POLYMERIC SUBSTANCES AND MICROBIAL COMMUNITIES IN SOURCE WATER AND IN BIOFILTRATION PROCESSES IN THE TREATMENT FOR DRINKING WATER

by

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Abstract

The application of membrane filtration for drinking water treatment is limited by membrane fouling due to the accumulation of biopolymers. Biofiltration may be an effective pretreatment approach to reduce the biopolymers, thus improving the performance of membrane filtration. Biological treatment with the microbial community associated with drinking water treatment systems can potentially play a positive role on chemical contaminant removal and biopolymer reduction through biodegradation. However, extracellular polymeric substances (EPS) developed inside biofilters through microbial secretions may have adverse effects on downstream membrane filtration if an EPS sloughing event occurs. In this study, the efficacy of biofiltration to remove biopolymers was investigated. Methods developed for the analyses of EPS associated with microbial flocs and biofilms in wastewater were adapted to analyze surface waters where biopolymers are typically found at lower concentrations.

Freeze-drying was found to be an effective method for concentrating water samples and to recover and analyze neutral and acidic polysaccharides (PS), but was ineffective for protein (PN) quantification. EPS extracted from the biofilter media was analyzed as part of the characterization of the microbial community associated with biofiltration. Results of water samples suggest that a passive biofilter was not effective for PS removal at the Peterborough drinking water pilot plant (DWPP). However, coupled with a roughing filter, a consistent removal of PS was observed in the passive biofilter at the Mannheim DWPP. Nutrient addition, hydrogen peroxide supplementation, inline coagulation and GAC were found to have impacts on PS removal and total EPS. Transparent exopolymer particles (TEP) are a group of acidic PS, potentially playing an important role in membrane fouling. A significant reduction (over 20%) of TEP was observed in the passive biofilter at the Mannheim DWPP, suggesting the positive role of biofilter in removing TEP. Subsequently, microbial community analysis using denaturing gradient gel electrophoresis (DGGE) and 16S rRNA sequencing have been undertaken to understand the microbial stability of biofiltration system by studying the microbial community association between source water and biofilter systems. High similarities of microbial communities (30-65%) based on DGGE analysis were found between source waters and biofilters, suggesting microbial community shift inside biofilters are due to the changes in the microbial community of source water. Biofilters do not appear to develop into stable compartmentalized communities. It appears these are subject to fluctuation or shifts linked to the conditions of the source water. Therefore, fluctuation of biofilter performance may be expected.

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List of Abbreviations

AOC –Assimilable organic carbon

ATP –Adenosine triphosphate

BDOC –Biodegradable organic carbon

BSA –Bovine serum albumin

CER –Cation exchange resin

COD –Chemical oxygen demand

DGGE –Denaturing gradient gel electrophoresis

DOC –Dissolved organic carbon

DOM –Dissolve organic matter

DWPP –Drinking Water Pilot Plant

EPS –Extracellular polymeric substances

FEEM –Fluorescence excitation–emission matrix

GA –Gluocoronic acid

GAC –Granular activated carbon

HA –Humic acid

HS –Humic substances

LC-OCD -Liquid chromatography-organic carbon detection

MF –Microfiltration

MW–Molecular weight

NF –Nanofiltration

NOM –Natural organic matter

PCR –Polymeric chain reaction

PS –Polysaccharides

PN –Proteins

POM –Particulate organic matter

TEP –Transparent Exopolymer Particles

TOC –Total organic carbon

RW –Raw water

RO –Reverse osmosis

UF –Ultrafiltration

XG –Xanthan gum

Chapter 1 Introduction

1.1 Background

Flemming et al. (1997) introduced a perspective that biofouling during membrane filtration is a result of the bioreactor in the wrong place (on the membrane surface). Biofouling could be reduced by having a bioreactor in the right place where biofilm growth is encouraged to remove the nutrient content in the influents. Hence, biofilm formation at an undesired location could be restricted. Biofiltration can be such a bioreactor where microbial communities produce biofilms on a support filter media and sequester the nutrients from the water-phase through biodegradation. As such, biofiltration is often used as such a pretreatment that helps minimize membrane fouling by reducing organic matter that either contributes directly to membrane fouling or provides the carbon source for the development of biofilm on the membrane surface (Wend et al., 2003). Compared to other pretreatments for membrane fouling control, biofiltration has been recognized as a high-performance, cost-effective, and (can be) chemical-free (when operated as passive biofiltration) drinking water pretreatment method (Hallé et al., 2009).

High effectiveness of fouling control by biofiltration has been observed by a number of researchers (e.g. Wend et al., 2003; Hallé et al., 2009). For example, fouling thickness was reduced by half (from 21.8 µm to 10.5 µm) in the presence of a biofilter using granular activated carbon (GAC) as filter media (Wend et al., 2003). When a reverse osmosis (RO) system was operated without biofiltration, it only took 72 hours to have a significant impact on performance of RO membrane. With the presence of biofilter, the operation time extended to more than 300 hours (Hu et al., 2005).

Extracellular polymeric substances (EPS) play an important role during biofiltration process. They mainly consist of high molecular weight (MW) organic substances produced through microbial excretions (Tsuneda et al., 2003). Other processes that lead to the extracellular localization of these polymeric substances includes the shedding of the cell surface materials, cell lysis, and adsorption from the environment (Wingender et al., 1999). EPS are composed of a variety of organic substances, mainly polysaccharides (PS) and proteins (PN), although lipids, humic substances (HS), nucleic acids and other inorganic constituents may also be present (Frølund et al., 1996). EPS bind the microbes together in a three dimensional-matrix, which leads to a change in physicochemical characteristics of microbial aggregates in terms of mass transfer, adsorption ability, and stability (Nguyen et al., 2012). From an ecological perspective, EPS are the construction materials of biofilm that allows microorganisms to form stable aggregates leading to the development of a synergistic microbial community, facilitating the sequential degradation of organic substances that are not readily biodegradable by single species of microorganisms (Wingender et al., 1999).

EPS can play multiple roles in biological systems due to the abundance of the charged groups present (e.g., carboxyl, phosphoric, phenolic and hydroxyl groups) and nonpolar groups (e.g., aromatic and aliphatic groups in PN, and hydrophobic regions in PS) (Nguyen et al., 2012). Therefore, EPS have wetting and cross-linking capabilities as EPS contain many hydrophilic and hydrophobic sites in their structure that enable them to adhere to both hydrophilic and hydrophobic surfaces. The hydrophobic components of EPS promote selective adsorption of organic matter from water. Therefore, under low nutrient conditions, the presence of EPS serves as a competitive advantage as they help sequester the nutrients from the environment to support bacterial growth (Decho, 1990). Based on this mechanism, even a low concentration of biopolymers present in the water can be removed, and a membrane fouling reduction can be expected through biofiltration.

Statements of Problems

EPS facilitate cell aggregation and biofilm adhesion to supporting media, thus promoting biodegradation. However, EPS can be problematic for downstream membrane filtration when microorganisms produce excess amounts of EPS inside a biofilter and causes EPS sloughing off. EPS that sloughs from the biofiltration system and is introduced into membrane filtration through feedwater will accumulate on membrane and facilitate undesirable microbial colonization, leading to biofouling. Biofouling potential of EPS is significantly higher than some of natural organic matter (NOM) (Fonseca et al., 2007). As the EPS make contact with the membrane, they form a gel layer by cross-linking with the membrane surface (Nguyen et al., 2012). The binding of EPS to the membrane will become stronger in time due to the flexibility and cross-linking features of EPS. In addition, during membrane cleaning EPS can act as a diffusion barrier effectively slowing the transport of some antimicrobial agents and protecting the microorganisms inside biofilms (Nguyen et al., 2012).

1.2 Objectives

The aim of this study was to investigate biofiltration as an approach for membrane fouling control. From an engineering perspective, it is very important to determine the effectiveness of this pretreatment in terms of the removal of organic matter responsible for membrane fouling. The performance of the biofilter is strongly related to the dynamic of the EPS. Therefore, the characterization of biofilter-associated EPS is also of strong interest. Due to the biological nature of a biofilter, it is essential to understand the microbiological aspects associated with the biofiltration system. This understanding could be obtained through microbial community analysis through denature gel gradient electrophoresis (DGGE) analysis to investigate the microbial compositions.

To summarize, the first objective of this study was to evaluate the removal efficiency of biofiltration for membrane fouling control by quantifying and comparing the amounts of organic substances (PN and PS) present in raw water (RW) and biofilter effluent samples. The second task was to characterize the EPS of biofilters. The final objective is to identify the microorganisms present and determine the microbial community composition of the RW, biofilter effluent and biofilter biofilms.

1.3 Thesis Outline

The thesis includes a review of literature related to this study (Chapter 2). It provides an introduction to membrane filtration technologies and their applications in drinking water treatment, wastewater reclamation and seawater desalination for water crisis alleviation. This chapter also introduced membrane fouling, the biggest disadvantage of membrane filtration. Membrane foulants are identified with an emphasis on their potential roles and relative importance in membrane fouling. Different analytical and quantification techniques of biopolymers are discussed and compared. Additionally, two other common pretreatment methods for membrane fouling reduction are reviewed, including coagulation, ozonation.

Experimental methods are described in Chapter 3 with details on the sources of biofilter feedwater, effluent and filter media samples, and descriptions of multiple biofiltration systems operated under different conditions at two different drinking water pilot plants (DWPPs) are provided. The experimental approaches applied in this study and the chemical and microbial techniques used are also described. In Chapter 4, the results obtained through chemical analyses and microbial analyses are presented. Chapter 5 contains a discussion of the results with comparisons to other biofiltration studies. Chapter 6 summarizes the conclusions of this study and the engineering significance of this research. Chapter 7 provides recommendations for future research.

Chapter 2 Literature Review

2.1 Membrane Technologies Employed in Water and Wastewater Applications

Membranes employed in water and wastewater treatment are porous or nonporous water permeable polymeric films or ceramic matrices that are designed to create a barrier to retain contaminants primarily through size exclusion (Huang et al., 2009). During filtration processes, pressure difference between the feed water and permeate side is used as the driving force to transport water through the membrane. Pressure-driven membrane processes can be classified by three main criteria: the pore size of the membrane, size of the retained particles or molecules, and pressure applied on the membrane (Van der Bruggen et al., 2003). These differentiate microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO).

Table 2-1 Properties of pressure-driven membrane processes (Van der Bruggen et al., 2003)

	MF[a]	UF[b]	NF[c]	RO[d]
Permeability (1/h.m^2.bar)	>1,000	10-1,000	1.5-30	0.05-1.5
Pressure (bar)	0.1-2	0.1-5	3-20	5-120
Pore size (nm)	100-10,000	2-100	0.5-2	<0.5
Rejection	Particles	Particles and macromolecules	Small organic compounds and multivalent ions	Multivalent ions and monovalent ions
Separation mechanism	Sieving	Sieving	Sieving, Charge effects	Solution-Diffusion

[a]Microfiltration; [b]ultrafiltration; [c]nanofiltration; [d]reverse osmosis

Compared to the other three membranes, MF membranes have the largest pores with the highest permeability so that a sufficient water flux can be obtained at a relatively low pressure. During MF process, substances bigger than the pore size are removed by a sieving mechanism (Van der Bruggen et al., 2003). This process is suitable for the removal of suspended solids (Drexler and Yeh, 2014). UF membranes have smaller pores, and the permeability is considerably lower than in MF, thus requiring higher pressure. UF is usually applied to remove viruses and large dissolved molecules that constitute the largest molecules of NOM (Van der Bruggen et al., 2003). NF membranes have pore sizes equivalent to the dissolved compounds with MW of about 300 g/mol. They are used to remove small organics, including organic micropollutants and color from surface water or groundwater, as well as degradation products from biologically treated effluents. Additionally, NF membranes have a net negative surface charge as the membranes contain ionizeable groups, such as carboxylic or sulfonic acid groups. Therefore, some ions can be removed by NF based on the mechanism characterized by the electric potential between charged membrane and feed solution (Van der Bruggen et al., 2003). RO membranes are dense membranes without predefined pores. Hence, the permeation is slower and the rejection through the RO membranes is not the result of sieving. Instead, the retention of solutes is due to solution-diffusion mechanism. RO membranes require high pressure, consequently higher energy consumption (Van der Bruggen et al., 2003).

Membrane filtration processes can also be classified according to different operating conditions. Based on the applied trans-membrane pressure, membranes can be broadly divided into two classes: high pressure driven membranes and low pressure driven membranes (Huang et al., 2009). Low pressure driven membrane processes (LPMs) are typically operated at a pressure less than 1-2 bar. LPMs include MF and "loose" UF membranes (pore size larger than 10nm), which are commonly applied in water and wastewater treatments. With pore size ranging from 10 to 100 nm, LPMs are effective in eliminating turbidity and pathogens but not effective for removing disinfection by-products and organic micropollutants.

Using pressure-driven processes, untreated water is processed either through dead-end filtration or cross-flow filtration, which are the two most frequently used operations in industry (Drexler and Yeh, 2014). Typical flow patterns during dead-end and cross-flow filtration are illustrated in Figure 2-1.

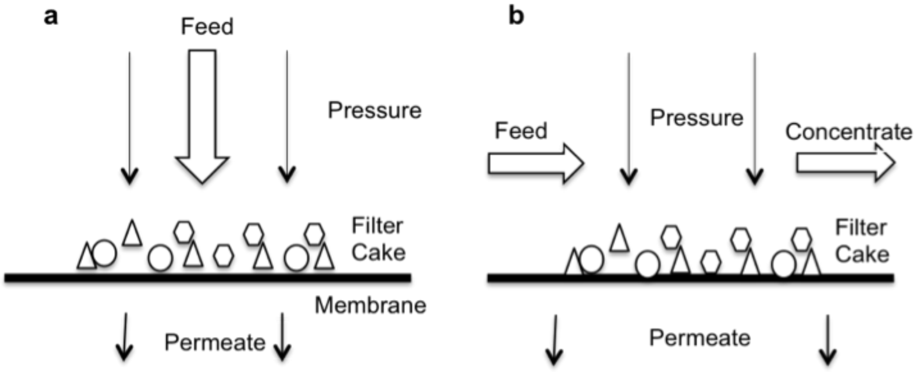

Figure 2-1 Schematic of (a) dead-end filtration and (b) cross-flow filtration, modified from: Drexler and Yeh (2014)

During dead-end filtration process, water flows perpendicular to the membrane, where substances with sizes larger than membrane pores accumulate on the membrane surface. During dead-end filtration, all the energy applied is transmitted to the membrane forcing the water across the membrane. Dead-end filtration has an appealing feature that energy loss during the process is less than the application of cross-flow filtration. However, the disadvantage is that this filtration mode is prone to membrane fouling, and consequently results in greater resistance for water to pass through (Liang et al., 2014). Therefore, dead-end filtration requires frequent backwash to clean the surface and restore the filtration capability. When membrane cleaning is performed (physically, chemically or hydraulically), the membrane module is temporarily out of service; hence, dead-end filtration is a considered as a discontinuous process. Therefore, membrane fouling control is of vital importance for dead-end filtration to extend the filtration time and to minimize recovery cleaning or maintenance. In practice, dead-end filtration is often applied to source water with low concentrations of particles, such as in the drinking water industry.

On the other hand, cross-flow filtration (also known as tangential-flow filtration) features the parallel flow of feed water to membrane. Compared to dead-end filtration, cross-flow produces higher flux as a parallel flow pattern creates shear force restricting the growth of foulants. The majority of the solutes end up flowing with the concentrate instead of accumulating on the membrane. In addition, the filtration efficiency of this filtration mode is relatively low as the largest part of feed water will leave the module and be recycled, and only small part is used for permeate production. Consequently, cross-flow operation has a higher energy cost.

2.2 Membrane Applications in Drinking Water Treatment

Millions of preventable deaths in the world, mostly in developing countries, are caused by waterborne pathogens that come from polluted drinking water (Gao et al., 2011). Membrane filtration systems are an approach to eliminate the risk of drinking water-associated diseases or deaths by removing the microbial contaminants from the drinking water. The application of LMPs in drinking water treatment has been drastically increased in the past decade mainly due to their high level of pathogen removal (Guo et al., 2010). Compared to conventional surface water treatment system, which consists of coagulation, flocculation, sedimentation, filtration, and chlorination, LMPs present many advantages, including high quality water production, decrease reliance on chemical consumption during treatment process, smaller footprint, lower energy consumption, and the capability of handling wide fluctuations in feed quality.

Among different membrane technologies, UF has been increasingly accepted as a primary option for drinking water treatment as UF membrane has higher selectivity when compared to MF and lower filtration energy consumption when compared with NF and RO (Guo et al., 2010). With the decreasing cost of UF membrane, this process has also become affordable and gradually accepted by developing countries (Gao et al., 2011).

2.3 Membrane Applications in Desalination & Wastewater Reclamation

With the climate change and population growth, water scarcity has become a worldwide concern. It has been projected that about over 1 billion people are living without clean drinking water, and about 2.3 billion people are living in the regions with water shortage (Service, 2006). In an attempt to reduce the pressure from increasing demand of potable water, different strategies are being investigated, and great effort has been put into exploitation of water supplies.

Wastewater reuse represents a promising solution, where highly treated effluent are discharged directly into groundwater or surface water with the purpose of augmenting drink water supplies (Rodriguez et al., 2009). The reclamation of wastewater can serve as a shortcut within the natural water cycle, and allow for a more rapid delivery system to increase the availability of drinking water. Constant water production can also be expected, as its source water is not dependent on precipitation (Wintgens et al., 2005). Saline water, 96.5% of the water on this planet, is another important source of potable water supplies. Seawater desalination presents an alternative for irrigation, industrial and municipal use, and an important source for drinking water production (Greenlee et al, 2009). The first countries using desalination on a large scale for municipal drinking water production were in the Middle East, whereas the first practices of wastewater recycling for potable water supply was observed in a full-scale wastewater reclamation plant in Namibia.

Desalination is a general term for a treatment process removing salt from water to produce fresh water that is defined as containing less than 1000 mg/L of salts or total dissolved solids (Greenlee et al, 2009). Above the defined concentration, properties of the water including taste, color, corrosion propensity and odor can be adversely affected. For seawater desalination, RO, NF, and electrodialysis are the three available membrane processes. Electrodialysis relies on an electric current that causes ions to move through parallel membranes, and is typically only applied for brackish water desalination (Reahl, 2006). Research has shown that NF is not able to desalt seawater to meet drinking water standards (Greenlee et al, 2009). However, NF membrane has been coupled with RO to treat seawater, where NF membrane functions as a barrier for divalent ions, including calcium and magnesium, as well as dissolved organic substances. On the other hand, RO is able to reject monovalent ions such as sodium and chloride. Typical RO membranes are able to remove more than 99% of salt, where some membranes has shown as high as 99.7%-99.8% salt rejection when operated under standard test conditions (Greenlee et al, 2009). RO membrane filtration has expanded to approximately 44% of global desalting production, and an 80% share in the total number of desalination plants installed worldwide due to the decrease of costs and improvement of materials (Greenlee et al, 2009).

The most common concern for the reuse of wastewater is the high concentration of potentially pathogenic microorganisms frequently present in secondary wastewater effluents.

Incorporating membrane filtration into water reuse schemes provides a strong barrier for all the microorganisms and viruses (Wintgens et al., 2005). Membrane processes are considered as key elements of advanced wastewater reclamation and reuse systems and have already been implemented into several full-scale reclamation plants including the most well-known, New Goreangab Water Reclamation Plant, located in the capital of Namibia, one of the most arid countries in the world (Lahnsteiner and Lempert, 2007).

In the Old Goreangab Water Reclamation Plant, dam water is treated by a conventional treatment process that consists of flocculation, dissolved air flotation, rapid sand filtration, granular activated carbon filtration and chlorine disinfection. Treated water from the old plant is not biological safe and sustainable for human consumption, and it is only used for irrigation purpose (mainly for recreational fields) (Lahnsteiner and Lempert, 2007). The insufficient removal of chemical and microbial contaminants from wastewater using conventional treatment indicates that more advanced treatments are required for wastewater reuse (Ho et al., 2011). In order to address the problem and meet the potable water demand, the New Goreangab Water Reclamation Plant, was designed and constructed. Secondary effluent is treated in the following processes including pre-ozonation, coagulation & flocculation, dissolved air flotation, dual media filtration, main ozonation, activated carbon filtration, ultrafiltration and chlorination. After initiation of the new reclamation plant in December 2002, 24,000 m^3/day of reclaimed water was produced, making up an average of 25% of the total potable water in the city. The amount of reclaimed water reaches a maximum of 50% of the water available during periods of water scarcity (Lahnsteiner and Lempert, 2007).

2.4 Membrane Fouling and Associated Foulants

2.4.1 Membrane Fouling Associated with Water Treatment Applications

Although membrane filtration has many superior and attractive features, fouling issues that arise during long-term operations restrict its widespread application (Zularisam et al., 2006). During the filtration process, inorganics, organics and microorganisms in the influent/feed water are rejected to a variable but generally significant degree (> 99%), and accumulate on the membrane. This clogs the membranes and results in the long-term flux decline of the system. This phenomenon is known as membrane fouling (Wiesner and Apte, 1996).

Membrane fouling can be further divided into two types: physically reversible and physically irreversible fouling. Reversible fouling can be depicted as the deposition of retained substances on the membrane surface that exists as a gel-like cake layer, whereas irreversible fouling refers to the adsorption of pore clogging solutes building up within the membrane matrix (Zularisam et al., 2006). After a certain amount of filtration time, foulants accumulated on the membrane start to cause a severe reduction of membrane permeability, and cleaning of membrane is required to restore its filtering capability. Physically reversible fouling caused by particulate and colloidal foulants without affinity to the membrane surface can be eliminated by physically cleaning, including backwashing, hydraulically flushing and scouring with air bubbles. Foulants with affinity to the membrane surfaces leading to physically irreversible fouling, need to be removed through chemical cleaning, often referred to recovery cleaning when comparing with operational strategies including backwashing etc. Recovery cleaning involves reaction between chemicals and foulants to mitigate the affinities of foulants to the membrane surface. Some colloidal organic substances possess strong affinities to the membrane regardless of solution chemistry, making them particularly crucial to irreversible fouling (Huang et al., 2009). Recovery cleaning is disadvantageous for several reasons (i.e. increased production downtime, energy usage, chemical consumption and waste generation) but, most importantly, it can deteriorate the membrane material reducing the lifetime of the membrane (Kennedy et al., 2008).

2.4.2 Membrane Foulants

In surface water treatment, foulants include particulate and dissolved organic and inorganic substances, as well as microorganisms and microbial products (Zularisam et al., 2006). They are collectively defined as a group known as NOM. NOM fouling is attributed to large particles that are rejected by the membrane, and accumulating on the membrane surface to form a cake or gel layer, and the adsorption of non-retainable substances in the inner pores of the membrane (Van Geluwe et al., 2011). Based on size, NOM can be categorized as particulate organic matter (POM, size >0.45μm) and dissolved organic matter (DOM, size < 0.45μm). Reversible fouling is caused by the accumulation of POM whose sizes are larger than membrane pore size (Chang et al., 2002). On the other hand, DOM causes more detrimental effects on membrane performance as this material is able to diffuse through the pores, and accumulate within the membrane causing irreversible fouling (Zularisam et al., 2006).

Specific organic substances have been identified as important membrane foulants (Hallé et al., 2009). Early investigations suggested that hydrophobic fractions of NOM (i.e. humic substances) were more responsible for membrane clogging. Intermolecular hydrophobic interations (Yuan and Zydney, 1999) and electrostatic interactions (Li and Elimelech, 2004) are the two mechanisms causing the binding between hydrophobic NOM and membranes. More recent studies have revealed hydrophilic NOM (carbohydrate or protein) to be the major foulants

(Hallé et al., 2009). Hallé et al. (2009) reported that humic substances were found to be irrelevant to either reversible or irreversible fouling, whereas protein was reported to be highly related to irreversible fouling of polymeric UF membrane under normal operating conditions. In a study of a long term UF membrane operation with surface water, it was also observed that protein-like substances were strongly correlated to irreversible fouling (Peldszus et al., 2011). However, the role of protein in irreversible fouling and the exact mechanism still remain unknown in the membrane filtration of surface water. On the other hand, PN were found to have a poor correlation with reversible fouling. Therefore, Peldszus et al. (2011) suggested that proteins played a different role in reversible and irreversible fouling in membrane filtration of surface water. In a long-term membrane filtration study at a drinking water facility, Kimura et al. (2004) using Fourier-transformed infrared spectra analysis found that polysaccharides played an important role in irreversible fouling. Yamamura et al. (2007) reported that carbohydrates were the dominant membrane foulant regardless of the type of the membrane. A two-step fouling proposed by Yamamura et al. (2007) suggests that hydrophobic (humic-like) substances attach to the membrane and narrow the pore size of the membrane and facilitate the hydrophilic (carbohydrate-like) compounds to further absorb to membrane surface.

With regard to PS, transparent exopolymer particles (TEP) are a class of organic particles that are abundantly present in oceans as well as in freshwater, wastewater and groundwater systems, which have been overlooked for many years in membrane industry due to their transparent nature (Kennedy et al., 2009; Berman and Parparova, 2010; Discart et al., 2013). Being unveiled by staining with alcian blue, TEP are deformable, gel-like, sticky particles of acid mucopolysaccharides from phytoplankton and bacterioplankton. They are suspended in the aquatic environments in different forms, with increasing size of aggregates from particles to gel webs to gel matrix (Valladares Linares et al., 2012). They are often divided into two categories based on their sizes: particulate TEP (> 0.4 µm) and colloidal TEP (0.05-0.4 µm) (Kennedy et al., 2009).

Berman and Holenberg (2005) suggested a link between TEP and membrane fouling during filtration processes. Their concept led to an increased focus on TEP to reveal its roles in diverse filtration systems, including reverse osmosis, ultrafiltration, and membrane bioreactors. TEP are suspected to induce biofouling on surfaces by attaching to the membrane surface, and subsequently serving as attachment sites and as a nutrient for microbial growth (Berman and Holenberg, 2005). Berman et al. (2011) stated that early deposition of EPS on membranes only originated from TEP in the feed water instead of being excreted by the active bacteria developing a biofilm. In all the membrane systems, TEP was suggested playing a role in membrane fouling. Therefore, TEP could be one of the important parameters to evaluate pretreatment performance for membrane fouling control. According to the latest study evaluating different methods for TEP quantification (Discart et al., 2014), the accurate measurement of the particles still remains as a major challenge associated with TEP-related research, due to their dynamic microgel properties with highly changeable sizes and shapes under subtle shift of physical or chemical conditions.

2.4.3 NOM Quantification

As popular pretreatment methods, coagulation, ozonation and biofiltration have been reported for their capabilities to improve membrane performance by removing various amounts of NOM from surface water. In order to optimize and improve these processes, the characterization and quantification of NOM during pretreatment processes are very important.

2.4.3.1 Direct NOM Quantification

Low concentration of NOM present in natural water makes fouling and fouling associated studies challenging due to a lack of adequate techniques to quantify specific organic fractions (Peldszus et al., 2011). Peldszus et al. (2011) pointed out that spectrophotometric methods applied in wastewater studies for protein and polysaccharide quantification, are not sensitive enough to quantify such low concentrations of NOM associated with surface water. Up to date, liquid chromatography-organic carbon detection (LC-OCD) and fluorescence excitation–emission matrix (FEEM) are the two most successful methods used to evaluate the removal of NOM from surface water for different purposes, including reducing disinfection by-products. With a detection limit of 10 µg/L, they are able to detect and quantify different fractions of NOM at very low concentration (Henderson et al., 2009; Huber et al., 2011).

LC-OCD can measure biopolymers, humic substances and some lower molecular weight fractions (Huber et al., 2011), whereas FEEM can differentiate humic and fulvic acid-like substances, protein like substances and colloidal/particulate substances (Henderson et al., 2009). According to the literature, LC-OCD is reported to be able to quantify six different fractions of organic carbon in water samples: (1) biopolymers composed of polysaccharides and proteins, (2) humics (and fluvics), (3) building blocks that correspond to breakdown products of humics, (4) low-molecular-weight organic acids, and (5) low-molecular-weight neutrals (alcohols, aldehydes, ketones, and amino acids), (6) hydrophobic organic carbon (Huber et al., 2011). It has several applications including NOM investigation in drinking water, wastewater, marine water, and quality control in ultra-pure water used in power plants and semi-conductor industry. LC-OCD has also been widely used in the application of membrane based water treatment to characterize different constituents of NOM in source waters that may contribute to membrane fouling (Kennedy et al., 2008; Hallé et al, 2009).

FEEM is a method that generates a large number of fluorescence intensity readings at different combinations of emission and excitation wavelengths (Peiris et al., 2010a). FEEM provides more detailed information and better sensitivity for recording and capturing subtle fluorescence features that correspond to humic substance- and protein-like materials (Peiris et al., 2010b). FEEM has been applied in drinking water research to identify surface water constituents causing hydraulically reversible and irreversible fouling (Peldszus et al., 2011).

2.4.3.2 Indirect NOM Quantification

Although a low concentration of biopolymers in drinking water samples can be measured as a group by LC-OCD or FEEM, concentration and measurement of individual components (including polysaccharides and proteins) still remain a challenge. Direct quantification of proteins and polysaccharides could improve the identification and understanding of important foulants in membrane filtration. In addition, it can further reveal and compare the treatment efficiencies of different pretreatment methods for membrane fouling mitigation. Studies show traditional colorimetric or spectrophotometric methods applied in wastewater studies for PN and PS quantification are not sensitive enough to quantify such low concentrations of NOM (i.e. Drews, 2010). However, if the aqueous samples could be concentrated and/or the constituents could be isolated, substances present in the water samples can be chemically analyzed (Jolley and Suffet, 1987).

For chemical quantification to be meaningful, a main consideration that should be taken into account when choosing concentration methods is that separation or concentration of the organic constituents should result in minimal chemical alteration (Jolley and Suffet, 1987). Options include vacuum distillation, lyophilization (also known as freeze-drying), and membrane processes such as reverse osmosis and ultrafiltration. One common issue associated with concentration process is that inorganic substances present in the water are concentrated along with organic constituents.

The principle of freeze concentration is that solutes will be excluded by ice during the freezing process (Croue et al., 2000). This is a very gentle concentration technique and is particularly good for the recovery of semi-volatile solutes that may be lost during evaporative techniques. However, freeze concentration is a time-consuming process and is not widely applied for concentrating water. Although vacuum distillation has been used to concentrate sample volume wastewater effluent samples (Pitt et al., 1974), the related experimental process is labour intensive (Jolley and Suffet, 1987).

Freeze-drying is generally accepted as the best method for producing dry NOM isolates that have been minimally altered from their dissolved state while having physical properties that are relatively easy for subsequent sample handling (Croue et al., 2000). This process is able to concentrate a few liters of water samples per day using a typical freeze-dryer. Jolley and Suffet (1987) suggested that lyophilization was a powerful concentration technique that was able to concentrate waters up to 3000-fold.

2.5 Pretreatment Processes for Controlling Biofouling

In order to cope with membrane fouling, single or integrated pretreatment is required for minimizing membrane fouling and improving membrane performance. Pretreatment in this context refers to processes or operations that are conducted upstream of membrane filtration, which are designed to effectively modify feed water quality and properties of certain aquatic constituents (Huang et al., 2009). Integration of pretreatment with low-pressure membranes has been widely employed at full-scale for fouling control and/or certain contaminant removal. Huang et al. (2009) proposed three main mechanisms in which pretreatment can affect membrane performance: alternation of size distribution of contaminants, changing mutual affinities of contaminants or their affinities to membranes and removing of biodegradable contaminants. Presently, other than biofiltration, common pretreatment options include coagulation and oxidation, which are described in more detail in the following sections.

2.5.1 Coagulation

Coagulation is a physiochemical process based on the principle of decreasing or neutralizing the negative electric charge on suspended colloids or NOM (Shammas, 2005). Before coagulation, similar electric charges on small particles in waters result in natural repulsion between each other, which hold the small, colloidal particles apart and keep them in suspension in the system. During coagulation, colloidal particles are brought together by positively charged coagulants, and agglomerated to form larger particles that can be removed more easily (Shammas, 2005). Based on this mechanism, coagulation helps the separation of particulate species in downstream processes such as sedimentation and filtration (Shammas, 2005).

Coagulation has been intensively studied as part of conventional water treatment process, and optimum use of coagulants is well established. On the other hand, the performance of coagulation-membrane filtration is still not well understood (Kimura et al., 2008). Optimum conditions for coagulation for a membrane process may not be the same as that for the conventional water treatment due to the additional complexity of involving membranes (Gao et al., 2011). Applying the same optimal dosage of coagulants for conventional systems often results in adverse effect on membrane fouling alleviation (Gao et al., 2011), where excess amount of coagulants have been reported to cause severe irreversible fouling (Kimura et al., 2008). As a part of the conventional treatment process, coagulation is used for particle removal, whereas as a pretreatment approach for membrane filtration, the final goal of coagulation is to improve membrane performance. Therefore, the current view of operating conditions, such as coagulant dosage, the type of coagulants, and mixing condition, needs to be transformed from optimized classical coagulation to that of coagulation/membrane filtration system.

Kim et al. (2005) identified the influence of coagulant dosage on membrane filtration performance by applying different alum concentrations (1, 10 and 100 mg/L) in secondary effluent from wastewater treatment plant involving a rotating biological contactor. When alum as a coagulant was applied at 1 mg/L, membrane performance was improved by 42% in terms of permeation rate compared to control without any pretreatment. As alum concentrations were increased to 10 mg/L and 100 mg/L, permeation rates were enhanced by 64% and 83% respectively. TOC removal efficiencies were 4% with 10 mg/L alum addition, and 56% with 100 mg/L alum addition. Alum at 10 mg/L was found to improve the organic removal by about 10%, but permeation rate improved by 64% suggested that TOC removal by coagulant was not the only reason for enhancement of membrane performance with respect to permeation rate. The sizes of particle were increased, and zeta potential was decreased due to the increase alum concentration. Kim et al. (2005) concluded that even at low coagulant dosage, coagulation can reduce membrane fouling by changing particle characteristics. At high coagulant dosage, membrane fouling can be further controlled by both changing particle characteristics and reducing contaminant loading.

The type of coagulants has been recognized as another key factor affecting the efficiency of coagulation. Available coagulants can be divided into two groups: inorganic salts and organic macromolecules. Inorganic salts, such as aluminum and ferric salts, are more frequently used in pretreatment of UF, compared to organic coagulants. In a study where UF was operated under dead-end filtration mode, the efficacies of three different aluminum salts were tested, including alum, polyaluminum chloride and sodium aluminates (Kabsch-Korbutowicz, 2006). Alum and polyaluminum chloride effectively increased the removal rates and resulted in a considerable fouling reduction, whereas sodium aluminates had no effect on fouling control. Under the same operating condition and environment (i.e. pH, mixing time, membrane type), some salts appear to be superior to others due to their chemical properties.

Pre-coagulation in UF systems can be performed in two different ways, standard coagulation and in-line coagulation, where standard coagulation includes sedimetation, and in-line coagulation operates in the absence of sedimentation (Gao et al., 2011). During inline coagulation, coagulation is added to the feed immediately before the membrane (Konieczny et al., 2009). Omitting the stage of sedimentation, inline coagulation requires a smaller amount of coagulants and shortens the duration of the treatment process. In Guigui et al. (2002), inline coagulation was found to improve not only the quality of membrane permeate but also slow down membrane fouling. From an economic perspective, inline coagulation seems to be a better solution, where the efficiency and the quality of the permeate is insignificantly worse but smaller doses of coagulant are required (Guigui et al., 2002). However, under certain circumstances, coagulation with sedimentation has demonstrated a better performance than inline coagulation. Liang et al. (2007) compared the pretreatment efficiencies of in-line coagulation, standard coagulation (coagulation-sedimentation) and standard coagulation-filtration, prior to UF treating feed water coming from algae-rich reservoir to produce drinking water. The result suggested in-line coagulation was the least effective approach based on the permeate water quality and specific flux of membrane. Liang et al. (2007) also indicated that, with the absence of sedimentation, although coagulation destabilized algae cells and brought them together to form large flocs of algae, algae accumulating on the membrane surface could still release EPS that caused membrane fouling. The gel layer of EPS was difficult to be removed through the process of backwashing. Moreover, by comparing to coagulation-sedimentation, coagulation-sedimentation-filtration resulted in a faster reduction of specific flux within the same time course, indicating that filtration step played a negative role on the membrane fouling control (Liang et al., 2007). The adverse effect from filtration step may have come from trapped algae in the filter, which may be still biologically active and able to released TEP, thus producing membrane fouling. On the other hand, Kimura et al. (2005) reported that in-line coagulation with aluminum salt effectively alleviated reversible fouling; however irreversible fouling attributed to proteins/polysaccharides like substances could not be effectively removed by this approach.

In conclusion, although coagulation is believed to be an effective and promising pretreatment for UF in terms of fouling reduction (i.e. Gao et al., 2011; Huang et al., 2009), inconsistent results with different studies suggested that more research is still needed to optimize and establish a particular coagulation method directly aiming at fouling control. Several issues that remains to be resolved before coagulation can be integrated with UF system, includes the compatibility of inorganic salts with membrane materials, overall cost and benefit of coagulation to the UF treatment process and the optimum conditions for coagulation (Farahbakhsh et al., 2004).

2.5.2 Ozonation

Ozone is a powerful oxidant that can efficiently change the physio-chemical characteristics of NOM, which leads to a reduction of membrane fouling (Van Geluwe et al., 2011). Ozone reacts with dissolved organic substances through two major pathways: highly selective attacks on organic molecules at low pH by ozone molecules directly, and the non-selective reaction of free radicals from ozone decomposition with the organic compounds. In addition, free radicals can be generated in the aqueous treatment system by pH modification, the addition of hydrogen peroxide together with ozone or UV-irradiation from a high pressure mercury lamp (Agustina et al., 2005).

Many studies have shown that ozone can effectively reduce membrane fouling. In a pilot plant study with ozone-MF system, 3-4 times higher permeate flux was achieved compared to a filtration system without ozone; hence ozone was believed to prevent foulants from adhering to membrane surface (Hash ino et al., 2000). Another study with a UF system, with a residual ozone at 4 mg/L, the permeate flux was able to be maintained at 90%, whereas without ozone the permeate flux dropped to 60% in one hour of treatment (You et al., 2007).

Unlike photocatalysis that has a relatively low oxidation rate, ozone has a greater capacity for selective destruction of recalcitrant compounds; therefore it is more frequently used as a pre-treatment step before conventional biological treatment (Agustina et al., 2005). Ozonation could increase the biodegradable organic carbon that can be more easily removed by biofiltration. Lee et al. (2005) reported that when ozone was applied, most of the high molecular weight (MW) (~24,400 g/mol) organic compounds were degraded to low MW (20–438 g/mol) organic compounds. Wang et al. (2008) investigated the removal efficiency of dissolved organics in the combination of ozonation and biofiltration for treating the secondary effluent from sewage treatment plant. The chemical oxygen demand (COD) removal by ozone alone and by biofiltration alone was high as 20–30% and 17–25%, respectively. When biofiltration was combined with ozonation, the total removal efficiency was increased to 46–63%, which is higher than the sum of both combined (Wang et al., 2008). In addition, short ozone contact time was found to be effective to reduce organic substances when ozonation and biofiltrations is combined. At an ozone dose of 10 mg/L, DOC and COD were reduced from 38.2 and 10.92 mg/L to 29.6 and 8.11 mg/L respectively, with 4 min of contact time. However, when the contact time was extended to 30 min, only 5% increase in efficiency was found. The result indicates that ozonation combined with biofiltration could be an effective and low cost alternative for reducing membrane fouling as only 4 min ozone contact time can significantly increase the biofilter removal efficiency.

When considering ozonation as a pretreatment for membrane filtration, the compatibility of ozone with membrane material needs to be considered. In a review of oxidants compatibility with low-pressure membrane, ozone was found to be incompatible with the most commercially available polymeric membranes, whereas it works well when combined with ceramic membrane (Huang et al., 2009).

Chapter 3 Materials and Methods

3.1 Materials

This section provides an overview of the biofiltration systems with different engineering set-ups located at Peterborough Drinking Water Pilot Plant (DWPP) and Mannheim DWPP. These systems had been established by collaborating research groups at the University of Toronto (Professor R.C. Andrews, Department of Civil Engineering) and the University of Waterloo (Professor P. Huck, Department of Civil and Environmental Engineering), respectively.

3.1.1 Biofitration systems at Peterborough DWPP, Peterborough, Ontario

The conventional and biological filters located at the Peterborough DWPP treats water from the Otonabee River. In this study, water and filter media samples were collected from two large-scale conventional filters and six biofilters including two large-scale and four small-scale biofiltration units (Figure 3-1). For both conventional filters and biofilters, influents from constant head tank had not been pretreated prior to filtration. The empty bed contact time (EBCT) was 10 minutes for all the filters.

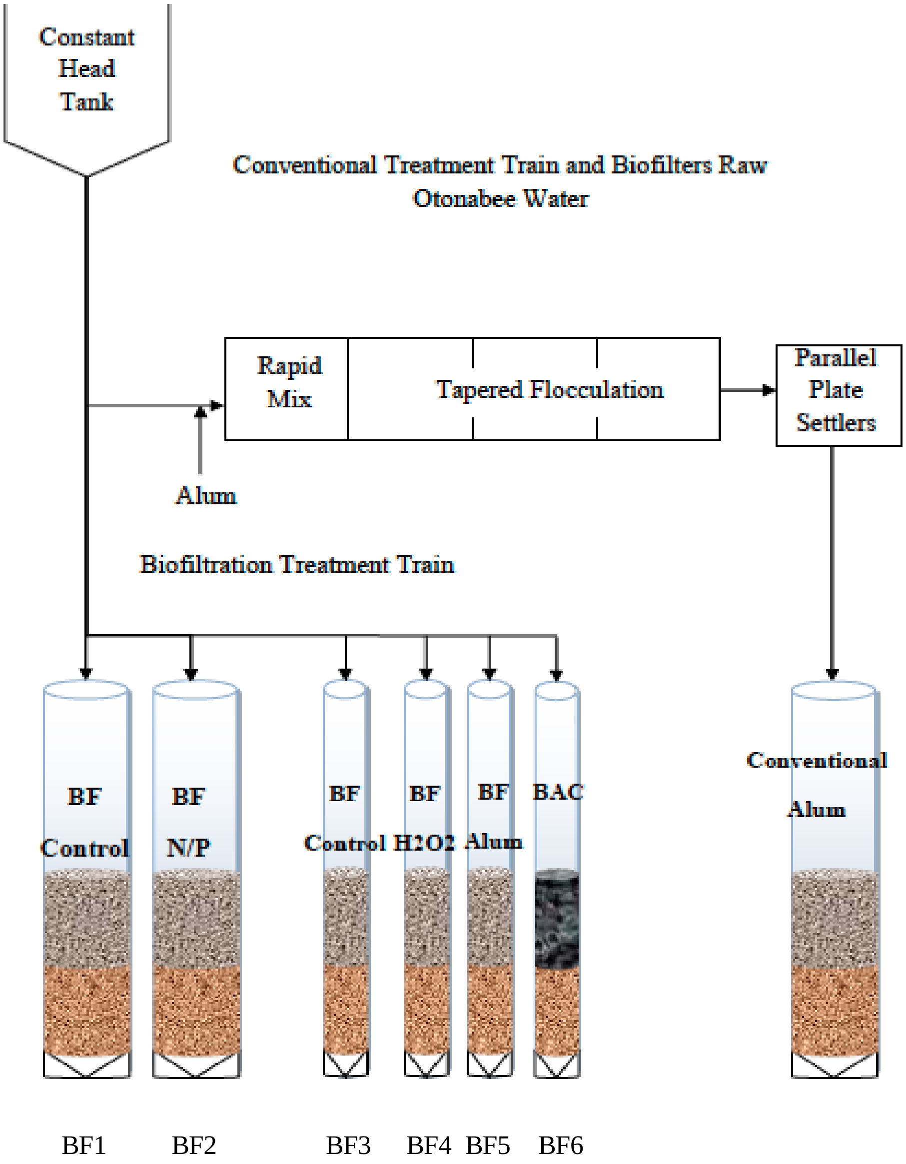

Figure 3-1 Illustration of experimental biofilters (BFs) operated at Peterborough DWPP, Ontario (diagram courtesy of Dr. R.C. Andrews, Department of Civil Engineering, University of Toronto).

For the large-scale conventional filters, each of the two conventional trains includes coagulation, flocculation, sedimentation, and filtration. The last step filtration was carried out with non-biological filters that were backwashed with chlorinated water on a weekly basis. These two filters have the width of 152.4 mm. Each filter consists of 50 cm of anthracite over 50 cm of sand, except BF6 which had 50 cm of GAC (8 years old) over 50 cm of sand.

The six experimental biofiltration systems include: two large-scale biofilters with the same dimension as those of conventional filters, and four small-scale biofilters with a smaller width of 76.2 mm. Between the two large-scale biofilters, one was used as control biofilter 1 (BF1) operated in the passive mode, and the other one was used for a nutrient enrichment test, biofilter 2 (BF2). BF2 was fed with phosphoric acid and ammonia at a concentration of 0.5 mg/L. The first small biofilter, biofilter 3 (BF3), was also operated in a passive mode as a control. Biofilter 4 (BF4) was treated hydrogen peroxide at a dose 0.2 mg/L. Biofilter 5 (BF5) was operated with inline coagulation using alum as the coagulant at a dose of 0.2 mg/L. Biofilter 6 consisted of GAC and sand as a filter media, which was different from BF1 to BF5 that contained anthracite and sand as the filter media. In this study, samples included raw water (RW) and all six biofilter effluents and filter media from the six biofilters.

3.1.2 Biofitration system at Mannheim DWTP, Kitchener, Ontario

The passive biological filters located at the Mannheim DWPP treats water from the Grand River (Figure 3-2). Biofilter A (BFa) contains 20 cm of anthracite and 20 cm of sand with an EBCT of 7.8 minutes. Biofilter B (BFb) contains 20 cm of anthracite and 60 cm of sand. (BFb) is connected to biofilter C (BFc), where effluent from filter B flows into filter C, leading to a combined EBCT of filter B + C of 23.5 minutes. For sample collected on July 24th and August 7th, 2014, media samples were taken from the sample port in BFc. The raw water (BFc influent) had been pretreated by a roughing filter. The roughing filter was designed to reduce peak concentrations of suspended materials prior to the biofiltration systems, due to the highly varying and sometimes challenging raw water quality of the Grand River (Peldszus et al., 2012). The effluent samples were collected at the outlet of BFc, which had travelled through BFb and BFc with a total EBCT of 23.5 minutes.

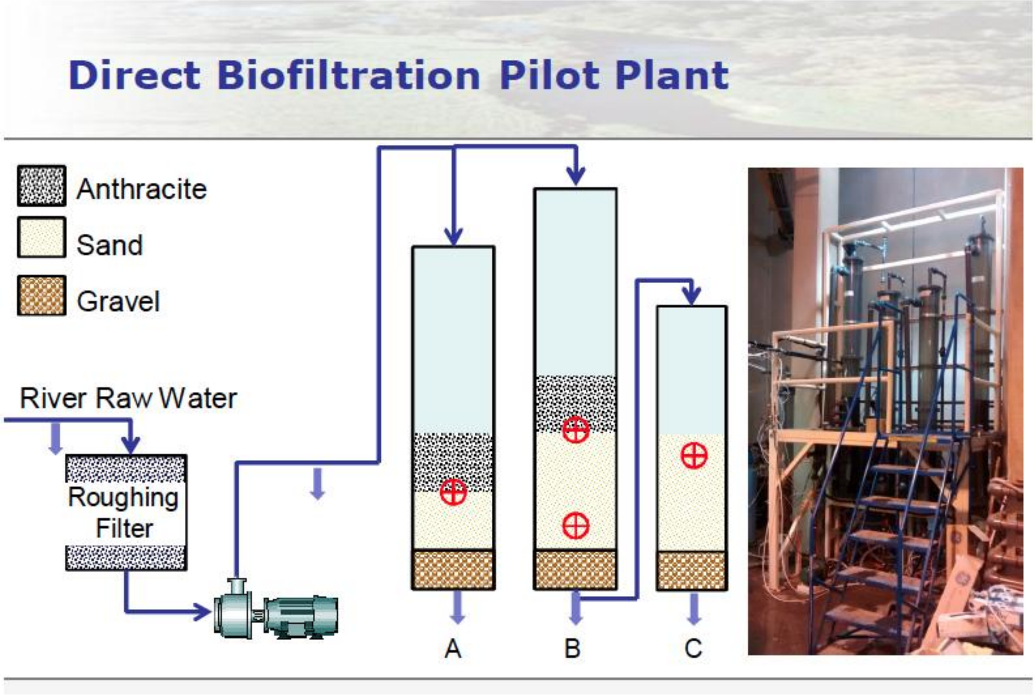

Figure 3-2 Illustration of experimental passive biofilters operated at Mannheim DWPP, Kitchener, Ontario (diagram courtesy of Brad Wilson, Department of Environmental and Civil Engineering, University of Waterloo). 4 sample ports were also shown in the diagram, including 1 port each for biofilter A (BFa) and biofilter C (BFc), and 2 ports in biofilter B (BFb).

3.2 Methods

3.2.1 Concentration of Water Samples by Freeze Drying

Freeze-drying is a drying process during which water is eliminated from the system by transforming the frozen water directly into vapor without the formation of liquid water. The mechanisms involved in this sublimation process include the adsorption of heat by the frozen sample to allow vaporization of ice; the use of vacuum pump to promote the removal of water vapor from the system; the transfer of water vapor to a collector; and the removal of the heat by the collector with the purpose of condensing the water vapor. Freeze-drying is an important technique commonly applied in sample preparation and for the preservation and storage of biologicals, pharmaceuticals and foods. Compared to other dehydration techniques, freeze-drying process is particularly suited for substances that are heat-sensitive (Jolley and Suffet, 1987).

Theoretically, freeze-drying could also be used as a concentration approach for water samples analysis where organic substances present at low concentrations can be increased to the point of detection. Due to limited information from literature on freeze-drying applied for such a purpose, a new protocol has been designed, tested and modified during this study, which is shown as below.

During freeze-drying experiments, 30 mL of water samples were transferred into 50 mL conical tubes and kept at -20˚C overnight. In order to prevent tubes from cracking due the expansion of water during the freezing process, caps were kept loose on the top of the conical tubes. Conical tubes with frozen water samples inside were covered with parafilm. Subsequently, 50 holes were made in each parafilm cover by using a syringe needle to poke through each parafilm in order to allow for sublimation to occur, while protecting the samples from contamination. After water sample preparation, frozen samples were transferred to a freeze dryer (Labconco Corporation, Kansas, USA). Under normal conditions, 30 hours were required to reduce the volume from 30 ml to less than 5 mL for 12 water samples. After freeze-drying, water samples were thawed in the water bath at room temperature. Each sample was then centrifuged at 10,000 *g* at 4˚C to remove precipitates that would interfere with downstream analysis. The supernatant was decanted into new a centrifuge tube and brought up to the final volume of 5 ml. Hence, water samples were concentrated by 6-fold for downstream analysis.

3.2.1.1 Validation of Freeze Drying Concentration Approach

Validation experiments were conducted in order to support that freeze drying is not only able to effectively increase PN and PS concentrations in water samples so that they can be detected and quantified by traditional colorimetric methods but also truly reflect original PN and PS presented in the water samples.

Reference water samples were prepared by adding known amounts of standard analytical grade chemicals (glucose for neutral PS, glucuronic acid for acidic PS, bovine serum albumin for PN) from Sigma-Aldrich, Canada Ltd. (Oakville, Canada) into original water samples, which gave a final concentration of 2 mg/L standard chemicals in addition to naturally present biopolymers. The concentration of 2 mg/L was chosen based on the rough estimation of the amounts of PN, PS presented in the water samples, according to available literature. In addition, an extra 2 mg/L of standard contributes 12 mg/L after 6-fold concentration process after freeze drying, which can effectively increase the total concentration above the detection limits of downstream colorimetric analyses. A minimum of triplicate original and reference water samples were taken for freeze-drying. After concentration, PN and PS concentrations in original and reference water sample were measured and compared. If freeze-drying is effective and reliable, the estimated concentration of PN or PS in reference water samples should be 2 mg/L higher than that in the original water samples.

3.2.2 EPS Extraction from Biofilter Biofilms

The cation exchange resin (CER) method was adopted for EPS extraction (Frølund et al., 1996; Liao et al., 2001; Mahendran et al., 2013) from biofilter biofilms. For each sample, 0.5 g of wet filter media was used. The extraction was performed by transferring filter media to a clean 15 mL centrifuge tube with addition of 5 mL EPS extraction buffer (2 mN Na_3PO_4, 4 mN NaH_2PO_4, 9 mN NaCl, 1 mN KCl in 1 L of deionized water at pH 7) and 0.5 g of CER (DOWEX® HCR-W2 Cation Exchange Resin, Sigma-Aldrich, Canada Ltd, Oakville, Canada). The mixture of each sample and CER was vortex at a constant rate (5) for 60 minutes at +4 ˚C. Each sample was then centrifuged for 15 minutes at 10,000 *g* at 4˚C. The supernatant was decanted into a new centrifuge tube and stored at -20˚C for future analyses of PN, PS and HS.

3.2.3 Chemical analysis

3.2.3.1 Neutral Polysaccharides (PS)

In this study, the anthrone assay was employed to quantify the neutral PS presented in the biofilter associated water samples and EPS extracted from biofilter biofilms, as described by Gaudy (1962). A standard solution of glucose (grade≥ 99.5%, Sigma-Aldrich, Canada Ltd, Oakville, Canada) was used to prepare a calibration curve. The anthrone reagent was prepared fresh for each experiment (2 hours prior to use) by dissolving 0.2 g anthrone (analytical grade, Sigma-Aldrich, Canada Ltd, Oakville, Canada) in 100 mL H_2SO_4 (grade≥ 96.4%, Sigma-Aldrich, Canada Ltd, Oakville, Canada). For each sample, a 0.2 mL aliquot was added to a 2 mL centrifuge tube in triplicate. 1 mL of anthrone reagent was added to each centrifuge tube and well mixed by vortexing for 30 seconds, and placed in a boiling water bath for 15 minutes. After 15 minutes, each samples was placed into an ice bath sequentially to cool down to room temperature. At the last step, samples were analyzed by reading the absorbance at 625 nm in a Multiskan GO Microplate Spectrophotometer (Thermo Scientific, Vantaa, Finland). The tested detection limit of this method is found to be 2.5 mg/L.

3.2.3.2 Acidic Polysaccharides (PS)

Analysis of acidic PS was carried out by using a colorimetric method described by Filisetti-Cozzi and Carpita (1991). A calibration curve using glucuronic acid (GA, grade≥98%, Sigma-Aldrich, Canada Ltd, Oakville, Canada) was prepared for each analysis. For each tested sample, a 0.2 mL aliquot was added to a 2 mL centrifuge tube in triplicate. 20 µL of 4 M sulfamic acid-potassium sulfamate (pH=1.6, adjusted with saturated KOH) was added to each tube and well mixed by vortexing for 15 seconds. In the next step, a 1.2 mL aliquot of sulfuric acid (grade≥ 96.4%, Sigma-Aldrich, Canada Ltd, Oakville, Canada) containing 75 mM sodium tetraborate was added to each tube, and mixed again with vortexing for another 15 seconds. After all the tubes are well mixed, they were placed into a water bath at 100 ˚C for 20 minutes. Each sample was placed into an ice bath sequentially to cool down to room temperature. At the final step, 40 µL of 0.15% (w/v) m-hydroxydiphenyl in 0.5% (w/v) NaOH at 4˚C was added to each sample and mixed by vortexing for 15 seconds. After 10 minutes incubation time at room temperature, samples were analyzed by reading the absorbance at a wavelength of 525 nm. The determined detection limit of this method for acidic PS quantification was found to be 5 mg/L.

3.2.3.3 Transparent Exopolymer Particles (TEP)

Originally, TEP measurement was performed by following the dyeing method described by Tokarczyk et al. (1995) due to its relative simplicity and less time required compared to other methods. For particulate TEP (particle size > 0.40 µm) quantification, 0.45 µm filter (filter type: HV) was used. For colloidal TEP (particle size: 0.05-0.40 µm), a filter with pore size of 0.1 µm (filter type: VVPP) was used. In Tokarczyk et al. (1995), only the particulate TEP concentration was quantified using 0.4 µm polycarbonate filters. In order to replicate previous experiments conducted by Tokarczyk et al. (1995), TEP experiments were started with particulate TEP measurements. Polycarbonate filter of 0.45 µm pore size (DURAPORE® MEMBRANE FILTER, Merck Millipore Ltd, Cork, Ireland) was used due to commercial availability.

However, due to the unsuccessful replicates of xanthan gum (XG) standard curve with 0.45 µm pore size filters, attempts to improve the method include modifying the volume of alcian blue dye applied to the filter, the volume of water applied to wash off the excessive dye, de-staining time and using a smaller pore size polycarbonate filter (0.1 µm). A new protocol of TEP quantification was developed by modifying the method from Tokarczyk et al. (1995) and is described as follow:

A standard solution of XG (analytical grade, Sigma-Aldrich, Canada Ltd, Oakville, Canada) was used to prepare a standard calibration curve. 0.02 % Alcian blue stain was prepared by dissolving 0.02 g of alcian blue dye (8XG, analytical grade, Acros Organics, New Jersey, USA) in 100 mL 0.06 % acetic acid (pH 2.5). As the dye coagulates, the stain solution was pre-filtered with 0.1 µm filter to avoid the interference due to the alcian blue particles. After the stain was ready, the sample was first filtered through a 0.1 µm polycarbonate filter (DURAPORE®

MEMBRANE FILTER, Merck Millipore Ltd, Cork, Ireland) at low and constant pressure (150 mm of Hg). Following filtration of the sample, the vacuum pressure was turned off. With the filter still secured in the vacuum apparatus, 2 mL of pre-filtered alcian blue stain was added ensuring the entire surface of the filter was covered by the stain). Vacuum filtration was resumed at a low and constant pressure until all of the dye was filtered through. With the filter still secured in the vacuum apparatus, the filter was washed with 5 mL of deionized water to remove any excess dye that was not bound to TEP. Subsequently, stained filters were transferred to a 25 mL beaker and completely submerged in 6 mL of 80 % H_2SO_4. Each filter was soaked for 1 hour, during which the beaker was gently shaken 3-5 times. After 1 hour of de-staining, absorbance of each sample was read in a Multiskan GO Microplate Spectrophotometer (Thermo Scientific, Vantaa, Finland) at wavelength of 787 nm.

3.2.3.4 Proteins (PN) & Humic Substances (HS)

The contents of PN and HS in water and EPS samples were measured by modified Lowry method described by Frølund et al. (1995) based on Folin reaction. For PN measurement, BSA (grade≥ 98%, Sigma-Aldrich, Canada Ltd, Oakville, Canada) was used to prepare standard calibration curve. For each sample, 0.2 mL aliquot was added to each 2 mL centrifuge tube in triplicate. 1 mL of a prepared reagent (20 g Na_2CO_3 in 1 L of 0.1 N NaOH, mixed with 0.25 g $CuSO_4 \bullet 5H_2O$ dissolved in 50 mL of 1% (w/v) aqueous solution of sodium tartrate, in a ratio of 25:1) was added to each tube and mixed by vortexing for 15 seconds. Subsequently, samples were allowed to stand for 10 minutes at room temperature. Then, to each tube was added 0.1 mL Folin reagent (Folin and Ciocalteu's phenol reagent (analytical grade, Sigma-Aldrich, Canada Ltd, Oakville, Canada) diluted to a ratio of 1:1 with deionized water), mixed by vortexing for 15 seconds, allowed to stand for 30 minutes at room temperature. After 30 minutes incubation time, absorbance of each sample was read at a wavelength of 750 nm.

For HS quantification, humic acid (HA) (analytical grade, Sigma-Aldrich, Canada Ltd, Oakville, Canada) was used to quantify HS concentration. Same procedures were followed except that the first reagent added to each sample was different (20 g Na_2CO_3 in 1 L of 0.1 N NaOH). The determined detection limits of this method for PN and HS measurements were the same, which is 5 mg/L.

3.2.4 Microbial Community Analysis

3.2.4.1 Deoxyribonucleic acid (DNA) Extraction

In this study, DNA from source water and biofilter effluents, as well as biofilter media were extracted for microbial community analysis. For raw water and effluent samples, 500 mL of water of each sample was prefiltered with 0.45 µm pore size filter (Filter membrane for microbial analysis, Sigma-Aldrich, Canada Ltd, Oakville, Canada) to capture the microorganisms. After filtration, filters were cut into pieces and transferred to 5ml conical tubes for DNA extraction using PowerBiofilm DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, Canada). For filter media, PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, Canada) was used to extract the DNA from 0.3 gram of biofilter media to study the biofilm microbial community associated with each biofilter.

3.2.4.2 PCR-DGGE

Molecular methods provide the opportunity to understand the microbial composition of RW and natural biofilms on filter media without cultivation. Compared to cultivation methods, characterization of biofilms using polymeric chain reaction (PCR)-DGGE is rapid and more comprehensive (Emtiazi et al., 2004). Therefore, PCR-DGGE was applied to investigate the microbial similarity between water and filter media samples to undestand what relationship, if any existed, between source water and biofilters, between source water and biofilter effluents, as well as between sampling times.

16S-rRNA genes from the purified DNA obtained through DNA extraction were amplified using polymerase chain reaction (PCR) with a set of PCR primers for DGGE analysis. The primer set specific to the bacteria domain was 341F (5’- CCTACGGGAGGCAGCAG-3’) with a GC-clamp (5’- CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G -3’) and 907R (5’- CCGTCAATTCCTTTGAGTTT -3’). The PCR program was performed in a C1000 Thermal Cycler (BioRad) using the following program: initial denaturation at 98°C for 10 sec; 30 cycles of denaturation (95°C, 30 seconds), annealing (55°C for 40 secconds) and extension (72°C, 45 seconds); and final extension at 72°C for 5 min for Bacteria specific primers. PCR products were examined with EZ Vision (Amresco) DNA staining on agarose gels (1%) and were used for DGGE analysis.

Parallel DGGE of PCR amplified extracted 16s rDNA was performed using D-Code Universal Mutation Detection System (Bio Rad) as described by Muyzer et al. (1998). The gel contained a linear gradient of denaturant ranging from 35% to 70% (100% denaturant is 7 M urea and 40% formamide). DGGE was run at 70 V for 18 hours at 60 °C. After electrophoresis, the gel was stained with ethidium bromides (0.5 mg/L) and visualized by UV transillumination. The gel image was captured using a CCD camera and Multi Image Light Cabinet (Alpha Innotech). Image analysis was done using Alpha EaseFc (Alpha Innotech), which allows fragment detection and quantification. For each sample, a densitometric profile was generated to determine the relative contribution of each band to the total signal in the lane sample. DGGE bands were scored as present or absent from DGGE gel analysis. DGGE gels were further analyzed using an image analysis software for gel application, called GeneTools (Syngene, A Division of Synoptics Ltd). This software is able to detect more patterns that are not visualized clearly when gel image was taken under UV transilluminator

3.2.5 Statistical Analysis

Student *t*-tests were performance to determine the significance of the concentration differences of acidic PS, neutral PS and TEP between RW and biofilter effluents, to evaluate the effects of passive biofilter, biofilter with nutrient enhancement, biofilter with hydrogen peroxide supplementation, biofilter with inline coagulation and biofilter using GAC as filter media by comparing the average concentration (neutral PS, acidic PS and TEP) in between RW and each biofilter effluent. All statistical analyses were performed at 95% confidence level.

Chapter 4 Results

4.1 Validation of Freeze Drying as a Method for Concentrating Biopolymers in Surface Waters

4.1.1 The Importance of Biopolymeric Materials in Membrane Filtration Systems

Biopolymeric materials, also known as biopolymers, have been identified as key foulants in LMPs. This group of organic substances is mainly comprised of PS and PN (Hallé et al., 2009). Hence, due to the important role of biopolymers playing in membrane fouling, biopolymer concentrations were determined in biofilter influent and effluent samples to evaluate the treatment efficiency of biofiltration system as a pretreatment approach for membrane fouling control. Colorimetric methods used in wastewater studies were adopted and combined with freeze-drying to quantify the individual components, including total PS, acidic PS and PN (Filisetti-Cozzi and Carpita, 1991; Frølund et al., 1995; Gaudy, 1962).

4.1.2 Neutral PS Measurement Based on Anthrone Method

Originally, the anthrone method was applied to measure total PS concentration in water and EPS samples. However, preliminary results of EPS samples (Table 4-1) showed that total PS concentrations were lower than the acidic PS concentration concentrations.

Table 4-1 Total PS and acidic PS concentrations presented in EPS samples.

Filter media	mg total PS/ g WW	mg acidic PS/ g WW
BF1	0.069	0.371
BF2	0.080	0.423
BF3	0.066	0.350
BF4	0.075	0.373
BF5	0.062	0.228
BF6	0.074	0.205

Therefore, it raised the question whether the anthrone method has the same sensitivity to acidic PS as to neutral PS. In order to answer this question, an experiment using pure glucose, pure GA and a 1:1 mix of both sugars as standards with same concentration range (0-100 mg/L) was conducted to compare the variation in sensitivity of the anthrone method to glucose and GA. Based the results in Figure 4-1, although all three standards showed linear correlation between optical density (OD) and concentration, color production due to the presence of glucose and GA were different, where the color intensity was highest using pure glucose standard and lowest with GA standard. The pure GA standard curve indicates no big change in OD reading with the increase of GA concentration with the presence of the anthrone reactant, even when GA reached 100 mg/L. Standard curve from mixtures with equal amounts of glucose and GA generated about close to half of the OD compared to the color produced by pure glucose.

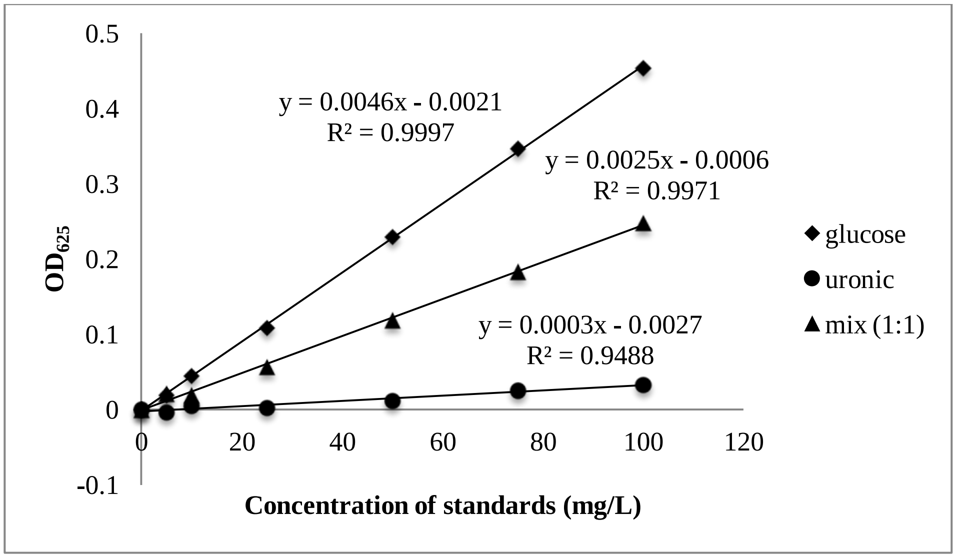

Figure 4-1 Standard curve comparion using anthrone method with pure glucose, pure GA and a mix (glucose: GA= 1: 1) as standards with same concentration range of 5-100 mg/L. Absorbance was read at 625 nm with microplate in triplicate.

Hence, it suggests acidic PS has little impact on the color intensity when the anthrone method is applied. Consequently, polysaccharide concentration detected using the anthrone method should be defined as neutral PS in contrast to acidic PS being quantified by modified m-hydroxydiphenyl sulphuric acid (Filisetti-Cozzi and Carpita, 1991). In this section, PS that was quantified using the anthrone method was defined as neutral PS.

4.1.3 Neutral PS

Neutral PS contents were quantified using the anthrone method in original and reference water samples, where original water samples were referred to RW and biofilter effluent samples that contained neutral PS naturally presented in the samples. Reference water samples were the group of RW and effluent samples with an additional 2 mg/L of glucose before freeze-drying. If freeze-drying is a valid method for neutral PS quantification, after freeze-drying and correcting the concentration factor (6-fold), the concentration of neutral PS in reference water samples should be 2 mg/L higher than that in original water samples.

After freeze-drying (FD), neutral PS concentrations were sufficiently increased. As illustrated in Table 4-2 and 4-3, the detected neutral PS concentrations of seven tested samples were over 5 mg/L and 15 mg/L in original and reference water samples respectively, which were above the detection limit (2.5 mg/L) of the applied Gaudy method (1962), suggesting concentration factor of 6 was sufficient enough.

Table 4-2 Analysis of concentrations (Conc) of neutral PS in original water samples following concentration by freeze-drying (FD).

Sample	Glucose (mg/L)		
	Conc. After FD[a]	Conc. Before FD[b]	SD[d]
RW	5.1	0.9	0.1
BF1[c]	5.2	0.9	0.5
BF2	6.2	1.0	0.1
BF3	5.5	0.9	0.3
BF4	5.7	1.0	0.3
BF5	5.5	0.9	0.1
BF6	6.5	1.1	0.1

[a] Detected glucose concentrations in samples concentrated by freeze-drying

[b] Estimated glucose concentrations in samples before freeze-drying by correcting the concentration factor

[c] BF1-BF6 represent effluents from biofilter 1-6 respectively

[d] Standard deviation of estimated glucose concentration among triplicate samples of each original water used for freeze-drying validation experiment.

Table 4-3 Analysis of concentrations (Conc) of neutral PS in reference water samples following concentration by freeze-drying (FD).

Sample	Glucose (mg/L)		
	Conc. After FD[a]	Conc. Before FD[b]	SD[d]
Feed Ref	19.2	3.2	0.3
BF1[c] Ref	16.8	2.8	0.4
BF2 Ref	18.9	3.2	0.2
BF3 Ref	17.4	2.9	0.2
BF4 Ref	17.1	2.9	0.3
BF5 Ref	16.8	2.8	0.3
BF6 Ref	18.0	3.0	0.2

[a] Detected glucose concentrations in samples concentrated by freeze-drying
[b] Estimated glucose concentrations in samples before freeze-drying by correcting the concentration factor
[c] BF1-BF6 represent effluents from biofilter 1-6 respectively
[d] Standard deviation of estimated glucose concentration among triplicate samples of each reference water used for freeze-drying validation experiment.

For the seven tested water samples, six samples demonstrated no statistically significant difference ($P > 0.05$) between original and reference water samples (Table 4-4), which suggests that freeze-drying is a reliable concentration approach with the capability of reflecting original neutral PS concentrations. The significant difference ($P = 0.01$) of neutral PS concentrations between original and reference feed water samples might be due to experimental errors during the reference water samples preparation. In addition, the fairly small concentration difference (0.3 mg/L) between original and reference feed water samples failed to minimize the effectiveness of freeze-drying for neutral PS measurement in surface water samples.

Table 4-4 Comparison of estimated neutral PS concentrations (Conc) in original (Org) and reference (Ref) water samples.

Sample	Glucose (mg/L)			
	Conc In Org [a]	Conc In Ref [b]	Difference	P (2-tails, ≥ 0.05)
Feed	0.9	1.2	0.3	0.01
BF1	0.9	0.8	0.1	0.86
BF2	1.0	1.2	0.2	0.29
BF3	0.9	0.9	0	0.67
BF4	1.0	0.9	0.1	0.56
BF5	0.9	0.8	0.1	0.52
BF6	1.1	1.0	0.1	0.15

[a] Estimated neutral PS concentration in original water samples after correcting the concentration factor

[b] Estimated neutral PS concentration in reference water samples after correcting the concentration factor

4.1.4 Acidic PS

In a fresh water environment, many organisms (e.g. algae, bacteria, plants) produce carbohydrate-rich substances that facilitate their growth and survival. Some of these substances are acidic PS. These poly-anionic extracellular polymers have the capability of aggregating together to form colloidal fibrils (Leppard, 1997). In particular, acidic PS has a high tendency of initiating biofilm formation, which may cause detrimental effects to water treatment facilities involving membranes. Thus, removal of acidic PS by biofiltration is of strong interest.

As indicated in Table 4-5 and 4-6, three water samples were used to test freeze-drying efficiency for acidic PS quantification. After freeze-drying, acidic polysaccharides (PS) concentrations were increased to 6 mg/L and 19 mg/L in original and reference water samples respectively, which were above the detection limit (5 mg/L) of the applied quantification method.

Table 4-5 Analysis of concentrations (Conc) of acidic PS in original water samples following concentration by freeze-drying (FD).

Sample	GA (mg/L)		
	Conc. After FD[a]	Conc. Before FD[b]	SD[d]
Feed	8.8	1.5	0.7
BF1[c]	10.6	1.8	0.3
BF2	6.6	1.1	0.4

[a] Detected GA concentrations in samples concentrated by freeze-drying.
[b] Estimated GA concentrations in samples before freeze-drying, by correcting the concentration factor.
[c] BF1-BF2 represent effluents from biofilter 1-2 respectively.
[d] Standard deviation of estimated GA concentration among triplicate samples of each original water used for freeze-drying validation experiment.

Table 4-6 Analysis of concentrations (Conc) of acidic PS in reference water samples following concentration by freeze-drying (FD).

Sample	GA (mg/L)		
	Conc. After FD[a]	Conc. Before FD[b]	SD[d]
Feed Ref	20.3	3.4	0.3
BF1[c] Ref	20.9	3.5	0.3
BF2 Ref	19.3	3.2	0.8

[a] Detected GA concentrations in samples concentrated by freeze-drying.
[b] Estimated GA concentrations in samples before freeze-drying, by correcting the concentration factor.
[c] BF1-BF2 represent effluents from biofilter 1-2 respectively.
[d] Standard deviation of estimated GA concentration among triplicate samples of each reference water used for freeze-drying validation experiment.

In Table 4-7, for all three tested samples, no significant differences ($P > 0.05$) of acidic PS concentrations were observed; indicating freeze-drying is also a feasible approach to concentrate water samples with low concentrations of acidic PS detection.

Table 4-7 Comparison of estimated acidic PS concentrations (Conc) in original (Org) and reference (Ref) water samples.

Sample	GA (mg/L)			
	Conc. In Org	Conc. In Ref	Difference	P (2-tails, ≥ 0.05)
Feed	1.5	1.4	0.1	0.87
BF1	1.8	1.5	0.3	0.70
BF2	1.1	1.2	0.1	0.96

[a] Estimated acidic PS concentration in original water samples after correcting the concentration factor

[b] Estimated acidic PS concentration in reference water samples after correcting the concentration factor

4.1.5 Proteins

As indicated in Table 4-8 & 4-9, although protein (PN) concentrations have been efficiently increased after freeze-drying and could be detected by the colorimetric method, expected concentration differences between original and reference samples were not observed. PN concentrations in original and reference water samples after freeze-drying were considerably small, compared to the results of the validation experiment for neutral PS and acidic PS measurements.

Table 4-8 Analysis of concentrations (Conc) of PN in original water samples following concentration by freeze-drying (FD).

Sample	BSA (mg/L)		
	Conc. After FD[a]	Conc. Before FD[b]	SD[c]
Feed	24.7	4.1	0.3
BF1	16.1	2.7	0.3

[a] Detected BSA concentrations in samples concentrated by freeze-drying.
[b] Estimated BSA concentrations in samples before freeze-drying, by correcting the concentration factor.
[c] Standard deviation of estimated BSA concentration among triplicate samples of each original water used for freeze-drying validation experiment.

Table 4-9 Analysis of concentrations (Conc) of PN in reference water samples following concentration by freeze-drying (FD).

Sample	BSA (mg/L)		
	Conc. After FD[a]	Conc. Before FD[b]	SD[c]
Feed Ref	27.1	4.5	0.4
BF1 Ref	19.6	3.3	0.4

[a] Detected BSA concentrations in samples concentrated by freeze-drying.
[b] Estimated BSA concentrations in samples before freeze-drying, by correcting the concentration factor.
[c] Standard deviation of estimated BSA concentration among triplicate samples of each reference water used for freeze-drying validation experiment.

For two tested samples, significant differences ($P < 0.01$) of PN concentrations were shown in Table 4-10. The result suggests that original protein concentrations could not be estimated by colorimetric methods after freeze-drying. One possible reason is that protein might precipitate out with an increase in the concentration of salt in the concentrated water samples during the freeze-drying process.

Table 4-10 Comparison of estimated PN concentrations (Conc) in original (Org) and reference (Ref) water samples.

Sample	BSA (mg/L)			
	Conc. In Org[a]	Conc. In Ref[b]	Difference	P (2-tails, ≥ 0.05)
Feed	4.1	2.5	1.6	<0.01
BF1	2.7	1.3	1.4	<0.01

[a] Estimated PN concentration in original water samples after correcting the concentration factor

[b] Estimated PN concentration in reference water samples after correcting the concentration factor

Therefore, another experiment was conducted to test the hypothesis that if the observed protein concentration difference between original and reference water samples was caused by high salt concentrations after freeze drying, by preparing pure protein standards with known concentrations using deionized water for freeze-drying.

However, as shown in Table 4-11, expected concentrations in pure standard samples after freeze-drying were not observed. The estimated original protein concentrations, determined by correcting for the concentration factor, were different from the known original concentrations. Possible physiochemical changes might occur during the concentration process.

Table 4-11 Comparison of estimated PN concentration and original concentration in deionized water.

BSA (mg/L)	Conc. After FD	Conc. Before FD
0.25	1.26	0.26
0.5	0.33	0.07
1	18.49	3.70
1.5	20.55	4.11
2	26.53	5.31

4.1.6 Summary

To summarize, validation experiment results for using freeze-drying as a concentration approach for low concentration organic matters measurement, suggest that freeze drying is an effective concentration method for low concentration neutral and acidic PS quantification but does not provide any aid for low concentration protein measurement. Meanwhile, preliminary results of applying the modified Lowry method to water samples without freeze-drying, PN concentrations in RW and biofilter effluents were not detected, which indicates PN concentrations were below the detection limit (5 mg/L). In addition, based on the preliminary results of confocal imaging scanning microscopy of fouled membrane treating both RW and biofilter effluents, PN concentrations in RW and biofilter effluents were less abundant than PS, and appeared to be largely cell associated (results not shown). Consequently, free PN were believed to play a minor role in membrane fouling than PS in this study and the focus shifted to polysaccharides as a main biopolymeric material likely contributing to fouling. Therefore, in this study, neutral and acidic PS were quantified and compared between raw water and biofilter effluent samples, and PN in water samples was not measured.

4.2 Analysis TEP

TEP are a class of organic particles ubiquitous in aquatic environments, which can be considered as free-floating EPS that are composed of acidic PS with specific properties of gel-like structure, transparency, stickiness and flexibility (Passow, 2002). The unique properties of TEP are recently linked to membrane fouling in a variety of membrane processes. Therefore, TEP quantification was undertaken to evaluate the treatment efficiency of biofiltration.

Unlike other chemical analyses performed in this study, the sticky, deformable properties of TEP also make the quantification challenging. Following previously published descriptions of the quantification of TEP (Tokarczyk et al., 1995), as indicated in Figure 4-2, it was found that it was not possible to generate standard curves with XG concentrations ranging from 0-100 mg/L and poor correlation was found between the absorbance and XG concentration over the concentration range of 0-50 mg/L.

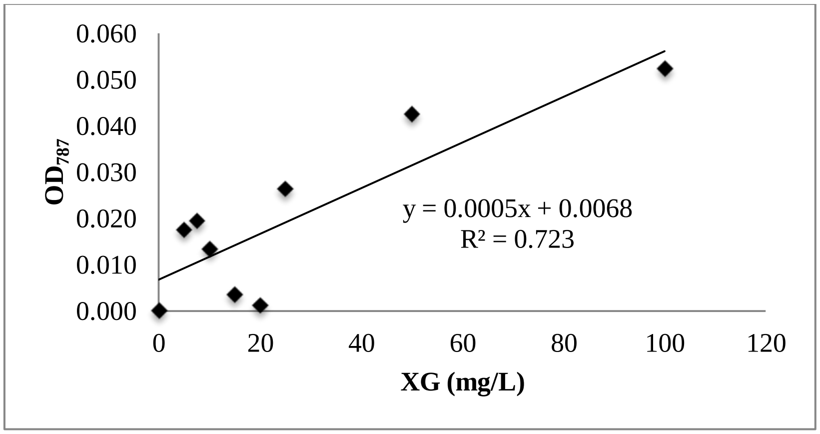

Figure 4-2 Typical TEP standard curve with XG as standard using 0.45 µm polycarbonate filters over a concentration range of 5 to 100 mg/L. Absorbance was read at 787 nm.

During the dyeing process, 500 µl of alcian blue dye suggested by Tokarczyk et al. (1995) was found to be insufficient to cover the entirety of the filter surface and a volume of 2 mL alcian blue dye was found to be enough to cover the filter surface and make sure all the TEP particles on the filter could be stained. Each stained filter was washed by pipetting 5 mL of deionized water to remove any excess dye that was not bound to TEP. Different de-staining times using 80% sulfuric acid had been tested. One hour proved to be sufficiently long enough to wash off the dye from the filter.

To minimize the complexity, time consumption and reliability of the test, the volume of dye, the volume of water to wash off the excess dye and the de-staining time were optimized. However,little improvement with respect to linearization of standard curve was observed.

It was suspected that the failure of establishing a standard curve with a high linear correlation might be due to: 1) the low amounts of particulate TEP present in the standard solution 2) 0.45 µm pore size filter might not be able to capture the particulate fraction of TEP due to their high flexibility 3) or a combination of 1) and 2). Therefore, another experiment was conducted with an increased concentration range of 0-1000 mg/L. Figure 4-3 shows a better linear correlation ($R^2 > 0.95$) when the XG concentration range was increased to 1000 mg/L, compared to XG standard curve ranging from 0 to 100 mg/L.

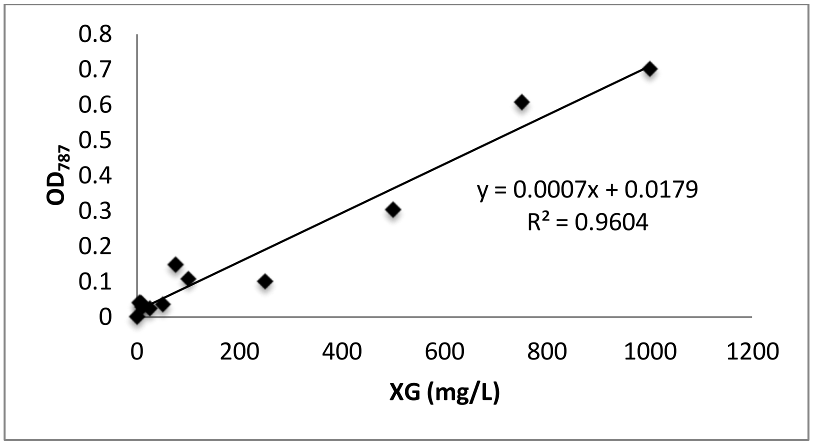

Figure 4-3 Typical TEP standard curve with XG as a standard using 0.45 µm polycarbonate filters with a concentration range of 5 to 1000 mg/L. Absorbance was read at 787 nm.

Although XG standard curves, at the concentration range of 0-1000 mg/L, could be reproduced, the small amounts of TEP present in the RW and biofilter effluents required larger volumes of water samples (more than 1 L) to capture enough TEP to be estimated using a high concentration range standard curve. The increase of required water sample volume would greatly extend the filtration time; thus, making the TEP quantification experiment impractical due to the increased time.

During the experiments of preparing high concentration range XG standard curves, a significant limitation of using 0.45 µm filters was discovered as the alcian blue stained XG complex was lost through the 0.45 µm filters and accumulated with the filtrate (see Figure 4-4).

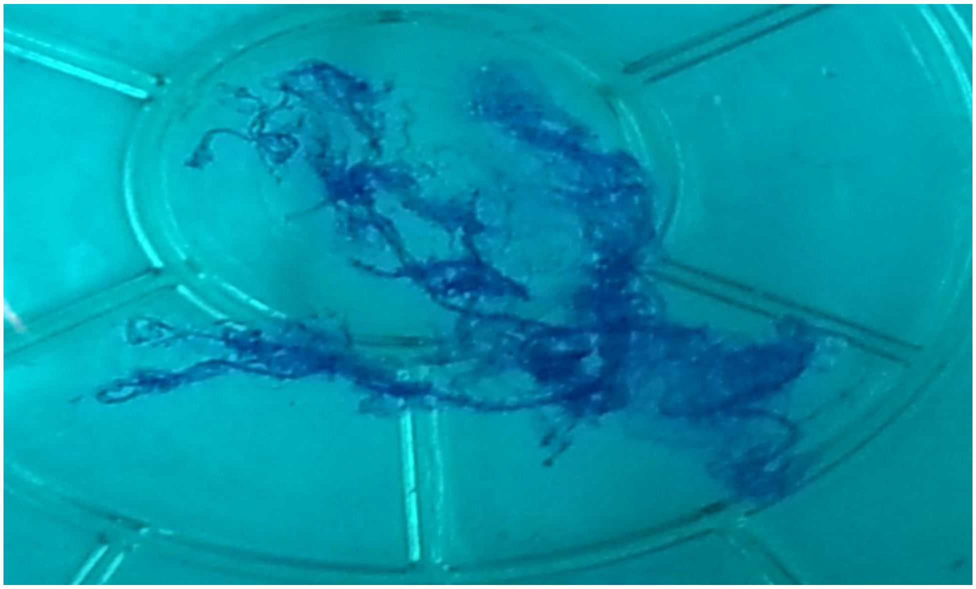

Figure 4-4 XG complexes that escaped through 0.45 µm filter during filteration step and presented in the filtrate. The blue color of the particles was due to the binding with alcian blue dye.

This observation indicates that at a low concentration range (i.e. < 100 mg/L), the small amounts of particulate TEP escaped through 0.45 µm pore size filters, and resulted in poor linear correlations. When the concentration range was increased to 1000 mg/L, the loss of TEP might be minimal, compared to the total amounts of TEP available to be stained and quantified. Thus, a better standard curve can be achieved with increased XG concentration range (0-1000 mg/L).

After the observation of particulate TEP loss through 0.45 µm filters, it was hypothesized that a 0.1 µm filter would trap the complex and in fact concentrate both the particulate (particle sizes >0.4 µm) and colloidal (particle sizes over the range of 0.05- 0.4 µm) fractions. Thus, experiment of XG standard curve of 0- 100 mg/L was conducted applying 0.1 µm polycarbonate filter. Good XG standard curves were achieved with a typical example shown in Figure 4-5. Reproducibility of the standard curve was also tested by conducting the same experiment for more than three times.

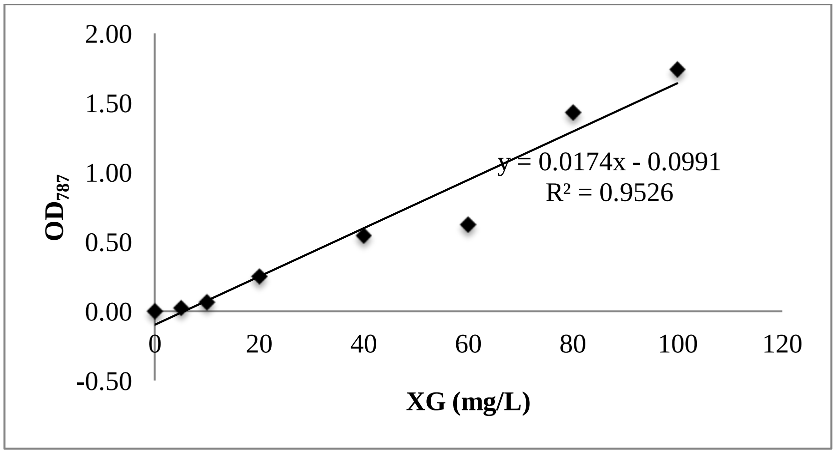

Figure 4-5 Typical TEP standard curve using 0.1 µm polycarbonate filters with a concentration range of 0 to 100 mg/L.

To summarize, it proved difficult to quantify the particulate fraction of TEP using a 0.45 µm filter and using the filtrate passing through a 0.45 µm filter to measure the colloidal TEP. In this study, particulate and colloidal TEP was measured together as a group (i.e. total TEP) by using 0.1 µm filters and directly filtering water samples to capture both fractions.

4.3 Chemical Analysis of Water Samples

4.3.1 Water Samples from the Peterborough Drinking Water Pilot Plant (DWPP)

Raw water without any pretreatment prior to biofiltration (source: the Otonabee River) and six biofilter effluent samples were collected on May 15th, 2014, from the Peterborough DWPP. Neutral and acidic PS were quantified using the methods described by Gaudy (1962) and Filisetti-Cozzi and Carpita (1991). BF2 showed a statistically significant reduction of acidic PS (from 2.7 ±0.3 to 1.9 ±0.3 mg/L, $P < 0.01$), which accounted for about 30% removal. On the other hand, a small increase in acidic PS was observed in BF5 effluent, where the biofilter was operated with inline coagulation (from 2.7 ±0.3 to 3.0 ±0.7 mg/L). Although the increase of acidic PS in BF5 effluent was not significant ($P = 0.46$), the increase trend of acidic PS may indicate a potential adverse effect caused by inline coagulation. In addition, acidic PS accounted for a larger proportion of total PS than neutral PS in all seven water samples, where the concentrations of acidic PS were about 3-fold greater than the neutral PS detected.

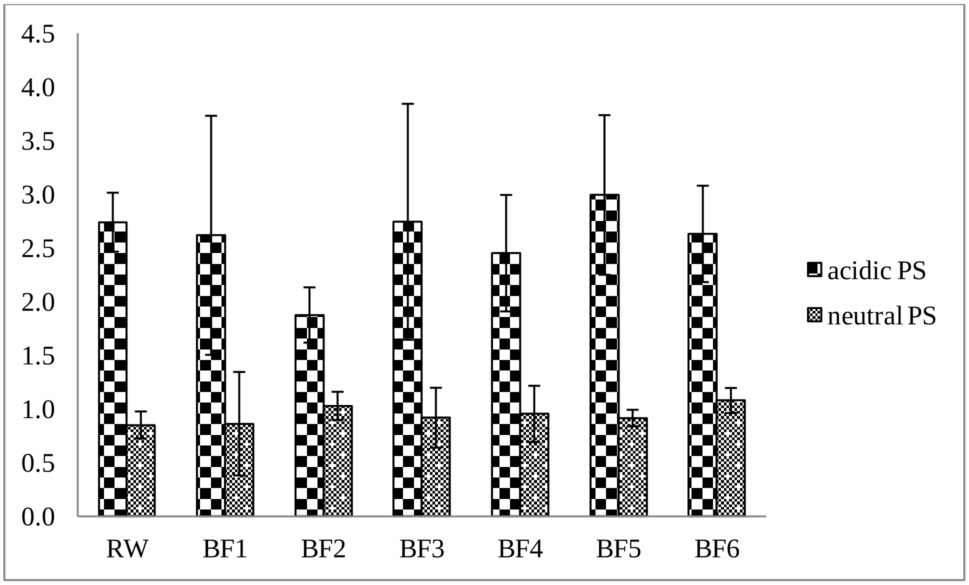

Figure 4-6 Neutral PS and Acidic PS presented in water samples collected from the Peterborough DWPP, May 15th, 2014. RW and BF1-BF6 indicate raw water and effluent samples obtained from BF1 to BF6. Error bars represent standard deviation among triplicate water samples with duplicate absorbance measurements (n = 6).

In the June samples collected from the Peterborough DWPP, compared to RW (1.5 ±0.7 mg/L), there were small increases of acidic PS in effluents from BF1 (1.8 ±0.3 mg/L), BF3 (1.9 ±0.3 mg/L), BF4 (1.8 ±0.1 mg/L) and BF5 (1.9 ±0.2 mg/L), which accounted for 120%, 126%, 122% and 130% of acidic PS in RW. Small reductions of acidic PS were shown in BF2 (1.1 ±0.4 mg/L, which accounted for 74% of acidic PS in RW) and BF6 (1.2 ±0.5 mg/L, which accounted for 82% of acidic PS in RW) effluents. In BF2 effluent, the concentration of neutral PS was almost two-fold greater than that of neutral PS concentration in RW, where the increase of neutral PS was significant ($P < 0.01$). Looking at the relative abundance of neutral and acidic PS, except BF2 effluent, all water samples have relatively higher concentrations of acidic PS compared to neutral PS. For BF2 effluent, almost equal amounts of neutral and acidic PS were found.

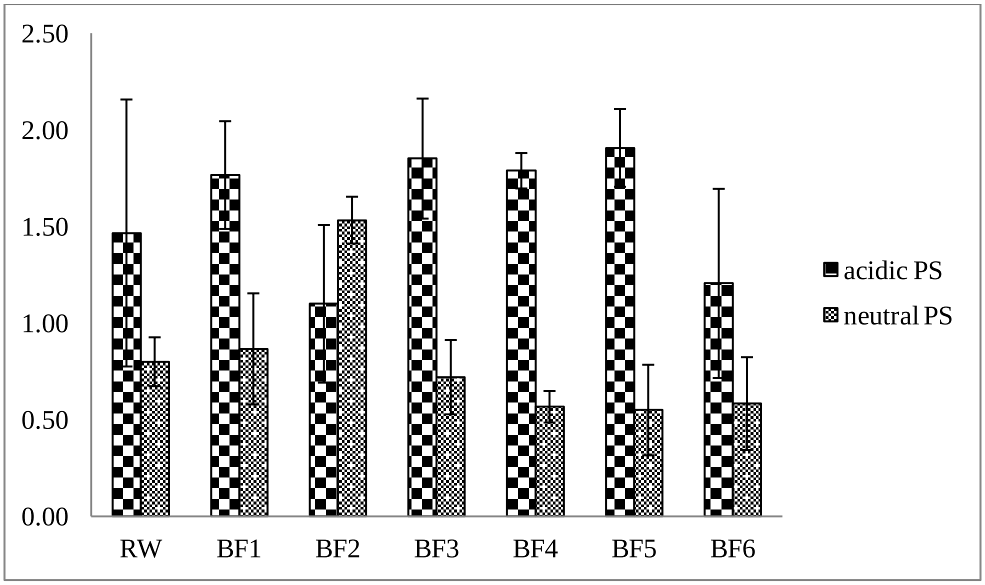

Figure 4-7 Neutral PS and acidic PS presented in water samples collected from the Peterborough DWPP, June 23rd, 2014. RW and BF1-BF6 indicate raw water and effluent samples from BF1 to BF6. Error bars represent standard deviation among triplicate water samples with duplicate absorbance measurements (n = 6).

4.3.2 Water Samples from the Mannheim DWPP

Figure 4-8 shows the concentrations of neutral PS and acidic PS presented in RW (pretreated by a roughing filter prior to biofilter) and biofilter effluent samples collected on July 24th, 2014, from Mannheim DWPP. Small reductions were observed for neutral PS (from 4.3 ±0.3 mg/L to 4.2 ±0.3 mg/L) and acidic PS (2.0 ±0.2 mg/L to 1.8 ±0.4 mg/L) following biofiltration. However, the concentration differences were not statistically significant between RW and biofilter effluent for both neutral PS (P=0.55,) and acidic PS (P=0.36).

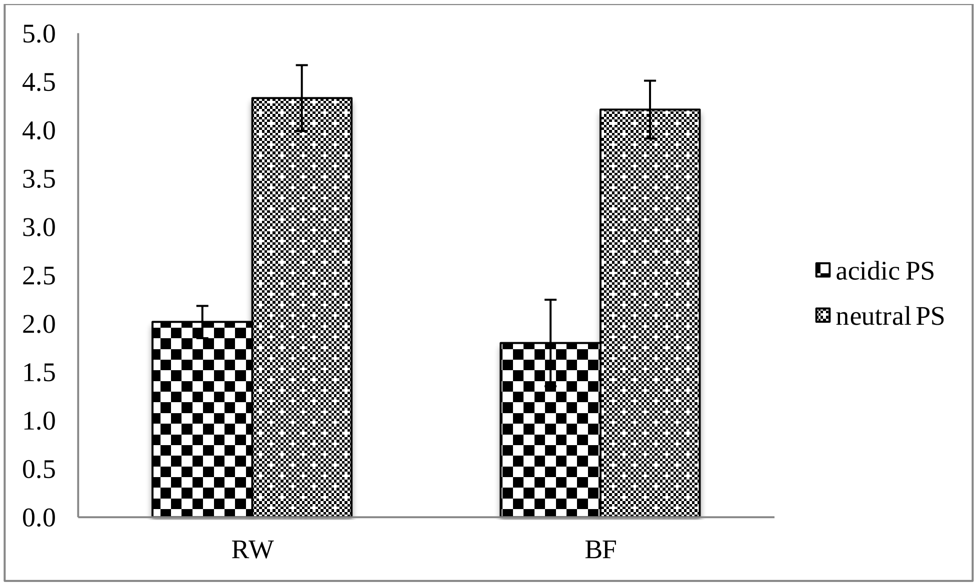

Figure 4-8 Neutral PS and Acidic PS presented in water samples collected from the Mannheim DWPP, July 24th, 2014. RW and BF indicate raw water and BFc effluent. Error bars represent standard deviation among triplicate water samples with duplicate absorbance measurements (n = 6).

As shown in Figure 4-9, for samples obtained on August 7th, 2014, in additional to neutral and acidic PS, TEP was also quantified following the successful establishment of TEP analysis. Similar to July samples, small reductions were observed for neutral PS (from 3.5 ±0.1 to 3.4 ±0.1 mg/L) and acidic PS (from 2.3 ±0.4 to 1.9 ±0.3 mg/L), as well as for TEP (from 2.9 ±0.9 to 2.3 ±0.7 mg/L). Statistical analysis suggests no significant differences of neutral PS (P = 0.09) and acidic PS (P = 0.12) between RW and biofilter effluent. However, TEP concentrations were significantly different between RW and biofilter effluent (P= 0.01).

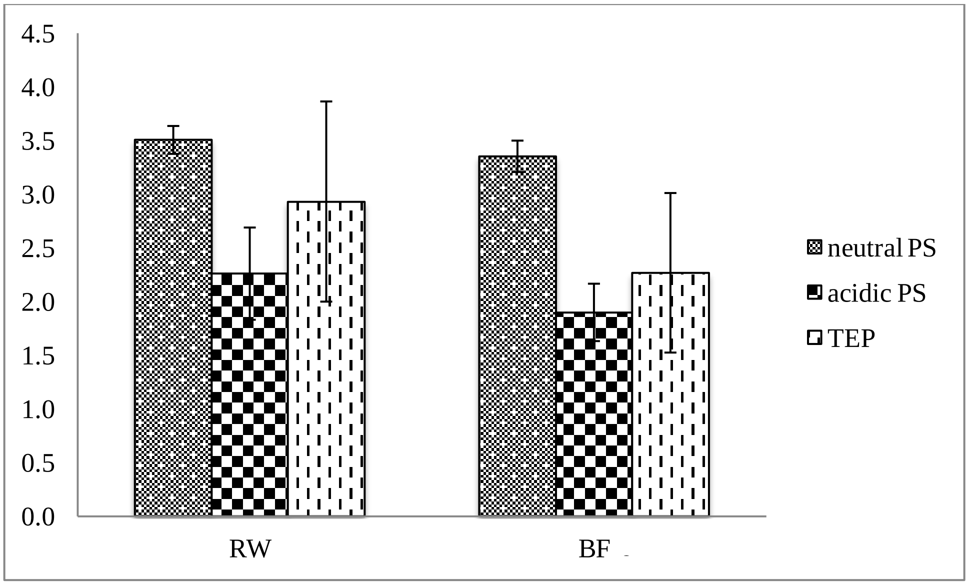

Figure 4-9 Neutral PS, Acidic PS and TEP presented in water samples collected from the Mannheim DWPP, August 7th, 2014. RW and BF indicate raw water and BFc effluent. Error bars represent standard deviation among triplicate water samples with duplicate absorbance measurements (n = 6).

4.4 EPS Compositions of Biofilters

The treatment efficiency of biofilter is strongly influenced by the dynamic of EPS developed inside the biofilter. Stable EPS can help sequester the nutrients from the environment for bacterial growth and promote the removal of biopolymeric materials through microbial degradation. However, unstable EPS will lead to an EPS sloughing event, which has adverse effects on downstream membrane filtration. Therefore, the purpose of studying the nature of EPS present in biofilters was of interest to help understand the biofiltration systems from a microbiological perspective. EPS characterization was done by analyzing individual components of EPS, including PN, PS (acidic and neutral fractions), and HS.

The EPS from the biofilms established in two large-scale biofilters located at the Peterborough DWPP, were extracted and the constituents, including neutral PS, acidic PS, PN and HS were analyzed. BF2 was fed with a nutrient supplement consisting of phosphoric acid and ammonia at a concentration of 0.5 mg/L, and BF1 was the control without nutrient amendment. The result of the analyses on samples collected on May 15th, 2014, is shown in Table 4-12. BF2 yielded a slightly higher level of total EPS compared to the control biofilter (BF1). For both biofilters, the acidic PS and HS fractions were the most dominant EPS constituents.

Table 4-12 EPS compositions of biofilter biofilms in two large-scale biofilters located at the Peterborough DWPP, May 15th, 2014

Large-scale Biofilter	BF1	BF2
mg Neutral PS/ g WW	0.069 ±0.008	0.080 ±0.007
mg Acidic PS/ g WW	0.371 ±0.007	0.423 ±0.006
mg PN/ g WW	0.138 ±0.002	0.154 ±0.005
mg HS/ g WW	0.388 ±0.010	0.395 ±0.014
Total EPS	0.966 ±0.027	1.052 ±0.032

As shown in Table 4-13, for samples collected on June 23rd, 2014, all EPS components had similar concentrations in both of biofilters (BF1 and BF2), and the amounts of total EPS were very similar in between BF2 (nutrient addition) and BF1 (control).

Table 4-13 EPS compositions of biofilter biofilms in two large-scale biofilters located at the Peterborough DWPP, June 23rd, 2014

Large-scale Biofilter	BF1	BF2
mg Neutral PS/ g DW	0.065 ±0.007	0.068 ±0.004
mg Acidic PS/ g DW	0.444 ±0.028	0.434 ±0.036
mg PN/ g DW	0.134 ±0.006	0.138 ±0.001
mg HS/ g DW	0.290 ±0.047	0.272 ±0.012
Total EPS	0.934 ±0.089	0.912 ±0.053

BF3 to BF6 are the four small-biofilters, located at the Peterborough DWPP. BF3 was designed as a control biofilter using anthracite as filter media. BF4 with anthracite was supplemented with 0.2 mg/L hydrogen peroxide. BF5 with anthracite was combined with inline coagulation, where alum was used as the coagulant at a dose of 0.2 mg/L. BF6 used GAC as filter media instead of anthracite.

In Table 4-14, for filter media collected from small-scale biofilers on May 15th, 2014, the biofilter with inline coagulation (BF5) had the lowest levels of total EPS. Compared to the control biofilter (BF3), addition of hydrogen peroxide slightly reduced the total EPS content in BF4. BF6 with GAC as filter media also have a lower level of total EPS compared to that in BF3.

Table 4-14 EPS compositions of biofilter biofilms in small-scale biofilters located at the Peterborough DWPP, May 15th, 2014

Small-scale Biofilter	BF3	BF4	BF5	BF6
mg Neutral PS/ g WW	0.066 ±0.007	0.075 ±0.021	0.062 ±0.008	0.074 ±0.019
mg Acidic PS/ g WW	0.350 ±0.019	0.373 ±0.021	0.228 ±0.001	0.205 ±0.024
mg PN/ g WW	0.113 ±0.007	0.115 ±0.004	0.100 ±0.006	0.271 ±0.005
mg HS/ g WW	0.419 ±0.016	0.356 ±0.038	0.359 ±0.021	0.216 ±0.035
Total EPS	0.948 ±0.049	0.919 ±0.084	0.748 ±0.036	0.765 ±0.083

As shown in Table 4-15, total EPS was different in biofilm samples from four small-scale biofilters collected on June 23rd, 2014. With hydrogen peroxide addition, an increased of total EPS was observed in BF4, compared to the control biofilter (BF3). Inline coagulation (BF5) effectively reduced the total EPS. BF6 also had a lower total EPS content than that in BF3.

Table 4-15 EPS compositions of biofilter biofilms in small-scale biofilters located at the Peterborough DWPP, June 23rd, 2014

Small-scale Biofilter	BF3	BF4	BF5	BF6
mg Neutral PS/ g DW	0.055 ±0.013	0.051 ±0.004	0.043 ±0.004	0.060 ±0.026
mg Acidic PS/ g DW	0.267 ±0.009	0.289 ±0.038	0.226 ±0.015	0.164 ±0.012
mg PN/ g DW	0.106 ±0.001	0.076 ±0.005	0.107 ±0.005	0.139 ±0.009
mg HS/ g DW	0.138 ±0.015	0.208 ±0.027	0.102 ±0.057	0.036 ±0.016
Total EPS	0.566 ±0.038	0.624 ±0.074	0.478 ±0.081	0.399 ±0.063

As indicated in Table 4-16, relative low EPS contents were found in biofilms in the filter media collected from the Mannheim DWPP, compared to the EPS concentrations from biofilters located at Peterborough DWPP. Over the course of 9 days between the two sampling times, total EPS content remained the same. However, EPS composition changed, where neutral PS and PN increased and acidic PS decreased.

Table 4-16 EPS compositions of biofilter biofilms in biofilter located at Mannheim DWPP on July 24th and August 7th, 2014

	July 24th BFc	August 7th BFc
mg Neutral PS/ g WW	0.018 ±0.022	0.027 ±0.006
mg Acidic PS/ g WW	0.065 ±0.026	0.046 ±0.032
mg PN/ g WW	0.034 ±0.010	0.046 ±0.006
mg HS/ g WW	N/D	N/D
Total EPS	0.117 ±0.058	0.118 ±0.044

N/D indicated concentration below detection limit.

4.5 Microbial Community

The microbial communities in raw water and in biofilters, as well as effluents, were examined. This was done to first illustrate whether there was a relationship between the source water and the community that develops in the biofilter. Secondly, the stability of the community structure was of interest to establish whether a highly compartmentalized community, distinct and relatively stable, formed within the biofilters. Samples were collected and DNA was extracted and 16S RNA genes were amplified as outlined in the Chapter 3 (Methods and Materials). After amplification, 16S RNA genes were collected for both DGGE and sequence analysis, where only DGGE results are shown and discussed, and the latter results are not being presented in this thesis.

DGGE has been used a basis for characterizing microbial communities (Muyzer et al., (1998). A complex banding pattern produced by DGGE analysis reflects the diversity of the microbial communities in these samples. There are limitations associated with DGGE analysis (i.e. only the major population of the analyzed community are represented by the DGGE patterns). However, it is possible to illuminate clear differences of microbial communities between the samples. Hence, changes of microbial community in the samples over time or due to changes of environment can be monitored (Boon et al., 2002). Cluster analysis of DGGE banding profiles of amplified 16S RNA genes are conducted to generate dendrograms that are used to depict the similarities in banding pattern, reflecting the similarities of microbial communities.

For the samples collected on May 15th, 2014, from the Peterborough DWPP, the banding patterns observed in biofilter media and water samples were clearly different. Fewer bands were observed in BF1-BF3 media sample, indicating less microbial diversity in those three biofilters. Compared to biofilter media biofilm samples, water samples had higher magnitude of diversity.

Dendrogram of DGGE banding of May 15th, 2014, samples is shown in Figure 4-10, where 13 samples were grouped into two main clusters. In the main cluster that only consists of water samples, effluents from BF5 formed one subset by itself, joined with a highly complex subset formed by RW and effluents from BF1, BF2, BF3, BF4 and BF6. In the bottom main cluster that only includes biofilter media samples, media from BF4 and BF5 formed one subset and joined with a complex subset formed by media from BF1, BF2, BF3 and BF6. Microbial similarity with over 40% was observed between the two main clusters. Furthermore, biofilter water samples showed a microbial similarity of over 48%, and 6 biofilter media biofilm samples had microbial similarity of about 52%.

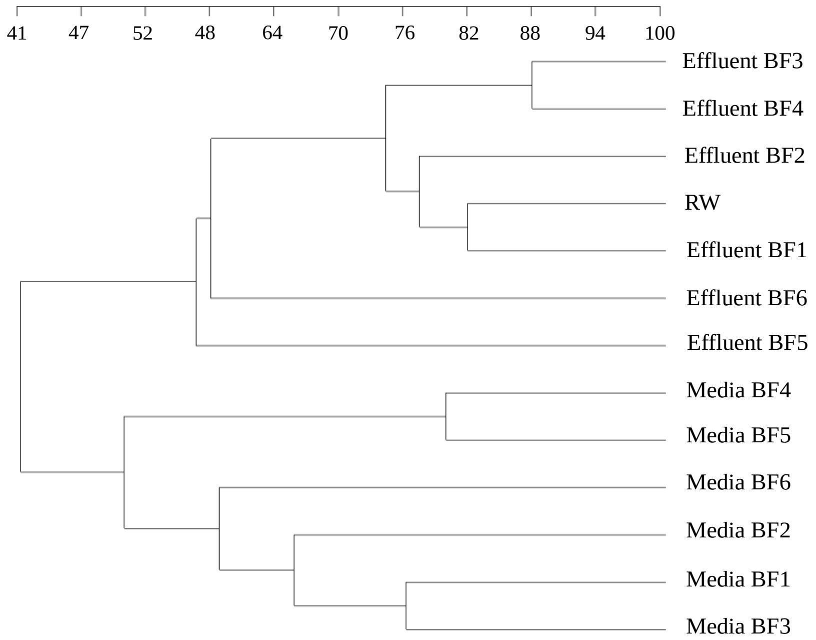

Figure 4-10 Dendrogram showing the relatedness of PCR-DGGE fingerprints from biofilter media, raw water and biofilter effluents collected from Peterborough DWPP, May 15th, 2014.

For the samples collected on June 23rd, 2014, from the Peterborough DWPP, with a total of 16 samples being analyzed, 13 samples with bands appearance indicated the presence of 16s rRNA genes, where no bands were generated from 3 samples including filter media from BF1 and BF2, as well RW. Agarose gel image of PCR products had shown the presence of amplified 16S rRNA genes of all 16 samples. Therefore, no bands shown for BF1 and BF2 media and RW might be the result of the lost 16S rRNA genes during DGGE analysis. Similar banding patterns were observed among effluents samples except effluent from BF1. Although the banding pattern of BF1 effluent differed from the other effluent samples, its pattern was similar to those of media samples.

Figure 4-11 shows the result of cluster analysis of DGGE banding profile of June 23rd samples. Although 16 samples were analyzed by DGGE, no bands were detected in three lanes, corresponding to media from BF1 and BF1, and RW samples. Therefore, only13 samples with detected bands were grouped into two main clusters. Effluent samples formed one main cluster, and biofilter media together with effluent from BF1 formed another main cluster. A microbial similarity of over 30% was observed between the majority of effluents samples and biofilter media. June effluent samples shared a microbial similarity of 77%. Among effluents samples, the microbial communities present in effluents from small-scale biofilters had a high similarity of over 86%, joining BF2 forming a bigger subset. Compared to effluent samples, a lower similarity of 45% was observed among biofilter media, including effluent from BF1.

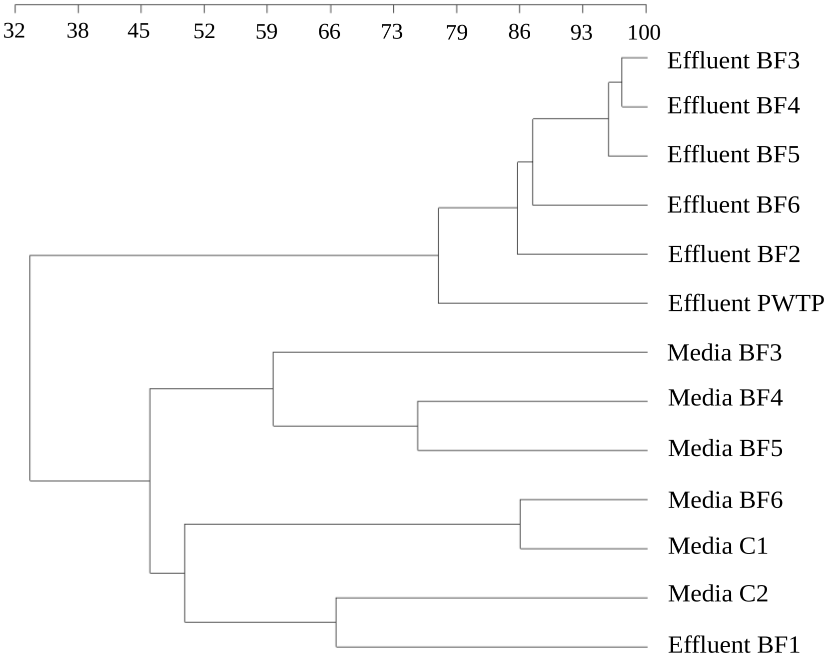

Figure 4-11 Dendrogram showing the relatedness of PCR-DGGE fingerprints from biofilter media, raw water and biofilter effluents, collected from Peterborough DWPP, June 23rd, 2014.

Based on the result of cluster analysis as shown in Figure 4-12, a very high similarity was observed between RW and effluent samples from BFc, forming a main cluster. The microbial communities in biofilms extracted from duplicate BFc (B5e & B5c) were also very similar, with a similarity value of 82%. The two main clusters formed by media samples and water samples separately shared a similarity of 65%.

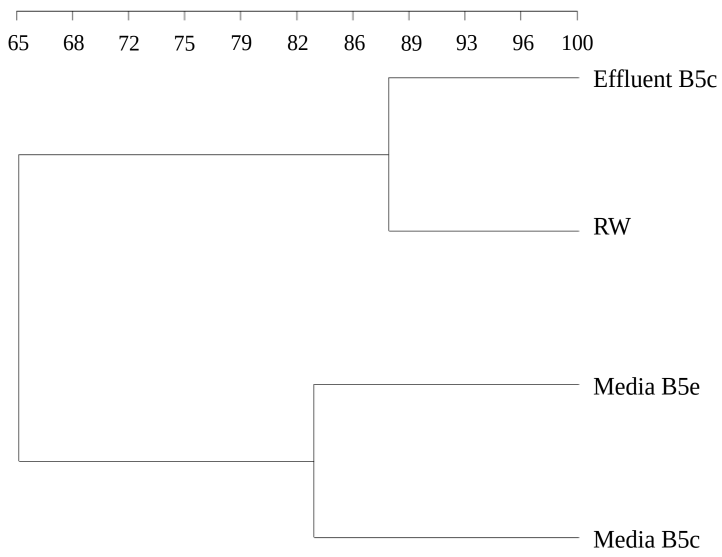

Figure 4-12 Dendrogram showing the relatedness of PCR-DGGE fingerprints from biofilter media, raw water and biofilter effluent collected from Peterborough DWPP, August 7th, 2014.

Chapter 5 Discussions

Direct biofiltration, or passive biofiltration (without prior coagulation, nutrient amendment, or ozonation) has been reported as an effective pretreatment approach for membrane fouling control in surface water treatment (Hallé et al., 2009; Peldszus et al., 2012). As a fixed-film bioreactor, a biofilter and its performance is also closely associated to EPS, which are the main constituents of biofilms developed inside biofilter. Clogging of biofilter has been reported, mainly due to the build-up of biomass (including EPS), as a result of microbial activity. EPS associated with biofilm are important to headloss of a biofilter since they can occupy as much as 1,000 times the void space of filter media compared to bacteria (Mauclaire et al., 2004). EPS sloughing from a biofitler also has detrimental effect on downstream membrane filtration. As EPS make contact with the membrane, they form a gel layer on the membrane surface and facilitate undesirable microbial colonization (biofouling) (Nguyen et al., 2012). Thus, biofiltration systems in drinking water treatment are operated with backwashing step to control biofilm. Lauderdale et al. (2012) proposed two alternatives of control biofilm aiming at improving biofilter performance in drink water treatment, by 1) managing EPS production through nutrient supplementation, and 2) direct removal of EPS through hydrogen peroxide (H_2O_2) supplementation. Other enhancement strategies for biofilter performance include inline coagulation and using GAC as filter media.

In this study, two biofiltration systems with different operational designs were investigated, located at the Peterborough and Mannheim DWPPs. At the Peterborough DWPP, 6 biofilters are operated to study the effects different operational conditions on biofitler performance, including nutrient enrichment, H_2O_2 supplementation, inline coagulation, as well as using GAC as filter media. At the Mannheim DWPP, passive biofilters are operated without any chemical addition to study the treatment efficiency of direction biofiltration. The performances of passive biofilters (direct biofiltration) and biofilters operated under different conditions were investigated by comparing the concentrations of neutral and acidic PS and TEP in RW and biofilter effluents. Due to the low concentrations of biopolymers presented in surface water, available colorimetric methods for PN and PS quantification mainly employed in wastewater studies are not sensitive enough to detect the small amounts of PN and PS presented in biofilter associated water samples (Drews, 2010). Therefore, a new approach using freeze-drying process was developed to tackle the low concentration challenge of biopolymers.

5.1 Direct Biofiltration Performance

The effect of direct biofiltration was investigated by analyzing samples from three passive biofilters (no enhancement), two located at the Peterborough DWPP and one located at Mannheim DWPP.

For acidic and neutral PS removal, paired t-test showed no significant reduction through two passive biofilters (BF1 and BF3) located at the Peterborough DWPP on the first sampling date. On the second sampling date, 20% and 27% increases of acidic PS were observed in BF1 and BF3. These results suggest that direct biofiltration was not effectively removing PS over the course of 5 weeks at the Peterborough DWPP. In a previous study conducted by Azzeh et al. (2014) using the same biofiltration systems at the Peterborough DWPP, 17% of biopolymer (including PS and PN) and 5% of DOC removal were observed through one of the passive biofilter. As different removal rates in terms of biopolymers and DOC were observed, Azzeh et al. (2014) suggested that DOC measurements do not provide an effective information about the biopolymer removal. Similarly, biopolymer measurements may not be an effective way to determine PS removal. The increase of acidic PS observed may indicate the occurrence of EPS sloughing. This explanation is provided based on the observation that acidic PS accounted for the largest portion of total EPS (36-47%) for both the passive biofilters. Therefore, if EPS slough off the biofilters and leave the system with the effluent, the likelihood of EPS sloughing leading to a noticeable increase of acidic PS in a biofilter effluent is high.

On the other hand, a positive impact was observed in the direct biofiltration system located at the Mannheim DWPP. Consistent removals of acidic PS and neutral PS were observed in the passive biofilter, and acidic removal (11-15%) was higher than neutral PS removal (3-4% removal). A similar removal rate of DOC (<15%) was reported by Peldszus et al. (2012) using the same direct biofiltration system at the Mannheim DWPP. Latter developed TEP quantification in this study shows a 22% removal of TEP by the same biofilter. Although TEP is one type of acidic PS, higher removal rate of TEP (22%) than acidic PS (11-15%) indicate a gap between acidic PS and TEP quantification. Hence, acidic PS measurement may not efective to predict TEP removal.

The better performance of direct biofiltration at the Manheim DWPP than that at that Peterborough DWPP might be the result of the installation of a roughing filter prior to the passive biofilter at the Manheim DWPP. According to Peldszus et al. (2012), the roughing filter was designed to tackle the variations of turbidity and remove the particulate matters (including biological materials) present in river water. Therefore, the pre-screening process of a roughing filter prior to a passive biofilter might result in the better performance of the passive biofilter in terms of PS (neutral, acidic and TEP) removal.

5.2 Impact of Nutrient Enhancement

Phosphorus plays an essential role in cell growth and function. The impact of limiting phosphorus availability to biological wastewater treatment systems has been reported, for example, morphological changes of in floc structure and the composition of EPS can result from the depletion of phosphorus (Liu et al., 2006). Studies show that phosphorus addition to engineered bioreactor systems can improve bioreactor performance by promoting microbial growth (Nishijima et al., 1997; Li et al., 2010). In this study, with nutrient supplementation (phosphoric acid and ammonia at a concentration of 0.5 mg/L), changes of PS composition were observed, as 25-32% reduction (0.37-0.8 mg/L) of acidic PS and 14-92% increase (0.1-0.7 mg/L) of neutral PS was found in BF2 effluent. Although the role of neutral PS in membrane fouling is still a mystery, acidic PS has been recognized as problematic substances for membrane filtration due to their capability of aggregating and high tendency of biofilm formation (Leppard, 1997). This preliminary finding indicates that the removal of acidic PS can be promoted by providing the biofilter with phosphorus and nitrogen supplementation.

Lauderdale et al. (2012) reported that nutrient enrichment was found to reduce the headloss of the biofilter by 15%, which might be the result of a decrease of EPS production from microorganisms. In this study, results show that nutrient enrichment did not reduce EPS production. On contrast, an increase of EPS (including PN, PS and HS) was observed on the second sampling date, which suggests that nutrient enrichment is not capable of controlling EPS production. Azzeh et al. (2014) who investigated the same biofiltration system with nutrient enrichment suggested that neither phosphorous nor nitrogen was limited across filter. Instead, biodegradable organic carbon was limited. Hence, providing phosphorus and nitrogen may promote the degradation of acidic PS but it does not help control the EPS production.

5.3 Impact of H_2O_2 Supplementation

With the purpose of improving the operational performance of a biofilter, 0.2 mg/L of H_2O_2 was added in-line to biofilter influent. Low doses of peroxide (≤ 1 mg/L) are believed be an approach that can effective oxidize and remove EPS and inactive biomass without negatively affecting the biological activity inside biofilters (Lauderdale et al., 2012).

Azzeh et al. (2014) reported that EPS contents were 6% and 52% lower than the control biofiler, when 0.5 mg/L and 1 mg/L H_2O_2 was applied, respectively, whereas 0.1 mg/L of peroxide resulted in an increase of EPS by 30%. However, at a dose of 1 mg/L H_2O_2, biopolymer removal decreased by 6% indicating a high dose of peroxide might oxidize the biofilm and cause the release of biopolymers. In this study, the impact of H_2O_2 addition at a dose of 0.2 mg/L on effluent quality differed from two sampling times, whereas 11% decrease of acidic PS and 12% increase of neutral PS on the first sampling date and 20% increase of acidic PS and 29% decrease of neutral PS. Compared to control biofilter (BF3), a lower EPS content of was observed on the first sampling date, and higher on the second sampling date. Interestingly, a similar trend can be found between acidic PS and EPS content. Peroxide demand was 0.1-0.3 mg/L prior to the biofilter (Azzeh et al., 2014). Therefore, the changes of acidic and neutral PS removal, as well as EPS contents, might link to the fluctuation of river water quality. This suggests 0.2 mg/L of H_2O_2 may not be always sufficient enough.

5.4 Impact of Inline Coagulation

As pretreatment strategies, both coagulation and biofiltration have been shown to effectively reduce membrane fouling in drinking water treatment (Chen et al., 2007; Hallé et al., 2009; Peldszus et al., 2012). Therefore, a combination of coagulation and biofiltration has the potential to further improve membrane performance. Studies have demonstrated a synergistic impact when combining coagulation and biofiltration in drinking water treatment and advance treatment of sewage (Zhang et al., 1998; Hidaka et al., 2003).

In Azzeh et al. (2014), additional 3% and 4% of biopolymer removals were found, when 0.1 mg/L and 0.25 mg/L of alum were added, respectively. In this study, analyses were performed to look at the impact of inline alum addition (0.2 mg/L) on acidic PS and neutral PS removal, as well as EPS composition in biofilter biofilms. With inline coagulation, poor performances in terms of acidic PS removal were observed on both sampling dates over a course of 5 weeks, where acidic PS were 9-30% higher than those presented in RW. Neutral PS removals were inconsistent, as a 7% decrease and 31% increase were observed on the first and second sampling date, respectively. Although inline alum addition at a dose of 0.2 mg/L did not show consistent removal of acidic PS and neutral PS removal, consistent reductions of EPS were observed in the biofiler with alum addition, where EPS content was 16-21% lower than that in the control biofilter. This result is similar to Azzeh et al. (2014) observation, where 0.1 mg/L of alum effectively reduced the headloss of the biofilters by 40%.

5.5 Impact of Employing GAC as Filter Media

GAC has been reported as a more robust support media than anthracite to support biological growth in drinking water treatment (Nyfennegger et al., 2013), mainly due to its high surface area and porous nature (Dussert and Tramposch, 1997).

Biofilter with GAC removed on average an additional 0.35 mg/L of acidic PS and 0.05 mg/L of neutral PS, when compared to the control biofilter using anthracite as filter media. In Azzeh et al. (2014) study, GAC showed on average an additional 0.1 DOC removal. However, no improvement of biopolymer removal was observed when compared to anthracite. The differences between the observation from this study and Azzeh et al. (2014) study may indicate the gap among PS (including acidic PS and neutral PS), biopolymer and DOC measurements. In addition, 19-29% less of EPS were found in GAC biofilter, compared to anthracite biofilter, suggesting biofilters using GAC as filter media may be less prone to biofilter clogging.

5.6 Microbial Community Analysis

As indicated from the cluster analysis of DGGE profiles, a high similarity (65%) of microbial community between RW and effluent was observed in the direct biofiltration system located at the Mannheim DWPP. At the Peterborough DWPP, relatively high similarities (32-41%) were also observed between RW and effluents from the biofilters operated under different conditions. This result underlines that a biofilter microbial community is susceptible to the changes of biofilter environmental including the source water. Physiochemical properties (i.e. nutrients, temperature) of source water have a strong influence on the structure of a microbial community. For example, Pang and Liu (2006) studied the influence of the organic carbon content on the composition and succession of biofilm community, and found that the differences of organic carbon in two water samples led to the selection of distinct biofilm communities. Interestingly, peroxide addition and inline alum addition were found to result in a over 75% similarities in terms of microbial community, indicating peroxide and alum might have the same impact on microbial community.

Chapter 6 Conclusions

In this study, a combination of freeze-drying with colorimetric analysis has been developed and proved to be a reliable and cost-effective method to measure low concentrations acidic and neutral PS presented in surface water samples. This method provides a means to evaluate biofilter performance in terms of PS removal. 0.45 µm pore size polycarboante filters were found to be ineffective for TEP quantification. When TEP was present at low concentrations, the majority of the particles can escape through the filter pores with diameter of 0.45 µm. Filters with reduced pore size to 0.1 µm were found to be suitable for TEP measurement with high accuracy.

Through the chemical and microbial analysis of RW, biofilter effluents and EPS extracted from biofilter, the main findings of this study are summarized as follows:

- Passive biofiltration performance varied when treating different water from different sources. When directly treating water from the Otonabee River, passive biofiltration systems located at the Peterborough DWPP did not effectively reduce PS contents. However, the passive biofiltration system at Mannheim DWPP treating water from the Grand River demonstrated a consistent removal of acidic PS (11-15%) and neutral PS (3-4%). A significant reduction of TEP (22%) was also observed in Mannheim passive biofilter. The better performance of passive biofilter at Manheim DWPP might attribute to the installation of a roughing filter prior to the passive biofilter.

- Nutrient enhancement with phosphoric acid and ammonia at a concentration of 0.5 mg/L result in a decrease of acidic PS (0.37-0.8 mg/L) and an increase of neutral PS (0.1-0.7

mg/L), indicating a change of PS composition in the biofilter effluent due the addition of nutrient. No EPS reduction was observed in this biofilter.

- Biofilter with a 0.2 mg/L hydrogen peroxide addition did not show consistent acidic PS, neutral PS and EPS removal. Hence, a dose 0.2 mg/L of peroxide was not sufficient enough, and might not have the same impact on the biofilter with the fluctuation of the source water chemistry.

- Inline coagulation with 0.2 mg/L of alum effectively reduced EPS content by 16%-21%. However, addition of alum caused increase of acidc PS in the effluent. In addition, no consistent neutral PS was observed.

- Biofilter using GAC as filter media showed a better removal of PS compared to the control biofilter using anthracite as filter media. Lower EPS production was also observed.

- Microbial community analysis, using DGGE, showed relatively high similarities of microbial community between biofilters and their source waters (30-65%) at both the Peterborough and Mannheim DWPPs. It suggests that biofilters did not develop into stable compartmentalized communities. Therefore, biofilers are subject to fluctuation or shifts linked to the conditions of the source water.

Compared to the RW samples, the evidence of higher acidic PS and neutral PS concentrations in some effluent samples could be the result of EPS sloughing. Biofilter is capable to remove some of the biopolymers present in the source water. Meanwhile, microorganisms inside the biofilter also generate EPS during the biofiltration process, which is the main reason that periodically backwashing is required to restore the hydraulic capacity by removing the EPS accumulated in a biofilter. If EPS develop inside the biofilter is not removed in time, some of the EPS may detach from the filter media and leave the filter with the effluent. A model is proposed as shown in Figure 6-1, depicting the occurrence of EPS sloughing during a biofiltration process.

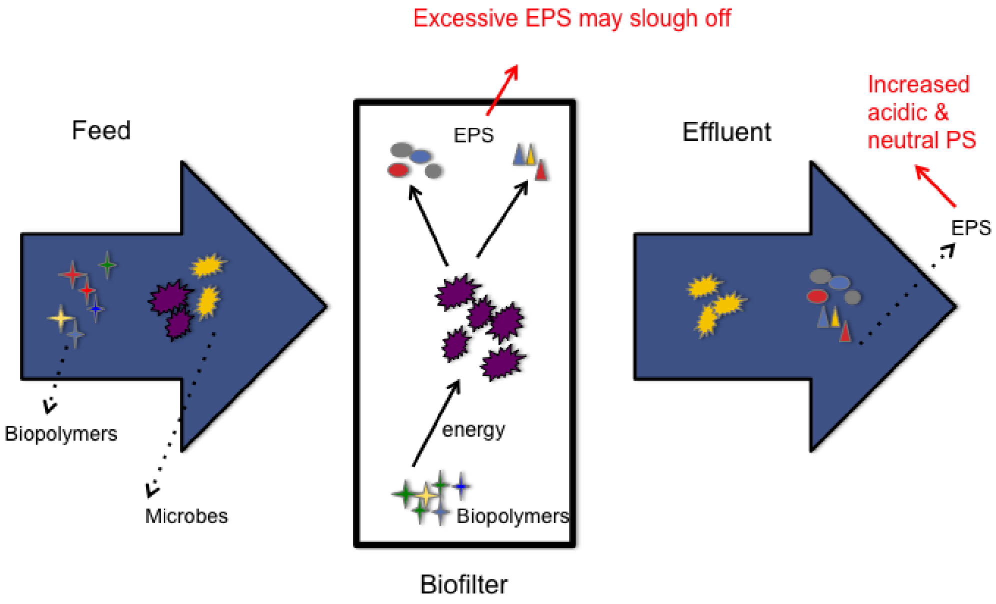

Figure 6-1 Bofiltration model highlighting EPS sloughing during biofiltration process.

Chapter 7 Future Research

- Further analyses of water samples employing freeze-drying and colorimetric methods should be performed to have a better understanding of the impacts of different operating conditions on neutral and acidic PS removal.
- With the availability of TEP quantification method developed in this study, the impacts of different operating conditions on TEP can be evaluated.
- Changes of PS composition were observed in different engineered biofilters (i.e. nutrient enrichment caused decrease of acidic PS and increase of neutral PS). This finding raises the question about the roles that neutral PS and acidic PS play in membrane fouling. Are neutral PS and acidic PS equally important to membrane fouling?

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Appendix: Typical Standard Curves & DGGE Gel Images

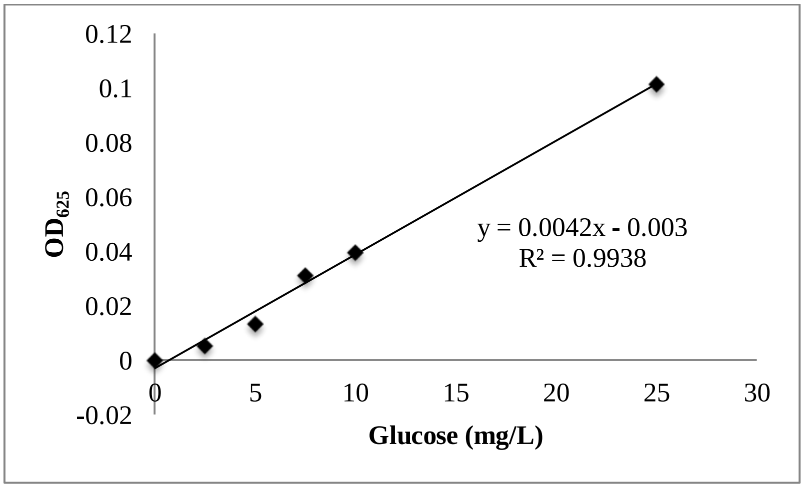

Figure 0-1 Typical standard curve of anthrone method for total PS measurement using glucose as standard with concentration ranged from 2.5 to 25 mg/L. Absorbance was read at 625 nm in triplicate.

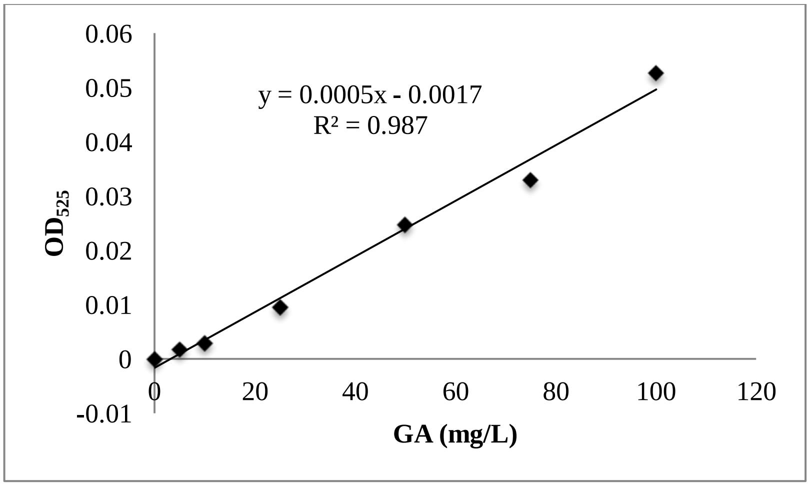

Figure 0-2 Typical acidic PS standard curve using method described by Filisetti-Cozzi and Carpita (1991) with GA as standard with concentration ranged from 5 to 100 mg/L. Absorbance was read at 525 nm in triplicate

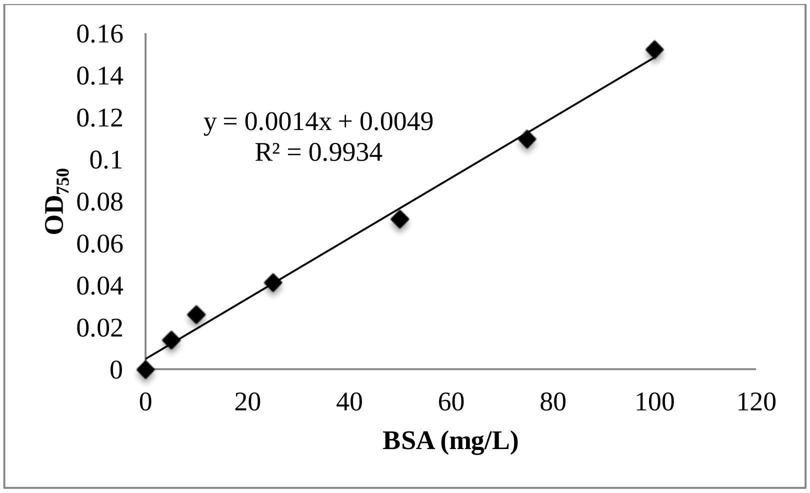

Figure 0-3 Typical standard curve of Lowry method for PN measurement using BSA as standard with concentration ranged from 5 to 100 mg/L. Absorbance was read at wavelength of 750 nm in triplicate.

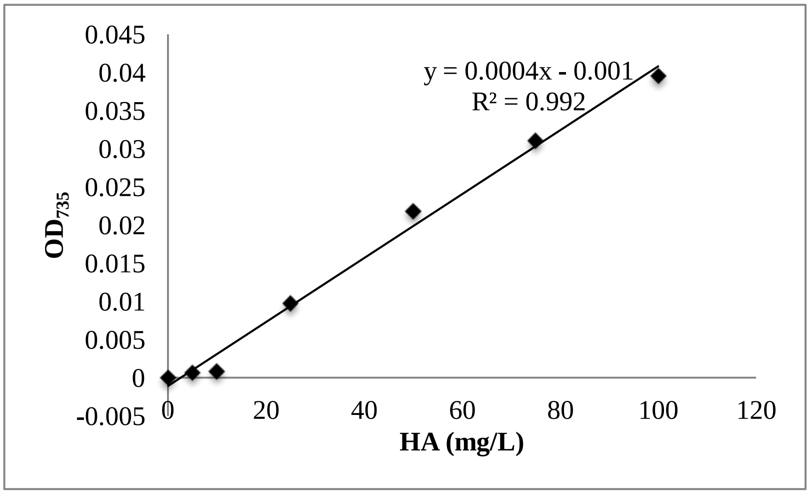

Figure 0-4 Typical standard curve of Lowry method using a microplate for HS measurement using HA as standard with concentration ranged from 5 to 100 mg/L. Absorbance was read at wavelength of 735 nm.

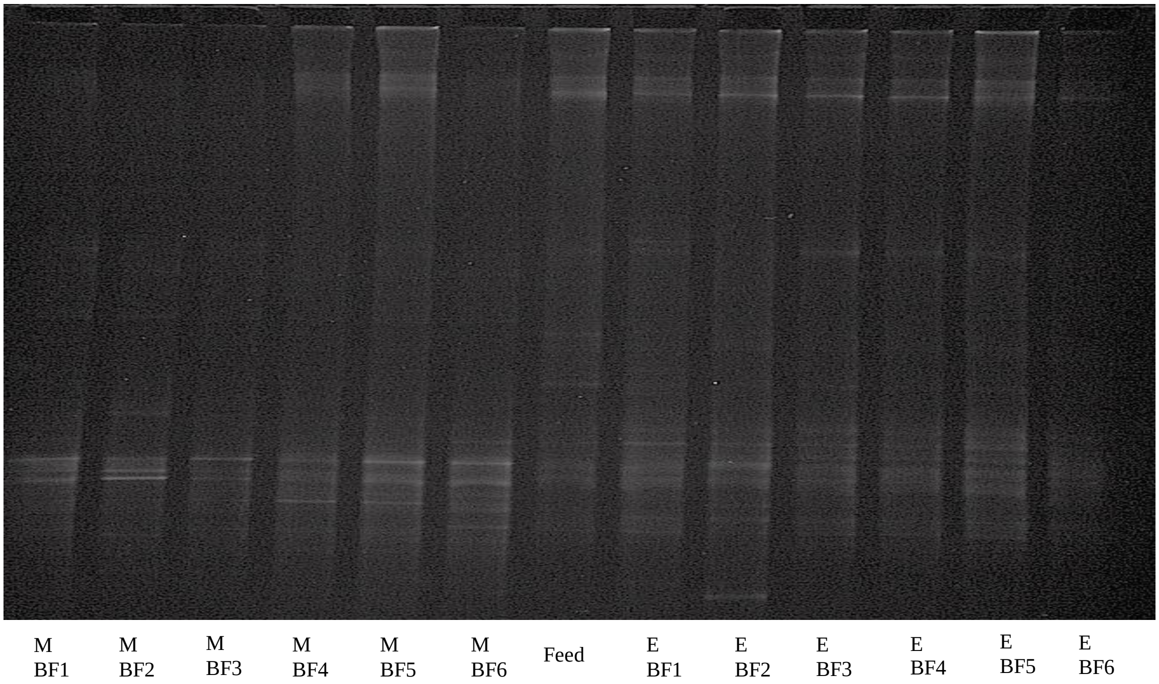

Figure 0-5 Denaturing Gradient Gel Electrophoresis (DGGE) profile of biofilter media, raw water and six biofilter effluents collected from Peterborough DWPP, May 15th, 2014.

MBF1- MBF6 represent filter media from BF1-BF6; RW represents raw water; EBF1-EBF6 represent efflunets from BF1-BF6.

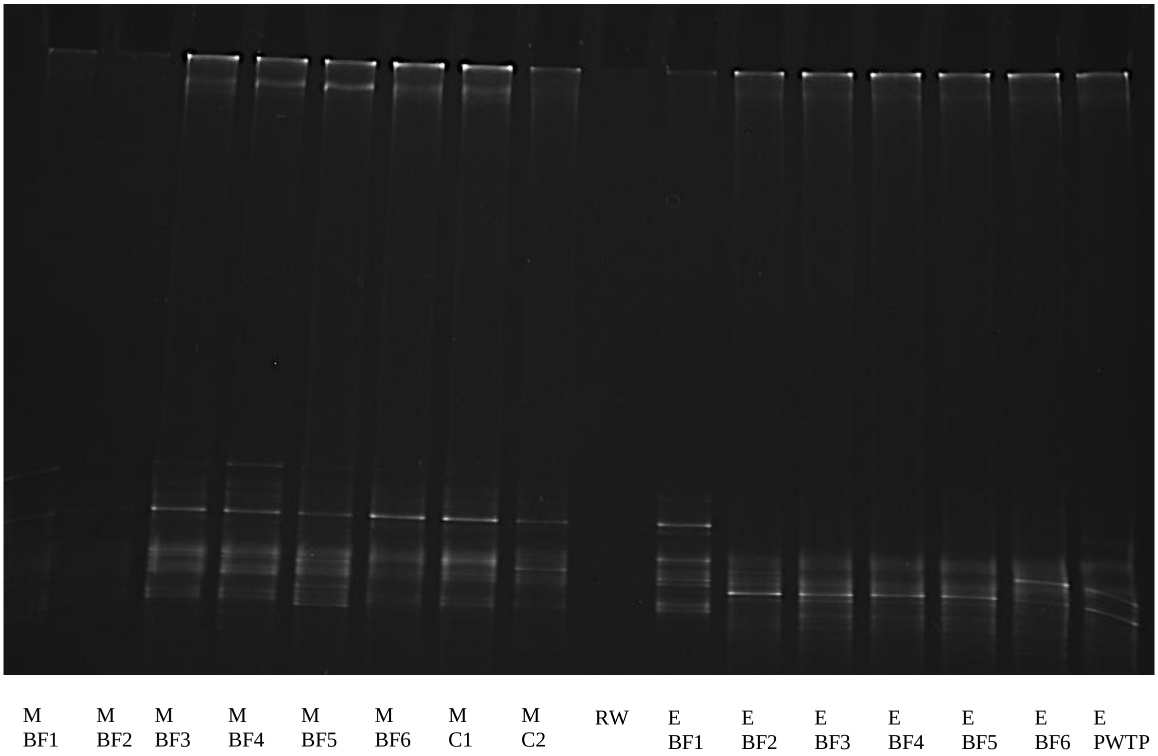

Figure 0-6 Denaturing Gradient Gel Electrophoresis (DGGE) profile of biofilter media, raw water and biofilter effluents collected from Peterborough DWPP, June 23rd, 2014

MBF1- MBF6 represent filter media from BF1-BF6; MC1-MC2: filter media from conventional filter 1-2; EBF1-EBF6 represent effluents from BF1-BF6; EPWTP represents effluent from full-scale conventional filter.

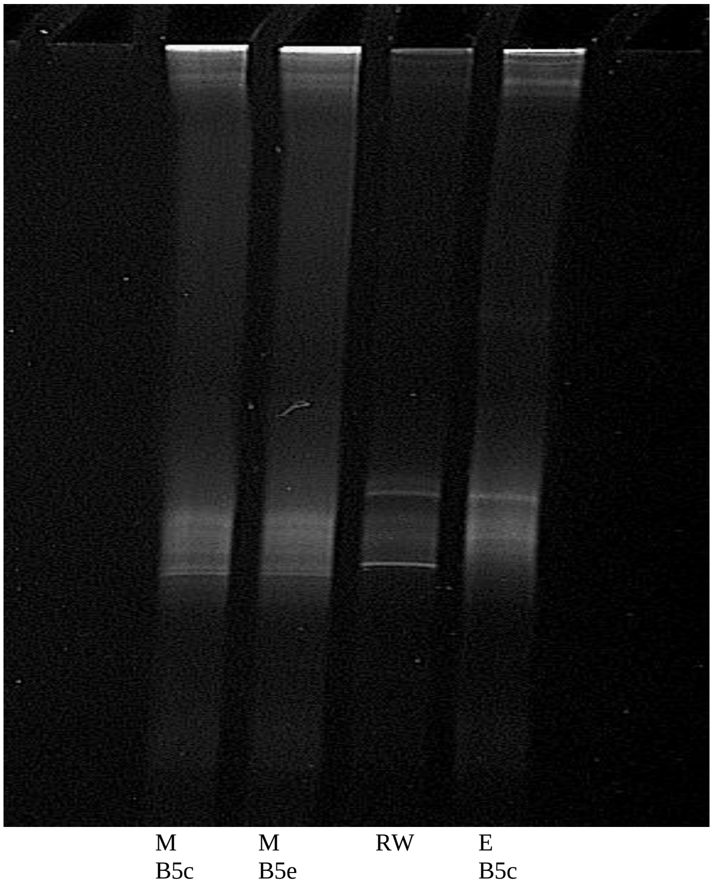

Figure 0-7 Denaturing Gradient Gel Electrophoresis (DGGE) profile of biofilter media, raw water and biofilter effluent collected from Mannheim DWPP, August 7th, 2014

Note: MB5c and MB5e represent filter media from duplicate BFc (B5c and B5e); RW represents raw water; EB5c represents effluent from B5c.