NANOFIBER AS FLOCCULANT OR MODIFIER IN MEMBRANE BIOREACTORS
FOR WASTEWATER TREATMENT

A Thesis
Presented to
The Graduate Faculty of The University of Akron

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

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December, 2005
NANOFIBER AS FLOCCULANT OR MODIFIER IN MEMBRANE BIOREACTORS FOR WASTEWATER TREATMENT

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Thesis

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ABSTRACT

The more stringent regulations for wastewater discharge present new technology challenges to wastewater treatment (WWT) plants. There is a particular pressing need for improving the hygienic quality of the treated water. Membrane bioreactors (MBRs) represent one of the most innovative approaches to restrain the release of pathogens from WWT plants. Using membranes with a pore-size of 0.1~0.5 μm or less, not only bacteria but also viruses are virtually completely retained.

However, membrane fouling is a very serious problem faced by MBRs. Cake layer formation generates largest resistance for membrane filtrations. It was well known that adding flocculants could flocculate small sludge flocs and soluble EPS (Extracellular Polymeric Substances) into large flocs. Flocculated sludge flocs can form a more porous cake, which would enable a higher permeate flux. A procedure was developed to prepare glutaraldehyde-crosslinked chitosan nanofibers as a flocculant that is insoluble but well dispersible in water. Polyacrylonitrile (PAN) nanofiber serves as the reference for evaluating the nanofiber structure contribution to membrane filtration, because of its poor flocculation ability. Another commercial soluble flocculant MPE50 (Nalco Company, Naperville, Illinois) was also compared. The toxicity/inhibition test, turbidity reduction test, and short-time filtration test were conducted to evaluate the flocculant performance.

There was no obvious inhibition to the growth of microorganisms by addition of 50~100 mg/L of any of the tested flocculants. It was also demonstrated that adding
dissolved chitosan and MPE50 could help reduce the turbidity of the supernatant up by 80% and 55% respectively after allowing the sludge to settle for 45 minutes. Chitosan was effective in the pH range of 5-8, while MPE50 was effective in the wider range of 4-9. PAN nanofiber and crosslinked chitosan nanofiber showed low turbidity reduction ability at concentrations higher than 25 and 50 mg/L, respectively, after adjusting for the turbidity of nanofibers themselves.

Total filtration resistance $R_t$ and membrane fouling rate were calculated from the short-term filtration tests. Both dissolved chitosan and MPE50 could improve the filtration performance with lower transmembrane pressure (TMP) and higher permeate flux. However, the performance of PAN and crosslinked chitosan nanofibers was not very consistent from replicate experiments. It is demonstrated that adding nanofiber would not harm the filtration system but the crosslinked chitosan did not improve the filtration process as expected. The possible reasons are discussed for future improvements.
DEDICATION

To my parents
ACKNOWLEDGEMENT

First and foremost, I would like to thank my advisor, Dr. Lu-Kwang Ju, for his encouragement, support, understanding and providing critical comments whenever necessary throughout the course of this study. He has been a good friend as well as a great advisor all through my work and study.

My appreciation also goes to my committee members, Dr. William B. Arbuckle and Dr. Darrell H. Reneker for their valuable advice on this thesis and time to serve as my committee members.

I’m extremely grateful to Dr. Woraphon (Tony) Kataphinan in Polymer Science for his great help and support on all nanofiber electrospinning in this study.

I would like to thank Ms. Elizabeth A. Amaddio, who was my research assistant, for her great assistance in the experiments.

I would also like to thank Dr. David Black for his assistance and help in taking ESEM pictures.

It has been my pleasure to work with my group members, Ms. Lin Huang, Mr. Fan Chen, Ms. Qin Zhang, Ms. Nessia Pinzon, Ms.Qing Xia, Mr. Chi-Ming Lo and Ms. Sabrina Restrepo. Specially, I would like to thank Ms. Lin Huang for her continual help with wastewater analysis and experimental problems that I had come across over the years.
Finally, I would like to express a special appreciation to my family, Mon, Dad Shuping and Yuanwei, for their love, their support and encouragement. Without you all, I would not be able to pursue my goals.
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CHAPTER I

INTRODUCTION

1.1. Biological Wastewater Treatment Using Membrane Bioreactors

The more stringent regulations for wastewater discharge present new technology challenges to wastewater treatment (WWT) plants, which have to not only limit the amount of nutrients but also ensure the hygienic quality of the discharge [1, 2]. WWT plants have been considered as significant sources for pathogens because of the growing epidemiological evidence for a correlation between the use of surface waters for recreational purposes, especially bathing and diving, and the occurrence of infections of the ears and eyes [3, 4]. There is a pressing need for improving the hygienic quality of the treated water. Membrane bioreactors (MBRs) represent one of the most innovative approaches to restrain the release of pathogens from WWT plants. Using micro-filtration (MF) or ultra-filtration (UF) membranes with a pore-size of 0.1~0.5 µm or less, not only bacteria but also viruses are virtually completely retained, rendering the treated water meeting bathing water requirements in bacterial counts [5].

The advantages offered by the MBRs over conventional wastewater treatment methods were significant. The effluent quality of activated sludge process was susceptible to large fluctuations as a result of solid-liquid separation problems, such as bulking and foaming [6, 7]. In addition, the process also generates large quantities of
excess sludge. MBR technologies have emerged as one of the innovative and promising solutions for wastewater treatment and reclamation, by replacing clarifier with membrane separation unit in an activated sludge process. MBRs have a smaller footprint and less sludge production through maintaining a high microbial concentration from 10,000 to 50,000 mg/L [8, 9]. The MBR system is also capable of handling high or shock loadings, and achieves higher effluent quality.

1.2. Problem Specification and Hypothesis

Notwithstanding the significant advantages, the widespread application of the MBR process is constrained by membrane fouling [10]. Membrane fouling is the most serious problem affecting system performance and become the bottleneck of its development. The membrane fouling by surface deposits and pore blockage of complex materials present in wastewater will significantly decrease the permeate flux or increase the transmembrane pressure (TMP), which remains a serious challenge to the successful and economical implementation of MBRs.

The cake layer formation seriously deteriorates the filtration process by limiting the permeate flux in MBRs [10]. Porosity and pore size are the two primary factors that govern the resistance to liquid flow through cake layer. Studies have shown that the mixed liquor with smaller particles and broad particle size distribution could form a more dense cake layer than only large articles present, for the smaller particles trapped in the cake layer leading to smaller pores and lower porosity, which increase filtration resistance. Consequently, the size and size distribution of the particles in the wastewater to be filtered affect the performance of the filtration. Especially in the continuous-fed
systems, the smaller particles (<10µm) suspended will create greater filtration resistance and rapidly deteriorate the effluent flux [10-13]. Decreased porosity and pore size of biofilms also result from the compressibility of biological materials under the filtration pressure. The biofilm materials deform and collapse to fill the void space, causing significant increase in filtration resistance.

Since the particle size and the presence of organic solutes were significant factors influence the membrane fouling, more and more researchers are interested in studying the effect of flocculation on MBR performance [14, 15]. Flocculation is an aggregation process of colloidal particles involved in a solid-liquid separation [16]. The net surface charges of flocs and EPS are typically negative. Positively charged flocculants can effectively flocculate and/or adsorb them by forming “bridges” or “nets” among them. The flocculant can flocculate small particles into large ones, and help to get rid of the soluble organics in mixed liquor. Flocculated particles can form a highly porous filtration cake and prevent the deposition of particles on the membrane, which would enable a higher permeate flux [17]. Polymeric flocculants are increasingly more attractive than inorganic metal flocculants because of its advantages such as the small dosage required, formation of large cohesive floc, lower sludge volume to handle, and being comparatively inert to pH changes [18-20]. Polymeric flocculants made by natural polymers are biodegradable and very shear stable [21]. However the biodegradability may cause the natural flocculants to have shorter shelf life than the synthetic polymers, and require larger dosages. However, the biodegradability prevents introducing new contaminants to the system and also makes water reutilization easier, and the separated solids can be directly recovered [18, 22, 23]. Among the main natural polymeric
floculants, polysaccharides are promising for its mostly moderately efficient (due to their low molecular weights), shear stable, cheap and easily available from natural resources [21]. Chitosan, a cationic biopolymer, is the deacetylation product of the natural polymer chitin, which is the second most abundant natural polymer after cellulose in nature [22, 24]. The cationic chitosan is a demonstrated biopolymer flocculant in water treatment, which can bind with negatively charged sludge flocs and EPS to form larger particles even with a very small dosage [25, 26].

Nearly all the flocculants used in wastewater treatment field are water-soluble. Water-soluble flocculants requires rather precise dosages to be added; otherwise, too low dosages are not enough for flocculation and too high dosages will cause excessive amounts in the effluent. In any case, it introduces potential contaminants into the effluent and needs frequent or continuous supplementation to compensate for the loss in the effluent. MBRs have the special characteristics of complete solid retention and long SRT (Solid retention time). Consequently, it may be beneficial to use insoluble solid flocculants so that there would be no loss due to dissolution during the process. This property not only minimizes the costs due to flocculant loss (particularly when long SRT is used with minimal sludge wastage) but also eliminates the introduction of additional chemicals into the receiving water environments. However, the insoluble flocculants cannot be completely dispersed in the water (not at the molecular level) to interact with the colloids and sludge flocs, and thus will have inherently lower flocculation efficiency. The nanofiber structure may be a good way to supply high contact surface for the flocculant to interact with the particles and to be held inside the bioreactor by membrane. In addition, by entangling with the bioflocs, nanofibers may enhance the rigidity of the
cake layer formed on the membrane surface and make the cake layer less compressible under the filtration pressure. Less compressible filter cakes should be able to maintain larger pores and higher porosity for better filtration performance.

Accordingly, we hypothesized that the membrane fouling can be effectively controlled by addition of nanofibers flocculant that (1) have the suitable chemical functional groups and large surface areas to flocculate and/or adsorb the biological materials in wastewater, and (2) form rigid, porous structures of cake layers on the membranes. To separately evaluate these different effects, nanofibers of chitosan and polyacrylonitrile (PAN) were included in the study. While chitosan contains primary and secondary amine groups, PAN contains only tertiary amines and has no (or very weak) ionic interaction with the biomaterials. Consequently, PAN nanofibers are expected to exhibit predominantly the effects of biofilm-structure modification. For further comparison, we have also included the non-fiber, soluble chitosan and a non-chitosan-based, commercial, soluble flocculant MPE50 (Nalco Company, Naperville, Illinois) in the study.

Toxicity/inhibition test, turbidity reduction test, and short-time filtration test are designed to evaluate the ability of nanofibers flocculant from inducing flocculation/adsorption and reducing biofilm compressibility. The Toxicity/inhibition test will tell the flocculant effect on biological activity, turbidity reduction test could evaluate the flocculation and adsorption abilities of the flocculant, and short-time filtration test would present the modification on biofloc compressibility and flocculation/adsorption ability together. As a comparison, commercially available water-soluble polymeric flocculants MPE50, PAN nanofiber and water-soluble chitosan are all evaluated the same
way to enhance fundamental understanding on the interactions of the nanofibers with sludge flocs and EPS in wastewater and effect on membrane biofouling.

1.3. Research Objectives and Signification

The specific research objectives of the research are:

1. To design and operate the continuous running MBR system for synthetic domestic wastewater treatment in lab scale and evaluate its performance.
2. To develop a procedure to prepare water-insoluble chitosan nanofiber flocculant
3. To characterize the properties of the developed flocculant
4. To evaluate the performance of developed flocculant and to understand the mechanism of flocculants interacting with sludge.
CHAPTER II

LITERATURE REVIEW

2.1. Biological Wastewater Treatment

Activated sludge process is the most widely used biological method for wastewater treatment, for both municipal and industrial wastewaters. Activated sludge process is designed to use microorganism for degradation of organic pollutant. The conventional activated sludge process requires an aeration tank and sedimentation tanks or a clarifier. The aeration tank is for organic compound breakdown and microorganism growth, and the clarifier is the place where the solids settle and separate with decanted clear supernatant.

2.2. Descriptions of Membrane Bioreactor (MBR)

The effluent quality of activated sludge process is susceptible to large fluctuations as a result of solid-liquid separation problems, such as bulking and foaming [6, 7]. In addition, the process also generates large quantities of excess sludge. By replacing clarifier with membrane separation unit in an activated sludge process, membrane bioreactor (MBRs) technologies have emerged as one of the innovative and promising solutions for wastewater treatment and reclamation.
The advantages offered by the MBR over conventional treatment are significant. They include a smaller footprint and less sludge production through maintaining a high microbial concentration from 10,000 to 50,000mg/L [8, 9]. The MBR system is also capable of handling high or shock loadings, and achieves higher effluent quality than conventional wastewater treatment. The high quality water can be reused directly for non-potable purposes by effective separation of bacteria and viruses using microfiltration (MF) or ultrafiltration (UF) membrane, generally with pore size in 0.1-0.5µm range. Moreover, an increased rate of nitrification can be achieved since the membrane completely keeps the slow-growing nitrifying autotrophs in the aeration tank, and these nitrifiers could proliferate without any loss [8, 10]. For the presence of high cell concentration in MBR, complete sludge retention and extra long SRT, bacteria face a condition with very low unit food supply. So the microorganism population in MBR activated sludge is varying as in conventional activated sludge [27, 28]. The membrane sludge population is shown to be more substrate-limited than the conventional activated sludge, and the majority of the cells in MBR are not in a physiological state characteristic for growth [27].

Figure 2.1 Schematic of side flow mode MBR
The membrane filtration unit in wastewater treatment may work in cross-flow mode (Figure 2.1), or submerged mode (Figure 2.2), and the membrane unite could be parallel flat membrane or bundled hollow fibers. The cross-flow type is also called side flow system, which is located outside the bioreactor, and wastewater is circulated through the module by an external pump. A high cross-flow velocity provided by a circulation pump can achieve relatively higher permeation fluxes over a longer period of operation, and tangentially flow is also served to reduce deposition of suspended solids at the membrane surface [7, 10]. Side flow mode MBR can be operated in both aerobic and anaerobic conditions.

![Figure 2.2 Schematic of submerged mode MBR process](image)

In submerged membrane process (Figure2.2), the membrane modules are submerged in an aeration tank, permeate of which is sucked out of the aeration tank by a pump. In contrast, submerged process is an energy conserving system since there are no circulation pumps but lower permeation fluxes [29, 30]. To improve the filtration performance, an air-scouring technique is often used. The coarse rising bubble provides a turbulent crossflow velocity over the surface of the membrane, which will help to maintain the flux through the membrane and to reduce the extent of cake formation on
the membrane surface. [7] However, the coarse bubble diffuser cannot offer very efficient oxygen transfer. In the side flow mode, the aeration is usually through a fine bubble diffuse, which offers a much more efficient oxygen transfer. Because of the higher crossflow velocity and air-scouring for side stream and submerged mode respectively, the reactor can be expected to show uniform and well-mixed behavior.

Though, operation MBR with long SRT is an often-stated advantage due to higher biomass concentration and fewer problems of solids management and disposal [9], MBRs also offer a different approach available when the treatment plants are striving to reduce external energy consumption and become energy self-sufficient [31]. The performance of MBR operated under extremely short SRT <2.5d is good in excellent effluent qualities in terms of organics removal and free of suspended solid [9]. Tertiary wastewater treatment with membrane does not have any beneficial effects compared with MBR. Even though the MLSS concentration is significantly lower in the secondary effluent than the MBR, the flux improvement is not better due to the small particle size in the secondary effluent [11].

2.3. Membrane Fouling

2.3.1. Membrane Biofouling Mechanism

Notwithstanding the significant advantages, the widespread application of the MBR process is constrained by membrane fouling [10]. Membrane fouling is the most serious problem affecting system performance and become the bottleneck. The membrane fouling by surface deposits and pore blockage of complex materials present in wastewater will significantly decrease the permeate flux or increase the transmembrane
pressure (TMP), which remains a serious challenge to the successful and economical implementation of MBRs. The interactions between the membrane surface and the components in the activated sludge liquor lead to the membrane fouling [10]. As soon as the membrane surface comes to contact with the biofluid, the biosolid deposition takes place to induce the flux decline [10]. There’s no fixed composition for the suspended solids, containing both organic and inorganic component. In general it includes cells, substrate components, cell debris, extracellular polymeric substances (EPS), and cell metabolites. Membrane fouling phenomena can be described in three ways, as shown in Figure 2.3 [30], (A) the adsorption on the membrane and pore surfaces, (B) pore clogging by small particles, blockage or significant reduction of the water permeation through the porous membrane and (C) cake layer formation over the membrane surface.

![Figure 2.3 Three mechanisms of membrane fouling phenomena](image)

Most of the cake layer can be removed by hydraulic wash, which is usually called reversible fouling. The pore clogging and absorption on the inside pore surface can only be get rid of by backwash and chemical cleaning, which is also called irreversible fouling. However, there’s no restricting categorization of membrane fouling to be reversible or irreversible. For instance, some gel layer formed on over the surface layer is almost irreversible although it is in a form of cake layer and it is notional reversible. Some kind
of pore clogging and adsorption may partially wash off for the low strength of adhesion, which is the reversible fouling [32].

The cake layer formation mostly deteriorates the filtration process and limiting the permeate flux in MBRs, which is also called biofilm [10]. The biofilm developed on the surface of the membrane beginning with the deposition of microorganisms. In spite of the crossflow or bubble wash, the colloids and microorganisms are proportionally transported to the membrane surface where they adsorb, forming a fouling layer [32]. Once attached, microorganisms may grow and multiply at the expense of feed water nutrients, developing a biofilm, and that is why biofouling of membrane surface is more problematic than abiotic colloidal fouling or mineral scaling [32, 33]. Under anaerobic condition, membrane fouling is mainly attributed to external fouling, which is most related to the movement of cell to membrane surface and inorganic precipitation at the membrane surface [34].

Biofilm can be defined simply and broadly as communities of microorganisms that are attached to a surface, which can comprise a single microbial species or multiple microbial species entrapped in extracellular products and can form on a range of biotic and abiotic surfaces. Biofilm formation involves the accumulation of microorganisms at a phase transition interface, which may be solid-liquid, gas-liquid or liquid-liquid. The transportation of microorganism may occur by diffusion, gravitational settling, or bulk fluid convection. Studies indicate that biofilm is a stable point in a biological cycle that includes initiation, maturation, maintenance, and detachment, shown in Figure 2.4 [35].
Figure 2.4 Diagram showing the development of a biofilm as a five-stage process.


Bacterial attachment is the earliest step in biofilm formation, and the initial stage will influence the subsequent biofilm development and maturation. Primary adhesion refers to the attachment of cells to the substratum, while secondary adhesion involves attachment of cells to a pre-established biofilm. During this period the EPS is intrigued to produce and firm the adhesion [32]. The primary attachment is usually reversible, and a reversible attachment is always weak in which the cell is only transported to the membrane surface and poised within the viscous sub-layer. The fluid shear stress can easily disrupt them off. Once the cells sense certain environmental conditions, which
triggers the transition of the mode of growth from a planktonic to a biofilm [36], it comes to an irreversible attachment phase. Moderate fluid shear forces cannot remove the cells.

The irreversible attachment is mediated by the biosynthesis and extrusion of EPS [32]. A number of changes in gene regulation are involved, which cause the adhering cells to become phenotypically and metabolically distinct from their planktonic counterparts. The cell may grow and multiply using nutrients transported to the surface from the bulk flow [37]. There’re at least three mechanisms occurring in biofilm growth. One is the distribution of the attached cells by surface motility [38], second is the binary division of the attached cells, and the third mechanism is recruiting of new cells from bulk fluid to the biofilm. The relative contribution of each mechanism depends on the organisms involved, the physical and chemical condition of the environment and the nature of the surface colony [35]. The biosynthesis of adhesive extracellular biopolymer increases the adhesion of binding to the membrane as time increases. The biopolymers are frequently produced and form an envelop outside the cells and the EPS matrix reinforces cellular bonding to membranes and other solids [32]. During membrane filtration, EPS and cells were co-deposited with EPS filling the voids between the cells, which form a barrier to prevent permeate going through [39].

2.3.2. Factors Effect Membrane Fouling in MBR

There are lots of factors affecting the membrane fouling in MF. It is obvious that the air scouring or circulation flow generate a cross-flow velocity, which directly affect the cake layer formation over the membrane. The cross-flow velocity increases the shear and so shear-induced diffusion, which affects the mass transportation of particles away
from the membrane surface and thus resultant cake layer thickness [10]. Both experimental and empirical studies have revealed the influence of the cross-flow velocity or aeration on membrane fouling [40, 41], but it is still unknown when air flow rates increasing how much shear at the membrane surface increase in a submerged system. Certainly it will be different between flat sheet and hollow fiber configurations [3].

Lots of studies demonstrate that there is a critical value for the cross-flow velocity or aeration rate, beyond which the influence on cake layer removal is not increased [41]. One reason is that as the mechanical forces works on preventing the membrane fouling, they also affect the particle size and the size distribution of the sludge floc in wastewater. The shear force arising from pumping, crossflow and aeration bubble during filtration process results in the breakup of biological flocs, generating fine colloids, small particles, and the release of EPS which then form a denser cake layer on the membrane [7, 10, 42-44]. As Figure 2.5 shows, mixed liquor with smaller particles and broad particle size distribution could form a denser cake layer than only large particles present, because the smaller particle can be trapped into the space of cake layer increase its resistance.

![Figure 2.5 Schematic of the cake layer formation for mixed liquor with different particle size and size distribution](image)
From the study of Altmann (1997), small particles are easier to be brought to the membrane surface because the balance between the lift force and the drag force of the filtrate flow determines the particle transport to the layer. Small particles can attach to the membrane to form a layer much easier than large ones [12]. So the size and size distribution of the particles in the wastewater to be filtered consequently affect the performance of the filtration. Especially in the continuous-fed system, the suspended smaller particle (<10µm) will create greater filtration resistance and rapidly deteriorate the effluent flux [10-13]. The smaller particles in the mixed liquor also approach the membrane pores and result in pore blocking [13]. The flux decay is caused by several fouling mechanisms together, and the relative importance of each fouling mechanisms changes with time. From the model fitting, system with more small particles will cause the transition of fouling mechanisms from pore blocking to cake formation, which takes longer time [13].

The MLSS concentration in MBRs is not a dominant factor influencing the overall membrane fouling unlike the dead-end type membrane filtration [11], but also directly related to cake layer resistance and flux decrease as MLSS concentration increases [10]. However, some studies show that when MLSS concentrates up to 30,000mg/L\(^{-1}\), it will not be direct responsible for irreversible fouling [45]. Viscosity and dissolved matter impact more significantly on filtration flux. The dissolved organics and colloidal play a more important role in membrane fouling, which is mostly from dissolved EPS, metabolites and influent components in aerobic MBRs. Many studies reveal that EPS is the significant factor responsible for membrane fouling in MBR [46, 47]. The accumulation of EPS both in the aeration tanks and on the membrane surfaces
might have caused an increase of viscosity of the mixed liquor and an increase in the filtration resistance [29]. No matter what are the composition of the influent, the viscosity of the mixed liquor influences the filtration resistance [29, 48]. The interaction between the soluble and colloidal fractions with the membrane material consequently increases the membrane’s hydraulic resistance over certain time, which depends on the quantity of soluble and colloidal compounds the supernatant contains [48]. Unlike particulate, dissolved matters impact on both internal and external fouling. Many researchers investigated the contribution of specific species of mixed liquor to membrane fouling, such as suspended solids (SS), colloids, and dissolved matter. Some of them identified EPS as being the main component contributing to fouling resistance [42, 49, 50]; some showed suspended solids contribute more than 50% of fouling problem [51].

The operation conditions like SRT, HRT and DO also influence the membrane fouling during filtration indirectly by changing the component of the suspended solids and dissolved organics. The study focused on the physicochemical and biological characteristics of sludge in submerged membrane bioreactors at various SRT reveal that the contribution of the supernatant to the overall membrane fouling is higher at SRT = 20d than SRT = 40, 60ds, where the overall fouling resistance increases as SRT prolongs. Longer sludge age also leads to decreased microbial activity in the MBRs, as reported that prolonged SRT could result in lower microbial activity [52]. However, the reduction of microbial activity and variation of microbial quantity has no significant effect on the COD removal and the nitrification in the MBRs [8].
2.3.3. Controlling of Membrane Fouling

From the study of factors influencing the membrane fouling in MBR, there’re several ways to solve the problem respectively. Generally speaking, there’re three ways, process improvement, chemical method and operation condition control. Bubbling wash, backwash and intermediate operation are the most widely used technique to improve the MBRs process. Bubbling wash for submerged mode and control cross-flow velocity for side stream mode already became necessary for the MBR process. Backwash is a another widely used technique for membrane clean especially hollow fibers, which is pumping the effluent or air through the filtration pipeline opposite [53]. Backwash is demonstrated that it can recover some of the membrane permeation in MBR by getting rid of the pore blockage and loosing the cake layer. Intermittent operation is to turn on and off the effluent suction pump intermittently upon different system, which could keep the system continuously running for a longer time than constantly turning on the pump. This technique allows long-term sustainability even when operating above the critical flux. Because when the transmembrane pressure (TMP) is during the suspension of permeation, the force that holds the particles is negated, the particles deposited on the membrane will be removed by the crossflow [3]. Sometimes, intermittent operation can combined with backwash. The operation conditions of MBR, like HRT, SRT, MLSS and F/M influence bunch of factors related to membrane fouling. Lots of studies are carried out, but there are still only few consistent conclusions on how to optimize the operation condition and get a less fouling system operation due to the complexity of the system.

Application of flocculants was accepted as an efficient way for solid-liquid separation in wastewater treatment processing due to economic and environmental
considerations [21, 32, 54, 55]. Adding flocculants into the clarifier is an efficient way to improve the sludge settleability and effluent quantities in conventional activated sludge processes. Flocculants could help the small particles and colloidal in liquid to coagulation or flocculation into large particles, which can get rid of the free particles in supernatant and make the sludge have a faster settle speed. Since the particle size and organic solute are the significant reasons leading the membrane fouling, more and more researchers are interested in studying the effect of flocculation on MBR performance [14, 15].

2.4. General Aspects of Flocculants Used in Wastewater

2.4.1. Flocculants in MBR System

It is well known that the cake layer formatted on the surface of the membrane during the MF process mostly limits the permeate flux. At the beginning, the membrane retains the particles, but later the cake layer retains the particles. This bond layer provides additional resistance to the permeate flow [56]. Thus, the porosity of the cake layer is critical to the membrane fouling. Flocculated particles can form a highly porous filtration cake. In crossflow filtration the flocculation somehow prevent the deposition of particles on the membrane, which would enable a higher permeate flux [17]. Some researchers also find out that effect of flocculants on the filtration process can be positive or negative, and a critical dosage does exist for certain system [56, 57]. The adding flocculant may also adsorb on the surface of the membrane and wall of membrane pores as well as flocculate the fine particles. The previous activity can lead to an increase in hydraulic resistance [56]. Various attempts have been done to achieve better membrane
permeability by addition of different kind of flocculant or coagulant into MBR for both industrial and municipal wastewater treatment.

2.4.2. General Aspects of Flocculants

Flocculation is termed as an aggregation process of colloidal particles involving in a solid-liquid separation [16]. Both inorganic and organic flocculant are used in various kinds of flocculation phenomena [58]. Inorganic flocculants are usually used in large quantities, formation of large amount of sludge and are very sensitive to pH changes [16, 18-20, 58]. Otherwise, polymeric flocculants are used in a very small dosage, form large cohesive floc, lower sludge volume to handle and comparatively inert to pH changes [18-20].

Inorganic Flocculants

The salt of multivalent metals, such as aluminum, iron and calcium, are mostly used inorganic flocculants, like alum, iron salts or lime [14, 18, 20, 59]. The metal flocculant is normally based on precipitation of phosphates as well as coagulation of colloids, which is the reason that leads to excessive sludge production [20]. It also remains high total dissolved solids in the effluent with little potential for reutilization [23]. The inorganic polymers, such as polyaluminium chloride (PAC), polymerized ferrous sulfate (PFS) and copolymers of Al and Fe, are used as coagulant for their high flocculation effect and small dosage [60-62]. Powdered activated carbon is another inorganic flocculant used in wastewater treatment process. Several authors reported that powdered activated carbon addition improves the organic removal efficacy by adsorption and coprecipitating the smaller and less-biodegradable organic compounds. The sludge
floc formed by addition of powdered activated carbon releases less amounts of fine colloids and EPS than conventional activated sludge does when they are under the pattern of floc breakage by similar pumping shear. The powdered activated carbon in the biological sludge floc also adsorbs and entraps some microfloc components into the floc and enhanced the membrane permeability [44].

Organic Flocculants

The organic flocculants are most electrolytes and essentially polymeric nature [18, 59]. Both natural and synthetic water soluble polymers are used as flocculants [21, 54, 55]. They can act as an agent for floc growth through binding already formed small flocs into larger ones and a establishing agent via a charge neutralization/precipitation mechanism (coagulation) as well [59].

The synthetic polymers are highly effective at very small dosages and have high tailorability, but poor shear stability [18]. The synthetic flocculants are available in all three ionic forms, i.e. cationic, anionic and nonionic. Some of the widely used synthetic polymers are polyacrylamide, polyacrylic acid, poly (styrene sulphonic acid), poly (diallyldimethyl ammonium chloride) (DADMAC) etc [18]. Due to the tailorability, synthetic flocculant can be designed for versatile conditions by controlling the molecular weight, molecular weight distribution, chemical structure of polymer, nature and ratio of functional groups on the polymeric backbone [18]. However, they are not biodegradable and shear resistant [18].

Nature polymers are biodegradable and are effective at comparatively large dosages but they are very shearing stable. Among the natural polymers, starch, guar gum,
alginates and products based on chitin, like chitosan, glue and gelatin are used as flocculants and retention aids. Polysaccharides are one of the mainly nature polymeric flocculants, which are moderately efficient due to their low molecular weights, shear stable, cheap and easily available from nature resources [21]. Nature polymeric flocculant solutions and flocs lose stability and strength because of biodegradability, which reduces their shelf life and needs to be suitably controlled [21]. However, biodegradability of nature polymeric flocculant avoid introducing new contaminants to the system and the separated solids can be recovered, some of them can be safely used as animal feedstuff [22, 23]. Flocculation Characteristics of Chitosan

2.4.3. Flocculation Characteristics of Chitosan

Chitosan, the product of deacetylation of the natural polymer chitin (Figure2.6 [63]), the biopolymer that forms the invertebrate exoskeleton of insects, crustaceans, and the fungal cell wall, is the second most abundant natural polymer after cellulose in nature [22, 24].

The Chitosan has an amino (NH$_2$) group and two hydroxyl groups on each glucose ring, which make it a highly versatile molecule with potential applications to vastly diverse fields ranging from waste management, medicine to food processing and biotechnology [23]. The active amino groups can also be protonated with H$^+$ in water to form a cationic polyelectrolyte. The cationic nature from highly reactive amino group at the C-2 position makes chitosan an ideal candidate for applications as adsorbent in waste management [64, 65]. The cationic chitosan is easy to form complexes with a variety of polyanions, like the negatively charged sludge floc and EPS. Chitosan is a well-known
widely applied flocculant in water and wastewater treatment, especially for the treatment of food processing and textile industry wastewater. The ability of chitosan and its derivatives flocculate proteins and other soluble organic matter are well studied and demonstrated that they are as good as synthetic polymeric flocculant under lots of conditions [22, 23, 25, 26]. Chitosan can also be modified to form complexes which could have both positive and negative charged groups depending on the process spatiality [23]. However, the application of chitosan as flocculant in MBR for controlling the membrane fouling is rarely studied.

Figure 2.6 Molecular structure of chitin and chitosan, Deacetylation of chitin to get chitosan
CHAPTER III

MATERIALS AND METHODS

3.1. Chemicals and Apparatus

All of the chemicals used in the experiments were reagent grade.

All of the glassware used in the experiments were completely cleaned, rinsed thoroughly with DI water, and dried in the oven. For some analysis, the glassware was washed separately according to the procedure required, which is described later in the Analytical methods section.

3.2. Membrane Module

The SURM0334 hollow-fiber membrane module panel from Mitsubishi Rayon (city, state) with panel dimension: \( W \times L = 3'' \times 4'' \), was used for the entire test. Some important membrane module characteristics are shown in Table 3.1.

<table>
<thead>
<tr>
<th>Table 3.1 SURM0334 Hollowfiber membrane module characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtration Area</td>
</tr>
<tr>
<td>Pore Size</td>
</tr>
<tr>
<td>Material</td>
</tr>
<tr>
<td>Frame</td>
</tr>
</tbody>
</table>
3.3. Membrane Bioreactor (MBR) Setup

3.3.1. Continuous Running MBR Setup

One 12-L ABS (Acrylonitrile Butadiene Styrene) rectangular reactor

(H×W×L=20×25×30 cm) was maintained continuously during this study. Four panels of membrane modules were submerged in the reactor in parallel, as shown in Figure 3.1. A peristaltic pump was used to withdraw permeate through the membrane. The pressure on the permeate side of the membrane was measured by a pressure transmitter (PX271 Omega, Stamform, CT). The effluent flowrate was also monitored by an electronic balance, which was connected to a computer for the data collection. Another peristaltic pump controlled by the liquid level sensor (50195K94, McMaster-Carr Supply Company)
pumps the feed into the reactor. pH value was controlled between 6.8 and 7.5 by a pH meter (Mettler Toledo S/N 4124042) and controller (Alpha pH200, EUTECH Instruments Pte Ltd. Ayer Rajah Crescent, #04-14/24 Singapore), through automatic addition of base (1N NaOH) and acid (1N HCl). Four long strips of air stones, each beneath one membrane panel, were installed to generate coarse bubbles to scrape the membrane surface as well as to provide aeration and mixing. DO, measured by an YSI DO probe and meter (YSI 58, Yellowsprings Instrument Co. INC, Yellowspring, OH), and was kept between 4.5 and 6 mg/L by adjusting the air flowrate. In order to keep the reactor well mixed, two 1” stirring bars were placed on the bottom and stirred by two magnetic stir plates. The data from pressure transmitter and electronic balance were computer logged by Fermac and Labview. (Ferrmac V:132-21, Biochem, Technoloy Inc.,Great Valley Corportate Center, Malvern, PA. LabView6.0, National Instruments Corporation, Austin, TX).

3.3.2. Short-time Filtration Test Setup

Figure 3.2 Schematic of filtration test experimental setup
A simplified version of MBR was used for the short-time filtration test, as shown in Figure 3.2. There is only one piece of membrane module used in a 1.5-L rectangular reactor. The other measuring devices were the same as those used in the continuous MBR.

3.4. Sequencing Batch Reactor (SBR) Process Setup

The aim to run the Sequencing Batch Reactor (SBR) process (Figure 3.3) in this study was to prepare fresh activated sludge for the short-time test studies, including the short-time filtration tests and. Ideally, the sludge was to be kept the same so that the results from the test studies would not be affected by the differences in the sludge employed. The above requirement might be best met by maintaining a continuous treatment reactor at the desirable steady state. Sequencing batch reactor (SBR) process was chosen because of its flexibility and easier operation in the laboratory. Although the activated sludge in an SBR varies in its properties within each cycle, the sludge collected at a fixed stage of the operating cycle should be relatively constant once the cyclic operation has been maintained long enough to reach a “stable” state. It has been reported that an SBR is capable of mimicking the operation of continuous systems, if the HRT and SRT of the SBR are equal to the HRT and SRT of the continuous systems [6]. For the purpose of this study, when TSS at the end of the “react” period had little change between cycles, the SBR was considered to reach the “stable” state and the wasted sludge could be used in the short-time filtration test.

The influent composition is listed in Table 3.2. The sequencing batch reactor was maintained at 6-h cycles (Figure 3.3): Fill (4.5 h) \(\rightarrow\) React (0.5 h) \(\rightarrow\) Settle (0.5 h) \(\rightarrow\) Draw (0.4 h) \(\rightarrow\) Idle (0.1 h). The aeration is turned on during the Fill and React period.
and turned off during the Settle, Draw and Idle period. Note that the milk-based synthetic influent had high content of proteins, which, if accumulated, would cause severe foaming. The long “fill” period was therefore used to slow down the medium addition, for preventing the foaming. In addition, the slow feeding was more similar to the condition encountered in the conventional continuous-fed systems. The aeration rate is 6L/min in the react period, and pH was controlled between 6.8~7.5. Two liters of mixed liquor was wasted once a day (i.e., every 4 cycles). The TSS/VSS was measured everyday. When stable TSS values were reached, the drawn effluent samples were analyzed for ammonia concentrations to make sure that the stable operation was indeed achieved. The system needs around 2~3 weeks to reach pseudo steady state.

![Figure 3.3 Sequencing Batch Reactor Process.](image)

3.5. Synthetic Wastewater

All wastewater fed to reactor in this study was synthetic wastewater to ensure consistent feed quality. The synthetic wastewater was chosen to simulate real wastewater in COD and sources of carbon, nitrogen and trace metals. Table 3.2 listed all composition and characteristics of the synthetic wastewater used in this study, which is milk protein based media.
Table 3.2 Synthetic wastewater components concentrations

<table>
<thead>
<tr>
<th>Component and Unit</th>
<th>MBR</th>
<th>SBR</th>
<th>Toxic-1</th>
<th>Toxic-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH4Cl (mg/L)</td>
<td>32</td>
<td>48</td>
<td>96</td>
<td>480</td>
</tr>
<tr>
<td>CaCl2·2H2O (mg/L)</td>
<td>4</td>
<td>6</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>FeCl·6H2O (mg/L)</td>
<td>0.16</td>
<td>0.24</td>
<td>0.48</td>
<td>2.4</td>
</tr>
<tr>
<td>MnCl·4H2O (mg/L)</td>
<td>0.12</td>
<td>0.18</td>
<td>0.36</td>
<td>1.8</td>
</tr>
<tr>
<td>MgSO4·7H2O (mg/L)</td>
<td>6</td>
<td>9</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td>ZnSO4·7H2O (mg/L)</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
<td>3</td>
</tr>
<tr>
<td>K2HPO4 (mg/L)</td>
<td>8</td>
<td>12</td>
<td>24</td>
<td>120</td>
</tr>
<tr>
<td>NaCH3COO (mg/L)</td>
<td>12</td>
<td>18</td>
<td>36</td>
<td>180</td>
</tr>
<tr>
<td>Milk (g/L)</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Peptone (g/L)</td>
<td>0.03</td>
<td>0.09</td>
<td>0.18</td>
<td>0.9</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>840</td>
<td>880</td>
<td>1670</td>
<td>8350</td>
</tr>
</tbody>
</table>

Note: Toxic-1, 2 are toxicity/inhibition test, and the details are in chapter 5.

3.6. Analytical Methods

1. Total Solids (TS) and Volatile Solids (VS)

   Total Solids (TS) and Volatile Solids (VS) (19th Edition) were measured daily according to Method 2540 B of Standard Methods (19th Edition).

2. Chemical Oxygen Demand (COD)

   Chemical Oxygen Demand (COD) is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. In this study, COD was measured for the influent, supernatant (sludge samples taken from reactor and take the supernatant after centrifuge with 6000rpm), and effluent samples taken from various experiments, according to Method 5220 C of Standard Methods (19th Edition).
3. **Ammonia (NH₄⁺)**

An ammonia-selective electrode method (Standard Methods, 19th Edition 4500-NH₃ D) was used to measure the ammonia concentrations of the influent and effluent samples of the continuous MBR and SBR processes.

4. **Turbidity**

Turbidity was measured with Hach 2900N turbidimeter. Great care was taken to have clean and smooth surface of the turbidity measurement cell.

5. **Proteins**

Concentrations of intracellular proteins and MBRs supernatant proteins were analyzed by the Total Protein Kit (Sigma TP0200 and B 3934) according to the Micro Lowry, Onishi method with Barr Modification as the following procedures. The sample preparation of intracellular proteins is as following: a) take 1ml mixed liquor into centrifuge tube and centrifuge at 9000rpm for 5min, b) dump the supernatant, adding DI water back to 1ml and vortex mix the pallet, then centrifuge (9000rpm for 5min) again, and repeat once, c) dump the supernatant and add 1N NaOH till 1ml, d) vortex mix the pallet and heat at 100C for 20min, e) after cooling down, centrifuge (9000rpm for 5min) the sample, take the supernatant protein concentration measurement.
CHAPTER IV

PERFORMANCE OF CONTINUOUS RUNNING MBR

4.1. Summary

A laboratory scaled MBR is designed and continuously operated a month as described in section 3.3.1. The performance of MBR was evaluated from nutrient removal and membrane fouling.

The COD (Chemical Oxygen Demand), ammonia concentration of the MBR influent and effluent was measured and compared. The TMP and effluent flowrate was monitored to indicate the membrane fouling. The COD removal efficiency was very high, averagely at 95% and high up to 98%. The ammonia concentration in the effluent was lower than 10ppm, most of them lower than 5ppm. No matter how the operation conditions change during the operation, the MBR always has very good nutrient removal efficiency. TMP increase speed depends on the aeration rate. A higher aeration rate could slow down the membrane fouling speed at certain range. A permeate flux decrease was observed during TMP increasing.

4.2. Summary of Experimental Protocols

The laboratory scale MBR system with four pieces of submerged hollow fiber membrane modules was operated under the condition of controlled permeation flowrate.
The COD and Ammonia concentrations of the influent, aeration tank supernatant (centrifuge the sample of mixed liquor form aeration tan, take the supernatant for measurement), and effluent were measured. Other properties measured included total solids (TS), volatile solids (VS), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), TMP, and effluent flowrate. The results of these measurements were used to describe the performance of the MBR.

4.3. Results and Discussion

4.3.1. MBR Performance Results

The initial sludge in the tank was taken form Akron Wastewater Treatment Plant (Akron, OH) with TS 4100mg/L. Figure 4.1 shows the results of various solids concentrations.

![Figure 4.1 The TS, VS, MLSS, MLSS values of continuous running MBR](image)

The time was counted from the effluent pump on. The green lines indicate the
time of chemical backwash by back pumping sodium hypochlorite solution through the effluent pipeline and membrane. The red line is chemical washing by stop the MBR and take the membrane module out of the tank flush the surface by As shown in Figure 4.1, after each membrane washing time, all of the solids concentrations (TS, VS, MLSS, and MLVSS) first decreased because the released chemicals caused some cells to die, but then grew faster in the following days. The faster growth was at least partly because the cleaned membrane allowed for higher permeation fluxes, which provided more influent nutrients for faster cell growth. The value of TS/MLSS began to decrease after running a while, especial when the TMP dramatically increased. The higher TMP shows that membrane fouling became very serious, the effluent was found decrease a lot. When the TMP goes higher than 70kPa, the effluent permeation flowrate becomes only 1/3 of the initial value. At that time, less food was supplied, that’s why the cell concentration (TS/MLSS) began to decrease.

Figure 4.2 The COD, ammonia concentration of MBR effluent and supernatant.

The time profiles of COD, ammonia and nitrate concentrations in the samples of membrane effluent and mixed liquor supernatant are shown in Figure 4.2. The COD
profile of the effluent was much more stable than the profile of the mixed liquor supernatant. In addition, the effluent COD was consistently lower than the supernatant COD. This finding agreed with the results reported [41, 46, 66], indicating that the sludge deposited on the membrane helped to degrade or retain organics not readily degraded by the population in mixed liquor. The COD removal efficiency was very high, around 95% and up to 98%.

4.3.2. Results of Transmembrane Pressure and Permeate Flux

The Transmembrane Pressure (TMP) and permeation flowrate was monitored during the operation to detect how membrane fouling affects MBR performance. Figure 4.3 and 4.4 show the TMP change with time in different ways. When the pressure drop increased to certain value the membrane cleaning should be performed. The pressure drop will come back to origin after cleaning and the flux will also be recovered (Figure 4.3). The new membrane is more resistible for the biofilm growing at the beginning reversible adhesion, which can last longer time without obvious pressure drop increasing. Once the irreversible attachment occurs, the pressure drop increases rapidly. The increasing speed of the pressure drop is laid on the aeration rate during certain range, which determines the scouring force to clean the membrane. Figure 4.4 also tells that the higher the aeration rate supplies better washing effect. However if the aeration rate higher than certain value, it won’t help to prevent the pressure drop increasing anymore[34].
4.3.3. Conclusions

Membrane fouling speed was affected by the bubbling wash intensity. Increasing the aeration rate prolonged the time before the increase in TMP was observed. From the observation, under higher aeration rate condition, cake layer formed on the membrane was much more rigid and compressed than it is under lower aeration rate. When the TMP
began to increase, very obvious effluent flowrate decreased was observed, the effluent flowrate was measured at that point. When the TMP goes higher than 60kPa, the flowrate will decrease from 20ml/min to only 1/3 of the original setup flowrate.
5.1. Introduction

Adding flocculant may have short time and/or long time effects on the activated sludge bioactivity. The long time effect is very complicated and wont effect the flocculation ability immediately. In this study only the short time effect was investigated. In order to detect the short-time effects, experiments were designed to test the toxic or inhibitory effects of the flocculants. When the toxic/inhibiter compound was introduced to the system, it will directly affect the microorganism growth [6]. In order to study the immediate toxicity/inhibition effects of the flocculant on the system, the cell growth profile was studied with all the candidate flocculant material. Because it is multi-culture, in this study, intracellular protein concentration changing with time was chosen to express the cell growth rate.

5.2. Summary of Experimental Protocols

The adding the flocculant would inhibit the cell growth [6], a batch process cell growth profile would well present the short-time effects. The nutrients were added in three times during 24 hours in order that the cell growth was not too fast to take samples and not to be inhibited by high substrate concentration. Table 5.1 shows the nutrient
components and their concentration in the media used. The final concentrations given in Table 5.1 do not include the slim volume change by taking samples and adding fresh media. Two flocculant concentrations were tested, high and low, corresponding to 100 ppm and 50 ppm, respectively.

Table 5.1 Substrate components concentrations

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Dilute M</th>
<th>Final M</th>
<th>Fresh M</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH4Cl(mg/L)</td>
<td>48</td>
<td>96</td>
<td>480</td>
</tr>
<tr>
<td>CaCl2·2H2O(mg/L)</td>
<td>6</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>FeCl·6H2O(mg/L)</td>
<td>0.24</td>
<td>0.48</td>
<td>2.4</td>
</tr>
<tr>
<td>MnCl·4H2O(mg/L)</td>
<td>0.18</td>
<td>0.36</td>
<td>1.8</td>
</tr>
<tr>
<td>MgSO4·7H2O(mg/L)</td>
<td>9</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td>ZnSO4·7H2O(mg/L)</td>
<td>0.3</td>
<td>0.6</td>
<td>3</td>
</tr>
<tr>
<td>K2HPO4(mg/L)</td>
<td>12</td>
<td>24</td>
<td>120</td>
</tr>
<tr>
<td>NaCH3COO(mg/L)</td>
<td>18</td>
<td>36</td>
<td>180</td>
</tr>
<tr>
<td>Milk (g/L)</td>
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<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Peptone(g/L)</td>
<td>0.09</td>
<td>0.18</td>
<td>0.9</td>
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<tr>
<td>COD (mg/L)</td>
<td>880</td>
<td>1670</td>
<td>8350</td>
</tr>
</tbody>
</table>

Note: M= Media

Table 5.2 First batch process experiment procedures

<table>
<thead>
<tr>
<th>No.</th>
<th>Procedure</th>
<th>Mark</th>
<th>V(ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Take 10ml sample inoculation from the preculture flask</td>
<td>Sample 1</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>Dilute prepared media 10 times into Dilute media</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Add 40ml Dilute media, and 5ml different flocculant solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Take 1ml sample at 12:00PM</td>
<td>Sample 2</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>Take 1ml sample at 3:00PM</td>
<td>Sample 3</td>
<td>54</td>
</tr>
<tr>
<td>6</td>
<td>Add 3ml fresh media right after taking sample at 3:00PM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Take 1ml sample at 6:00 (optional)</td>
<td>Sample 4</td>
<td>56</td>
</tr>
<tr>
<td>8</td>
<td>Take 1ml sample at 9:00PM and then add 3ml fresh media</td>
<td>Sample 5</td>
<td>55</td>
</tr>
<tr>
<td>9</td>
<td>Take 1ml sample at 9:00AM</td>
<td>Sample 6</td>
<td>57</td>
</tr>
<tr>
<td>10</td>
<td>Take 1ml sample at 3:00PM</td>
<td>Sample 7</td>
<td>56</td>
</tr>
</tbody>
</table>
Table 5.2 shows the detailed experimental procedure. The 10 times diluted sludge sample from Akron Wastewater Treatment Plant was used to inoculate. Sludge cells (1ml) were inoculated into 250ml beaker flasks with 50ml Diluted Media for batch culture and very high stirring speed was used for mixing and oxygen supply. A second batch was also tested. The purpose of the second run was to increase the nutrient concentrations to get higher cell concentrations.

All the medium concentrations were 5 times of those in the first batch of test (i.e., 5 times of those given in Table 5.1). The high and low flocculant concentrations were kept the same. The procedure was listed in Table 5.3. During the second test, the culture color was found to change and a strong smell was detected. The final pH was therefore tested to detect if anaerobic fermentation occurred during the culture. A third batch of test was then carried out with increased buffer strength, by including 2 g/L of NaHCO₃. The pH value was monitored along with the intracellular protein concentration.

Table 5.3 Second batch experiment procedures

<table>
<thead>
<tr>
<th>No.</th>
<th>Procedure</th>
<th>Mark</th>
<th>V(ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Take 5ml sample inoculation from the preculture flask</td>
<td>Sample 1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Dilute prepared media 10 times into Dilute media</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Add 40ml Dilute media and 5ml flocculant solution at 11:30AM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Take 1ml sample at 2:30PM</td>
<td>Sample 2</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>Take 1ml sample at 6:30PM and Add 3ml fresh media</td>
<td>Sample 3</td>
<td>49</td>
</tr>
<tr>
<td>6</td>
<td>Take 1ml sample at 11:30PM and then add 3ml fresh media</td>
<td>Sample 4</td>
<td>52</td>
</tr>
<tr>
<td>7</td>
<td>Take 1ml sample at 10:30AM</td>
<td>Sample 5</td>
<td>54</td>
</tr>
</tbody>
</table>
5.3. Results and Discussion

5.3.1. Toxicity/Inhibition Test Results

Figure 5.1 shows the results of the intracellular protein analysis of the first run, where the profiles given included the control system and the systems with low (L, 50 ppm) and high (H, 100 ppm) concentrations of three flocculants. In the first several hours, there were no significant differences among the systems. After the cell growth slowed down, minor differences appeared. The resumed growth at 6 h and 12 h were because of the supplementary addition of fresh media. Despite the differences seen at longer time, there were no clear trends that allowed for concluding the effects of flocculants on the growth of sludge. The strange profiles for the systems with 100 ppm of MPE50 and 50 ppm of chitosan likely resulted from the analytical errors. Nonetheless, the results during the period of Hour 2 to Hour 6 suggested that there might be some effects after the sludge population ran out of food for a while with positive effects from PAN nanofibers and negative effects from MPE50 and chitosan. These potential effects were investigated further in the study.

During the second run with higher media concentration, the broth changed color and spread a strong smell in the end of the test. The final pH was tested to detect if anaerobic fermentation had occurred. Figure 5.2 shows the intracellular protein profiles and Table 5.4 gives the final pH values of each systems. The higher nutrient concentrations sustained longer growth and much higher intracellular protein concentrations (2.3-3.2 g/L, vs. 0.5~0.6 g/L in the first run of study). The trend remained the same as the culture time increased, the differences between systems increased but,
when considered together with the results of the first batch, the differences can not conclude clear toxic or inhibitory effects of any of the three flocculants examined.

![Figure 5.1 First batch intracellular protein concentration results](image)

It was surprising to see the widely different final pH values reached in different systems. The metabolism and, probably, population involved were clearly different, although the intracellular protein concentrations were similar. The system with 50ppm MPE50 and 100ppm PAN have the same final pH 4.13–4.24 range with control. Higher concentration (100ppm) MPE50 and Chitosan have the highest pH around 8.2, while 50ppm Chitosan and PAN got to the same pH 7.59. There is no obvious relationship between the flocculants and concentration. In order to minimize the pH effect during the bacteria growth, the third batch of study was conducted in shaker instead of stirring plates.

<table>
<thead>
<tr>
<th>pH</th>
<th>Control</th>
<th>L MPE50</th>
<th>H MPE50</th>
<th>L Chitosan</th>
<th>H Chitosan</th>
<th>L PAN</th>
<th>H PAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.16</td>
<td>4.13</td>
<td>8.23</td>
<td>7.59</td>
<td>8.18</td>
<td>7.59</td>
<td>4.24</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.4 Final pH value culture under higher media concentration
The use of shaker was expected to keep the condition for each system more similar and stable. The pH was measured and adjusted periodically in all of the systems throughout this batch of experiments.

The results of pH and intracellular protein concentrations are shown in Figure 5.3. The growth (< 18 h) again showed the two-stage profiles, with corresponding changes in the pH profiles. The two-stage behavior probably reflected primarily the change in C-substrate utilized. The milk powders used in preparing the media contained proteins and carbohydrates. It is hypothesized that the milk proteins and peptone (Table 5.1) were consumed first (0-5 h), which caused the release of the excess ammonia and the observed pH increase. A brief period of lag phase for metabolic (and potentially population) adjustment followed before the cells could switch to grow on the carbohydrates rapidly (12-18h). The utilization of carbohydrates caused the pH to decrease, as a result of organic acid production.
The cells began to die after 25-30 hours. At the same time, pH rose to higher than 8.5 and was adjusted back to 7.0 at 47h and 59h. There were two possible limitations, i.e., nutrient and pH, which prohibited the cells from continuing to grow. However, because the cell decay continued after the pH adjustment, the pH increase being the cause of cell death can be excluded.

The autotrophs mostly the nitrifiers only contribute a very small amount of cell concentration in the sludge. However nitrifiers are the most sensitive species in the activated sludge. The initial rapid increase in pH is typical to growth of heterotrophic organisms on protein-based media. Proteins are nitrogen-rich. During the metabolism, the excess nitrogen (beyond the required amount for biomass synthesis) is released in the form of ammonia, causing the medium pH to increase. Interestingly, the pH decreased subsequently (after 6 h) and the cell growth slowed, before resuming to faster growth.
again (between 12-18 h). A plausible explanation is that the group of autotrophs grows fast after the first period pH increase to 8. The metabolism subsequently shifted from heterotrophy-dominant to nitrification-dominant, resulting to a net trend of pH decrease. During that heterotrophs “lag phase,” the nutrient concentration decreased and the heterotrophs beat the growth rate of autotrophs, leading to the second phase of rapid growth and pH increase. The growth eventually ceased when all carbon substrates were depleted. As ammonia concentrations were not measured, it is unclear when the much slower growth of nitrifying population stopped. However from the pH curve we can tell that there are no big differences among each system. The effects of adding flocculant are not obvious.

5.3.2. Conclusions

The results of all the toxicity/inhibition studies indicated that adding the three flocculants (including the PAN nanofibers), at concentrations up to 100 ppm, had no insignificant effects on the cell growth and, presumably, would not decrease the bioactivity.
CHAPTER VI

CHITOSAN NANOFIBER PREPARATION

6.1. Introduction

Normally two kinds of nanofiber are used as flocculant in the study, i.e. Polyacrylonitrile (PAN) and chitosan. Nanofibers are expected to exhibit predominant effects of biofilm-structure modification.

Chitosan nanofiber is considered interesting for various kinds of applications because of their high specific surface area, high porosity and other useful proprieties [19]. Examples are for filter applications, biomedical applications, such as fibrous scaffolds for tissue engineering, wound healing and drug delivery [67, 68], and sensing applications [19]. Some researchers got electrospinning chitosan nanofibers by 1) blending or composing chitosan with other polymers, such as electrospinning of chitosan/poly(ethylene oxide) (PEO) blend aqueous acetic acid solution [69], chitosan/poly(vinyl alcohol) (PVA) blend formic acid aqueous solution [70, 71], 2) employed chitosan derivatives, complex of poly (lactide-co-glycolide) (PLGA) and poly(ethylene glycol)-g-chitosan (PEG-g-CHN) dissolved in Dimethylformamide (DMF) with a weight ratio of 90:10, was successfully electrospun into fine nanofibers [72]. Very broad solvents and wide concentration range were tested for prepare the chitosan solution and failed to directly electrospinning into fibers [19, 71]. The reason might be that amino groups in
chitosan are ionizable under the acidic or neutral pH conditions [70]. During electrospinning process, chitosan is under the high electric field, and the repulsive force between ionic groups within polymers backbone is expected to inhibit the formation of continuous fiber, especially during the jet stretching by whipping and bending. Therefore, the ionic polymer results in fine particles but not fine fibers [19]. Few publications talk about successfully directly electrospinning chitosan into nanofibers. Trifluoroacetic acid (TFA) is the only solvent that has been tried to get pure chitosan fiber after electrospinning with concentration 7~8 % (w/w). The study also mentioned that adding dichloromethane (MC) into the chitosan TFA solution could prepare a more homogenous solution and optimize the electrospinning conditions [71]. In our experiments, the chitosan TFA solution concentration that could get fine fiber during electrospinning process is 2.5~3% (w/w) dissolved in pure TFA. The possible reasons that electrospinning chitosan is successful using TFA are: a) TFA could form salts with the amino groups of chitosan, which destroys the rigid interaction between the chitosan molecules, making them ready to be electrospun [73]; b) the high volatility of TFA is advantageous for the jet of chitosan TFA solution rapid solidification [71].

6.1.1. Electrospinning of Nanofiber

6.1.2. PAN Nanofiber Preparation

The electrospinning PAN solution is prepared by dissolving PAN into DMF with concentration of 10% (w/w). Figure6.1 shows the schematic of the electrospinning setup. Because PAN is not soluble in water, to obtain well-dispersed fiber, the electrospun fiber is collected in water with aeration in the bottom. The bubbling air could make sure the
fibers dispersed in water instead of compacting together. The collected fiber is washed by water again to get rid of the remained solvent from PAN. The long PAN fiber is chopped into small pieces by blender with a high speed for 5min, and then the fiber is shown dispersed very well in water. The PAN fiber used for all the tests in this study is prepared as above.

6.1.3. Chitosan Nanofiber Preparation

For the intended application in MBRs, it is desirable to make the chitosan nanofibers insoluble but readily dispersible in water. We tried to cross-link the chitosan nanofibers to make them insoluble in water. The crosslink reaction is to let the amine groups on chitosan react with di-aldehyde. In this study only glutaraldehyde was tried. The difficulty of this approach lies in confining the crosslinking within individual fibers, instead of crosslinking multiple neighboring fibers into non-dispersible mass. From the
preliminary study, the cross linked chitosan fibers changed color from grayish white to yellowish brown and appeared as a mesh under microscope. Crosslinking the electrospun chitosan is a feasible way to get insoluble chitosan nanofibers.

There are two cross linking methods that we tried: a) in ethanol, For the cross linking in ethanol solution of glutaraldehyde, pieces of the electrospun chitosan fibers were soaked in absolute ethanol added with different concentrations of glutaraldehyde, i.e., 1%, 5%, 10%, and 20% (w/w) and observe the reaction phenomena. The glutaraldehyde use for crosslinking is prepared as follows. Taking the same amount of glutaraldehyde water solution (50% w/w) mixed with absolute ethanol. Then adding enough silicon molecular sieves into the mixed solution, the container was sealed and shaken for a 5min. After that, the solution stays in refrigerator for 48hours, meanwhile, shaking the solution a 5 minutes every 12hours. The molecular sieves absorb most of the water from glutaraldehyde, and the solution becomes glutaraldehyde in ethanol, which is called extracted glutaraldehyde in this study. For crosslinking in glutaraldehyde vapor, put the extracted glutaraldehyde in the bottom of a jar, then put the electrospun chitosan fiber on a mesh holder right above the liquid, seal the container and put it in room temperature to check the fiber under microscope after washed by DI water. After crosslinking, the non-crosslinked chitosan would dissolve in water but the crosslinked fibers would not. After washing, the fibers are checked under microscope.

Figure6.2 shows that there is some of un-cross-linked chitosan dissolved in water. The problem is that it cannot be dispersed in water. The cross linked chitosan fiber changes color from gray white to yellow brown and looks like a mesh under microscope. To cross link the electrospun chitosan is a feasible way to get non-water-soluble chitosan
The individual nanofiber structure does not exist any more after cross-linking. From the preliminary study, the cross linked chitosan fiber changed color from gray white to yellow brown and look like a mesh under microscope (Figure 6.3). So crosslinking the electrospun chitosan is a feasible way to obtain non-water-soluble chitosan nanofiber.

Figure 6.2 Cross linked chitosan nanofiber, picture after DI water washing, a) cross linked in ethanol (x1000), b) cross linked in glutaraldehyde vapor (x400)

Figure 6.3 Microscopic pictures of electrospun chitosan nanofiber (X1000) a) Original fiber, b) after cross-linking
It is nearly impossible to get dispersible cross linked chitosan nanofibers if the fibers are compacted together during the fiber collection. How to get well-dispersed chitosan nanofibers became the most significant step. Liking PAN can be collected in bubbling water, so using liquid to collect the fibers might be a way to be considered. In order to well disperse the electrospun nanofibers, a feasible way is to spin the fiber into gently stirred liquid. Because the chitosan nanofibers will dissolve in water regardless the pH condition, some suitable organic solvents have to be used. Ethanol, pentanol and light mineral oil (company, city, state) are characterized for chitosan fiber solubility, swelling and cross-link reaction occurring possibility.

1. Ethanol: Two experiments were done to evaluate the possibility of using ethanol as the dispersion liquid. In the first experiment, the spun fibers were soaked in pure ethanol for 24 h. The pictures taken under the microscope for the soaked fibers revealed that the fibers absorbed ethanol (evident in the enlarged diameters) but still kept the basic configuration as long-stranded fibers. The observation confirmed that the spun fibers did not dissolve in pure ethanol.

On the other hand, because the fibers were spun from a chitosan solution in Trifluoroacetic acid (TFA), (2.8-3) % w/w, it was necessary to consider the effect of the presence of TFA. In the second experiment, ethanol was gradually added to the chitosan solution in TFA. Under stirring, chitosan did not precipitate out until the added ethanol volume was twice of the original volume of the chitosan solution. Ethanol is therefore not a perfect choice as the dispersion liquid, because the electrospun fibers might dissolve in it if the fibers reached the liquid surface with TFA not completely evaporated.
Pentanol: The two experiments done on pentanol followed the same designs and procedures as those on ethanol. The findings were somewhat different. As expected, pure pentanol, being more hydrophobic than ethanol, was found incapable of dissolving the electrospun chitosan fibers. Furthermore, when added to the chitosan solution in TFA, pentanol formed a separate phase from the TFA solution and caused chitosan to precipitate out of solution. Pentanol was clearly more suited than ethanol as the dispersion liquid. Nevertheless, the chitosan fibers still swelled substantially in pentanol during the electrospinning, and most of the fibers gradually lost their morphology and turned into gelled beads. Pentanol, although better than ethanol, was still not ideal as the suspension liquid.

3. Light Mineral Oil: Light mineral oil was more hydrophobic than pentanol. Adding the mineral oil into the chitosan solution in TFA did not cause any changes to the solution, and the interface between the two layers was very clear. The glutaraldehyde did not dissolve in mineral oil but could form emulsion when added as a solution in ethanol. It was therefore feasible to cross-link chitosan nanofibers in the emulsion. Unfortunately, the use of mineral oil was excluded at the end because of its poor conductivity, which would demand special equipment to enhance the electric force required for successful electrospinning.

4. The new electrospinning method was to use two electrodes as shown in Figure6.4. One pipette holding the polymer solution was connected to the positive electrode, and the other one was connected to the negative electrode. The two streams of fibers spun from the pipettes met to neutralize the charges on the fibers before they reached the collector. The vacuum drawn under the collector pulled the fibers down onto
a collection mat. The fibers thus prepared were very loosely packed, much more than the fibers prepared by the typical method where only one pipette was connected to the (positive or negative) electrode while the collector was grounded. The loosely packed structure had fewer contact points among fibers and allowed for easier subsequent dispersion in ethanol, which may also minimize the cross linking reaction between fibers.

6.2. Results and Discussion

6.2.1. ESEM Characterization of PAN Nanofiber

Figure 6.5 is ESEM picture of electrospinning PAN nanofiber. The fiber diameter was measured by ESEM operation software. The average diameter of PAN fiber is 308 nm and they are distributed in the range 133~722 nm based on 121 samples. More than 85% of them are distributed in the range of 200~450 nm.
6.2.2. ESEM characterization chitosan fiber

Figure 6.6 shows the ESEM picture of electrospinning chitosan fiber. The average diameter of the dry chitosan fiber is 303nm and distributed in the range 64–654nm. Around 75% of the fibers are distributed in the range of 150–450nm. When dispersing the electrospinning nanofiber into ethanol, the fibers swell a little bit. So after soaked in pure ethanol for two hours, the chitosan fibers was taken out for 15 min and then taken pictures by ESEM (Figure 6.8). The average diameter of the moisturized fibers is 408nm, and they are distributed in a much larger range 136–1117nm. The moisturized fibers have a 105nm larger diameter than the dry one and the swelling ratio based on the average diameter is 34.8%. There are no significant different between the dried fiber from ethanol and the original one from ESEM pictures.

Two crosslinking methods described previously were both tried on the fluffy chitosan fiber, i.e. a) crosslinking in ethanol, and b) in vapor. The first method was chosen to deal with the final product because the one cross linked in vapor cannot be dispersed in water. From the picture taken by ESEM (Figure 6.7) we can tell that the cross-linked nanofibers still form a network between different layers. Even chopped by a commercial blender, the piece of tangled mat cannot come back to individual fibers. After putting the electrospinning fiber into ethanol with glutaraldehyde, the container was kept in a shaker with a 270rpm shaking speed to insure the fiber was dispersed well during the reaction. Cross-linked chitosan fiber was washed by DI water for 48 hours after the crosslinking reaction to get rid of the un-reacted glutaraldehyde and ethanol. To washing the crosslinked nanofiber, first, centrifuge the reaction mixer and dump the supernatant, second, adding DI water to resuspension the fibers, third, put the
resuspended fibers into a dialysis bag, which will stay in DI water with air bubbling for 48 hours. The dialysis water was changed every 12 hour. After then, the nanofiber was transferred into DI water for later test use. The concentration of the nanofiber is based on the dry weigh of crosslinked chitosan. As we can see from the pictures (Figure 6.9) of the cross linked chitosan fiber, some of the fibers are separated from the others, while some of them also form into networking. But the pictures cannot show how much vertical cross-linking occurs.

Figure 6.5 ESEM picture of PAN Nanofiber
Figure 6.6 ESEM picture of the electrospinning fluffy chitosan nanofibers
Figure 6.7 ESEM Picture of cross linked fluffy nanofiber in glutaraldehyde vapor and washed by DI water
Figure 6.8 ESEM picture of chitosan nanofiber swelling in ethanol
Figure 6.9 ESEM chitosan nanofiber Cross-linked in ethanol
7.1. Introduction

The turbidity in wastewater is caused by suspended solids and colloidal matters, such as flocculated and dispersed organisms, clay, silt, finely divided organic and inorganic matters, and complex substrates or biological products [74], which also cause the membrane fouling. Among the three membrane fouling mechanisms, (A) the adsorption on the membrane and pore surfaces, (B) pore clogging, and (C) cake layer formation over the membrane surface. The cake layer formation induced by particle deposition and biofilm adhesion was reported to cause the largest resistance during the filtration [10, 30]. The particle size is very critical to effect the deposition on the membrane surface. Several researchers reported that only small particles could be deposited on the cake layer at low filtration rates [12, 75]. With the addition of flocculants to the feed, the particle size can be increased and the size distribution changed. At the same time, a more porous cake layer, which leads to a higher permeability, would be obtained [56]. Accordingly, a possible approach to enhance the permeate flux in micro filtration (MF) would be the creation of a flocculated feed.

As flocculants were introduced in the filtration system, many processes might occur: (a) flocculation of the particles due to the adsorption of flocculants on the particle
surfaces, (b) adsorption of flocculants on the membrane surface and probably within the membrane pore surface, or (c) presence of residual free flocculants in the suspension [56]. The mechanisms of flocculation can be different, it may (a) flocculant binding with the extracellular polysaccharides substrate (EPS) to help the small sludge floc form into large particles through, (b) flocculate individual cells together and (c) coagulate the dissolved organic and colloidal materials. No matter which mechanism dominates the process, the flocculation will reduce the water turbidity. So the turbidity reduction test has become a way widely used to evaluate the flocculation activity. Adding flocculant may also change the sludge compressibility, which might significantly affect the filtration process. The hypothesis was that if the cake layer formed by deposition of sludge flocs was more porous and less compressible, the decrease in permeate flux due to membrane fouling would be less and/or slower. The sludge volume index (SVI) was measured in this work to indicate the sludge compactness.

7.2. Summary of Experimental Protocols

The turbidity reduction experiments were carried out in 500-mL test jars with the following procedures [26, 76]. 1) Take 250 mL of mixed-liquor sample from the well-mixed sequencing batch reactor (SBR) to each test jar. 2) Add different amounts of the flocculant under investigation and deionized (DI) water to adjust the flocculant concentration and the total liquid volume to 300 mL. A control system was included adding only DI water. 3) Stir the flask rapidly using Speed setting 9 on a Corning stirring plate (PC-210) for 10 min to well mix the flocculant with the mixed liquor sample. 4) Slow the stirring speed to Setting 5 for 10 more minutes, to promote the flocculation. 5)
Let the mixed liquor settle for 45 min. 6) Take the supernatant of the settled sludge and immediately measure its turbidity (expressed as nephelometric turbidity units (NTU)) using a turbidimeter (HACH, 2100P). 7) Measure the volume of the settled sludge according to the level marks on the jar.

The flocculation activity was quantified as the percentage of turbidity reduction with the control as the reference for comparison [14], i.e.,

$$\text{Flocculation Activity}_{\text{Sample}} = \frac{\text{NTU}_{\text{Control}} - \text{NTU}_{\text{Sample}}}{\text{NTU}_{\text{Control}}}.$$ 

Calculated as such, the flocculation activity indicates the extent (and mechanism, as described in Results and Discussion) of the interaction (flocculation and/or adsorption) between the flocculants and the sludge (and other wastewater particles). The stronger interaction leads to more turbidity reduction, i.e., a larger difference ($\text{NTU}_{\text{Control}} - \text{NTU}_{\text{Sample}}$) and a larger value of the flocculation activity. The SVI change of settled sludge was also calculated, as the percentage of change in SVI from that of the control, which is the reciprocal of compactness, i.e.

$$\text{SVI Change}_{\text{Sample}} = \frac{\text{SVI}_{\text{Sample}} - \text{SVI}_{\text{Control}}}{\text{SVI}_{\text{Control}}}.$$ 

Conceptually, this compactness may include some information regarding the effects of flocculant on the porosity and packing speed of the sludge floc.
7.3. Results and Discussion

7.3.1. Turbidity Reduction Test Result of Flocculant

The turbidity reduction test was conducted on all of the candidate flocculants (and the control). Figure 7.1 shows the flocculation activity and Figure 7.2 shows the SVI Change results in different flocculant concentrations. As the concentration increased from several ppm to 100ppm, the dissolved chitosan and MPE50 both showed very good ability in reducing the turbidity of the supernatant. The chitosan solution and chitosan nanofiber dissolved in water both appeared to reach plateaus in the flocculation activity at higher concentrations, while the flocculation activity of MPE50 continued to increase in the range of concentration tested.

Figure 7.1 Flocculation activity result of the flocculants in different concentrations
Figure 7.2 SVI Changes of flocculants in different concentrations

Note: CH.S. is chitosan solution, MPE50 is Nalco’s flocculant, PAN is PAN nanofiber dispersed in water, CH.F.D. is chitosan nanofiber dissolved in water, and CH.F. is insoluble cross-linked chitosan nanofiber. It is same for other figures in this study.

Note that the MPE50 concentration was based on the added weight of the commercial solution sample, while the concentration of the two chitosan systems was based on the dry weight of chitosan. The dry weight concentration of the liquid MPE50 sample was later determined as 15.5% (w/w). From the two replicates test of PAN, we can see when PAN concentration became higher the sludge will pack more loose after settle down. However, the turbidity of the supernatant was not consistent for the two sets of experiment. The PAN nanofiber itself doesn’t have many active sites as chitosan and the polymers in MPE50. Sometimes, PAN seems to increase the turbidity of the wastewater. The cross-linked chitosan fiber showed the same result as PAN. In order to know whether the increased turbidity was induced by the fibers or because they can’t
combined with biological material, the high concentration was tested and self-flocculation test was carried out to explain the mechanism behind.

![Graph showing turbidity removal and SVI change with chitosan concentration](image)

**Figure 7.3** Chitosan turbidity removal test results summary

From the result we can tell the trends of the effects of flocculant addition on the sludge, but cannot tell exactly which one was the best performer because sludge samples used in the tests were taken at different days for different flocculants. There’s no obvious trend of SVI change as flocculant concentration increasing from the figures. The ability of MPE50 to reduce the turbidity of the supernatant seemed to be proportional to its ability of enhancing the sludge packing. Adding chitosan will loose the sludge packing in some extent no matter the chitosan morphology. After putting all the chitosan result together in Figure 7.3, we can tell there are some relations between the turbidity reduction and SVI Change. It seems that the better turbidity reduction also gets settled sludge more compressed.
7.3.2. Turbidity Study of Nanofibers

Chitosan was evaluated a good candidate material for the flocculant, which shows promising flocculant activity and looses the settled sludge at certain concentration range when it dissolved into water. From the two replicates test of PAN, we can see when PAN concentration became higher the sludge will pack more loose after settle down. However, the turbidity of the supernatant was not consistent for the two sets of experiment. The PAN nanofiber itself doesn’t have many active sites as chitosan and the polymers in MPE50. Sometimes, PAN seems to increase the turbidity of the wastewater. The cross-linked chitosan fiber showed the same result as PAN. In order to know the increased turbidity was induced by the fibers or because they can’t combine with biological material, the high concentration was tested and self-flocculation test was carried out to explain the mechanism behind. Figure 7.4 shows the original flocculant activity of the fibrous flocculant and the Delta NTU, Figure 7.5 show fiber turbidity of the self-flocculation test result. Delta NTU was considering the turbidity of the fiber itself to evaluate the interaction of the fibrous flocculant and sludge floc. It was calculated as this:

\[ \text{Delta NTU} = (\text{NTU}_{\text{sludge}}) + (\text{NTU}_{\text{Fiber}} - (\text{NTU}_{\text{sludge+Fiber}})) \]

\( \text{NTU}_{\text{sludge}} \) was from the control turbidity value of each experiment, and \( \text{NTU}_{\text{Fiber}} \) is the turbidity of fibrous flocculant in water with the same concentration and undergoes the same procedure as the turbidity reduction test that was described before. \( \text{NTU}_{\text{sludge+Fiber}} \) was the turbidity from the turbidity test in certain flocculant concentration. The turbidity was supposed additive during the test range (0~50NTU), then if the Delta NTU is positive that means there are interaction between the flocculant and sludge floc, the larger NTU value means better turbidity reduction efficiency.
Figure 7.4 Fibrous flocculant flocculation activity and Delta NTU result

Figure 7.5 Fibrous flocculant turbidity with different settling time
From Figure 7.4, it seems both PAN and Chitosan fiber contribute to remove the turbidity when the fiber concentration increase to 25ppm and 50ppm respectively and the higher fiber concentration would get better removal performance. While increasing the concentration, the Delta NTU of PAN will go to a plateau with certain value. The tested concentration didn't tell that about chitosan fibers.

However, from the three flocculation mechanisms (a) flocculation of the particles due to the adsorption of flocculants on the particle surfaces, (b) adsorption of flocculants on the membrane surface and probably within the membrane pore surface, or (c) presence of residual free flocculants in the suspension [56]. The results can’t tell us which mechanism was dominant the turbidity reduction during the flocculant interacting with sludge. It may be different for different flocculant and different sludge. Some microscope pictures (Figure 7.6, 7.7) were taken to investigate the floc morphology change after interaction with flocculant. Indian ink was used for dyeing the sludge, which was expected to show the EPS around the cells and floc.

The black part is sludge floc and particles. The white edge around floc is supposed to be EPS attached on cells. Addition of chitosan and MEP50 both flocculate the sludge flocs into large pieces, and there is a little thinker white edge around the sludge floc with them. One possible reason is that more EPS is adsorbed on floc than dissolved in water. The PAN nanofibers are entangled with sludge floc and form larger piece, but it is hard to tell they’re flocculated together or just mixed.
Figure 7.6 Microscope picture of India ink dyed a) sludge, b) with chitosan solution
7.3.3. pH Effect of Turbidity reduction

pH is an important factor that affects the flocculant changing and flocculation activity. To detect the optimal pH value of the flocculant is necessary to get better flocculation performance and investigate the flocculation mechanism. Two sets of pH

![Microscope picture of India ink dyed sludge](image)

c) with MPE 50

d) with PAN nanofiber

Figure 7.7 Microscope picture of India ink dyed sludge a) MPE50, b) PAN
effect tests were carried out with different references. The first set of experiment was done by change the sludge pH of each jar into 4, 5, 6, 7, 8, and 9, after that, and then adding the same amount of flocculant, all the procedure was the same as the previous turbidity reduction test described in section 7.2. Each flocculant was only tested for one concentration. Chitosan is 2ppm, MPE50 is 100ppm and PAN is 100ppm. The flocculation activity result is plotted in Figure 7.8.

![Figure 7.8 Flocculation activity based on neutral sludge](image)

The concentration used for this test was based on the optimum concentration of first turbidity reduction test. Because when the test was carried on chitosan concentration was calculated based on chitosan solution, when convert to chitosan concentration 2ppm for here it is too low compared to the later turbidity reduction test optimum value 50ppm. MPE50 shows a peak reduction ability at pH=7, and have turbidity reduction ability for
the tested pH range 4~8. PAN and chitosan solution have the same trend as pH change from 4 to 8, increase first and then decrease, the peak value is around 6-7. Flocculation activity is very low even in the peak area. One phenomenon was observed during the experiment, and it was that pH adjusting would affect the sludge settling and supernatant turbidity. So, that the calculation of flocculation activity referred to pH=7 is not reasonable enough to explain the pH effect on the flocculation ability of flocculant, especially for PAN. It includes two effect factors, one is the pH effect on sludge properties and the other is pH effect on interactions between flocculant and sludge.

![Figure 7.9 pH effect on supernatant turbidity of sludge](image)

When this was realized, another turbidity reduction experiment was done only with sludge in different pH and the sludge underwent the same procedure as turbidity reduction test. Figure 7.9 shows two replicate experiments. The turbidity of sludge supernatant change with pH adjusting, both of them have a large turbidity at low and high pH, neutral pH have smaller turbidity. Consequently, the PAN and chitosan flocculation result in figure 7.8 may be mainly induced by the pH effect on sludge change. One extra
experiment of adding different flocculant to the same sludge was done for pH=5, 6, 7, 8, and 9 (Figure 7.10).

MPE50 has turbidity reduction ability above 25% for all the tested pH range. The pH change will not affect the flocculation ability of MPE50 much. Chitosan do a good job at pH=5-8, which shows more than 45% flocculation ability. When the pH increases to 9, the flocculation activity of chitosan dramatically decreases to negative, which has a supernatant turbidity even higher than only sludge. The reason is chitosan is only dissolved in acidic environment, when pH increases, some of them will precipitate out. The precipitated chitosan lost their of flocculation/absorption activity. There is no turbidity removed by chitosan, but turbidity added by the precipitation. PAN result in Figure is that the flocculation ability of PAN is not affected by pH, but the ability of PAN reduce the turbidity is not very good though.

![Figure 7.10 Flocculation activity based on sludge with different pH](image-url)
7.4. Conclusions

It was also demonstrated that adding dissolved chitosan and MPE50 could help reduce the turbidity of the supernatant after allowing the sludge to settle for 45 minutes. The flocculation activity was up to 80% and 55% for chitosan and MPE50, respectively. Chitosan was efficient (more than 45% flocculation activity) in the pH range of 5-8, while MPE50 was efficient (more than 25% flocculation activity) in the wider range of pH 4-9. PAN nanofiber and crosslinked chitosan nanofiber showed low turbidity reduction ability at concentrations higher than 25 and 50 mg/L, respectively, after adjusting for the turbidity of nanofibers themselves. Note the concentration of MPE50 is based on the solution but not the effective polymers, a higher MPE50 concentration is recommended to try.
CHAPTER VIII

FILTRATION TEST

8.1. Summary

Filtration test could more comprehensively evaluate the flocculant performance under conditions similar to those in the continuous MBRs. The short-time filtration test, instead of the continuous MBRs, was used to eliminate the uncontrolled factors for higher reproducitivy. There are lot of advantages of doing short-time test instead of continuous MBRs, for instant, one short-time test can be done in 10hours, the microorganism population, the pH value, the DO level would not change too much from the beginning to the end of the test. Another significant advantage is that short-time test can be easily repeated than continuous MBRs. The filtration tests were conducted with all of the flocculants investigated. Since each test took one day, the test for control and each flocculant was repeated once on a different day to accommodate the inevitable differences of the sludge used in the tests.

Among the flocculants tested, adding dissolved chitosan and MPE50 improved the filtration performance by postponing the TMP increase and slowing down the decrease of permeates flux. PAN at a high concentration of 100 mg/L gave better filtration performance than at 50 mg/L. On the other hand, the glutaraldehyde-crosslinked chitosan nanofibers, prepared according to the procedure described in a previous chapter
(Chapter 6), did not yield appreciable improvement of the filtration performance. Nonetheless, the filtration test results were consistent with the results from the turbidity reduction test: The systems with better turbidity reduction had larger filtration improvement.

8.2. Summary of Experimental Protocols

8.2.1. Evaluation of Resistances

Darcy’s Law was used to describe the membrane filtration process:

\[
J = \frac{\Delta P}{\mu R_m}
\]

where \(J\) (m\(^3\) m\(^{-2}\) s\(^{-1}\)) is the permeate flux, \(\Delta P\) (Pa) the transmembrane pressure, \(\mu\) (Pa s) the permeate viscosity, and \(R_m\) (m\(^{-1}\)) the resistance of the membrane.

For suspension filtration, the flux decline is a result of increase in filtration resistance to the permeate flow, resulting from membrane fouling [13]. The resistance-in-series model modified from Darcy’s Law has been widely used to describe the membrane fouling phenomena in MBRs [13, 34, 46, 77]:

\[
J = \frac{\Delta p}{\mu(R_m + R_c + R_p)}
\]

\[R_t = R_m + R_c + R_p\]

\(R_t\) (m\(^{-1}\)) is the total resistance, \(R_m\) (m\(^{-1}\)) the resistance of the membrane, \(R_c\) (m\(^{-1}\)) the resistance due to cake layer, and \(R_p\) (m\(^{-1}\)) the resistance arising from blocking of membrane pores and adsorption of organic matters on the wall of membrane pores. All these resistance values can be estimated by the following equations:
\[ J_0 = \frac{\Delta P}{\mu R_m}, \quad J_r = \frac{\Delta p}{\mu(R_m + R_c + R_p)}, \quad J_{hd} = \frac{\Delta p}{\mu(R_m + R_p)}, \quad J_{ch} = \frac{\Delta p}{\mu(R_m' + R_{ir})}, \]

\[ R_m' = R_m + R_{ir}, \]

\( R_m' (m^{-1}) \) is the membrane resistance after the sample filtration test is also called irreversible fouling membrane resistance and \( R_{ir} (m^{-1}) \) is the resistance due to irreversible fouling.

### 8.2.2. Experimental Procedures

The filtration experiments were designed upon the way to evaluate the membrane performance described above. Before the filtration test, each membrane was soaked in deionized (DI) water in the refrigerator overnight, which was reported to help stabilize the membrane permeate flux and pressure during filtration [77]. The permeate flux in pure ware \( (J_0) \) was measured for each membrane to get the intrinsic membrane resistance \( (R_m) \). Then the filtration test was carried out with the activated sludge added with the flocculant under investigation at a specific concentration.

The filtration experiments were operated with multiple step-increases in the no-load pump rate of the pump used to draw permeate through membrane. It is usually used to determine the critical flux for the membrane filtration [77, 78]. At each step, the same pump rate was set for a same period of time, and then increased to next step at a fixed pace. Before the step-increase test, a fast biofilm buildup process was performed by using a very high flowrate, at 16 ml/min (flux = 32 LMH\(^1\)), for 1–2 hours, till the increase of

\(^1\) LMH: The unit of flux used in membrane filtration, Lm\(^2\)h\(^{-1}\) where, L is volume of permeate, m\(^2\) is the effective filtration area of membrane, and h is hour.
transmembrane pressure (TMP) verses time slowed significantly. Given the manufacturer recommended permeate flux of 0.25 m/day (10.4 LMH), the set no-load pump rate was step-wise increased from 2 ml/min (4 LMH) to about 16 ml/min (32 LMH) in 8 steps (i.e., 2, 4, 6, 8, 10, 12, 14, and 16 ml/min). The filtration was performed for 60 min for each step, and during the filtration, both the TMP change and actual filtrate flowrate were continuously monitored by means of a pressure transmitter installed in the permeate line.

After filtration, the membrane was rinsed by tap water to remove the visible cake layer and soaked in DI water for 2 hours to remove any possible labile particles from the surface of the membrane. Another pure water filtration was then performed on the membrane to evaluate the resistance after the hydraulic rinse, which is the resistance without cake layer. So the resistance of cake layer ($R_c$) can be calculated from the modified Darcy's law. The same membrane was next cleaned by back-flush of DI water for 30 min at the flowrate of 6 ml/min, with air bubbles scrubbing the membrane surface. The adsorbed matters were mostly cleaned up from the pores and membrane surface. After soaked in DI water for another 12 hours, the third pure water filtration test was conducted to evaluate the resistance ($R_p$) [77].

8.3. Results and Discussion

The filtration test was conducted twice for each candidate flocculant with the same concentration as that used in the study for the pH effect on turbidity reduction. For the same reason, the concentration chosen for each flocculant has the best turbidity reduction performance during the tested range. The MLSS concentrations in MBRs were typically higher than those in the conventional processes. To have more representative
MLSS for the filtration test, the daily wasted sludge from the SBR was batch cultured for 23 hours in a richer medium, as described below. The mixed liquor (1.2L) taken out of the SBR was first concentrated by removing 200 ml of the supernatant after 30 min of settling. The remaining mixed liquor was transferred into a 2-L flask and added with 200 ml of the medium whose composition was given in Table 8.1.

<table>
<thead>
<tr>
<th>Component</th>
<th>Media (g/L)</th>
<th>Component</th>
<th>Media (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH4Cl(g/L)</td>
<td>11.7</td>
<td>ZnSO4·7H2O (mg/L)</td>
<td>15</td>
</tr>
<tr>
<td>CaCl2·2H2O (mg/L)</td>
<td>300</td>
<td>K2HPO4 (mg/L)</td>
<td>600</td>
</tr>
<tr>
<td>FeCl3·6H2O (mg/L)</td>
<td>12</td>
<td>NaCH3COO (g/L)</td>
<td>25</td>
</tr>
<tr>
<td>MnCl·4H2O (mg/L)</td>
<td>9</td>
<td>Milk (g/L)</td>
<td>5</td>
</tr>
<tr>
<td>MgSO4·7H2O (mg/L)</td>
<td>450</td>
<td>Peptone (g/L)</td>
<td>1.8</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>39.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The medium was pumped into the flask at the rate of 1 ml/min, which had been tested to be slow enough to avoid serious foaming. The batch cultivation was carried out with an aeration rate of 2 L/min, through an air stone placed near the bottom of the flask, and with an agitation speed of 600 rpm, using a magnetic stirrer. The operation condition was confirmed to keep the mixed liquor well mixed and DO in the flask around 4~6 mg/L throughout the cultivation.

The sludge MLSS values for all the filtration tests were measured at both the beginning and the end of the filtration test. The average MLSS between the two values are listed in Table 8.2.
Two sample filtration test results are shown in Figures 8.1 and 8.2, where TMP and permeate flux are plotted against time, the other run data are listed in Appendix A.

<table>
<thead>
<tr>
<th>Run No.</th>
<th>404S</th>
<th>405S</th>
<th>406S</th>
<th>407MPE</th>
<th>408PAN</th>
<th>409CHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLSS(mg/L)</td>
<td>7333</td>
<td>6850</td>
<td>7120</td>
<td>7665</td>
<td>5100</td>
<td>7835</td>
</tr>
<tr>
<td>Run No.</td>
<td>410PAN</td>
<td>411PAN</td>
<td>414CHS</td>
<td>0508S</td>
<td>513PAN</td>
<td>516CHF</td>
</tr>
<tr>
<td>MLSS(mg/L)</td>
<td>6145</td>
<td>6675</td>
<td>6650</td>
<td>4910</td>
<td>4935</td>
<td>7665</td>
</tr>
<tr>
<td>Run No.</td>
<td>518CHFD</td>
<td>523S</td>
<td>525MPE</td>
<td>526CHF</td>
<td>527CHF</td>
<td></td>
</tr>
<tr>
<td>MLSS(mg/L)</td>
<td>6155</td>
<td>5055</td>
<td>5625</td>
<td>5400</td>
<td>5410</td>
<td></td>
</tr>
</tbody>
</table>

Note: The run number is composed of the test date (e.g. 404 correspond to April 04, 2005) and the flocculant abbreviation: S is for the control (sludge only) and the others are the same as those in Chapter 7.

Two sample filtration test results are shown in Figures 8.1 and 8.2, where TMP and permeate flux are plotted against time, the other run data are listed in Appendix A.
Figure 8.2 04/09 Filtration test with 50ppm dissolved chitosan adding to sludge

It can be seen from all these filtration test results that when the TMP began to increase, the permeate flux started to show dramatic decrease with time. During the one-hour operation period for each step, there is no TMP curve bending to plateau was observed and it clearly increasing as time going. At the first one or two TMP-increasing steps, after an initial period of faster TMP increase (TMP curve bending part) the TMP increased roughly linearly with time after the short time bending. The initial TMP curve bending maybe induced by the suddenly increase of effluent flowrate and the increased driving force compressed the biofilm on the membrane surface to increase the filtration resistance. The coming linear ship is presumably due to the increasing deposit of particles on the existent cake layer with the increasing flux. However, in the subsequent steps, the linear trend was not obvious anymore and the bending part becomes longer than the first step. As the filtration running longer, the thickness and rigidity of biofilm on the
membrane surface increases the compression period takes longer time. The chitosan solution, MPE50 and non-crosslinked chitosan nanofiber gave obviously and consistently better results compared to the control. These flocculants somehow postponed the onset of detectable TMP increase (and the associated decrease in permeate flux). On the other hand, adding PAN nanofiber and crosslinked chitosan nanofiber did not give consistent improvement in the filtration performance. It is hard to conclude only from the TMP and permeate flux figures shown in Figure 8.1, 8.2. A further analysis of the data is performed to gain more information from the results. No TMP was detected from any of the pure water filtration tests. The pressure transmitter used in the study could only detect pressure differences large than 0.5 kPa. The resistance of membrane $R_m$ and that potentially resulting from pore plugging $R_p$ were therefore very small compared to the resistance of cake layer. Only the total resistance $R_t$ was calculated for the filtration tests, and plotted in Figure 8.3 (a-d) versus the filtrate volume $V$.

Figure 8.3 Total resistance $R_t$ change with filtrate volume, a) control,
Figure 8.3 continued, b) chitosan (Including chitosan solution, non-crosslinked chitosan nanofiber and crosslinked chitosan nanofiber)

Figure 8.3 continued, d) PAN
Note that a slower \( R_t \) increase with \( V \) means a better, longer-lasting filtration process with less serious fouling. Figure 8.3 thus again indicated that MPE50 and dissolved chitosan improved the filtration performance over the control. However, the five control results shown in Figure 8.3 (a) varied considerably. The likely reason was that the sludge used on different days had different filtration properties despite our best effort to maintain the SBR at a pseudo-steady state and culture the wasted sludge with the same media under the same condition. As shown in Table 8.2, the average MLSS values of the sludge used in different runs were not the same.

A way to reduce the effects of these uncontrollable parameters was considered. As the rate of resistance increase with filtrate volume reflects the rate of membrane fouling, the average slope of the \( R_t \) vs. \( V \) curves in Figure 8.2 was taken to represent the fouling rate in a filtration test. Three parameters, i.e., MLSS, concentration of dissolved
organics in mixed liquor, and permeate viscosity, were then evaluated for their effects on membrane fouling, as given in the following equation:

\[
FoulingRate(F.R.) = \frac{dR_t}{dV} = k(MLSS)^a (VS - MLVSS)^b \mu^c
\]

where \((VS-MLVSS)\) is used to reflect the concentration of dissolved organics (mg/L), \(\mu\) is viscosity (Pa·s), and \(k\), \(a\), \(b\), and \(c\) are empirical correlation constants. The fouling rate (FR) was calculated using the results from the first step with detectable TMP increase, since the relationship of the \(R_t\) vs. \(V\) was liner in this step.

Figure 8.4 Fouling rate versus the potential factors

The calculated F.R. values are plotted separately against the three potential factors in Figure 8.4 for the five control tests. From Figure 8.4, the fouling rate changed randomly with either MLSS or viscosity but appeared to increase with increasing concentration of dissolve organics. The correlation was therefore simplified to include only \((VS-MLVSS)\), as in the following equation:
Fitting the control data with this model, we get the value of the two empirical constants: $k = 436.3$ and $b = 1.30$, with $R^2 = 0.77$. As a comparison, we also used the following exponential model to fit the data, and get $m = 3.91 \times 10^5$ and $k = 0.0023$, with $R^2 = 0.86$.

\[
\text{Fouling Rate (F.R.)} = \frac{dR_t}{dV} = k(V_S - MLVSS)^b
\]

\[
\text{Fouling Rate (F.R.)} = \frac{dR_t}{dV} = m \exp(k(V_S - MLVSS))
\]

![Figure 8.5 Data fitting of the control test results](image)

The exponential model showed a better fit than the power-law model. Hence, the exponential model was chosen to evaluate the other experimental result. After plotting the fouling rate of each system versus dissolved organic concentration (Figure 8.6), we can tell whether adding flocculant would improve the filtration performance or not. Table 8.3 list the data plotted in Figure 8.6.
There are three points above the control line, 0408PAN 50ppm, 0516CHF 50ppm, 0527CHF 100ppm. One point is on the line, 0407MPE50 100ppm. They other results are all below the line. Comparing the Rt–V lines in Figures8.3, which doesn’t consider the dissolved organic effect. R_t of 0527CHF 100ppm is larger than most of the control Rt with the same V, which means worse performance than the control sludge. R_t of 0408PAN 50ppm and 0516CHF 50ppm lies in the middle of control results. The conclusions drawn from these two ways for 0407MPE50 100ppm performance were
contradicted with each other. From Figure 8.3, we can see that the performance of 0407MPE50 100ppm is nearly the best run among all these runs.

The point far way below is suppose to have better filtration performance. These are 0414CHS 50ppm, 0518CHFD 100ppm, 0525MPE 100ppm, and 0409CHS 50ppm. It is agree with Figure 8.1, 8.2 and 8.3 that the results of these four runs are obvious better than control sludge. Comparing the results of chitosan nanofiber with PAN, system with PAN shows a little better filtration performance than with chitosan nanofiber. The two runs with 100ppm PAN all give better result than controls. There is only one run of 100ppm chitosan fiber gives a weakly good performance.

The average dissolved organic value for all these tests are 670mg/L, then all the control values were calculated into the same dissolved organic concentration by the exponential model (Figure 8.7). The curved values at 670mg/L have a very similar F.R. range than the original F.R., which is dramatically different with each other.

![Figure 8.7 Curved control F.R. at Disso. Org. =670mg/L](image-url)
Figuer8.8 Curved F.R. for all filtration tests at Disso. Org. =670mg/L

The same method used to curve the flocculant system F.R. based on the same dissolved organic concentration 670mg/L, plotting them together with the average curved control F.R. value as reference (Figure8.8). Chitosan solution and non-c-L chitosan fiber both get a very low F.R. value compared to the control result, adding dissolved chitosan can improve the filtration performance. The F.R. of two MPE50 test are both smaller than control, we can say that MPE50 will better the filtration process. Chitosan fiber didn’t show a consistent better result, but 100ppm has a lower Fouling rate than 50ppm. The four F.R. value of PAN system were not compatible with each other, from the average, adding 100ppm was better than 50ppm. Considering the nanofiber Fouling Rate together, it reveals that the higher nanofiber concentration have a preferable result. The nanofiber concentration we tested maybe not enough to exhibit its structure modification ability. The chitosan nanofiber didn’t obtain a good result as expected. The reasons may be as following.
The non-crosslinked chitosan nanofiber has a compatible turbidity reduction test and filtration performance as the chitosan solution, so that the electrospinning process would not influent the chitosan flocculation ability. Then the procedure of crosslinking may cause the poor results of chitosan nanofiber both in turbidity reduction test and filtration test.

8.4. Conclusion

Dissolved chitosan and MPE50 contribute to the filtration process with a postponed TMP increase and smaller permeate flux decrease. They also have a small R_t and fouling rate. PAN results are not consistent with each other, but seem to improve the filtration performance with 100ppm concentration. The filtration test result is well related to the turbidity reduction test. The one with better turbidity reduction will also get better filtration performance improvement.

The developed insoluble chitosan nanofiber didn’t present obvious contribution to the turbidity reduction and filtration as dissolve chitosan did. Because the cross linking degree was not measured, the reason maybe the cross linking reaction occupied too many amino groups, which are the active flocculation sites to bond with sludge floc and EPS. The fibers still with a lot amino groups are dissolved into water during the washing and dialysis procedure. There is another possibility. Since the crosslinking reaction took place from the outside of the nanofibers, the easy reach amino groups were mostly been reacted with glutaraldehyde and loose the flocculation ability. Even there are still amino groups on the fibers, maybe they are all hiding inside, and the flocs are hard to get into it.
CHAPTER IX

SUMMARY

9.1. Conclusions

A laboratory-scale continuous running membrane bioreactor (MBR) was designed and operated. The performance of MBR was evaluated from nutrient removal, transmembrane pressure drop (TMP) and permeate flux changes. A procedure was developed to prepare glutaraldehyde-crosslinked chitosan nanofiber, which is insoluble but well dispersible in water and works as flocculant and sludge floc modifier. The insoluble nanofibers do not leave the system with membrane permeate. This property not only minimizes the costs due to flocculant loss but also eliminates the introduction of additional chemicals into the receiving water environments. The performance of four types of flocculants, soluble chitosan, crosslinked chitosan nanofiber, PAN nanofiber, and soluble MPE50 (a commercial flocculant from Nalco Company, Naperville, Illinois) was compared according to the toxicity/inhibition test, turbidity reduction test, and short-time filtration test.

Toxicity/inhibition tests were carried out with two culture media concentrations. There was no obvious inhibition to the growth of microorganisms by addition of 50~100 mg/L tested flocculants. It was also demonstrated that adding dissolved chitosan and MPE50 could help reduce the turbidity of the supernatant after allowing the sludge to
settle for 45 minutes. The flocculation activity was up by 80% and 55% for chitosan and MPE50, respectively. Chitosan was effective in the pH range of 5-8, while MPE50 was effective in the wider range of 4-9. PAN nanofiber and crosslinked chitosan nanofiber showed low turbidity reduction ability at concentrations higher than 25 and 50 mg/L, respectively, after adjusting for the turbidity of nanofibers themselves. Possible explanations were given.

Step-increase filtration test operating under constant flux mode was carried out for all flocculant systems. The TMP and permeate flowrate were monitored online. When the TMP started increase, the permeate flux decreasing were observed for all the filtration system. Total filtration resistance $R_t$ and membrane fouling rate were calculated from the short-term filtration tests, which have been used to evaluate the performance of flocculant. Both dissolved chitosan and MPE50 could improve the filtration performance with lower transmembrane pressure (TMP) and higher permeate flux. However, the performance of PAN and crosslinked chitosan nanofibers was not very consistent from replicate experiments. The idea of using nanofiber as the biofilm structure modifier was not proved an efficient way to contribute the membrane filtration. It is demonstrated that adding nanofiber would not do harm to the filtration system. The crosslinked chitosan did not improve the filtration process as expected the possible reasons are addressed. One reason is that the crosslinking reaction occupies too many active site of chitosan; the other is that the amount of chitosan nanofiber used for filtration test is too small to change the sludge floc properties.
9.2. Future Research

The future work to extend the results and knowledge from this study includes:

1. To modify the chitosan nanofiber by crosslinking the hydroxyl group, and keep amino groups free, or mix chitosan with other material, which make chitosan insoluble in water.

2. To try one-step filtration test, develop mathematical models to get rid of the sludge effect and predict the performance of the flocculant.

3. To measure the sludge floc size and size distribution change after adding flocculant.

4. To measure the water soluble EPS and sludge extracted EPS concentration of the mixed liquor.

5. To increase the nanofiber concentration for filtration test, to investigate the sludge floc property changes.

6. To try other materials in nanofiber structure, like polyethylene imine (PEI) and polyacrylic acid.
REFERENCES


63. Website (2005).


Overall results of short time filtration test

04/04 Control filtration test only with sludge
04/05 Control filtration test only with sludge

04/06 Control filtration test only with sludge
04/07 Filtration test with 100ppm MPE50 adding to sludge

04/08 Filtration test with 50ppm PAN adding to sludge
04/09 Filtration test with 50ppm dissolved chitosan adding to sludge

04/10 Filtration test with 50ppm PAN adding to sludge
04/11 Filtration test with 100ppm PAN adding to sludge

04/14 Filtration test with 50ppm dissolved chitosan adding to sludge
05/08 Control filtration test only with sludge

05/13 Filtration test with 100ppm PAN adding to sludge
05/16 Filtration test with 50ppm cross linked chitosan nanofiber adding to sludge

05/18 Filtration test with 100ppm non-cross linked chitosan nanofiber adding to sludge
05/23 Control filtration test only with sludge

05/25 Filtration test with 100ppm MPE50 adding to sludge
05/26 Filtration test with 100ppm cross linked chitosan nanofiber adding to sludge

05/27 Filtration test with 100ppm cross linked chitosan nanofiber adding to sludge