considered as the most challenging problem due to its severe impact on membrane performance and life span (Baker and Dudley, 1998). Membrane biofouling starts with the deposition of single cell or cluster of cells on membrane surface, which leads to the formation of biofilm layer (Nguyen et al., 2012). The cells reproduce and grow into biofilms embedded with extracellular polymeric substance (EPS) matrix.

Membrane fouling can be affected by a lot of factors, including: 1) membrane properties, such as membrane surface hydrophilicity/hydrophobicity, membrane pore size and distribution, membrane material, and etc.; 2) solution properties, such as concentration, the nature of the components and particle size distribution; 3) operating conditions, such as pH, temperature, flow rate and pressure.

Periodic cleaning can be provided for membrane biofouling control which aims to weaken the interaction between microorganisms and membrane surface. The current

membrane cleaning methods can be classified into physical, chemical, physico-chemical and biological cleaning, etc. (Nguyen et al., 2012).

2.3.2. Membrane fouling in FO process

The growing attention to FO from various disciplines raises the demand of systematic study on membrane fouling in FO process (Mi and Elimelech, 2008). Currently, only a few publications touched on the fouling of FO membranes (Tang et al., 2010). Mild fouling phenomena in FO process are observed in some literature, since there is little or no hydraulic pressure applied during FO process (Holloway et al., 2007; Lay et al., 2010). Nevertheless, fouling is still a critical problem in prolonged FO applications (Liu and Mi, 2012). Therefore, the systematic research on FO fouling and fouling control are further required (Tang et al., 2010; Xiao et al., 2011; Zou et al., 2011).

FO fouling, which mainly reflects in the form of flux reduction, could be strongly affected by membrane structures, properties, orientations, foulants properties and operational conditions (Mi and Elimelech, 2008; Wang et al., 2010c; Zou et al., 2011; Jin et al., 2012). Mi and Elimelech (Mi and Elimelech, 2008) showed that fouling is governed by the coupled influence of chemical and hydrodynamic interactions. In addition, calcium binding, permeation drag, and hydrodynamic shear force are the major factors governing the development of a fouling layer on the membrane surface, although the dominating factors controlling membrane fouling vary from foulant to foulant. Tang et al. (Tang et al., 2010) pointed out that internal concentration polarization (ICP) is one of the major drawbacks of FO, which significantly limits the FO flux efficiency. And FO membrane flux can be adversely affected by membrane fouling. Moreover, their results indicated that pore clogging enhanced ICP mechanism probably played a dominant role in FO flux reduction at higher flux levels. The effects of back diffusion on FO fouling process were also investigated by Zou et al. (Zou et al., 2011). The results indicate the reverse diffusion of Mg^{2+} from the draw solution into the feed solution, which led to an unfavorable interaction between the divalent ion and the algal biomass in the feed water.

2.3.3. Membrane biofouling in FO process

Biofouling is a major type of membrane fouling in FO operation. One of the most frequent examples is OMBR. As introduced previously, OMBR is one of the most promising applications of FO membranes in wastewater treatment (Cornelissen et al., 2008; Qin et al., 2010; Zhang et al., 2012b).

Cornelissen et al. (Cornelissen et al., 2008) investigated the effect of temperature, membrane type, membrane orientation, type and concentration of draw solution to optimize FO performance. They also studied the relationship lying in membrane fouling and draw solution leakage by using the activated sludge solution from MBR. A pilot OMBR system composing anoxic/aerobic FO processes was operated by Qin et al. (Qin et al., 2010). The effects of draw solution on water flux and air scouring on fouling reversibility were tested. Their results show that better water quality in OMBR could be achieved over the conventional MBR. Zhang et al. (Zhang et al., 2012b) studied the multiple mechanisms of OMBR fouling caused by the biomass, dissolved organic and inorganic compounds retained in the bioreactor. Their results suggest that FO fouling is governed by the coupled influences of biofilm formation and inorganic scaling when the active layer facing the draw solution (AL-DS), while a negligible flux decline was obtained by applying intermittent tap water flushing to the membrane surface when the active layer facing the feed solution (AL-FS). This may suggest that the AL-FS orientation is favorable for OMBR operation.

2.4. Bactericidal activities of nanoparticles (NPs)

2.4.1. Antimicrobial NPs and their antimicrobial mechanisms

Several natural and engineered NPs have demonstrated strong antimicrobial properties through diverse mechanisms including photocatalytic production of reactive oxygen species (ROS) that damage cell components (i.e. TiO_2 and ZnO), compromising the bacterial cell envelope (i.e. peptides, chitosan, carboxyfullerene, carbon nanotubes, ZnO and silver nanoparticles (AgNPs)), interruption of energy transduction (i.e. AgNPs and aqueous fullerene nanoparticles (nC_{60})), and inhibition of enzyme activity and DNA synthesis (i.e. chitosan) (Li et al., 2008b). A summary of the antimicrobial mechanisms of above NPs are presented in Table 2-1.

Due to the relatively high antimicrobial activity of silver against a variety of aquatic organisms and microbial communities (Borm and Berube, 2008), as well as the high degree of commercialization of AgNPs in various applications (Chen and Schluesener, 2008), toxicity of AgNPs draws extraordinary attention compared to other engineered NPs. The antimicrobial properties of silver compounds and silver ions have been historically recognized and applied in a wide range of applications from disinfecting medical devices and home appliances to water treatment (Li et al., 2008b). Great efforts have been put into studying the antimicrobial mechanisms of AgNPs in the past few years. Possible mechanisms that have been raised and widely accepted include: 1) extracellular release of silver ions or other dissolved silver species followed by cellular uptake and accumulation, or a cascade of intracellular reactions; 2) direct interaction with cell membranes by damaging membrane integrity or impacting its protein function, disrupting the proton gradient, and increasing membrane permeability, as a result, regulation of material transport, energy transduction, and intercellular communication will be destroyed; 3) generation of ROS which potentially impact DNA replication, destabilize and oxidase protein, damage the cell component and interrupt of energy transduction (Klaine et al., 2008; Jin et al., 2010; Marambio-Jones and Hoek, 2010); 4) Trojan horse mechanism, which suggests NPs enter a cell by bypassing its barriers as “normal”-sized silver, and then release silver ions that damage cell machinery (Lubick, 2008; Park et al., 2010; Service, 2010).

Table 2-1. Antimicrobial mechanisms of several commonly used NPs (table reproduced from the Ref. (Li et al., 2008b)).

NPs	Antimicrobial mechanism
AgNPs	Release of Ag ⁺ ions, disruption of cell membrane and electron transport, DNA damage
TiO ₂	Production of ROS, cell membrane and cell wall damage
ZnO	Intracellular accumulation of nanoparticles, cell membrane damage, H ₂ O ₂ production, release of Zn ²⁺ ions
CNTs	Physically compromise cell envelope
Chitosan	Cell membrane damage, chelation of trace metals

2.4.2 Influencing factors of NPs bactericidal properties

Toxicity of NPs against bacteria are influenced by various factors, such as the characteristics of NPs (including the kinds, concentrations, particle sizes, shapes, and surface charges of NPs), the bacteria, the testing media, the initial concentration of bacterial population, and pure culture or mixed population used for the test (Dasari and Hwang, 2010). The presence of specific ions may also affect the bactericidal properties of NPs (Jin et al., 2010). In addition, the oxidation and reduction reactions of certain metal NPs in waters can be another significant influencing factor.

2.4.2.1. Effects of different kinds of NPs

The bactericidal activity could vary among different kinds of NPs, which may due to the mechanisms by which NPs act on bacterial cells. For example, it was found that the bactericidal activity of SiO₂, TiO₂ and ZnO generally increased in their respective water suspensions (i.e. antibacterial activity of SiO₂ < TiO₂ < ZnO) towards both *B. subtilis* and *E. coli* (Adams et al., 2006). Dissolution of NPs, which may greatly affected by water chemistry conditions, also plays an important role in nanotoxicity. Previous study indicated that ZnO dissociation disrupts cellular zinc homeostasis, leading to lysosomal and mitochondria damage and ultimately cell death; In contrast, the antioxidant properties of CeO₂ protect cells from oxidant injury (Xia et al., 2008). Therefore, the intrinsic chemical compositions, ion release, and generation of ROS may all contribute to the differences of NPs bactericidal activity.

2.4.2.2. Effects of particle size

It is widely acceptance that the bactericidal activity of NPs is highly size-dominated (Morones et al., 2005; Powers et al., 2007; Klaine et al., 2008; Bae et al., 2010); and some studies also show that the bactericidal effect of NPs generally increases as the particle size decreases (Choi and Hu, 2008; Zhang et al., 2011c). Despite of various original size of NPs, similar antibacterial activity might be observed if similarly sized particles can be formed through aggregation (Adams et al., 2006). In addition,

particle sizes may also relate to the ion release of NPs, which is another pathway to inhibit bacterial growth or viability besides the direct penetration and ROS generation. It was found that Ag^+ release rates were highly depend on primary particle size and concentration when other environmental factors (such as dissolved oxygen and protons) were kept constant (Zhang et al., 2011c). Consequently, the key processes that may govern NPs behavior and bactericidal activity in the aquatic environments are aggregation and dissolution of NPs, driven by size and surface properties of the materials (McLaughlin, 2010).

2.4.2.3. *Effects of test medium*

Dramatic different toxicity of the same NPs are observed in various aqueous media, which may due to the water chemistry conditions affect the physicochemical properties of NPs. Li et al. (Li et al., 2011b) investigated the effects of five commonly used aqueous media with various chemical properties on the toxicity of nano-ZnO against *E. coli*, including ultrapure water, 0.85% NaCl, phosphate-buffered saline (PBS), minimal Davis, and Luria-Bertani (LB). It was found that the antibacterial property of nano-ZnO tested in five media was mainly due to the free zinc ions and labile zinc complexes. The toxicity of nano-ZnO in the five media deceased by following order of ultrapure water > NaCl > MD > LB > PBS.

Sondi and Salopek-Sondi (Sondi and Salopek-Sondi, 2004) studied the growing of *E. coli* on agar plates and liquid LB medium using AgNPs. In their study, distinct differences were obtained through those two medium. When AgNPs were presented on LB agar plates, they could completely inhibit bacterial growth. However, the inhibition depends on concentration of AgNPs as well as the CFU of bacteria used in the experiments. In contrast, AgNPs in liquid LB medium only delayed the growth of *E. coli*, even at high concentrations. As the concentration of AgNPs decreasing, resuming of bacterial cells growth was observed.

2.4.2.4. *Effects of solution chemistry*

Water chemistry can be a major factor affecting the toxicity of NPs in aquatic medium. For example, the presence of divalent cations, such as Ca^{2+} and Mg^{2+} ,

significantly enhanced the aggregation of AgNPs thus reduced their toxicity (Jin et al., 2010; Huynh and Chen, 2011). Influences of pH and NOM were also investigated, and the results show that extensive aggregation of NPs began at pH 5 to 6 and reached a maximum at approximately pH 8.5, whereas with additional SRHA, aggregation was shifted to lower pH values of 4 to 5 and was affected by SRHA concentration (Baalousha et al., 2008). Influence of dissolved oxygen or the redox potential on the stability of NPs in aqueous environments were also studied, which suggested the aggregation rates became much faster with the present of dissolved oxygen rather than without it (Zhang et al., 2011b).

2.4.2.5. Effects of light

The presence of light was an important factor under most testing conditions, presumably due to its role in promoting the generation of ROS (Adams et al., 2006). It has been proved that illumination may enhance the antibacterial activity of certain NPs, especially TiO₂ NPs. In contrast, it did not affect the ROS generation of ZnO or SiO₂ NPs (Adams et al., 2006). This difference also suggested that the antibacterial mechanism(s) of NPs might differ from one another depending on their types.

2.4.2.6. Effects of single cell and biofilm

Most existing studies focus on the effect of NPs on individual bacteria cells (Adams et al., 2006; Baalousha et al., 2008; Choi et al., 2008; Choi and Hu, 2008). On the other hand, studies of the nanotoxicity effect on biofilms are quite limited (Fabrega et al., 2009b; Fabrega et al., 2011; Peulen and Wilkinson, 2011; Applerot et al., 2012). Most microorganisms exist in nature in the form of biofilm where cells are bound to each other and/or to a surface. These adherent cells are frequently embedded within a self-produced matrix of EPS. Compared to individual cells in suspension, cells in biofilm tend to be more resistant to external stress (Choi et al., 2010). Biofilm may obtain some properties that different from individual cells. The past decades wit a dramatic development of applications of biofilm in water purification and wastewater treatment.

2.4.3. Challenges of nanotechnology in water and wastewater treatment

Potential applications of antimicrobial NPs have been increasingly used in hybrid processes combined with the existing disinfection technologies, such as the photosensitive/photocatalytic TiO₂ NPs applied in UV reactors which enhance disinfection efficiency of some pathogenic microorganisms that are highly resistant to UV disinfection (Li et al., 2008b). However, several challenges exist for the efficient utilization of antimicrobial NPs in drinking water treatment, primarily concern including the dispersion and retention of NPs, and the sustainability of antimicrobial activity (Li et al., 2008b). One barrier of efficient utility of NPs is aggregation. Previous studies indicate that NPs will aggregate severely in aquatic environment, which depends on the size, structure, chemical composition and surface coatings of NPs, and the water chemistry conditions (Chen and Elimelech, 2009; Hotze et al., 2010; Chowdhury et al., 2012). Aside from aggregation, well-dispersed NPs of small sizes applied in the form of slurry are very hard to retain and recycle, which may not only lead to the increase of cost associated with the loss of NPs, but also the potential impacts on human health and ecosystems caused by the NPs escaping from the treatment system and entering the product water.

The incorporation of antimicrobial NPs and membrane technology could serve multiple purposes with one step, the fabrication of nanocomposite membranes. Nanocomposite membranes are likely to overcome the limitations of both nanotechnology and membrane technology that introduced above.

2.5. Nanocomposite membranes — a combination of NPs and membrane technology

2.5.1. Advantages of nanocomposite membranes

Based on the awareness of advantages of NPs, currently there are three major approaches to combine NPs with membrane technology: 1) the hybrid system of suspended NPs and membrane separation process (i.e. hybrid photocatalysis-membrane reactor) (Molinari et al., 2002; Le-Clech et al., 2006b), 2) the neat nanomaterial membranes (inorganic membranes fabricated by using NPs) (Alberti et al., 2006), and 3) fabrication of nanocomposite membranes by incorporating the NPs in polymeric membrane structure (including membrane surface modification using NPs) (Hinds et al., 2004; Ouyang et al., 2010; Diagne et al., 2012).

Among above three approaches, fabrication of nanocomposite membranes by incorporating NPs in polymeric membrane structure has received most attention and interest mainly because: 1) The footprint of whole treatment process can be shortened compared with the hybrid photocatalysis-membrane system. 2) The nanocomposite membranes can be loaded in the current available membrane modules and systems. No special module and system is required for using the nanocomposite membranes. 3) Different from the neat nanomaterial membranes (inorganic membranes), nanocomposite membranes are more flexible and can be scaled up very easily.

Compared with the conventional NPs-free membranes, nanocomposite membranes have several significant advantages. 1) The functionalized nanocomposite membranes can be used in versatile applications, such as the photocatalytic process (Goei and Lim, 2014). 2) Nanocomposite membranes may exhibit excellent antifouling properties, especially in membrane biofouling control (Diagne et al., 2012; Liu et al., 2013b). 3) Nanocomposite membranes may enhance the membrane water permeability due to the porous nature and hydrophilicity of NPs. For example, the incorporation of NaY zeolite NPs in polyamide rejection layer significantly changed the separation properties of a thin film composite (TFC) membrane (Ma et al., 2012). 4) Nanocomposite membranes can solve the retention and recycle

problems of using the slurry-form NPs. The leaching and escaping of NPs into downstream can also be controlled.

2.5.2. Fabrication of nanocomposite membranes

A variety of nanocomposite membranes have been successfully fabricated by using different methods for versatile environmental applications including MF, UF, NF, RO and FO.

2.5.2.1. Fabrication of nanocomposite MF membranes

Taurozzi et al. (Taurozzi et al., 2008) synthesized the flat sheet porous polysulfone–silver nanocomposite membranes by using wet phase inversion process. The effects of casting mixture composition and NPs incorporation route on membrane morphological and separation properties were investigated. Similar studies were also conducted by Basri et al. (Basri et al., 2010) and Alpatova et al. (Alpatova et al., 2013). The results indicate that the nanocomposite membranes exhibited enhanced permeability and anti-fouling properties with AgNPs incorporation. Diagne, Malaisamy et al. (Diagne et al., 2012) developed the nanocomposite membranes by modifying the commercially available polyethersulfone (PES) MF membranes using polyelectrolytes and AgNPs. Filtration and cleaning studies confirmed that the modification may significantly reduce organic and biological fouling of the membranes.

2.5.2.2. Fabrication of nanocomposite UF membranes

Zhang et al. (Zhang et al., 2012c) investigated the anti-biofouling characteristics of PES UF membranes with biogenic silver nanoparticles (bio-Ag⁰). Different amounts of bio-Ag⁰ were embedded in PES membranes using phase inversion method. Bio-Ag⁰ incorporation slightly increased the hydrophilicity of PES membrane, thus increased the membrane permeate flux. In addition, the nanocomposite membranes exhibited excellent antibacterial activity by preventing the bacterial attachment to membrane surface and the biofilm formation. The antifouling nanocomposite UF PES membranes were synthesized with mesoporous silica particles and achieved by phase inversion method (Huang et al., 2012b). Functionalized multi-walled carbon

nanotubes (MWNTs) can also be blended with polyacrylonitrile (PAN) to prepare nanocomposite UF membranes by phase inversion process (Majeed et al., 2012; You et al., 2013).

2.5.2.3. Fabrication of nanocomposite NF membranes

To obtain the nanocomposite NF membranes, Wu et al. (Wu et al., 2013) developed the MWNTs/polyester thin film nanocomposite (TFN) membranes through an improved interfacial polymerization on polysulfone (PSf) supporting membrane in the presence of MWNTs. The effects of MWNTs concentration and reaction time of interfacial polymerization on membrane properties were investigated. The membrane water permeability also increased due to the MWNTs incorporation. Another kind of TFN NF membrane was fabricated by embedded the TiO₂ NPs in membrane polyamide selective layer using similar interfacial polymerization process (Rajaeian et al., 2013). PES was coated on a porous α -Al₂O₃ ceramic hollow fibre membrane to form the membrane substrate layer in this study. Results indicated that the incorporation of TiO₂ NPs improved the selectivity of TFN NF membrane.

2.5.2.4. Fabrication of nanocomposite RO membranes

Several studies have investigated the synthesis of nanocomposite RO membranes by incorporating the zeolite NPs (Fathizadeh et al., 2011; Kim et al., 2013c; Pendergast et al., 2013), single-walled nanotubes (SWNTs) (Baroña et al., 2013) and MWNTs (Zhao et al., 2014) in membrane selective layer or support layer. Membrane water permeability of the nanocomposite RO membranes has been significantly enhanced, and these membranes are promising for potential applications such as desalination and advanced water purification.

2.5.2.5. Fabrication of nanocomposite FO membranes

Ma et al. (Ma et al., 2012) prepared the zeolite-polyamide TFN FO membranes by embedding the zeolite NPs in membrane selective layer. The zeolite-polyamide TFN FO membranes were characterized and evaluated in comparison with a NPs-free TFC membrane. The results indicated that the zeolite incorporation exhibited a

concentration-dependent effect on membrane water permeability as well as FO performance. In the range of 0.02–0.1 wt./v% zeolite loading, the incorporation of zeolite-polyamide exhibited enhanced water permeability of membrane, however, further increase in zeolite loading led to a reduction in water permeability (Ma et al., 2012). This study is also the first report on zeolite-polyamide based TFN membranes for FO application. The usage of zeolite-PSf nanocomposite substrates for controlling the ICP and improving FO water flux was investigated by the same group of people (Ma et al., 2013). The incorporation of zeolite NPs in PSf substrate can improve membrane water permeability and prevent the ICP by reducing the substrate structural parameter (Ma et al., 2013). Afterwards, a few studies have been seen to investigate the fabrication and performance (including the reduction of ICP and membrane organic fouling control) of the TiO₂ nanocomposite FO membranes by using similar methods (Emadzadeh et al., 2013; Emadzadeh et al., 2014a; Emadzadeh et al., 2014b; Emadzadeh et al., 2014c).

2.5.3. Potential approaches for antimicrobial nanocomposite NF and FO membrane fabrication

According to the literature investigation presented in Section 2.4.2, most current nanocomposite NF membranes were obtained by embedding different NPs in membrane selective layer via interfacial polymerization (Rajaeian et al., 2013; Wu et al., 2013). And there are only a few studies investigating the synthesis of nanocomposite FO membranes. Moreover, those studies mainly focused on the effects of NPs incorporation on membrane water permeability and ICP (Ma et al., 2013; Emadzadeh et al., 2014b). The fabrication and characterization of nanocomposite NF and FO membranes with antimicrobial properties have not been studied yet. Since the fabrication of high performance FO membrane is a relatively new topic and there is a significant gap between the demand and supply of high performance FO membrane, therefore, it is worthwhile to review the possible approaches for FO membrane fabrication.

A few studies have been carried out to fabricate membranes with improved FO performance. In this section, promising technologies of FO membrane fabrication

are reviewed to gain the insight for synthesizing antimicrobial nanocomposite NF and FO membranes.

2.5.3.1. Asymmetric FO membrane via phase inversion

Phase inversion is a membrane formation process whereby a polymer solution inverts from a dissolved state into a solid state under controlling manner (Mulder, 1996). It is a very important method for asymmetric membrane preparation (Lonsdale, 1987). Polymeric membranes for different environmental applications have been fabricated via this process by using a variety of materials since its invention.

The commercial FO membrane HTI (Hydration Technologies Inc., OR, USA), which is made of celluloses triacetate (CTA) supported by polyester woven or nonwoven porous fabric to enhance the membrane mechanical strength, is produced based on this method. The resultant membrane has significantly reduced ICP compared with the conventional RO and NF membranes (Cath et al., 2006; Tang et al., 2010). However, membranes prepared via phase inversion are limited in terms of material. There are still some limitations of FO membranes made of cellulose acetate and its derivatives, including: 1) relatively low water permeability (Wang et al., 2010a; Zhang et al., 2010), 2) low biological and chemical resistance to hydrolysis which required a narrow pH range for membrane usage (Romsaiyud et al., 2009) and 3) rigid-range materials to form membrane.

Microporous substrate could be prepared via phase inversion, which is shown as follows. The polymer solution is prepared by stirring the pelletized resin in the solvent. Heat may be provided to speed up the dissolution of resin. The solution should be de-gassed, either by standing for several hours or by vacuum evacuation in desiccator or bell jar. A portion of this solution is poured onto a clean glass plate at room temperature, and is spread by means of a blade having a gap. Immediately upon coating, the liquid film on the glass plate should be quickly but smoothly immersed into a pan of water. The liquid film is converted to a thin film layer by precipitation of the polymer. This film automatically detaches from the surface of the glass plate in one to two minutes of immersion. The film should be soaked for

several minutes, followed by rinsing with solvent-free water, before inspection and use. The films should be visually inspected for pinholes or defects (Petersen, 1993). Nanocomposite microfiltration membranes could be fabricated by simply adding the NPs in dope solution. For example, Zhang et al. succeeded in embedding different amounts of biogenic AgNPs (bio-Ag⁰) in PES membranes using phase inversion method (Zhang et al., 2012c).

2.5.3.2. Interfacial Polymerization

A number of techniques could be used to prepare the selective layer. Among them, interfacial polymerization is dominant in composite membrane synthesis. Interfacial polymerization provides a method for forming a thin layer upon a porous support. In principle, two reactive monomers react at interface of two immiscible solvents (aqueous phase and organic phase) supported by MF or UF membrane. The support was first immersed into an aqueous solution containing one reactive monomer. Then the membrane is immersed into an organic solution containing the other reactive monomer. Finally, two monomers react at the interface to form a dense and thin selective layer (typical thickness is ~ 200 nm) (Mulder, 1996).

The composition and morphology of the interfacially formed polymer film will be dependent on several variables, including: 1) concentration of reactants; 2) partition coefficients of the reactants (Wei et al., 2011a); 3) reactivity ratios where blends of reactants are employed; 4) solubility of nascent polymer in the solvent phase as it is formed; 5) the overall kinetics and diffusion rates of the reactants; 6) presence of by-products (such as hydrogen chloride in the case of reaction of amine with an acyl chloride); 7) hydrolysis or other potentially competitive by-reactions; 8) crosslinking reactions; and 9) post-reactions or treatments of the resulting interfacial films. The initial five factors should be considered for membrane formation and subsequent properties. The latter four factors may affect the performance of fabricated membranes.

The TFC FO flat sheet or hollow fibers membranes with an ultra-thin polyamide-based skin layer present good rejection for monovalent salt (i.e. NaCl) and relatively high water flux (Chou et al., 2010; Wang et al., 2010b; Yip et al., 2010;

Tiraferri et al., 2011b; Wei et al., 2011c). Ma et al. (Ma et al., 2012) synthesized and characterized the zeolite-polyamide TFN membranes using interfacial polymerization which significantly enhanced the membrane separation properties.

2.5.3.3. Layer-by-layer (LbL) assembly

Layer-by-layer (LbL) assembly is also called electrostatic self-assembly (ESA) or polyelectrolyte multilayers (PEMs). With roots in the Langumir-Blodgett technique (Blodgett 1934) and self-assembled monolayers (Bigelow, Pickett et al. 1946), the modern period of research multilayer films on solid substrate began at 1990s (Decher 1997). A large amount of review articles on this topic show great interest in using the simple LbL assembly method for fabricating ultrathin films (Sano et al., 1996; Ulman, 1996; Bertrand et al., 2000; Schlenoff et al., 2000; Kharlampieva and Sukhishvili, 2006; Ariga et al., 2008; Schlenoff, 2009). Compared to other membrane fabrication methods such as interfacial polymerization, LbL assembly is a highly versatile membrane synthesis method, which may have several advantages including high water flux, low operation pressure, less energy consumption, low operation costs, high thermal stability, good solvent resistance, and etc. (Bertrand et al., 2000; Hilal et al., 2004; Qiu et al., 2011; Liu et al., 2012).

Ultra-thin rejection layers could be formed by using LbL assembly of oppositely charged polyelectrolytes (Saren et al., 2011). The fabrication process is simply soaking the charged substrate into the two opposite charged polyelectrolyte solutions in turn. The process is self-limiting and the surplus polyelectrolyte is removed by washing (Bertrand et al., 2000). Such LbL FO membranes showed a remarkable FO performance (i.e. 15 L/m²·h in AL-FS using a draw solution of 0.1 M MgCl₂) (Saren et al., 2011).

The traditional procedure of LbL assembly consists four basic steps shown in Figure 2-8. A negative charged substrate is first submerged in a polycation solution ((1) in Figure 2-8) and then a polyanion solution ((3) in Figure 2-8) consecutively. After step (1) and (3), surplus polyelectrolyte solution is removed by washing ((2) and (4) in Figure 2-8). The predetermined cycle is repeated to gain a film with desired thickness and performance.

Recently Diagne et al. (Diagne et al., 2012) successfully fabricated AgNPs modified MF membranes by using LbL assembly which mitigated the organic and bacterial fouling on membranes.

Basic principle of Layer-by-Layer (LbL) assembly

Figure 2-8. Schematic of the film deposition process using LbL assembly method (figure reproduced from Ref. (Saren et al., 2011) with copyright permission).

2.6. Summary

A comprehensive literature review is presented in this chapter to look into: 1) The principle and potential environmental applications of membrane separation processes, especially the NF and FO processes; 2) Membrane fouling, especially the irreversible membrane biofouling, and the influencing factors and controls in membrane separation processes; 3) The antimicrobial properties of NPs and their influencing factors, and 4) the fabrication of antimicrobial nanocomposite NF and FO membranes as a potential membrane fouling control approach.

Currently, little is known about the bactericidal activity of NPs against bacteria and its influencing factors in environmentally relevant freshwater matrices. In order to take the advantages of NPs bactericidal activity and fabricate the antifouling (especially anti-biofouling) nanocomposite FO membranes, it is essential to study and understand the toxicity of NPs first.

Membrane fouling remains a serious issue in membrane applications. Biofouling is a major type of fouling which is irreversible and may affect the membrane performance greatly, such as reducing water flux, increasing backwash frequency, shortening life span of membrane module and increasing operational cost. The incorporation of NPs and membrane may not only be able to prevent membrane biofouling by using the toxicity of NPs, but also enhance membrane water permeability. Unfortunately, research in this field is still lacking. The possible fabrication approaches of nanocomposite FO membranes were investigated in literature review.

Most existing nanocomposite FO membranes are focused on asymmetric structure membranes, which are formed by a phase inversion porous support layer incorporated by interfacial polymerized selective layer (Chou et al., 2010; Wang et al., 2010b; Yip et al., 2010; Tiraferri et al., 2011b; Wei et al., 2011c; Ma et al., 2012; Ma et al., 2013). The LbL assembly method has been investigated to fabricate FO membranes (Saren et al., 2011; Setiawan et al., 2011; Qi et al., 2012c), however, the studies to use this method for fabricating the nanocomposite FO membranes are still lacking.

CHAPTER 3

Materials and Methodology

3.1. Chemicals and materials

3.1.1. Chemicals and materials for membrane fabrication

Unless otherwise specified, all reagents and solutions were prepared using chemicals of ACS reagent grade or analytical grade and Milli-Q water (Millipore Integral 10 water purification system, Singapore). Polyacrylonitrile (PAN, weight-averaged molecular weight $M_w \approx 150\,000$, Sigma–Aldrich) and the pore former lithium chloride (LiCl, 99%, Sinopharm or Merck) were dissolved in the solvent N,N-dimethylformamide (DMF, $\geq 99.8\%$, Sigma–Aldrich or Merck) for membrane substrate fabrication. Alkali solution prepared by sodium hydroxide (NaOH, $\geq 98\%$, Sigma–Aldrich) was used for post-treatment of PAN or PAN-Ag substrates to introduce negative charges and enhance surface hydrophilicity. Poly(allylamine hydrochloride) (PAH, $M_w \approx 120\,000$ – $200\,000$, Sigma–Aldrich) and poly(sodium 4-styrene-sulfonate) (PSS, $M_w \approx 70\,000$, 30 wt.% in H_2O , Sigma–Aldrich) were used for layer-by-layer (LbL) assembly. Ionic strength of polyelectrolyte solutions was adjusted using sodium chloride (NaCl, 99.5%, Merck). Commercial AgNPs (~ 100 nm powder, contains poly(vinyl pyrrolidone) as dispersant, 99.5% trace metals basis, Sigma–Aldrich) were incorporated in membrane selective layers based on LbL assembly and substrates by adding in dope solutions to achieve desirable silver concentration. Glutaraldehyde (GA, 25% in water, Sigma–Aldrich) was used for crosslinking of membrane selective layers.

3.1.2. Chemicals and materials for membrane characterization

Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, 99.0–101.0%, Merck) was used to determine membrane intrinsic separation properties and forward osmosis (FO) performance. Milli-Q water and 10 mM NaCl solution were used as feed solutions in FO test. Dextran samples with different molecular weight ($M_w \approx 6\,000$ – $500\,000$,

Fluka and Sigma–Aldrich) were used to prepare the dextran solution for determining molecular weight cut off (MWCO). Live/Dead® BacLight™ bacterial viability kit (Molecular Probes, Eugene, OR) was used to determine bacterial viability by high-throughput screening analysis.

3.1.3. Chemicals and materials for bacterial cultivation

Gram-positive bacteria *Bacillus subtilis* (ATCC 6633) and Gram-negative bacteria *Escherichia coli* (ATCC 8739) were obtained from American Type Culture Collection (ATCC) and used as model organisms to evaluate the toxicity of AgNPs against bacteria with the presence of natural organic matter (NOM) and chelating agent, ethylenediaminetetraacetic acid (EDTA). Both bacteria were also used to evaluate the antibacterial activities of silver nanocomposite nanofiltration (NF) and FO membranes fabricated via LbL assembly. Nutrient broth (BD DIFCO 234000) and agar powder (BD BACTO 214010) were adopted to prepare the solution for bacterial cultivation and agar plates for bactericidal tests. 0.9 % NaCl solution and environmental relevant freshwater (shown in Table 4-1 in Section 4.2.1) were used to wash and resuspend the bacterial cells for further tests.

3.1.4. Chemicals and materials for bactericidal activity test of AgNPs

The humic substances representative, humic acid (HA), was Suwannee River Humic Acid Standard II obtained from International Humic Substances Society (IHSS), St. Paul, MN. The protein representative, bovine serum albumin (BSA, lyophilized powder, ≥98%), and polysaccharides representative, alginic acid (AA, alginic acid sodium salt from brown algae), were obtained from Sigma–Aldrich, St. Louis, MO. Ethylenediamine-tetraacetic acid (EDTA, 99.4–100.6%, Sigma–Aldrich; St. Louis, MO) was used to observe the effects of chelating agent on bactericidal activity of AgNPs. Commercial silver nanoparticle suspensions (10 nm, 40 nm and 100 nm AgNPs, 20 mg/L in 0.059% sodium citrate aqueous buffer) used in the tests were purchased from Sigma–Aldrich, St. Louis, MO.

3.2. Fabrication of silver nanocomposite membranes

3.2.1. Fabrication of silver nanocomposite NF and FO membranes with AgNPs in membrane selective layer based on LbL assembly

Procedures of PAN substrate preparation, post treatment, LbL assembly and crosslinking were developed based on previous studies (Zhang et al., 2007; Qiu et al., 2011; Saren et al., 2011; Qi et al., 2012b) and will be introduced in details in Section 3.2.2. Membrane selective layer was formed based on LbL assembly by soaking the substrate with 1 g/L positively charged PAH solution and then with 1 g/L negatively charged PSS solution for 30 min (Decher et al., 1992; Bertrand et al., 2000; Zhang et al., 2007; Saren et al., 2011). After each soaking step, the substrate surface was rinsed with Milli-Q for 5 min to remove excess polyelectrolytes (Bertrand et al., 2000). For the fabrication of silver nanocomposite membranes, controlled amount of AgNPs (0.01 wt.%) was added in PSS solution, Milli-Q water, or PAH solution respectively after ultrasonicated (Ultrasonic Bath S450H, 50/60Hz, 2000W, Fisher Scientific Pte. Ltd.; Singapore) for 30 min at room temperature (23 ± 1 °C). The AgNPs suspensions were continuously stirred during LbL process. 2.5 PAH/PSS bilayers (i.e., twice PAH/PSS coating procedures with an additional PAH coating on the outermost layer) was adopted based on previous optimization results (Qi et al., 2012b). The membranes were crosslinked by immersing them in 0.1 wt.% GA solution at room temperature (23 ± 1 °C) for 2 h followed by Milli-Q water washing (Qiu et al., 2011) to prepare crosslinked LbL (xLbL) membranes. The detailed designation and compositions of nanocomposite membranes can refer to Table 5-1.

3.2.2. Fabrication of silver nanocomposite NF and FO membranes with AgNPs in membrane substrate followed by LbL assembly

In order to incorporate AgNPs in membrane substrate, the AgNPs (0~0.10 wt.%) were suspended in DMF and ultrasonicated (Ultrasonic Bath S450H, 50/60Hz, 2000W, Fisher Scientific Pte. Ltd.; Singapore) for 30 min at room temperature (22 ± 1 °C) before using. Dope solutions were prepared by dissolving PAN and LiCl (18 wt.% and 2 wt.% respectively) in DMF (without or with certain amount of

AgNPs) in sealed containers at 60 °C with continuous stirring until the solutions became homogeneous and transparent. The cooled (22 ± 1 °C) and degassed dope solutions were cast on a clean glass plate with a 150 μm gate height of the casting knife (Elcometer Pte. Ltd., Asia). All membrane substrates, which can be independently used as the nanocomposite ultrafiltration (UF) membranes, were obtained by solidifying the casting films in tap water bath at room temperature. The nascent substrates were kept in flowing water bath to remove residual solvent and then stored in Milli-Q water separately before tests. Detailed compositions of dope solutions and designations of the UF membranes were shown in Table 6-1. 3.0 PAH/PSS bilayers was used to fabricate the selective layers on membrane substrates using LbL assembly. As a result, the silver nanocomposite NF and FO membranes with AgNPs in membrane substrates were obtained.

3.3. Characterization of silver nanocomposite membranes

3.3.1. Membrane intrinsic separation properties

Unless otherwise specified, all results of membrane intrinsic separation properties were obtained from 3 replicate measurements of 3 independent membrane samples. Membrane intrinsic separation properties were evaluated by using a high pressure crossflow filtration setup (Tang et al., 2010; Saren et al., 2011) at an applied pressure of 5 bar. Pure water permeabilities (A) of the silver nanocomposite UF membranes (substrates without LbL selective layers) and NF membranes were determined based on pure water flux (J_v) obtained by weighting permeate water samples. 1 h compaction was applied under the target pressure using Milli-Q water before the measurements were taken. The test was conducted at a crossflow velocity of ~ 20 cm/s.

Molecular weight cut off (MWCO) of nanocomposite UF membranes were determined using a 2000 ppm dextran feed solution at an applied pressure of 1 bar under crossflow conditions. The dextran solution was made by a mixture of several different molecular weights ranging from 6000~500,000 Da following the

procedures described in previous literatures (Wei et al., 2006; Setiawan et al., 2011). Dextran concentrations were analyzed by gel permeation chromatography (GPC) on a PL-GPC 50 Plus system (Polymer Laboratories, Varian Inc.; USA). Double PL Aquagel OH Mixed-M 8 μm columns (Polymer Laboratories, Varian Inc.; USA) with packing material of 7.5 mm diameter were used in the system.

Salt rejections of silver nanocomposite NF membranes (R) were determined based on conductivity measurements of 5 mM MgCl_2 feed solution and permeate water using conductivity meter (Ultrameter II, Myron L Company; CA, USA).

3.3.2. FO performance evaluation

FO performance, including water flux (J_v), solute flux (J_s), and solute flux over water flux (J_s/J_v), was evaluated using a cross-flow FO setup reported previously (Saren et al., 2011; Qi et al., 2012b). Both feed solution (FS) and draw solution (DS) were circulated at a cross flow rate of 500 mL/min (cross flow velocity ~ 10 cm/s) at room temperature (23 ± 1 °C). FO water flux was determined by measuring the weight change of the feed tank using a digital mass balance connected to a computer data logging system. Salt flux through the FO membrane was determined by monitoring the conductivity change in the FS. 0.5 M MgCl_2 solution was used as DS. Milli-Q water (for J_s measurement) or 10 mM NaCl (for J_v measurement) was used as FS. Both AL-DS and AL-FS membrane orientations were tested. All FO performance results were obtained from 3 replicate measurements of 3 independent membrane samples.

3.3.3. Membrane surface properties

3.3.3.1. Scanning electron microscope (SEM) with energy-dispersive X-ray (EDX)

Surface morphologies of nanocomposite NF and FO membranes were obtained by using a Field Emission Scanning Electron Microscope (FE-SEM, JSM-7600F, JEOL Ltd.; Japan) combined with energy-dispersive X-ray (EDX) spectroscopy (X-MaxN, Oxford Instruments; England). All membrane samples were dried and coated with platinum using a vacuum electric sputter coater (JEOL JFC-1600, JEOL Ltd.; Japan) before SEM/EDX examination.

3.3.3.2. Atomic force microscopy (AFM)

Atomic force microscopy (AFM, Park Systems XE-100; USA) was also used to measure the surface morphologies and roughness of membrane samples (Tang et al., 2010; Saren et al., 2011; Ma et al., 2012). The scan area was $25\ \mu\text{m}^2$, and the scan rate was varied from 0.5 to 1.0 Hz. The reported AFM results were obtained from at least 3 measurements of 3 independent membrane samples.

3.3.3.3. Contact angle measurements

Contact angle measurements were performed with the sessile drop method (Tang et al., 2010) using OCA Contact Angle System (DataPhysics Instruments GmbH; Germany). The membrane samples were completely dried before the measurements. During the test, a droplet of 5 μL of Milli-Q water was transferred to membrane surface. After stabilizing, image of droplet on membrane surface was captured by a CCD camera. Contact angle calculation was obtained by using a software. The reported contact angle is the average of at least 10 measurements obtained from 3 independent membrane samples.

3.3.3.4. Membrane surface zeta-potential

Membrane surface zeta-potential was measured by eletrokinetic analyzer (SurPASS, Anton Paar GmbH; Austria) using a 10 mM NaCl background solution with an applied pressure of 300 mbar and a membrane gap of 120 μm . Zeta potential values were given by the best fittings with Helmholtz–Smoluchowski equation (Qi et al., 2012a). The reported zeta-potentials were the average of 4 measurements.

3.3.4. Membrane porosity measurements

Membrane porosity (ϵ) was determined by measuring the dry mass (m_{dry}) and wet mass (m_{wet}) of membrane samples on a digital analytical balance (AB204-S/FACT, Mettler-Toledo Pte Ltd; Singapore) and calculated using following equation (Sukitpaneemit and Chung, 2009; Wei et al., 2011c):

$$\varepsilon = \frac{(m_{wet} - m_{dry}) / \rho_w}{(m_{wet} - m_{dry}) / \rho_w + m_{dry} / \rho_m} \times 100\% \quad (3-1)$$

where ρ_w and ρ_m are the densities of water (1.00 g/cm³) and PAN (1.18 g/cm³) respectively. It is assumed that all pores in membrane samples were fully filled with water after immersing in water for 24 h. The reported membrane porosities are average of 3 measurements obtained from 3 independent membrane samples

3.3.5. Dope solution viscosity

The viscosities of dope solutions were measured using Modular Compact Rheometer (Physica MCR 101, Anton Paar; USA). 100 μ L sample of dope solution was placed in the central of sample measuring plate at 22 °C. The reported dope solution viscosities are the values at shear rate of 10 s⁻¹ and the average of at least 3 measurements obtained from 3 independent samples.

3.3.6. Membrane antibacterial tests

Before antibacterial tests, all freshly fabricated membranes were immersed in Milli-Q water for 48 h to remove the potential residual chemicals. Gram-positive *B. subtilis* and Gram-negative *E. coli* were used as model organisms to perform membrane antibacterial tests with the cell density of 1.0×10^7 cells/mL (Tchobanoglous and Schroeder, 1985; Van Hoecke et al., 2011). Three membrane antibacterial tests, colony-forming units (CFU) test, bacterial cell membrane integrity test, and diffusion inhibition zone (DIZ) test, were used to evaluate the membrane antibacterial properties.

3.3.6.1. Colony-forming units (CFU) test

CFU test was performed by serial diluting the bacterial suspensions which contacted with a piece of membrane sample with an area of 7.60 cm² for 24 h according to CLSI M07-A9 (CLSI, 2012a). 100 μ L diluent was inoculated and spread on a nutrient agar plate and incubated for another 24 h. CFU results were obtained from 9 replicates by mixing 3 batches of independently cultivated bacteria with 3

independently fabricated membranes respectively (3 agar plates were used as parallel samples in each measurement).

3.3.6.2. Bacterial cell membrane integrity test

After 24 h exposure to membranes, 100 μ L of bacterial suspensions were completely mixed with the solutions of Live/Dead[®] BacLight[™] bacterial viability kit into specific wells of a 96-well F-bottom polystyrene microplate (Greiner Bio-One; Monroe, NC, USA). Percentages of cell membrane integrity bacteria were obtained using the green/red fluorescence ratios detected by Synergy 2 Multi-Mode Microplate Reader (BioTek Singapore, Singapore) and calculated by the calibration curves obtained by blending different known ratios of live-to-dead bacteria (detailed procedures are shown in Section 3.5) (Jin et al., 2010; Liu et al., 2013b; Liu et al., 2014). 3 independent batches with at least 18 replicates were used to determine the calibration curves. The reported percentages are based on 27 measurements in 9 individual plates using 3 independent batches of bacteria and 3 independently fabricated membranes.

3.3.6.3. Diffusion inhibition zone (DIZ) test

Diffusion inhibition zone test of silver nanocomposite NF/FO membranes was performed by following a modified CLSI document M02-A11 (CLSI, 2012b). 100 μ L bacterial inoculums were spread on the agar plates evenly. Different nanocomposite membranes were punched into circle discs with a diameter of 12.7 mm using circle disc punch (Ted Pella, Inc.; Redding, CA, USA), and placed on agar plates with the active layers facing up and incubated at optimal bacterial growth conditions for 24 h. The DIZ formed around the nanocomposite membranes were observed and measured.

3.3.7. Membrane silver leaching test

To quantify silver release from nanocomposite membranes, the membranes were cut into circles using circle disc punch. The membrane samples were subsequently immersed into Milli-Q water with sustained shaking at room temperature. After determined time frames, the silver released in the water was digested using 7%

HNO₃ according to a modified U.S. EPA method 3050B (USEPA, 1996a) and analyzed by Inductively Coupled Plasma Mass Spectrometer (ICP-MS, ELAN DRC-e, Perkin Elmer; MA, USA). Error bars of reported results were obtained by 3 replicate measurements.

Total silver incorporated in corresponding nanocomposite membranes was determined by completely digesting the membrane sample in concentrated HNO₃ solution using Microwave Reaction System (Multiwave 3000, Anton Paar GmbH; Austria) (USEPA, 1996b). Silver concentrations in the collected samples were quantified using the ICP-MS described above.

3.4. Bacterial cultivation

Planktonic bacterial cultures of *B. subtilis* and *E. coli* were prepared in nutrient broth (BD DIFCO 234000) with shaking (150 rpm) at their respective optimum growth temperature, *i.e.*, 30 °C for *B. subtilis* and 37 °C for *E. coli*. Bacterial cells were harvested at mid-exponential growth phase (24 h for *B. subtilis* and 12 h for *E. coli*, respectively) and then were washed twice with 0.9% NaCl and specific freshwater matrices sequentially, and then resuspended in specific freshwater matrices to achieve certain cell densities for further tests.

3.5. AgNPs bactericidal activity test

100 µL of AgNPs suspensions of different concentrations (from 0.01 µg/L to 10 mg/L) and 100 µL of bacterial suspensions were added and completely mixed into specific wells of a 96-well F-bottom polystyrene microplate (Greiner Bio-One; Monroe, NC). Each combination was tested in 2–4 independent batches with at least 9 replicates, therefore, 18–36 replicates were used to determine one testing condition. All plates with bacteria were incubated for 24 h under respective optimum growth temperature. After 24 h exposure, stain solutions with a mixture of SYTO9, green-fluorescent nucleic acid stain that labels all bacteria in a population,

and propidium iodide, a red-fluorescent nucleic acid stain that only affects the bacteria with membrane damage, were added into each well.

The green/red fluorescence ratio detected by Synergy 2 Multi-Mode Microplate Reader (BioTek Singapore, SG) provided the percentage of live bacterial cells in each well (excitation at 485 nm and emission at 635 and 500 nm) according to calibration curves obtained by blending different known ratios of live-to-dead bacteria (3–4 independent batches with at least 18 replicates). Silver-free controls contained only bacteria and stain mixture. Percentages of live bacteria cells calculated by calibration curves were normalized by the silver-free controls and analyzed by fitting the biostatistics software Prism 5 “Dose-response Inhibition, log(inhibitor) vs. normalized response-variable slope” (GraphPad Software, San Diego, CA) with 95% confidence intervals. Half maximal inhibitory concentration (IC_{50}) value, usually referring to the effectiveness of chemicals in inhibiting biological functions or viability, was adopted to determine the nanotoxicity of AgNPs suspensions to both bacteria with different water chemistry conditions with 95% confidence intervals in the expression of mass of silver existed in per liter of water matrix. It corresponds to the AgNPs concentrations at 50% live bacteria cells in respective “Percentages of live bacteria vs. Log concentration of AgNPs suspension” curves obtained after 24 h incubation with 19 different concentrations (from 0.01 $\mu\text{g/L}$ to 10 mg/L) of AgNPs suspensions.

CHAPTER 4

Bactericidal Activity of Silver Nanoparticles in Environmentally Relevant Freshwater Matrices: Influences of Organic Matter and Chelating Agent

4.1. Introduction

Nanomaterials are defined as materials with at least one dimension on nanoscale, usually ranging from 1 to 100 nm in size (Fabrega et al., 2009a; Nowack, 2010; Nowack et al., 2011). Different from the traditional bulk materials of same composition, nanomaterials exhibit unique properties stemming from their nanoscale dimensions, which enables their widespread applications in almost all industrial sectors including materials, healthcare, transport, energy, water remediation, and information and communications technologies (Rickerby and Morrison, 2007; Das et al., 2012). According to some estimates, nanotechnology is likely to develop into a \$1 trillion industry by 2015 (Nel et al., 2009).

While the frontier of nanotechnology is expanding rapidly, new challenges in understanding, predicting and controlling potential adverse effects of nanomaterials also arise. For example, silver nanoparticles (AgNPs) are the most widely used nanomaterial among all commercial nano-products (Ma et al., 2011; Wang et al., 2011). They can be released into the environment through variety routes during their production, usage, and disposal, which inevitably leads to their accumulation, transformation and degradation in the atmosphere, water, soils, or organisms (Wiesner et al., 2006; Benn and Westerhoff, 2008). AgNPs could be dangerous to the environment, especially in aquatic environments, due to the relatively high toxicity of silver against some aquatic organisms and microbial communities (Borm and Berube, 2008; Liu and Hurt, 2010). Hence, understanding the potential risks of AgNPs imposed to the environment is as important as studying their novel physicochemical properties used in promising applications.

Previous studies suggested that toxicity of AgNPs in different matrices may differ greatly (Jin et al., 2010; Li et al., 2011b). While most currently documented ones were conducted in bio-relevant medium that is rich of various nutrients and with a high concentration of salts. Little is known about the transport, fate, and toxicity of AgNPs in natural environments, especially in natural freshwaters (Jin et al., 2010). Natural freshwaters are enriched with natural organic matter (NOM) with varied structures and compositions, which may adsorb on the surface of AgNPs and produce a core-shell structure (Ghosh et al., 2011). Stability and toxicity of AgNPs could be affected during this process. In addition, relatively subtle changes in water chemistry conditions can significantly alter interfacial morphology, hydrophobicity, and charge of AgNPs, which dictate their stability, reactivity, bioavailability and potential toxicity in aquatic environments (Li et al., 2011a; Zhang et al., 2012a). Therefore, studies on stability and bactericidal activity of AgNPs with the presence of different NOM in environmentally relevant freshwater matrices is important to elucidate the fate, reactivity and impacts of AgNPs on microorganisms in ecosystems.

Chelating agents may also enter natural water system through the effluents of a variety of industrial applications (Means et al., 1980). Their concentrations can be comparable to those of NOM (Nowack, 2002). They are a series of polydentate ligands that may change the water chemistry conditions (Choi et al., 2009a) and form a variety of complexes with different kinds of metals. Among them, EDTA has been one of the most widely used chelating agents with high extraction efficiency (Tandy et al., 2003). EDTA may significantly affect the size, shape, surface charge, bioavailability and toxicity of AgNPs during the formation of complexes (Xiu et al., 2011). However, to the best of our knowledge, no systematic research has been conducted on learning the effects of chelating agent on AgNPs toxicity in environmentally relevant freshwater matrices.

The objective of this chapter was to illustrate the influences of NOM and chelating agent on the stability and bactericidal activity of AgNPs in environmentally relevant freshwater matrices.

4.2. Materials and methods

4.2.1. Chemicals and solution chemistry

Humic acid (HA), bovine serum albumin (BSA) and alginic acid (AA) were used as model NOM to represent humic substances, protein and polysaccharides in natural streams. HA was Suwannee River Humic Acid Standard II obtained from International Humic Substances Society (IHSS), St. Paul, MN. Both BSA (lyophilized powder, $\geq 98\%$) and AA (alginic acid sodium salt from brown algae) were obtained from Sigma–Aldrich, St. Louis, MO. Ethylenediamine-tetraacetic acid (EDTA, 99.4–100.6%, Sigma–Aldrich; St. Louis, MO) was used to observe the effects of chelating agent on bactericidal activity of AgNPs. Commercial silver nanoparticle suspensions (10 nm, 40 nm and 100 nm AgNPs, 20 mg/L in 0.059% sodium citrate aqueous buffer) used in all experiments were purchased from Sigma–Aldrich, St. Louis, MO.

Environmentally relevant freshwater matrices were prepared by using Milli-Q water referring to the water quality parameters reported for the fresh river water in Wheeling, West Virginia (Tchobanoglous and Schroeder, 1985) and The White River in Muncie, Indiana (Van Hoecke et al., 2011). The total ionic strength in each solution was maintained at a constant level of 5.6 mM by replacing specific cation or anion with ionic strength equivalent, NaCl, unless specified. In order to simulate realistic situation, 5 mg/L (Tchobanoglous and Schroeder, 1985; Van Hoecke et al., 2011; Zhang et al., 2012a; Zhao et al., 2013) was chosen as the concentration of three model NOM (HA, BSA and AA) and chelating agent (EDTA) in test solutions, unless otherwise specified. The pH value of each solution was adjusted to 7.0 by using 0.1 M NaOH or HCl. All solutions were either autoclaved or disinfected using 0.2 μm syringe filters with Supor membrane (Pall Corporation, Ann Arbor, MI). All chemicals and reagents were either ACS reagent grade or analytical grade unless specified. Compositions, pH, and conductivity of each solution are shown in Table 4-1.

Table 4-1. Compositions and water quality of each environmentally relevant freshwater matrix

Solution	Concentration of model organic matter or chelating agent, mg/L				pH	Ionic strength, mM	Conductivity, $\mu\text{S}/\text{cm}$
	HA	BSA	AA	EDTA			
1					6.61 ± 0.14	5.6	678.2
2	5				6.42 ± 0.08	5.6	717.9
3		5			6.47 ± 0.04	5.6	720.6
4			5		6.46 ± 0.06	5.6	682.1
5				1	6.99 ± 0.04	5.6	661.0
6				5	6.63 ± 0.05	5.6	732.0
7				20	6.88 ± 0.01	5.6	766.1

4.2.2. Preparation and characterization of AgNPs suspensions

AgNPs (20 mg/L in 0.059% sodium citrate aqueous buffer) with different particle sizes (nominal diameters of 10 nm, 40 nm and 100 nm) were tested to investigate the influence of particle size on their toxicity. The AgNPs stock solutions were prepared by simply diluting the products to a silver concentration of 10 mg/L. In order to make sure that AgNPs were uniformly dispersed in stock solutions, 15 min ultrasonication (Ultrasonic Bath S450H, 50/60Hz, 2000W, Fisher Scientific Pte. Ltd.; SG) was employed before experiments. Hydrodynamic diameters and ξ -potentials of AgNPs in respective water matrix were determined using Zetasizer (Nano ZS, Malvern Instruments; Worcestershire, UK).

To determine the concentration of dissolved silver, AgNPs suspensions (same conditions as those used in nanotoxicity studies) were filtered through a 3 kDa ultrafiltration centrifugal filter units (Amicon Ultra-4 3K, Millipore; Billerica, MA) by centrifuging for 40 min at $4000 \times g$. The filtrate was digested in 7% HNO_3 according to a modified U.S. EPA method 3050B (U.S.EPA, 1996) and measured by using Inductively Coupled Plasma Mass Spectrometer (ICP-MS, ELAN DRC-e, Perkin Elmer; Waltham, MA).

4.2.3. Preparation of bacterial cultures

Gram-positive bacteria *Bacillus subtilis* (ATCC 6633) and Gram-negative bacteria *Escherichia coli* (ATCC 8739) were used as model organisms to evaluate the toxicity of AgNPs against bacteria in various water chemistry conditions. Both genera are widely existed in environment (Jin et al., 2010). Planktonic bacterial cultures were prepared in nutrient broth (BD DIFCO 234000) with shaking (150 rpm) at their respective optimum growth temperature, *i.e.*, 30 °C for *B. subtilis* and 37 °C for *E. coli*. Bacterial cells were harvested at mid-exponential growth phase (24 h for *B. subtilis* and 12 h for *E. coli*, respectively) and then were washed twice with 0.9% NaCl and specific freshwater matrices sequentially, and then resuspended in specific freshwater matrices to achieve a cell density of 1.0×10^7 cells/mL for bacterial viability tests.

4.2.4. Bacterial viability assay by high-throughput screening analysis

Live/Dead BacLight bacterial viability kit (Molecular Probes, Eugene, OR) was used to determine bacterial viability by high-throughput screening analysis (Mortimer et al., 2008; Jin et al., 2010; Thomas et al., 2011; Tong et al., 2013). 100 μ L of AgNPs suspensions of different concentrations (from 0.01 μ g/L to 10 mg/L) and 100 μ L of bacterial suspensions were added and completely mixed into specific wells of a 96-well F-bottom polystyrene microplate (Greiner Bio-One; Monroe, NC). Each combination was tested in 2–4 independent batches with at least 9 replicates, therefore, 18–36 replicates were used to determine one testing condition. All plates with bacteria were incubated for 24 h under respective optimum growth temperature. After 24 h exposure, stain solutions with a mixture of SYTO9, green-fluorescent nucleic acid stain that labels all bacteria in a population, and propidium iodide, a red-fluorescent nucleic acid stain that only affects the bacteria with membrane damage, were added into each well.

The green/red fluorescence ratio detected by Synergy 2 Multi-Mode Microplate Reader (BioTek Singapore, SG) provided the percentage of live bacterial cells in each well (excitation at 485 nm and emission at 635 and 500 nm) according to calibration curves obtained by blending different known ratios of live-to-dead bacteria (3–4 independent batches with at least 18 replicates). Silver-free controls contained only bacteria and stain mixture. Percentages of live bacteria cells calculated by calibration curves were normalized by the silver-free controls and analyzed by fitting the biostatistics software Prism 5 “Dose-response Inhibition, log(inhibitor) vs. normalized response-variable slope” (GraphPad Software, San Diego, CA) with 95% confidence intervals. Half maximal inhibitory concentration (IC₅₀) value, usually referring to the effectiveness of chemicals in inhibiting biological functions or viability, was adopted to determine the nanotoxicity of AgNPs suspensions to both bacteria with different water chemistry conditions with 95% confidence intervals in the expression of mass of silver existed in per liter of water matrix. It corresponds to the AgNPs concentrations at 50% live bacteria cells in respective “Percentages of live bacteria vs. Log concentration of AgNPs

suspension” curves obtained after 24 h incubation with 19 different concentrations (from 0.01 µg/L to 10 mg/L) of AgNPs suspensions.

4.3. Results and discussion

4.3.1. Size-dependent bactericidal activity of AgNPs

IC₅₀ value is commonly used to determine bacterial viability under different water chemistry conditions (Jin et al., 2010; Li et al., 2011a; Kim et al., 2013b). A high IC₅₀ value indicates low toxicity and vice versa. Figure 4-1 presents typical live bacterial percentage curves of *B. subtilis* and *E. coli*, obtained by exposing each microorganism to a series of 10 nm AgNPs suspensions of different concentrations (0.01 µg/L - 10 mg/L). Clearly, the percentage of live bacteria cells decreased with increasing AgNPs concentration. The concentration corresponding to 50% live bacteria cells is the IC₅₀ value: 0.32 ± 0.04 mg/L for *B. subtilis* and 0.71 ± 0.01 mg/L for *E. coli*.

Live Bacterial Percentage Curves of 10 nm AgNPs

Figure 4-1. Live bacterial percentage curves (*B. subtilis* and *E. coli*) of 10 nm AgNPs suspensions without model NOM (HA, BSA and AA) or chelating agent, EDTA, (total ionic strength = 5.6 mM as NaCl, pH = 7.0) after 24 h of incubation at respective optimal growth temperature. The percentages were calculated with 95% confidence intervals, and the error bars represent the standard deviation of 18–36 replicates.

Figure 4-2 presents IC_{50} values of both *B. subtilis* and *E. coli* after 24 h exposure to three AgNPs with different particle sizes (10 nm, 40 nm and 100 nm). IC_{50} values of three AgNPs followed the order of 10 nm AgNPs < 40 nm AgNPs < 100 nm AgNPs. This clearly indicates toxicity of 10 nm AgNPs is the highest among the three AgNPs tested. The strong size-dependency observed in the current study is consistent with previous studies that report reduced toxicity for large particle size (Choi et al., 2008; Zhang et al., 2011c; Kim et al., 2012; Xiu et al., 2012; Zhang et al., 2012a; Zhao and Wang, 2012; Kim et al., 2013b). As a result, 10 nm AgNPs was selected for further studying the influences of three model NOM and chelating agent (EDTA) on silver nanotoxicity.

Figure 4-2. IC_{50} values (*B. subtilis* and *E. coli*) of 10 nm, 40 nm and 100 nm AgNPs suspensions as mg/L silver (total ionic strength = 5.6 mM as NaCl, pH = 7.0) after 24 h of incubation in respective optimal growth temperature. The values were calculated with 95% confidence intervals, and the error bars represent the standard deviation of 18–36 replicates.

In Figure 4-2, it is also observed that IC_{50} values of Gram-positive *B. subtilis* were generally lower than those of Gram-negative *E. coli* under the same testing conditions. This difference might be attributed to different outer cell membrane structures between Gram-positive and Gram-negative bacteria. A thick peptidoglycan layer with teichoic acid, functional protein and embedded lipid wraps the single bilayer outside the cell membrane of Gram-positive bacteria. While a thin

peptidoglycan layer lies between two lipid bilayers and further coated by lipopolysaccharide (LPS) layer constitutes the cell wall outside Gram-negative cell membrane. LPS contributes significantly to bacterial surface property and structural integrity, and plays an important role in protecting varieties of substances permeating into the bacteria (Kučerka et al., 2008). Therefore, Gram-positive *B. subtilis* without LPS layer was more sensitive to AgNPs than Gram-negative *E. coli*. This agrees with the evidences observed in several previously publications (Jin et al., 2010; Li et al., 2011a; Liu et al., 2013b).

4.3.2. Influences of NOM on AgNPs toxicity and stability

NOM may change the surface chemistry and thus dispersion of nanoparticles. Table 4-2 presents a summary of previous studies focusing on the influences of NOM on nanoparticles stability and toxicity. In general, most existing studies used humic substances as a surrogate NOM. In addition, the effect of NOM on nanotoxicity is not conclusive. In this study, BSA and AA were selected in addition to HA to investigate the influences of organic macromolecules on AgNPs stability and toxicity.

IC₅₀ values (both *B. subtilis* and *E. coli*) of 10 nm AgNPs suspensions in 5.6 mM NaCl with and without three model NOM after 24 h of incubation are shown in Figure 4-3(a). There is little difference observed between IC₅₀ values with and without three model NOM. This indicates NOM representatives (HA, BSA and AA) had negligible effect on the toxicity of 10 nm AgNPs under the experimental conditions of this study. This is consistent with the results obtained by previous study on investigating the interactions of AgNPs with *Pseudomonas putida* biofilms using Suwannee River fulvic acid (Fabrega et al., 2009b). However, other studies also showed that the presence of NOM could mitigate the bactericidal activity of AgNPs (Fabrega et al., 2009a; Liu and Hurt, 2010; Zhang et al., 2012a; Kim et al., 2013b). The discrepancy on NOM influences might be attributed to different effects of NOM on the particle stability and aggregation behavior of AgNPs (Kvitek et al., 2008; Liang et al., 2013). In the current study, we did not observe significant influences on dissolved Ag species (Table 4-3) and particle sizes with the addition of different model NOM in 5.6 mM NaCl. In all conditions, sizes of 10 nm AgNPs

fall in the narrow range of 9.4 nm to 13.9 nm (Figure 4-3(b)), and the corresponding ξ -potential of 10 nm AgNPs only changed slightly in the presence of different model NOM.

(a)

(b)

Figure 4-3. (a) IC_{50} values (*B. subtilis* and *E. coli*) of 10 nm AgNPs suspensions

(total ionic strength = 5.6 mM as NaCl, pH = 7.0) with different kinds of model NOM after 24 h of incubation in respective optimal growth temperature. The values were calculated with 95% confidence intervals, and the error bars represent the standard deviation of 18–36 replicates. (b) Effects of different model NOM (HA, BSA and AA) on hydrodynamic diameters and ξ -potentials of 10 nm AgNPs in respective water matrix. The concentration of 10 nm AgNPs used in the experiment was 10 mg/L as silver. The error bars represent the standard deviation of 9 replicates.

To further investigate the effect of particle stability on nanotoxicity, divalent calcium ion (1.0 mM) was added to the AgNPs/NOM suspension to induce aggregation of the nanoparticles (Jin et al., 2010; Akaighe et al., 2012) and the resulting IC_{50} values are reported in Figure 4-4(a). The total ionic strength of matrices was kept constant at 5.6 mM by reducing NaCl concentration correspondingly. In the presence of both $CaCl_2$ and HA, the IC_{50} value of AgNPs increased greatly to 5.17 ± 1.55 mg/L for *B. subtilis* and 4.16 ± 1.74 mg/L for *E. coli*, which indicated significantly mitigated toxicity. Corresponding to the reduced toxicity, we observed severe aggregation of AgNPs (particle size increasing from 10.5 ± 1.1 nm to 70.5 ± 20.0 nm) when both HA and Ca^{2+} were added to the AgNPs suspension, which was likely a result of reduced electrostatic repulsion between the particles (ξ -potential shifted from -53.7 ± 2.2 mV to -31.1 ± 1.9 mV). Calcium ions, which has little adverse effect on bacterial viability, can form complex with carboxylic groups in HA that coats on AgNPs surface (noting that calcium alone had minimal effect on particle size of 10 nm AgNPs, see Figure 4-5) and thus partially neutralize the particle surface charge (Li and Elimelech, 2004; Lee and Elimelech, 2006; Huynh and Chen, 2011; Wang and Tang, 2011; Calder et al., 2012; Romanello and Fidalgo de Cortalezzi, 2013). The current study suggests that nanotoxicity may be strongly linked to particle aggregation behavior (Zhang et al., 2009; Chae et al., 2012; Kim et al., 2013b). This may explain the different observations of NOM influences on toxicity of AgNPs obtained in this study and some previous ones listed in Table 4-2, especially when natural waters or certain nutrient solutions, which usually contain a relatively high concentration of calcium, were adopted in the investigations.

(a)

(b)

Figure 4-4. (a) IC_{50} values (*B. subtilis* and *E. coli*) of 10 nm AgNPs suspensions without HA or Ca (total ionic strength = 5.6 mM as NaCl), with HA but without Ca (total ionic strength = 5.6 mM as NaCl), and with both HA and Ca (total ionic strength = 5.6 mM, including 1 mM $CaCl_2$ and 2.6 mM NaCl) after 24 h of incubation in respective optimal growth temperature. The values were calculated

with 95% confidence intervals, and the error bars represent the standard deviation of 18–36 replicates. (b) Effects of Ca (with the existence of HA) on hydrodynamic diameters and ξ -potentials of 10 nm AgNPs in water matrix. The concentration of 10 nm AgNPs used in the experiment was 10 mg/L as silver. The error bars represent the standard deviation of 9 replicates.

Figure 4-5. FE-SEM images of (a) original 10 nm AgNPs without HA or CaCl₂, (b) 10 nm AgNPs with 5 mg/L HA, (c) 10 nm AgNPs with 1 mM CaCl₂, and (d) 10 nm AgNPs with both 5 mg/L HA and 1 mM CaCl₂ (total ionic strength = 5.6 mM, pH = 7.0). (e) Size distributions (by volume) of original AgNPs without HA or CaCl₂, AgNPs with HA, AgNPs with CaCl₂, and AgNPs with both HA and CaCl₂ (obtained by using Zetasizer (Nano ZS, Malvern Instruments; Worcestershire, UK)).

4.3.3. Influence of EDTA on AgNPs toxicity and stability

Figure 4-6(a) presents the IC₅₀ values of 10 nm AgNPs for both bacteria, *B. subtilis* and *E. coli*, with different concentrations of EDTA. The hydrodynamic diameters and ξ -potentials of 10 nm AgNPs with different EDTA concentrations are shown in Figure 4-6(b). IC₅₀ values first increased at EDTA concentration of 1 mg/L, which indicates the toxicity of 10 nm AgNPs was reduced at low EDTA concentration; however, IC₅₀ values then decreased again as EDTA concentration further increasing to 5 mg/L and 20 mg/L, suggesting toxicity of 10 nm AgNPs was increased at relatively high EDTA concentrations.

(a)

(b)

Figure 4-6. (a) IC_{50} values (*B. subtilis* and *E. coli*) of 10 nm AgNPs suspensions (total ionic strength = 5.6 mM as NaCl, pH = 7.0) with different concentrations of EDTA (1 mg/L, 5 mg/L and 20 mg/L) after 24 h of incubation in respective optimal growth temperature. The values were calculated with 95% confidence intervals, and the error bars represent the standard deviation of 18–36 replicates. (b) Effects of different EDTA concentrations on hydrodynamic diameters and ξ -potentials of 10 nm AgNPs in respective water matrix. The concentration of 10 nm AgNPs used in the experiment was 10 mg/L as silver. The error bars represent the standard deviation of 9 replicates.

At concentrations of 1 mg/L, 5 mg/L and 20 mg/L, EDTA itself has no significant adverse effects on both *B. subtilis* and *E. coli* (Figure 4-7). However, according to IC_{50} values presented in Figure 4-6(a), different concentrations of chelating agent (EDTA) did affect the bactericidal activity of 10 nm AgNPs under specific water chemistry conditions. At low concentration (1 mg/L), EDTA significantly mitigated the toxicity of 10 nm AgNPs. Nevertheless, the addition of the small amount of EDTA had little effects on the AgNPs aggregation (Figure 4-6(b)) nor the dissolved silver concentration (Table 4-4). Instead, it may cause transformations of dissolved silver species. Since silver ion has a strong interaction with EDTA (the formation

constant of $\text{Ag}(\text{EDTA})^{3-} = 2.1 \times 10^7$), the addition of EDTA may convert the more toxic species such as Ag^+ and Ag-Cl complexes (Choi et al., 2008; Jin et al., 2010) into more stable Ag-EDTA complexes, which may have relatively low bactericidal activity. Therefore, bactericidal activity of 10 nm AgNPs was mitigated at low chelating agent concentration (1 mg/L) indicating the chelating agent-induced mitigation of nanotoxicity. Findings agree with previous studies on influences of relatively low concentrations of EDTA and tannic acid on nanotoxicity (Choi et al., 2009a; Li et al., 2011a; Romanello and Fidalgo de Cortalezzi, 2013). Similar results were also observed in studies of mitigation of NPs toxicity by using other kinds of chelators, such as cysteine for AgNPs and bathocuproine for CuO NPs (Dimkpa et al., 2011; He et al., 2012). When EDTA concentration increased to ≥ 5 mg/L, toxicity of AgNPs increased. Since EDTA in the experiment has no obvious influence on AgNP stability (Figure 4-6(b) and Table 4-4), the toxicity enhancement may due to the disassembling of membrane structures cause by high concentration of EDTA. EDTA can react with divalent cations (such as Ca^{2+} and Mg^{2+}) on bacterial cell membrane, which play an important role in preserving stability of bacterial outer membrane structures (Nikaido and Vaara, 1985). EDTA is also used as an extractant in extracellular polymeric substances (EPS) isolation from biofilm (Nagai and Oita, 2004; Cao et al., 2011a; Cao et al., 2011b). As the compromise and disassembling of cell membrane structures, bacteria membranes became more permeable. However, damages of cell membrane structures caused by EDTA cannot directly result in significant inactivation of bacteria without the existence of AgNPs. This in turn enhanced the contact, penetration and finally the toxicity of 10 nm AgNPs and its dissolved species. The possible concentration-dependent effect by which different EDTA concentrations influencing the toxicity of 10 nm AgNPs is proposed and shown in Figure 4-8.

Figure 4-7. Percentages of live bacterial cells (*B. subtilis* and *E. coli*) in water matrices containing different concentrations of chelating agent (EDTA) after 24 h incubation in respective optimal growth temperature without Ag (total ionic strength = 5.6 mM as NaCl, pH = 7.0).

Table 4-2. Summary of previous studies of NOM influences on nanoparticles (NPs) toxicity and stability

Nanomaterial(s) investigated	NOM investigated	Conclusion	Reference
Multiwalled carbon nanotubes (MWNTs) from the MER Corporation, USA	100 mg C/L Standard Suwannee River NOM (SRNOM) from International Humic Substances Society (IHSS), USA	Carbon-based NPs in natural aqueous environment occur to an unexpected extent following a mechanism that has not been considered in environmental fate and transport studies	(Hyung et al., 2007)
Self-fabricated iron oxide NPs	0–25 mg/L of Suwannee River humic acid (SRHA) from IHSS, USA	SRHA cause significant aggregation of iron oxide NPs depending on the concentration and solution pH	(Baalousha et al., 2008)
C ₆₀ from SES Research, Houston, or Materials and Electrochemical Research, USA	0–10 mg/L HA from Sigma-Aldrich, SRHA, and Suwannee River fulvic acid (SRFA) from IHSS	NOM mitigate the potential impacts of nC ₆₀ on microbial activities	(Li et al., 2008a)
Nanometer-sized zerovalent iron (NZVI) from Toda America Inc., USA	0–200 mg/L NOM	Different concentrations of NOM may significantly affect the mobility of NZVI in variety ways	(Johnson et al., 2009)
AgNPs from Stanford Materials Corp., USA	10 mg/L SRHA from IHSS	SRHA mitigated the bactericidal action	(Fabrega et al., 2009a)
AgNPs from Stanford Materials Corp., USA	10 mg/L SRFA from IHSS	No changes in cell viability were detected; biofilm behavior was affected by the addition of NOM with changes in surface chemistry and dispersion	(Fabrega et al., 2009b)

NZVI particles from Toda Kogyo Corporation, Japan	100 mg/L HA from Aldrich	NOM mitigates the toxicity of NZVI at 0.1 to 0.5 g/L depending on the coating	(Li et al., 2010)
Self-fabricated AgNPs	0–40 mg/L standard terrestrial HA from Sigma–Aldrich and SRHA from IHSS	Cytotoxicity of AgNPs was affected by HA concentration	(Dasari and Hwang, 2010)
Self-fabricated citrate stabilized AgNPs	0–50 mg/L SRHA and SRFA from IHSS	Ion release rates of AgNPs decrease with increasing addition of HA and FA	(Liu and Hurt, 2010)
NZVI particles from Toda Kogyo Corporation, Japan	10 mg/L SRHA from IHSS	SRHA significantly mitigated toxicity of NZVI particles	(Chen et al., 2011)
ZnO NPs from Meliorum, USA	10 mg/L HA, FA, AA, tannic acid (TA), and BSA	By combining the most free Zn^{2+} ions, TA decreased toxicity of ZnO NPs more than HA, FA, and AA	(Li et al., 2011a)
γFe_2O_3 NPs from Nanoamor, USA	500 mg/L HA extracted from Amherst peat soil, USA	Weakly polar HA-coated γFe_2O_3 NPs could obtain a higher stabilization than for PAA-coated γFe_2O_3 NPs	(Ghosh et al., 2011)
a) Commercial AgNPs stabilized with casein from Argenol laboratories b) Self-fabricated AgNPs stabilized with dextrin and PVP	5 mg/ L HA	Rapid coating of HA on the surface of AgNPs creates a physical barrier that impedes AgNPs-bacteria interactions and reduces the toxicity	(Zhang et al., 2012a)
Self-fabricated AgNPs	1% w/v BSA premixed MH agar	BSA decreases antibacterial effect of AgNPs by binding silver and silver ions	(Grade et al., 2012)
Self-fabricated AgNPs	30 mg/L SRNOM and SRHA from IHSS	SRNOM and SRHA contributed to the NPs instability at high ionic strength in divalent metallic cation solutions	(Akaighe et al., 2012)

ZnO NPs from Zhejiang Hongsheng Material Technology Co., China	1 mg TOC/L SRHA from IHSS and alginate from Sigma–Aldrich	NOM decreased the transportation of ZnO NPs	(Jiang et al., 2012)
Four fullerene NPs from MER, USA and BuckyUSA, USA)	1–10 mg/L SRHA from IHSS	HA increased the size of aggregates and reduced transport of fullerene aggregates and their ROS production	(Chae et al., 2012)
Citrate-capped AgNPs from NanoComposix, USA	5 mg DOC/L SRFA and SRHA from IHSS	HA has a greater capability to enhance the transport of AgNPs by reducing aggregation and deposition compared to FA	(Furman et al., 2013)
Self-fabricated AgNPs	4–5.5 mg C/L HA from Sigma–Aldrich and SRHA from IHSS	NOM mediated Ag ion photoreduction under ambient environmental light conditions	(Hou et al., 2013)
Self-fabricated citrate-coated AgNPs	SRFA from IHSS	SRFA enhances the stability of AgNPs and shifts the critical coagulation concentrations to higher electrolyte concentrations for all electrolytes	(Baalousha et al., 2013)
CuO NPs from Beijing Nachen S&T Ltd., China	5 mg/L SRFA from IHSS	FA mitigated the damage of CuO NPs due to the enhanced electrostatic repulsion and surface coating	(Zhao et al., 2013)
Aeroxide P25 TiO ₂ NPs from Evonik Degussa Corporation, USA	a) 0.1–1.0 mg/L TA from Carlo Erba Reagents, Italy b) 1.0 mg/L HA from Aldrich	NOM adsorbed to the particles and made their zeta potential more negative and stabilizing them by lowering the pH of maximum aggregation	(Romanello and Fidalgo de Cortalezzi, 2013)

PVP stabilized gold NPs	20 mg/L SRFA from IHSS	FA had no observable changes to the PVP stabilised gold NPs	(Hitchman et al., 2013)
ZnO NPs Shenzhen Nanotech Port Co., Ltd., China	HA from Wako, Japan	DOM reduced the concentration of free Zn^{2+} released from ZnO NPs and lowered their toxicity	(Li et al., 2013)
C_{60} from MER Corp., USA	4–11 mg/L SRHA and SRFA from IHSS	NOM stabilized C_{60} , and SRHA was more effective than SRFA in suppressing C_{60} aggregation	(Zhang et al., 2013)
Modified AgNPs from Sigma–Aldrich	HA from Sigma–Aldrich	HA may cause agglomeration of AgNPs and reduce their nanotoxicity	(Kim et al., 2013a)

Table 4-3. Dissolved Ag concentration in corresponding water matrix with different NOM after 24 h of incubation without bacteria

Conditions	Dissolved Ag concentration (average $\pm$ standard deviation), $\mu\text{g/L}$	
	30°C (temp. for <i>B. subtilis</i> growth)	37°C (temp. for <i>E. coli</i> growth)
10 nm AgNPs (10 mg/L) without NOM or EDTA	34.3 ± 0.9	54.4 ± 7.5
10 nm AgNPs (10 mg/L) with 5 mg/L HA	37.3 ± 6.0	57.5 ± 6.7
10 nm AgNPs (10 mg/L) with 5 mg/L BSA	37.8 ± 6.7	54.2 ± 0.4
10 nm AgNPs (10 mg/L) with 5 mg/L AASS	37.5 ± 5.4	54.8 ± 4.5

Table 4-4. Dissolved Ag concentration in corresponding water matrix with different EDTA concentrations after 24 h of incubation without bacteria

Conditions	Dissolved Ag concentration (average $\pm$ standard deviation), $\mu\text{g/L}$	
	30°C (temp. for <i>B. subtilis</i> growth)	37°C (temp. for <i>E. coli</i> growth)
10 nm AgNPs (10 mg/L) without NOM or EDTA	34.3 ± 0.9	54.4 ± 7.5
10 nm AgNPs (10 mg/L) with 1 mg/L EDTA	36.4 ± 4.3	53.3 ± 0.6
10 nm AgNPs (10 mg/L) with 5 mg/L EDTA	34.5 ± 2.4	53.5 ± 0.5
10 nm AgNPs (10 mg/L) with 20 mg/L EDTA	40.9 ± 3.7	55.0 ± 3.3

Figure 4-8. (a) Possible mechanisms by which AgNPs affected bacterial cells. (b) Low concentration (1 mg/L) of EDTA decreased the toxicity of AgNPs likely by converting the relatively higher toxic dissolved Ag species into lower toxic Ag-EDTA complex. (c) High concentrations (5 and 20 mg/L) of EDTA increased the nanotoxicity of AgNPs probably by disassembling cell membrane structure and enhancing the direct penetration of AgNPs into bacterial cells.

4.4. Conclusions

Influences of model NOM and chelating agent EDTA on bactericidal activity and stability of AgNPs in environmentally relevant freshwater matrices were investigated. Main conclusions can be drawn from this study are as follows:

- Within the range of particle size investigated (10–100 nm), for the AgNPs of same kind, the smaller ones had higher nanotoxicity.
- Under the experimental conditions, three model NOM had no significant effects on the bactericidal activity and stability of AgNPs in the absence of Ca^{2+} . When introducing additional Ca^{2+} into the freshwater matrices, stability of AgNPs decreased greatly thus its bactericidal activity decreased significantly. This may due to aggregation of AgNPs as a result of Ca^{2+} -NOM-AgNPs interaction partially neutralized the negative charge of AgNPs.
- Chelating agent, EDTA, exhibited a concentration-dependent effect on AgNPs bactericidal activity: at low concentration (1 mg/L), EDTA decreased the nanotoxicity of AgNPs by converting the relatively higher toxic dissolved Ag species, such as Ag^+ and Ag-Cl complex, into a series of lower toxic Ag-EDTA complex; at high concentrations (5 and 20 mg/L), EDTA increased the toxicity of AgNPs probably by disassembling cell membrane structures and enhancing the direct penetration of AgNPs and dissolved Ag species into bacterial cells.
- Results also imply that water chemistry conditions can affect the toxicity of AgNPs greatly. Potential applications, such as nanotoxicity control or nano-disinfection, could be developed based on the findings obtained from this study.

Taken together, this study demonstrated the importance of interactions between AgNPs and dissolved organics such as NOM and chelating agents in controlling bactericidal activity of AgNPs in naturally relevant freshwater matrices. Results also showed that chelating agent (EDTA) exhibited a concentration-dependent effect on nanotoxicity of AgNPs. Molecular mechanisms underlying the observations warrant further investigations.

CHAPTER 5

Synthesis and Characterization of Novel Antibacterial Silver Nanocomposite Nanofiltration and Forward Osmosis Membranes based on Layer-by-Layer Assembly

5.1. Introduction

The versatile structures fabricated by layer-by-layer (LbL) assembly are very promising for many potential applications (Jiang and Tsukruk, 2006). In LbL assembly, the membrane selective layer can be formed by depositing oppositely charged polyelectrolytes in an alternate sequence on a substrate via electrostatic interactions and van der Waals forces (Hammond, 1999). As an important membrane fabrication technology, LbL assembly method has attracted increasing attention and been successfully used in synthesizing high-performance nanofiltration (NF) (Malaisamy and Bruening, 2005; Ouyang et al., 2008) and NF-like forward osmosis (FO) membranes (Saren et al., 2011; Qi et al., 2012b). LbL NF membranes are applicable to various applications (Eriksson, 1988) by offering several advantages such as high water flux, low operation pressure, less energy consumption, and low operation and maintenance costs (Hilal et al., 2004). Meanwhile, NF-like FO membranes fabricated based on LbL assembly (Saren et al., 2011; Liu et al., 2012) can also obtain higher water permeability and expanded applications compared with those RO-like ones (Wang et al., 2007; Tang et al., 2009; Geise et al., 2011; Setiawan et al., 2011; Wei et al., 2011a). Moreover, the NF-like LbL FO membranes have high thermal stability, good solvent resistance, and etc. (Hammond, 1999; Bertrand et al., 2000; Qiu et al., 2011). They may show great promise in wastewater treatment (such as biomass concentrating, and sludge dewatering) and food processing with a range of potential benefits (Cath et al., 2006; Zhao et al., 2012b). Despite above advantages and potential applications, membrane fouling, especially biofouling, remains an obstacle in NF membrane and FO membrane operations (Ridgway et al., 1985; Flemming, 1997; Hong and Elimelech, 1997; Vrijenhoek et al., 2001; Xu et al., 2006; Herzberg et al., 2009; Gao et al.,

2010; Wei et al., 2013). To reduce membrane biofouling, fabrication of membranes containing biocides or antibacterial materials has attained great attention (Diagne et al., 2012; Zhang et al., 2012c).

Silver nanoparticles (AgNPs) are one of the most extensively studied biocides, which are effective against various aquatic microorganisms including bacteria, fungi, algae, and etc. (Sondi and Salopek-Sondi, 2004; Li et al., 2008b; Choi et al., 2010). They may affect the cell integrity and metabolism by direct interaction with cell membranes, releasing dissolved silver species, and/or generating reactive oxygen species (ROS) (Klaine et al., 2008; Jin et al., 2010; Marambio-Jones and Hoek, 2010). AgNPs have been incorporated into nanocomposite microfiltration (MF) (Gunawan et al., 2011; Diagne et al., 2012), ultrafiltration (UF) (Taurozzi et al., 2008; Zodrow et al., 2009; Huang et al., 2012a), NF (Lee et al., 2007) and reverse osmosis (RO) (Yang et al., 2009a) membranes via various methods including membrane surface modification (Diagne et al., 2012), phase-inversion (Lee et al., 2007; Taurozzi et al., 2008; Zodrow et al., 2009; Huang et al., 2012a) and interfacial polymerization (Yang et al., 2009a). Compared to the existing method, LbL assembly is a highly versatile membrane fabrication method. The layered structure can potentially allow the incorporation of AgNPs with great flexibility in controlling the loading conditions. Nevertheless, LbL-Ag nanocomposite membranes with antibacterial properties have not been reported for NF and FO preparation.

The objectives of this chapter were: 1) to synthesize novel antibacterial nanocomposite NF and FO membranes using LbL assembly method; 2) to characterize membrane surface properties and performance of the membranes under NF and FO conditions, and 3) to evaluate their antibacterial activity against Gram-positive *B. subtilis* and Gram-negative *E. coli*. Influences of different AgNPs doping routines on membrane performances were also systematically investigated. To the best of our knowledge, this is the first study reporting the synthesis and characterization of LbL-assembly-based silver nanocomposite NF and FO membranes with antibacterial properties.

5.2. Materials and methods

5.2.1. Chemicals and Solution Chemistry

Polyacrylonitrile (PAN, weight-averaged molecular weight $M_w \approx 150\,000$, Sigma–Aldrich) with additional lithium chloride (Sinopharm) as pore former were dissolved in the solvent N,N-dimethylformamide (DMF, $\geq 99.8\%$, Sigma–Aldrich) for membrane substrate fabrication. Alkali solution prepared by sodium hydroxide (Sigma–Aldrich) was used for post-treatment of PAN membrane substrate to introduce negative charges and enhance surface hydrophilicity. Polycation poly(allylamine hydrochloride) (PAH, $M_w \approx 120\,000$ – $200\,000$, Sigma–Aldrich) and polyanion poly(sodium 4-styrene-sulfonate) (PSS, $M_w \approx 70\,000$, 30 wt.% in H_2O , Sigma–Aldrich) were used for LbL assembly. Ionic strength of polyelectrolyte solutions was adjusted using sodium chloride (Merck). Commercial AgNPs (~ 100 nm powder, 99.5% trace metals basis, Sigma–Aldrich) were suspended in polyelectrolyte solutions or Milli-Q water (Millipore Integral 10 water purification system; Singapore) to achieve desirable silver concentration and incorporated in membrane selective layers via LbL assembly. Glutaraldehyde (GA, 25% in water, Sigma–Aldrich) was used for crosslinking of membrane selective layers. Magnesium chloride (Merck) was used to determine membrane intrinsic separation properties and FO performance. Live/Dead BacLight bacterial viability kit (Molecular Probes) was used to determine the percentage of bacteria with compromised cell membranes. Unless otherwise specified, all reagents and solutions were prepared using chemicals of analytical grade and Milli-Q water.

5.2.2. Synthesis of Nanocomposite NF and FO Membranes via LbL assembly

The detailed procedures of PAN substrate preparation, post treatment, LbL assembly and crosslinking have been reported elsewhere (Qiu et al., 2011; Saren et al., 2011; Qi et al., 2012b). Briefly, top surface of substrates was firstly soaked with 1 g/L positively charged PAH solutions and then with 1 g/L negatively charged PSS solution for 30 min. After each soaking step, the substrate surfaces were rinsed with Milli-Q for 5 min to remove excess polyelectrolytes and AgNPs. Specifically, for LbL process of nanocomposite membranes, controlled amount of AgNPs (0.01 wt.%)

unless otherwise specified, see Figure 5-4 in Section 5.3.2.1) was added in PSS solution, Milli-Q water, or PAH solution respectively after ultrasonicated (Ultrasonic Bath S450H, 50/60Hz, 2000W, Fisher Scientific Pte. Ltd.; Singapore) for 30 min at room temperature (23 ± 1 °C). The AgNPs suspensions were continuously stirred during LbL process. In our current study, 2.5 PAH/PSS bilayers (i.e., twice PAH/PSS coating procedures with an additional PAH coating on the outermost layer) was adopted based on previous optimization results (Qi et al., 2012b). The membranes were crosslinked by immersing them in 0.1 wt.% GA solution at room temperature (23 ± 1 °C) for 2 h followed by Milli-Q water washing according to our previously published procedures (Qiu et al., 2011) to prepare crosslinked LbL (xLbL) membranes. The AgNPs-free membrane was labeled as xLbL2.5. Silver nanocomposite membranes were labeled as xLbL2.5-Ag(XXXN), where XXX indicates the solution incorporating AgNPs and N indicates the layer numbers containing them (i.e. xLbL2.5-Ag(PSS1) means Ag being incorporated into PSS solution and coated only once). The detailed designation and compositions of nanocomposite membranes can refer to Table 5-1.

Table 5-1. Designation and synthesis conditions of xLbL-Ag nanocomposite membranes.^a

Membrane ID	Synthesis conditions of xLbL-Ag membranes
xLbL2.5	PAH + PSS + PAH + PSS + PAH
xLbL2.5-Ag(PSS1)	PAH + PSS + PAH + PSS(Ag) ^b + PAH
xLbL2.5-Ag(PSS2)	PAH + PSS(Ag) + PAH + PSS(Ag) + PAH
xLbL2.5-Ag(W1)	PAH + PSS + PAH + H ₂ O(Ag) ^c + PSS + PAH
xLbL2.5-Ag(W2)	PAH + H ₂ O(Ag) + PSS + PAH + H ₂ O(Ag) + PSS + PAH
xLbL2.5-Ag(PAH1)	PAH + PSS + PAH + PSS + PAH(Ag) ^d
xLbL2.5-Ag(PAH2)	PAH + PSS + PAH(Ag) + PSS + PAH(Ag)

Notes:

a. 0.01% AgNPs (w/w, as silver) were used in membrane fabrication unless specified

- b. PSS(Ag) indicated that AgNPs were suspended in PSS solution during membrane fabrication
- c. H₂O(Ag) indicated that AgNPs were suspended in Milli-Q water during membrane fabrication
- d. PAH(Ag) indicated that AgNPs were suspended in PAH solution during membrane fabrication

5.2.3. Characterization of Nanocomposite NF and FO Membranes

5.2.3.1. Membrane Surface Properties

Surface morphologies of nanocomposite NF and FO membranes were obtained by using a Field Emission Scanning Electron Microscope (FE-SEM, JSM-7600F, JEOL Ltd.; Japan) combined with energy-dispersive X-ray (EDX) spectroscopy (X-Max^N, Oxford Instruments; England). All membrane samples were dried and coated with platinum using a vacuum electric sputter coater (JEOL JFC-1600, JEOL Ltd.; Japan) before SEM/EDX examination. Atomic force microscopy (AFM, Park Systems XE-100; USA) was also used to measure the surface morphologies and roughness of membrane samples following similar procedures reported elsewhere (Tang et al., 2010; Saren et al., 2011; Ma et al., 2012). The reported AFM results were obtained from at least 3 measurements of 3 independent membrane samples. Contact angle measurements were performed with the sessile drop method using OCA Contact Angle System (DataPhysics Instruments GmbH; Germany). The reported contact angle is the average of at least 10 measurements obtained from 3 independent membrane samples.

5.2.3.2. NF Performance Evaluation

NF performance of nanocomposite NF and FO membranes were evaluated by using a high pressure crossflow filtration setup reported previously (Saren et al., 2011) at an applied pressure of 5 bar. Pure water permeability (A) was determined based on pure water flux (J_v) obtained by weighting permeate water samples. Salt rejection (R) was determined based on conductivity measurements of 5 mM MgCl₂ feed solution and permeate water using conductivity meter (Ultrameter II, Myron L

Company; CA, USA). Salt permeability coefficient B was calculated using following equation (Tang et al., 2010):

$$B = \left(\frac{1}{R} - 1 \right) \cdot A(\Delta P - \Delta \pi) \quad (5-1)$$

where ΔP is the trans-membrane pressure, $\Delta \pi$ is the osmotic pressure difference across the membrane, and A was determined in pure water tests. NF performance results were obtained from 3 replicate measurements of 3 independent membrane samples.

5.2.3.3. FO Performance Evaluation

FO performance, including water flux (J_v), solute flux (J_s), and solute flux over water flux (J_s/J_v), was evaluated using a cross-flow FO setup reported previously (Saren et al., 2011; Qi et al., 2012b). Both feed solution (FS) and draw solution (DS) were circulated at a cross flow rate of 500 mL/min (cross flow velocity ~ 10 cm/s) at room temperature (23 ± 1 °C). FO water flux was determined by measuring the weight change of the feed tank using a digital mass balance connected to a computer data logging system. Salt flux through the FO membrane was determined by monitoring the conductivity change in the FS. 0.5 M MgCl_2 solution was used as DS. Milli-Q water (for J_s measurement) or 10 mM NaCl (for J_v measurement) was used as FS. Both AL-DS and AL-FS membrane orientations were tested. All FO performance results were obtained from 3 replicate measurements of 3 independent membrane samples.

5.2.4. Quantification of Silver Leaching

To quantify silver release from nanocomposite membranes, the membranes were cut into circles with a diameter of 12.7 mm (area ≈ 1.27 cm²) using circle disc punch (Ted Pella, Inc.; Redding, CA, USA). 25 pieces of membrane samples (total area ≈ 31.67 cm²) were subsequently immersed into 50 mL Milli-Q water with sustained shaking at room temperature. After determined time frames, the silver released in the water was digested using 7% HNO_3 according to a modified U.S. EPA method 3050B (USEPA, 1996a) and analyzed by Inductively Coupled Plasma Mass

Spectrometer (ICP-MS, ELAN DRC-e, Perkin Elmer; MA, USA). Error bars of reported results were obtained by 3 replicate measurements.

Total silver incorporated in corresponding nanocomposite membranes was determined by completely digesting 0.1 g membrane sample (area $\approx 50.28 \pm 6.23$ cm²) in 9 mL concentrated HNO₃ solution for 15 min using Microwave Reaction System (Multiwave 3000, Anton Paar GmbH; Austria) (USEPA, 1996b). Silver concentrations in the collected samples were quantified using the ICP-MS described above. The percentage of silver α remained in membrane after leaching test was calculated using following equation:

$$\alpha = \left(1 - \frac{\text{Silver released in membrane leachate}}{\text{Total silver incorporated in membrane}} \right) \times 100\% \quad (5-2)$$

5.2.5. Membrane Antibacterial Tests

After fabrication, all nanocomposite membranes were immersed in Milli-Q water for 48 h before membrane antibacterial tests. Gram-positive *Bacillus subtilis* (ATCC 6633) and Gram-negative *Escherichia coli* (ATCC 8739) were used as model organisms to perform membrane antibacterial tests. The harvested cells were suspended in environmentally relevant freshwater (water quality parameters were determined by referring to fresh river water conditions reported previously (Tchobanoglous and Schroeder, 1985; Van Hoecke et al., 2011)) to achieve a cell density of 1.0×10^7 cells/mL. For colony-forming units and bacterial cell membrane compromise tests, bacterial suspensions were contacted with nanocomposite membranes for 24 h before the tests. AgNPs-free xLbL2.5 membranes were also investigated as controls.

5.2.5.1. Plate count of colony-forming units (CFU)

Bacterial suspensions (after 24 h exposure to membranes) were agitated for 1 min using Vortex shaker (Vortex-Genie 2, Scientific Industries, Inc.; NY, USA) in order to desorb the bacterial cells from membranes. A serial dilution of bacterial suspensions was performed according to CLSI M07-A9 (CLSI, 2012a). 100 μ L diluent was inoculated and spread on a nutrient agar plate and incubated for another

24 h. CFU results were obtained from 3 measurements of 3 independent membranes, and each measurement was based on 3 replicates (3 agar plates) of the same condition.

5.2.5.2. Bacterial cell membrane compromise tests

To obtain the percentages of bacteria with compromised cell membrane, 100 μ L of bacterial suspensions (after 24 h exposure to membranes) were completely mixed with the solution of Live/Dead BacLight bacterial viability kit into specific wells of a 96-well F-bottom polystyrene microplate (Greiner Bio-One; Monroe, NC, USA). Percentages of live bacterial cells were obtained using the green/red fluorescence ratios detected by Synergy 2 Multi-Mode Microplate Reader (BioTek Singapore, Singapore) and calculated by the calibration curves obtained by blending different known ratios of live-to-dead bacteria (3 independent batches with at least 18 replicates) (Jin et al., 2010). The reported percentages are based on 27 measurements in 9 individual plates using 3 independent batches of bacteria and 3 independently fabricated membranes.

5.2.5.3. Diffusion inhibition zone (DIZ)

Diffusion inhibition zone test of nanocomposite membranes was performed following a modification of CLSI document M02-A11 (CLSI, 2012b). 100 μ L bacterial inoculums were spread on the agar plates. Different nanocomposite membranes were punched into circle discs using the disc punch described previously, and placed on agar plates and incubated at optimal bacterial growth conditions for 24 h. The diffusion inhibition zones formed around the nanocomposite membranes were observed.

5.3. Results and discussion

5.3.1. Membrane Characterization

5.3.1.1. Membrane Surface Properties

Figure 5-1 presents the FE-SEM and EDX images of AgNPs-free control, xLbL2.5 membrane (Figure 5-1(a) and (b)), and three xLbL2.5-Ag nanocomposite NF and FO membranes fabricated via different synthesis routines (Figure 5-1(c)-(h)). Agglomerates of AgNPs embedded in membrane rejection layer can be clearly observed. Silver nanocomposite membranes were synthesized via three AgNPs doping routines (Table 5-1). Silver incorporation of xLbL2.5-Ag(PSS1) membranes (0.22 wt.% as silver, Figure 5-1(c) and (d)) was significantly less than that of xLbL2.5-Ag(W1) (1.19 wt.% as silver, Figure 5-1(e) and (f)) and xLbL2.5-Ag(PAH1) membranes (1.04 wt.% as silver, Figure 5-1(g) and (h)). The percentage of silver incorporation is obtained via EDX analysis. This can be attributed to the severe aggregation of AgNPs in PSS solution (Appendix Section B Figure B-1), resulting in more AgNPs loss during the soaking and water rinsing steps of membrane fabrication.

AFM micrographs of top surfaces of xLbL2.5 membrane and three xLbL2.5-Ag nanocomposite NF and FO membranes are shown in Figure 5-2. The average roughness of AgNPs-free membrane xLbL2.5 (the control) was 20 ± 3 nm (Figure 5-2(a)), while surface roughness of xLbL2.5-Ag(PSS1), xLbL2.5-Ag(W1) and xLbL2.5-Ag(PAH1) membranes was 25 ± 2 nm, 34 ± 3 nm and 28 ± 1 nm, respectively (Figure 5-2(b), (c) and (d)). This suggested an increase in surface roughness with the incorporation of AgNPs in membranes, and a positive correlation between the amount of AgNPs incorporated in and the roughness of membrane surfaces. The increased roughness of AgNPs incorporated membranes may be partially attributed to the agglomerate of AgNPs on membrane surface.

Figure 5-1. FE-SEM and EDX images of xLbL2.5 and xLbL2.5-Ag nanocomposite NF/FO membranes. (a) FE-SEM image of xLbL2.5 membranes. (b) EDX image of xLbL2.5 membranes. (c) FE-SEM image of xLbL2.5-Ag(PSS1) membranes. (d) EDX image of xLbL2.5-Ag(PSS1) membranes. (e) FE-SEM image of xLbL2.5-Ag(W1) membranes. (f) EDX image of xLbL2.5-Ag(W1) membranes. (g) FE-SEM image of xLbL2.5-Ag(PAH1) membranes. (h) EDX image of xLbL2.5-Ag(PAH1) membranes. Silver is depicted by light green to highlight the distribution and aggregation of AgNPs on membrane surface in EDX images.

Figure 5-2. AFM images of (a) xLbL2.5 membranes, (b) xLbL2.5-Ag(PSS1) membranes, (c) xLbL2.5-Ag(W1) membranes, and (d) xLbL2.5-Ag(PAH1) membranes. Errors were based on the standard deviations of 3 replicate measurements of 3 independent membranes.

5.3.1.2. Hydrophilicity

Contact angles of all membrane samples are presented in Figure 5-3. The AgNPs-free control, xLbL2.5 membrane, had a contact angle of $69.1 \pm 3.2^\circ$. The incorporation of AgNPs decreased contact angles, i.e., increased membrane surface hydrophilicities. The more AgNPs embedded in, the more hydrophilic membrane surfaces were obtained (Figure 5-3). Higher surface hydrophilicities could significantly decrease bacterial adhesion and thus mitigate membrane biofouling (Pasmore et al., 2001; Marmur, 2006; Mazumder et al., 2010; Zhang et al., 2012c). Among the tested membrane samples, xLbL2.5-Ag(PAH2) was most hydrophilic (contact angle of $51.3 \pm 2.8^\circ$). This could be because the PAH suspension containing AgNPs formed the outmost layer of the membranes. These results are consistent with previous studies (Diagne et al., 2012; Zhang et al., 2012c).

Figure 5-3. Contact angles of xLbL2.5 and xLbL2.5-Ag nanocomposite NF/FO membranes.

5.3.2. Membrane Performances

5.3.2.1. Membrane NF performance

The effect of AgNPs loading on membrane performance is shown in Figure 5-4. In general, both water permeability and MgCl_2 were not affected for AgNPs concentrations up to 0.01 wt.%. At higher AgNPs loadings, the membrane permeability increased while the rejection decreased, indicating defects formation in the LbL rejection layer possibly due to excessive aggregation. Similar loading effects have been reported for other types of nanocomposite membranes (Huang et al., 2012a; Zhang et al., 2012c). Consequently, an optimal loading of 0.01 wt.% was used for the rest of the experiments.

(a)

(b)

(c)

Figure 5-4. Effect of AgNPs concentrations in nanocomposite membranes on membrane NF performance: (a) water permeability coefficient, evaluated in the RO testing mode over an applied pressure range of 5 bar with Milli-Q water as feed solution; (b) salt rejection, evaluated in the RO testing mode at 5 bar with 5 mM MgCl_2 as feed solution; and (c) B/A ratio. Error bars were based on the standard deviations of 3 replicate measurements of 3 independent membranes.

Figure 5-5 presents effect of synthesis routines of silver nanocomposite NF and FO membranes on membrane NF performance. Generally, the NF performance of our LbL-assembly-based silver nanocomposite membranes was superior to most NF-like FO membranes reported in the literature (Appendix Section C Table C-1), especially considering the relatively high water permeability coefficients ($7.2\sim 10.9\times 10^{-12}$ $\text{m/s}\cdot\text{Pa}$) and excellent membrane separation properties (small B/A ratios, 4.8~5.8 kPa). Water permeability coefficients of silver nanocomposite membranes fabricated by doping AgNPs in polyelectrolyte solutions (both PSS and PAH) remained comparable with the AgNPs-free controls (Figure 5-5(a)). However, water permeability of the membranes synthesized by doping AgNPs in Milli-Q water, i.e., xLbL2.5-Ag(W1) and xLbL2.5-Ag(W2), was slightly lower than the

others (Figure 5-5(a)). The decrease in water permeability after AgNPs addition could be caused by increased thickness of membrane selective layer, which could be partially supported by the greater membrane surface roughness obtained for these membranes (Hyder et al., 2008). The high performance of xLbL2.5 membranes (including both AgNPs-free control and silver nanocomposite xLbL2.5-Ag membranes) was likely due to the superior separation properties of LbL rejection layer as well as the excellent properties of PAN support layer, such as high porosity, low tortuosity, good hydrophilicity, thin cross section and finger-like pores (Saren et al., 2011).

MgCl₂ rejections of all the nanocomposite membranes except xLbL2.5-Ag(PAH2) were at comparable level as that of the AgNPs-free controls (Figure 5-5(b)). In xLbL2.5-Ag(PAH2) membranes, MgCl₂ rejections decreased substantially. Correspondingly, *B/A* ratio of xLbL2.5-Ag(PAH2) membranes was significantly higher than the controls (Figure 5-5(c)). The reduced rejection and increased *B/A* ratio may be attributed to the larger amount of AgNPs incorporated in these membranes that caused more defects in selective layers. This observation is consistent with the effect of AgNPs loading on membrane rejection (Figure 5-4).

(a)

(b)

(c)

Figure 5-5. Effect of synthesis routines of xLbL2.5-Ag nanocomposite NF/FO membranes on membrane NF performances: (a) water permeability coefficient, evaluated in the RO testing mode over an applied pressure range of 5 bar with Milli-Q water as feed solution; (b) salt rejection, evaluated in the RO testing mode at 5 bar with 5 mM MgCl_2 as feed solution; and (c) B/A ratio. Error bars were based on the standard deviations of 3 replicate measurements of 3 independent membranes.

5.3.2.2. Membrane FO performance

Figure 5-6 presents the effect of synthesis routines of silver nanocomposite NF and FO membranes on membrane FO performance. Water fluxes (J_v) of silver nanocomposite membranes were comparable to the AgNPs-free controls with both membrane orientations (AL-DS and AL-FS), which were superior to most NF-like FO membranes reported previously (Appendix Section C Table C-2, also see RO-like FO membrane performance in Table C-3). Solute fluxes (J_s) and J_s/J_v values of both xLbL2.5-Ag(PSS) membranes with different doping times (once and twice) remained the same with the AgNPs-free controls. This indicated the small amount of AgNPs incorporated in xLbL2.5-Ag(PSS) membranes had little effect to membrane structures, which helped the membranes retain their original separation

properties. Similar results were also observed from the solute fluxes (J_s) and J_s/J_v values of xLbL2.5-Ag(W1) and xLbL2.5-Ag(PAH1) membranes. Nevertheless, the solute fluxes (J_s) and J_s/J_v values of xLbL2.5-Ag(W2) and xLbL2.5-Ag(PAH2) membranes increased significantly. The observed FO performance are consistent with corresponding NF results, (e.g., the FO J_s/J_v trend matched well with the NF B/A trend (Wei et al., 2011b)). As discussed in Section 5.3.2.1, the large amount of AgNPs in these membranes may decrease membrane selectivity due to excessive defects creation.

(a)

(b)

(c)

(d)

(e)

(f)

Figure 5-6. Effect of synthesis routines of xLbL2.5-Ag nanocomposite NF/FO membranes on membrane FO performances: (a) water flux, AL-DS orientation; (b) water flux, AL-FS orientation; (c) solute flux, AL-DS orientation; (b) solute flux, AL-FS orientation; (e) solute flux over water flux, AL-DS orientation; and (f) solute

flux over water flux, AL-FS orientation. Testing conditions: 0.5 M MgCl_2 as draw solution, and 10 mM NaCl as feed solution in water flux tests; 0.5 M MgCl_2 as draw solution, and Milli-Q water as feed solution in solute flux and solute flux over water flux tests. Error bars were based on the standard deviations of 3 replicate measurements of 3 independent membranes.

5.3.3. Membrane Silver Leaching Studies

Silver leaching was analyzed by ICP-MS and the results are presented in Figure 5-7 & Table 5-2. Silver released from all the silver nanocomposite membranes, and the leaching effect increased with more incorporated AgNPs in the membranes. At the initial 12 h, silver concentrations increased dramatically; after 48 h, the silver concentrations varied slowly. Although the antibacterial mechanism of AgNPs has not been fully understood, most researchers believed that it highly relies on the dissolved silver species released from AgNPs (Taurozzi et al., 2008; Yang et al., 2009a; Diagne et al., 2012; Zhang et al., 2012c). The dissolved silver species formed by released silver may damage bacteria upon contact, cellular uptake and accumulate, generate reactive oxygen species which prevent DNA replication and other metabolic activities in bacterial cells (Marambio-Jones and Hoek, 2010; Diagne et al., 2012). According to the regulation of USEPA and WHO guideline, the highest tolerable silver concentration in drinking water is 0.1 mg/L (USEPA, 2009; WHO, 2011), which is 10 times higher than the highest silver concentration released from nanocomposite xLbL2.5-Ag membranes. In addition, the released silver in the experimental conditions was overestimated compared to real membrane operations, and it only occupied 0.01~0.04% of the total silver in the membranes (Table 5-2). This suggested most silver remained inside the membranes, and the release of silver as well as the antibacterial properties of the nanocomposite membranes may last for a very long time.

Figure 5-7. Silver concentrations in the leachate of the silver nanocomposite NF/FO membranes.

Table 5-2. Silver release from the nanocomposite NF/FO membranes.

Membranes	Total leaching Ag (μg) ^{a,b}	Total Ag in membranes (μg) ^{a,b}	Percentage of Ag remained in nanocomposite membranes ^a
xLbL2.5	0.014 ± 0.001	0.177 ± 0.009	--
xLbL2.5-Ag(PSS1)	0.171 ± 0.004	1631.8 ± 51.9	99.99%
xLbL2.5-Ag(PSS2)	0.365 ± 0.020	1816.7 ± 20.4	99.98%
xLbL2.5-Ag(W1)	0.329 ± 0.010	1436.9 ± 40.1	99.98%
xLbL2.5-Ag(W2)	0.673 ± 0.020	3320.5 ± 54.7	99.96%
xLbL2.5-Ag(PAH1)	0.435 ± 0.011	1949.6 ± 24.2	99.98%
xLbL2.5-Ag(PAH2)	0.531 ± 0.011	3401.6 ± 41.4	99.98%

Notes:

a. The leaching Ag, total Ag in membranes and the percentage of Ag remained in nanocomposite membranes were obtained by testing a piece of membrane with an area of 32 cm^2 for 96 h.

b. Both the leaching Ag and total Ag in membranes were calculated by using the concentrations of Ag obtained from ICP-MS test $\times$ volume of solution in which membrane was submerged in. In this case, 50 mL Milli-Q water was adopted.

5.3.4. Membrane Antibacterial Studies

5.3.4.1. Colony-forming units test

The antibacterial activities of nanocomposite xLbL2.5-Ag membranes against *B. subtilis* and *E. coli* were tested using colony-forming units (CFU) method, and the results are shown in Figure 5-8. The reduced CFU indicated decrease of live bacteria after 24 h exposure to the nanocomposite membranes. Since AgNPs-free xLbL2.5 membranes had little effects on CFU number (Figure 5-9), the antibacterial activities of nanocomposite membranes were caused by incorporated AgNPs in the membranes. The LbL-assembly-based nanocomposite membranes xLbL2.5-Ag(PSS2), xLbL2.5-Ag(W2), and xLbL2.5-Ag(PAH2) exhibited excellent bactericidal activities: 2–3 Log reduction for *B. subtilis* and ~2 Log reduction for *E. coli*. Slightly better bactericidal activities of the nanocomposite membranes against *B. subtilis* were achieved, which might be because the cell wall structure of Gram-negative bacteria makes them more resistant to Ag toxicity due to the lipopolysaccharide (LPS) layer present outside the Gram-negative bacterial cell membrane (Kučerka et al., 2008). The LPS layer contributes significantly to bacterial structural integrity and plays an important role in protecting varieties of substances permeating into the bacteria constitutes the cell wall outside (Jin et al., 2010; Li et al., 2011a).

(a)

(b)

Figure 5-8. Effect of synthesis routines of xLbL2.5-Ag nanocomposite NF/FO membranes on colony forming units count: (a) Gram-positive *Bacillus subtilis*, and (b) Gram-negative *Escherichia coli*, after 24 h exposure to the membranes. Error bars were based on the standard deviations of 3 replicate measurements obtained from 3 batches of independent cultivated bacteria mixed with 3 independent fabricated membranes respectively.

Figure 5-9. Effect of AgNPs-free xLbL2.5 NF/FO membranes on bacterial colony forming units (after 24 h exposure). Error bars were based on the standard deviations of 3 replicate measurements obtained from 3 batches of independent cultivated bacteria mixed with 3 independent fabricated membranes respectively.

5.3.4.2. Bacterial cell membrane compromise test

Effect of synthesis routines of silver nanocomposite NF and FO membranes on bacteria with integrated cell membranes is shown in Figure 5-10. Membranes fabricated by doping AgNPs twice exhibited more severe antibacterial activities than those doping only once. For different doping routines, membranes synthesized by doping AgNPs in PAH solution provided the best antibacterial activities. These results are consistent with silver incorporation and release of silver nanocomposite membranes, which indicate the higher silver concentrations provide better antibacterial performance. The xLbL2.5-Ag (PAH2) membranes have the highest activity against both *B. subtilis* and *E. coli* (90.8% to 5.8%, and 87.6% to 12.2%, respectively). Consistent with the results of CFU test, Gram-positive *B. subtilis* was more sensitive than *E. coli*.

(a)

(b)

Figure 5-10. Effect of synthesis routines of xLbL2.5-Ag nanocomposite NF/FO membranes on bacterial cell membrane compromise test: (a) Gram-positive *Bacillus subtilis*, and (b) Gram-negative *Escherichia coli*, after 24 h exposure to the membranes. Error bars were based on the standard deviations of 3 replicate measurements obtained from 3 batches of independent cultivated bacteria mixed with 3 independent fabricated membranes respectively.

5.3.4.3. Diffusion inhibition zone test

Diffusion inhibition zones (DIZ) of nanocomposite NF and FO membranes are presented in Figure 5-11. The AgNPs-free xLbL2.5 membranes did not show any inhibition effect against the growth of *B. subtilis* or *E. coli*. All silver nanocomposite membranes showed inhibition effect against bacteria around them. The DIZs for *B. subtilis* were generally larger than those for *E. coli*. In addition, nanocomposite membranes incorporated with more AgNPs showed clearer and wider inhibition zones: 1) the membranes fabricated by doping AgNPs twice show significant inhibition effect compared with those synthesized by doping AgNPs only once; 2) the inhibition zones around xLbL2.5-Ag(W) and xLbL2.5-Ag(PAH) membranes were more obvious than the xLbL2.5-Ag(PSS) membranes (i.e., 13.9–14.3 mm for xLbL2.5-Ag(W2), 14.3–14.6 mm for xLbL2.5-Ag(PAH2), and 12.8–13.4 mm for xLbL2.5-Ag(PSS2)).

Figure 5-11. Diffusion inhibition zones of nanocomposite NF/FO membranes: (a) against Gram-positive *B. subtilis*; (b) against Gram-negative *E. coli*, after 24 h exposure.

5.3.5. Environmental implications

Biofouling is considered as the most prevalent and problematic issue in prolonged membrane operation. Synthesis of advanced silver nanocomposite membranes with antibacterial properties based on LbL assembly provides a promising approach for nanocomposite membrane fabrication and fouling control. This study put forward new understanding on synthesis and characterization of silver nanocomposite membranes based on flexible LbL assembly method. The results could be used for:

1) providing a novel method of nanocomposite membrane fabrication; 2) offering an advanced membrane biofouling control approach; 3) investigating the novel properties that nanocomposite membranes may obtain; 4) guiding the further investigations on synthesis and characterization of nanocomposite membranes. Although the current study primarily focused on NF-like FO membranes, AgNPs doping can be potentially applied to RO-like FO membranes as well as pressure retarded osmosis (PRO) membranes for biofouling control.

5.4. Conclusions

In this study, synthesis and characterization of novel antibacterial silver nanocomposite NF and FO membranes based on LbL assembly were investigated. Main conclusions can be drawn from this study are as follows:

- Silver nanocomposite membranes can be fabricated via LbL assembly. Performances of silver nanocomposite membranes are highly depended on the silver incorporation in the membranes, which could be controlled by using different membrane synthesis routines and the doping times of AgNPs.
- The silver nanocomposite membranes could be adopted in both NF and FO processes with comparable performances to AgNPs-free controls. Moreover, the performances were superior to most NF-like FO membranes reported in previous literature.
- The silver nanocomposite membranes exhibit excellent antibacterial properties against both Gram-positive and Gram-negative bacteria.
- Results also imply that LbL assembly can be used as a promising and flexible method for synthesizing other nanocomposite membranes. Membranes with novel properties or multiple applications could be synthesized using this method.

CHAPTER 6

Synthesis and Characterization of Novel Silver Nanocomposite Membranes with Enhanced Water Permeabilities and Excellent Antimicrobial Properties

6.1. Introduction

Membrane separation processes, such as ultrafiltration (UF), nanofiltration (NF) and forward osmosis (FO), have been used in a wide range of environmental applications including water and wastewater treatment, power generation, seawater desalination, food processing and pharmaceutical industry (Eriksson, 1988; Geckeler and Volchek, 1996; Goosen et al., 2004; Cath et al., 2006; Zhao et al., 2012b). In these operations, membrane fouling, especially the irreversible membrane biofouling, remains a major drawback in prolonged membrane applications (Fane and Fell, 1987; Hong and Elimelech, 1997; Li and Elimelech, 2004; Herzberg and Elimelech, 2007; Zou et al., 2011; Zou et al., 2013). It may result in severe water flux decline and shortened membrane life, which increases the overall energy consumption and the costs of membrane operation (Fane and Fell, 1987; Vrijenhoek et al., 2001; Li and Elimelech, 2006; Herzberg et al., 2009; Gao et al., 2011; Zou et al., 2011).

Several methods have been used to mitigate membrane biofouling, such as the fabrication of antifouling membranes (Fan et al., 2008; Zodrow et al., 2009; Zhang et al., 2012c; Liu et al., 2013b), membrane surface modification (Contreras et al., 2011; Tiraferri et al., 2011a; Diagne et al., 2012; Nikkola et al., 2013) and membrane cleaning (including both chemical and physical cleaning) during the operations (Lim and Bai, 2003; Li and Elimelech, 2004; Le-Clech et al., 2006a). Among them, the fabrication of nanocomposite membranes with bactericidal nanoparticles (NPs) has attained great attention and been widely used in membrane fouling control (Diagne et al., 2012; Zhang et al., 2012c; Liu et al., 2013b). Besides the antifouling properties, nanocomposite membranes may also exhibit various advantages, such as the enhanced water permeability and separation capacity

(Huang et al., 2012c; Ma et al., 2013), improved mechanical strength (RamanathanT et al., 2008), and good thermal stability (RamanathanT et al., 2008; Huang et al., 2012c; Zhang et al., 2012c).

Silver nanoparticles (AgNPs) are effective against various aquatic microorganisms (Sondi and Salopek-Sondi, 2004; Li et al., 2008b; Choi et al., 2010) by affecting the cell integrity and metabolism via direct interaction with cell membranes, releasing dissolved silver species, and/or generating reactive oxygen species (ROS) (Klaine et al., 2008; Jin et al., 2010). In addition, they can be used to effectively modify membrane surface properties (Diagne et al., 2012; Liu et al., 2013b). As a result, they are selected as the biocide to synthesize the antibacterial nanocomposite membranes in current study.

Various kinds of nanocomposite membranes have been successfully fabricated, including microfiltration (MF) (Diagne et al., 2012), ultrafiltration (UF) (Taurozzi et al., 2008; Zodrow et al., 2009; Huang et al., 2012c), nanofiltration (NF) (Lee et al., 2007; Liu et al., 2013b), reverse osmosis (RO) (Yang et al., 2009a) and forward osmosis (FO) (Liu et al., 2013b) membranes. Routines used to obtain above nanocomposite membranes include membrane surface modification (Yang et al., 2009a; Diagne et al., 2012), phase inversion (Taurozzi et al., 2008; Zodrow et al., 2009), interfacial polymerization (Lee et al., 2007), and layer-by-layer (LbL) (Liu et al., 2013b) assembly. However, effects of nanoparticles in membrane support layer on the formation of rejection layer thus the overall performance of the membrane have not been investigated yet.

In this study, the widely used one-step phase inversion method was adopted to form the porous membrane substrates (those contain AgNPs can be used as nanocomposite UF membranes) with decent water permeabilities (Taurozzi et al., 2008; Zodrow et al., 2009). Based on these substrates, flexible LbL assembly method was used to form the membrane selective layer of nanocomposite NF and NF-like FO membranes. Properties and performances of both PAN-Ag nanocomposite UF membranes and xLbL3.0-Ag nanocomposite NF/FO membranes were systematically evaluated. Effects of AgNPs incorporation on them were also elucidated.

Therefore, the objectives of this chapter were: 1) to synthesize silver nanocomposite UF membranes using phase inversion method, and to fabricate the silver nanocomposite NF/FO membranes with additional LbL assembly process on them; 2) to characterize membrane surface properties and their performance under UF, NF and FO conditions, 3) to evaluate the effects of silver incorporation on the formation of membrane rejection layer thus the membrane overall performance, and 4) to evaluate their antibacterial activity against Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli*. Influences of different silver incorporation concentrations on membrane performances were also systematically investigated.

6.2. Materials and methods

6.2.1. Chemicals and Solution Chemistry

Polyacrylonitrile (PAN, weight-averaged molecular weight $M_w \approx 150\,000$, Sigma–Aldrich) was dissolved in N,N-dimethylformamide (DMF, $\geq 99.8\%$, Merck) with additional lithium chloride (LiCl, 99%, Merck) as pore former for dope solutions preparation. Different amount of commercial AgNPs (~100 nm powder, contains poly(vinyl pyrrolidone) as dispersant, 99.5% trace metals basis, Sigma–Aldrich) were also added in dope solutions for fabricating the silver nanocomposite membranes. Sodium hydroxide (NaOH, $\geq 98\%$, Sigma–Aldrich) was used to prepare the alkali solution for substrates post-treatment to introduce negative charges and enhance surface hydrophilicity for LbL assembly. Poly(allylamine hydrochloride) (PAH, $M_w \approx 120\,000$ – $200\,000$, Polysciences, Inc.) and poly(sodium 4-styrene-sulfonate) (PSS, $M_w \approx 70\,000$, 30 wt.% in H_2O , Sigma–Aldrich) were used as polycation and polyanion respectively for LbL assembly. Ionic strength of polyelectrolyte solutions was adjusted using sodium chloride (NaCl, 99.5%, Merck). Glutaraldehyde (GA, 25% in water, Sigma–Aldrich) was used for crosslinking of the selective layers after the LbL assembly. Dextran samples with different molecular weight ($M_w \approx 6\,000$ – $500\,000$, Fluka and Sigma–Aldrich) were used to prepare the dextran solution for determining molecular weight cut off (MWCO). Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, 99.0–101.0%, Merck) was used to

determine NF membrane intrinsic separation properties and membrane FO performance. Live/Dead[®] BacLight[™] bacterial viability kit (Molecular Probes) was used to determine the percentages of integrated bacterial cells after exposure to membranes. Unless otherwise specified, all reagents and solutions were prepared using chemicals of analytical grade and Milli-Q water (Millipore Integral 10 water purification system, Singapore).

6.2.2. Synthesis of Nanocomposite UF, NF and FO Membranes

6.2.2.1 Synthesis of nanocomposite UF membranes via phase inversion

AgNPs (0~0.10 wt.%) were suspended in DMF and ultrasonicated (Ultrasonic Bath S450H, 50/60Hz, 2000W, Fisher Scientific Pte. Ltd.; Singapore) for 30 min at room temperature (22 ± 1 °C) before using. Dope solutions were prepared by dissolving PAN and LiCl (18 wt.% and 2 wt.% respectively) in DMF (without or with certain amount of AgNPs) in sealed containers at 60 °C with continuous stirring until the solutions became homogeneous and transparent. The cooled (22 ± 1 °C) and degassed dope solutions were cast on a clean glass plate with a 150 μ m gate height of the casing knife (Elcometer Pte. Ltd., Asia). All UF membranes (including AgNPs-free PAN membrane and silver nanocomposite PAN membranes) were obtained by solidifying the casting films in tap water bath at room temperature. The nascent membranes were kept in flowing water bath to remove residual solvent and then stored in Milli-Q water separately before tests. In our current study, the UF membranes were labeled as PAN-Ag(C), where C indicates the concentration of AgNPs in membrane dope solutions (i.e. PAN-Ag(0) indicates the AgNPs-free PAN control membranes, while PAN-Ag(0.01) means 0.01 wt.% AgNPs were incorporated into corresponding dope solution). Detailed compositions of dope solutions and designations of the UF membranes were shown in Table 6-1.

Table 6-1. Compositions of dope solutions and the designations of corresponding membranes.

Membrane ID		Compositions of dope solutions (wt.%)			
After phase inversion (UF membranes)	After LbL assembly (NF/FO membranes)	PAN	LiCl	DMF	AgNPs
PAN-Ag(0)	xLbL3.0-Ag(0)	18.00	2.00	80.00	0
PAN-Ag(0.01)	xLbL3.0-Ag(0.01)	18.00	2.00	79.99	0.01
PAN-Ag(0.02)	xLbL3.0-Ag(0.02)	18.00	2.00	79.98	0.02
PAN-Ag(0.05)	xLbL3.0-Ag(0.05)	18.00	2.00	79.95	0.05
PAN-Ag(0.10)	xLbL3.0-Ag(0.10)	18.00	2.00	79.90	0.10

6.2.2.2 Synthesis of nanocomposite NF and FO membranes via LbL assembly

LbL assembly method was adopted to synthesize nanocomposite NF and FO membranes based on the UF membranes obtained in Section 6.2.2.1. Detailed procedures of post treatment of the UF membranes, LbL assembly and crosslinking have been reported elsewhere (Qiu et al., 2011; Saren et al., 2011; Qi et al., 2012b). Briefly, after immersed in 1.5 M NaOH solution at 45 °C for 90 min, top surface of the UF membranes was firstly soaked in 1 g/L positively charged PAH solutions (with 1.0 M NaCl) and then in 1 g/L negatively charged PSS solution (with 1.0 M NaCl) for 30 min. After each soaking step, the surfaces were rinsed with Milli-Q for 5 min to remove excess polyelectrolytes. In our current study, 3.0 PAH/PSS bilayers (i.e., three times PAH/PSS coating procedure) was adopted to mitigate potential fouling in real membrane operations based on previous results (Qi et al., 2012b). The NF and FO membranes were crosslinked by immersing them in 0.1 wt.% GA solution at room temperature (22 ± 1 °C) for 2 h followed by Milli-Q water washing according to our previously published procedures (Qiu et al., 2011) to prepare the crosslinked LbL (xLbL) membranes. The NF and FO membranes were labeled as xLbL3.0-Ag(C), where C indicates the concentration of AgNPs in membrane dope solutions (i.e. xLbL3.0-Ag(0) indicates the AgNPs-free control membranes, while xLbL3.0-Ag(0.01) means 0.01 wt.% AgNPs were incorporated

into the membrane substrate). Detailed designations of the NF/FO membranes were shown in Table 6-1.

6.2.3. Characterization of Nanocomposite UF, NF and FO Membranes

6.2.3.1. Characterization of membrane morphology, contact angle, ξ -potential and porosity

Surface and cross section morphologies of the PAN-Ag UF membranes were obtained by using a Field Emission Scanning Electron Microscope (FE-SEM, JSM-7600F, JEOL Ltd.; Japan) combined with energy-dispersive X-ray (EDX) spectroscopy (X-Max^N, Oxford Instruments; England). All membrane samples were dried and coated with platinum using a vacuum electric sputter coater (JEOL JFC-1600, JEOL Ltd.; Japan) before SEM/EDX examination. Contact angle measurements were performed with the sessile drop method using OCA Contact Angle System (DataPhysics Instruments GmbH; Germany). The reported contact angles are average of 10 measurements obtained from 3 independent membrane samples. Membrane surface zeta-potential was measured by eletrokinetic analyzer (SurPASS, Anton Paar GmbH; Austria) using a 10 mM NaCl background solution with an applied pressure of 300 mbar and a membrane gap of 120 μm . Zeta potential values were given by the best fittings with Helmholtz–Smoluchowski equation (Qi et al., 2012a). The reported zeta-potentials were the average of 4 measurements. Membrane porosity (ε) was determined by measuring the dry mass (m_{dry}) and wet mass (m_{wet}) of membrane samples on a digital analytical balance (AB204-S/FACT, Mettler-Toledo Pte Ltd; Singapore) and calculated using following equation (Sukitpaneenit and Chung, 2009; Wei et al., 2011c):

$$\varepsilon = \frac{(m_{\text{wet}} - m_{\text{dry}}) / \rho_w}{(m_{\text{wet}} - m_{\text{dry}}) / \rho_w + m_{\text{dry}} / \rho_m} \times 100\% \quad (6-1)$$

where ρ_w and ρ_m are the densities of water (1.00 g/cm³) and PAN (1.18 g/cm³) respectively. It is assumed that all pores in membrane samples were fully filled with water after immersing in water for 24 h. The reported membrane porosities are average of 3 measurements obtained from 3 independent membrane samples.

6.2.3.2 Viscosity of dope solution

The viscosities of dope solutions (with and without AgNPs) were measured using Modular Compact Rheometer (Physica MCR 101, Anton Paar; USA). 100 μL sample of dope solution was placed in the central of sample measuring plate at 22 $^{\circ}\text{C}$. The reported dope solution viscosities are the values at shear rate of 10 s^{-1} and the average of at least 3 measurements obtained from 3 independent samples.

6.2.3.3. UF Performance Evaluation

Pure water permeability of the nanocomposite PAN-Ag UF membrane was evaluated by using a high pressure crossflow filtration setup reported previously (Saren et al., 2011; Qi et al., 2012b). Pure water permeability (A) was determined based on weighting permeate water samples and the following equation:

$$A = \frac{J_w}{\Delta P - \Delta \pi} \quad (6-2)$$

where A is the water permeability coefficient ($\text{m/s}\cdot\text{Pa}$), J_w is the water flux, ΔP is the trans-membrane pressure, and $\Delta \pi$ is the osmotic pressure difference across the membrane ($\Delta \pi = 0$ under our testing conditions) (Mulder, 1996). The reported PAN-Ag UF membrane water permeabilities were obtained from 3 replicate measurements of 3 independent membrane samples.

Molecular weight cut off (MWCO) of nanocomposite PAN-Ag UF membrane was determined using a 2000 ppm dextran feed solution at an applied pressure of 1 bar under crossflow conditions. The dextran solution was made by a mixture of several different molecular weights ranging from 6000~500,000 Da following the procedures described in (Wei et al., 2006; Setiawan et al., 2011). Dextran concentrations were analyzed by gel permeation chromatography (GPC) on a PL-GPC 50 Plus system (Polymer Laboratories, Varian Inc.; USA). The system used double PL Aquagel OH Mixed-M 8 μm columns (Polymer Laboratories, Varian Inc.; USA) with packing material of 7.5 mm diameter.

6.2.3.4. NF Performance Evaluation

NF performance of the nanocomposite xLbL3.0-Ag NF/FO membranes was evaluated by using the high pressure crossflow filtration setup in Section 6.2.3.3. Pure water permeability (A) was determined using Equation (6-2). Salt rejection (R) was determined based on conductivity measurements of 5 mM MgCl_2 feed solution and permeate water using conductivity meter (Ultrameter II, Myron L Company; CA, USA) and the following equation:

$$R = \left(1 - \frac{C_p}{C_f} \right) \times 100\% \quad (6-3)$$

where R is the salt rejection of MgCl_2 , C_p and C_f are the concentrations (in terms of conductivity) of MgCl_2 in permeate and feed solution respectively.

Salt permeability coefficient B and the B/A value at a given pressure were calculated using following equation (Tang et al., 2010):

$$B = \left(\frac{1}{R} - 1 \right) \cdot A(\Delta P - \Delta \pi) \quad (6-4)$$

NF performance results were obtained from 3 replicate measurements of 3 independent membrane samples.

6.2.3.5. FO Performance Evaluation

FO performance, including water flux (J_v), solute flux (J_s), and solute flux over water flux (J_s/J_v), was evaluated using a crossflow FO setup reported previously (Saren et al., 2011; Qi et al., 2012b). FO water flux was determined by measuring the weight change of the feed tank using a digital mass balance connected to a computer data logging system. Salt flux through the FO membrane was determined by monitoring the conductivity change in the feed solution (FS). 0.5 M MgCl_2 solution was used as draw solution (DS). Milli-Q water (for J_s measurement) or 10 mM NaCl (for J_v measurement) was used as FS. Both membrane orientations, active layer facing draw solution (AL-DS) and active layer facing feed solution (AL-FS),

were tested. All FO performance results were obtained from 3 replicate measurements of 3 independent membrane samples.

6.2.4. Membrane Antibacterial Tests

Before antibacterial tests, all freshly fabricated membranes were immersed in Milli-Q water for 48 h to remove the potential residual chemicals. Gram-positive *Bacillus subtilis* (ATCC 6633) and Gram-negative *Escherichia coli* (ATCC 8739) were used as model organisms to perform membrane antibacterial tests. The cell density of 1.0×10^7 cells/mL was achieved by suspending the harvested cells in environmentally relevant freshwater (Tchobanoglous and Schroeder, 1985; Van Hoecke et al., 2011). Three membrane antibacterial tests, colony-forming units (CFU) test, bacterial cell membrane integrity test, and diffusion inhibition zone (DIZ) test, were used to evaluate the membrane antibacterial properties. The detailed procedures of these tests were reported elsewhere (Liu et al., 2013b; Nikkola et al., 2013; Liu et al., 2014). For CFU and bacterial cell membrane integrity tests, bacterial suspensions were contacted with membranes for 24 h before tests. AgNPs-free xLbL3.0-Ag(0) membrane was used as a control.

6.2.4.1. Colony-forming units (CFU) test

CFU test was performed by serial diluting the bacterial suspensions which contacted with the membrane samples for 24 h according to CLSI M07-A9 (CLSI, 2012a). 100 μ L diluent was inoculated and spread on a nutrient agar plate and incubated for another 24 h. CFU results were obtained from 9 replicates by mixing 3 batches of independently cultivated bacteria with 3 independently fabricated membranes respectively (3 agar plates were used as parallel samples in each measurement).

6.2.4.2. Bacterial cell membrane integrity test

After 24 h exposure to membranes, 100 μ L of bacterial suspensions were completely mixed with the solutions of Live/Dead[®] BacLight[™] bacterial viability kit into specific wells of a 96-well F-bottom polystyrene microplate (Greiner Bio-One; Monroe, NC, USA). Percentages of cell membrane integrity bacteria were obtained using the green/red fluorescence ratios detected by Synergy 2 Multi-Mode

Microplate Reader (BioTek Singapore, Singapore) and calculated by the calibration curves obtained by blending different known ratios of live-to-dead bacteria (3 independent batches with at least 18 replicates) (Jin et al., 2010; Liu et al., 2013b; Liu et al., 2014). The reported percentages are based on 27 measurements in 9 individual plates using 3 independent batches of bacteria and 3 independently fabricated membranes.

6.2.4.3. Diffusion inhibition zone (DIZ) test

Diffusion inhibition zone test of xLbL3.0-Ag nanocomposite NF/FO membranes was performed by following a modified CLSI document M02-A11 (CLSI, 2012b). 100 μ L bacterial inoculums were spread on the agar plates evenly. Different nanocomposite membranes were punched into circle discs with a diameter of 12.7 mm using circle disc punch (Ted Pella, Inc.; Redding, CA, USA), and placed on agar plates with the active layers facing up and incubated at optimal bacterial growth conditions for 24 h. The DIZ formed around the nanocomposite membranes were observed and measured.

6.3. Results and discussion

6.3.1. Membrane Characterization

6.3.1.1. Membrane FE-SEM images

Figure 6-1 presents the FE-SEM images of top surface of AgNPs-free control membrane (PAN-Ag(0) UF membrane) and PAN-Ag(0.10) nanocomposite UF membrane. In Figure 6-1(b), nanosized particles appeared on the top surface of PAN-Ag(0.10) nanocomposite UF membrane, while no such particles were observed on the control membrane (Figure 6-1(a)). Enlarged FE-SEM image of PAN-Ag(0.10) nanocomposite UF membrane (Figure 6-2(a)) and its corresponding EDX image (Figure 6-2(b)) clearly indicated the particles on membrane surface are AgNPs. EDX spectra of PAN-Ag(0) and PAN-Ag(0.10) membranes (Figure 6-2(c)) also suggested the successful incorporation of AgNPs in PAN-Ag(0.10) nanocomposite UF membrane. In addition, no severe aggregation of AgNPs was observed on surface of the nanocomposite membrane even at the highest silver incorporation concentration, 0.10 wt.% as silver (see the FE-SEM image of PAN-Ag(0.10) nanocomposite UF membrane in Figure 6-1(b)).

Figure 6-1. FE-SEM images of the top surface of PAN-Ag(0) UF membrane and PAN-Ag(0.10) nanocomposite UF membrane. (a) Top surface of PAN-Ag(0) membrane; (b) Top surface of PAN-Ag(0.10) nanocomposite membrane, where the white dots represent the AgNPs on membrane surface.

Figure 6-2. FE-SEM image and corresponding EDX image and spectra of PAN-Ag(0.10) nanocomposite UF membrane. (a) FE-SEM image of the top surface of PAN-Ag(0.10) nanocomposite membrane. (b) Corresponding EDX image of the top surface of PAN-Ag(0.10) nanocomposite membrane, where silver is depicted by light green to highlight the distribution of AgNPs on membrane surface. (c) EDX spectra of AgNPs-free PAN-Ag(0) membrane and PAN-Ag(0.10) nanocomposite membrane.

6.3.1.2. Membrane hydrophilicity and surface charge of PAN-Ag nanocomposite UF membranes

Contact angles of PAN-Ag nanocomposite UF membranes are presented in Figure 6-3(a). The smaller contact angle indicates a more hydrophilic membrane surface. Among all membranes, the AgNPs-free control membrane, PAN-Ag(0) UF membrane, had the highest contact angle of $60.6 \pm 2.3^\circ$. As AgNPs concentrations increased from 0 to 0.02 wt.%, contact angles of nanocomposite UF membranes decreased significantly ($43.1 \pm 1.2^\circ$ for PAN-Ag(0.02) UF membrane). Similar

enhanced hydrophilicity effects were also observed in previous studies of various nanocomposite membranes incorporating nanoparticles of hydrophilic nature (Luo et al., 2005; Diagne et al., 2012; Tiraferri et al., 2012; Zhang et al., 2012c; Liu et al., 2013b; Pendergast et al., 2013). However, further increasing AgNPs concentration from 0.02 to 0.10 wt.% was less effective in improving membrane hydrophilicity, noting that the contact angles of these membranes ($\sim 43^\circ$) were only marginally greater than the characteristic contact angles of silver surfaces of $\sim 38^\circ$ (Bartell and Cardwell, 1942).

Zeta potentials of PAN-Ag nanocomposite UF membranes in 10 mM NaCl solution are shown in Figure 6-3(b). Isoelectric points of PAN-Ag nanocomposite UF membranes decreased as increase of AgNPs concentrations. This may due to the significant negative charged AgNPs (Figure 6-4(a)) brought down the overall surface charges of nanocomposite membranes.

(a)

(b)

Figure 6-3. (a) Contact angles of PAN-Ag nanocomposite UF membranes. Error bars were based on standard deviations of 10 measurements obtained from 3 independent membrane samples. (b) Zeta potentials of PAN-Ag nanocomposite UF membranes in 10 mM NaCl solution with the pH ranging from 3 to 10. Error bars were based on standard deviations of 4 measurements.

(a)

(b)

Figure 6-4. Zeta potentials of (a) AgNPs in Milli-Q water and (b) xLbL3.0-Ag nanocomposite NF/FO membranes in 10 mM NaCl solution with the pH ranging from 3 to 10. Error bars of zeta potentials of AgNPs were based on the standard deviations of 6 measurements, while those of xLbL3.0-Ag nanocomposite NF/FO membranes were based on the standard deviations of 4 measurements.

6.3.1.3. Membrane hydrophilicity and surface charge of xLbL3.0-Ag nanocomposite NF/FO membranes

The hydrophilicity and zeta potential of the xLbL3.0-Ag nanocomposite NF/FO membranes were also characterized in the current study (Figure 6-5 and Figure 6-4(b)). No significant changes of these surface properties (membrane contact angles remained the same, and the zeta potentials fluctuated slightly) were observed between the control (xLbL3.0-Ag(0) NF/FO membrane) and the AgNPs loaded membranes, consistent with the fact that membrane hydrophilicity and surface charge are mainly depended on its outmost surface (Qi et al., 2012a).

Figure 6-5. Contact angles of xLbL3.0-Ag nanocomposite NF/FO membranes. Error bars were based on the standard deviations of 10 measurements obtained from 3 independent membrane samples.

6.3.2. Membrane UF Performance

Effect of AgNPs incorporation in PAN-Ag UF membranes on water permeability is shown in Figure 6-6(a). When AgNPs concentration ≤ 0.02 wt.%, water permeability of PAN-Ag UF membrane was improved as AgNPs concentration increasing. This may due to the changes of membrane physicochemical property (i.e. increase of membrane hydrophilicity in Figure 6-3(a) and its porous structure (see this section)) affected by AgNPs incorporation. Compared with the AgNPs-free control membrane, 14.3 % enhancement of water permeability was achieved for the membrane PAN-Ag(0.02). Water permeability of PAN-Ag UF membranes decreased as further increasing AgNPs concentration. In addition to water permeability, molecular weight cut off (MWCO), which reflects the membrane rejection properties (and its surface pore sizes) (Cho et al., 1999), was also characterized. In Figure 6-6(b), MWCO results of PAN-Ag UF membranes with different AgNPs incorporations are highly correlated with the water permeability results of PAN-Ag nanocomposite UF membranes (Figure 6-6(a)). When AgNPs concentration ≤ 0.02 wt.%, MWCOs of PAN-Ag UF membranes increased as

AgNPs concentration increasing, which may suggest the increase of membrane water permeability and decrease of membrane rejection of certain macromolecules. When AgNPs concentration > 0.02 wt.%, both MWCOs and water permeability of PAN-Ag UF membranes decreased.

The impact of AgNPs loading on the membrane water permeability can be explained by the combined effects of MWCO (Figure 6-6(b)) and membrane bulk pore changes at various particle loadings (Figure 6-6(c) and (d)). According to Figure 6-6(c), the membrane bulk pores also first increased (when AgNPs concentration ≤ 0.02 wt.%) and then decreased (when AgNPs concentration > 0.02 wt.%), showing an identical trend to those for water permeability and MWCO. FE-SEM cross-section images of PAN-Ag UF membranes in Figure 6-6(d) provided a direct observation of the changes of membrane bulk pores with increase of AgNPs concentration. At the AgNPs concentration of 0.02 wt.%, nanocomposite UF membrane appeared to have larger MWCO, greater porosity and more uniformly distributed pore size (Figure 6-6(d)iii), which explains its better permeability compared to the other membranes. Similar, the reduction in water permeability after further increasing AgNPs loading above 0.02 wt.% was due to the smaller MWCO and less porous membrane structures (Figure 6-6(d)iv and v). The latter can be better understood by studying the viscosity of the polymer dope solutions used for casting membranes. In the current study, the dope solution viscosity started to increase when AgNPs concentration was greater than 0.02 wt.% (Figure 6-7). Since high dope solution viscosity may hinder membrane pore formation in phase inversion process (by prohibiting the immediate convective type solvent exchange in an instantaneous liquid–liquid phase separation process) (Smolders et al., 1992; Sukitpaneenit and Chung, 2009), both membrane surface pore sizes and bulk pore densities of PAN-Ag(0.05) and PAN-Ag(0.10) nanocomposite UF membranes decreased correspondingly.

Taken together, the highest membrane water permeability appeared at AgNPs incorporation of 0.02 wt.%, which is likely due to the competing effects of 1) the increased membrane hydrophilicity at higher AgNPs concentration that promotes water permeation (note that this effect also resulted in the changes of membrane

structures, for example, the increase of MWCO and bulk pore sizes, and became weaker at AgNPs loading above 0.02 wt.%); and 2) the higher polymer dope solution viscosity may hinder the pore formation (both surface and bulk pores) in phase inversion process.

(a)

(b)

(c)

(d)

Figure 6-6. Effects of AgNPs incorporation in PAN-Ag nanocomposite UF membranes on membrane UF performance: (a) water permeability of PAN-Ag UF membranes, evaluated under an applied pressure of 5 bar with Milli-Q water as feed solution; (b) molecular weight cut off (MWCO) of PAN-Ag UF membranes, evaluated under an applied pressure of 1 bar with dextran solution ($M_w \approx 6\text{--}500$ kDa) as feed solution; (c) porosity of PAN-Ag UF membranes. Error bars were based on standard deviations of 3 measurements of 3 independent membranes. (d) FE-SEM images of the cross-sections of PAN-Ag UF membranes: (i) PAN-Ag(0) membrane, (ii) PAN-Ag(0.01) membrane, (iii) PAN-Ag(0.02) membrane, (iv) PAN-Ag(0.05) membrane, and (v) PAN-Ag(0.10) membrane.

Figure 6-7. Effect of AgNPs incorporation on dope solution viscosities. Error bars were based on the standard deviations of 3 measurements of 3 independent samples.

6.3.3. Membrane NF and FO Performances

6.3.3.1. Membrane NF performance

Effects of AgNPs incorporation on membrane NF performance are shown in Figure 6-8. Water permeabilities of xLbL3.0-Ag nanocomposite NF/FO membranes (Figure 6-8(a)) were highly correlated with those of PAN-Ag UF membranes

(Figure 6-6(a)), which suggest membrane substrate contributed greatly to the water permeability (Wei et al., 2011c). It may also due to the porosity changes of PAN-Ag UF membranes have some impact on the LBL assembly structure thus the permeabilities of xLbL3.0-Ag NF/FO membrane. Water permeability of xLbL3.0-Ag nanocomposite NF/FO membrane enhanced most significantly at AgNPs concentration of 0.02 wt.% (10.1 % improvement of water permeability compared with the AgNPs-free control). Effects of AgNPs incorporation on MgCl_2 rejection and B/A ratios of xLbL3.0-Ag NF/FO membranes are shown in Figure 6-8(b) and (c) respectively. The additional AgNPs increased membrane MgCl_2 rejection slightly. Decreased B/A ratios also suggest better selectivities of the nanocomposite membranes (Wei et al., 2011c; Liu et al., 2013b). These may due to the porosity changes of PAN-Ag UF membranes and the enhancement of membrane surface charges caused by AgNPs incorporation benefits the selective layer formation in LbL assembly process (Zhang et al., 2007).

(a)

(b)

(c)

Figure 6-8. Effects of AgNPs incorporation in xLbL3.0-Ag nanocomposite NF/FO membranes on membrane NF performance: (a) water permeability, evaluated in RO testing mode over an applied pressure of 5 bar with Milli-Q water as feed solution; (b) salt rejection, evaluated in the RO testing mode at 5 bar with 5 mM MgCl₂ as feed solution; and (c) *B/A* ratio. Error bars were based on standard deviations of 3 measurements of 3 independent membranes.

6.3.3.2. Membrane FO performance

Figure 6-9 presents the effects of AgNPs incorporation on membrane FO performance. Water fluxes (J_v) and solute flux over water flux values (J_s/J_v values) of xLbL3.0-Ag nanocomposite membranes with both membrane orientations (i.e. AL-DS and AL-FS) are shown in Figure 6-9(a) and (b) respectively. Water fluxes with both orientations exhibited the same trend of water permeabilities of nanocomposite UF and NF membranes (shown in Figure 6-6(a) and Figure 6-8(a)), which also suggest membrane substrate contributed greatly to water permeability in FO operations. AgNPs incorporation determined S values of nanocomposite membranes due to their effect on membrane porosity (shown in Figure 6-6(b), (c) and (d)). The J_v of the nanocomposite membranes were superior to most NF-like FO membranes reported previously (Liu et al., 2013b). At AgNPs concentration of 0.02 wt.%, J_v of xLbL3.0-Ag(0.02) nanocomposite NF/FO membrane increased by 24.4 % for AL-DS orientation and 17.0 % for AL-FS orientation. The J_s/J_v values of all xLbL3.0-Ag nanocomposite membranes remained the same with the AgNPs free control, which indicated the additional AgNPs in membrane substrates had little adverse effect on membrane separation property. J_s/J_v in AL-FS orientation is always greater than that in the other orientation. It is because the dilutive ICP in AL-FS orientation is more severe compared with the concentrative ICP in AL-DS orientation, which usually results in greater water flux decline.

(a)

(b)

Figure 6-9. Effects of AgNPs incorporation in xLbL3.0-Ag nanocomposite NF/FO membranes on membrane FO performance: (a) water flux under AL-DS and AL-FS orientations, evaluated by using 0.5 M MgCl₂ as draw solution and 10 mM NaCl as feed solution; (b) solute flux over water flux under AL-DS and AL-FS orientations, evaluated by using 0.5 M MgCl₂ as draw solution and Milli-Q water as feed solution. Error bars were based on standard deviations of 3 measurements of 3

independent membranes.

6.3.4. Membrane Antibacterial Studies

6.3.4.1. Colony-forming units test

Antibacterial activities of xLbL3.0-Ag nanocomposite NF/FO membranes against Gram-positive *B. subtilis* and Gram-negative *E. coli* were tested using colony-forming units (CFU) method, and the corresponding results are shown in Figure 6-10. The reduced CFU indicated the decrease of live bacteria after 24 h exposure to the membranes. Since the AgNPs-free xLbL3.0 membrane had little effect on the CFU number (Figure 6-11), the antibacterial activities of xLbL3.0-Ag nanocomposite membranes could be attributed to the AgNPs incorporation in membrane substrates. The bactericidal activity of nanocomposite membrane was enhanced (the CFU number decreased) as AgNPs incorporation increased. At the highest AgNPs incorporation concentration (0.10 wt.%), the nanocomposite xLbL3.0-Ag(0.10) membrane exhibited excellent bactericidal activities: ~6 Log CFU reduction for *B. subtilis* and ~5 Log CFU reduction for *E. coli*. The bactericidal effect of xLbL3.0-Ag nanocomposite membranes against *B. subtilis* was better than that against *E. coli*, which might be due to different cell wall structures of the bacteria (Kučerka et al., 2008). The lipopolysaccharide (LPS) layer outside the Gram-negative bacterial cell membrane contributes significantly to bacterial structural integrity and plays an important role in protecting varieties of substances permeating into the bacteria (Jin et al., 2010; Li et al., 2011a; Liu et al., 2013b; Liu et al., 2014). Effect of AgNPs incorporation in PAN-Ag nanocomposite UF membranes on CFU test was shown in Figure 6-12. Comparable bactericidal effect was observed for AgNPs incorporated UF membranes. This suggested the additional selective layers in xLbL3.0-Ag membranes had limited impeding effect upon membrane bactericidal activity under the current experimental conditions.

Compared with the membrane antibacterial activities presented in Chapter 5 (Liu et al., 2013b), the xLbL3.0-Ag(0.10) nanocomposite NF/FO membrane exhibited much better antibacterial performance (~6 Log reduction vs. ~3 Log reduction for *B.*

subtilis, and ~5 Log reduction vs. ~2 Log reduction for *E. coli*). This may be due to the membrane fabrication routine adopted in current study allowed a larger amount of AgNPs embedded in membranes, which enhanced silver leaching and membrane bactericidal activities. Detailed silver leaching results of xLbL3.0-Ag NF/FO membranes and the comparisons are presented in Appendix D.

Figure 6-10. Effect of AgNPs incorporation in xLbL3.0-Ag nanocomposite NF/FO membranes on colony- forming units (CFU) test against Gram-positive *B. subtilis* and Gram-negative *E. coli* after 24 h exposure to the membranes. Error bars were based on standard deviations of 9 replicates obtained from 3 batches of independent cultivated bacteria mixed with 3 independent fabricated membranes respectively.

Figure 6-11. Effect of AgNPs-free xLbL3.0-Ag(0) NF/FO membrane on bacterial CFU test (after 24 h exposure). Error bars were based on the standard deviations of 9 replicates obtained from 3 batches of independent cultivated bacteria mixed with 3 independent fabricated membranes respectively.

Figure 6-12. Effect of AgNPs incorporation in PAN-Ag nanocomposite UF membranes on colony-forming units (CFU) test against Gram-positive *B. subtilis* and Gram-negative *E. coli* after 24 h exposure to the membranes. Error bars were

based on standard deviations of 9 replicates obtained from 3 batches of independent cultivated bacteria mixed with 3 independent fabricated membranes respectively.

6.3.4.2. Bacterial cell membrane integrity test

Effect of AgNPs incorporation on bacterial cell membrane integrity test is shown in Figure 6-13. The incorporation of AgNPs significantly decreased the amount of bacteria with integrated cell membranes. Results of bacterial cell membrane integrity test were also highly consistent with the CFU results: 1) xLbL3.0-Ag(0.10) nanocomposite membrane exhibited the best bactericidal activity against both *B. subtilis* and *E. coli* (decreased from 100 % to 6.2 % and 7.4 %, respectively); 2) Gram-positive *B. subtilis* was more sensitive than *E. coli* (the percentages of *B. subtilis* with integrated cell membranes are less than those of *E. coli*); 3) the additional selective layers in xLbL3.0-Ag membranes had little effect on membrane bactericidal performance under the experimental conditions (comparable percentages of bacteria with integrated cell membranes contacted with PAN-Ag nanocomposite UF membranes are shown in Figure 6-14).

Figure 6-13. Effect of AgNPs incorporation in xLbL3.0-Ag nanocomposite NF/FO membranes on bacterial cell membrane integrity test against Gram-positive *B.*

subtilis and Gram-negative *E. coli* after 24 h exposure to the membranes. Error bars were based on standard deviations of 27 replicates obtained from 3 batches of independent cultivated bacteria mixed with 3 independent fabricated membranes.

Figure 6-14. Effect of AgNPs incorporation in PAN-Ag nanocomposite UF membranes on bacterial cell membrane integrity test against Gram-positive *B. subtilis* and Gram-negative *E. coli* after 24 h exposure to the membranes. Error bars were based on the standard deviations of 27 replicates obtained from 3 batches of independent cultivated bacteria mixed with 3 independent fabricated membranes respectively.

6.3.4.3. Diffusion inhibition zone test

Diffusion inhibition zone (DIZ) images of xLbL3.0-Ag nanocomposite NF/FO membranes were presented in Figure 6-15. The radius of bacterial-free area formed around the nanocomposite membranes indicated the relative toxicity of membranes against the bacteria. The DIZ results are consistent with those of CFU test and bacterial cell membrane integrity test. As the loading of AgNPs increased, distinct inhibition zones of ~14.4–15.3 mm and ~15.3–15.7 mm were observed for the xLbL3.0-Ag(0.05) and xLbL3.0-Ag(0.10) nanocomposite membranes, respectively.

Figure 6-15. Effect of AgNPs incorporation in xLbL3.0-Ag nanocomposite NF/FO membranes on diffusion inhibition zone (DIZ) test against Gram-positive *B. subtilis* and Gram-negative *E. coli* after 24 h exposure to the membranes.

6.3.5. Environmental Implications

Membrane fouling, especially the irreversible biofouling, remains a critical obstacle for prolonged membrane operation. This study investigates the fabrication of silver nanocomposite membranes with potentially enhanced water permeability and excellent bactericidal activity. Those membranes could be used in UF, and NF or FO process, respectively. Results of this study could be used for: 1) providing a new aspect of fabrication the high water permeability nanocomposite membranes (in addition to the use of porous nanoparticles); 2) investigating various factors that may affect the properties of nanocomposite membranes; 3) guiding the further investigations on synthesis and characterization of nanocomposite membranes. Although the current study primarily focused on UF, NF and NF-like FO membranes and their potential applications, AgNPs incorporation in membrane dope solution can be potentially applied to fabricate other types of nanocomposite membranes including reverse osmosis. In addition, the silver release from the silver nanocomposite membranes will not cause any adverse effect to human health and the environment, and the antibacterial properties of the nanocomposite membranes may last for a relatively long time (Appendix Section D). The incorporation of AgNPs in membranes is also economically viable (Appendix Section E), especially by considering the potential benefits of AgNPs in membrane biofouling control and the long term membrane operation.

6.4. Conclusions

In this study, synthesis and characterization of silver nanocomposite UF, NF and FO membranes with enhanced water permeabilities and excellent antimicrobial properties based on “phase inversion + LbL assembly” were investigated. Main conclusions can be drawn from this study are as follows:

- Silver nanocomposite UF membranes can be fabricated via phase inversion method; based on them, silver nanocomposite NF/FO membranes can be obtained with additional LbL assembly process.

- The silver nanocomposite membrane exhibited enhanced water permeability in UF, NF and FO applications by 14.3 %, 10.1 % and 24.4 % respectively, at the optimal AgNPs incorporation concentration of 0.02 wt.%.
- Enhanced water permeability of UF membranes may mainly be due to the AgNPs incorporation affecting both membrane surface and bulk pore sizes together with membrane hydrophilicity; while possible explanations for the variation in water permeability of NF/FO membranes might be the changes in UF membrane properties (including the water permeability and the porosity changes which may further affect LbL assembly) brought by AgNPs.
- The silver nanocomposite membranes exhibit excellent antibacterial properties against both Gram-positive *B. subtilis* and Gram-negative *E. coli* in three membrane antibacterial tests conducted by using environmentally relevant freshwater (e.g. ~6 Log CFU reduction for *B. subtilis* and ~5 Log CFU reduction for *E. coli*).

CHAPTER 7

Conclusions and Recommendations

7.1. Conclusions

The fabrication, characterization, intrinsic membrane transport properties, forward osmosis (FO) performance and bactericidal activities of the silver nanocomposite nanofiltration (NF) and FO membranes based on layer-by-layer (LbL) assembly were systematically investigated. Different synthesis routines (incorporating the silver nanoparticles (AgNPs) in membrane selective layer or substrate) and the influencing factors (such as the effects of doping amounts and doping times of AgNPs on membrane performances) were evaluated. The major findings and conclusions are summarized as follows:

In Chapter 4:

- (1) Within the range of particle size investigated (10–100 nm), for the AgNPs of same kind, the smaller ones had higher nanotoxicity.
- (2) Under the experimental conditions, three model natural organic matter (NOM) had no significant effects on the bactericidal activity and stability of AgNPs in the absence of Ca^{2+} . When introducing additional Ca^{2+} into the freshwater matrices, stability of AgNPs decreased greatly and its bactericidal activity decreased significantly as well. This may due to aggregation of AgNPs as a result of Ca^{2+} -NOM-AgNPs interaction partially neutralized the negative charge of AgNPs.
- (3) Chelating agent, EDTA, exhibited a concentration-dependent effect on AgNPs bactericidal activity: at low concentration (1 mg/L), EDTA decreased the nanotoxicity of AgNPs by converting the relatively higher toxic dissolved Ag species, such as Ag^+ and Ag-Cl complex, into a series of lower toxic Ag-EDTA complex; at high concentrations (5 and 20 mg/L), EDTA increased the toxicity of AgNPs probably by disassembling cell

membrane structures and enhancing the direct penetration of AgNPs and dissolved Ag species into bacterial cells.

- (4) Results also imply that water chemistry conditions can affect the toxicity of AgNPs greatly. Potential applications, such as nanotoxicity control or nano-disinfection, could be developed based on the findings obtained from this study.

In Chapter 5:

- (5) Silver nanocomposite membranes can be fabricated via LbL assembly. Performances of silver nanocomposite membranes are highly depended on the silver incorporation in the membranes, which could be controlled by using different membrane synthesis routines and the doping times of AgNPs.
- (6) The silver nanocomposite membranes could be adopted in both NF and FO processes with comparable performances to AgNPs-free controls. Moreover, the performances were superior to most NF-like FO membranes reported in previous literature.
- (7) The silver nanocomposite membranes exhibit excellent antibacterial properties against both Gram-positive and Gram-negative bacteria.
- (8) Results also imply that LbL assembly can be used as a promising and flexible method for synthesizing other nanocomposite membranes. Membranes with novel properties or multiple applications could be synthesized using this method.

In Chapter 6:

- (9) Silver nanocomposite ultrafiltration (UF) membranes can be fabricated via phase inversion method; based on them, silver nanocomposite NF/FO membranes can be obtained with additional LbL assembly process.

- (10) The silver nanocomposite membrane exhibited enhanced water permeability in UF, NF and FO applications by 14.3 %, 10.1 % and 24.4 % respectively, at the optimal AgNPs incorporation concentration of 0.02 wt.%.
- (11) Enhanced water permeability of UF membranes may mainly due to the AgNPs incorporation affected both membrane surface and bulk pore sizes together with membrane hydrophilicity; while possible explanations to the variation in water permeability of NF/FO membranes might be the changes of UF membrane properties (including the water permeability and the porosity changes which may further affect LbL assembly) brought by AgNPs.
- (12) The silver nanocomposite membranes exhibit excellent antibacterial properties against both Gram-positive *B. subtilis* and Gram-negative *E. coli* in three membrane antibacterial tests conducted by using environmentally relevant freshwater (e.g. ~6 Log CFU reduction for *B. subtilis* and ~5 Log CFU reduction for *E. coli*).

7.2. Recommendations

This study has systematically investigated the fabrication, characterization and potential applications of the silver nanocomposite NF and FO membranes obtained via LbL assembly. Based on the current study, further research work could be conducted in order to obtain the antibacterial nanocomposite membranes with enhanced performances and versatile environmental applications. Better understanding of the bactericidal mechanism of nanocomposite membranes in real membrane operations is also worthy of further studies. The suggestions include:

- (1) The prolonged membrane operations under NF and FO processes of the silver nanocomposite NF and FO membranes fabricated via LBL assembly have not been studied yet. The effects of operation conditions (such as pH, ionic strength, applied pressure and etc.) on membrane performances (including the separation and antibacterial performances) are also worthwhile to investigate.

- (2) Fabrication and characterization of the antibacterial nanocomposite pressure retarded osmosis (PRO) membranes based on LbL assembly. The silver nanocomposite NF and FO membranes with AgNPs incorporated in membrane substrate (support layer) were successfully synthesized in this study. The antibiofouling silver nanocomposite PRO membranes could be developed based on current study. Moreover, the LbL FO membranes may have potential high water permeability in PRO process.
- (3) The synthesis conditions of the nanocomposite membranes based on LbL assembly are worthwhile to investigate. Pretreatment of the substrate and post-treatment of the LbL membrane (such as thermal and chemical crosslinking and UV curing) may improve membrane performance and enlarge its applications.
- (4) Double-skinned design can effectively mitigate membrane fouling in certain circumstances. The fabrication of double-skinned nanocomposite membranes based on LbL assembly may further benefit membrane fouling control.
- (5) Biofouling behavior as well as the antibiofouling mechanisms of silver nanocomposite NF/FO LbL membranes in NF and FO processes have not been investigated yet.
- (6) The silver leaching issue and life cycle evaluation of the silver nanocomposite membranes are also important and challenging topics.
- (7) Besides AgNPs, other nanomaterials with specific properties can also be used to fabricate the nanocomposite membranes based on LbL assembly method.

APPENDIX

Section A

Effects of NOM on live bacterial percentages

Three model NOM (HA, BSA and AA) did not cause any significant adverse effects on live bacterial percentages (both *B. subtilis* and *E. coli*) under the experimental conditions.

Figure A-1. Percentages of live bacterial cells (*B. subtilis* and *E. coli*) in water matrices containing three model NOM (HA, BSA and AA) after 24 h incubation in respective optimal growth temperature without Ag (total ionic strength = 5.6 mM as NaCl, pH = 7.0).

Section B***Dispersion and aggregation of AgNPs in PSS solution, Milli-Q water and PAH solution***

Figure B-1 presents the images of AgNPs (0.01 wt.% as silver) in PSS solution (Figure B-1(a)), Milli-Q water (Figure B-1(b)) and PAH solution (Figure B-1(c)) respectively. Images show that severe aggregation of AgNPs (black precipitations) appeared in PSS solution in Figure B-1(a), while there was no precipitation of AgNPs observed in Milli-Q water or PAH solution (Figure B-1(b) and Figure B-1(c)).

Figure B-1. Images of AgNPs (0.01 wt.%) in (a) PSS solution, (b) Milli-Q water and (c) PAH solution.

Section C

Comparison of the NF and FO performances of the silver nanocomposite membranes with membranes in literature

*C-1. Comparison of NF performances of the silver nanocomposite membranes with membranes in literature***Table C-1.** Comparison of membrane NF performance of the silver nanocomposite NF/FO membranes with membranes in literature

Membranes	A ($\times 10^{-12}$ m/s Pa)	MgCl ₂ rejection (%)	B/A (KPa)	Reference
xLbL2.5	9.8	95.4	4.8	current study
xLbL2.5-Ag(PSS1)	10.9	94.5	5.8	
xLbL2.5-Ag(W1)	7.2	95.4	4.9	
xLbL2.5-Ag(PAH1)	9.1	95.9	4.8	
E-23-5-50K ^a	44.2	89.0	12.4	(Setiawan et al., 2012)
Double-skinned CA ^b	2.1	73.5	36.0	(Su et al., 2012)
PAI#1-NF ^c	25.8	83.9	19.2	(Fang et al., 2012)
PAI#2-NF ^c	25.0	90.4	10.6	
xLbL3-3 ^d	8.9	93.2	15.9	(Qi et al., 2012b)
xLbL3 ^e	19.2	94.2	13.3	(Qiu et al., 2011)
3#LbL FO ^f	28.4	64.0	33.8	(Saren et al., 2011)
ST#1 ^g	4.8	94.4	5.9	(Setiawan et al., 2011)

ST#2 ^g	6.2	92.7	7.9	(Setiawan et al., 2011)
ST#3 ^g	6.1	91.1	9.8	
CA-#3 ^h	1.3	96.7	3.4	(Su et al., 2010)
DI-PBI ⁱ	4.8	87.2	14.6	(Yang et al., 2009b)
PBI ^j	0.5	91.0 ^m	900.0	(Wang et al., 2007)

C-2. Comparison of FO performances of the silver nanocomposite membranes with membranes in literature

Table C-2(a). Comparison of membrane FO performance of the silver nanocomposite NF/FO membranes with NF-like FO membranes in literature

Membranes	J_v (L/m ² h)	J_s (g/m ² h)	J_s/J_v (g/L)	FS	DS: MgCl ₂ (M)	orientation	Temp. (°C)	Reference
xLbL2.5 ⁿ	17.2	2.0	0.120	10 mM NaCl /DI	0.5	AL-FS	23 ± 1	current study
	39.3	3.1	0.071	10 mM NaCl /DI	0.5	AL-DS	23 ± 1	
xLbL2.5-Ag(PSS1) ⁿ	17.9	2.8	0.157	10 mM NaCl /DI	0.5	AL-FS	23 ± 1	
	41.8	2.9	0.067	10 mM NaCl /DI	0.5	AL-DS	23 ± 1	

xLbL2.5-Ag(W1) ⁿ	14.9	2.5	0.149	10 mM NaCl /DI	0.5	AL-FS	23 ± 1	current study
	34.0	4.6	0.115	10 mM NaCl /DI	0.5	AL-DS	23 ± 1	
xLbL2.5-Ag(PAH1) ⁿ	16.8	2.3	0.131	10 mM NaCl /DI	0.5	AL-FS	23 ± 1	
	37.8	4.0	0.093	10 mM NaCl /DI	0.5	AL-DS	23 ± 1	
E-23-5-50K ^a	27.5	5.5	0.2	DI	0.5	AL-FS	23	(Setiawan et al., 2012)
	15.5	84.7	5.4	DI	0.5	AL-DS	23	
Double-skinned CA ^b	9.0	1.0	0.11	DI	0.5	AL-FS	Room temp.	(Su et al., 2012)
	15.0	0.9	0.06	DI	0.5	AL-DS	Room temp.	
xLbL3-3 ^d	42.3	12.0	0.284	DI	0.5	AL-DS	22 ± 0.5	(Qi et al., 2012b)
	35.5	N.A.	N.A.	10 mM NaCl	0.5	AL-DS	22 ± 0.5	
xLbL ^e	30.0 ^m	5.8	0.193 ^m	DI	0.5	AL-FS	23	(Qiu et al., 2011)
	60.0 ^m	3.5	0.058 ^m	DI	0.5	AL-DS	23	
3#LbL FO ^f	28.7	10.5	0.366	DI	1	AL-FS	23 ± 1	(Saren et al., 2011)
	25.3	N.A.	N.A.	10 mM NaCl	1	AL-FS	23 ± 1	
	31.7	28.6	0.903	DI	1	AL-DS	23 ± 1	
	23.8	N.A.	N.A.	10 mM NaCl	1	AL-DS	23 ± 1	
HTI ^k	15.8	1.2	0.074	DI	1	AL-FS	23 ± 1	(Saren et al., 2011)

HTI ^k	36.3	7.6	0.209	DI	1	AL-DS	23 ± 1	(Saren et al., 2011)
ST#1 ^g	4.2	1.9	0.46	DI	1.5	AL-FS	23	(Setiawan et al., 2011)
	6.3	3.0	0.48	DI	1.5	AL-DS	23	
ST#2 ^g	10.4	1.9 ^m	0.187 ^m	DI	1	AL-FS	23	
	15.4	8.6 ^m	0.555 ^m	DI	1	AL-DS	23	
ST#3 ^g	11.0	2.7 ^m	0.245 ^m	DI	1	AL-FS	23	
	14.6	15.7 ^m	1.075 ^m	DI	1	AL-DS	23	
CA-#3 ^h	3.1	N.A.	N.A.	DI	1	AL-FS	Room temp.	(Su et al., 2010)
	4.1	0.058	0.014	DI	1	AL-DS	Room temp.	
PBI 2 h ^l	7.5	N.A.	N.A.	DI	1	AL-FS	23 ± 1	(Wang et al., 2009)
	14.5	N.A.	N.A.	DI	1	AL-DS	23 ± 1	
DI-PBI ⁱ	12.5 ^m	0.234 ^m	0.019 ^m	DI	1	AL-FS	23 ± 0.5	(Yang et al., 2009b)
	15.7 ^m	0.349 ^m	0.022 ^m	DI	1	AL-DS	23 ± 0.5	
PBI ^j	5.2	N.A.	N.A.	DI	2	AL-FS	22.5	(Wang et al., 2007)
	9.0	N.A.	N.A.	DI	2	AL-DS	22.5	

Notes:

^a Dual-layer FO hollow fiber membranes

^b Double-skinned CA hollow fiber FO membranes

^c Double-skinned hollow fiber membranes

^d Cross-linked double-skinned NF-like flat sheet FO membranes

^e Cross-linked NF-like flat sheet FO membranes

^f Flat sheet FO membranes

^h Cellulose acetate (CA) NF hollow fiber membranes

ⁱ Dual layer NF hollow fiber membranes

^j Polybenzimidazole (PBI) NF hollow fiber membranes

^k Commercial flat sheet thin film FO membranes

^l Cross-linked PBI hollow fiber membranes

^m These values were estimated from published figures.

ⁿ J_v values were obtained by using 0.5 M $MgCl_2$ as draw solution and 10 mM NaCl as feed solution. J_s and J_s/J_v values were obtained by using 0.5 M $MgCl_2$ as draw solution and Milli-Q water as feed solution.

Table C-2(b). Comparison of membrane FO performance of the silver nanocomposite NF/FO membranes with NF-like FO membranes in literature

Membranes	J_v (L/m ² h)	J_s (g/m ² h)	J_s/J_v (g/L)	FS	DS: NaCl (M)	orientation	Temp. (°C)	Reference
PPSU ^a	10.0	2.3	0.23	DI	2.0	AL-FS	22 ± 0.5	(Widjojo et al., 2013)
(non-sulfonated)	10.0	2.1	0.21	DI	2.0	AL-DS	22 ± 0.5	
sPPSU-2.5 ^b	48.0	7.6	0.16	DI	2.0	AL-FS	22 ± 0.5	
(2.5 mol% sDCDPS)	54.0	8.8	0.16	DI	2.0	AL-DS	22 ± 0.5	
sPPSU-5 ^c	62.8	24.5	0.39	DI	2.0	AL-FS	22 ± 0.5	
(5 mol% sDCDPS)	81.9	24.2	0.30	DI	2.0	AL-DS	22 ± 0.5	
TFN0.1 ^d	17.6 ^t	7.8 ^t	0.44 ^t	DI	1.0	AL-FS	Room temp.	(Ma et al., 2012)
	38.0 ^t	15.0 ^t	0.39 ^t	DI	1.0	AL-DS	Room temp.	
	15.0 ^t	3.3 ^t	0.22 ^t	10 mM NaCl	1.0	AL-FS	Room temp.	
	31.0 ^t	7.0 ^t	0.22 ^t	10 mM NaCl	1.0	AL-DS	Room temp.	
PAN ^e	30.0 ^t	8.3 ^t	0.28 ^t	DI	1.5	AL-FS	25 ± 0.5	(Bui and McCutcheon, 2012)
	52.0 ^t	3.3 ^t	0.06 ^t	DI	1.5	AL-DS	25 ± 0.5	
PAN without PET ^f	35.0 ^t	8.2 ^t	0.23 ^t	DI	1.5	AL-FS	25 ± 0.5	
	62.0 ^t	5.2 ^t	0.08 ^t	DI	1.5	AL-DS	25 ± 0.5	

Double-skinned CA ^g	6.0 ^t	16.8 ^t	2.80 ^t	DI	0.5	AL-FS	Room temp.	(Su et al., 2012)
	8.0 ^t	16.8 ^t	2.10 ^t	DI	0.5	AL-DS	Room temp.	
TFC-1 ^h	17.4	N.A.	N.A.	10 mM NaCl	2.0	AL-FS	23	(Wei et al., 2011b)
	41.3	N.A.	N.A.	10 mM NaCl	2.0	AL-DS	23	
	9.5	2.4	0.25	10 mM NaCl	0.5	AL-FS	23	
	18.1	6.3	0.35	10 mM NaCl	0.5	AL-DS	23	
TFC-2 ^h	25.0	N.A.	N.A.	10 mM NaCl	2.0	AL-FS	23	
	53.7	N.A.	N.A.	10 mM NaCl	2.0	AL-DS	23	
	12.0	4.9	0.41	10 mM NaCl	0.5	AL-FS	23	
	20.5	5.9	0.29	10 mM NaCl	0.5	AL-DS	23	
CTA-HW ⁱ	9.0	5.3	0.59	10 mM NaCl	0.5	AL-FS	23	
	15.4	9.4	0.61	10 mM NaCl	0.5	AL-DS	23	
CTA-W ^j	5.0	2.9	0.58	10 mM NaCl	0.5	AL-FS	23	
	6.6	4.8	0.73	10 mM NaCl	0.5	AL-DS	23	
CTA-NW ^k	4.4	0.6	0.14	10 mM NaCl	0.5	AL-FS	23	
	8.2	2.8	0.34	10 mM NaCl	0.5	AL-DS	23	
BW30 ^l	0.8	0.2 ^t	0.25 ^t	10 mM NaCl	0.5	AL-FS	23	
	0.4	0.4 ^t	1.00 ^t	10 mM NaCl	0.5	AL-DS	23	
BW30-o ^m	1.1	0.4 ^t	0.36 ^t	10 mM NaCl	0.5	AL-FS	23	

	1.3	0.3 ^t	0.23 ^t	10 mM NaCl	0.5	AL-DS	23	
#C-FO membrane ⁿ	42.6	4.0	0.09	DI	0.5	AL-DS	23	(Chou et al., 2010)
	32.9	2.9	0.09	8.6 mM NaCl	0.5	AL-DS	23	
	24.2	2.0	0.08	0.17 M NaCl	2.0	AL-DS	23	
	12.4	1.0	0.08	0.59 M NaCl	2.0	AL-DS	23	
	3.9	N.A.	N.A.	0.05 M NaCl	2.0	AL-DS	Room temp.	
CA-#3 ^o	1.5	N.A.	N.A.	0.10 M NaCl	2.0	AL-DS	Room temp.	(Su et al., 2010)
	1.2	N.A.	N.A.	0.25 M NaCl	2.0	AL-DS	Room temp.	
	1.0	N.A.	N.A.	0.60 M NaCl	2.0	AL-DS	Room temp.	
HTI CA ^p	43.2 ^t	N.A.	N.A.	DI	1.5	AL-FS	20	(McCutcheon and Elimelech, 2008; Wang et al., 2009)
CE ^q	36.0 ^t	N.A.	N.A.	DI	1.5	AL-FS	20	
SW30 XLE ^r	8.1 ^t	N.A.	N.A.	DI	1.5	AL-FS	20	
HTI ^s	5.1	7.4	0.69	DI	0.5	AL-FS	20 ± 2	(Cornelissen et al., 2008)
	8.5	7.4	0.87	DI	0.5	AL-DS	20 ± 2	

Notes:

^a Non-sulfonated polyphenylenesulfone thin film composite (TFC) FO membranes^b Sulfonated polyphenylenesulfone TFC FO membranes with 2.5 mol% sDCDPS monomer^c Sulfonated polyphenylenesulfone TFC FO membranes with 5 mol% sDCDPS monomer

- ^d Zeolite-polyamide thin film nanocomposite (TFN) membranes with 0.1 wt./v% zeolite loading
- ^e TFC polyacrylonitrile (PAN) nanofiber electrospinning membranes
- ^f TFC PAN nanofiber electrospinning membranes without PET backing layer
- ^g Double-skinned CA hollow fiber FO membranes
- ^h Two types of TFC FO membranes
- ⁱ Commercial asymmetric flat-sheet cellulose triacetate (CTA) Hydrowell[®] module
- ^j Commercial asymmetric flat-sheet CTA supported by a polyester woven fabric
- ^k Commercial asymmetric flat-sheet CTA supported by a non-woven fabric
- ^l Commercial TFC polyamide RO membrane supported by a non-woven fabric
- ^m Commercial TFC polyamide RO membrane with the non-woven fabric support layer removed
- ⁿ Hollow fiber membranes without an outer UF skin
- ^o Cellulose acetate (CA) NF hollow fiber membranes
- ^p Commercial Hydration Technologies, Inc. (HTI) FO flat sheet membranes
- ^q Commercial RO membranes from GE Osmonics
- ^r TFC RO membranes from Dow Filmtec used in seawater desalination
- ^s Commercial HTI FO membranes
- ^t These values were estimated from published figures.

Section D

Silver leaching test of PAN-Ag nanocomposite UF membranes

Figure D-1 presents the silver concentrations in membrane leachate of different PAN-Ag nanocomposite UF membranes. The membrane leachate was obtained by submerging a piece of membrane with an area of 12.7 cm² in 50 mL Milli-Q water at room temperature (22 ± 1 °C). Continuous stirring was provided during the test. 1 mL sample was taken at 0, 1, 3, 6, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h respectively. The leachate samples were digested in 7% HNO₃ according to a modified U.S. EPA method 3050B and measured by using Inductively Coupled Plasma Mass Spectrometer (ICP-MS, ELAN DRC-e, Perkin Elmer; Waltham, MA). Error bars of reported results were obtained by 3 replicate measurements.

Total amount of silver in different PAN-Ag nanocomposite UF membranes were obtained by digesting 0.10 g membrane samples (with an area of 50.28 ± 6.23 cm²) in 9 mL concentrated HNO₃ solution for 15 min using Microwave Reaction System (Multiwave 3000, Anton Paar GmbH; Austria). Silver concentrations in the collected samples were quantified using the ICP-MS described above. The percentage of silver α remained in membrane after leaching test was calculated using following equation:

$$\alpha = \left(1 - \frac{\text{Silver released in membrane leachate}}{\text{Total silver incorporated in membrane}} \right) \times 100\%$$

The silver release time β (year) was estimated by using the percentage of silver remained in membrane after 240 h leaching test (α_{240h}) and the following equation:

$$\beta = \frac{1}{1 - \alpha_{240h}} \times \frac{240}{24 \cdot 365}$$

The total amount of silver in different PAN-Ag nanocomposite UF membranes and their corresponding silver release time (year) are shown in Table D-1. Results show

that the silver release of the nanocomposite membranes was relatively slow. The silver concentrations in membrane leachate were lower than the highest tolerable silver concentration in drinking water (0.1 mg/L) suggested by USEPA and WHO. Moreover, the released silver in the experimental conditions was overestimated compared to real membrane operations, and the estimated silver release time was reasonable long compared to the ordinary membrane life time. These indicated that the silver release from the silver nanocomposite membranes will not cause any adverse effect to human health and the environment, and the antibacterial properties of the nanocomposite membranes may last for a relatively long time.

Figure D-1. Silver concentrations in the leachate of PAN-Ag nanocomposite UF membranes (240 h).

Table D-1. Silver release from PAN-Ag nanocomposite UF membranes.

Membrane ID	Ag conc. in 240 h membrane leachate ($\mu\text{g/L}$) ^a	Total Ag in 0.10 g membrane sample (μg) ^b	Ag percentage in 0.10 g membrane sample	Total Ag in membrane sample for leaching test ($\mu\text{g/L}$) ^a	Percentage of Ag remained in membrane (α)	Ag release time (β , year)
PAN-Ag(0)	0.22 ± 0.02	6.12 ± 0.26	0.001%	62.24 ± 2.68	--	--
PAN-Ag(0.01)	2.81 ± 0.30	32.26 ± 0.51	0.006%	328.29 ± 5.16	99.14%	3.18
PAN-Ag(0.02)	7.84 ± 0.21	109.15 ± 11.95	0.019%	1110.89 ± 121.59	99.29%	3.86
PAN-Ag(0.05)	20.63 ± 1.02	314.56 ± 8.46	0.056%	3201.60 ± 86.15	99.36%	4.28
PAN-Ag(0.10)	36.53 ± 0.80	888.49 ± 3.43	0.154%	9042.88 ± 34.90	99.60%	6.85

Notes:

^a The Ag concentration in membrane leachate and total Ag in membrane sample for leaching test were obtained by testing a piece of membrane with an area of 12.7 cm^2 in 50 mL Milli-Q water at room temperature for 240 h.

^b The total Ag in 0.10 g membrane sample was calculated by using the concentrations of Ag obtained from ICP-MS test $\times$ volume of solution in which membrane was submerged in.

Section E

Evaluation of the increased costs of PAN-Ag nanocomposite UF membrane fabrication

Table E-1 presents the evaluation of the increased costs of PAN-Ag nanocomposite UF membrane fabrication. To simplify the calculation and make the comparison straight forward, only PAN and AgNPs were considered for the evaluation. This estimation does not include the costs of other materials for membrane fabrication, for example, the pore former LiCl, the solvent DMF, and the polyelectrolytes PAH and PSS. In other words, the real increased cost of PAN-Ag and xLbL3.0-Ag nanocomposite membranes will be even less than the estimated percentages in Table E-1. According to the estimation, for the highest AgNPs loading in PAN-Ag nanocomposite membranes (PAN-Ag(0.10)), the increased cost is only 3.22% compared with the AgNPs-free control membrane (PAN-Ag(0)). Moreover, as the development of nanotechnology, the additional cost brought by AgNPs could be further cut down in the future.

Taken together, the cost of AgNPs will not hinder the use of silver nanocomposite membranes, especially by considering the potential benefits of AgNPs in membrane biofouling control.

Table E-1. Evaluation of the increased costs of PAN-Ag nanocomposite UF membrane fabrication.

Membrane ID	PAN (SGD 402.50/100g)	AgNPs (SGD 116.50/5g)	Cost (SGD)	Percentage of increased cost
PAN-Ag(0)	18.00 g	0	72.45	--
PAN-Ag(0.01)	18.00 g	0.01 g	72.68	0.32%
PAN-Ag(0.02)	18.00 g	0.02 g	72.92	0.64%
PAN-Ag(0.05)	18.00 g	0.05 g	73.62	1.61%
PAN-Ag(0.10)	18.00 g	0.10 g	74.78	3.22%

Notes:

^a The currency used in the evaluation is Singapore dollar (SGD).

^b The weights of PAN and AgNPs in above table are based on the preparation of 100 g dope solution.

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