Enhanced PVDF membrane antifouling performance via surface modification by functional polymers

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NANYANG
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**ENHANCED PVDF MEMBRANE ANTIFOULING
PERFORMANCE VIA SURFACE MODIFICATION BY
FUNCTIONAL POLYMERS**

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NANYANG ENVIRONMENT AND WATER RESEARCH INSTITUTE**

2016

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PERFORMANCE VIA SURFACE MODIFICATION BY
FUNCTIONAL POLYMERS**

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LIST OF SYMBOLS

3D	three-dimensional
AFM	atomic force microscopy
ATRP	atom transfer radical polymerization
BSA	bovine serum albumin
CBMA	carboxybetaine methacrylate
CFU	colony forming unit
CLSM	confocal laser scanning microscopy
COD	chemical oxygen demand
EPS	extracellular polymeric substances
FRR	flux recovery ratio
FTIR	Fourier transformed infrared spectroscopy
HA	humic acids
HRT	hydraulic retention time
LB	lysogeny broth
LCST	lower critical solution temperature
MBR	membrane bioreactor
MF	microfiltration
NF	nanofiltration
PAC	powdered activated carbon
RAFT	reversible addition-fragmentation chain transfer
PBS	phosphate buffered saline

PE	polyethylene
PEG	polyethylene glycol
PEO	poly(ethylene oxide)
PES	polyethersulfone
PHEMA	poly(hydroxyethyl methacrylate)
PMAA	poly(methacrylic acid)
PNIPAAm	poly(N-isopropylacrylamide)
PSF	polysulfone
PTFE	polytetrafluoroethylene
PVDF	poly(vinylidene fluoride)
RMS	root-mean square
RO	reverse osmosis
SBMA	sulfobetaine methacrylate
SEM	scanning electron microscopy
SMPs	soluble microbial products
SRT	solid retention time
TGA	thermo-gravimetric analysis
THF	tetrahydrofuran
UF	ultrafiltration
YPD	yeast extract-peptone-dextrose
XPS	X-ray photoelectron spectroscopy

ABSTRACT

In the past several decades, membrane technology is widely used in separation process in various fields. In particular, membrane bioreactors (MBRs) as an application of membrane technology has attracted increasing attention from the researchers worldwide. However, there still exists many obstacles in membrane widespread application and the major challenge is membrane fouling. Recently, antifouling membrane fabrication by functional polymers is regarded as a promising and effective strategy to inhibit membrane fouling. Thus, this research work mainly focuses on poly(vinylidene fluoride) (PVDF) membrane modification by thermosensitive polymer PNIPAAm [poly(N-isopropylacrylamide)] and its copolymer with other functional polymers. Meanwhile, the objective of this thesis is to investigate the application potential of antifouling membrane prepared and to provide a feasible and effective strategy to achieve better antifouling performance for wide application, thereby providing a meaningful reference for future study.

Firstly, functional polymer PNIPAAm was successfully bonded onto PVDF membrane via surface-initiated atom transfer radical polymerization (ATRP). The hydrophilicity of PVDF membrane was dramatically enhanced due to the incorporation of PNIPAAm chains. The microfiltration experiments indicated that PNIPAAm modified membrane exhibited a flux recovery ratio (FRR) as high as 91.59%. In addition, bacteria adhesion test revealed that the attachment of *Escherichia coli* on the modified membrane was reduced by 75% compared to the original membrane. Hence, the fouling tests demonstrated that PNIPAAm modified membrane exhibited obvious thermosensitive

property and good antifouling ability compared with pure PVDF membrane. In order to further improve not only fouling resistance but also fouling release property of membrane, three types of modified membrane were well designed by grafting PNIPAAm, PHEMA [poly(hydroxyethyl methacrylate)] and P(HEMA-co-NIPAAm) respectively. Minimum protein adsorption and bacterial adhesion were both obtained on PVDF-g-P(HEMA-co-NIPAAm) membrane, with reduction by 44% and 71% respectively compared to the pristine membrane. The filtration testing indicated that the copolymer modified membrane exhibited high resistance to protein fouling and the foulant on the surface was removed easily by washing, suggesting easy-cleaning capacity.

The biofouling on the membrane surface was analyzed using Gram-negative *E. coli* and Gram-positive *Staphylococcus epidermidis*. According to biofilm study using *E. coli* and *S. epidermidis*, it is inferred that microorganism species played an essential role in the formation and structure of biofilm as the species influenced the bacterial adhesion each other owing to their characteristics. Besides, the modified membrane exhibited higher fouling resistance during filtration and higher flux recovery after hydraulic cleaning compared with the original membrane for long-term use. Furthermore, for thermosensitive PVDF-g-P(HEMA-co-NIPAAm) membrane prepared in this thesis, water cleaning with temperature control provide an auxiliary and feasible strategy to promote the fouling release and to enhance the efficiency of fouling removal except for classical cleaning methods for wastewater treatment.

This thesis was one of the limited studies to achieve higher antifouling property focusing on not only fouling resistance but also fouling release for PVDF membrane via grafting functional polymers P(HEMA-co-NIPAAm). This research study provided a

feasible and effective strategy of antifouling membrane preparation which can be also used for other types of the membrane in wide range of applications.

CHAPTER 1: Introduction

1.1 Background

It is well known that membrane technology plays a very important role in separation process in the past several decades owing to its advantages, such as no phase changes, easy scale-up in modular, simple operation and relatively low energy consumption [1]. Thus there are widespread applications of membrane technology in the fields such as water purification [2, 3], wastewater treatment [4, 5], desalination [6], gas separation [7, 8], food industry [9], etc. In recent years, more attention has been made to membrane technology due to increased water scarcity. Especially, one of important applications of membrane technology is membrane bioreactors (MBRs) for wastewater treatment which has gradually become a promising and popular technology and has attracted increasing attention all over the world [10, 11], and the development of MBR in wastewater treatment has achieved great progress in recent years. Compared to conventional activated sludge system for wastewater treatment, MBRs hold many advantages such as small footprint, less sludge production and high effluent quality [12-14]. Specifically, MBRs provide independent regulation of the solid retention time (SRT) and hydraulic retention time (HRT) which can allow a larger volume of wastewater to be treated in a smaller bioreactor. Besides, the solid and liquid separation can be conducted effectively using membranes during wastewater treatment, which can improve the treatment efficiency compared to conventional activated sludge. Thus it can provide an effective wastewater treatment process with a small footprint, which has important meanings for a country with limited land such as Singapore. Even for the whole world,

MBRs technology is increasingly widespread according to market analyst reports. For example, in Europe, MBRs market value was reaching \$57 million and the market was roughly occupied by six countries in 2004 (Figure 1-1) [15]. Additionally, at present, the individual MBR membrane suppliers has been over 50 and there are hundreds of large-scale MBR plants which have the treatment capacity larger than 10,000 m³/d [16-18].

Figure 1- 1. European membrane bioreactors market [15].

1.2 Challenges in application of membrane technology

At present, even though the numerous advantages provided by membrane technology, there still exist several challenges which are urgent to be solved for wider application of membrane technology as well as MBRs. It is known that one of the major obstacles limiting the potential of this technology is membrane fouling which significantly reduces membrane filtration flux, shorter lifespan of the membrane, and increases energy consumption and overall costs [19, 20]. Consequently, many researchers have focused on membrane fouling in the application of membrane technology and MBR, and especially around 30% of all MBRs articles published deal with fouling [21]. Therefore, it requires effective and efficient methods to inhibit membrane fouling and

improve membrane performance.

1.3 Antifouling membrane as a solution

There are several strategies to relieve and control membrane fouling, for example, pre-treatment of influent, optimization of operation condition, cleaning using chemical reagents and physical methods (Figure 1-2). Another effective approach is to develop antifouling membrane and improve the antifouling property of membranes via chemical modification by functional materials. According to previous literature [22-24], it is well acknowledged that the membrane fouling is mainly caused by soluble extracellular polymeric substances (soluble EPS) and soluble microbial products (SMPs) in MBRs. They mainly consist of microbial substance including proteins and carbohydrates produced by microorganisms during MBRs operation. It is worth noting that a research paper successfully identified that proteins played a significant role in membrane fouling in MBRs treating municipal wastewater [25]. Meanwhile, some articles reported that the attachment of bacterial clusters attributed to cake layer formation further leading to membrane fouling, and particularly microbial cells of foulant layer contributed to fouling directly [26, 27]. Therefore, it is considered that the functional polymers with high ability of resistance to bacteria adhesion and protein adsorption are promising materials for antifouling membrane preparation.

Figure 1- 2. The targets and strategies of previous studies to address membrane fouling challenge.

In the past decades, stimuli-responsive polymers have attracted increasing interests as they exhibit responsive property to environmental stimuli including temperature, pH, ionic strength, pressure and certain reagents [28]. These polymers generally undergo a conformation change or physiochemical change in response to external stimuli. Due to the special property, the interfacial interactions between these polymers and external stimuli play an essential role in biochemical and biomedical applications including controlling cell adhesion, selective membrane, biosensors and drug delivery [29-32]. It is known that poly(*N*-isopropylacrylamide) (PNIPAAm) is the most extensively investigated thermosensitive polymer. It is reported that PNIPAAm experiences a reversible conformational transition in an aqueous medium across lower critical solution temperature (LCST) at around 32 °C. The PNIPAAm chains show a random coil structure with hydrophilic property below the LCST and the PNIPAAm chains become

hydrophobic with a compacted globular structure above LCST due to conformational transition [33]. Owing to the tunable hydrophilicity, PNIPAAm brushes and its modified surface have been widely applied in controlling protein adsorption and bacterial adhesion. Meanwhile, PNIPAAm modified surfaces have been successfully utilized as microbial cell-culture substrates that control the reversible attachment and detachment of cells in response to temperature change. In fact, previous literature [34-37] reported that PNIPAAm had been successfully employed in membrane preparation to inhibit fouling, and as a result, PNIPAAm endowed membrane thermosensitive property and the antifouling property of membranes was improved to a certain extent accordingly. Thus, thermosensitive polymer PNIPAAm is regarded as a promising material for antifouling membrane fabrication to effectively reduce and control membrane fouling.

1.4 Aims and Objectives

It is reported that most membranes employed in industry processing are made from polymeric materials as a matrix, including polyethylene (PE), polysulfone (PSF), poly(ether sulfone) (PES), poly(vinyl chloride) (PVC), polyamide, poly(vinylidene fluoride) (PVDF), polytetrafluoroethylene (PTFE), etc (summarized in Table 1-1). In comparison to many polymeric membranes, PVDF membrane is widely utilized in various fields owing to its outstanding chemical resistance, high thermal stability, strong mechanical stress and well-controlled fabrication [1, 38]. Therefore, this work focuses on the study of PVDF membrane modification and its antifouling property.

Table 1- 1. Comparison of properties of different polymeric membranes

Polymeric membrane	Glass transition temperature (°C)	Thermal stability	Chemical stability	Mechanical stress	Hydrophilicity
PE	-120	low	medium	low	low
PSF	190	high	medium	high	high
PES	230	excellent	high	low	high
PVC	80	low	medium	high	high
PTFE	115	excellent	excellent	high	high
PVDF	-35	excellent	excellent	excellent	high

It is also reported that some functional polymers have successfully modified hydrophobic PVDF membrane to increase hydrophilicity and permeability, leading to less fouling and improved performance, for example, methacrylic acid (MAA), poly(ethylene glycol) (PEG), and poly(ethylene glycol) methacrylate (PEGMA), poly(2-(N,N-dimethylamino) ethyl methacrylate) (PDMAEMA), poly(2-oligo (ethylene glycol) monomethyl ether methacrylate) (POEGMA), and poly(2-hydroxyethyl methacrylate) (PHEMA). Besides, there are other functional materials such as nanoparticles/nanomaterials used in membrane modification to reduce fouling and biofouling on the surface, for example, protein adsorption and bacterial adhesion. However, the severe membrane fouling caused by foulant such as proteins, cells and bacteria still existed in a wide range of application. At present, the antifouling property of membrane generally includes fouling resistance and fouling release, and the former means the property to prevent foulant deposition onto the membrane while the later represents the property to release and remove foulant from membrane surface under certain condition. It is well known that biofilm on the membrane usually starts with

bacterial adhesion followed by gradual bacterial proliferation, and it is difficult to be completely removed after formation. The fouling resistance property of membrane is capable of preventing bacteria adhesion to the surface to some extent, and fouling release property assists to remove bacteria adsorption from the surface before biofilm formation. However, the membrane with fouling resistance cannot exhibit all advantages to inhibit biofouling effectively since the subsequent accumulation of bacteria are affected significantly by the interaction between the fouled surface and new foulant after formation of a biofouling layer on the surface [39]. Thus, the fouling release/cleaning property of membrane plays an essential role in prevention the biofilm formation on the membrane surface. The study in a combination of fouling resistance and fouling release is very crucial, and it is a promising strategy to improve membrane antifouling performance and has drawn increasing attention.

PNIPAAm, a typical thermosensitive polymer, has been regarded as a promising material to form a fouling-resistant and fouling-release surface in a wide range of applications because of its tunable hydrophilicity in response to temperature [31, 40]. Recently, many studies reported that the PNIPAAm could enable membrane exhibit thermos-sensitive property to obtain high separation efficiency of $\geq 99.26\%$ for practical oil-water separations [41], outstanding permeability [42] and excellent antifouling property [43]. Thus, it is supposed that PVDF membrane can be modified by incorporating PNIPAAm chains to resist protein fouling and control bacterial behavior (attachment/detachment) under temperature regulation, leading to inhibit the further formation of biofilm on the substrate surface. This information is supposed to give us an idea on how to prevent membrane biofouling formation, which is an advantage of

incorporation of PNIPAAm polymers compared to other functional groups. As mentioned above, in this study, much effort is being devoted to improve the antifouling performance of membrane not only in fouling resistant property but also fouling release property, and an effective strategy is to design and prepare antifouling membrane via grafting functional PNIPAAm to reduce membrane fouling and inhibit biofilm.

To conclude, the aim of this work is to prepare antifouling PVDF membrane, and we plan to achieve the specific objectives in three chapters as following: Firstly, in chapter 3, antifouling PVDF membrane is to be prepared by grafting PNIPAAm via surface-initiated ATRP in order to improve performance, and both fouling resistance and fouling release property are to be evaluated via protein adsorption, bacterial attachment/detachment and filtration tests. Secondly, it is supposed that copolymer of PNIPAAm with other hydrophilic polymer can be used to modify membrane to further improve antifouling property owing to a synergistic effect of multiple functional groups. Hydrophilic PHEMA has been applied in membrane technology to enhance the performance due to its high hydrophilicity, anti-biofouling property and good physical properties, and PHEMA grafted membrane exhibited high permeability and excellent protein resistance. Hence, the aim of chapter 4 is to design antifouling PVDF membrane by grafting copolymer of PHEMA and PNIPAAm in order to obtain higher antifouling performance, which is one of limited works of study in both fouling resistance and fouling release, and also provide a feasible and effective strategy in fouling resistance/release enhancement focused on PVDF membrane modification by copolymer P(HEMA-co-NIPAAm). In addition, one of points is to find the factors which influent the fouling resistance and release property via membrane characterization and fouling tests.

Thirdly, the aim of chapter 5 is to investigate and explain the effect of these functional polymers on antifouling property of membrane through fouling tests analysis and filtration tests, especially biofilm analysis. The antifouling property of modified membrane is to be investigated by biofilm analysis through bacterial behavior (attachment and detachment), and the effect of bacteria species on the biofilm formation and structure is supposed to be analyzed, which can assist the researcher to know how to inhibit the biofilm formation effectively. In conclusion, the aim of this study is to provide a feasible and effective strategy to achieve better antifouling property of membrane, thereby providing a meaningful reference for future study.

CHAPTER 2: Literature review

2.1 Introduction

As proposed project mentioned previously, this research work mainly aims to address the important challenge in the widespread application of membrane technology, that is, membrane fouling. Thus, this chapter summarizes the mechanism of membrane fouling. Besides, in this study, functional polymer PNIPAAm is applied to fabricate antifouling membrane because of its specific property. Most importantly, the phase transition mechanism of PNIPAAm and its polymerization/grafting methods should be understood clearly. It is also important to have a good command of the development of PNIPAAm polymer/copolymers and its application in antifouling membrane preparation. They are all reviewed and summarized comprehensively in the following sections based on previous studies and literature published.

2.2 Membrane fouling

2.2.1 Mechanisms of membrane fouling

The membranes are mainly divided into microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) membrane based on the pore characteristics of the membrane (pore size, pore-size distribution, and porosity). Accordingly, various types of membrane are also applied in the different area depending on characteristics of membranes and contaminants. MF and UF process are usually utilized to remove bacteria, suspended solids, protein, colloids, viruses and other large molecular weight substance in

wastewater treatment or the pre-treatment of wastewater [1], and they are also typically employed in the MBR for water treatment [44]. For MF and UF membrane, fouling commonly occurs through the following mechanisms: adsorption, pore blocking and cake layer formation [45, 46]

During the filtration process, adsorption generally occurs because of different molecular interaction between membrane surface and the specific group of compounds in foulant. These interactions include van der Waals forces, electrostatic attraction, or chemical bonding respectively based on the functional groups involved. The adsorption between solutes and membrane also occurs even in the static state, further leading to a monolayer of foulant on the membrane surface. This phenomenon is common when the feed contains high concentration of proteins and humic acids (HA) [47]. Adsorption or affinity between these macromolecules and membrane substrate can be reduced by changing physicochemical characteristics and property of membrane surface. It is worth noting that the fouling caused by such macromolecules adsorption is usually irreversible or hard to be removed as it occurs spontaneously and the inverse process is thermodynamically unfavorable.

Pore blocking/plugging usually arises rapidly in the initial stage of filtration owing to deposition of colloids and particles on the membrane surface and within pores, and closure of membrane pores by pore-sizes foulant [48, 49]. Fouling caused by pore plugging mechanism is irreversible to a certain extent owing to foulant trapped within the internal structure of membrane [50-52]. Hence, the membrane fouling resulting from pore blocking is hardly restored by typically physical cleaning methods.

Cake layer is usually formed gradually due to foulant attachment and solutes

deposition layer by layer on the membrane surface, resulting in an extra resistance towards the permeation flux which is called cake resistance. The interaction between cake layer formed and the membrane material affects the reversibility of membrane fouling [53], and generally, cake layer is considered as a typical external fouling and reversible fouling which can be partially swept away by physical cleaning methods, for example, surface hydraulic washing and backwash. In case of long term operation and inadequate cleaning the reversible fouling may lead to an irreversible form.

2.2.2 Foulants

There are three different types of fouling: inorganic, organic and biofouling or biofilm, which mainly depends on the types and characteristic of foulant. Inorganic fouling is mainly caused by the precipitation of inorganic crystals and complexation of foulant in the presence of certain ions such as Ca^{2+} and Mg^{2+} [54]. Organic fouling commonly results from adsorption and deposition of organic solutes such as proteins, humic acids, polysaccharides, colloids from feed solution/sludge or products from microorganisms. Specifically, as mentioned in section 1.3, microorganisms-produced EPS and SMP are much important factors for membrane fouling in MBR, and their adsorption and deposition directly results in organic fouling and they are also responsible for biofouling. Biofouling or biofilm formed on the substrate surface are ubiquitous and unwanted in a submerged aqueous environment, and it is mainly caused by attachment of microbial cells [55]. It is reported that conglomeration of different types of EPS on the substrate surface supports the biofilm and is responsible for the surface adhesion and cohesion of the biofilm. Generally, the biofouling on material surface is induced by the

interfacial interactions between the cells/organic molecule (protein) and the underlying substrate, which starts with the primary and secondary attachment of microbial cells. Thus removal of unwanted biofouling requires a good command of initial attachment of cells, and requires a clear understanding of the supporting substrate properties that are significant in the biofilm formation. The Figure 2-1 and 2-2 show that a thin yellow foulant layer resulting in membrane fouling are mainly bacterial cell and EPS which covers the membrane pores, and the dark layer mainly consists of EPS and other components in the wastewater [27].

Figure 2- 1. (a) The mixed liquor separated into supernatant, light solids and dark solids after centrifugation (b) Photograph of the fouled cPVDF membrane [27].

Figure 2- 2. (a) AFM image of the surface of a virgin cPVDF membrane. (b) AFM image of membrane fouling results from cells and EPS in the yellow foulant layer that covers the membrane pores beneath. The scale of the AFM scan is $5\mu\text{m} \times 5\mu\text{m}$ [27].

For protein adsorption, the process is complicated and the interactions between

protein and surface are affected by some factors such as the physicochemical property of the material, the properties of attached protein and solution environment. However, the important point is that surface hydrophobicity has a major effect on protein adsorption onto the surface and the process is usually accompanied by protein denaturation. The hydrophobic character generally leads to simultaneous dehydration of the hydrophobic domains of the protein and release of bound water from hydrophobic surfaces [56]. Meanwhile, there are two types of adhesion of microbial cell leading to biofouling. Microbial attachment to the original surface is affected mainly by the macroscopic properties of the underlying surfaces such as surface charge and hydrophobicity, and this kind of interaction is called non-specific interaction. In addition, microbial adhesion usually involves protein-receptor interactions at the interface might via the binding of external domain of a cell protein to the extracellular matrix as “ligand-receptor” bond, and this kind of interaction is commonly regarded as the specific interaction. Although the interactions between the microbial cell and substrate surface are also complicated, and not completely known at the molecular level to date, it is known that surface physicochemical property and topography affect microbial cell adhering onto membrane surface.

2.3 Antifouling membrane preparation

2.3.1 Antifouling membrane prepared with functional materials

As mentioned in the previous section, fouling is considered as a severe challenge in the widespread application of membrane technology since it results in a decline of permeation flux, shorter lifespan of the membrane, more energy consumption and an

increase in overall costs. Recently, antifouling membrane fabrication is regarded as an effective strategy to resist foulant and reduce fouling, and the antifouling membrane is generally fabricated using functional materials as one component or additive of the membrane bulk during fabrication process or as the agents for surface modification in following membrane fabrication.

Although PVDF membrane is widely employed in various industry process, its surface is hydrophobic and is very susceptible to membrane fouling due to interaction with foulant such as bacteria and protein. It is reported that the antifouling property of PVDF membrane is improved significantly by increasing its hydrophilicity [57]. According to previous literature [58-60], the hydrophilicity of PVDF membrane has been usually enhanced via surface modification by incorporating hydrophilic, amphiphilic and other functional materials. In Liu's review work [61], it is also summarized that some functional polymers have successfully modified hydrophobic PVDF membrane to improve the performance, for example, methacrylic acid (MAA) [62], poly(ethylene glycol) (PEG) [58], and poly(ethylene glycol) methacrylate (PEGMA) [63]. In addition, three functional polymers poly(2-(N,N-dimethylamino) ethyl methacrylate) (PDMAEMA), poly(2-oligo (ethylene glycol) monomethyl ether methacrylate) (POEGMA), and poly(2-hydroxyethyl methacrylate) (PHEMA) were incorporated onto PVDF membrane in order to increase hydrophilicity and permeability [64]. Recently, Venault and coworkers [65] reported that the antifouling PVDF membrane with mixed-charge copolymers of [2-(methacryloyloxy) ethyl] trimethylammonium (TMA) and sulfopropyl methacrylate (SA) was designed via plasma-induced surface modification. As a result, the hydrophilicity of membranes increased and the mixed-charge property

was introduced into PVDF membrane, thus resulting in remarkable enhancement of antifouling ability of PVDF membrane with high flux recovery ratio (FRR). In Han's study [66], antifouling property and permeation flux of PVDF membrane were improved effectively by grafting sulfobetaine methacrylate (SBMA) via surface modification. These functional materials information and the performance/antifouling property of modified membrane were summarized in Table 2-1.

Hydrophobic PVDF membrane also can be modified by inorganic particles such as titanium dioxide (TiO_2) [67] and zinc oxide (ZnO) [68] to inhibit membrane fouling significantly and to obtain enhanced membrane permeability. Many studies also reported that the antifouling property of membrane has been improved by incorporating with different types nanomaterials/nanoparticles. In a previous study [69], a coating of silver nanoparticles/ multi-walled carbon nanotubes (Ag/MWNTs) was deposited on polyacrylonitrile (PAN) hollow fiber membrane surface, and the resultant membrane exhibited excellent antimicrobial and antifouling properties via bacterial feed filtration. The polyethersulfone (PES) membrane blended with reduced graphene oxide (rGO)/ TiO_2 was prepared in order to enhance hydrophilicity in a previous study [70], and the results indicated $\text{rGO/TiO}_2/\text{PES}$ membrane exhibited better overall properties including the water permeability, fouling resistance and protein rejection than original PES membrane, TiO_2/PES and GO/PES membrane respectively. Chung *et al.* [71] also reported that nanohybrid membranes incorporated with ZnO and ZnO-GO nanoparticles were fabricated via wet phase inversion method, and the resultant membranes presented significant enhanced properties such as high hydrophilicity and permeability, improved humic acid rejection as well as high antifouling and resistance to bacterial attachment.

Table 2- 1. The functional material used for PVDF membrane modification via various method in previous studies, and the membrane performance and antifouling efficiency.

Functional material	Surface modification method	Membrane performance and antifouling efficiency
PEG	argon plasma-induced grafting	γ -globulin adsorption and water filtration after protein adsorption; much lower protein adsorption was detected on the modified membrane; 66.7% higher water flux of modified membrane was obtained compared with original membrane [58].
PEGMA	electron beam-induced grafting	BSA filtration; the grafted membrane exhibited a better antifouling property compared with the original hydrophobic PVDF membrane; the flux was as high as 85% of the initial flux for modified membrane in 3 h while it declined to 30% for the original membrane [59].
PNIPAAm- <i>b</i> -PPEGMA	surface-initiated ATRP	filtration using synthetic produced water contained soybean oil, NaCl; the modified membrane exhibited higher antifouling property than the original membrane for long-term operation; modified membranes achieved 100% flux recovery, while only 81% of the initial flux was recovered for the unmodified membrane [60].
PDMAEMA or POEGMA	activators generated by electron transfer ATRP	Water filtration; the flux of modified membrane was higher than that of original membrane [64].
TMA/SA	plasma-induced surface grafting	protein (BSA and lysozyme) adsorption, biofouling (bacterial and blood cells) attachment and BSA filtration; the modified membranes exhibited excellent resistance to biofouling in static conditions (with 70% reduction in protein adsorption); the FRR of modified membrane was excellent in comparison with original membrane during BSA filtration [65].
SBMA	Argon plasma-induced ATRP	BSA filtration; the modified membrane exhibited better fouling resistance to protein; the FRR and flux of modified membrane were higher than pristine membrane [66].
TiO ₂	atomic layer deposition	BSA adsorption and filtration; the modified membrane exhibited enhanced hydrophilicity and better resistance to protein; all the modified membrane displayed reduced amounts of adsorbed BSA compared with pristine membrane [67].
ZnO	atomic layer deposition	sodium alginate filtration; less fouling propensity of polysaccharide was detected after ZnO modification during filtration [68].

Besides, it is reported in Huang *et al.* work [72] that the PES membrane was modified by Ag–SiO₂ nanocomposites, resulting in improved filtration performance and excellent antibacterial and antifouling properties. Table 2-2 also describes other several inorganic and polymeric nanoparticles applied in membrane modification for improved performance.

Table 2- 2. Recent advances in water treatment membranes with several inorganic and polymeric nanoparticles and their properties.

Base membrane	Nanoparticles	Properties
PES	Mg(OH) ₂	Improved permeability, high egg albumen rejection (94.58%) and recovery flux ratio (70%) [73].
Poly(acrylic acid)-grafted polypropylene	CaCO ₃	Superhydrophilic surface; high water flux under ultralow operational pressure [74].
PVDF	Polydopamine (PDA)	Significantly enhanced hydrophilicity; improved water permeability and antifouling properties; satisfying long-term stability in aqueous environment [75].
PVDF	Polyethyleneimine (PEI)	Uniform particle distribution; particles depositing range from 27% to 48% [76].
PVDF	PEGylated PEI	High algae retention (100%); 50% higher water flux of modified membrane than a commercial hydrophilic PVDF membrane [77].
PES	Quaternized PEI	Obviously enhanced hydrophilicity and water permeability; better antifouling property and rejection of reactive dyes [78].

However, the severe membrane fouling caused by foulant such as proteins, cells and bacteria still existed in a wide range of application, and espacilly, biofilm commonly occurred and formed on the membrane surface during the filtration process with microorganisms involved. The biofilm on the substrate surface usually starts with

bacterial adhesion followed by gradual bacterial proliferation, and it is difficult to be completely removed after formation. Besides, the membrane with fouling resistant property cannot exhibit all advantages to prevent biofilm formation effectively since the subsequent accumulation of bacteria are affected significantly by the interaction between the fouled surface and new foulant after formation of a biofouling layer on the surface [39]. Thus, the fouling release/cleaning property of membrane is very crucial in prevention the biofilm formation on the membrane surface. Currently, there are few of research works focusing on membrane modification to combine fouling resistance and fouling release/cleaning property. Wang and coworkers [39] well designed a reverse osmosis membrane with combined “anti-adhesive” and “anti-microbial” property to achieve better anti-biofouling effect via grafting zwitterionic polymer carboxybetaine methacrylate (CBMA), and the results showed that FRR of the final modified membrane was higher than 92% with high mortality of bacteria attachment of 99%. A recent study [79] also reported a modified thin-film composite membrane with complementary “defending” and “attacking” capabilities against biofouling by incorporating zwitterionic polymer, demonstrating low biofouling property and excellent bacterial inactivation. Furthermore, Zhao and coworkers prepared a polyethersulfone (PES) blend membrane with amphiphilic block copolymer hydrophilic poly(ethyleneoxide) (PEO) and low surface energy polydimethylsiloxane (PDMS), exhibiting fouling resistance and fouling release property simultaneously [80]. Therefore, the study in a combination of fouling resistance and fouling release is very crucial, and it is a promising strategy to improve membrane antifouling performance and has drawn increasing attention.

Except for the polymers mentioned in previous studies [39, 79, 80], PNIPAAm has

been considered as a promising polymer used in membrane modification to exhibit a fouling-resistant and fouling-release property owing to its tunable hydrophilicity in response to temperature [31, 40]. In addition, it is reported that the PNIPAAm could enable membrane exhibit thermos-sensitive property to obtain high separation efficiency of $\geq 99.26\%$ for practical oil-water separations [41], outstanding permeability [42] and excellent antifouling property [43]. Therefore, PVDF membrane has been modified by incorporating PNIPAAm chains to resist protein fouling and control bacterial adhesion under temperature regulation, leading to inhibit the further formation of biofilm on the substrate surface. This information also gives us an idea on how to prevent membrane biofouling formation, which is an advantage of incorporation of PNIPAAm polymers compared to other functional groups.

2.3.2 The property of PNIPAAm chains

Previous studies have reported that hydrophilic surfaces provide great property for control of biofouling due to their stability in aqueous solution and resistance to initially attachment of living cells [81, 82]. Therefore, the hydrophilicity of material interface can be regulated via surface modification by grafting functional polymers in order to obtain low-adhesive and fouling-release surface. Besides, the surface physicochemical characteristics, such as electrostatic and Lifshitz-van der Waals forces, charge, surface chemical composition and adsorbed water, are considered as main factors affecting microbial attachment and behavior, and also have an important effect on the interactions at the material interface. Thus, it is also significant to investigate the properties and characteristics of the fouling-release material surface.

With the change of temperature across LCST, the hydrophobicity of PNIPAAm varies, and the conformational transition between hydrophilic state and hydrophobic state is showed as Figure 2-3 [83]. Below LCST (about 32 °C), water provide a good aqueous environment for PNIPAAm chains so that interactions between polymer and water are stronger than that of polymer-polymer. Under this situation, hydrogen bonds are formed between the water molecule and the hydrophilic moieties of PNIPAAm chains, and hydration of water also exists in this environment and modifies the interaction between hydrophobic moieties. As a consequence of these phenomena, the polymer chains exhibit an extended coil structure which allows maximum interaction between the water molecule and the polymer. With the increase of temperature, the water becomes a non-favorable solvent for the polymer, leading to a stronger interaction between polymer surface and proteins or hydrophobic compounds. Above LCST of PNIPAAm, the hydrogen bonds between the water molecule and polymeric chains are disrupted, and the water molecule is expelled from the polymeric coils owing to increase of hydrophobic interactions. As a result, a compact globules structure of polymeric chains is formed as PNIPAAm chains hide the hydrophilic amide groups and expose the hydrophobic isopropyl groups. The phase transition is very similar to that of biological counterpart such as protein, and can be considered as the process of reversible cold denaturation of the protein. Due to the unique property, PNIPAAm also presents a certain potential in bioengineering and biomedical applications. Note that, well-defined PNIPAAm brushes and PNIPAAm-coated substrate have been commonly applied in the preparation of temperature-sensitive surfaces for controlling bacteria adhesion and protein adsorption.

Figure 2- 3. Reversible conformational transition of PNIPAAm chains [83].

A great number of papers reported that the attachment and detachment of tissue cells on PNIPAAm-coated substrates can be controlled by temperature stimuli, without any enzymatic reaction [84, 85]. Generally, above the LCST of PNIPAAm, cells can adhere, grow and proliferate on hydrophobic PNIPAAm-coated surfaces. As the temperature decreases below the LCST, the cellular adhesion is weakened owing to the structure change of polymer chains and the cells are released spontaneously from the hydrophilic PNIPAAm layer. Additionally, the attachment and detachment of bacterial cells on PNIPAAm film is also switchable by tuning the operating temperature [86].

Temperature-responsive polymer PNIPAAm have been exploited its fouling release property in a lot of studies. Ista [86] examined the bacterial release property using PNIPAAm-coated polystyrene and the results showed that the attached bacterial cells released over 90% from the polymer thin film after 2, 18, 36, 72 h incubations. It also demonstrated that fouling release occurred when the wettability of the polymer was changed from the attachment-favorable state to less attachment-favorable state. A research article [87] showed that surface grafted PNIPAAm release attached bacterial cells upon

induction of temperature-triggered phase transition, and it also demonstrated that altering the physicochemical characteristics of PNIPAAm thin film by changing the composition of the layer. David and coworkers also investigated the effect of phase transition of surface-grafted PNIPAAm copolymers on protein and bacterial attachment [88]. In this case, the amount of attached protein was affected by a copolymer of PNIPAAm with different functional polymers. The results showed that protein adsorption/release and bacterial adhesion/detachment correlated well with properties changes of the copolymer surface.

Recently, the properties of membrane with PNIPAAm can be determined and analyzed using some measurements and instrument to describe protein and bacterial adhesion/detachment, for instance, surface hydrophobicity via contact angle measurements, topography and morphology analysis using atomic force microscopy (AFM), and analysis of surface chemical properties via Fourier-transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). The studies show that the roughness, thickness and chemical composition of underlying layer also affect the fouling-resistant effectiveness of antifouling surface with PNIPAAm [84, 87, 89]. For example, Kong and coworkers [90] prepared the copolymer brushes of PNIPAAm on glass surfaces and studied the effect of film thickness and polymer chemical compositions on the cells attachment and detachment behavior. The results indicated that cells behavior most likely correlated with contact angles of the surfaces, and a slight change in thickness or chemical compositions significantly affected the cells adhesion and detachment behavior on the surface.

2.4 Surface modification by grafting PNIPAAm

Chemical and physical surface modification have been attempted for coating polymer brushes. However, the physical sorption is a reversible process and leads to unstable modification due to the weak interaction [33]. There are two typical strategies for covalent coating of polymer brushes, “grafting to” and “grafting from” respectively. For the “grafting to” technique, polymer chains terminated with reactive groups and functionally molecules or on the side chains covalently anchor and bind to the surfaces. On the other hand, the “grafting from” approach is a heterogeneous polymerization process whereby active groups existing on the surfaces initiate the polymerization of monomers from the solution. In comparison with “grafting to”, the “grafting from” technique has been verified to be more flexible and versatile more likely due to initiator sites on the surface. In addition, the “grafting from” method can effectively generate functional polymer layers with appropriately high thickness and density under a controllable model. Meanwhile, the polymer grafting density is affected by molecular weight (MW) of the polymer chain using “grafting to” strategy, while polymer chain density is independent to the molecular weight in “grafting from” approach. For the overall performance of modified surface, the grafting density has a significant influence on fouling release property. For instance, low grafting density results in low antifouling ability, and over high grafting yields can weaken the response to temperature stimuli and also reduce antifouling ability because the polymer chains are trapped in an extensive construction [91].

Surface-initiated polymerization (“grafting from”) generally can be conducted in

two steps: firstly, the initiator of polymerization is immobilized onto the surface; secondly, the monomer can be added to the initiator sites and then polymer grow from this site. There are several grafting methods for polymerization, including plasma-initiated polymerization, photo-initiated polymerization, thermal-initiated grafting, redox-initiated grafting, and controlled radical grafting methods including atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT) polymerization [91]. To date, surface-initiated ATRP is a typical grafting method to incorporate functional groups to membrane, and it provides some advantages to polymerization, including better control to design the structure and length of polymer chains which result in controlled pore size and the distribution, and well-designed composition of polymer chains.

It is reported that ATRP has been utilized to graft different functional groups to PVDF membrane for surface modification [64, 92]. Additionally, a great number of studies reported that PNIPAAm layers can be fabricated successfully onto target substrate surface via surface-initiated ATRP. Xu and coworkers [93] obtained well-defined functional PNIPAAm brushes from silicon surfaces through surface-initiated ATRP to control cell adhesion/detachment, and the results indicated that the limited step of cell substrate recovery rate depended on the hydration of the grafting PNIPAAm brushes. In this case, copolymer brushes of NIPAAm-PEGMA (poly(ethylene glycol) methacrylate) were also synthesized via surface-initiated ATRP, and for this copolymer brushes, the hydration of the cell-attached surfaces increased due to incorporation of PEGMA units with PNIPAAm chains compared with PNIPAAm brushes. As a consequence, rate of cell detachment increased from copolymer brushes. Chen and coworkers [94] successfully

prepared PNIPAAm brushes through surface-initiated ATRP to examine the relationships between the cells adhesion/detachment and the physiochemical properties of PNIPAAm brushes. The results indicated that the morphology of polymer brushes was affected by polymerization time so that the initial rate of cells detachment increased as the increase of polymerization time. Moreover, the average energy of cell attachment decreased with the increase of PNIPAAm brushes density during 40 min of detachment. Additionally, Daniel and coworkers [60] also prepared grafted PNIPAAm-*block*-PPEGMA (poly(oligoethylene glycol methacrylate) brushes from ultrafiltration membrane surface via surfaced-initiated ATRP. The properties of membrane surface were examined and the performance of modified membrane during wastewater treatment process was also studied. The results illustrated that roughness of membrane surface was reduced following PNIPAAm-*b*-PPEGMA compared to the unmodified membrane. Finally, the results also showed that almost 100% flux was recovered for PNIPAAm-*b*-PPEGMA-grafted and PNIPAAm-grafted membrane while the flux recovery achieved about 81% for the unmodified membrane. Therefore, this can provide an easy, chemical-free method to remove membrane fouling for wastewater treatment.

2.5 Functional copolymers of PNIPAAm

There are many other polymeric materials which have been studied and reported to reduce biofouling, and the polymers include poly(ethylene glycol) (PEG) [95], poly(2-hydroxyethyl methacrylate) (HEMA) [96], poly(hydroxypropyl methacrylate) (PHPMA) [97], poly(sulfobetaine methacrylate) (PSBMA) [98], poly(carboxybetaine methacrylate) (PCBMA) [99], poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC)

[100], poly(N-hydroxyethyl acrylamide) (PHEAA) and poly(2-hydroxyethyl acrylate) (PHEA) [101] and so on. It is considered that we can incorporate another hydrophilic polymer with PNIPAAm onto PVDF membrane so as to investigate both fouling resistance and fouling release property, thus achieving better performance, since grafted copolymer probably further enhance antifouling performance owing to a synergistic effect of multiple functional groups [80].

Many studies report that copolymers of PNIPAAm with other functional monomers have been fabricated successfully and carry better property compared to homopolymer of PNIPAAm. In an article [102], the effect of length and ratio of poly(ethylene oxide) (PEO) in copolymerization with PNIPAAm on the micellar structure and thermo-sensitivity was studied. The results showed that long PEO chains (MW 1900 g/mol) could increase LCST of PNIPAAm whereas the short PEO block (MW 550 g/mol) had no obvious effect on LCST of PNIPAAm. Meanwhile, LCST of PNIPAAm reduced with the increase in the ratio of PNIPAAm/PEO for long PEO block. Ebara and coworkers [103] successfully prepared the copolymer of NIPAAm with CIPAAm (2-carboxyisopropylacrylamide) for control cell adhesion and de-adhesion. The side chain structure of CIPAAm was very similar to that of NIPAAm and also had a functional carboxylate group. As a consequence, LSCT and thermo-sensitivity of the copolymer were very similar to that of the NIPAAm homopolymer. However, the copolymer exhibited better cell detachment capacity and accelerated cell detachment time due to the introduced carboxylic acid moiety. The results indicated that PNIPAAm homopolymer released about 90% cells from the surface in 120 min while P(NIPAAm-co-CIPAAm) accomplished the same target in 60 min. There are dual pH-thermal responsive copolymers that have the property in response to both pH and

temperature, such as poly(N-isopropylacrylamide-*co*-acrylic acid), copolymers of poly(N-isopropylacrylamide-*co*-propylacrylic acid), copolymers of PNIPAAm and Poly((2-dimethyl amino)ethyl methacrylate) [P(DMAEMA)]. It is showed that great species of bacteria have negative charge under environmental pH so that the pH-thermal responsive copolymers can be used to control bacterial adhesion. In an article [104], the authors successfully prepared pH-thermal sensitive copolymers of PNIPAAm with poly(methacrylic acid) (PMAA), i.e. PMAA-*b*-PNIPAAm, and the results demonstrated that responsive behavior of copolymer to external stimuli was different from that of PMAA and PNIPAAm homopolymer, respectively. More specific, the phase transition and responsive behavior were broadly controlled by pH and temperature stimuli.

It is well known that poly(ethylene glycol) (PEG) is usually employed in application of controlling protein adsorption and microbial adhesion. Thus, materials in biomedical and bioengineering can be modified by PEG brushes or coating owing to its antifouling properties. Some studies reported that incorporation of PEG into PNIPAAm could accelerate cell detachment from the surface compared to PNIPAAm homopolymer [105, 106]. In a study [107], the author fabricated co-grafted polymer of PEG and PNIPAAm onto porous membrane to achieve rapid cell detachment. The results showed that cells detachment could be accomplished completely from membrane grafted only PNIPAAm during approximately 35 min while all cells could be removed almost from co-grafted membrane in only 19 min. This phenomenon might be due to the fact that grafted PEG chains should accelerate the water molecules diffusion to PNIPAAm chains for co-grafted membrane. In addition, poly((3-(methacryloylamino)propyl)-dimethyl(3-sulfopropyl)- ammonium hydroxide)

(PMPDSAHA) was reported as a zwitterionic polymers that could resist to fouling. The PMPDSAHA brushes grafted onto surfaces could minimize the nonspecific protein adsorption due to zwitterionic moieties of the polymer chains. Kong and coworkers [90] fabricated copolymers brushes of PNIPAAm and PMPDSAHA onto a glass surface through surface-initiated ATRP, and the effect of copolymers on cell attachment and detachment was investigated. The results showed that the copolymer could well control the cell behavior of adhesion/detachment, and the thickness of film layer and ratio of NIPAAm/MPDSAHA both affected the cell detachment.

2.6 Thermosensitive membrane with PNIPAAm

Recently, fast development has been achieved in the field of thermosensitive membrane via modification by PNIPAAm and its copolymers with other functional materials. The thermosensitive membranes generally exhibit the changes in polymers chain conformation leading to changes in surface property and overall performance. It is acknowledged that thermosensitive membrane has good property to reduce membrane fouling due to the change in hydrophilicity. Therefore, many studies in terms of thermosensitive membrane have been conducted to develop their antifouling property and overall performance such as permeability and rejection.

Peng and Cheng [108] designed porous PNIPAAm-g-PE (polyethylene) membrane by grafting PNIPAAm onto PE microfiltration membrane and examined the effect of grafting density on vitamin B₁₂ permeability of the modified membrane. The results showed that increase in temperature could increase the permeability of modified membrane with low graft yield of PNIPAAm whereas the permeability increased as

temperature decrease in high graft yield membrane. For membrane with high graft density, the membrane pores were filled by PNIPAAm polymers and a grafting surface layer was formed to regulate the permeability. Wan and coworkers [109] prepared a thermo-responsive membrane via surface-initiated ATRP method. The functional brushes formation was performed in two steps: at first, 2-hydroxyethyl methacrylate (HEMA) grafting was carried out from the polypropylene membrane surface and then NIPAAm was introduced to HEMA grafting layer via ATRP. The polymer composition and surface morphology of the regenerated membrane were analyzed by SEM, FTIR and XPS, and the property was examined through contact angle and water flux measurement. The results indicated that water contact angle exhibits sharp change during phase transition of PNIPAAm in response to temperature change. At the same time, the water flux of modified membrane showed a significant enhancement as temperature increased over its LCST, which was also due to the fact that the PNIPAAm chains collapsed above LCST. In a study [110], a temperature sensitive membrane was fabricated by grafting PNIPAAm brushes via ATRP approach. The effect of length and density of PNIPAAm chains on membrane characteristic was examined. As a result, the graft length and density were regulated by polymerization time, grafting temperature and NIPAAm monomer concentration. The results indicated that the length of PNIPAAm brushes was a very important factor affecting the properties of modified membrane.

Guo and coworkers [111] successfully prepared a PNIPAAm grafted porous polyethylene membrane which could resist biofouling effectively. In this study, the modified membrane was fouled by bovine serum albumin (BSA) at 35 °C and the fouling layer could be cleaned from the membrane surface with water washing at 25 °C. As a

result, 97.6% flux was recovered. At the same time, after membrane cleaning, the secondary BSA fouling could be cleaned by alkali solution wash with temperature change, and the flux recovery reached up to 99.1%. In another study [112], a pH and thermosensitive membrane was prepared based on a commercial RO membrane using N-isopropylacrylamide-*co*-acrylic acid copolymers (P(NIPAm-*co*-AAc)) via ATRP method. The results showed that the hydrophilicity and surface charge at neutral pH of the modified membrane were increased, and (P(NIPAm-*co*-AAc)) grafted film could improve the antifouling capacity and foulant cleaning efficiency. This was owing to the fact that the foulant on the membrane surface could be released by copolymers layer during the phase transition across LCST. Furthermore, a study [113] reported that an amphiphilic membrane was well designed by a copolymer of PVDF backbones and PNIPAAm side chains using phase inversion. The PNIPAAm side chains addition increased the hydrophilicity of the membrane compared to initial PVDF membrane. As described previous, Daniel and coworkers [60] modified a commercial cellulose ultrafiltration membrane by grafting copolymer PNIPAAm-*b*-PPEGMA. The performance of modified membrane was evaluated by permeation flux measurement, flux recovery and organic removal using synthetic produced water as feed. It was observed that removal of total organic carbon using modified membrane was higher than that of the unmodified membrane. At the same time, flux recovery of modified membrane almost reached 100% after a cold water rinse while only 81% flux could be recovered for the original membrane. These studies demonstrated that the property and performance of membrane could be improved significantly by grafting thermosensitive and amphiphilic polymer PNIPAAm and its copolymers.

CHAPTER 3: Antifouling PVDF membrane prepared by PNIPAAm¹

3.1 Introduction

As mentioned in previous section, it is well known that PVDF membrane is widely utilized in various field even in commercial application due to several advantages compared with many polymeric membranes, such as outstanding chemical resistance, high thermal stability, strong mechanical stress and well-controlled fabrication [1, 38]. However, the membrane fouling easily occurs on the PVDF membrane owing to its high hydrophobicity. Many studies reported the hydrophobicity of PVDF membrane can be decreased via modification by PNIPAAm and other functional materials in order to reduce membrane fouling [58, 59, 114], and there are some products of commercial hydrophilic PVDF membrane already. Specifically, some literature reported that different functional polymers could be grafted directly from PVDF membrane via ATRP [35, 64, 92], and in the further studies, the functional chains also could be grafted from PVDF membrane activated by pretreatment or incorporation of active groups [109, 115, 116]. However, the severe membrane fouling caused by foulant such as proteins, cells and bacteria still existed in a wide range of application. The fouling resistance of membrane cannot exhibit all advantages to prevent biofilm formation effectively since the subsequent accumulation of bacteria are affected significantly by the interaction between the fouled surface and new foulant after formation of a biofouling layer on the surface. Thus, the fouling release property of membrane plays an important role in inhibition of the biofilm formation on

¹ (This chapter is reproduced with permission from G. Zhao, W.N. Chen, *Enhanced PVDF membrane performance via surface modification by functional polymer poly(N-isopropylacrylamide) to control protein adsorption and bacterial adhesion*, *Reactive and Functional Polymers*, 97 (2015) 19-29. Copyright 2015. Elsevier)

the surface. Currently, it has drawn increasing attention in the research of membrane modification to combine fouling resistance and fouling release/cleaning property since it is considered as a promising strategy to improve antifouling property of membrane. PNIPAAm has been regarded as a promising material to form a fouling-resistant and fouling-release surface in a wide range of applications owing to its tunable hydrophilicity in response to temperature [31, 40]. Therefore, in this work, PNIPAAm was used to modify PVDF membrane to resist protein fouling and control bacterial behavior (attachment/detachment) under temperature regulation, leading to inhibit the further formation of biofilm on the substrate surface. This also assists us to know how to prevent membrane biofouling formation, which is an advantage of grafting PNIPAAm compared to other functional materials.

In this chapter, thermosensitive polymer PNIPAAm was prepared on PVDF membrane by using a modified method including an improved pretreatment process and followed by surface-initiate ATRP. More importantly, a dramatic enhancement in grafting density was observed and finally the modified membrane had a good thermosensitive property and obvious biofouling resistant capability.

3.2 Materials and methods

3.2.1 Materials

The PVDF membrane used in this study with a diameter of 50 mm and average pore size of 0.45 μm was supplied from ACME Research Support Pte Ltd., Singapore. The commercial hydrophilic PVDF membrane was purchased from Environmental

Express, USA. The following chemicals and reagents were purchased from Sigma-Aldrich, with the purity showed as wt.%: *N*-isopropylacrylamide (NIPAAm, >99%), 2-Bromoisobutyryl bromide (BIBB, 98%), 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA, 97%), copper (I) chloride (CuCl, 99.995%), copper (II) chloride (CuCl₂, 97%), triethylamine (TEA, ≥99%), tetrahydrofuran (THF, anhydrous, 99.9%), methanol (anhydrous, 99.9%), ethanol (anhydrous, 99.9%), sodium hydroxide (NaOH, 98%), ethylenediamine tetra-acetic acid (EDTA, 99%). Potassium permanganate (KMnO₄, 99%) and bovine serum albumin (BSA, >99%, *M_n*=67 kDa) were both obtained from the Chemicals Testing & Calibration Laboratory Pte Ltd., Singapore. The water used in this study was double distilled water.

3.2.2 Preparation of PNIPAAm grafted PVDF membrane

The PVDF membranes were firstly immersed in anhydrous methanol for 30 min to remove any organic impurity on the surface and then rinsed completely with water. After that, the washed membranes were dried using vacuum oven overnight for further use. The PVDF-*g*-PNIPAAm membranes were then prepared as the following protocol showed in Figure 3-1. In detail, PVDF membranes were pretreated using a different concentration of NaOH aqueous solution respectively at 70 °C for 24 h and then rinsed thoroughly with water. Thereafter, the membranes were immersed into KMnO₄ aqueous solution (5wt. %) at 40 °C for 6 h, and then exhaustively washed with water and dried under vacuum overnight. A previous study [116] reported that surface-initiated ATRP reaction was first carried out using BIBB as initiator from the PTFE membrane which has the same chemical bond C-F as PVDF membrane. In this study, the ATRP initiator BIBB was

immobilized onto the membrane surface as following steps: one piece of membrane was put into a glass vial with 20 ml THF followed by 0.5 ml TEA, and then 1 ml BIBB was added into the mixture dropwise at 0 °C under an argon atmosphere; the reaction was conducted under 0°C for another 2 h, followed by stirring at room temperature for 24 h. Subsequently, the obtained membrane with initiator was washed extensively with methanol and dried by the high purity of argon stream. After that, the activated membrane with initiator was put into a glass vial with 15 ml NIPAAm solution (with 0.20 g/ml) that had been already degassed, and then the ligand HMTETA was added into the solution. After purging with argon for 30 min, a certain amount of CuCl and CuCl₂ were added into the mixture under an argon atmosphere and the glass vial was sealed to start the polymerization. The mixture solution of polymerization consisted of monomer, catalyst complex and ligand as the mole ratio of [NIPAAm]:[CuCl]:[CuCl₂]:[HMTETA] at 100:1:0.2:1.2. After 24 h reaction, the membrane was taken out from the solution and rinsed successively with methanol and distilled water. In addition, the obtained modified membranes were also washed by EDTA solution to elute Cu²⁺ on the membrane surface and then rinsed with water. Finally, the PVDF-*g*-PNIPAAm membranes were dried at room temperature in a vacuum oven for further use. In this chapter, the modified membrane pretreated by 5M, 10M, and 12M NaOH aqueous solution were described as M5, M10 and M12 PVDF-*g*-PNIPAAm membrane, respectively.

Figure 3- 1. Schematic illustration of preparation of modified membranes

The grafting yield of PNIPAAm layer on the membrane surface was described as the weight of PNIPAAm brushes per unit area of the membrane, and the grafting degree (GD) was calculated gravimetrically according to the following equation Eq. 3-1:

$$GD = \frac{M_{PNIPAAm} - M_{Br}}{M_{Br}} \times 100\% \quad (\text{Eq. 3-1})$$

where M_{Br} and $M_{PNIPAAm}$ are the weight of initiator-immobilized membrane and PNIPAAm grafted membrane, respectively. The data was calculated based on three measurements for each sample.

3.2.3 Characterization of membranes

1) Surface chemical composition

The surface chemistry information on the original and modified PVDF membrane surface was characterized by attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR, PerkinElmer, USA). Each spectrum was scan over the range of 550–4000 cm⁻¹ at a resolution of 8 cm⁻¹ using an ATR unit.

2) Thermal gravimetric analysis

Thermo-gravimetric analysis (TGA) was performed on a TGA instrument (Diamond TGA analyzer, PerkinElmer, USA). The analysis condition was showed as following: the heating temperature was from 40 °C to 800 °C at a heating rate of 10

°C/min and under an air flow of 200 ml/min.

3) *Hydrophilicity of membranes*

Water contact angle was measured with a contact angle instrument (FTA 200 Dynamic Contact Angle analyzer, USA) equipped with video camera and digital image analysis software. 5 μ l sessile drop of water was vertically deposited onto the membrane surface at 5 different positions of each sample and the average value was calculated as its contact angle. The result was recorded exactly 3 seconds after the water drop had been deposited on the membrane surface in order to reduce variations of droplet shape along with time.

Hydration capability (g/cm^3) of membranes was also measured. Dry membrane sample with a certain area and thickness was first weighed and then immersed in pure water for 24 h. After that, the water on the surface was gently wiped out using a tissue and recorded the new weight of the sample. Hydration capability was calculated from the difference weight of the dry and wet sample. At least three tests were conducted for each sample.

4) *Surface morphology of membranes*

The surface morphology and cross-section of the original PVDF membrane and PNIPAAm grafted membrane were analyzed using scanning electron microscopy (SEM, JSM-6701F, JEOL Asia Pte. Ltd.). The membrane samples were cut into about 0.5 cm $\times$ 0.5 cm pieces and coated with platinum to make them conductive. The cross-sectioned samples were prepared by fracturing membranes in liquid nitrogen and then dried completely. Each sample was scanned at 5 (at least) different locations.

Atomic force microscopy (AFM, XE-100, Park Systems Corp., Korea) was used to

analyze the surface topology of the original and modified membrane, and the surface roughness of the samples was also obtained. The measurement was performed in the non-contact mode and the average root-mean-square (RMS) roughness was calculated according to 5 different points.

In order to further compare the surface morphology of membrane before and after modification, the pore size distributions on the top surface of unmodified and modified membrane were analyzed by picture analysis software (ImageJ: Image Processing and Analysis in Java) based on SEM images, which was referred to the microscopic analysis method showed in previous publications [117, 118]. The pore size on the membrane top surface was analyzed by ImageJ software according to SEM pictures and the pore size distributions were calculated accordingly. Measurements were taken at more than three different locations for each sample and the pore size distribution was calculated based on at least 200 pores.

5) Mechanical stability measurement of membranes

The mechanical stability of membranes was detected by tensile meter (Instron 5543, USA) with a load cell of 100 N at room temperature (25 °C). Three times were tested and the average value was taken for each sample.

3.2.4 Protein adsorption measurement

In order to evaluate the protein resistant ability of the pristine and modified PVDF membrane, protein adsorption measurement was conducted using bovine serum albumin (BSA) as a model protein. The pristine membrane and modified membrane with the same area (3.6 cm²) were both immersed in anhydrous ethanol and subsequently 0.9% NaCl

solution for 30 min, respectively. After that, the membranes were immersed into 3 ml BSA solution with the concentration of 0.5 mg/ml in a shaker incubator at 25 °C and 37 °C respectively for 22 h. The concentration of BSA solution after and before membrane adsorption was detected using Bradford method with UV spectrophotometer at 595 nm [119]. The amount of protein adsorbed on the membrane surface was calculated by the comparison of BSA concentration before and after membrane adsorption. The measurement was conducted three times for each membrane sample.

3.2.5 Microfiltration experiments

1) Permeation performance of membranes

A cross-flow cell filtration system was designed to measure water flux of the original and modified membrane to evaluate filtration performance, and the effective area of filtration cell was 3.8 cm². The filtration experiment results were recorded at a steady filtration after 60 min operation. Pure water flux of virgin and modified membranes were measured under a pressure of 1 bar with flow rate of 400 ml/min at 25 °C and 37 °C, respectively. The flux was determined by the following equation Eq. 3-2:

$$J = \frac{V}{A \times t} \quad (\text{Eq. 3-2})$$

where J is the initial flux, V is the total volume of permeation, A is the effective membrane area for filtration, and t is the tested time. Furthermore, in order to investigate the membrane permeation and fouling caused by protein, the flux of the original and modified membrane was also detected using 1.0 g/L BSA solution in 0.01 mol/L Phosphate Buffered Saline (PBS) (pH=7.4) under a pressure of 0.8 bar with flow rate of

300 ml/min at 25 °C and 37 °C, respectively. The filtration test was carried out three times for each membrane sample.

2) *The flux recovery ratio of membranes*

Membrane performance and antifouling property were evaluated by several runs of BSA solution filtration and then membranes were washed with water after each run of filtration. The microfiltration experiments were carried out under the processes as following: the membrane was initially compacted using pure water for 1 h at 1 bar and the J_{w1} was obtained; then flux J_{BSA} was recorded at 1 bar every 5 min using 1.0 g/L BSA filtration for 30 min; after that, membrane was washed and cleaned thoroughly using water for 1 h and the flux was retested and expressed as J_{w2} ; all the experiments were conducted at 25 °C. The water flux recovery ratio (FRR) was calculated by the following equation Eq. 3-3:

$$FRR = \frac{J_{w2}}{J_{w1}} \times 100\% \quad (\text{Eq. 3-3})$$

and the higher FRR values mean better antifouling property in the protein filtration experiment.

In order to further evaluate the thermosensitive property of membranes under temperature control, protein filtration testing was carried out at 37 °C (above LCST) using 1.0 g/L BSA solution, and then the membrane was washed with cold water at 20 °C below the LCST. The water flux of cleaned membrane was recorded and FRR was also obtained.

3.2.6 Bacteria adhesion test

In order to further evaluate the antifouling property of membranes, bacterial

adhesion behavior on the membrane surface was tested using *Escherichia coli* which is regularly used to assess the antifouling property of membranes [120, 121]. *E. coli* was cultivated in LB (lysogeny broth) medium which consists of 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L sodium chloride. The culture was incubated at 37 °C under shaken speed of 200 rpm for 5 h, and finally, the bacterial concentration reached 10^8 cells/ml. The original membrane was immersed in methanol for 0.5 h and then adequately washed with water. Next, the original membrane was dried under vacuum. After that, dry original and modified membrane samples were immersed in 5 ml bacteria suspension in 6-well plate and incubation at 25 °C or 37 °C for 5 h. Thereafter, the membrane samples were washed with phosphate buffered saline (PBS) solution three times at the same temperature. The bacterial attachment on the membrane samples was examined by SEM (JSM-6701F, JEOL Asia Pte. Ltd.) referring to previous work [122, 123]. To obtain quantitative data for each condition, bacterial cells were counted on at least eight SEM images at a 5K magnification with an around $453.3 \mu\text{m}^2$ surface area. The bacteria number of per unit surface area could be calculated and the average value was also obtained.

Biofouling associated *E. coli* or *Pseudomonas fluorescens* was also visualized by confocal laser scanning microscopy (CLSM). Images were acquired with a laser-scanning microscope (Nikon, A1R hybrid spectral imaging confocal, Japan) equipped with 7 laser excitation lines (405, 457, 477, 488, 514, 561, 638 nm) and a motorized upright microscopy 90i. The bacteria adhesion experiment was similar to the previous section. After that, the different membrane samples with *E. coli* or *P. fluorescens* attachment were stained by SYTO 63 (Molecular Probes, Thermo Fisher Scientific). A laser was set at 638 nm to detect the red fluorescence and three measurements were conducted for each

sample.

3.3 Surface modification of PVDF membrane

Preparation of PVDF-*g*-PNIPAAm membrane via surface modification was carried out as the process showed in Figure 3-1. According to previous studies [124, 125], the formation of hydroxyl groups (-OH) and carbonyl bond (C=O) were observed on the PVDF sample surface after aqueous alkaline treatment, and in our work, PVDF membrane was activated via a modified pretreatment method including strong alkaline solution processing and followed by oxidizing agent treatment [125]. After the reaction, PVDF membrane was successfully incorporated with hydroxyl groups which are active to react with the ATRP initiator BIBB. As a result, the initiator was immobilized onto membrane surface through a chemical reaction between -Br groups and -OH groups. Thereafter, PNIPAAm chains were fabricated onto PVDF membrane surface from -Br active site via atom transfer radical polymerization.

Table 3- 1. Effect of alkali pretreatment on grafting density of PNIPAAm chains. Each data point represents the mean value $\pm$ the standard deviation (SD) calculated from three replicates.

Membrane name	Grafting density (mg/cm ²)	Grafting degree (%)
M5	0.90 $\pm$ 0.38	24.41 $\pm$ 11.40
M10	0.71 $\pm$ 0.15	22.31 $\pm$ 5.47
M12	0.39 $\pm$ 0.03	12.87 $\pm$ 0.50

The effect of alkali pretreatment on grafting density and grafting degree of PNIPAAm was investigated and the results are displayed in Table 3-1. It can be seen that the grafting density of M5 modified membrane was higher than that of M10 and M12

membrane, increased by 26.8% and 130.8% respectively. Meanwhile, the grafting degree of M5 and M10 modified membrane were both higher than 20%. Some literature [126, 127] reported that the PVDF membrane would degrade and lose its integrity after NaOH solution treatment at a relatively high temperature, and the effect would increase with the increase of alkaline concentration. Thence, the grafting density of M10 and M12 membrane were lower than that of M5 probably due to certain membrane degradation after treatment at a higher alkaline concentration. Therefore, the concentration of the alkaline solution of 5M was chosen for the membrane pretreatment in the subsequent study.

Referring to some previous studies [64, 111, 115, 128-130], the grafting density of PNIPAAm chains onto different polymers surface or grafting density of some polymers onto PVDF membrane surface generally ranged from 0.1 to 0.6 mg/cm², while the grafting density of PNIPAAm was significantly improved up to 0.90 mg/cm² through the modified method in this study. As mentioned above, even though different functional polymers can be grafted directly from PVDF membrane from the C-F initiating site via ATRP method, the grafting density is usually not high as the high C-F bond energy is not susceptible for direct ATRP, thereby limiting the antifouling property and performance of the membrane. Thus, in the recent studies, the functional chains also could be grafted from PVDF membrane activated by pretreatment or incorporation of active groups. According to the mechanism of treatment showed in previous papers [119, 124, 125], carbon double bond, carbonyl groups, and hydroxyl groups are formed in the membrane after using the modified pretreatment method. More importantly, grafting density of membrane prepared using the modified method is also enhanced to a certain extent. As a

result, the biofouling resistance and membrane performance would be affected and improved with high grafting density of PNIPAAm chains.

3.4 Characterization of membranes

3.4.1 Chemical composition of membranes

The chemical information of pristine PVDF membrane and PNIPAAm-modified membrane were analyzed by ATR-FTIR and the typical spectra are showed in Figure 3-2. Compared to the spectrum of original membrane, two new and obvious adsorption peaks appeared in that of modified membrane at about 1650 cm^{-1} and 1540 cm^{-1} as marked by dashed line, which were attributed to the secondary amide C=O stretching and N-H stretching of amide (O=C-NH) groups of grafted PNIPAAm chains, respectively, as well as mentioned in previous literature [30, 60]. The ATR-FTIR results proved that PNIPAAm chain was prepared onto the PVDF membrane surface via surface-initiate ATRP. Furthermore, from Figure 3-2, it also can be seen that the characteristic peaks of the M5 membrane were stronger than that of M10 and M12 membrane, which were consistent with the preliminary results of grafting density.

Figure 3- 2. ATR-FTIR spectra of the pristine and modified PVDF membranes: M5, M10 and M12 membranes with grafting density of 1.17, 0.60 and 0.43 mg/cm² respectively.

3.4.2 Thermal stability of membranes

The thermo-gravimetric analysis of unmodified and PNIPAAm-modified membrane was carried out and the related TGA curves are presented in Figure 3-3. There was only one-step degradation process starting at about 400 °C for pristine and pure PVDF membrane, which was due to decomposition of PVDF beginning at this temperature. In comparison to TGA curve of the unmodified membrane, two distinct weight loss stages were observed from TGA curve of modified membrane. It was due to the fact that grafted PNIPAAm layer and PVDF main chains in the modified membrane were decomposed at different temperatures, respectively. The first weight loss began at about 309 °C, which was attributed to the degradation of PNIPAAm chains. According to the analysis mentioned above, the second weight loss was caused by decomposition of PVDA main chains starting at around 400 °C. The results further demonstrated that PNIPAAm brushes were successfully grafted from the PVDF membrane surface.

Figure 3- 3. TGA curves of original PVDF membranes and PNIPAAm-modified membranes (grafting degree: 26%).

3.4.3 Hydrophilicity of membranes

As mentioned in the previous section, it is currently considered that the hydrophobic surface is susceptible to adsorption of biofouling compounds such as protein and bacteria. Thus, the hydrophilicity of membrane surface plays a crucial role in membrane antifouling property and filtration performance, and it is generally characterized by evaluating the water contact angle. In this study, the contact angle of the pristine membrane and modified membrane were detected at 25 °C and 37 °C, respectively, as shown in Figure 3-4 (a). The contact angle of virginal PVDF membrane was very high (about 106°) at both 25 °C and 37 °C owing to its high hydrophobicity, and the unmodified membrane exhibited no significant change in contact angle under different temperature. On the contrary, after incorporation of PNIPAAm, the modified membrane became much hydrophilic at 25 °C with a sharp decrease in water contact angle (below 77°). The results also indicated that the water contact angle of modified membrane experienced a significant increase along with the increasing of temperature. It is due to

the fact that PNIPAAm chains had hydrophilic and extended conformation resulting in the reduction of contact angle at 25 °C below the LCST, while the PNIPAAm chains showed a hydrophobic and compact structure leading to block water diffusion in the membrane surface at 37 °C above the LCST. The phenomenon revealed that the modified membrane exhibited obvious thermosensitive and tunable wettability after modification, which was ascribed to the grafting of amphiphilic PNIPAAm brushes.

Figure 3- 4. Water contact angle of pristine PVDF membrane and PNIPAAm modified membrane at different (a) temperature ($n=3$) and (b) drop age under 25°C.

The water contact angle of the original and modified membrane was also detected at different drop age under 25 °C and the results are shown in Figure 3-4 (b). It illustrated that the contact angle of the original membrane was rather stable at a high value of 106° within 45 s, while that of modified membrane dropped quickly and sharply in short time and reached only 53° at 45 s. The results indicated that the hydrophilicity of membranes was dramatically improved by incorporating PNIPAAm. Moreover, the PNIPAAm chains were probably not only grafted onto the PVDF membrane surface but also present inner the membrane pores so that the modified membrane had high water affinity and was

rapidly infiltrated by water within short time. The similar changes in contact angle were also obtained in the study of Wan and coworkers [109].

3.4.4 Surface morphology of membranes

In order to examine the effect of surface modification on membrane characterization as well as membrane property, structures and morphology of pristine and modified membranes were investigated by conducting SEM observation, and obtained images of surface morphology and cross-section are presented by Figure 3-5. The image (a) shows that the original PVDF membrane exhibited a porous structure on the top surface and the pores were open to creating continuous channels, while there was a thin brushes layer covered on the membrane surface after incorporation of PNIPAAm chains to the membrane (Image b). In addition, grafting polymer could be obviously observed in the SEM micrograph of the modified membrane, and the pore size of modified membrane top surface was smaller than that of the unmodified membrane (Figure 3-6), which was attributed to the fact that porous structure was covered with PNIPAAm brushes. Herein, SEM image (a) and (b) evidenced certain variation in the surface morphology of PVDF membranes after modification.

Figure 3-5 (c) and (d) present the cross-section of unmodified PVDF membrane and PNIPAAm modified PVDF membrane, respectively. The original and modified PVDF membranes both showed a sponge-like structure. However, comparing image (c) with (d), it can be seen that a denser structure was formed for the modified membrane, which might be due to the fact that part of PNIPAAm polymers also existed in membrane pores. Besides, the thickness of membrane which probably affect the filtration

performance increased significantly after surface modification (Table 3-2). Hence, the changes of membrane morphology evidenced that hydrophilicity of modified PVDF membrane rapidly increased when contacting with water drop in 45 s, mentioned in Section 3.2.3.

Figure 3- 5. SEM images of PVDF membranes: (a) surface morphology of the pristine membrane; (b) surface morphology of the modified membrane; (c) cross-section of the pristine membrane; (d) cross-section of the modified membrane.

According to SEM images of membranes, the pore size distributions on the top surface of the unmodified and modified membrane were analyzed and showed in Figure 3-6. The results indicated that the pore size distribution was narrowed, leading to a more uniform pore size distribution, and average pore size decreased after surface modification which probably affect the filtration performance negatively and reduce the flux of membrane. The morphology of original and modified membrane was also analyzed by AFM in order to detect the membrane roughness which plays an essential role in antifouling property, and the images are shown in Figure 3-7. According to AFM tests

results at 5 different points, the roughness of membrane surface decreased slightly after modification, which was 135.06 ± 40.59 nm for original membrane and 113.81 ± 28.84 nm for the modified membrane. It showed that the surface of modified membrane became smoother compared with the pristine membrane. This phenomenon also might cause less biofouling on the modified membrane surface which is discussed later.

Figure 3- 6. Pore size distributions on the top surface of unmodified PVDF membranes and PVDF-g-PNIPAAm membranes. Measurements were taken at more than three different locations for each sample and the pore size distribution was calculated based on at least 200 pores.

Figure 3- 7. AFM images of original and modified membrane: (a) the morphology of original membrane; (b) the morphology of modified membrane. The scale of the AFM scan is $5\mu\text{m} \times 5\mu\text{m}$.

Table 3- 2. The mechanical stability of original and PNIPAAm modified membrane. Each data point represents the mean value $\pm$ the standard deviation (SD) calculated from three replicates.

	Tensile stress at maximum tensile extension (MPa)	Tensile strain at maximum tensile extension (%)	Thickness (μm)
PVDF membrane	4.13 ± 0.21	43.44 ± 0.01	70.78 ± 1.34
PVDF-g-PNIPAAm membrane (10% grafting degree)	4.70 ± 0.75	23.36 ± 0.06	92.27 ± 2.02

3.4.5 Mechanical stability of membranes

In order to evaluate the mechanical stability of modified membrane, the tensile stress and tensile strain were detected using the tensile meter (shown in Table 3-2). The results showed that the tensile strain of membrane decreased significantly after surface modification, which demonstrated that the elasticity of original membrane was much higher than that of modified membrane. However, the tensile stress of membrane increased slightly with the incorporation of PNIPAAm, which revealed that the membrane could endure higher mechanical strength per unit area due to grafting PNIPAAm polymers.

3.5 Antifouling property and filtration performance of membranes

3.5.1 Protein static adsorption of membranes

The anti-protein static adsorption onto membrane surface had an important effect on the antifouling performance during protein filtration. Thus, in this work, the fouling resistant capability of pristine and modified PVDF membranes was evaluated by nonspecific protein adsorption experiment using a model protein BSA. As shown in

Figure 3-8, it is observed that BSA adsorption amount of modified membrane at 25 °C and 37 °C were both much lower than that of the unmodified membrane at the same temperature, respectively. Specifically, the amount of BSA adsorption for pristine membrane was 84 $\mu\text{g}/\text{cm}^2$ while the amount of BSA adhered to modified membrane descended dramatically to only 8.9 $\mu\text{g}/\text{cm}^2$ at 25 °C, with reduction by 89%. Meanwhile, the protein deposited on PNIPAAm-modified membrane was reduced by 16% at 37 °C in comparison with the unmodified hydrophobic membrane. Additionally, comparing Figure 3-4 (a) with Figure 3-8, it can be seen that the BSA adsorption amount of membranes declined with the increasing of the surface hydrophilicity of membranes. It tends to be demonstrated that the protein was effectively inhibited to attach to membrane surface after grafting PNIPAAm polymer. It also indicated that the protein adsorption on the original membrane decreased with the increase in temperature, which was probably due to higher thermal motion of protein molecular at higher temperature; while the BSA amount adhering to the modified membrane enhanced as the temperature increased, which was mainly caused by the hydrophobic property of the modified membrane at 37 °C.

Figure 3- 8. BSA adsorption amount of the virgin membrane and PNIPAAm modified membrane at different temperatures for 22 h incubation ($n=3$).

Table 3- 3. Comparison of PNIPAAm modified membrane prepared in this study and a commercial hydrophilic PVDF membrane at 25 °C ($n=3$).

	Water contact angle (°)	Hydration capability (g/cm ³)	BSA adsorption amount (µg/cm ²)
PVDF-g-PNIPAAm membrane (15% grafting degree)	53.68 ± 2.58	0.32 ± 0.02	28.75 ± 2.9
Commercial hydrophilic PVDF membrane	41.14 ± 1.89	0.58 ± 0.01	32.49 ± 0.44

In order to evaluate the property and performance of PNIPAAm modified membrane, comparison of the modified PVDF membrane and a commercial hydrophilic PVDF membrane was carried out in this chapter (shown in Table 3-3). The results indicated that the protein adsorption amount of modified membrane was slightly less than that of the commercial membrane. Consequently, it can be regarded that modified membrane also exhibited a higher anti-fouling performance compared to the commercial hydrophilic PVDF membrane. Additionally, in comparison with the commercial membrane, PNIPAAm modified membrane exhibited thermosensitive property which could assist us to investigate bacterial behavior (attachment/detachment) on the membrane surface in the future study, thereby leading to further examine how to control and prevent membrane biofouling.

3.5.2 Permeation performance of membranes

The permeate flux experiments were also conducted using pure water and BSA so as to evaluate the filtration performance of original and modified PVDF membranes. Figure 3-9 (a) and (b) shows the water flux of membranes using pure water filtration at 25

°C and 37 °C, respectively. It is observed that permeate flux of PNIPAAm-modified membrane were obviously much higher than the original membrane both at 25 °C and 37 °C during 5-6 h filtration. Specifically, as shown in Figure 3-9 (a), the flux of modified membrane was about 1.5 times that of the pristine membrane, which was attributed to the increasing of hydrophilicity and variation of membrane structure after modification as mentioned in the previous section. The Figure 3-9 (b) indicates that the permeate flux of the modified membrane experienced a remarkable decline after several hours of operation at 37 °C, which was mainly caused by hydrophobic property, but it was still much higher than that of the pristine membrane. The Figure 3-9 (a) and (b) also illustrate that the grafted PNIPAAm brushes brought a thermosensitive property to the modified membrane, which was also presented by the water flux difference between 25 °C and 37 °C. Even though the PNIPAAm chains were swollen and extended at the temperature below the LSCT resulting in the decrease of pore size [113, 131], the hydrophilicity still played a more important role in water permeation performance in this study. This is probably owed to a higher grafting density of PNIPAAm polymer prepared in this work.

Figure 3- 9. The flux of original PVDF membrane and modified PVDF membrane using a pure water filtration at (a) 25 °C and (b) 37°C; (c) using a 1 g/L BSA solution filtration at different temperatures ($n=3$); (d) for several runs of protein fouling-water washing processes: run 1 of fouling-washing process is indicated by R-1 in the legend.

In order to further examine the membrane resistance to fouling, a BSA solution filtration was conducted using original and modified membranes at different temperatures below and above the LCST of PNIPAAm, respectively. SEM images of protein fouled membrane were also showed in Figure 3-10. As shown in Figure 3-9 (c), the permeate fluxes at the start of experiment decreased dramatically for all membranes owing to protein fouling and concentration polarization on the membrane surface and protein adsorbed into the membrane pores (Figure 3-10) [132], and the similar phenomenon was also reported in previous studies [133, 134]. We compared the flux value of

PNIPAAm-modified membrane under different temperatures and found that the flux descended slowly and was much higher at 25 °C than that of filtration operated at 37 °C. This phenomenon demonstrated that the PNIPAAm chains on the membrane surface exhibited phase transition under the temperature range. At the same temperature, the flux through the modified membrane always exceeded permeate flux of the unmodified membrane even for several hours filtration, which indicated that PNIPAAm-modified membrane had a good antifouling property to resist protein adhesion on the surface. It is due to the fact that PNIPAAm brushes constructed a hydration layer between the membrane and bulk solution, and it is difficult for hydrophobic proteins to cross the hydration layer and deposit on the membrane surface.

Figure 3- 10. SEM images of membrane fouling caused by protein: (a) original PVDF membrane; (b) protein fouled membrane.

3.5.3 Flux recovery ratio (FRR) of membranes

Figure 3-9 (d) shows the permeation performance of original and modified membrane under several runs of filtration using BSA solution. It obviously indicates that the permeation flux of modified membrane was always much higher than that of the

pristine membrane. It is also observed from Figure 3-9 (d) that the flux of modified membrane can be recovered to a high value after four runs of fouling-washing processes while the permeation flux of original membrane dropped to a quite low value after only two times filtration. The results demonstrated that the fouling on the modified membrane caused by protein was removed easily compared to the original membrane, thereby providing an improvement of filtration performance for the modified membrane. The FRR values of membranes are presented in Figure 3-11. It can be seen that the FRR of original membrane declined to 70.89% after three times fouling-washing processes while it was still above 80% after three runs of protein filtration for the modified membrane. Moreover, the maximum FRR value 91.59% was obtained for the modified membrane, suggesting a high antifouling ability towards protein occurs on the membrane surface and protein fouling was almost swept away. According to above discussion, the results indicate that the modified membrane has the great potential of long-run utilization and operation reliability.

Figure 3-11 also shows the FRR of the modified membrane using BSA filtration at 37 °C and cold water washing at 20 °C. According to Section 3.4, it is known that the protein fouling is more serious at a higher temperature. However, the FRR still reached a high value, nearly 90%, which indicated that protein foulant attached on the membrane surface was easily removed and released by cold water washing.

Figure 3- 11. Water flux recovery ratio (FRR) of original and modified membranes using 1 g/L BSA solution filtration and water washing.

3.5.4 Bacteria adhesion test

Generally, biofouling on the substrate surface is induced by the interfacial interactions between the cells/organic molecule (protein) and the underlying substrate, which starts with the primary and secondary attachment of microbial cells, and proteins adsorption usually favors bacteria adhesion [122]. Once protein adsorption and first bacteria attachment were formed on the membrane surface, this surface was prone to undergo bacteria colonization, and leading to biofouling. Figure 3-12 displays bacterial adhesion behavior on the membrane surface by SEM analysis. The images show that much fewer bacteria were observed on the modified membrane surface at 25°C compared with the original membrane at 25°C and the modified membrane at 37°C. The bacterial cells more easily attached to the original membrane with a hydrophobic surface. From quantitative data indicated in Figure 3-13, bacteria adhesion on the modified membrane at 25 °C was reduced by 75% and 72% respectively compared to the virgin surface at 25°C

and the modified surface at 37°C, which was attributed to the hydrophilic property of modified membrane at 25°C below its LCST. It also can be seen from the confocal 3D images (Figure 3-14) that both *E. coli* and *P. fluorescens* adhesion on the PNIPAAm modified membrane were much less than the bacteria attachment on the original membrane. Moreover, the thickness of biofilm on the PVDF membrane was around 30.82 μm for *E. coli* and 34.25 μm for *P. fluorescens*, respectively, while the thickness of biofouling on PVDF-g-PNIPAAm were both approximately 30.82 μm for *E. coli* and *P. fluorescens*. The results further demonstrated that the modified membrane exhibited good anti-biofouling property to resist bacterial adhesion and good thermosensitive property.

Figure 3- 12. The SEM images of *Escherichia coli* adhesion on membrane surfaces for 5 h incubation at different temperatures.

Figure 3- 13. Quantitative attachment of *Escherichia coli* onto unmodified PVDF membrane and modified membrane after 5 h incubation at different temperatures ($n \geq 6$).

Figure 3- 14. In situ confocal images after 24 h adhesion of *E. coli* or *P. fluorescens* at 25 °C on original PVDF membrane (a, c) and PVDF-g-PNIPAAm membrane (b, d).

3.6 Conclusion

PVDF membrane had been well modified by PNIPAAm via an improved modification method, and finally, grafting density was significantly enhanced thereby effectively improving membrane performance, which was one advantage of this work. The results showed that PNIPAAm chains grafted from the membrane enhanced the hydrophilicity and brought a thermosensitive property to the membrane. Consequently, less amount of protein adsorption and bacterial attachment were observed on the modified membrane in comparison to the unmodified membrane, and the flux of modified membrane was always higher than that of the pristine membrane. The protein fouling formed on the modified membrane was more susceptible to be removed compared with the pristine membrane. Meanwhile, a relative higher antifouling property was also obtained on the modified membrane even compared with a commercial hydrophilic PVDF membrane. In conclusion, the modified membrane prepared in this chapter had the good antifouling capability and high performance for long-run utilization. Another advantage of the PNIPAAm modified membrane was its thermosensitive property which played a dominant role in the behavior of bacteria attachment/detachment on the membrane surface, leading to assist us to study how to prevent and release biofouling in the future work.

CHAPTER 4: Design of PVDF-g-P(HEMA-co-NIPAAm) membrane for enhanced antifouling property

4.1 Introduction

The preliminary results of this study showed that the antifouling property of PVDF membrane is significantly improved by surface-initiated ATRP of PNIPAAm. However, there are some problems in our previous work. For example, the polymerization of PNIPAAm lasted for 24 h which prolonged the membrane preparation process, and the filtration performance of modified membrane with grafted PNIPAAm at high temperature was not excellent. Hence, it is considered that we can incorporate another hydrophilic polymer with PNIPAAm into PVDF membrane so as to investigate both fouling resistance and fouling release property owing to a synergistic effect of multiple functional groups. Hydrophilic PHEMA has been applied in membrane technology to enhance the performance due to its high hydrophilicity, anti-biofouling property and good physical properties [109, 133], and PHEMA grafted membrane exhibited high permeability and excellent protein resistance [135]. Meanwhile, it is well known that the polymerization rate of acrylate monomers is quite rapid, and the acrylate monomers were used to form grafting copolymers with low glass transition temperatures to endow good contacting to the membrane [136]. Hence, in this chapter, copolymer of PHEMA and PNIPAAm was grafted onto PVDF membrane in order to obtain higher antifouling performance, which is one of limited works of study in both fouling resistance and fouling release, and also provide a feasible and effective strategy in fouling resistance/release enhancement

focused on PVDF membrane modification by copolymer P(HEMA-co-NIPAAm) compared to other studies [39, 79, 80].

The aim of this chapter is to prepare modified PVDF membrane so as to further improve antifouling property via surface-initiated ATRP. The modified membrane obtained is capable of inhibiting biofilm before complete formation on the membrane surface through investigation of bacteria behavior (attachment and detachment), which gives us information on how to control and remove fouling effectively in future work. Moreover, the improvement of antifouling property of membrane was justified by physicochemical properties analysis, including hydrophilicity, roughness, and adhesive force.

4.2 Materials and methods

4.2.1 Materials

The chemicals and materials used in this chapter are referring to the section 3.2.1. In addition, Hydroxyethyl methacrylate (HEMA, purity: 97%) was purchased from Sigma-Aldrich.

4.2.2 Membrane modification

In this chapter, the membrane modification process was improved compared with the section 3.2.2 since the membrane pretreatment using alkali solution and KMnO_4 required time as much as 30 h. Figure 4-1 shows the modified membrane preparation process. Briefly, a piece original PVDF membrane was treated by plasma cleaner (model PRC-32G Harrick, US) for both sides under the following conditions: low RF level 7 W;

pressure 0.2 Torr; flow rate of argon 60 mL/min, reaction time 2 min. After plasma treatment, the membrane sample was directly immersed into 20 mL THF mixed with 0.5 mL TEA, and then 1 mL BIBB was added into the solution dropwise at 0 °C under an argon atmosphere for introducing active -Br groups. The chemical reaction was carried out under 0 °C for 2 h, and for more 24 h at room temperature. The PVDF membrane was drawn out, washed extensively with methanol, and dried under argon stream resulting in PVDF-Br membrane. Thereafter, obtained active sample was put into a glass vial with 15 mL mixture of distilled water and methanol (1:1). After being degassed by a continuous stream of dry argon, the monomer HEMA or/and NIPAAm with different concentration, followed by the ligand HMTETA, were added to the mixture solution. CuCl and CuCl₂ used as catalysts were also put into the mixture under an argon atmosphere after the system purging with argon for 30 min, and the glass vial was sealed to start the polymerization at the temperature 40 °C. After the reaction, the membrane sample was rinsed thoroughly with methanol and followed by distilled water. The resulting modified membrane was dried under vacuum at room temperature for further use.

Figure 4- 1. Schematic illustration of the preparation of modified membranes.

In this chapter, three types of modified membrane, PVDF-g-PHEMA, PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane, were designed respectively with different monomer concentration, and they are further described as M1 (with low concentration) and M2 (with high concentration) membrane for the same type of membrane. Specifically, monomer HEMA concentration was 0.025 and 0.05 mol/L respectively for PVDF-g-HEMA membrane preparation; NIPAAm concentration was 0.075 and 0.125 mol/L respectively for PVDF-g-PNIPAAm membrane; HEMA concentration was 0.025 mol/L and NIPAAm concentration was 0.05 and 0.1 mol/L respectively for PVDF-g-P(HEMA-co-NIPAAm) membrane. The ATRP reaction solution consisted of monomer, catalyst complex and ligand as the mole ratio of [monomer]:[CuCl]:[CuCl₂]:[HMTETA] at 100:1:0.2:1.2. In addition, the polymerization

time lasted for 0.5 h, 24 h and 3 h respectively for PVDF-g-PHEMA, PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane preparation.

The grafting density of functional polymers on the modified membrane surface was described as the weight of polymer brushes per unit area of membrane, and the grafting degree (GD) was calculated according to the weight difference of membrane before and after ATRP reaction, showed as the following equation:

$$GD = \frac{M_m - M_{Br}}{M_{Br}} \times 100\% \quad (\text{Eq. 4-1})$$

where M_{Br} and M_m are the weight of PVDF-Br membrane and the resulting modified membrane, respectively. The data was calculated from three replicates for each membrane.

4.2.3 Membrane characterization

The membrane characterization was carried out partially according to the section 3.2.3. In particular, ATR-FTIR was used to characterize the changes in chemical information of membrane before and after surface modification. XPS surface analysis was performed on a spectrometer (XPS Quantera II, company) equipped with Al $K\alpha$ excitation radiation ($h\nu = 1486.6$ eV). The survey spectra (from 0 to 1300 eV) and the core level spectra were both recorded, and all binding energies were referred to the C 1s hydrocarbon peak at 284.6 eV. The elemental composition on the membrane surface was calculated by element analysis. Hydrophilicity of membranes was measured by a contact angle analyzer. Five tests were carried out at different locations with respect to the same

sample. Hydration capacity (g/cm^3) of membranes was also detected to evaluate the hydrophilicity and the test was conducted three times for each sample.

The surface morphology of pristine and modified membranes was observed by SEM and at least 5 scans were conducted for each sample. The surface roughness analysis and adhesive force measurement of membranes were conducted by atomic force microscopy AFM, and the average root-mean-square (RMS) roughness was calculated according to three tests.

4.2.4 Protein static adsorption test

The adsorption testing of protein was performed to evaluate the fouling resistant ability of membranes using BSA as a model protein, and the process was conducted according to section 3.2.4.

4.2.5 Bacterial attachment and detachment test

In order to further evaluate fouling resistant ability, the bacterial adhesion testing was also performed on the membrane surface using *Escherichia coli* according to bacterial adhesion test in section 3.2.6. Briefly, the *E. coli* was cultured in an LB medium placed in a shaker for 5 h incubation at 37 °C. The unmodified and modified membrane samples were immersed into 5 mL bacterial culture in a 6-well plate and then incubated for 5 h at 25 °C and 37 °C respectively. After rinsed with 0.01M PBS solution, bacteria adhering to the membrane surface were observed by SEM. The quantitative data of bacterial attachment on the sample's surface was obtained by counting the bacterial cells on a certain area of membrane according to at least 6 SEM images. Besides, after 5 h

bacterial adhesion at 37 °C on PNIPAAm and P(HEMA-co-NIPAAm) modified membrane (bacterial attachment number is recorded as N_1), the bacterial detachment from membranes was carried out in PBS solution. Specifically, the modified membranes attached with bacteria were put into PBS solution at 25 °C and 37 °C respectively for 5 h to release biofouling, and then they were rinsed with PBS. The bacteria adhering to the membranes were observed by SEM again, and the number of bacterial adhesion on the membrane is recorded as N_2 . The bacterial detachment rate (DR) was calculated by the following equation based on at least 3 measurements:

$$DR = \frac{N_1 - N_2}{N_1} \times 100\% \quad (\text{Eq. 4-2})$$

The microbial fouling on the membranes surface caused by *E. coli* was also visualized using CLSM according to tests in section 3.2.6. The biofilm was formed after bacteria deposition on the membranes surface for 24 h and this process was the same as bacterial adhesion experiment. Thereafter, *E. coli* biofilm on the different membrane samples were stained with SYTO 63 for 10 min and followed by washing with 0.9% NaCl solution. The samples were observed by CLSM and a laser was set at 638 nm to detect the fluorescence for three replicates.

4.2.6 Microfiltration performance of membranes

The permeation experiment was carried out using a cross-flow filtration system to characterize the filtration performance of pristine and modified membranes, and the effective permeation area of membrane cell was 3.8 cm². The system was filtered with

distilled water first for 60 min at a pressure of 1 bar to ensure a steady state. Then the permeation of membranes was measured under 1 bar at 25 °C using 1 mg/mL BSA solution (in 0.01 mol/L PBS, pH = 7.4) as filtration feed. The flux of membranes was calculated from the following equation:

$$J = \frac{V}{A \times t} \quad (\text{Eq. 4-3})$$

where J is the flux, V is the total volume of the permeate, A is the effective permeation area of the filtration system, and t is the permeation time, respectively. Moreover, bacteria challenge tests were conducted with *E. coli* suspension (10^7 cells/mL) in a cross flow filtration at a pressure of 1 bar and 25 °C, referring to a previous study [120] and a revised testing process. The flux and retention of membranes were detected during 30 min operation. The filtration was carried out three times for each membrane.

Filtration performance and antifouling property of membranes were also investigated by several runs of fouling-washing using 1 mg/mL BSA solution filtration and followed by pure water washing. Specifically, the microfiltration of membranes was conducted lasting for 0.5 h with 1 mg/mL BSA at 1 bar and 25 °C after 1 h pre-filtration, recording the flux as J_{w1} after 1 h pre-filtration using pure water and as J_{BSA} for BSA filtration. Afterward, the membrane was washed and cleaned thoroughly using water for 1 h, and the flux was re-evaluated and expressed as J_{w2} , herein one fouling-washing process completed. The fouling-washing process was also carried out at different temperatures with the fouling process at 37 °C and washing process using cold water at 15 °C. According to previous studies [137, 138], the antifouling capacity of membranes could be further evaluated by total fouling resistance ratio (R_t) which is divided into reversible

fouling ratio (R_r) and irreversible fouling ratio (R_{ir}). R_r describes the recoverable flux decrease caused by cake layer, while R_{ir} expresses the non-reversible fouling resulted from pore clogging and deposition of foulant on the membrane surface and inner pores.

The three ratios are calculated according to the following equations:

$$R_r = \frac{J_{w2} - J_{BSA}}{J_{w1}} \times 100\% \quad (\text{Eq. 4-4})$$

$$R_{ir} = \frac{J_{w1} - J_{w2}}{J_{w1}} \times 100\% \quad (\text{Eq. 4-5})$$

$$R_t = R_r + R_{ir} \quad (\text{Eq. 4-6})$$

4.3 Surface modification of PVDF membrane

In this chapter, in order to further improve the antifouling property of hydrophobic PVDF membrane, functional monomer HEMA and NIPAAm were grafted from PVDF membrane via surface modification by an ATRP method, resulting in designed PVDF-g-PHEMA, PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane. The simplified preparation process can be divided into three steps illustrated in Figure 4-1. First, the hydroxyl groups were introduced onto PVDF membrane surface via plasma treatment. Second, chemical reaction with hydroxyl groups was performed to immobilize the initiator BIBB of the ATRP process onto the membrane. Last, functional polymers were incorporated into PVDF membrane via ATRP from the initiator site. The polymerization time was 0.5, 24 and 3 h for PHEMA, PNIPAAm and the copolymer modified membrane, respectively. The grafting density and grafting degree of modified membranes were displayed in Table 4-1. It can be seen that, with respect to the same type of modified membrane, the grafting density/degree of the M2 membrane was higher than

that of the M1 membrane due to higher monomer concentration during the M2 membrane fabrication. Meanwhile, among which the grafting density of PHEMA modified membrane was also higher than PNIPAAm modified membrane, even though polymerization time of PHEMA modified membrane was much shorter compared to the PNIPAAm modified membrane. It is due to the fact that the polymerization of HEMA was very fast [136] and probably PHEMA is easier to grafted onto PVDF membrane than PNIPAAm. In the synthesis process of PVDF-g-P(HEMA-co-NIPAAm) membrane, the polymerization time decreased to 3 h from 24 h and resulted in a high grafting density after incorporating PHEMA polymer compared with PVDF-g-PNIPAAm membrane preparation, leading to decrease in time consumption and production costs. In this work, the grafting density was also improved to a certain extent via this synthesis process as compared to previous studies reported in the literature [111, 128, 129], which fall within the range of 0.1 to 0.6 mg/cm². It is worth noting that grafting density of functional polymers have a crucial effect on antifouling ability and performance of membranes and it will be discussed in the later section.

Table 4- 1. The grafting property and hydrophilicity property of membranes ($n=3$).

	Grafting density (mg/cm ²)	Grafting degree (%)	Water contact angle (t=3s, °)		Hydration capacity at 25 °C (g/cm ³)
			25 °C	37 °C	
PVDF	--	--	110.73 ± 5.42	110.48 ± 1.12	0.027 ± 0.005
PVDF-g-PHEMA M1	0.72 ± 0.10	27.55 ± 2.61	39.78 ± 6.00	--	0.256 ± 0.043
PVDF-g-PHEMA M2	0.88 ± 0.07	35.08 ± 5.98	40.39 ± 3.91	--	0.438 ± 0.023
PVDF-g-PNIPAAm M1	0.46 ± 0.12	17.42 ± 2.59	88.21 ± 4.52	97.81 ± 1.10	0.389 ± 0.022
PVDF-g-PNIPAAm M2	0.66 ± 0.19	22.83 ± 5.30	91.17 ± 4.26	98.7 ± 0.98	0.427 ± 0.016
PVDF-g-P(HEMA-co- NIPAAm) M1	0.59 ± 0.15	23.01 ± 5.01	82.34 ± 4.67	97.51 ± 2.59	0.398 ± 0.016
PVDF-g-P(HEMA-co- NIPAAm) M2	0.82 ± 0.14	30.81 ± 5.56	81.19 ± 1.67	88.08 ± 1.69	0.408 ± 0.011

4.4 Membrane characterization

4.4.1 Chemical composition of membranes

Chemical information of original and modified membranes was analyzed by ATR-FTIR as illustrated in Figure 4-2. In comparison with the original membrane, there is one obvious peak found in PHEMA modified membrane at around 1730 cm⁻¹, which was the characteristic signal of O-C=O functional group. This provides evidence for the existence of the PHEMA chains as similarly reported by a previous study [133]. Moreover, the signal intensity increased slightly with HEMA monomer concentration. Similarly, with the incorporation of PNIPAAm chains, PVDF-g-PNIPAAm membrane exhibited two obvious peaks associated with the amide groups of PNIPAAm chains at about 1650 and 1540 cm⁻¹ [130, 138]. At the same time,

these three peaks were also observed on PVDF-g-P(HEMA-co-NIPAAm) membrane, and it indicated that the copolymer of PHEMA and PNIPAAm chains was successfully prepared onto the PVDF membrane via surface-initiate ATRP.

In order to test the relative content of PHEMA and PNIPAAm on the surface of PVDF-g-P(HEMA-co-NIPAAm) membrane, XPS analysis was carried out to calculate the composition of P(HEMA-co-NIPAAm). Figure 4-3 (a) and (b) shows XPS survey spectrum of PVDF-g-P(HEMA-co-NIPAAm) membrane M1 and M2, respectively, proving the existence of PNIPAAm chains by the presence of *N* element. In addition, *C* core-level 1s spectra of PVDF-g-P(HEMA-co-NIPAAm) membrane M1 and M2 is displayed in Figure 4-3 (c) and (d) respectively, fitting with three or four peak components. Specifically, the peak components with binding energies at 284.2 eV, 286.1 eV, 288.3 eV and 290.0 eV are attributed to C–C/C–H, C–N, C–O=O and CF₂ species, respectively [130, 133, 138]. Additionally, with respect to M2 membrane, no binding energies at 290.0 eV assigned to CF₂ were observed probably due to the high content of grafted copolymer P(HEMA-co-NIPAAm) totally covering membrane surface. The peak at 288.3 eV for C–O=O species also confirmed the presence of PHEMA chains on the membrane surface.

Figure 4- 2. ATR-FTIR spectra of the pristine and modified PVDF membranes.

Figure 4- 3. XPS survey spectrum of PVDF-g-P(HEMA-co-NIPAAm) membrane M1 (a) and M2 (b); and C 1s core-level spectra of PVDF-g-P(HEMA-co-NIPAAm) membrane M1 (c) and M2 (d).

The elements content of M1 and M2 membrane surface and composition of P(HEMA-co-NIPAAm) copolymer are shown in Table 4-2. The XPS-derived atomic ratio was used to calculate the composition of the copolymer on the membrane surface. It is known that *N* is only from PNIPAAm while *O* stems from both PNIPAAm and PHEMA, so the composition of P(HEMA-co-NIPAAm) on membrane surface was obtained from molar ratio of [*N*] and ([*O*]-[*N*]) referring to the following equation [138]:

$$\left(\frac{[PNIPAAm]}{[PHEMA]} \right) \propto \left(\frac{[N]}{[O]-[N]} \right) \quad (\text{Eq. 4-7})$$

It can be seen from Table 4-2 that the ratio of PNIPAAm and PHEMA on the membrane surface increased from M1 to M2 with the increased molar ratio of monomer NIPAAm to HEMA in the polymerization. In addition, the ratio of PNIPAAm and PHEMA was approximately 1:2 and 1:1 for M1 and M2 membrane.

Table 4- 2. Elements content (%) of PVDF-g-P(HEMA-co-NIPAAm) membrane M1 and M2 and composition of the copolymer on the surface.

	C (%)	N (%)	O (%)	F (%)	PNIPAAm : PHEMA
M1	68.2	3.2	21.7	6.9	0.519
M2	71.7	5.4	21.8	1.1	0.988

4.4.2 The hydrophilicity of membranes

Hydrophilicity of the virgin and modified membranes generally was assessed by water contact angle measurement and hydration capacity testing, and the results are presented in Table 4-1 and Figure 4-4. As shown in Table 4-1, contact angle (taken in 3 s)

of original PVDF membrane was rather high at both 25 °C and 37 °C (about 110°), while contact angle experienced a significant decline after surface modification. This indicates an enhancement in surface hydrophilicity of membranes due to the incorporation of the functional polymers. The trend was also consistent with the results from the hydration capacity tests. Additionally, the results also showed that the contact angle of PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane increased obviously along with an increase in temperature. It is due to the fact that PNIPAAm chains exhibit a hydrophilic and extended structure to improve water affinity on the surface at 25 °C below the LCST, while PNIPAAm chains shrink to a compact and hydrophobic conformation, resulting in a rise of contact angle at 37 °C above the LCST. The result demonstrated that the wettability of PNIPAAm and copolymer modified membrane can be tuned by simply varying the temperature, indicating an obvious thermosensitive property of the membranes. In the meantime, as shown in Figure 4-4, contact angle of PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane experienced a remarkable decline with increasing drop age, dropping to less than 55° within 60 s, while the contact angle of the original membrane was still higher than 100° at 60 s. After dropping onto the surface, the water droplet gradually diffused from membrane surface to the inner membrane pores, and finally infiltrated into the pores of the membrane in a short time and leading to a gradual decrease in contact angle.

Figure 4- 4. Water contact angle of the pristine and modified membrane at different drop age under 25 °C.

4.4.3 Thermal stability of membranes

The thermal stability of pristine and modified membranes was detected by the thermo-gravimetric analysis and the curves are shown in Figure 4-5. The thermos-gravimetric analysis also assisted to check if the functional polymers were successfully grafted on PVDF membrane and to confirm the composition of the membranes. It can be seen that only one-step degradation starting at about 410 °C was observed for virgin and pure PVDF membrane owing to decomposition of PVDF. In comparison to the original membrane, there were two major thermal degradation stages for three types of modified membrane respectively, which was attributed to the decomposition of grafted functional polymers and PVDF main chains at different temperatures. In the case of the PVDF-g-PNIPAAm membrane, the first weight loss which appeared at 309 °C caused by the thermal degradation of PNIPAAm chains. In addition, the first weight loss which started at around 270 °C with respect to

PVDF-g-PHEMA and PVDF-g-P(HEMA-co-NIPAAm) membrane was attributed to the thermal degradation of the PHEMA chains. Moreover, the first degradation step of PVDF-g-P(HEMA-co-NIPAAm) membrane was always lagging behind that of PVDF-g-PHEMA but was always ahead of the first weight loss stage of PVDF-g-PNIPAAm. This provides evidence for the degradation difference between a homogeneous polymer and the copolymer. The TGA curves further proved that functional polymers were successfully prepared onto the PVDF membrane surface.

Figure 4- 5. TGA curves of original PVDF membrane and modified membranes.

4.4.4 Surface morphology of membranes

The SEM analysis was used to investigate the morphology of membranes, and the surface and cross-section structure are displayed as SEM images in Figure 4-6. It can be seen from Figure 4-6 (a) that a porous structure was observed on the surface of pristine PVDF membrane. There was no significant difference in surface morphology between pristine PVDF membrane and plasma-treated membrane, shown as Figure 4-6 (a) and (b),

but the after modification, while the modified membrane with grafting polymers displays different surface morphology from the original membrane, comparing Figure 4-6 (c), (d) and (e) with (a) respectively. The images of modified membrane indicated there was a coating layer covered partially on the membrane surface, covering some membrane pores on the surface and leading to many fake pores as well. Thus, it is difficult to distinguish the membrane pores and measure the pore size on the top surface directly. It is verified that a dense layer formed on the membrane surface after grafting functional polymers comparing the cross-section image (g), (h), (i) with (f) respectively, leading to high hydrophilicity of modified membrane and excellent wettability even in 60 s. It can be seen from Figure 4-6 that the thickness of membrane increased significantly after modification. Besides, it is found that there was no obvious difference in the surface morphology between modified membrane M1 and M2, while the grafting monomer concentration significantly affect the membrane thickness. Table 4-3 shows that a denser layer formed on the M2 membrane grafted with high concentration monomer compared with M1 membrane.

Table 4- 3. The thickness of membranes ($n \geq 3$)

	Thickness (μm)	
	M1	M2
PVDF	67.06 ± 0.75	67.06 ± 0.75
PVDF-g-PHEMA	73.67 ± 0.53	90.17 ± 1.13
PVDF-g-PNIPAAm	78.10 ± 0.61	85.45 ± 1.00
PVDF-g-P(HEMA-co-NIPAAm)	75.53 ± 0.50	86.21 ± 0.26

Figure 4- 6. SEM images of original and modified membranes: surface morphology of (a) pristine membrane; (b) plasma-treated membrane; (c) PVDF-g-PHEMA membrane; (d) PVDF-g-PNIPAAm membrane; (e) PVDF-g-P(HEMA-co-NIPAAm) membrane. Cross-section structure of (f) pristine membrane; (g) PVDF-g-PHEMA membrane; (h) PVDF-g-PNIPAAm membrane; (i) PVDF-g-P(HEMA-co-NIPAAm) membrane.

The roughness of membranes was also characterized by AFM analysis and the results are presented in Table 4-4 and Figure 4-7. According to the results shown in Table 4-4, pristine PVDF membrane had relatively rough surface compared to the smoother surface of modified membranes. Furthermore, PVDF-g-P(HEMA-co-NIPAAm) membrane exhibited the smoothest surface with the lowest roughness among these three types of modified membranes. It is due to that the pits on the surface became leveled up and the pore dimension decreased after grafting functional polymers, leading to the decline of surface roughness [64]. The similar results were also reported that the

roughness of membrane surface decreased by surface-initiated polymerization of functional polymers [60, 139], which probably results in less protein adsorption and biofouling on the membrane surface.

Figure 4- 7. AFM images of PVDF membranes: surface morphology of (a) pristine membrane; (b) PVDF-g-PHEMA membrane; (c) PVDF-g-PNIPAAm membrane; (d) PVDF-g-P(HEMA-co-NIPAAm) membrane. The scale of the AFM scan is $20\mu\text{m} \times 20\mu\text{m}$.

Table 4- 4. Roughness and adhesive force of membranes ($n \geq 3$).

	Roughness (μm)	Adhesive force (nN)
PVDF	0.343 ± 0.067	240.51 ± 73.73
PVDF-g-PHEMA	0.298 ± 0.011	175.79 ± 41.68
PVDF-g-PNIPAAm	0.299 ± 0.021	168.11 ± 46.82
PVDF-g-P(HEMA-co-NIPAAm)	0.256 ± 0.047	109.21 ± 29.47

Figure 4- 8. Adhesive force analysis of original and modified membranes.

The study of adhesive force with AFM had led to a deeper understanding of many biological and physical processes, for example, study in the interaction of foulant (proteins, colloids and bacteria) and membrane surface. In our work, the adhesive force of membranes was obtained according to 32 measurements at different locations of membrane surface for each sample. Figure 4-8 shows the typical force curves of the original and modified membranes, including an approach and a retract curve between the probe and the membrane surface. The difference in force between the approach and the retract curve (as marked in Figure 4-8) as the probe departs the membrane surface was a direct measurement of the adhesive force [140, 141]. As shown in Table 4-4, it can be seen that the adhesive force of modified membranes was smaller than that of the virgin PVDF membrane, with the lowest adhesive force formed on PVDF-g-P(HEMA-co-NIPAAm) membrane, suggesting that

PVDF-g-P(HEMA-co-NIPAAm) membrane has a lowest attraction with the probe compared to other membrane surfaces. This was probably attributed to the difference between the interaction of PVDF with the probe and interaction of grafted polymer with the probe.

4.5 Antifouling property of membranes

4.5.1 Protein static adsorption of membranes

To study the antifouling property of membranes, the protein static adsorption tests were carried out, as protein adsorption on the membrane surface not only negatively affect membrane permeation but also provide bacteria with the nutrient source to form a biofilm. Figure 4-9 (a) presents the protein adsorption amount of membranes using BSA as a model protein. The results indicated that protein adsorption capacity of modified membranes exhibited a significant decline in comparison to the unmodified membrane. Besides, in the case of the same type of modified membrane, BSA adsorption amount of M2 membrane was lower than that of M1 membrane, which was ascribed to higher grafting density of functional polymers on the M2 membrane. Hence, M2 membrane was used in the subsequent tests to further evaluate its antifouling property. Specifically, the minimum adsorption of BSA was observed on PVDF-g-P(HEMA-co-NIPAAm) M2 membrane with the value of $35.20 \mu\text{g}/\text{cm}^2$, which was reduced by 44% compared to the value of $62.71 \mu\text{g}/\text{cm}^2$ for pristine membrane. The results revealed that functional polymers grafted to the membrane resisted protein adsorption onto the membrane surface effectively, and the PVDF-g-P(HEMA-co-NIPAAm) M2 membrane exhibited better

antifouling property in comparison to other modified membranes. Similar results were also reported that the protein deposited on the original PVDF membrane was about 135 $\mu\text{g}/\text{cm}^2$, and decreased to 79.6, 40 and 31 $\mu\text{g}/\text{cm}^2$, respectively, after membrane modification by grafting hydrophilic polymers with different density [115]. Zhao and coworkers [40] also reported that adsorption amount of protein on the modified membrane with PNIPAAm was reduced by 91% compared to the unmodified membrane, decreasing with the increase in hydrophilicity. It is widely considered that proteins easily attached to the hydrophobic surface through interaction between the proteins and membrane, and between proteins themselves. In addition, protein adsorption on the surface also easily favors further bacterial deposition via hydrophobic–hydrophobic interactions with their cell wall. It is generally regarded that almost no hydrogen bonding interactions are formed in the boundary layer between water and hydrophobic PVDF membrane interface, providing protein molecules tend to approach the boundary layer and deposit on the membrane with the repulsion of water molecules away from the hydrophobic surface at the same time. In contrast, the modified membrane with hydrophilic property enables to form a hydration layer on the surface via hydrogen bonds interaction between membrane interface and water, leading to preventing the hydrophobic substances, like some proteins, to approach the hydration layer and further adsorb onto membrane surface [61]. Moreover, previous studies [122, 142] reported that foulant like protein and bacteria often prefer to adsorb onto rough surface compared to the smooth surface, as well as confirming the results of BSA adsorption in this work. In addition, the protein adsorption measurement in this study was quite consistent with the results of the adhesive force of membrane.

Figure 4- 9. (a) BSA adsorption amount of virgin PVDF membrane and modified membrane for 22 h incubation at 25 °C ($n=3$); (b) Quantitative attachment of *Escherichia coli* onto unmodified and modified membranes for 5 h contact at 25 °C and 37 °C respectively ($n\geq 6$).

4.5.2 Antibacterial activity of membranes

It is generally accepted that the attachment of microorganisms on the surface gradually forms biofilm by undergoing colonization of cells. Hence, in this study, the modified membranes grafted functional polymers were designed to reduce biofouling and bacterial attachment, and further biofilm formation. Figure 4-10 shows the SEM analysis of *E. coli* adhesion on different membrane surfaces at 25 °C and 37 °C respectively, and the corresponding quantitative data is presented in Figure 4-9 (b). It can be seen that *E. coli* easily adhered to the pristine PVDF membrane with a hydrophobic surface which prefers the interaction with the bacterial hydrophobic cell wall, and the modified membranes attracted obviously less bacterial cells compared with original membrane both at 25 °C and 37 °C. Furthermore, among the bacteria attachment measurement of

modified membranes, the relatively lower value of bacterial adhesion was obtained on PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane at 25 °C (about 33000 and 30000 cells/mm²), with the reduction by 67% and 71% respectively compared to original PVDF membrane, which was a good indication of high resistance to bacteria adhesion on these modified membranes. The results also can be confirmed visibly from the confocal 3D images (shown in Figure 4-11) that biofouling on the modified membranes was much less than that of the virgin membrane. It is proven that antifouling capacity of membranes mainly affected by surface physicochemical properties such as hydrophilicity, roughness and adhesive force. A hydration layer formed on modified membranes prevents bacterial attachment onto the surface [39], providing there was a correlation between the increase in hydrophilicity and the decline in bacterial adhesion. It was also confirmed in a study [143] that bacterial adhesion on the membrane evidenced a reduction by 75% after incorporation of hydrophilic polymers. Meanwhile, it is reported that the modified surface with low roughness play a crucial role in resistance of bacterial adhesion [122]. Furthermore, the adhesive force of surface quantified by AFM significantly affect antifouling property so as to predict and assess membrane fouling quickly for this surface [140, 144].

Figure 4- 10. The SEM images of *Escherichia coli* adhesion on different membrane surfaces after 5 h contact at 25 °C and 37 °C respectively.

Figure 4- 11. The confocal 3D images after 24 h culture of *E. coli* at 25 °C on (a) original PVDF membrane; (b) PVDF-g-PHEMA membrane; (c) PVDF-g-PNIPAAm membrane; (d) PVDF-g-P(HEMA-co-NIPAAm) membrane.

Figure 4- 12. Quantitative attachment of *E. coli* onto (a) PVDF-g-PNIPAAm membrane, (d) PVDF-g-P(HEMA-co-NIPAAm) membrane after 5 h incubation at 37 °C; quantitative detachment and detachment rate of *Escherichia coli* from PVDF-g-PNIPAAm membrane at (b) 37 °C and (c) 25 °C, respectively; quantitative detachment and detachment rate of *Escherichia coli* from PVDF-g-P(HEMA-co-NIPAAm) membrane at (e) 37 °C and (f) 25 °C, respectively ($n \geq 3$).

4.5.3 Release of bacteria from membranes

The fouling release property of membranes was performed through bacterial detachment test using SEM observation (Figure 4-12). It was observed from Figure 4-9 (b) that the number of bacteria attached to PNIPAAm and copolymer modified membranes at 37 °C was much higher than that of adhesion at 25 °C, which was due to the tunable hydrophilicity of thermosensitive polymer PNIPAAm. The bacterial release ability of membranes caused by the thermos-sensitive property is also displayed in Figure 4-12. The results evidently indicated that the bacteria on the membrane surface detached at 25 °C more readily compared to that of detachment at 37 °C for the same membrane. The detachment rate of bacteria at 25 °C was 84% and 77% respectively with respect to PNIPAAm and copolymer modified membrane, much higher than that of detachment at 37 °C. This phenomenon was due to the fact that the hydrophilicity of membranes was tunable owing to the transition of amphiphilic polymer PNIPAAm under temperature regulation. Thus PNIPAAm chains brought fouling release property to the membrane in order to prevent biofilm formation on the surface. The results of bacterial attachment/detachment behavior were observed on the glass surface with a tunable property according to a previous study [87], in which microorganism cells detached from the tunable surface by varying the temperature, and the thermosensitive polymer PNIPAAm chains contributed to the behavior of cells. Other groups [60, 145] also verified that PNIPAAm chains help to release foulant which have been deposited onto membrane surface, providing higher recovery of the water flux compared with the pristine membrane. In summary, bacterial adhesion and release behavior on the membrane surface

was investigated in this work in order to examine fouling release property which contributed to inhibition of biofilm formation on the membrane surface, and the high bacterial detachment for the PVDF-g-P(HEMA-co-NIPAAm) membrane was probably beneficial to remove fouling easily and obtain high performance during microfiltration.

4.6 Permeation performance of membranes

The flux of membranes was also detected to evaluate antifouling performance using microfiltration, and the results are displayed in Figure 4-13. The protein filtration experiment indicated that the permeation fluxes declined significantly for all membranes in the initial state of measurement owing to protein adsorption on the membrane surface. In addition, the flux of membrane at the later stable state was enhanced 2-3 times after incorporation of functional polymers (not shown). As shown in Figure 4-13 (a), the flux decline of the modified membrane was much lower than that of the original membrane after 5 h, with 43% for PVDF-g-P(HEMA-co-NIPAAm) membrane and 75% for pristine membrane respectively, suggesting modified membrane could effectively resist protein fouling on the surface. Figure 4-13 (b) also shows that the flux of PVDF-g-P(HEMA-co-NIPAAm) membrane was about 2 times that of the original PVDF membrane during microfiltration using bacteria suspension. Besides, the retention was not significantly different between these two membranes, above 90% after 30 min, indicating high rejection of the membranes. It was reported in previous study [60] that the flux of unmodified membrane was higher than that of modified membrane grafted with PNIPAAm chains using synthetic produced water as feed, which was mainly caused by dense layer of grafting polymer leading to decrease in pore size after grafting, but the

permeation of the modified membrane exceeded that of the original membrane in long-term operation. The similar results was also found in another study [112] that the polyamide membrane was modified by coating N-isopropylacrylamide-co-acrylic acid copolymers P(NIPAAm-co-AAc) on the surface, and the permeability of modified membrane was less than that of virgin membrane mainly resulting from the additional resistance of the coating layer in spite of the increase in hydrophilicity. In our study, the effect of grafting layer on the pore size of membrane was not significant so that the grafting layer produced slight resistance to flux, while the hydrophilicity of membrane increased significantly after incorporating functional polymers and the hydrophilicity was a determinate factor on permeability, providing flux of the modified membrane always exceeded the original membrane.

Figure 4- 13. (a) The flux decline of unmodified and modified membranes using 1 g/L BSA solution filtration at 25 °C ($n=3$); (b) permeation flux and retention of microfiltration using bacteria suspension at 25 °C ($n=3$).

The membrane cleaning property of original and copolymer modified membrane was also tested lasting for several runs of protein fouling-washing operation at the same temperature and different temperatures respectively. As shown in Figure 4-14 (a), for the microfiltration at the same temperature, the permeability of modified membranes was always much higher than the original membrane, but the flux decline of modified membrane just exhibited slight superiority over the original membrane. For the operation at different temperatures, Figure 4-15 (a) and (b) show that not only the flux of modified membrane was much higher than pristine membrane but also the flux for modified membrane declined slowly and slightly between the adjacent cycles in comparison to the pristine membrane. In addition, it can be seen from Figure 4-14 (b) that the reversible fouling resistance ratio (R_r) of original membrane gradually decreased along with the increasing of filtration times, dropping to zero after four runs, and R_{ir} tend to increase significantly, while R_r of modified membrane experienced an obvious raise with the increasing in operation cycles although the difference in total fouling resistance ratio between original and modified membranes was not significant. Figure 4-15 (c) shows that modified membrane presented a much lower total fouling resistance ratio with lower R_{ir} and higher R_r than that of the original membrane for the operation at different temperatures, suggesting improved antifouling property both in fouling resistance and fouling release for the modified membrane. It indicated that high partial fouling on modified membrane could be removed only by simple water rinse, and the flux of pristine membrane could not be recovered by washing after three or four runs filtration leading to irreversible flux loss, proving P(HEMA-co-NIPAAm) provided enhanced fouling resistance and easy-cleaning property for the modified membrane. Also, it is confirmed

by flux recovery ratio tests shown in Figure 4-14 (c) and Figure 4-15 (d). Specifically, for the operation at the same temperature, even though FRR of original membrane during the first cycle was as high as 80%, higher than modified membrane with 65% of FRR, the FRR value of pristine membrane decreased to only 37% while it still maintained a stable value of 62% after four cyclic operations, suggesting that the modified membrane was beneficial to filtration for long-term use. Figure 4-15 (d) shows that the FRR of the modified membrane was always much higher than that of the original membrane after three cyclic filtration, demonstrating that fouling on the modified membrane was more readily removed by cold water cleaning. Less protein fouling was formed on the modified membrane which has large hydration barrier and steric repulsion of hydrophilic grafting chains to resist protein adsorption. In the meantime, the binding force between the protein and the modified membrane surface with P(HEMA-co-NIPAAm) chains was probably weak compared to the binding between protein and original membrane according to adhesive force testing, and the protein attracted to P(HEMA-co-NIPAAm) chains could be released via chains extension from compact conformation to expanded structure under temperature change. Thus the fouling formed on the modified membrane was easily released and removed by washing especially at low temperature. According to above discussion, the results demonstrated that copolymer modified membrane exhibited a better antifouling property to inhibit protein adsorption and to remove fouling, and the designed PVDF-g-P(HEMA-co-NIPAAm) membrane was a potential application to last for a long-term operation.

(a)

(b)

(c)

Figure 4- 14. Microfiltration using 1 g/L BSA solution at 25 °C for several runs of protein fouling-water washing processes: (a) the flux of unmodified and modified membranes; (b) the fouling resistance ratio of various membranes (R_r : reversible fouling ratio, R_{ir} : irreversible fouling ratio); (c) the flux recovery ratio of membranes. Run 1 of the fouling-washing process is indicated by R-1 in the legend.

Figure 4- 15. Microfiltration at 37 °C using 1g/L BSA solution followed by cold water washing for several runs of fouling-washing processes: (a) the flux of membranes; (b) the flux decline of membranes; (c) the fouling resistance ratio of membranes (R_t : reversible fouling ratio, R_{ir} : irreversible fouling ratio); (d) the flux recovery ratio of membranes. Run 1 of the fouling-washing process is indicated by R-1 in the legend.

4.7 Conclusion

In this chapter, the antifouling property of PVDF membrane was significantly improved via surface modification by grafting functional polymers PHEMA, PNIPAAm and P(HEMA-co-NIPAAm), respectively, and three types of modified membrane with different grafting density were obtained accordingly. FTIR and XPS analysis confirmed

that the functional groups were successfully grafted onto PVDF membrane. Besides, the hydrophilicity of membrane increased significantly after surface modification while the roughness and adhesive force decreased apparently after grafting functional polymers, all of which are responsible for enhanced antifouling performance of the PVDF membrane. Consequently, compared to the pristine membrane, the modified membranes exhibited high antifouling property to prevent protein adsorption and bacterial adhesion on a surface according to membrane fouling measurement. Especially, minimum protein adsorption was formed on the PVDF-g-P(HEMA-co-NIPAAm) membrane. Additionally, PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane had better resistance ability to protein and bacteria adhesion, and they also exhibited thermosensitive property which contributes to foulant release by adjusting the temperature to prevent biofilm formation on the surface. In addition, there were fewer bacteria attached on PVDF-g-P(HEMA-co-NIPAAm) membrane than PVDF-g-PNIPAAm membrane both at 37 °C and after detachment at 25 °C, which was attributed to the synergistic effect of the fouling resistance of PHEMA chains and fouling release of PNIPAAm chains. Besides, the polymerization time of PVDF-g-P(HEMA-co-NIPAAm) membrane was 3 h, much shorter than that of PVDF-g-PNIPAAm membrane with 24 h, leading to a decrease in time consumption and production costs. Thus, PVDF-g-P(HEMA-co-NIPAAm) membrane was well-designed to be used to further investigate biofouling formation and fouling release, which gives us information on how to effectively control fouling in future work. The permeation flux of PVDF-g-P(HEMA-co-NIPAAm) membrane always exceeded the pristine membrane, and was about 2 times that of the original membrane while their retention was nearly the same. The results of permeation performance

indicated that copolymer modified membrane showed good protein resistance and easy-cleaning property, leading to a reduction in operation cost and extended lifespan of the membrane for long-term use.

CHAPTER 5: Study of biofouling and biofilm formation on the membranes

5.1 Introduction

The preliminary results indicated that functional polymers PHEMA and PNIPAAm were successfully grafted from PVDF membrane leading to designed PVDF-g-PHEMA, PVDF-g-PNIPAAm, PVDF-g-P(HEMA-co-NIPAAm) membrane, respectively. As a result, the antifouling property of the three types of the modified membrane was remarkably enhanced in comparison to pure PVDF membrane, and especially, the PVDF-g-P(HEMA-co-NIPAAm) exhibited better resistance ability to protein adsorption and bacterial adhesion among the modified membranes. In the previous chapter, the biofouling on the membrane surface was investigated using Gram-negative *E. coli* as model biofoulant. It was reported that the high complexity of microbial communities in the activated sludge systems had an important effect on the antifouling efficiency of the membrane and increased the difficulty of the study [146]. Therefore, in this chapter Gram-positive *Staphylococcus epidermidis*, another well-studied model organism, was also utilized to investigate structure and formation of biofilm on the membranes. Moreover, the study of dynamic biofouling formation was more complicated than static bacteria adhesion, and dynamic filtration might reduce the antifouling efficiency of membranes. Hence, the filtration with the feed containing microorganisms was carried out to determine and evaluate the antibiofouling ability of membranes. Finally, the filtration experiments were also conducted using synthetic wastewater containing proteins and

humic acid to verify the great potential of the modified membrane for practical wastewater treatment.

5.2 Material and methods

5.2.1 Materials

Staphylococcus epidermidis DSM-20044 strain was purchased from DSMZ German Collection and of Microorganisms and Cell Cultures. LIVE BacLight™ Bacterial Gram Stain Kit L7005 which contains two dyes STYO 9 and hexidium iodide was purchased from Thermo Fisher Scientific, Life Technologies Holdings Pte Ltd. Singapore. Humic acid was obtained from Chemicals Testing & Calibration Laboratory Pte Ltd., Singapore.

5.2.2 Antibacterial activity and biofouling release test

The anti-microbial property and bacterial release property were also assessed through colony forming unit (CFU) test using a typical Gram-negative bacteria *E. coli*, the protocol of test was referred to previous work [39, 147]. In detail, the membrane samples were taken into 5 mL bacterial suspension (10^9 cells/mL) for 5 h contact 37 °C. After that, the membranes were taken out and washed slightly with sterile PBS solution, and then the parallel samples for the same membrane were put into 5 mL sterile PBS solution for another 5 h of bacterial detachment at 25 °C and 37 °C respectively. Afterward, the remaining bacterial cells adhering to membranes were collected thoroughly with rinse and the cleansed solution was diluted 100 fold. Then 0.1 mL diluent was inoculated on LB agar plate for 15 h culture at 37 °C. The CFU number of bacteria

collected from the membrane after 5 h detachment at different temperatures was used to evaluate biofouling release property. The CFU tests were performed using 3 independent samples for each measurement, and each measurement was carried out on 3 replicate agar plates.

In order to evaluate the fouling resistance of membranes to different bacterial strains, the bacteria adhesion test was also conducted on the membrane surface using a typical Gram-positive bacteria strain *Staphylococcus epidermidis* whose activity is different from Gram-negative bacteria like *E. coli*, and *S. epidermidis* is also usually utilized to assess the antifouling property of substrate surface [87]. The *S. epidermidis* was cultured in a YPD (yeast extract-peptone-dextrose) medium containing 10 g/L yeast extract, 20 g/L peptone and 20 g/L dextrose, and then the culture was placed on a shaking bed under a shaking speed of 200 rpm and 37 °C. After 5 h incubation, the cell concentration was up to 10^9 cells/mL. The next step of bacterial adhesion experiment was similar to section 3.2.6, and it was also carried out at 25 °C and 37 °C respectively. The bacteria attached to the membrane surface were also observed by SEM and the corresponding quantitative analysis of bacterial attachment on the sample's surface was same with the analysis in section 3.2.6 based on SEM images.

The biofouling release property was further evaluated by CFU test using Gram-positive bacteria *S. epidermidis*. The protocol was referred to the CFU test mentioned above using *E. coli*. It was noted that *S. epidermidis* was cultured in YPD medium. Specifically, the cleansed solution was diluted 1000-fold biofouling release test.

5.2.3 Biofilm analysis using confocal laser scanning microscopy

In order to study biofilm formation and construction caused by Gram-negative bacteria or Gram-positive bacteria, the biofilm of *E. coli* or *S. epidermidis* on the membrane surface was also visualized and analyzed by confocal laser scanning microscopy (CLSM). The biofilm was formed after bacteria deposition on the membranes for 12 h at different 25 °C and 37 °C respectively and this process was same as bacterial adhesion experiment mentioned in section 3.2.6. Thereafter, *E. coli* or *S. epidermidis* biofilm on the different membrane samples were stained with SYTO 63 for 10 min and followed by washing with 0.9% NaCl solution. The samples were observed by CLSM and a laser was set at 638 nm to detect the fluorescence. For analysis of biofilm of *E. coli* and *S. epidermidis* mixture, the membranes were immersed into 5 mL of bacterial suspension mixture (1:1) for 12 h contact at 25 °C and 37 °C respectively. After that, the membranes were taken out and rinsed with 0.9% NaCl solution. Then the samples were dyed using Gram Stain Kit L7005 (volume of solution A and B with 1:1) for 10 min and followed by washing with 0.9% NaCl solution. The samples were observed by CLSM with excitation laser at 488 nm to obtain green fluorescence at 500 nm emission for Gram-negative bacteria and red fluorescence at 625 nm emission for Gram-positive bacteria. The measurement was conducted three times for each membrane sample. The volume and construction of biofilm were analyzed by Imaris 8.1.2 (a software for 3D and 4D Real-Time Interactive Data Visualization and Management)

5.2.4 Microfiltration performance of membranes

In order to study the dynamic formation of biofilm and antifouling property of membranes, a cross-flow microfiltration was carried out with *E. coli* suspension (10^7 cells/mL) by several cycles of fouling-washing. The system was filtered with distilled water first for 60 min at a pressure of 1 bar to ensure a steady state. The next process was similar as filtration operation for several runs using BSA solution at 1 bar and 25 °C mentioned in section 3.2.5. The flux recovery ratio (FRR) and fouling resistance ratio (R_f , R_r and R_{ir}) were calculated by equation Eq. 3-3, Eq. 4-6, Eq. 4-4 and Eq. 4-5 respectively, with the high FRR values or low R_{ir} values representing the better antifouling ability. In addition, the fouling test was also investigated by the executed dynamic filtration using synthetic wastewater with three steps at 1 bar and 25 °C. Firstly, the stable water flux was obtained and recorded using pure water filtration for 0.5 h after 1 h pre-filtration. Secondly, the feed solution was replaced with synthetic wastewater and the flux was recorded as J_p during 1 h filtration. Thirdly, the membrane cell and filtration system were washed using pure water for 1 h, and then the filtration was conducted with pure water again and one cycle was completed. The synthetic wastewater consisted of 0.3 g/L BSA, 9 mM/L NaCl, 2 mM/L NaHCO₃, 1mM/L CaCl₂ and 1mg/L humic acid (HA) according to previous studies [148-150] and the suspension of the mixture was used as filtration feed after 2 h natural sedimentation.

5.3 Study of biofouling on membranes using Gram-negative and Gram-positive bacteria

According to literature, membrane anti-microbial property was generally detected by bacterial adhesion of Gram-negative and Gram-positive strain, and *E. coli* and *S. epidermidis* were used in this study respectively. The bacterial adhesion of *E. coli* was measured in chapter 3 and the results shown in section 4.5.2 indicated that the relatively lower value of bacterial adhesion was obtained on PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane at 25 °C, with the reduction by 67% and 71% respectively compared to original PVDF membrane, which was a good indication of high resistance to bacteria adhesion on these modified membranes. *E. coli* release activity was also confirmed by colony forming unit test as shown in Figure 5-1. The results indicated that the remaining amount of bacteria adhesion on the modified membranes after detachment at 25 °C was obviously less than that of groups at 37 °C, indicating good fouling release ability caused by thermosensitive property for modified membranes. Besides, after bacterial release at 25 °C, there were much less bacterial colonies observed on the plate of PVDF-g-P(HEMA-co-NIPAAm) membrane compared to original membrane and PNIPAAm modified membrane, which was mainly due to not only higher fouling resistant ability for copolymer modified membrane at 37 °C by grafting PHEMA (less bacterial attachment on copolymer modified membrane before detachment) but also better fouling release property under temperature change by grafting PNIPAAm.

Figure 5- 1. (a), (b), (c) The *E. coli* colonies after detachment at 37 °C observed on PVDF, PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane, respectively; (d), (e), (f) The *E. coli* colonies after detachment at 25 °C observed on PVDF, PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane, respectively.

S. epidermidis adhesion on the membranes was also observed by SEM and the results are shown in Figure 5-2 and Figure 5-3. It can be obviously seen that less amount of bacteria was observed on the modified membrane compared with the original membrane at both 25 °C and 37 °C for *S. epidermidis* adhesion, representing better antifouling property for modified membrane than the pristine membrane, and the results were similar as *E. coli* attachment test (Figure 4-10). Less bacterial adhesion on the original membrane at 37 °C was found in comparison to the attachment at 25 °C, which was probably attributed to lower viscosity of suspension at a higher temperature. However, the amount of *S. epidermidis* attachment on PNIPAAm and copolymer modified membrane at 25 °C was similar to the groups at 37 °C, and the results were

different from *E. coli* adhesion at different temperatures that less biofouling was formed on the surface at lower temperature owing to tunable hydrophilicity. The results indicated that the hydrophilicity have a significant effect on fouling resistance property through comparison of original and modified membranes, but tunable hydrophilicity caused by thermos-sensitive property affected fouling resistance slightly via comparison of the modified membrane between different temperatures. In a previous study [87], Ista and coworkers reported that *S. epidermidis* attached most to surfaces that are relatively hydrophilic under some condition, and the difference in the amount of *S. epidermidis* attachment on two surfaces was much significant when the hydrophilicity of these two surfaces was both very high but with a slight difference. It is also reported that the *S. epidermidis* adhesion on two surfaces became similar when the water contact angle of these two surfaces increased gradually but with a certain difference (hydrophilicity became lower and lower). According to the results of water contact angle shown in Table 4-1, the difference in hydrophilicity of pristine and modified membrane at same temperatures is much higher than the change of tunable hydrophilicity between same types of the modified membrane under temperature control. It is inferred that antifouling property resulting from high hydrophilicity was a determinant factor for *S. epidermidis* behavior when a large difference in hydrophilicity between different surfaces, which was similar to the results of *E. coli* adhesion. In Venault's study [122], it is also reported that both *E. coli* and *S. epidermidis* favored the pristine and hydrophobic PVDF surfaces more than modified membranes blended with hydrophilic functional polymers, with over 99% reduction of bacterial attachment on modified membranes. The results also were confirmed by fouling release test of *S. epidermidis* displayed in Figure 5-4. It can be seen

that the bacterial colonies on the plate for modified membranes were visibly less than that of original membranes after 5 h bacterial detachment in PBS solution according to colonies counting. Furthermore, with respect to the same type of modified membrane, there was no obvious difference in the amount of remaining bacterial cells on the membranes between detachment at 25 °C and 37 °C, suggesting that thermosensitive property affect lightly *S. epidermidis* release from membranes. Although the advantages of thermosensitive property of modified membranes were not obviously reflected on fouling resistance and fouling release in terms of *S. epidermidis* adhesion, the modified membranes, especially PVDF-g-P(HEMA-co-NIPAAm) membrane, still exhibited significantly better resistance to both Gram-negative and Gram-positive bacteria in comparison to original membrane, indicating higher antifouling property for PVDF-g-P(HEMA-co-NIPAAm) membrane.

Figure 5- 2. The SEM images of *Staphylococcus epidermidis* adhesion on different membrane surfaces for 5 h at 25 °C and 37 °C respectively.

Figure 5- 3. The quantitative analysis of *S. epidermidis* attachment on the membranes under SEM observation after 5 h incubation at different temperatures ($n \geq 3$).

Moreover, other Gram-positive bacteria can be used to evaluate the antifouling property of modified thermosensitive membranes in future work other than *S. epidermidis*. According to a previous study [39], Wang and coworkers designed a low-fouling RO membrane via surface modification by grafting zwitterionic polymer, and two model microorganisms *E.coli* and *Bacillus subtilis* (Gram-positive bacteria) were utilized to examine the anti-microbial property of membranes. They reported that the bacteria mortality of modified membrane was nearly 100% both for *E. coli* and *B. subtilis*, suggesting a high anti-microbial property for modified membrane against the Gram-negative and Gram-positive bacteria. Xu and coworkers [123] prepared antimicrobial polysulfone membrane blended with Ag/Cu₂O hybrid nanowires, and the anti-microbial tests revealed that the blended membranes showed excellent antibacterial performance against both Gram-negative *E. coli* and Gram-positive *Staphylococcus aureus* according to the inhibition zone of bacteria. It is also indicated from SEM images

that *E. coli* and *S. aureus* adhesion were resisted onto the blended membrane and their growth was also inhibited remarkably on the modified surface.

Figure 5- 4. (a), (b), (c) The *S. epidermidis* colonies after detachment at 25 °C observed on PVDF, PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane, respectively; (d), (e), (f) The *S. epidermidis* colonies after detachment at 37 °C observed on PVDF, PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane, respectively.

5.4 Study of biofilm on membranes using CLSM

5.4.1 Study of biofilm using Gram-negative and Gram-positive bacteria

In order to further study biofouling caused by bacterial species, we detected and analyzed the biofilm visibly under confocal microscopy using Gram-negative *E. coli* and Gram-positive *S. epidermidis* attachment respectively. The results of *E. coli* biofilm by confocal analysis are displayed in Figure 5-5 and Figure 5-6. It can be seen from Figure

5-5 that less green spots and weak fluorescence intensity were observed from the images of modified membrane compared with pure PVDF membrane, indicating that bacteria readily interacted with the hydrophobic surface of original PVDF membrane, which was also confirmed and directly observed in Figure 5-6. The biofilm structure on the pristine membrane was quite different from that of modified membrane. Specifically, a thick biofilm layer covered original membrane surface, with a thickness of 31.2 and 35.1 μm at 37 °C and 25 °C respectively, while small pieces of biofilm were scattered over PVDF-g-P(HEMA-co-NIPAAm) membrane surface with a thickness of 27.3 and 23.4 μm at 37 °C and 25 °C respectively. The results demonstrated that modified membranes exhibited high resistant ability toward *E. coli* attachment and biofilm formation. It is known that biofilm formation starts with bacterial attachment and subsequent proliferation on the membrane surface, while microorganisms usually produce extracellular polymeric substances (EPS) which mainly consists of protein and polysaccharide, and are responsible for microorganisms' growth and adhesion on the surface. Therefore, it is regarded that the good protein resistant property of modified membrane also contributed to good resistance towards microorganism attachment and biofilm formation. The quantitative analysis shown in Figure 5-7 also confirmed that minimum volume of *E. coli* biofilm was found on the PVDF-g-P(HEMA-co-NIPAAm) membrane at 25 °C attachment, proving its excellent anti-biofouling property and thermosensitive ability. Thus, biofilm formation of Gram-negative *E. coli* bacteria on the membrane surface was effectively inhibited after grafting functional polymers. In addition, it is inferred that the thick biofilm layer on the pure PVDF membrane is hardly released and removed by hydraulic cleaning due to high thickness, strong interaction between

biofilm and membrane through large contact interface, while the scattered pieces of biofouling on the PVDF-g-P(HEMA-co-NIPAAm) membrane is expected to be easily released and removed by cleaning.

Figure 5- 5. The resistance of original and modified membranes to *E. coli* adhesion under confocal observations after 12 h contact at different temperatures.

Figure 5- 6. Three-dimensional biofilm reconstructions for *E. coli* adhesion based on the confocal analysis after 12 h contact at different temperatures.

Figure 5- 7. The quantitative analysis of the volume of *E. coli* biofilm based on 3D reconstructions after 12 h contact at different temperatures ($n=3$).

The Gram-positive *S. epidermidis* was also used to obtain a wider overview of actual anti-biofouling and resistance of biofilm formation of our membranes, and the results are presented in Figure 5-8 and Figure 5-9. It is found from confocal observations that many bacterial species interacted with the virgin PVDF membrane and adhered to the surface, given its hydrophobicity and morphology. For the modified membrane, several fluorescence spots were observed on the relative images. It also can be seen from Figure 5-9 that the *S. epidermidis* biofilm structure was very similar to *E. coli* that a integrated and thick cake layer (biofilm) formed on the original membrane surface while biofouling spots scattered over the modified membrane surface. The biofilm density of original membrane were 35.07 and 31.18 μm at 37 °C and 25 °C respectively while the thickness of 23.4 μm was measured on the copolymer modified membrane both at 37 °C and 25 °C. In addition, no obvious difference in bacterial adhesion on the modified membrane was found between tests under different temperature, suggesting that the effect of thermosensitive property on *S. epidermidis* adhesion was not significant, which was consistent with the previous results. It is worth noting that bacterial adhesion is also strongly relied on the property of bacteria species, for example, different cell-walls and composition of EPS produced affecting the interaction with membrane material. It was reported by Carretier and coworker [151] that the different amount of bacterial adhesion was found on the same membrane between Gram-negative *E. coli* tests and Gram-positive *Streptococcus mutans* tests due to different nature of bacteria species.

Figure 5- 8. The resistance of unmodified and modified membranes to *S. epidermidis* adhesion under confocal observations after 12 h incubation at different temperatures.

Figure 5- 9. Three-dimensional biofilm reconstructions for *S. epidermidis* adhesion based on the confocal analysis after 12 h contact at different temperatures.

5.4.2 Study of biofilm using mixture of Gram-negative and Gram-positive bacteria

In order to study the effect of bacteria species on biofouling and mutual interaction of bacteria species and membrane, the simultaneous biofouling development and biofilm structure/community on the membranes of the two model microorganisms were subsequently investigated by bacteria adhesion tests under confocal observations. The 3D biofilm construction of a mixture of *E. coli* and *S. epidermidis* is shown in Figure 5-10. For the original membrane, a thick biofilm layer of mixture species covered completely the membrane surface, and both bacteria species were present uniformly and mixed throughout each other inside biofilm but a green signal of Gram-negative *E. coli* was more pronounced. Considering the modified membrane, the biofouling of mixture species was obviously less than that of the original membrane, and each species favored to assemble together to form their own biofouling layer. The biofilm thickness was approximately 35.4, 30.4, 24.4 and 24.4 μm for the PVDF, PVDF-g-PHEMA, PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane respectively at 25 $^{\circ}\text{C}$, and it was 33.7, 28.4, 28.4 and 28.4 μm respectively at 37 $^{\circ}\text{C}$. According to the quantitative analysis presented in Figure 5-11, total biofouling of the two model organisms on the pure PVDF membrane was quite more than the modified membrane, demonstrating obvious biofouling resistant ability for modified membranes. Additionally, the green signal of *E. coli* fouling was more than the red *S. epidermidis* fouling on the pristine membrane, while the biofouling of each species on the modified membrane was similar. Moreover, compared to biofilm study of mono-species, the total biofouling of mixture species on the modified membrane was more serious than the biofouling of each

species under the same condition (Figure 5-7 and Figure 5-11), suggesting that the biofouling behavior of each species was influenced by the presence of the other species. Therefore, the biofouling behavior and structure are expected to be quite complicated in the practical wastewater treatment in MBRs, as the microbial community and species in the sludge is quite rich and various, and the species would affect each other in terms of biofouling behavior. Besides, the biofilm structure was also influenced by species that a thin biofouling layer was observed on the modified membrane for two species mixture while bacterial cluster/spots formed for mono-species adhesion. It is inferred that biofouling layer is more hardly cleaned due to interaction with large surface area compared with the bacterial cluster. It is also considered that the type and characteristics of microorganisms, such as bacterial motility, EPS type produced, interaction with other microorganisms, play a crucial role in the formation of biofilms on the membrane surface [121]. In that case, Liu reported that a higher biofouling tendency of *Pseudomonas aeruginosa* was found compared with *E. coli* due to the difference in their characteristics. Also, it is reported that thicker biofilm was formed under the condition of two model microorganisms in comparison to mono-species, owing to the enhancement of *E. coli* attachment in the presence of *P. aeruginosa*, which was similar to our observation. It is suggested that inter-bacterial adhesion, rather than direct contact with the substrate, promote the progressive formation and maturation of biofilm, and it is confirmed by Kjærsgaard and coworkers that inter-bacterial adhesion enhanced the mixed biofilm formation [152].

Figure 5- 10. Three-dimensional biofilm reconstructions for *E. coli* and *S. epidermidis* mixture adhesion based on the confocal analysis after 12 h contact at different temperatures.

Figure 5- 11. The quantitative analysis of the volume of mixed biofilm based on 3D reconstructions after 12 h adhesion at different temperatures ($n=3$).

5.5 Microfiltration performance of membranes

5.5.1 Microfiltration using *E. coli* bacteria suspension

It is well known that activated sludge containing microorganisms plays a crucial role in biodegradation of wastewater compounds during MBR operation, and biofouling and biofilm are easily formed owing to early microorganism's attachment and their subsequent proliferation on the membrane surface. Therefore, in order to investigate the flux drop caused by biofilm, as well as antifouling property and flux recovery of PVDF-g-P(HEMA-co-NIPAAm) membrane, four cyclic filtration were carried out using *E. coli* bacteria suspension as pollutant feed and followed by water cleaning, and the result is displayed in Figure 5-12. The flux experienced a fast and significant decline at the beginning for both pristine PVDF membrane and the modified membrane probably

due to bacterial deposition on the surface and pore clogging, and the permeability of PVDF-g-P(HEMA-co-NIPAAm) membrane was much larger than that of pure PVDF membrane for each cycle of bacteria filtration. In addition, it can be found from Figure 5-12 that relative permeation flux of modified membrane was also very high compared to original membrane for every cycle of filtration, and the flux decline of modified membrane ranged from approximately 8% to 70% for last three cycles while the flux decreased from around 63% to 83% for original membrane. Higher flux value and less flux decline were both obtained for the modified membrane, proving better bacterial resistance and biofouling easy-cleaning property presented on modified membrane. The results also can be confirmed by flux recovery ratio (FRR) and fouling ratio shown in Figure 5-13. It is observed that the modified membrane always exhibited a higher FRR than virgin PVDF membrane for three cycles of filtration, corresponding to more serious bacterial fouling on the pure PVDF membrane. Figure 5-13 (b) shows that although total fouling ratio of PVDF-g-P(HEMA-co-NIPAAm) membrane was similar to that of original membrane, nearly 90%, the reversible fouling ratio of pure PVDF membrane decreased with filtration runs and dropped to 0% after three cycles of filtration while larger reversible fouling ratio was observed on modified membrane with lower irreversible fouling ratio, demonstrating that large partial of bacterial fouling could be swept away easily via water cleaning after grafting P(HEMA-co-NIPAAm). Additionally, as compared with the original membrane, the higher FRR value obtained on the modified membrane indicated a better antifouling property here. Moreover, the maximum FRR value of filtration using bacteria suspension as pollutant feed was approximate to 30%, and much less than that of BSA filtration, which was probably due to serious cake layer and

pore blocking by bacteria with large size. Therefore, it is inferred that backflush is able to effectively remove bacterial fouling from the membrane surface to improve flux recovery, and it can be performed in the future work. It is reported in Vanyacker's study [121] that the effect of membrane type and microorganism species on biofilm formation was examined via filtration operation on polyethylene (PE), polysulfone (PSF) and PVDF membrane using *E.coli* or/and *Pseudomonas aeruginosa* suspension as pollutant feed, and the result showed that relative permeability decreased with fluctuation between 55% and 85% after 24 h operation due to bacterial fouling. Additionally, the permeability of PVDF membrane was lower than PE and PSE membrane, indicating that biofouling was more easily formed on PVDF membrane probably owing to the higher interaction between membrane and biofoulant. It also reported that more internal pore blockage by bacteria cells easily occurs for membranes with large pores [153], and the PVDF membrane used in this study was exactly similar to this situation. It is also reported in Zhao and coworker's study [80] that filtration experiment was carried out to investigate the antifouling property of membranes using a yeast strain as model biofoulant., and as a result, both biofouling-resistant and biofouling-release ability of membrane were dramatically improved by introducing functional polymers.

Figure 5- 12. The relative flux of unmodified and modified membranes using bacteria suspension filtration at 25 °C for four cycles of fouling-cleaning processes. Run 1 of the fouling-washing process is indicated by R-1 in the legend.

Figure 5- 13. (a) The flux recovery ratio of the original and modified membrane using bacteria suspension filtration at 25 °C; (b) fouling ratio of membranes using bacteria suspension filtration at 25 °C (R_r : reversible fouling ratio, R_{ir} : irreversible fouling ratio). Run 1 of the fouling-washing process is indicated by R-1 in the legend.

5.5.2 Microfiltration using synthetic wastewater

During wastewater treatment in MBR, the feed sewage usually contains many types foulant, such as protein, humic substances and inorganic ions. Thus, in our study, synthetic wastewater was prepared by pollutant solution including three model foulant

BSA, humic acid (HA) and inorganic ions Ca^{2+} according to previous literature [148-150], and the filtration experiment was carried out using synthetic wastewater to examine antifouling performance of pure PVDF membrane and PVDF-g-P(HEMA-co-NIPAAm) membrane. Figure 5-14 (a) shows synthetic wastewater 5-fold concentrate solution used in the filtration process, and it is brown color resulting from HA and there are many bubbles caused by BSA. As shown in Figure 5-14 (b), the color of membranes became from white to dark brown after permeating synthetic wastewater, indicating fouling formed on the membrane surface. It can be confirmed by the results displayed in Figure 5-15. Specifically, although the permeation flux of original and modified membrane both experienced a dramatic decline at the initial stage of synthetic wastewater filtration owing to foulant deposition and cake layer, the modified membrane always exhibited much higher permeability than the pristine membrane. Then the flux of membranes became to a relatively constant value after 10 min filtration using pollutant solution. Furthermore, as shown in Figure 5-15 (b), although the total fouling ratio of modified membrane was similar to that of original membrane, modified membrane presented a higher reversible fouling ratio as high as 27% and a lower irreversible fouling ratio with 25% compared to virgin membrane with only 9% of R_r and R_{ir} as high as 45%. The significant decline of irreversible fouling for modified membrane demonstrated that the fouling resistance was enhanced, and the increase in reversible fouling ratio suggested that foulant were readily released and removed from modified membrane probably due to breaking weak interaction between foulant and membrane surface, proving that the antifouling property of membrane was significantly improved after grafting hydrophilic polymers [80]. It is mentioned that high hydrophilicity was able to decrease the adhesion forces between

typical organic pollutants and the hydrophilic surface of modified membrane [154, 155]. Besides, the FRR value of pure PVDF membrane was only 55% while the FRR value of PVDF-g-P(HEMA-co-NIPAAm) membrane increased to 75%, indicating a better antifouling property for modified membrane as well. It is inferred that the strong interaction between foulant and membrane surface and the pore clogging which could not be removed by flushing inhibited full recovery of flux. Furthermore, cake layer easily formed on the membrane surface in the microfiltration process using synthetic wastewater containing multiple foulant compound [156], and especially the complexation of pollutants usually occurred in the presence of Ca^{2+} , further promoting cake layer formation and aggravating membrane fouling [150]. Thus, the flux was not easily fully recovered by simple hydraulic cleaning.

Figure 5- 14. (a) Digital photo of synthetic wastewater 5-fold concentrate; (b) image of modified membrane before and after wastewater filtration.

Figure 5- 15. (a) The time-dependent flux of membranes using synthetic wastewater filtration at 25 °C; (b) dynamic fouling test of membranes using synthetic wastewater filtration at 25 °C (FRR: flux recovery ratio, R_t : total fouling resistance ratio, R_r : reversible fouling ratio, R_{ir} : irreversible fouling ratio). The entire filtration process consisted of three steps: pure water filtration, synthetic wastewater filtration (flux decline) and followed by pure water filtration again after hydraulic washing (flux recovery). The first step of the process is indicated by R-1 in the legend.

In order to investigate fouling release ability provided by the thermosensitive membrane prepared in this work, three cyclic filtration was conducted using synthetic wastewater as pollutant feed, and one cycle process consisted of 0.5 h pollutant filtration at 37 °C and followed by 1 h cold water cleaning at 15 °C. The flux of pure PVDF membrane and thermosensitive membrane PVDF-g-P(HEMA-co-NIPAAm) is shown in Figure 5-16. It is obvious that the modified membrane had higher permeability than pure PVDF membrane under three cyclic filtration due to the prevention of fouling after grafting hydrophilic polymers. Figure 5-17 shows flux recovery ratio and the fouling ratio of membranes. It can be seen that the modified membrane exhibited a FRR value as high as 94% while only that of 70% for the original membrane after the first cycle of fouling test, demonstrating that the flux of the modified membrane was very easily recovered by cold water cleaning. Moreover, the FRR of the modified membrane was always higher

than the pristine membrane for other two cycles of fouling test, suggesting better antifouling performance to the modified membrane. It is found from Figure 5-17 (b) that much higher irreversible fouling was formed on the pure PVDF membrane surface compared with PVDF-g-P(HEMA-co-NIPAAm) membrane, while higher reversible fouling deposited on the modified membrane and was further cleaned by cold water flushing in comparison to virgin membrane, proving higher fouling release ability to modified membrane due to tunable hydrophilicity under temperature change. Therefore, PVDF-g-P(HEMA-co-NIPAAm) membrane prepared in this work exhibited better antifouling performance (both fouling resistance and fouling release property) and good thermosensitive property. However, the result also showed that the FRR of modified membrane decreased to 41% after three cyclic filtration, indicating that flux was also not easily recovered by cold water washing. The phenomenon was probably due to two reasons. Firstly, the PNIPAAm chains were hydrophobic at high temperature 37 °C and progressive fouling was formed on the membrane surface during consecutive filtration runs. Secondly, as mentioned in above discussion, a heavy cake layer easily formed on the membrane surface in the presence of Ca^{2+} and other multiple compound pollutants leading to heavy membrane fouling, and internal fouling even might occur in this case. Thus, other multiple cleaning methods can be employed to combine with hydraulic cleaning below the LCST of PNIPAAm to promote removal of membrane fouling in the practical filtration process for long-term operation. In a literature review [156], Wang summarized that the membrane cleaning strategies was divided into four types, i.e., physical, chemical, physicochemical, and biological/biochemical cleaning, according to fouling removal mechanisms. Additionally, the time interval for in-situ cleaning in MBRs

is usually once for every 10 min, or several weeks or months based on the fouling conditions and cleaning methods used, and its frequency is much higher than ex-situ cleaning. Therefore, for the thermosensitive membrane, water cleaning with temperature control provide an auxiliary and feasible strategy to promote the fouling release and to enhance the efficiency of fouling removal except for classical cleaning methods for wastewater treatment.

Figure 5- 16. The flux of membranes for three cyclic fouling-cleaning processes. One cycle of fouling-cleaning consists of filtration using synthetic wastewater at 37 °C and followed by cold water cleaning at 15 °C. Run 1 of the fouling-washing process is indicated by R-1 in the legend.

(a)

(b)

Figure 5- 17. (a) The flux recovery ratio of membranes; (b) fouling ratio of membranes three cyclic filtration using synthetic wastewater at 37 °C and followed by water washing at 15 °C (R_t : total fouling resistance ratio, R_r : reversible fouling ratio, R_{ir} : irreversible fouling ratio). Run 1 of the fouling-washing process is indicated by R-1 in the legend.

5.6 Conclusion

In this chapter, biofouling formed on the membrane surfaces was investigated using bacterial adhesion test of Gram-negative *E. coli* and Gram-positive *S. epidermidis*, respectively. The results indicated that the number of bacteria adhesion both for *E. coli* and *S. epidermidis* on the modified membranes was much less than the original membrane, suggesting better fouling resistance ability for modified membrane. Additionally, colony forming test showed that the modified membrane exhibited higher bacterial release ability for *E. coli* compared to the original membrane but similar bacterial release property for *S. epidermidis* after bacteria detachment at 37 °C and 25 °C respectively. The biofilm formation and structure was further investigated by bacterial adhesion tests of two model microorganisms under confocal observation, and attachment experiment using mono-species and mixture species of Gram-negative *E. coli* and Gram-positive *S. epidermidis* were carried out respectively. The results indicated that less biofouling on the modified membrane was found in comparison to the original membrane for both mono-species tests and mixture species tests, which further confirmed that antifouling property of PVDF membrane was remarkably enhanced after surface modification by grafting functional polymers. Also, it is suggested that microorganism species played an essential role in the formation and structure of biofilm as the species influenced the bacterial adhesion and biofouling behavior each other owing to their type and characteristics. According to the results of membrane filtration using bacteria suspension and synthetic wastewater, it is known that the modified membrane exhibited higher fouling resistance during filtration and higher flux recovery after hydraulic

cleaning compared with the pure PVDF membrane. Furthermore, for the thermosensitive membrane prepared in this work, water cleaning with temperature control provide a feasible strategy to promote the fouling release and to enhance the efficiency of fouling removal in combination with classical cleaning strategies for wastewater treatment in MBRs. In conclusion, functional polymer PHEMA and PNIPAAm endow PVDF membrane great potential to prevent membrane fouling in wastewater treatment as well as a wide range of applications.

CHAPTER 6: Conclusions and future work

6.1 Conclusions

In the past decades, the membrane fouling has been regarded as the major challenge in the widespread application of membrane technology, leading to increasing attention paid on inhibition of membrane fouling. Antifouling membrane fabrication is considered as a feasible and promising strategy to address this problem except for traditional methods such as membrane cleaning technology and optimization of operation condition, where its advantage lies in resistance towards membrane fouling directly rather than membrane recovery after fouling. As mentioned in chapter 1, the aim of this thesis is to prepare antifouling PVDF membrane by grafting PNIPAAm and its copolymer with other functional polymers to achieve better performance. This thesis presents a certain achievement in the enhancement of antifouling property in terms of not only fouling resistance but also fouling release property for PVDF membrane. The major findings and achievement in our study are shown in the following Figure 6-1. The improvement of this thesis are briefly summarized into two points:

- a) The first one is that the not only fouling resistance but also fouling release property have been enhanced by thermos-sensitive copolymer of P(HEMA-co-NIPAAm) thereby providing better antifouling property for the membrane.
- b) The efficiency of membrane fouling removal has been improved by cleaning with temperature stimuli due to thermosensitive property of copolymer modified membrane, thereby enhancing the flux recovery.

Figure 6- 1. The achievement obtained in our study to address membrane fouling challenge.

In particular, the major conclusions of this thesis was summarized as following:

- 1) Thermosensitive polymer PNIPAAm was successfully grafted from PVDF membrane via ATRP, and finally grafting density was remarkably enhanced, thereby effectively improving membrane antifouling performance. In particular, the PNIPAAm modified membrane exhibited less protein adsorption and bacteria attachment on the surface compared with original PVDF membrane, which was attributed to the enhanced hydrophilicity and a thermosensitive property to the modified membrane. In addition, bacteria adhesion test revealed that the attachment of *E. coli* on the PNIPAAm modified membrane was reduced by 75% compared to the original membrane.
- 2) According to the fouling tests, the PNIPAAm modified membrane exhibited good antifouling capability and high performance for long-term operation, and a relative

higher antifouling property was also obtained on the modified membrane even compared with a commercial hydrophilic PVDF membrane, providing a meaningful reference for a feasible strategy in antifouling membrane preparation and practical application of the modified membrane. Specifically, the maximum of flux recovery ratio (FRR), 91.59%, was obtained for PNIPAAm modified membrane. It is evidently believed that protein foulant was removed easily from the modified membrane surface after water washing. Meanwhile, the thermosensitive property of PNIPAAm modified membrane played an important role in the behavior of bacteria attachment/detachment on the membrane surface, which could assist us to study the biofouling formation and inhibition biofouling.

- 3) Three types of the modified membrane were successfully designed by grafting functional polymers PHEMA, PNIPAAm and P(HEMA-co-NIPAAm), respectively, in order to achieve better antifouling property, leading to PVDF-g-PHEMA, PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane respectively. Consequently, the hydrophilicity of membrane increased obviously after surface modification while the roughness and adhesive force decreased apparently after grafting functional polymers, all of which are responsible for enhanced antifouling performance of the PVDF membrane.
- 4) The three modified membranes exhibited higher antifouling property to resist protein adsorption and bacterial adhesion than pristine PVDF membrane. In particular, minimum protein adsorption and bacterial adhesion were both obtained on PVDF-g-P(HEMA-co-NIPAAm) membrane, with reduction by 44% and 71% respectively in comparison to the pristine membrane. Meanwhile, the minimum

bacterial cells after detachment at 25 °C were observed on the PVDF-g-P(HEMA-co-NIPAAm) membrane with the detachment rate of 77%, indicating higher fouling release property due to the synergistic effect of the fouling resistance of PHEMA chains and fouling release of PNIPAAm chains.

- 5) PVDF-g-PNIPAAm and PVDF-g-P(HEMA-co-NIPAAm) membrane also exhibited thermosensitive property which contributes to foulant release by adjusting the temperature to prevent biofilm formation on the surface. Additionally, according to bacterial attachment and detachment tests, PVDF-g-P(HEMA-co-NIPAAm) membrane exhibited higher antifouling performance not only in fouling resistance but also fouling release property. Besides, the polymerization time of PVDF-g-P(HEMA-co-NIPAAm) membrane was 3 h, much less than that of PVDF-g-PNIPAAm membrane with 24 h, leading to a decrease in time consumption and production costs.
- 6) The filtration tests of protein solution indicated that the copolymer modified membrane exhibited high resistance to protein fouling and the foulant on the surface was released and removed easily by washing, suggesting high fouling release and easy-cleaning capacity, which leads to reduction in operation cost and extended lifespan of membrane for long-term use
- 7) In chapter 5, the study in biofouling of two model microbial species indicated that quite less biofouling of *E. coli* and *S. epidermidis* were both observed on the three modified membranes than the original membrane, suggesting better fouling resistance ability for modified membrane. Additionally, colony forming test showed that the modified membrane exhibited higher bacterial release ability for *E. coli* compared to

the original membrane but similar bacterial release property for *S. epidermidis* after bacteria detachment at 37 °C and 25 °C respectively.

- 8) According to biofilm study using *E. coli* and *S. epidermidis*, it is inferred that microorganism species played an essential role in the formation and structure of biofilm as the species influenced the bacterial adhesion and biofouling behavior each other owing to their type and characteristics. In particular, a thin biofouling layer was observed on the modified membrane for two species mixture while bacterial cluster/spots were found on the modified membrane for mono-species adhesion. It is inferred that the biofilm with former structure (a layer) would result in more severe flux drop owing to blocking more pores and would be more hardly removed by hydraulic cleaning due to interaction with large surface area compared with the bacterial cluster.
- 9) According to the results of membrane filtration using bacteria suspension and synthetic wastewater, it is known that the modified membrane exhibited higher fouling resistance during filtration and higher flux recovery after hydraulic cleaning compared with the pure PVDF membrane for long-term use. Furthermore, for thermosensitive PVDF-g-P(HEMA-co-NIPAAm) membrane prepared in this thesis, water cleaning with temperature control provide a feasible strategy to promote the fouling release and to enhance the efficiency of fouling removal in combination with classical cleaning strategies for wastewater treatment in MBRs.
- 10) This thesis was one of the limited studies to obtain higher antifouling property focusing on not only fouling resistance but also fouling release for PVDF membrane via grafting functional polymers P(HEMA-co-NIPAAm). This feasible and effective

strategy of antifouling membrane preparation can be also used for other types of the membrane in wide application.

6.2 Future prospects and plans

In order to achieve the ideal antifouling membrane for wastewater treatment, the modified PVDF membrane was well-designed by grafting P(HEMA-co-NIPAAm) in this thesis, thereby providing excellent fouling resistance, fouling release ability and thermosensitive property in fouling tests and synthetic wastewater treatment. As mentioned in the previous chapters, the thesis presents a feasible and promising strategy for antifouling membrane fabrication used in wastewater treatment and widespread application in other fields. It is also mentioned that the practical process of wastewater treatment in MBR is more complicated than the filtration in the small flat-sheet membrane module using synthetic wastewater. Hence, this section aims to provide greater insights for the future study to meet the demands of the practical application of antifouling membrane in the real wastewater treatment process.

It is mentioned in Chapter 5 that the microbial communities and species are very various and complicated in the activated sludge systems, and the microbial species have an important effect on biofilm formation and structure due to the interaction between different species. Besides, the biofilm formation speed and its structure determine the difficulty degree of biofilm removal under the same operation condition. Therefore, the biofouling formed in the practical wastewater treatment in MBR is usually difficult to remove by membrane cleaning owing to the complexity of microbial communities. Thus,

in the future research work, the antifouling membrane obtained in this thesis can be used to carry out the filtration experiments using real wastewater as feed.

It is well known that the operation condition also plays a very important role in the control of membrane fouling during MBR operation except for the nature and characteristic of membrane used. Therefore, in the practical process of wastewater treatment in MBR, we should adjust the parameters and optimize the operation condition depending on the MBR, membrane and feed involved in order to better control membrane fouling. The parameters generally include operation temperature, transmembrane pressure, shear stress, the adsorbent agent added, solid retention time (SRT) and hydraulic retention time (HRT), etc. For example, the temperature can affect the activity of microorganisms, and the production and degradation of SMP, which plays a role in membrane fouling and MBRs system performance [157]. In an anaerobic MBR, small bioreactor generally leads to low HRT which represents low costs, while there is low sludge biomass for treatment with long SRT. The lowest HRT occurred in a lab scale submerged anaerobic MBR as low as 3 h according to Hu and Stuckey's study [158], and the COD (chemical oxygen demand) removal reached as high as 90% for synthetic wastewater treatment. Furthermore, powdered activated carbon (PAC) is considered as an effective adsorbent used to absorb foulant in MBR in order to minimize membrane fouling. In a previous study, PAC and alum were utilized as a pre-treatment strategy for dye wastewater treatment in MBR and results indicated that the pre-treatment exhibited positive effect on the performance of MBR [159]. Hence, for the future prospects of membrane application, the antifouling membrane prepared in this thesis need to be verified in practical process of

wastewater treatment in MBR and the operation condition would be optimized so as to obtain better treatment performance.

Besides, for practical MBR operation, many membrane cleaning strategies are employed to remove membrane fouling and recover water flux. Wang and coworkers [156] summarized a wide variety of membrane cleaning methods, including physical cleaning, chemical cleaning and biological/biomedical cleaning methods. It is also mentioned in that review that the membranes with self-cleaning materials have been developed as a kind of self-cleaning membrane and are suitable for MBR operation. The thermosensitive PVDF-g-P(HEMA-co-NIPAAm) membrane prepared in this thesis also can be regarded as a self-cleaning membrane that the fouling would be easily removed and released to a certain extent by membrane cleaning with temperature control. It provides a feasible strategy to promote the fouling release and to enhance the efficiency of fouling removal in combination of temperature stimuli with classical cleaning strategies for wastewater treatment in MBRs. Thus, the temperature stimuli can be utilized in the practical process using thermos-sensitive membrane PVDF-g-P(HEMA-co-NIPAAm).

As mentioned in the previous section, it is known that this thesis provides a feasible and effective strategy to achieve better antifouling property of membrane, thereby providing great potential for wide application of different types of membrane. It is known that there are various types of the membrane in terms of membrane materials and different modules employed in MBR depending on the different purposes and feed samples. Therefore, we can try to modify the membranes made from other materials and improve their antifouling property so as to meet the demands of the membrane and MBR

application in the future work. This part of the work can assist us to further verify the great potential of this thesis. Hence this part of research work is considered to be carried out in the future.

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