MEMBRANE FOULING STUDY AND IDENTIFYING FOULING MICROORGANISMS IN MEMBRANE BIOREACTORS TREATING MUNICIPAL WASTEWATER

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAIʻI AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

CIVIL AND ENVIRONMENTAL ENGINEERING

DECEMBER 2011

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Keywords: membrane bioreactor, fouling, wastewater

DEDICATION

This dissertation is dedicated to my wife, Jing Meng, and my son, Jianxin Huang whose love and support has made my doctoral studies possible.

ACKNOWLEDGEMENTS

I would like to begin my acknowledging my family and especially my wife Jing Meng and my son Jianxin Huang, who has understood the sacrifices that must be made to ensure a successful pursuit of a doctoral degree. I would also like to acknowledge my parents, who have provided me with encouragement and support throughout my pursuit of education.

I would like to acknowledge the WateReuse Foundation, the Honolulu Board of Water Supply, and the Honolulu Department of Environmental Services and it could not have been completed without the insights, efforts, and dedication of many individuals. Special acknowledgements are in order for all of the operations personnel including Nic Musico, Mark Armas, Bud Reiter, Pat Roberts, Don Henderson, Gary Carolino, Robby Robinson, Bob Choate, Bob Nakamura, and Tod Matsushita.

I would like to thank my advisor, Roger Babcock, who provided valuable critiques and helps throughout the Ph.D. study. I would also like to thank my committee members, Chittaranjan Ray, Albert Kim, Roger Fujioka, and Ping-Yi Yang for their help. I would like to thank Bunnie Yoneyama, who provided valuable helps in laboratory work. I would like to thank Michael Cooney at Hawaii Natural Energy Institute, University of Hawaii at Manoa for his help on processing vacuum freeze-dried membrane samples.

I would like to acknowledge the project team of Honouliuli Pilot Membrane Study including Yingyot Chanthawornsawat, Sumon Kanpirom, Lauren Gannon, Anne Benvel, Jing Hu, Krishna Lamichhane, Yanling Li, Minghua Luo, Tian Liang, Russel Brain, and Jay Jolly. Without their helps, this pilot and bench-scale MBR study was unable to be done. The MBR vendors and their representatives made large contributions to this project

in terms of loaning their equipment, technical assistance and various other assistance including Enviroquip – Dennis Livingston, Kim Matheson, Ray Gauthier, Raymond Hoe, Elena Bailey, Jim DeWulf, and Alfonso Garza; Ionics – Michael Sparks and Gene Reahl; Huber – Christian Roedlich and Mike Elhoff; Koch – Alden Whitney, Antonia VanGottenburg, Ben Antrim, and Chinh Hoang; US Filter – Susan Pilgram, Mike Street, Paul Scott, Ken Windram; Zenon – Paul Schuler, Mark Murphy and Deo Phagoo.

I would like to give my special thanks to Renyong Jia and his advisor, Siqing Xia, at Tongji University, Shanghai, China. They made great contributions in work and funding for DNA sequencing, clone library analysis, and FTIR/SEM/EDX analysis.

ABSTRACT

Two bench membrane bioreactors (MBRs), Bench Enviroquip and Bench Ionics, and four pilot MBRs were operated at Honouliui wastewater treatment plant (WWTP) treating municipal wastewater at 10-d, 20-d, 30-d, 40-d mean cell residence time (MCRT), and no sludge wasting conditions for membrane fouling study and identification of fouling microorganisms. Results indicated that bench and pilot MBRs produced high quality effluent water. Higher critical flux was obtained for bench Ionics MBR (39.3 LMH) than for bench Enviroquip MBR (37.4 LMH). No sludge wasting condition showed higher conditioning and steady-state fouling rates. It was found that SMP concentration had no or little effect on membrane fouling process, while carbohydrate EPS concentration appeared to have a significant impact on steady-state fouling rate. Shock loading condition by glucose addition and no anoxic zone condition following the normal 20-d MCRT operation were also evaluated and results did not show any increase of membrane fouling, indicating bench MBRs are capable of handling shock loading and variance in operating schemes without compromising membrane performance.

Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) fingerprinting analysis and 16S rRNA clone library analysis were performed for the bench-scale MBRs. *Betaproteobacteria* was found to be the dominant bacterial group in MBR mixed liquors (47%). *Pseudomonas sp.* might involve in membrane fouling process since it was detected extensively in biofilm. The presence of *Rhodobacteraceae sp*. and *Brevundionas sp*. might increase membrane fouling too.

Laboratory bench MBR with polyvinylidene fluoride (PVDF) and polyvinyl chloride (PVC) ultrafiltration membrane modules were operated to study optimum ultrafiltation membrane cleaning methods. Majority of the membrane resistance at fouled condition was attributed to cake resistance. Vacuum permeation with Clorox® disinfectant + industrial alcohol or propylene glycol solution was found to be the best cleaning method with the highest membrane flux recovery and lowest membrane re-fouling rates. Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and Energy-dispersive X-ray spectroscopy (EDX) analysis indicated less contaminants were observed or detected in ultrafiltration membranes with the optimum cleaning method. DGGE fingerprinting and clone library analyses showed that bacteria responsible for fouling belonged to *Betaproteobacteria*, *Deltaporteobacteria* and *Bacteroidetes*.

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LIST OF ABBREVIATIONS

AFM	atomic force microscopy
BOD_5	5-day biochemical oxygen demand
BSA	bovine serum albumin
CA	cellulose acetate
CAS	conventional activated sludge
CER	cation exchange resin
COD	chemical oxygen demand
CT	capillary tube
DGGE	denaturing gradient gel electrophoresis
DNA	deoxynucleotide acid
DO	dissolved oxygen
DOTM	direct observation through the membrane
EDX	Energy-dispersive X-ray spectroscopy
EPS	extracellular polysaccharides
FC	pleated filter cartridge
FISH	fluorescence in-situ hybridization
F/M	food to microorganisms
FS	flat sheet
FTIR	Fourier transform infrared spectroscopy
HF	hollow fiber
HRT (θ_H)	hydraulic retention time
kb	kilo base
LMH	liter per minute per hour
M	mole/L
MBR	membrane bioreactor
MCRT	mean cell residence time
MF	microfiltration
ML	mixed liquor
MLSS	mixed liquor suspended solids
MT	multi tubular
NF	nanofiltration
NWRI	National Water Research Institute
OTU	operational taxonomic unit

Pa	Pascal
PAC	powdered activated carbon
PAN	polyacrylonitrile
PCR	polymerase chain reaction
PE	polyethylene
PES	polyetherfulfone
PP	polypropylene
PS	Polysulfone
PTFE	polytetrafluoroethylene
PVC	polyvinyl chloride
PVDF	polyvinylidene difluoride
R	total resistance
R_C	cake resistance
R_F	foulant resistance
R_M	membrane resistance
rRNA	ribosome ribonucleotide acid
RNA	ribonucleotide acid
RO	reverse osmosis
S	Svedberg
SRT	solids retention time
SEM	scanning electron microscopy
SMP	soluble microbial product
SRT	solid retention time
SW	spiral wound
Tm	melting temperature
TMP	transmembrane pressure
TN	total nitrogen
TOC	total organic carbon
TP	total phosphorus
TSS	total suspended solids
UF	ultrafiltration
UV	ultraviolet
UVT	UV transmittance
VSS	volatile suspended solids
WWTP	wastewater treatment plant

CHAPTER 1 INTRODUCTION

1.1 Introduction to Membrane Bioreactor (MBR)

A membrane bioreactor (MBR) is a combination of suspended growth activated sludge biological treatment and membrane filtration equipment performing the critical solids/liquid separation function that is traditionally accomplished using secondary clarifiers. Low-pressure membranes (either microfiltration MF or ultrafiltration UF) are commonly used in MBRs.

1.1.1 Membrane Classifications

Several types of membranes have been developed. Four key types of membrane separation processes primarily used in wastewater treatment include the following:

- Microfiltration (MF)
- Ultrafiltration (UF)
- Nanofiltration (NF), and
- Reverse osmosis (RO)

Spectrum of the four membrane separation processes is presented in Figure 1.1. Of the four membrane separation processes, microfiltration and ultrafiltration are applicable to MBR technologies. MF spectrum is approximately in the range between 0.1 and 1 μm and is capable of removing bacteria. UF spectrum is approximately in the range between 0.01 and 0.1 μm and is capable of removing bacteria and some viruses.

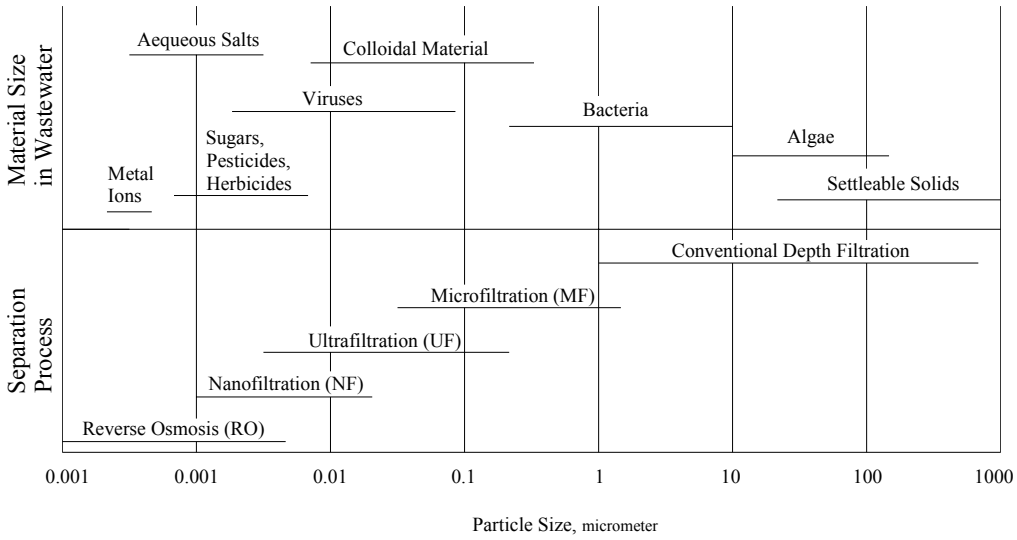


Figure 1.1 Membrane separation process spectrum (courtesy of American Membrane Technology Association)

1.1.2 Membrane Materials

Membranes generally comprise three layers including outside dense layer, middle irregular macrovoid support layer and internal dense layer. Two types of membrane material, polymeric and ceramic, are mainly used for MBR technology. Polymers that can be used for MF/UF membranes include fully hydrophilic polymer cellulose acetate (CA, C6H7O2(OH)3-m(OOCCH3)m, m = 0~3) and fully hydrophobic polymers such as polypropylene (PP, C3H6), polyethylene (PE, (C2H4)n) and polytetrafluoroethylene (PTFE, (C2F4)n) (Judd, 2011). Polysulfone (PS, C81H66O12S3X2), polyetherfulfone (PES, (C12H10O4S)n), polyacrylonitrile (PAN, (C3H3N)x) and polyvinylidene fluoride (PVDF, (C2H2F2)n) are in between hydrophilic and hydrophobic. Membranes used in current MBR technology are mainly made of PES, PVDF or derivatives of PE. PVDF and PS are much stronger than other type of membrane materials. PVDF also has the best flexibility which is required when air scouring of hollow fiber (HF) membrane is employed.

1.1.3 Membrane Configurations

According to the membrane module's structure, membrane processes can be classified into following categories (Judd, 2011):

- Flat sheet (FS)
- Hollow fiber (HF)
- Multi tubular (MT)
- Capillary tube (CT)
- Pleated filter cartridge (FC)
- Spiral wound (SW)

Of the six membrane configurations above, flat sheet and hollow fiber are suitable for MBR technologies. Toady, the leading global MBR suppliers are Zenon (hollow fiber) and Kubota (flat sheet) which rank as the top two of total installed plant capacity.

1.1.4 History of MBR Developments

The concept of an activated sludge process coupled with an ultrafiltration membrane for biomass separation was first developed and commercialized in the late 1960s by Dorr-Oliver (Bemberis et al, 1971; Bailey et al., 1971) and at around the same time by others (Hardt et al., 1970; Smith et al., 1969). In the 1970s the technology first entered the Japanese market through a license agreement between Dorr-Oliver and Sanki Engineering Co. Ltd, where MBRs had a rapid development. In 1980s MBRs were widely applied in Japan for domestic wastewater treatment and decentralized wastewater reuse in skyscrapers. During the early development, the side-stream MBR was the

original configuration, which used external membrane modules. However, MBRs were associated with high membrane cost and high energy consumption.

The submerged MBR was introduced in the late 1980s to reduce the high energy costs (Yamamoto et al., 1989). Since then the MBR technology has developed rapidly. A Japanese company, Kubota developed flat sheet MBRs and a Canadian company, Zenon Environmental, developed hollow fiber MBRs. As of 2009, these two MBR suppliers and Mitsubishi Rayon (hollow fiber MBR) had 4,400 installations around the world and held 85-90% of the municipal MBR market (Judd, 2011).

1.1.5 Configuration of MBRs

According to the configuration, MBR can be classified as side-stream and submerged MBRs as illustrated in Figure 1.2. In side-stream MBRs, the membrane module is separated from the main bioreactor. The sludge in the bioreactor is pumped into a membrane module, where a permeate stream is generated and a concentrated sludge stream is retained by the membrane and returned to the bioreactor. In the early development of side-stream MBRs, both of the transmembrane pressure (TMP) and crossflow velocity were generated by the recirculation pump. However, a few modifications were made to reduce the high energy consumption associated with the side-stream configuration. Firstly, a suction pump was added on the permeate side, which increased the operation flexibility and decreased the crossflow rate and energy consumption (Shimizu et al., 1996). The latest side-stream MBRs even introduced an air flow in the membrane module, which intensified the turbulence in the feed side of the membrane and reduced the fouling and operational cost.

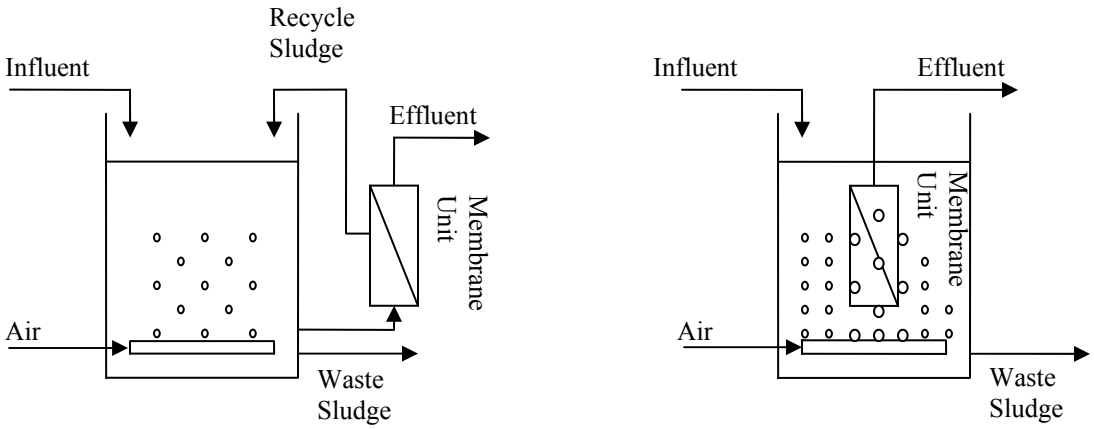


Figure 1.2 Configurations of sidestream MBR (left) and submersed MBR (right)

Aiming to reduce energy consumption associated with the recirculation pump in the sidestream configuration, the submerged BMRs were first introduced by Yamamoto (1989). A membrane module was directly submerged in the bioreactor, which avoided the recirculation pump. Consequently, only a suction pump was used on the permeate side to create the TMP. In some circumstances (eg., MF membrane and very low filtration fluxes), the permeate side is placed in a lower position, and the gravity itself is the only driving force for the filtration (Ueda and Hata, 1999).

The submerged MBR has a simpler configuration because it needs less equipment. The coarse bubble aeration in the membrane tank is multifunctional. In addition to membrane fouling control, it also supplies oxygen to the biological process (although the oxygen utilization efficiency is low). The biggest advantage of submerged over side stream configuration is the energy saving by using coarse bubble aeration and lower fluxes (10-30 L/m^2h), instead of high rate recirculation pump and high fluxes (40-100 /m^2h) in side stream MBRs. The hollow fiber membranes used in may submerged MBRs

have very high packing density and low cost, which make it feasible to use more membranes and operate at lower fluxes. However, typical tubular membranes used in sidestream MBRs have low packing density and they are every expensive. Gander et al. (2000) reviewed 4 side-stream and 4 submerged MBR systems and concluded that the side stream MBRs have a higher energy cost, by up to two orders of magnitude, mainly due to the high recycle flow velocity (1-3 m/s) and head loss within the membrane module. In addition to the energy saving, the submerged MBRs suffered less fouling and could be cleaned easier than the side stream MBRs (Gander et al., 2000).

However, the side stream MBRs have the advantage of having more robust physical strength, more flexible crossflow velocity control and hydraulic loading rate and allowing easier chemical cleaning. They are now mostly used in industrial wastewater treatment and small scale WWTPs, where influent flow rate and composition has larger variation and operational conditions are tough (e.g., at high temperature conditions).

In this study, Bench Enviroquip and Ionics MBRs and pilot Enviroquip, Ionics, and Huber MBRs are submerged MBRs, while the Koch MBR is side stream MBR.

1.1.6 Advantage and Disadvantage of MBRs

The MBR technology is actually a new development of the conventional activated sludge (CAS) process, where the bottle-neck is the biomass separation in the secondary clarifier. The secondary clarifier uses gravity to settle the flocs. However the specific gravity of the activities sludge (1.02) (Tchobanoglous et al., 2003) is so close to that of water that poor settling is a common phenomenon in the normal range of the hydraulic retention time (2-3 hrs) of a secondary clarifier. Small flocs (<10 μm), open structure

flocs and highly concentrated sludge (> 5 g/L) are often associated with the sludge setting problem. Sludge bulking is one of the most common settling problems in the operation of an activated sludge process. The causes are very complex, which include low loading rate, low dissolved oxygen concentration, nutrient deficient, nitric oxide or nitrite build up, toxic compounds, shock loading, denitrification in the secondary clarifier and process dynamics and process configuration, etc. (Casey et al., 1995; Eikelboom et al., 1998; Musvoto et al., 1999; Jenkins et al., 2004). The sludge settling is not a problem in MBR process due to the presence of MF or UF filtration process. Other advantages of MBR system include small foot print and high MLSS concentration. Disadvantages of MBR system include high membrane cost, high aeration requirement and high energy cost.

1.2 MBR Membrane Fouling

1.2.1 Membrane Fouling Mechanism

Membrane fouling is the undesirable deposition and accumulation of solutes, colloids, microorganisms, and cell debris within/on membranes. Membrane fouling is attributed to membrane pore blocking and sludge cake layer formation (Lee et al., 2001). Fouling mechanisms involved in membrane fouling include: 1) adsorption of solutes or colloids within/on membranes; 2) deposition of sludge flocs onto the membrane surface; 3) formation of a cake layer on the membrane surface; 4) detachment of foulants attributed mainly to shear forces; 5) the spatial and temporal changes of the foulant composition during the long-term operation (Meng et al., 2009); 6) and microbial community growth on membrane surface (biofilm).

1.2.2 Membrane Fouling Process

A three-stage fouling process is identified in MBR operation (Ognier et al., 2001; Wen et al., 2004; Cho and Fane, 2002; Zhang et al., 2006a; Judd, 2011). The three-stage processes are 1) an initial short-term rapid rise in TMP (conditioning fouling); 2) a long-term weak rise in TMP (steady fouling); 3) a sharp increase in dTMP/dt (TMP jump). The three-stage processes are described below. The initial conditioning fouling is caused by strong interactions between the membrane surface and the extracellular polysaccharides (EPS)/ soluble microbial products (SMP) in the mixed liquor such as initial pore blocking and passive adsorption of organics and colloids. After the initial conditioning fouling, the membrane surface is covered mostly by SMP, providing a favorable condition for attachment of particulate and colloidal biomass material, which will initiate cake formation on membrane surface. After an extended period of stage 2 operation, TMP jump might occur due to the following various reasons: 1) changes in local flux due to fouling eventually causing local fluxes to be higher than critical flux (Cho and Fane, 2002); 2) sudden changes of the biofilm or cake layer structure due to the death of bacteria in the inner of cake layer caused by oxygen transfer limitation and consequently release more EPS (Zhang et al., 2006a; Hwang et al., 2008).

1.2.3 Membrane Fouling Classification

Membrane fouling was traditional characterized by reversible and irreversible fouling. Reversible fouling can be physically removed by backflushing or relaxation, while irreversible fouling can only be removed by chemical cleaning. A more recent concept for the definition of membrane fouling is that it was defined by three types

fouling (Meng, et al., 2009): removable fouling, irremovable fouling and irreversible fouling. In this concept, removable fouling can be removed by physical cleaning such as backflusing, irremovable fouling can only be removed by chemical cleaning, while irreversible fouling is a permanent fouling which cannot be removed by any approaches.

Based on fouling components, membrane fouling can also be classified into three categories: biofouling, organic fouling, and inorganic fouling. Biofouling means the deposition, growth and metabolism of microorganisms on the membranes (Pang et al., 2005; Wang et al., 2005). Furthermore, SMP and EPS originated from microorganisms play important roles in biofouling (Flemming et al., 1997; Liao et al., 2004; Ramesh et al., 2007). The deposition of microorganisms on membrane surface can be visualized by techniques such as scanning electron microscopy (SEM), atomic force microscopy (AFM), confocal laser scanning microscopy, and direct observation through the membrane (DOTM) (Meng, et al., 2009; Li et al., 2003; Jin et al., 2006; Yun et al., 2006; Zhang et al., 2006a; Hwang et al., 2007; Lee et al., 2007). In addition, microbial community structures on membrane surfaces can be studied by microbial methods such as polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) and fluorescence in-situ hybridization (FISH) (Chen et al., 2004; Jinhua et al., 2006; Zhang et al., 2006c; Miura et al., 2007).

Organic fouling refers to the deposition of biopolymers such as proteins and polysaccharides on membrane surfaces. Deposited biopolymers can be investigated by some analytical methods such as Fourier transform infrared (FTIR) spectroscopy (Zhou et al., 2007).

Inorganic fouling refers to the deposition of cations and anions by chemical precipitation and biological precipitation (Meng, et al., 2009). In general, membrane fouling is governed more by biofouling and organic fouling than inorganic fouling in MBR operation.

1.2.4 Microbial Community Growth on Surfaces (Biofilm)

Biofilm was first defined as a collection of microorganisms, predominately bacteria, enmeshed within a three dimensional gelatinous matrix of extracellular polymers secreted by the microorganisms in 1984 (Marshal, 1984). Since then, the definition of a biofilm has evolved with development of technologies that have allowed more accurate observations using high-resolution microscopy and molecular detection of cell species and up-regulation of specific genes. In 2000, biofilm was defined as "A multi-species microbial community harboring bacteria that stay and leave with purpose, share their genetic material at high rates, and fill distinct niches within the biofilm. Thus, the natural biofilm is less like a highly developed organism and more like a complex, highly differentiated, multicultural community much like our own city" (Watnick and Kolter, 2000).

The process of biofilm growth begins with the transition of a bacterium from planktonic (free swimming) to surface attached growth. This planktonic-biofilm transition is a complex and genetically regulated process as the bacteria adapt to the new environmental conditions (Costerton et al., 2003). The genetic transition from planktonic to biofilm phenotype occurs across the life cycle of the biofilm, which is comprised of roughly seven distinct steps (O'Toole et al., 2000; Bryers, 2000): (1) conditioning: a

substratum is immediately conditioned by dissolved organic compounds and macromolecules absorbed rapidly from the liquid phase; (2) contact: bacterial cells in the fluid contact the substratum by mass transport mechanisms influenced strongly by the mixing in the bulk fluid; bacteria will also migrate through coordinate movement of their pili to the substratum surface; (3) adsorption: cells from bulk fluid phase accumulating directly on the surface of substratum and plays an important role only in the initial phase of colonization; (4) growth: microbial growth contributes to the creation of a biofilm as it increases the number of irreversibly adsorbed cells as a result of replication; (5) production of extracellular products: initiation of the expression of large quantities of EPS is controlled by genes that code for the production of products not observed for planktonic growth; (6) attachment: cells from bulk fluid stick to the existing biofilm; (7) detachment: the process of removal of biomass in the biofilm by erosion, sloughing, human intervention, predator grazing, abrasion, starvation.

Biofilms are composed of largely of cell biomass and the extracellular polysaccharides (EPS) they synthesis and excrete to create the biofilm. Encapsulation in a biofilm offers many advantages for microbial cells including: resistance to biocides; reduced impact of tangential surface shear force; and enhanced horizontal gene transfer (Watnick and Kolter, 2000).

Biofilm formation can cause many engineering related problems. For example, biofilms in cooling- or heating-water systems can reduce heat transfer (Characklis et al., 1981); biofilms in offshore oil pipelines can lead to substantial corrosion problems (Schwermer et al., 2008); biofilms in drinking water distribution system may impact drinking water quality and present a public health risk (EPA, 2002). In membrane

filtration system, biofilm formation on membrane surface will impact membrane filtration performance and lead to membrane biofouling.

1.2.5 EPS and SMP

Extracellular polysaccharides (EPS) and soluble microbial products (SMP) are two major sludge characteristics and are now considered as the predominant cause of membrane fouling. EPS are extracellular polysaccharides that are bound on the bacteria cells surface and consist of mainly proteins and polysaccharides. SMP are soluble microbial products that are released into solution from substrate metabolism and biomass decay (Barker and Stuckey, 1999). Increasing EPS levels were found to directly increase fouling resistance and hence impact membrane fouling (Drews et al., 2006; Cho et al., 2005b; Lesjean et al., 2005; Chae et al., 2006; Ramesh et al., 2007). On the contrary, some studies indicated SMP had greater impact on membrane fouling (Germain et al., 2005; Fan et al., 2006; Rosenberger et al., 2005, 2006; Jeong et al., 2007; Drews et al., 2008; Paul and Hartung, 2008) and it was found that polysaccharide SMP contribute more to membrane fouling than protein SMP (Rosenberger et al., 2006; Yigit et al., 2008).

1.2.6 Control of EPS and SMP in MBRs

In a MBR process, membrane fouling is directly governed by sludge characteristics, while operating conditions such as SRT, HRT and aeration indirectly affect membrane fouling through modifying sludge characteristics. Both EPS and SMP can be controlled by adjusting operating conditions such as SRT and aeration.

Research indicated that too short SRT (less than 10 days) might increase EPS concentration and membrane fouling (Cho et al., 2005a; Ng et al., 2006b; Masse et al., 2006) and that too long SRT (greater than 60 days) also resulted in excessive membrane fouling (Lee et al., 2003; Han et al., 2005). It can be concluded that the optimum SRT for MBR operation to control EPS and membrane fouling is between 20 and 50 days SRT. Short SRTs also have a significant impact on SMP concentration. Investigators observed that SMP concentrations decreased with increasing SRT (Shin and Kang, 2003; Lee et al., 2003; Zhang et al., 2006b; Liang et al., 2007; Rosenberger et al., 2006).

Aeration intensity directly impacts dissolved oxygen concentration in MBR. It was found that membrane permeability increased with increasing aeration intensity (Trussell et al., 2007). Sludge soluble COD concentrations were found to increase with limited dissolved oxygen concentrations (Kang et al., 2003; Jin et al., 2006). It was also reported that air scouring and variations of scouring operation are preferable for fouling control (Sofia et al., 2004; Phattaranawik et al., 2007; Wicaksana et al., 2006).

Another effective method of controlling membrane fouling is the addition of adsorbents such as powered activated carbon (PAC) into MBRs (Ng et al., 2006a; Hu and Stuckey, 2007; Akram and Stuckey, 2008). Adding PAC to MBR is simple and convenient. The PAC to MBRs can enhance floc formation and reduce floc breakage. As an alternative, coagulants can also be added to MBRs to remove SMP by charge neutralization and bridging (Wu et al., 2006; Kim and Jang, 2006). Different coagulants such as alum, ferric chloride and chitosan were investigated (Iversen et al., 2008; Ji et al., 2008; Koseoglu et al., 2008; Song et al., 2008).

1.3 Application of Molecular Biology Techniques in MBR Research

Study of microbial community within an environmental includes culture-dependent and culture-independent methods. Culture-dependent methods are tedious and time consuming due to the need for selective media for all species of interest. It is difficult to detect more than about 1% of the bacteria species by any conventional cultivation techniques (Ward et al., 1990). Microbial community in wastewaters is very complex and some microorganisms are not cultivable with traditional microbial isolation media. Recent advances in molecular biological techniques have overcome these difficulties. Analysis of total community DNA extracted from wastewater using culture-independent methods permits the detection of uncultured organisms. Some typical molecular biological techniques that are widely used in wastewater microbiological study include PCR-DGGE and clone library profiling.

1.3.1 PCR-DGGE

The polymerase chain reaction (PCR) is a technique to amplify community DNA extracted from an environmental sample by several orders of magnitude, generating millions of copies of the same DNA sequence. The amplified DNA sample can be subsequently used for DGGE and gene cloning analysis.

Denaturing gradient gel electrophoresis (DGGE) is an electrophoretic method to identify single base changes in a segment of DNA. In a denaturing gradient acrylamide gel, double-stranded DNA is subjected to an increasing denaturant environment and will melt in discrete segments called “melting domains.” The melting temperature (Tm) of these domains is sequence-specific. When the Tm of the lowest melting domain is

reached, the DNA reduces its mobility in a polyacrylamide gel. Since the Tm of a particular melting domain is sequence-specific, the presence of mutation will alter the melting profile of that DNA when compared to wild-type. DNA containing mutations will encounter mobility shifts at different positions in the gel than the wild-type. If the fragment complete denatures, then migration again becomes a function of size. Using DGGE, PCR products of the same length but with different sequences can be separated. The total number of DGGE bands provides an estimate of the microbial diversity within a given environment (Muyzer et al., 1993).

In DGGE, the denaturing environment is created by a combination of uniform temperature, typically between 50 and 65°C and a linear denaturant gradient formed with urea and formamide. A solution of 100% chemical denaturant consists of 7 M urea and 40% formamide. The denaturing gradient may be formed perpendicular or parallel to the direction of electrophoresis. A perpendicular gradient gel, in which the gradient is perpendicular to the electric field, typically uses a broad denaturing range, such as 0-100% or 20-70%. In parallel DGGE, the denaturing gradient is parallel to the electric field, and the range of denaturant is narrowed to allow better separation of fragments.

When running a denaturing gradient gel, both the mutant and wild-type DNA fragments are run on the same gel. This way, mutations are detected by differential migration of mutant and wild-type DNA. The mutant and wild-type fragments are typically amplified by the PCR to make enough DNA to load onto the gel. Optimal resolution is attained when the molecules do not completely denature and region screened is in the lowest melting domain. The addition of a 30-40 base pair (kb) GC clamp to one of the PCR primers insures that the region screened is in the lower melting domain and

that the DNA will remain partially double-stranded. The size of the DNA fragments run on a denaturing gel can be as large as 1 kb in length, but only the lower melting domains will be available for mutation analysis. For complete analysis of fragments over 1 kb in length, more than one PCR reaction should be performed.

1.3.2 Clone Library Profiling

Clone library profiling is a molecular biological technique used to obtain microbial community composition through constructing community gene library and statistical analyzing the frequency of each type of bacteria occurred in the clone library. 16S rRNA clone library is a normally used to study prokaryotes in an environmental system. 16S rRNA, consisting 1542 bp, is a component of bacterial 30S ribosomal subunit. Genetic information in the 16S rRNA is stored in a group of recipient bacterium via inserting a cloning vector carrying the genomic information of interest. This population of host bacteria is called clone library. The DNA sequence in clone library will be compared with known sequences in GeneBank and then a phylogenetic tree is generated.

Clone library technique was first used by Giovannoni in 1990 to study microbial diversity of sea bacterioplankton. Subsequently, 16S rRNA clone library was used to study microbial community in wastewater (Guieysse et al., 2001) and it was reported that *Betaproteobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteriodetes* and *Actinobacteria* are normally found in wastewater. In MBR system, *Bacteroidetes* (50%) and *Proteobacteria* (40%) were found to be dominant (Du et al., 2008). Microbial community on MBR membrane surface was studies by Miura (2007) and it was found that *Betaproteobacteria* and *Gammaproteobacteria* were dominant (61% and 22 %,

respectively), and that *Deltaproteobacteria* and *Alphaproteobacteria* were only account for 2.8% each. It was concluded that membrane fouling was mainly caused by *Betaproteobacteria*.

1.4 Current Research Status on MBRs

Over the past two decades (between 1990 and 2009), 1450 publications in MBRs for wastewater treatment were identified based on Scopus (Judd, 2011). Among those publications, the subject of membrane fouling has attracted the most attention from the academic community. Membrane fouling related to EPS and SMP was well studied, while recently membrane fouling and microbial diversity study with the application of molecular biological tools are gaining popularity (Duan, et al., 2009).

Up to date, different results were obtained for the correlation between EPS/SMP concentrations and membrane fouling. As mentioned in section 1.2.5, some research reported that increasing EPS levels were found to directly increase fouling resistance and hence impact membrane fouling while some other studies indicated SMP had greater impact on membrane fouling. Discrepancies also exist in the findings of fouling microorganisms in MBR. *Betaproteobacteria* were reported as the main fouling microorganisms in some research while *gammaproteobacteria* were also found to play a major role in development of membrane biofilms (Jinhua et al., 2006). The discrepancy results indicate membrane biofilm is a complex multicultural community and slight environment changes such as operating condition change, ambient environment change, and source wastewater change could substantially impact EPS/SMP and membrane biofilm induced fouling. Since Hawaii is located in a unique physical environment with

year round tropical climate and seawater intrusion, microbial communities in MBRs and fouling microorganisms in membrane biofilm might be different than those found by other researchers in a different ambient environment. Therefore, it is worth to obtain first hand information on microorganisms in membrane biofilms and correlation between EPS/SMP and membrane fouling for MBRs running in Hawaiian tropical environment.

Another research area that needs further systematic study is membrane chemical cleaning. Recovery chemical cleaning methods recommended MBR suppliers are all based on a combination of hypochlorite and organic acid such as citric acid for removing organic matters and inorganic foulants, respectively. Up to date, there has been no systematic study comparing the efficacy of a range of cleaning reagents or cleaning conditions on MBR permeability recovery. Therefore, it is imperative to study membrane flux recovery under different cleaning conditions with combinations of sodium hypochlorite, citric acid, and possibly organic cleaning reagents such as industrial alcohol and propylene glycol.

1.5 Objectives

The objectives of this study were to study membrane fouling, to identify biofouling microorganisms in bench-scale and pilot MBRs treating municipal wastewater, and to evaluate membrane cleaning methods. The main subjects of this study are summarized below.

A. Operate bench-scale MBRs at Honouliuli WWTP at different MCRT conditions including 10-d, 20-d, 30-d, 40-day, and no sludge wasting condition to study the impact of MCRT on membrane fouling. Shock loading condition with glucose

addition and no anoxic zone condition at 20-d MCRT following the normal 20-d MCRT operation were also operated to evaluate then impact on membrane performance. Four pilot MBRs were also operated at Honouliuli WWTP for a comparison study with bench-scale MBRs. Specific tasks are listed below.

(1) Analyze bench-scale MBR operating data and influent and effluent water quality data to see performance of bench-scale MBRs treating municipal wastewater.

(2) Following the normal 20-d MCRT condition, increase BOD strength by adding glucose and remove anoxic zone at 20-day MCRT condition to study reactor and membrane performance.

(3) Analyze pilot MBR operating data and influent and effluent water quality data to see performance of pilot MBRs treating municipal wastewater.

(4) Study bench-scale MBR mixed liquor properties (EPS and SMP concentrations) to explain reactor performance and membrane fouling.

(5) Perform membrane critical flux analysis by monitoring membrane flux and TMP response.

(6) Conduct long-term membrane performance of the bench-scale MBRs at each MCRT condition by monitoring the operating membrane flux and TMP response. Study the relationship between specific flux and time of operation to determine membrane fouling rates at each operating condition.

(7) Measure bench-scale MBR membranes at the end of each MCRT operating condition to determine resistance terms.

B. Conduct microbial community study of the bench-scale and pilot MBRs.

(1) Conduct DGGE fingerprinting analysis to identify dominant species in bench-scale MBRs at each operating condition and pilot MBRs.

(2) Conduct 16S rRNA clone library analysis for a selected bench-scale MBR operating condition to characterize overall microbial community composition.

C. Operate a laboratory bench-scale MBR with PVDF and PVC ultrafiltration membranes to study membrane fouling, flux restoration after different combinations of cleaning method, and microbial diversity studies of mixed liquor and membrane samples. Specific tasks are listed below.

(1) Obtain membrane resistance components prior to the end of MBR operation.

(2) Obtain flux recovery rate after membrane cleaning with different combinations of cleaning method

(3) Monitor post-cleaning membrane fouling process to determine an optimal ultrafiltration membrane cleaning method.

(4) Conduct FTIR, SEM and EDX analysis to study membrane surface structure properties.

(5) Conduct DGGE fingerprinting analysis to identify dominant species in both mixed liquor and membrane surface.

(6) Conduct 16S rRNA clone library analysis to characterize overall microbial community composition.

CHAPTER 2 MATERIALS AND METHODS

2.1 Honouliuli WWTP Bench-Scale and Pilot MBR Configurations

Two bench-scale MBRs, Enviroquip and Ionics, were setup at Honouliuli WWTP for the MBR fouling study. At the time of the bench-scale MBR fouling study, four pilot MBRs (Enviroquip, Ionics, Huber, and Koch) were also in operation and were included in the MBR fouling study. These four pilot MBRs were part of six pilot MBRs, provided by six vendors including Enviroquip, Huber, Ionics, Koch, US Filter, and Zenon, operated side-by-side at the Honouliuli WWTP for the Honolulu membrane bioreactor pilot study from 2003 to 2006. Figure 2.1 shows a schematic of the side-by-side bench-scale and pilot MBRs setup.

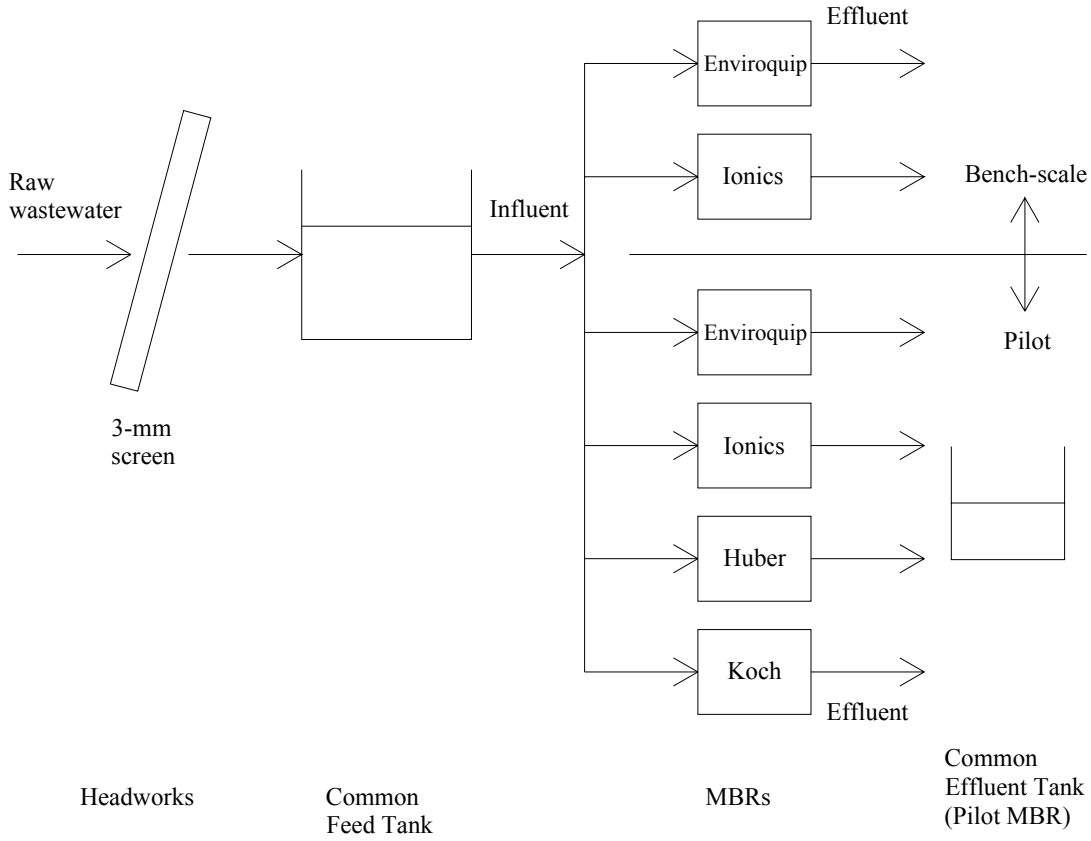


Figure 2.1 Schematic of bench-scale and pilot MBRs setup

The pilot MBR study setup included headworks with 3-mm fine screen, a open-top common feed tank, two bench-scale and four pilot MBRs, and a common effluent tank for pilot MBRs. A submersible pump was placed in a pre-aeration tank at Honouliuli WWTP to draw wastewater which had passed through the Honouliuli WWTP headworks (screening and degritting) only. Wastewater passed through the 3-mm fine screen was stored in a 1,000 gallon open-top common feed tank prior to being sent to each bench-scale and pilot MBR unit. Effluents of each pilot MBR unit were sent to a common effluent tank which houses four plastic buckets, respectively, to facilitate handling of grab and composite samples. Figures 2.2 to 2.4 show the setup of pilot MBRs.

Figure 2.2 Bench-scale and pilot MBRs headworks and feed tank at Honouliuli WWTP

Figure 2.3 Pilot MBRs at Honouliuli WWTP

Figure 2.4 Pilot MBRs common effluent tank at Honouliuli WWTP

The four Pilot MBR units in the membrane fouling study were provided by four vendors, Enviroquip, Ionics, Huber, and Koch, respectively. Each MBR system contained different membrane configurations and operations. The membrane specifications are presented in Table 2.1.

Table 2.1 Membrane specifications for pilot MBRs

Specification	Enviroquip	Huber	Ionics	Koch
Membrane configuration	Vertical flat panel	Rotating flat panel	Horizontal hollow fiber	Vertical hollow fiber
Membrane location	Aeration basin	Aeration basin	Aeration basin	Cell compartment
Membrane type	MF	UF	MF	MF
Pore size, μm	0.4	0.02	0.4	0.1
Membrane area, ft^2	630	775	1130	323
Flow rate, gpm	6.5	9.5	7.9	2.7
Flux, gfd	14.7	17.6	10	14.3
Peak flux, gfd	43	33.5	32.3	26.8
TMP, -psi	0.1 - 4	2 - 6	1 - 4	0.2 - 2
Operating mode	a	b	a	c

Operating mode:
a. Continuous air scour and permeation relax
b. Continuous air scour on one side and intermittent water scour
c. Cyclic air scour and intermittent backpulse

Figure 2.5 shows the setup of the bench-scale MBRs at Honouliuli WWTP. Membrane specifications are presented in Table 2.2. The bench-scale MBRs were comprised of anoxic tank and aeration tank. Fine air bubble aerators were placed in aeration tank to maintain DO above 2 mg/l. Coarse air bubbles were emitted continuously from the header at the bottom of the membrane module to scour the membrane surface. The coarse bubble aeration provides an efficient cross-flow velocity that removes solids from membrane surface. The membrane was operated with 9-min permeation cycle intervals. At the end of each cycle, the applied vacuum pressure was stopped for 1-min and membranes were under relaxation which allows the accumulated cake to be scoured from membrane surfaces. An internal recirculation pump was placed

in aeration tank to recirculate approximately 4-5Q of mixed liquor to anoxic tank for nitrogen removal.

Figure 2.5 Bench Enviroquip (right) and Ionics (left) MBRs at Honouliuli WWTP

Table 2.2 Membrane specifications for bench-scale MBRs

Specification	Enviroquip	Ionics
Membrane Configuration	Vertical flat panel	Horizontal hollow fiber
Membrane location	Aeration basin	Aeration basin
Membrane type	Microfiltration	Microfiltration
Pore size, µm	0.4	0.4
Membrane area, ft^2	3.9	1.3
Flow rate, ml/min	61	19
Flux, gfd	15.7	14.7
Operating mode	Continuous air scour and permeation relax	Continuous air scour and permeation relax

2.2 Feed Water

Honouliuli pilot-scale and bench MBRs were fed with wastewater from pre-aeration channel. Prior to entering a common feed tank, wastewater passed through a 3-mm fine screen to remove fibers and hairs that may be detrimental to membranes.

2.3 Determination of Calculated Parameters

2.3.1 Hydraulic Retention Time

The hydraulic retention time (HRT) for all reactors was calculated as follows:

$$\theta_H = \frac{V}{Q} \cdot \frac{24\,h}{1\,d} \tag{1}$$

where θ_H is the hydraulic retention time, [h], V the reactor volume, [L], and Q the flow of treated wastewater, [L/d].

2.3.2 Mean Cell Residence Time

The mean cell residence time (MCRT) or solids retention time (SRT) was calculated as follows assuming the concentration of suspended solids in the effluent is zero:

$$MCRT = \frac{V \cdot X_{MLSS}}{Q_W \cdot X_{MLSS}} = \frac{V}{Q_W} \tag{2}$$

where $MCRT$ is the mean cell residence time, [d], V the reactor volume, [L], X_{MLSS} the mixed liquor suspended solids concentration, [mg/L], and Q_W the flow of wasted mixed liquor, [L/d].

2.3.3 Food to Microorganism Ratio

The food to microorganism ratio (F/M) is defined as:

$$F/M = \frac{S_0 \cdot Q}{X_{MLVSS} \cdot V} = \frac{S_0}{X_{MLVSS} \cdot \theta_H} \quad (3)$$

where *F/M* is food to microorganism ratio, [gCOD/gVSS·d], S_0 the influent substrate, [mg COD/L], *Q* the wastewater flow, [L/d], *V* the aeration tank volume, [L], X_{MLVSS} the mixed liquor volatile suspended solids concentration, [mg/L], and θ_H the hydraulic retention time, [d].

2.3.4 Relationship between MCRT and F/M

A mass balance on the suspended solids (a surrogate for microorganisms) in the activated sludge process gives:

$$\mu_{net} = \mu' - k_d \quad (4)$$

where μ_{net} is net growth rate, [d^{-1}], μ' the growth rate, [d^{-1}], k_d the decay rate, [d^{-1}]. Since $\mu_{net} = 1/MCRT$ and $\mu' = Y \cdot U$, the above equation may be written as:

$$1/MCRT = Y \cdot U - k_d \quad (5)$$

where *Y* is the maximum yield coefficient, [gVSS/gCOD], *U* the substrate utilization, [gCOD/gVSS·d], and k_d the endogenous decay coefficient, [d^{-1}]. The terms U and F/M are related by the biological efficiency as follows: $U = (F/M) \cdot E$ and $E = \frac{S_0 - S}{S_0}$, where *E* is the biological efficiency, [%] and *S* the effluent substrate, [mg COD/L]. Combining the above two equations, the relationship between MCRT and F/M is as follows:

$$1/MCRT = Y \cdot (F/M) \cdot E - k_d \quad (6)$$

The equation above presents the effect of the MCRT on the F/M ratio as related by activated sludge kinetics. Longer MCRT results in lower F/M ratio for a given wastewater with constant biological efficiency.

2.3.5 Membrane Flux and Transmembrane Pressure

In membrane systems, flux is defined as the flow rate of water through a given area of membrane surface and was calculated as follows:

$$J = \frac{Q_P}{A} \tag{7}$$

where J is the membrane flux, [L/m^2·h (LMH)], Q_P the membrane permeate flow, [L/H], and A the outside membrane surface, [m^2]. The driving force applied to the membrane device, or transmembrane pressure (TMP), was calculated as follows:

$$\Delta P = P_{Vaccum} - \rho_w g \Delta h \tag{8}$$

where ΔP is the transmembrane pressure, [Pa], P_{Vaccum} the vacuum pressure, [Pa], ρ_w the density of water, [kg/m^3], g the gravitational acceleration, [m/s^2], and Δh the liquid level difference between top of tank and vacuum pressure measurement, [m].

2.3.6 Resistance Components

Membrane fouling was modeled using a resistance in-series model derived from Darcy's Law for flow through porous media:

$$v = \frac{Q}{A} = K \frac{\Delta h}{l} \tag{9}$$

where v is the Darcy flux, [m/s], Q the flow of water, [m^3/s], A the flow area perpendicular to flow path, [m^2], K the hydraulic conductivity, [m/s], Δh the difference in

hydraulic head, [m], l the flow path length, [m]. Because the water temperature is not constant in membrane filtration, permeability is substituted for hydraulic conductivity using the following equation:

$$K = \frac{\rho g k}{\mu} \tag{10}$$

where K is the hydraulic conductivity, [m/s], ρ the density of fluid, [kg/m^3], g the gravitational acceleration, [m/s^2], k the hydraulic permeability, [m^2], μ the absolute viscosity of fluid, [kg/m·s]. The resulting relationship is used to describe membrane filtration of water and Darcy's flux becomes equal to the membrane flux:

$$J = \frac{\rho g k}{\mu_w} \cdot \frac{\Delta h}{l_m} = \frac{k}{l_m} \cdot \frac{\rho g \Delta h}{\mu_w} = \frac{k}{l_m} \cdot \frac{\Delta P}{\mu_w} \tag{11}$$

where k *i*s the permeability, [m^2], l_m the membrane thickness, [m], and μ_w the absolute viscosity of water, [kg/m·s]. Since permeability, k, and the membrane thickness, l_m, are both intrinsic membrane properties, they can be combined into one term to account for filtration resistance, R, in the resistance in-series model:

$$J = \frac{k}{l_m} \cdot \frac{\Delta P}{\mu_w} = \frac{\Delta P}{\mu_w \cdot R} \tag{12}$$

The total resistance to filtration term is the sum of individual resistance terms:

$$R = \frac{\Delta P}{J \cdot \mu_w} = R_M + R_F + R_C \tag{13}$$

where R is the total resistance, [m^{-1}], R_M the membrane resistance, [m^{-1}], R_F the foulant resistance, [m^{-1}], and R_C the cake resistance, [m^{-1}].

2.3.7 Specific Flux

The specific flux or permeability is the membrane flux obtained from an applied driving force and is defined as follows:

$$L_P = \frac{J}{\Delta P} \tag{14}$$

where L_P is specific flux or permeability, [L/m^2·h·bar (LMH/bar)] or [m/s·Pa], J the membrane flux, [L/m^2·h (LMH)], and ΔP the transmembrane pressure, [Pa].

2.4 Reactor Operating Conditions

2.4.1 Bench-Scale MBR Operating Conditions

The bench-scale MBR was operated at 10-d, 20-d, 30-d, and 40-d MCRTs and without sludge wasting condition. The operation of each MCRT and HRT condition is summarized in Table 2.3. 18 hours of HRT was first operated for 20-d MCRT and no sludge wasting condition. At 20-d MCRT condition, glucose equal to approximately 50% of influent BOD_5 was added for one week operation to evaluate weather fouling condition will be impacted since glucose is a carbon media source that is ready to be used by microorganisms and may affect membrane fouling. In addition, anoxic zone was removed from the 20-d MCRT condition to evaluate the difference in membrane fouling between aerobic/anoxic scenario and aerobic only scenario. It should be noted that bench Ionics MBR was started up with no anoxic zone condition at 20-d MCRT. Partition baffles were then built in the aeration tanks of bench Enviroquip and bench Ionics MBRs to reduce the aeration tank volume to obtain a HRT of 12 hours without increasing membrane flux.

Table 2.3 Summary of operation schedule for bench-scale MBRs

MCRT, d	HRT, h	Days of operation	
		Enviroquip	Ionics
20	18	49	----
20[a]	18	7	----
20[b]	18	35	35
No sludge wasting	18	28	19
10	12	26	26
30	12	47	47
40	12	91	91

Note:
a. with glucose addition
b. without anoxic tank

2.4.2 pH Control

The feed to all reactors was supplemented with sodium bicarbonate solution so that the reactor pH was maintained at 7.0.

2.4.3 Dissolved Oxygen

The aeration tank dissolved oxygen (DO) concentration was maintained above 1 mg/L in all reactors using air blower (Yasumaga air pump, model LP-60A).

2.5 Analytical Methods

2.5.1 Total Suspended Solids

The total suspended solids (TSS) of ML samples were measured according to Standard Methods 2540 D (APHA, 1998).

2.5.2 Volatile Suspended Solids

The volatile suspended solids (VSS) of ML samples were measured according to Standard Methods 2540 E (APHA, 1998).

2.5.3 Chemical Oxygen Demand

The chemical oxygen demand (COD) was determined using Standard Method 5220 D (APHA, 1998).

2.5.4 Biochemical Oxygen Demand

The 5-day biochemical oxygen demand (BOD_5) was determined using Standard Method 5210-B (APHA, 1998).

2.5.5 Total Organic Carbon

The total organic carbon (TOC) was determined using Standard Method 5310-B (APHA, 1998).

2.5.6 pH

The pH of influent and effluent was measured using Standard Method 4500-H B (APHA, 1998).

2.5.7 Total Nitrogen

The total nitrogen (TN) of influent and effluent was measured by a cadmium colorimetric reaction (Nitraver 5 Reagent, Hach, Loveland, CO).

2.5.8 Ammonia Nitrogen

The ammonia nitrogen of influent and effluent was measured colorimetrically using Standard Method 4500-NH_3 D (APHA, 1998).

2.5.9 Total Phosphorus

The total phosphorus (TP) of influent and effluent was measured colorimetrically using Nessler reagents (Nessler Reagent, Hach, Loveland, CO).

2.5.10 Dissolved Oxygen

The dissolved oxygen (DO) concentrations of reactors were measured using Standard Method 4500-O G (APHA, 1998) with an YSI 500A dissolved oxygen meter.

2.5.11 Turbidity

Turbidity was measured using Standard Method 2130-B (APHA, 1998) with a Hach 2100N desktop nephelometer.

2.5.12 UV Transmittance

The UV transmittance at 254 nm (UVT_{254}) was measured using Standard Method 5910-B (APHA, 1998).

2.5.13 Extracellular Polymeric Substances and Soluble Microbial Products

Extracellular polymeric substances (EPS) concentrations were measured as carbohydrate and protein using a cation exchange resin (CER) extraction method

(Frolund et al, 1996). A mixed liquor sample was immediately cooled to 4°C to minimize microbial activity. The exchange resin (70 g of CER/g VSS) was added to a 50-mL sample and mixed at approximately 600 rpm using a magnetic stirring bar for 2 h at 4°C. The mixture (50 mL) was centrifuged for 15 min at 12,000 g to remove MLSS. Supernatant carbohydrate and protein concentrations were measured colorimetrically by the methods of Dubois et al (1956) and Lowry et al (1951), respectively. At the same time, 50 mL of untreated mixed liquor was centrifuged for 15 min at 12,000 g, and the protein and carbohydrate concentrations were determined on the supernatant to represent the soluble fraction (Soluble Microbial Products, SMP). The centrifuge supernatant of the mixed liquor sample represented the SMP concentration, and the centrifuged supernatant of the sample after CER extration represented the sum of SMP and EPS concentrations. The difference between these measurements is the EPS concentration. Bovine serum albumin (BSA) was used as a protein standard, and dextrose was used as a carbohydrate standard.

2.5.14 Carbohydrate Measurement

Carbohydrate concentrations were quantified using a phenol-sulfuric acid colorimetric method developed by Dubois (1956) and dextrose was used as the standard.

2.5.15 Protein Measurement

Protein concentrations were quantified using a Modified Lowry Protein Assay Kit. Bovine serum albumin (BSA) was used as the standard for the colorimetric test that used the Folin-phenol reaction of Lowry (1951).

2.6 Estimation of Resistance Terms

The R_C and R_F terms (section 4.3.6) of the fouled membranes were estimated at the completion of each operating condition. The resistance calculated from TMP and flux at the end of operation was total resistance (R). Then mixed liquor was diverted to a temporary storage tank and the aeration tank was filled with permeate. The resistance calculated from under this condition was the sum foulant resistance and membrane (R_F + R_M); the difference between R and R_F + R_M was cake resistance (R_C). Then the membrane was chemically cleaned with 0.02% NaOCl solution and the resistance measured for cleaned membrane in clean water was membrane resistance (R_M); the difference between R_F + R_M and R_M was foulant resistance (R_F).

2.7 DNA Extraction and PCR Amplification

Total genomic DNA was purified directly from mixed liquor samples and membrane samples using FastDNA Spin Kit (BIO 101; Vista, CA).

Two rounds of PCR amplifications were conducted. In the first round, the general 16S rRNA gene was amplified by PCR using the extracted genomic DNA as template and universal primers forward primer 27f (5'- AGA GTT TGA TCC TGG CTC AG-3') and reverse primer 1492r (5'-TAC GGT TAC CTT GTT CGC TT-3') (Mobarry et al., 1996). In the second round, the V3 region of 16S rRNA gene was amplified by PCR using the above 16S rRNA gene PCR product as template and forward primer 338f (5'-ACT CCT ACG GGA GGC AGC AG-3') and reverse primer 518r (5'-ATT ACC GCG GCT GCT GG-3') (Muyzer et al., 1993; Øvreås et al., 1997). A GC clamp of 40 bp was

added to the forward primer (Muyzer etal, 1993; Øvreås et al, 1997). The length of the expected amplified fragment with GC clamp was 236 bp.

The 50 µl PCR mixtures contained: 0.5 µM each primer, 200 µM each deoxynucloside triphosphate, 5 µl of thermophilic DNA polymerase, 10x reaction buffer (w/ 15 mM $MgCl_2$), 1.25 U of *Taq* DNA polymerase (Promega; Madison, WI), and 0.75 µl of extracted DNA. PCR amplifications were performed in iCycler (Bio-Rad Laboratories, Hercules, Calif). The 16S rRNA gene amplification had an initial denaturation at 94°C for 5 min; 32 cycles of denaturation (60 sec at 94 °C), annealing (90 sec at 57 °C) and extension (90 sec at 72 °C); and a final extension at 72 °C for 7 min. The V3 region of 16S rRNA gene amplification had an initial denaturation at 94°C for 5 min; 30 cycles of denaturation (45 sec at 94 °C), annealing (60 sec at 57 °C) and extension (60 sec at 72 °C); and a final extension at 72 °C for 7 min. Amplified DNA was examined by horizontal electrophoresis in 1.5% agarose with 2 µl aliquots of PCR products.

2.8 DGGE Analysis

DGGE was performed as described by Muyzer et al, 1993 using DCode system (Bio-Rad laboratories, Hercules, Calif) and are described in the following steps.

(1) Preparation of denaturing gradient gel. Prepare 0% and 100% denaturant. The 100% denaturant contains 7 M urea and 40% formamide). Add the final concentration of 0.09% (V/V) each of ammonium persulfate and TEMED solutions. Cast 8% denaturing gradient gel with 35-55% denaturant using the DCode system and let the gel polymerize for 1 h;

(2) Sample loading. Place the gel sandwich into the DCode container and heat the 1xTAE buffer to 60 °C. Load 30 μL of PCR product (V3 region of 16S rRNA gene) into each loading cell.

(3) Electrophoresis and staining. Run the system at 200 V for 5 min until all samples are in the gel and then lower the voltage to 80 V and run for 13 h. After electrophoresis is complete, stain the gel with 250 ml of 1x running buffer containing 25 μl of 10 mg/ml ethidium bromide for 10 min. Then destain the gel with 1x running buffer for 5 min and the gel is ready for visualization under UV transilluminator (Gel Doc 2000, Bio-Rad laboratories, Hercules, Calif.).

(4) Recovery of DNA from DGGE bands. Cut dominant and special bands, place the cut gel into eppendorf, rinse the gel with 70% ethanol two times, add 30 μl of autoclaved purified water and freeze and thaw for 3 times, the supernatant contains the DNA.

(5) Sequencing of DGGE bands. Use primer 338f and 518r to amplify the DNA and conduct DNA sequencing using primer 518r. The DNA sequences were determined by Map biotech Co., Ltd. (Shanghai, China) and were used to obtain information from GeneBank database.

2.9 16S rRNA Clone Library

Use the first round PCR product containing the whole 16S rRNA gene for 16S rRNA clone library analysis. The 16S rRNA clone library analysis was conducted by Map biotech Co., Ltd. (Shanghai, China). The procedures are described below.

(1) Purify PCR products using DNA purification kit (Ver.2.0 TAKARA);

(2) Plasmid ligation. Use pTG19-T TA cloning vector system to establish a 10 μL ligation reaction system comprised of 5 μL 2x clone buffer, 1 μL T4-DNA ligase and 4 μL purified PCR products, incubate overnight at 16 °C.

(3) Preparation of competent cell. Incubate DH5α strain of E.coli on media plate (no antibiotics), incubate 12-16 hr at 37 °C, pick a single colony and place into 1 mL LB liquid media tube, shaking incubate at 220 rpm at 37 °C overnight. Then place 1% of E. coli DH5α into a 250 mL flask containing 50 mL LB media. Shaking incubate at 220 rpm at 37 °C for 2 h until OD value reaches 0.3-0.4 and then place the flask in ice water bath for 10-15 min and then transfer the incubation to pre-ice cold 50 mL centrifuge tube. Centrifuge at 5000 rpm at 4 °C for 10 min, disregard supernatant, add 10 mL of 100 mM cold $CaCl_2$, resuspend pellet. Incubate on ice for 30 min and repeat the centrifugation step, again add 500 μL of 100 mM clod $CaCl_2$, resuspend pellet. Transfer cell suspension into 1.5 mL eppendorf, place on ice water bath for 2 h.

(4) Plasmid transformation. Pipette 50 μL of competent cell to 1.5 mL sterile eppendorf, add 2 μL of ligation mix and mix gently. Place on ice for 30 min, and immediately place in 42 °C water bath to heat shock for 60 s, immediately place on ice for 2 min, add 400 μL LB media (no antibiotics), shaking incubate at 37 °C for 1 h, spread cell suspension to f the plate (contains Amp, IPTG, X-Gal at 100 μg/mL, 0.5 mM, 80 μg/mL), incubate at 37 °C for 1 h and then 37 °C incubate with agar side up.

(5) Recombinant clones blue-white colony screening. Recombinant clones show white color in X-gal/IPTC media plate, while non-recombinant clones show blue color. Pick single clones and place in 2 mL LB tube, shaking incubate at °C overnight.

(6) Recombinant plasmid extraction. Use M13 primer pairs and 1 μL cell growth as template to amplify recombinant DNA segment and verify PCR products with agarose gel electrophoresis. Extract plasmid from positive clones.

(7) DNA sequencing of positive clones and clone library analysis. Pick 100 positive clones for DNA sequencing, then preliminarily group sequences with 97% similarity cutoff to one operational taxonomic unit (OTU) using Clustal-X software. Compare DNA sequences with GenBank database, and then create phylogenetic tree using Mega 4.0 software.

2.10 Lab Bench MBR for Ultrafiltration Membrane Cleaning Study

2.10.1 MBR Setup

The lab bench MBR setup (see Figure 5.1) adopted only one aerobic tank. No anoxic zone was implemented in the MBR so no denitrification process existed. The volume of the aerobic tank was 15 L. The MBR was seeded with sludge from Honouliuli WWTP and MLSS concentration was maintained at 5,000 mg/L. Both a PVDF UF membrane module and a PVC UF membrane module were utilized in the MBR with subcritical flux permeation. The membrane specifications are presented in Table 5.1. The PVDF and the PVC membrane had maximum flux of 20 LMH and 13 LMH, respectively.

Table 2.4 Specifications of UF membrane unit

Property	PVDF UF membrane	PVC UF membrane
Material	Polyvinylidene fluoride	Polyvinyl chloride
Type	Hollow fiber	Hollow fiber
Flow configuration	Outside-in	Outside-in
Pressure configuration	Immersed	Immersed
Fiber diameter, mm	ID：0.85；OD：1.45	ID：0.85；OD：1.45
Nominal pore size, μm	0.02	0.01
MW cutoff, dalton	50 k	50 k
Area, m^2	0.1	0.1
No. of membrane fibers	78	78
Effective length, cm	25	25
Maximum TMP, MPa	0.06	0.06
Working temperature, °C	5-38	5-38
pH tolerance	2.0-13.0	2.0-13.0

Figure 2.6 Lab bench MBR setup

2.10.2 Influent and Effluent Wastewater

The influent wastewater was taken from a sewer manhole located in UH Manoa campus near Dole Street. The source wastewater to the sewer manhole was primarily from Manoa Valley residential and commercial uses along with wastewater generated in upper UH Manoa campus. Wastewater was pumped from the manhole every evening after 6 pm to avoid the campus generated lab cleaning water. The characteristics of influent and effluent wastewater are summarized in Table 5.2. The influent was low to medium strength. Over 90% of COD removal and complete nitrification were obtained in the MBR, indicating the MBR was operated normally. It appears that the PVDF membrane module provided better performance than the PVC membrane module.

Table 2.5 Influent and effluent wastewater quality for lab bench-scale MBR

Parameter	Influent	Soluble contents in ML	Effluent	
			PVDF	PVC
COD, mg/L	106 - 204	31.3 - 52.8	5.1 - 21.2	14.2 - 24.6
TOC, mg/L	23.2 - 27.9	5.9 - 12.5	3.4 - 5.6	4.3 - 6.6
NH_4^+-N, mg/L	6.87 - 20.1	0.87 - 1.21	0.21 - 0.35	0.32 - 0.49
TN, mg/L	10.5 - 25.2	21.7 - 38.7	----	----
Phosphate, mg/L	4.15 - 6.42	----	----	----
Chloride, mg/L	73 - 109	----	----	----
Sulfate, mg/L	20.3 - 20.6	----	----	----

2.10.3 UF membrane Cleaning and Analytical Method

In MBR operation, membrane fouling is inevitable due to the direct contact of membrane with activated sludge. Membrane fouling could be mitigated by adding powdered activated carbon (Ng et al., 2006a; Hu and Stuckey, 2007; Akram and Stuckey, 2008) or coagulant (Wu et al., 2006; Kim and Jang, 2006) to increase floc size and thus to

reduce membrane fouling rate. But once the membrane is fouled to certain extent, chemical cleaning has to be implemented to restore membrane permeability. Hence, membrane cleaning maintenance after membrane fouling is a critical step in membrane operation.

Many studies on UF membrane cleaning have been done. Astudillo et al. (2010) utilized Ultrasil®11 and Ultrasil®10 to study ceramic membrane and PVDF membrane cleaning and proposed new membrane cleaning standards. Ahmad et al. (2002) applied electropulsing technique to study dead end filtration membrane cleaning. Many studies involved with acid and base type inorganic cleaning reagent were also conducted. So far, limited research has been done to apply organic cleaning reagents in membrane cleaning due to the treatment and disposal problem of waste organic byproducts generated from membrane cleaning process. In this study, physical cleaning and regular cleaning reagents such as NaOH, NaClO, citric acid and Clorox® disinfectant (6.0% Sodium Hypochlorite and other ingredient 94.0%), as well as ethanol and propylene glycol, were used. Cleaning byproducts from ethanol and propylene glycol cleaning can be easily disposed of and treated by biological treatment process.

The efficiency of membrane cleaning is represented by recovery of membrane specific flux:

$$r = {L_{P1}}/{L_{P0}} \times 100\% \qquad (15)$$

where r is the recovery of membrane specific flux, [%], L_{P1} the post-cleaning clean water membrane specific flux, [L/m^2·h·bar (LMH/bar)], and L_{P0} the pre-cleaning clean water membrane specific flux, [L/m^2·h·bar (LMH/bar)].

Four cleaning methods were applied to fouled PVDF and PVC membranes as follows:

- Method 1: (A) clean water rinsing, (B) mechanical cleaning (wet sponge), (C) citric acid cleaning (1% citric acid, soaked for 12 h), (D) NaOH cleaning (1% NaOH, soaked for 12 h), (E) NaClO cleaning (1% NaClO, soaked for 12 h).
- Method 2: (A) clean water rinsing, (B) mechanical cleaning (wet sponge), (C) citric acid cleaning (1% citric acid, soaked for 2 h), (D) NaOH cleaning (1% NaOH, soaked for 2 h), (E) Clorox® disinfectant cleaning (20% solution, pumping permeation for 30 min).
- Method 3: (A) clean water rinsing, (B) mechanical cleaning (wet sponge), (C) citric acid cleaning (1% citric acid, soaked for 2 h), (D) NaOH cleaning (1% NaOH, soaked for 2 h), (E) Clorox® disinfectant (20% solution) + industrial alcohol cleaning (pumping permeation for 30 min).
- Method 4: (A) clean water rinsing, (B) mechanical cleaning (wet sponge), (C) citric acid cleaning (1% citric acid, soaked for 2 h), (D) NaOH cleaning (1% NaOH, soaked for 2 h), (E) Clorox® disinfectant (20% solution) + propylene glycol (50% solution) cleaning (pumping permeation for 30 min).

It can be seen from the above cleaning methods that it takes at least 36 h to clean a membrane module utilizing the common industrial practice (method 1), whereas it takes only 5 h with the last three methods. The advantages of the short cleaning duration include minimum operation down time and carbon source (organic cleaning byproducts) for potential biological wastewater treatment process.

2.11 FTIR Spectroscopy

Fourier transform infrared spectroscopy (FTIR) analysis was conducted using Nicolet 5700 FTIR spectrometer at Tongji University, Shanghai, China. FTIR has been a useful technique for materials analysis in laboratory for tens of years. An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present.

Membrane material was vacuum freeze-dried for 24 h at Hawaii Natural Energy Institute, University of Hawaii at Manoa and stored at 4°C for subsequently FTIR analysis at Tongji University, Shanghai, China. The following technical parameters were adopted in FTIR analysis:

(1) Interferometer: digital interferometer, dynamic adjustment was up to 130,000Hz;

(2) Signal to noise ratio: 50000:1 (peak-to-peak value, 1 min scan); peak-to-peak noise: greater than 8.68×10^{-6}Abs (1 min scan); RMS noise: greater than 1.95×10^{-6}Abs (1 min scan); ASTM linearity: greater than 0.07%T;

(3) Spectrum range: 7800-50cm^{-1}; DTGS detector: 12,500-350cm^{-1};

(4) Resolution: greater than 0.09cm^{-1}; wavelength accuracy: 0.01cm^{-1}.

2.12 SEM Analysis

Scanning electron microscopy (SEM) analysis was conducted for surface and cross section of select UF membrane samples using Hitachi S-3400N SEM. The membrane material was vacuum freeze-dried for 24 hr at Hawaii Natural Energy Institute, University of Hawaii at Manoa and stored at 4°C for subsequently SEM analysis at Tongji University, Shanghai, China. The membrane sample was prepared with surface coating prior to SEM analysis.

Major technical parameters for the SEM include are as follows: accelerating voltage: 15KV; probe current: 30μm; working distance: 10mm; aperture size: #4; secondary electron detector resolution: 3.5nm; magnification: 20000x, 10000x, 5000x, and 1000x.

2.13 EDX Analysis

Energy-dispersive X-ray spectroscopy (EDX) analysis was conducted using Bruker Quantax 200 at Tongji University, Shanghai, China. EDX is an analytical technique for chemical characterization of the membrane sample through measuring X-ray spectroscopy generated in SEM. EDX is connected with SEM, and it can perform qualitative and near quantitative analysis for elements with atomic number greater than 4 (Boron 5-Uranium 92) in periodic table. EDX is a powerful tool for microanalysis of most elements.

Major technical parameters for the SEM include are as follows: EDX energy resolution: 130 eV, spatial resolution: $1\mu m^3$, analytical range of elements: Z5~Z~92; accelerating voltage: 20KV; probe current: 70μA; working distance: 10mm; aperture size: #1.

CHAPTER 3 PERFORMANCE OF BENCH-SCALE AND PILOT MBRS

3.1 Bench-Scale MBR Performance

3.1.1 Yield, k_d, and Food to Microorganism Ratio

The yield, Y, and decay coefficient, k_d, were determined for bench MBRs using average values of substrate utilization, U, under standard 10-d, 20-d, and 30-d MCRTs using equation 6 (Figure 3.1). Since COD and VSS data were not available for 40-d MCRT condition of both bench Enviroquip and bench Ionics and standard 20-d MCRT condition was not operated for bench Ionics, only three data points (10-d, 20-d, and 30-d MCRTs) were available for bench Enviroquip and two data points (10-d and 30-d MCRTs) were available bench Ionics for the estimation of cell yield and decay coefficient. Both Y and k_d for bench Enviroquip and bench Ionics MBRs were in the lower range of the typical values (Metcalf&Eddy, 2003) in municipal wastewater treatment as presented in Table 3.1. Low cell yield values for bench Enviroquip and bench Ionics indicates less sludge accumulation in the reactors, which can be verified by relatively low suspended solids concentration in bench reactors. Though limited data were available for the determination of cell yield and decay coefficient, it did provide a fairly accurate estimation of these two parameters.

The food to microorganism ratio (F/M) for the two bench-scale MBRs at the operating conditions in determining Y and k_d was calculated using Equation 3 as presented in Figure 3.2 and Table 3.2. As MCRT increases, more biomass was able to be maintained in reactors and therefore F/M ratio decreases. The highest and lowest F/M ratios in the bench Enviroquip MBR were 0.48 and 0.23 gCOD/gVSS.d at the 10-d and 30-d MCRT, respectively. The highest and lowest F/M ratios in the bench Ionics MBR

were 0.55 and 0.25 gCOD/gVSS.d at the 10-d and 30-d MCRT, respectively. Table 3.2 also summarized the F/M ratio for these operating conditions. It should be noted that there were high variations of F/M ratio due to occasionally high COD values at Honouliuli WWTP resulted from flushing of collection system by high wastewater flows due to inflow/infiltration when heavy rain event occurred.

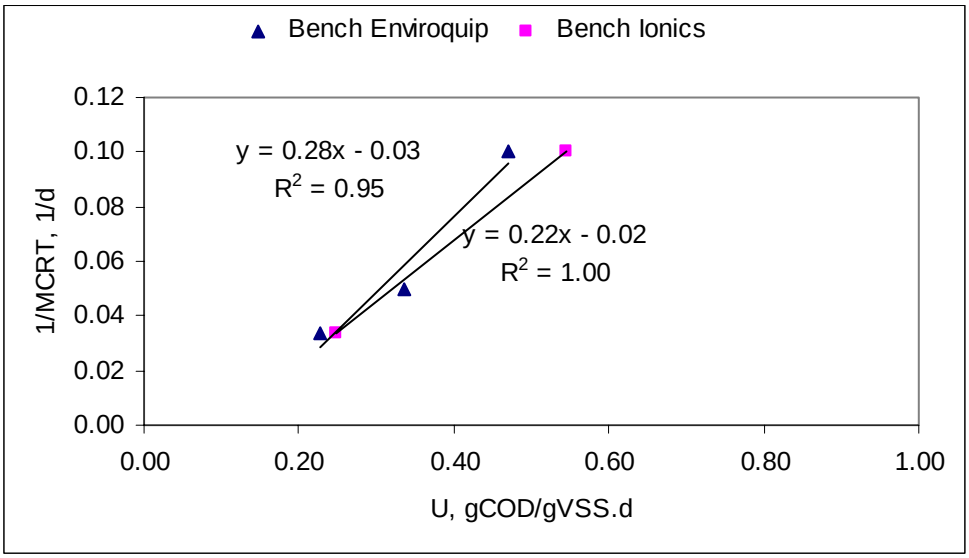


Figure 3.1 1/MCRT vs biological substrate utilization, U, to determine Y and k_d

Table 3.1 Summary of Y and K_d for bench Enviroquip and bench Ionics MBRs and typical values for municipal wastewater treatment

Source	Y, gVSS/gCOD	K_d, d^{-1}
Bench Enviroquip MBR	0.28	0.03
Bench Ionics MBR	0.22	0.02
Typical values at 20°C	0.25 – 0.4	0.02-0.1

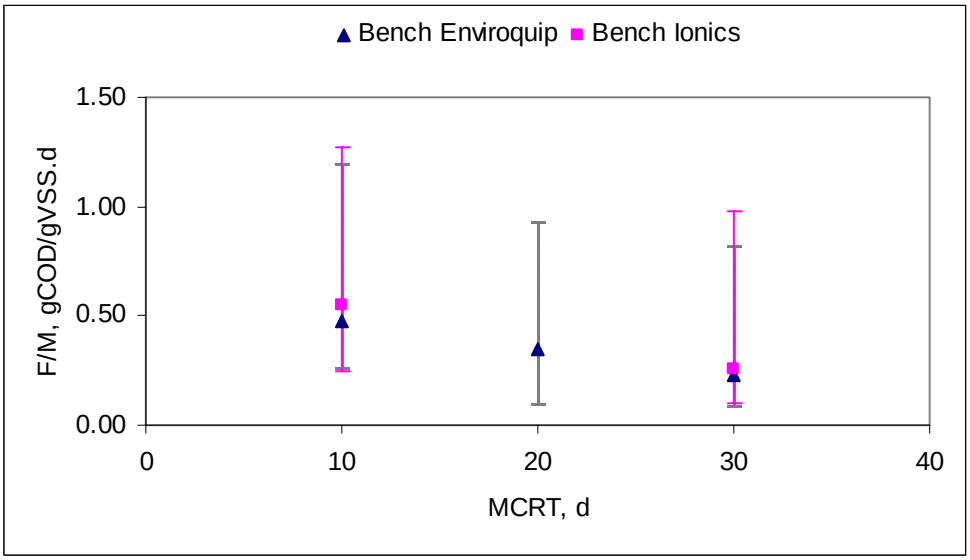


Figure 3.2 F/M ratio vs MCRT for Bench MBRs

Table 3.2 F/M for bench-scale MBRs

MCRT, d	F/M, gCOD/gVSS.d			
	Bench Enviroquip		Bench Ionics	
	Mean	Range	Mean	Range
10	0.48	0.27 - 1.19	0.55	0.25 - 1.28
20	0.35	0.1 - 0.93	----	----
30	0.23	0.09 - 0.82	0.25	0.10 - 0.98

3.1.2 Mixed Liquor Suspended Solids

The mixed liquor suspended solids (MLSS) for the bench Enviroquip and bench Ionics MBRs at all operating conditions are plotted in Figure 3.3. Figure 3.3 indicates that MLSS concentrations at all operating conditions, except 20-d MCRT with glucose addition and no sludge wasting condition, decreased during the course of operation of each condition. The MLSS of bench Enviroquip MBR in the no sludge wasting condition steadily increased to approximately 9 g/L and maintained at this level, while the MLSS of

bench Ionics MBR did not increase and maintained at around 6 g/L. The MLSS of bench Enviroquip MBR in 20-d MCRT with addition of glucose equivalent to 50% influent BOD_5, which is approximately 300 mg/L, increased continuously during 7 days of operation.

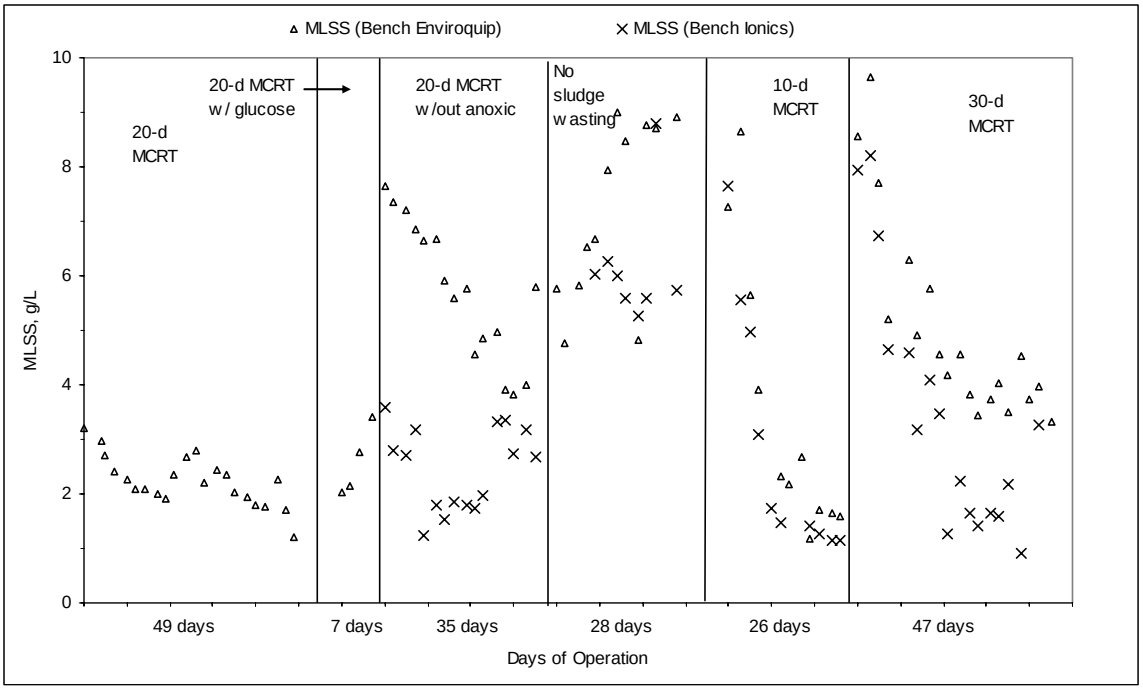


Figure 3.3 MLSS for bench Enviroquip and bench Ionics MBRs

Table 3.3 summarizes the MLSS concentrations for all operating conditions. Comparison of MLSS concentrations in both bench Enviroquip MBR and bench Ionics MBR indicates that MLSS concentration in bench Enviroquip MBR is relatively higher than that in bench Ionics MBR. The difference of MLSS can be explained by the relatively small reactor volume of bench Ionics MBR which is not as favorable for biomass accumulation as bench Enviroquip MBR. The MLSS concentration result is consistent with the result of the cell yield, Y, as presented in section 3.1. The Y value for bench Enviroquip MBR is 0.28 gVSS/gCOD, which is higher than that for bench Ionics MBR, 0.22 gVSS/gCOD.

Table 3.3 MLSS for bench-scale MBRs

MCRT, d	Bench Enviroquip MLSS, g/L			Bench Ionics MLSS, g/L		
	Days of Operation	Median	Range	Days of Operation	Median	Range
10	26	2.3	1.2 - 8.6	26	1.6	1.1 - 7.6
20	49	2.2	1.2 - 3.2	----	----	----
20[a]	7	2.5	2.0 - 3.4	----	----	----
20[b]	35	5.8	3.8 - 7.6	35	2.7	1.2 - 3.6
30	47	4.5	3.3 - 9.6	47	3.2	0.9 - 8.2
No sludge wasting	28	7.3	4.8 - 9.0	19	5.9	5.3 - 8.8

Note:
a. with glucose addition
b. without anoxic tank

Percent volatile SS in MLSS is summarized in Table 3.4. Results in Table 3.4 indicate that volatile SS percentage in MLSS was around 90% at 10 to 30 d MCRTs and decreased to low 80% range under the condition of no sludge wasting. When there is no sludge wasting for a biological wastewater treatment system, inorganic constituents will accumulate in sludge, resulting in the decrease of volatile SS percentage in MLSS.

3.1.3 TSS Removal

The influent and effluent TSS concentrations for the bench Enviroquip and bench Ionics MBRs at all operating conditions are plotted in Figure 3.4 and are summarized in Table 3.5. Influent TSS for all MCRTs was approximately 300 mg/L. Median effluent SS for both the bench Enviroquip and bench Ionics MBRs were less than 2 mg/L with a few values greater than 2 mg/L, which might be caused by slime layer growth in permeate line. Results indicate that both bench-scale MBRs can achieve 99% TSS removal.

Table 3.4 Percentage of volatile SS in MLSS for bench-scale MBRs

MCRT, d	Bench Enviroquip MLSS, % volatile			Bench Ionics MLSS, % volatile		
	Days of Operation	Median	Range	Days of Operation	Median	Range
10	26	90	81 - 94	26	94	81 - 97
20	49	93	89 - 98	----	----	----
20[a]	7	92	84 - 95	----	----	----
20[b]	35	84	81 - 85	35	87	84 - 96
30	47	87	85 - 94	47	89	87 - 97
No sludge wasting	28	82	79 - 83	19	84	53 - 91

Note:
a. with glucose addition
b. without anoxic tank

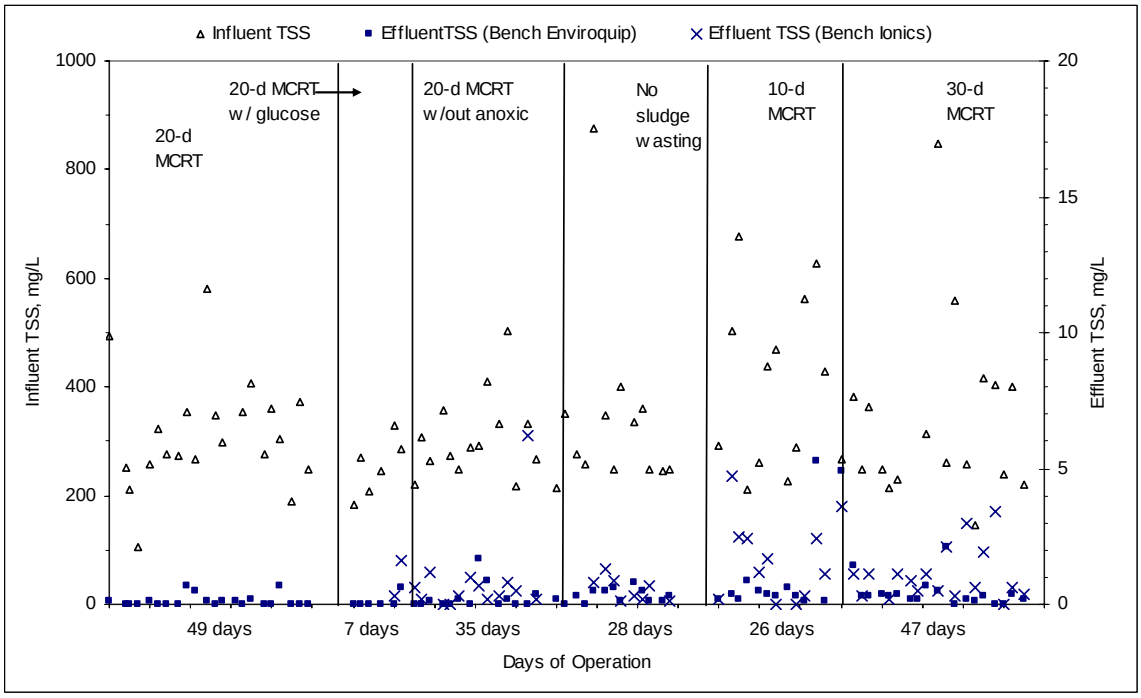


Figure 3.4 Influent and effluent TSS in bench Enviroquip and Ionics MBRs

Table 3.5 TSS for bench-scale MBRs

MCRT, d	Inf. TSS, mg/L	Effluent TSS, mg/L					
		Bench Enviroquip			Bench Ionics		
	Median	Median	Range	% Removal	Median	Range	% Removal
10	434	0.4	0.1 – 5.3	99.9	1.2	0.0 – 4.7	99.7
20	301	0.0	0.0 – 0.7	100	----	----	----
20[a]	227	0.0	0.0 – 0.0	100	----	----	----
20[b]	290	0.0	0.0 – 1.7	100	0.4	0.0 – 6.2	99.9
30	291	0.3	0.0 – 4.9	99.9	0.9	0.0 – 3.6	99.7
No sludge wasting	276	0.3	0.0 – 0.8	99.9	0.5	0.0 – 1.3	99.9

Note:
a. with glucose addition
b. without anoxic tank

3.1.4 BOD_5 Removal

The influent and effluent BOD_5 for the bench Enviroquip and bench Ionics MBRs at all operating conditions are plotted in Figure 3.5 and summarized in Table 3.6. Influent BOD_5 for all MCRTs was at medium strength level between 250 mg/L and 270 mg/L, except for the condition of 20-d MCRT with glucose addition. The 20-d MCRT with glucose addition had an influent BOD_5 of 441 mg/L, among which 150 mg/L equivalent BOD_5 of glucose was added to the influent to study the reactor performance in response to the shock loading. Results show that effluent BOD_5 for all MCRTs was less than 2.5 mg/L and greater than 99% of BOD_5 removal was achieved in both the bench Enviroquip and bench Ionics MBRs. Results indicate that both bench-scale MBRs performed normally during the course of BOD_5 shock loading operation with glucose addition.

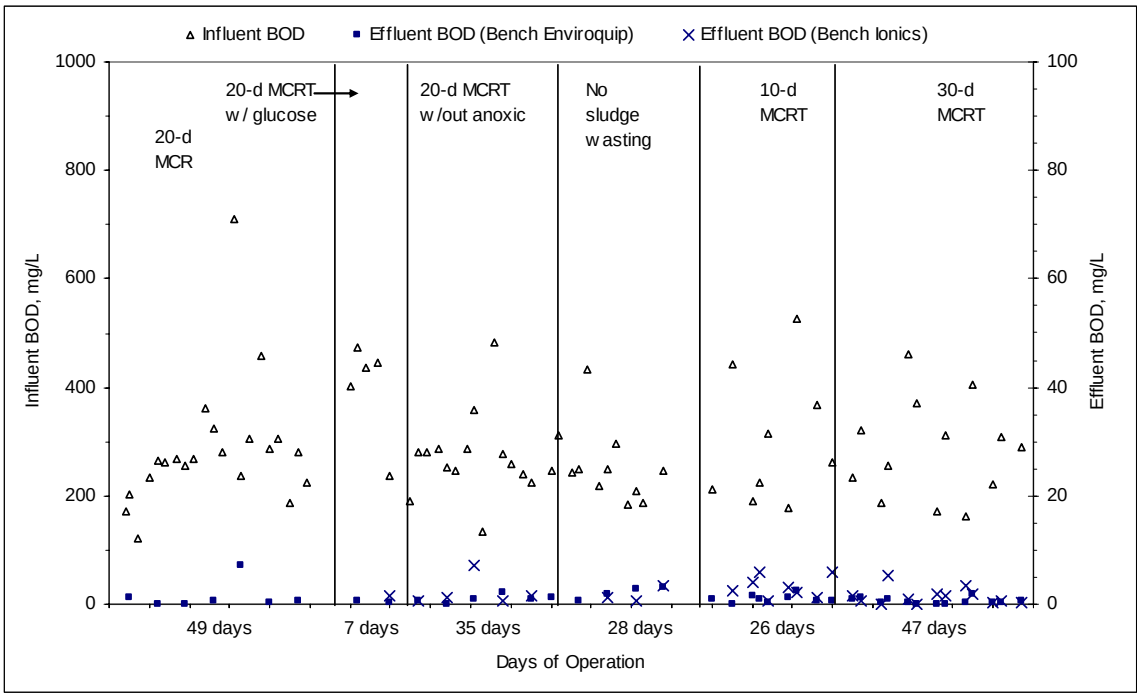


Figure 3.5 Influent and effluent BOD_5 for the bench Enviroquip and Ionics MBRs

Table 3.6 BOD_5 for bench-scale MBRs

MCRT, d	Inf. BOD_5, mg/L	Effluent BOD_5, mg/L					
		Bench Enviroquip			Bench Ionics		
	Median	Median	Range	% Removal	Median	Range	% Removal
10	270	0.9	0.0 – 2.5	99.7	2.5	0.5 – 6.0	99.1
20	268	0.5	0.0 – 7.3	99.8	----	----	----
20[a]	441	0.6	0.0 – 0.6	99.9	----	----	----
20[b]	257	0.6	0.0 – 2.1	99.7	1.4	0.6 – 7.1	99.4
30	276	0.4	0.0 – 1.9	99.9	1.2	0.0 – 6.1	99.6
No sludge wasting	248	1.9	0.0 – 3.1	99.2	1.3	0.7 – 3.3	99.5

Note:
a. with glucose addition
b. without anoxic tank

3.1.5 COD Removal

The influent and effluent COD for the bench Enviroquip and bench Ionics MBRs at all operating conditions are plotted in Figure 3.6 and summarized in Table 3.7. Influent COD for all MCRTs was between 360 mg/L and 760 mg/L. Effluent COD for all MCRTs was less than 20 mg/L. As shown in Figure 3.6, high COD influent wastewaters were experienced occasionally during the course of operation. As discussed in section 3.1.1, high COD wastewaters at Honouliuli WWTP were resulted from flushing of collection system by high wastewater flows due to inflow/infiltration when heavy rain event occurred. Results indicate that effluent COD values were generally less than 20 mg/L and greater than 97% of COD can be removed in both the bench Enviroquip and bench Ionics MBRs. The COD removal is slightly lower than the BOD removal due to the fact that some organic compounds in influent wastewater are not biodegradable and thus cannot be measured by BOD_5 analysis.

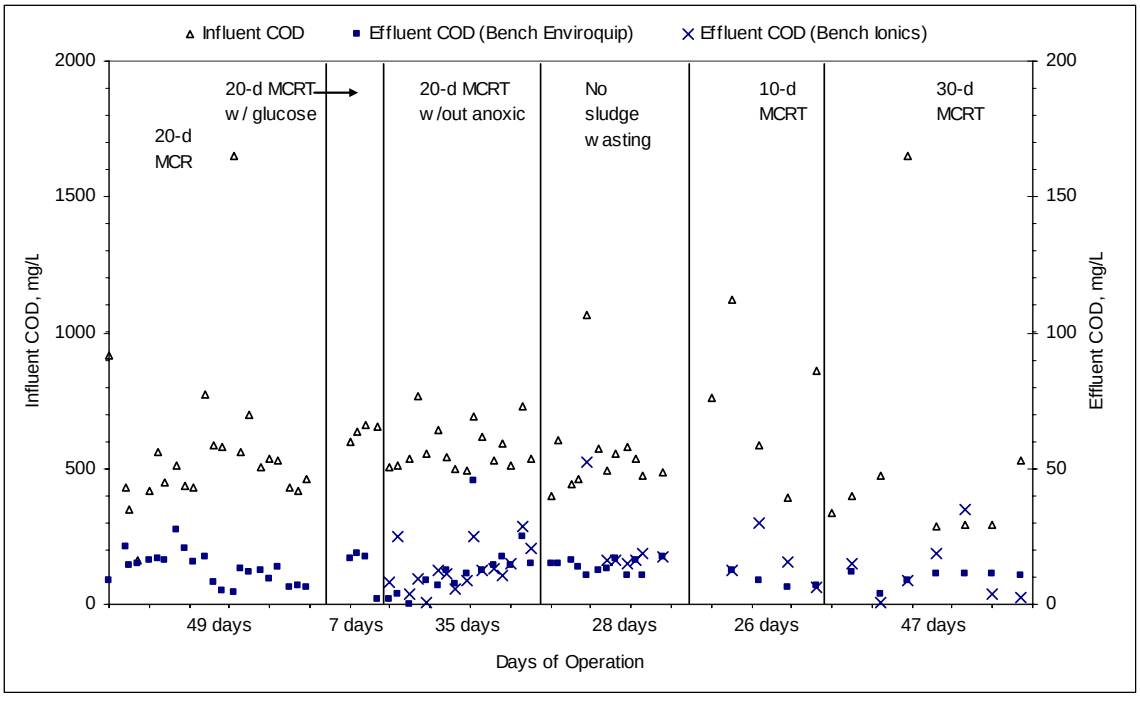


Figure 3.6 Influent and effluent COD for the bench Enviroquip and Ionics MBRs

Table 3.7 COD for bench-scale MBRs

MCRT, d	Inf. COD, mg/L	Effluent COD, mg/L					
		Bench Enviroquip			Bench Ionics		
	Median	Median	Range	% Removal	Median	Range	% Removal
10	760	8	7 - 12	99	14	6 - 30	98
20	507	13	5 - 28	97	----	----	----
20[a]	645	17	2 - 19	97	----	----	----
20[b]	541	12	1 - 46	98	12	1 - 29	98
30	367	11	4 - 12	97	9	1 - 35	98
No sludge wasting	515	14	10 - 17	97	16	15 - 52	97

Note:
a. with glucose addition
b. without anoxic tank

3.1.6 TOC Removal

The influent and effluent TOC for the bench Enviroquip and bench Ionics MBRs at all operating conditions are plotted in Figure 3.7 and summarized in Table 3.8. Influent TOC values for all MCRTs were between 32 mg/L and 61 mg/L. Effluent TOC for all MCRTs was between 4.5 mg/L and 9.8 mg/L. Results show that between 82% and 88% of TOC can be removed in both the bench Enviroquip and bench Ionics MBRs. Compared to BOD and COD results, the TOC removal is much lower. This means that a small amount of soluble organic matter that is not readily biodegradable passed through MBR membranes. TOC can be used as a rapid and accurate alternative to BOD and COD analyses for assessing pollution potential of wastewaters.

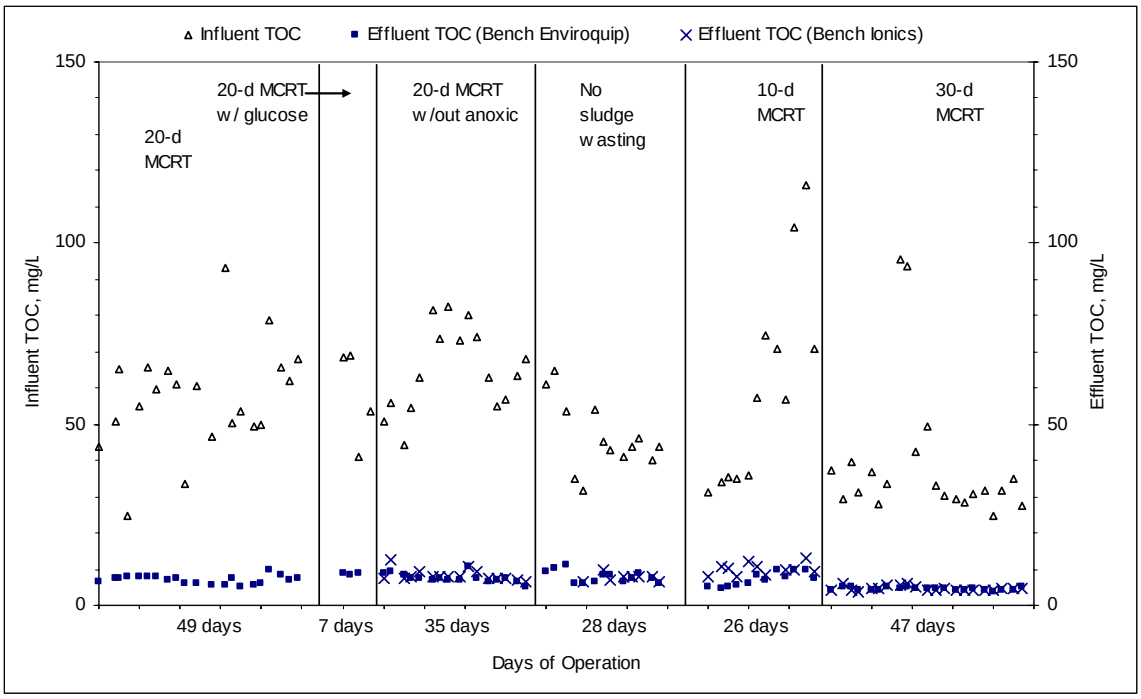


Figure 3.7 Influent and effluent TOC for the bench Enviroquip and Ionics MBRs

Table 3.8 TOC for bench-scale MBRs

MCRT, d	Inf. TOC, mg/L	Effluent TOC, mg/L					
		Bench Enviroquip			Bench Ionics		
	Median	Median	Range	% Removal	Median	Range	% Removal
10	57	7.2	4.7 – 9.9	87	9.8	7.7 –13.1	83
20	59	7.3	5.3 – 9.8	88	----	----	----
20[a]	61	8.7	8.2 – 8.7	86	----	----	----
20[b]	63	7.3	5.3 –10.9	88	7.7	6.3 –12.6	88
30	32	4.5	3.9 – 5.2	86	4.5	3.9 – 6.1	86
No sludge wasting	44	7.7	5.9 –11.1	82	7.7	6.5 –10.0	82

Note:
a. with glucose addition
b. without anoxic tank

3.1.7 TP Removal

The influent and effluent TP for the bench Enviroquip and bench Ionics MBRs at all operating conditions are plotted in Figure 3.8 and summarized in Table 3.9. Influent TP for all MCRTs was between 4.4 mg/L and 6.5 mg/L. Results show that higher TP removal was obtained in shorter MCRT and lower TP removal was obtained in longer MCRT. For the bench Enviroquip MBR, 60% of TP was removed in 10-d MCRT; approximately 40% of TP was removed in 20-d MCRT; and only 15% of TP was removed in 30-d MCRT. When there was no sludge wasting, essentially no TP was removed in the reactor. Similar trend was also observed in the bench Ionics MBR. The results can be explained by the fact that phosphorus is removed in the reactor via phosphate assimilation by microorganism and cell wasting thereafter.

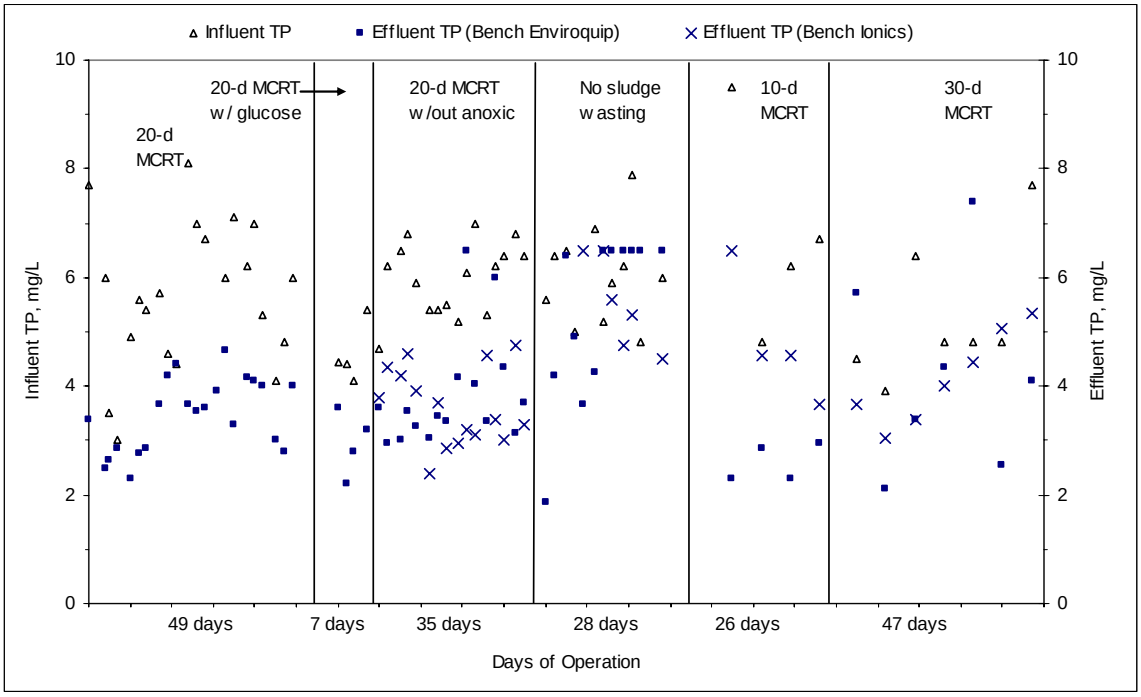


Figure 3.8 Influent and effluent TP for the bench Enviroquip and Ionics MBRs

Table 3.9 TP for bench-scale MBRs

MCRT, d	Inf. TP, mg/L	Effluent TP, mg/L					
		Bench Enviroquip			Bench Ionics		
	Median	Median	Range	% Removal	Median	Range	% Removal
10	6.5	2.6	2.3 – 3.0	60	4.6	3.7 – 6.5	29
20	5.9	3.6	2.3 – 4.7	39	----	----	----
20[a]	4.4	3.0	2.2 – 3.6	32	----	----	----
20[b]	6.2	3.5	3.0 – 6.5	43	3.6	2.4 – 4.8	15
30	4.8	4.1	2.1 – 7.4	15	4.0	3.1 – 5.4	17
No sludge wasting	6.1	6.2	1.9 – 6.5	-2	5.9	4.5 – 6.5	3

Note:
a. with glucose addition
b. without anoxic tank

3.1.8 TN Removal

The influent and effluent TN for the bench Enviroquip and bench Ionics MBRs at all operating conditions are plotted in Figure 3.9 and summarized in Table 3.10. Influent TN for all MCRTs was between 28 mg/L and 35 mg/L. For the bench Enviroquip MBR, only 36% TN removal was obtained under the condition of 20-d MCRT without anoxic tank, while TN removals for all other MCRTs were between 47% and 79%. Similar performance was also obtained in the bench Ionics MBR. The results indicate that the presence of anoxic tank can significantly improve TN removal but the removal efficiency fluctuated during the course of operation for each MCRT, which might be caused by inconsistent mixed liquor recirculation rate. The best recirculation rate should be controlled at approximately three to six times of influent flow rate (3Q to 6Q) to achieve approximately 80% TN removal. Low mixed liquor recirculation rate will not convey

sufficient nitrates from aerobic tank to anoxic tank to reduce the nitrates to nitrogen gas by denitrification process. However, high mixed liquor recirculation rate will carry too much dissolved oxygen from aerobic tank to anoxic tank, which will inhibit the performance of facultative heterotrophic denitrifying bacteria responsible for denitrification process. The presence of anoxic zone can also help alkalinity restoration in the reactor and hence reduce the amount of sodium bicarbonate solution addition to the reactor.

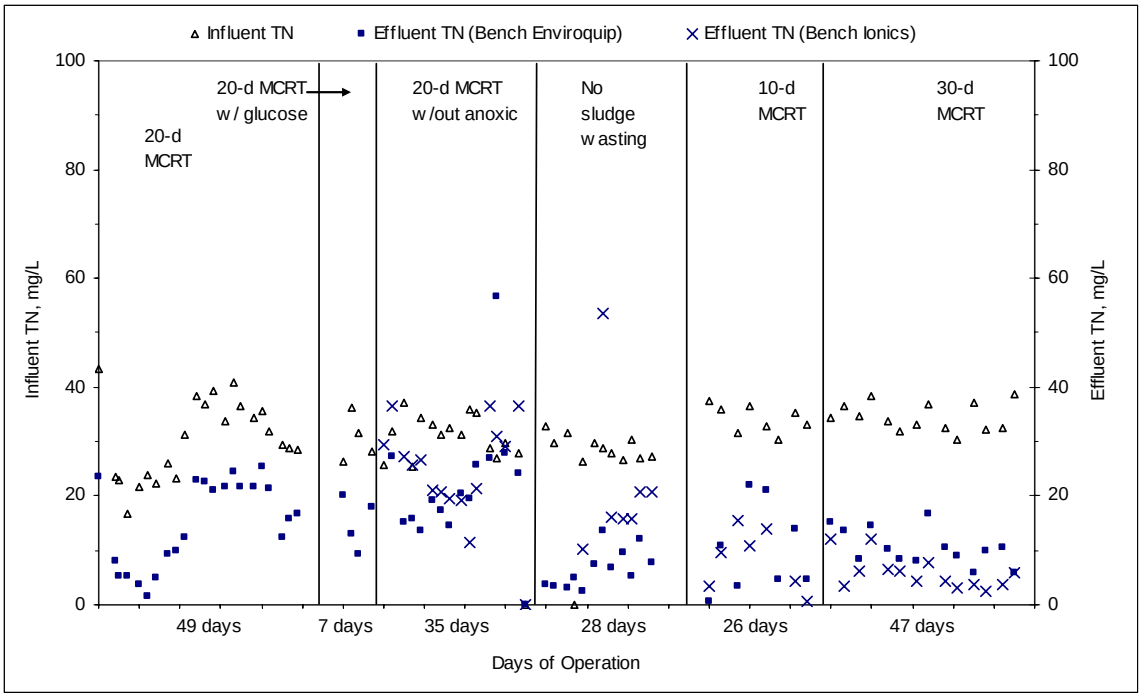


Figure 3.9 Influent and effluent TN for the bench Enviroquip and Ionics MBRs

Table 3.10 TN for bench-scale MBRs

MCRT, d	Inf. TN, mg/L	Effluent TN, mg/L					
		Bench Enviroquip			Bench Ionics		
	Median	Median	Range	% Removal	Median	Range	% Removal
10	34.2	7.8	0.7 –22.1	77	9.7	0.6 –15.5	72
20	30.4	16.2	1.6 –25.4	47	----	----	----
20[a]	29.9	15.6	9.2 –20.2	48	----	----	----
20[b]	31.2	20.0	13.5 –56	36	26.5	11.6 –36	15
30	34.0	10.0	5.8 –16.7	71	5.2	2.5 –12.2	85
No sludge wasting	28.7	6.2	2.6 –13.8	79	16.1	10.1 –53	42

Note:
a. with glucose addition
b. without anoxic tank

3.1.9 Ammonia Removal

The influent and effluent NH_4^+-N for the bench Enviroquip and bench Ionics MBRs at all operating conditions are plotted in Figure 3.10 and summarized in Table 3.11. Influent NH_4^+-N for all MCRTs was between 16 mg/L and 30 mg/L. Effluent NH_4^+-N concentrations for all MCRTs were mainly less than 1 mg/L. NH_4^+ was biologically converted to nitrate through nitrification process with the presence of aerobes, nitrifers (*Nitrosomonas* and *Nitrobacter*). Nitrification normally occurs after oxidation of other biodegradable organics since nitrification requires a long retention time, sufficient dissolved oxygen, and high MCRT. Results indicate that 10-d MCRT for MBR operation seems sufficient for nitrification, though NH_4^+-N concentration was slightly higher. NH_4^+-N concentration in no sludge wasting condition was also slightly higher, which might be attributed to its high MLSS and hence low oxygen transfer rate.

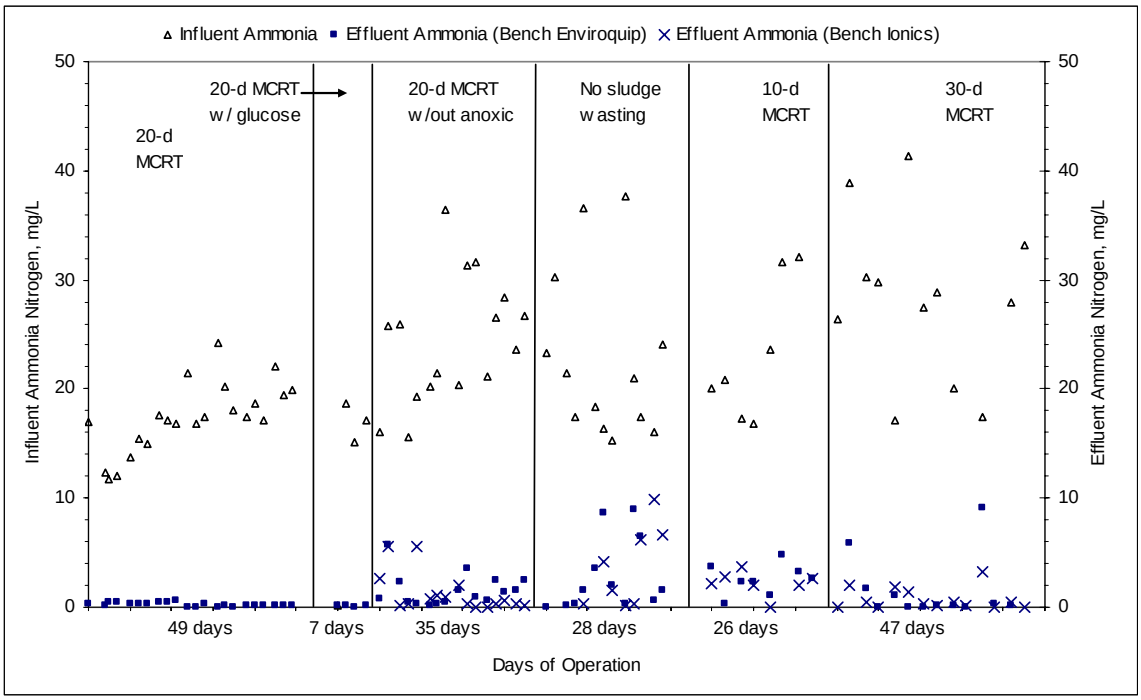


Figure 3.10 Influent and effluent NH_4^+ for the bench Enviroquip and Ionics MBRs

Table 3.11 NH_4^+-N for bench-scale MBRs

MCRT, d	Inf. NH_4^+-N, mg/L	Effluent NH_4^+-N, mg/L					
		Bench Enviroquip			Bench Ionics		
	Median	Median	Range	% Removal	Median	Range	% Removal
10	22.3	2.5	0.4 – 4.8	89	2.2	0.1 – 3.6	90
20	17.3	0.2	0.1 – 0.6	99	----	----	----
20[a]	16.2	0.1	0.1 – 0.2	99	----	----	----
20[b]	24.7	1.2	0.2 – 5.7	95	0.5	0.0 – 5.6	98
30	29.3	0.2	0.1 – 9.2	99	0.4	0.0 – 5.3	99
No sludge wasting	21.0	1.5	0.1 – 9.0	93	2.9	0.2 – 9.9	85

Note:
a. with glucose addition
b. without anoxic tank

3.1.10 Effluent Turbidity

The effluent turbidity for the bench Enviroquip and bench Ionics MBRs at all operating conditions are plotted in Figure 3.11 and are summarized in Table 3.12. For the bench Enviroquip MBR, effluent turbidities were less than 0.3 nephelometric turbidity units (NTUs), which was only slightly higher than effluent turbidity of 0.2 NTU for a well maintained MBR unit. The 0.2 NTU of turbidity is a significant value, since it is the cutoff for membrane-treated effluent intended for unrestricted wastewater recycling to qualify for a reduced dosage during UV disinfection based upon National Water Research Institute (NWRI) guidelines. However, effluent turbidities for the bench Ionics MBR were between 0.26 and 0.74 NTU, which was much higher than 0.2 NTU. The turbidity results are consistent with TSS results, which might be resulted from excessive slime layer growth in the permeate line of the bench Ionics MBR.

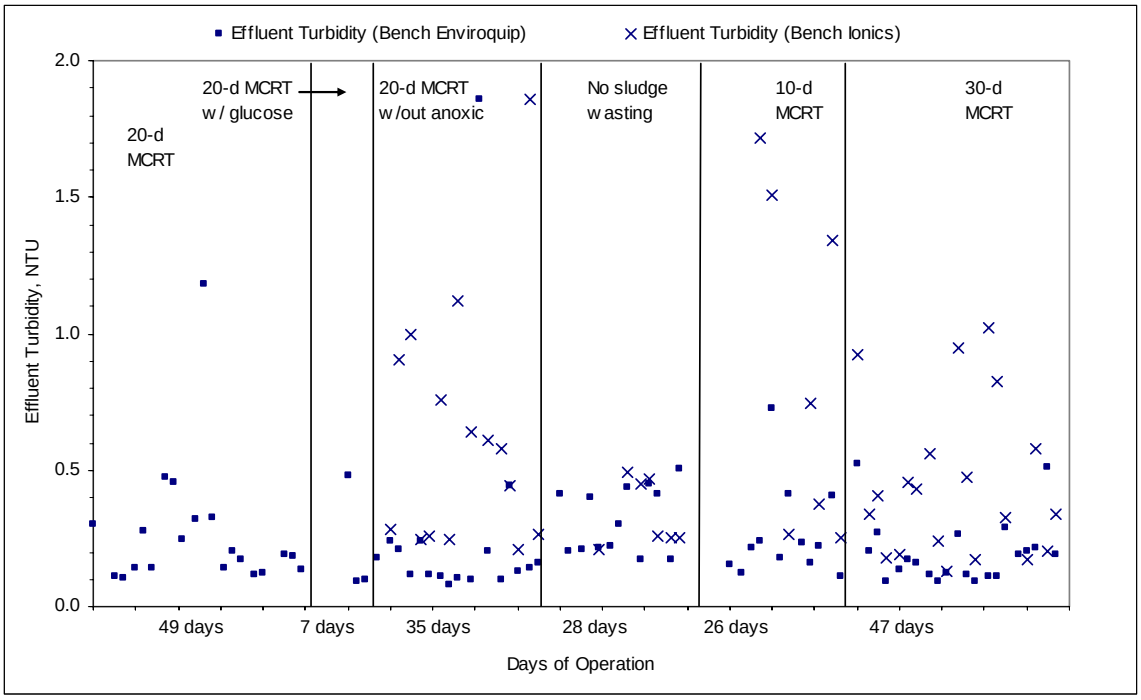


Figure 3.11 Effluent turbidity for the bench Enviroquip and Ionics MBRs

Table 3.12 Effluent turbidity for bench-scale MBRs

MCRT, d	Effluent turbidity, NTU			
	Bench Enviroquip		Bench Ionics	
	Median	Range	Median	Range
10	0.22	0.11 – 0.73	0.74	0.25 – 1.72
20	0.19	0.10 – 1.18	----	----
20[a]	0.14	0.10 – 0.48	----	----
20[b]	0.14	0.08 – 1.86	0.58	0.21 – 1.86
30	0.17	0.09 – 0.52	0.37	0.13 – 1.02
No sludge wasting	0.30	0.17 – 0.50	0.26	0.21 – 0.50

Note:
a. with glucose addition
b. without anoxic tank

3.1.11 UVT_{254}

The effluent ultraviolet transmittance (UVT) values for the bench Enviroquip and bench Ionics MBRs at all operating conditions are plotted in Figure 3.12 and are summarized in Table 3.13. Influent UVT_{254} for all MCRTs was between 42% and 50%. UVT is defined as the percentage of UV light at 254 nm not absorbed after passing through 1 cm of effluent sample. UVT is an important parameter for the design of UV disinfection. The UVT value of 65% is significant, since it is the cutoff for membrane-treated effluent intended for unrestricted wastewater recycling to qualify for a reduced dosage during UV disinfection based upon NWRI guidelines. Reduced UVT will lower the intensity of the UV light in water, thus requiring longer exposure time in order to deliver the proper UV dose. UV disinfection systems for secondary treated effluents are typically designed for 60% to 70% UVT. Results show that 70% to 80% of effluent

UVT_{254} was achieved for both the bench Enviroquip and bench Ionics MBRs, which is superior to traditional secondary treated effluent.

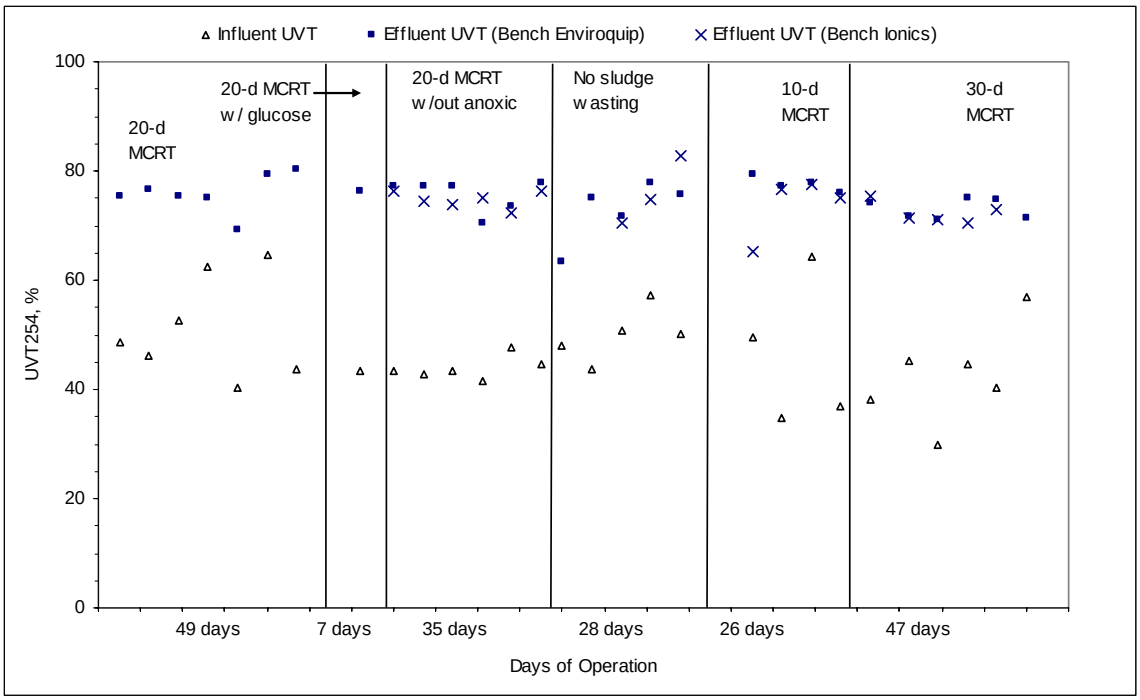


Figure 3.12 Influent and effluent UVT_{254} for the bench Enviroquip and Ionics MBRs

Table 3.13 Influent and Effluent UVT_{254} for bench-scale MBRs

MCRT, d	Influent UVT_{254}, %	Effluent UVT_{254}, %			
		Bench Enviroquip		Bench Ionics	
		Median	Range	Median	Range
10	43.3	77.6	76.0 – 79.3	75.9	65.4 – 77.5
20	48.5	75.5	69.4 – 80.3	----	----
20[a]	43.3	76.2	76.2 – 76.2	----	----
20[b]	43.4	77.2	70.4 – 77.9	74.8	72.3 – 76.3
30	42.6	72.8	71.2 – 75.0	71.3	70.5 – 75.5
No sludge wasting	50.1	75.1	63.4 – 77.9	74.9	70.5 – 82.8

Note:
a. with glucose addition
b. without anoxic tank

3.2 Pilot MBR Performance

Four pilot MBRs including Enviroquip, Ionics, Huber, and Koch were operated during the course of bench scale MBRs study. Influent water for both bench scale MBRs and pilot MBRs were drawn from the common feed tank, which makes permeates from bench scale MBRs and pilot MBRs comparable. Same water quality analyses were performed for bench scale and pilot MBRs. The pilot MBRs effluent water quality data were presented in Honolulu Membrane Bioreactor Pilot Study (Babcock, 2009). Table 3.14 summarizes the influent and effluent water quality data for the same water quality parameters that have been presented for bench scale MBRs.

Table 3.14 Average water quality data and removal efficiencies for pilot MBRs

Analyte	Influent	Pilot MBR effluent			
		Enviroquip (removal)	Ionics (removal)	Huber[a] (removal)	Koch[a] (removal)
TSS, mg/L	227	1.2 (99.5%)	1.5 (99.3%)	0.8 (99.8%	1.1 (99.7%)
BOD_5, mg/L	269	2.6 (99.0%)	2.3 (99.1%)	1.2 (99.7%)	1.5 (99.4%)
COD, mg/L	N/A	N/A	N/A	20 (98.6%)	14.3 (97.3%)
TOC, mg/L	57-130	9.0 (84.2%)	7.2 (87.5%)	6.5 (95.0%)	7.9 (86.6%)
Total P, mg/L	7.1	1.8 (75%)	2.4 (66%)	3.6 (65%)	3.5 (45%)
Total N, mg/L	39.9	15.8 (60%)	15.9 (60%)	14.5 (76%)	14.1 (62%)
Ammonia-N, mg/L	26.7	10.5 (61%)	2.7 (90%)	0.24 (99%)	0.2 (99%)
Turbidity, NTU	NA	0.07	0.02	0.14	0.13
UVT_{254}, %	30.1	71.6	71.2	71	75

Note:
[a]Effluent results and removal efficiencies determined based upon measured influent data from a different period of test of raw wastewater.

Results show that effluent TSS for pilot MBRs were all less than 2.0 mg/L and TSS removal rates were all greater than 99%. Effluent BOD_5 for pilot MBRs were all less than 3.0 mg/L and BOD_5 removal rates were all greater than 99%. Effluent COD for pilot Huber and Koch MBRs were less than 20 mg/L and COD removal rates were greater than 97%. The pilot MBR effluent TSS, BOD_5, and COD are comparable to bench-scale MBRs.

Effluent TOC for pilot MBRs were between 6.5 mg/L and 9.0 mg/L and TOC removal rates were between 84% and 95%. The pilot MBR effluent TOC is also comparable to bench-scale MBR effluent TOC, though 95% TOC removal was observed for pilot Huber MBR.

Effluent TP for pilot MBRs were between 1.8 mg/L and 3.5 mg/L and TP removal rates were between 45% and 75%. The pilot MBR effluent TP is comparable to bench Enviroquip MBR effluent TOC with 10-d and 20-d MCRTs, but is slightly lower than that with 30-d MCRT and apparently lower than that with no sludge wasting condition.

Effluent TN for pilot MBRs were between 14 mg/L and 16mg/L and TN removal rates were between 60% and 76%. The pilot MBR effluent TN is comparable to bench-scale MBR effluent TN with 10-d, 30-d, and no sludge wasting MCRTs. The pilot MBRs showed better removal efficiencies than bench-scale MBR with 20-d MCRT condition.

Effluent ammonia nitrogen for pilot MBRs varied substantially. Effluent ammonia nitrogen concentration in Ionics, Huber, and Koch MBRs are comparable to that in bench-scale MBRs, which were all in the range of 0.1 mg/L and 3 mg/L indicating a complete or near complete nitrification. However, effluent ammonia nitrogen concentration in pilot Enviroquip MBR is 10.5 mg/L, which is much higher than other

pilot MBRs and bench scale MBRs. High effluent ammonia nitrogen concentration indicates low nitrification occurred at various times due to high MLSS concentrations.

Effluent turbidities for pilot MBRs were all less than 0.2 NTU. It can be seen that pilot MBR performance in terms of turbidity was better than bench scale MBRs. The worse effluent turbidity performance of bench scale MBR might be attributed to slime layer growth in the small permeate line; however slime layer growth was not a problem for pilot MBR permeate lines since larger permeate line and turbulent flow within the lines did not provide the condition for slime layer growth.

Effluent UVT_{254} for pilot MBRs were between 71% and 75%. It appears that effluent UV transmittance in pilot MBRs is very similar to that in bench-scale MBRs, and reduced UV dose is anticipated in UV disinfection design.

3.3 Mixed Liquor Properties

The mixed liquor extracellular polymeric substances (EPS) and soluble microbial products (SMP) were analyzed in an attempt to explain reactor performance and membrane fouling.

3.3.1 Extracellular Polymeric Substances

EPS concentrations were quantified for carbohydrate and protein content and expressed on the basis of MLVSS (normalized concentrations). The normalized total EPS (carbohydrate and protein) concentrations for the bench Enviroquip and bench Ionics MBRs are plotted in Figure 3.13 and are summarized in Table 3.15. The total EPS concentrations for the bench Enviroquip MBR were around 30 mg/g VSS, except 18.1

mg/g VSS for the 20-d MCRT with glucose addition condition and 46.8 mg/g VSS for the no sludge wasting condition. Total EPS concentrations for the bench Ionics MBR were between 26 mg/g VSS and 39 mg/g VSS and the no sludge wasting condition did not show higher total EPS concentration as the no sludge condition of the bench Enviroquip MBR did.

The carbohydrate EPS and protein EPS concentrations for the bench Enviroquip and bench Ionics MBRs are plotted in Figure 3.14 and Figure 3.15. In Figure 3.14 and Figure 3.15 it appears that the three 20-d MCRT conditions of the bench Enviroquip MBR were high in protein EPS concentration and low in carbohydrate EPS concentration compared to other MCRT conditions, while the 20-d MCRT condition of the bench Ionics MBR was only high in protein EPS concentration compared to other MCRT conditions.

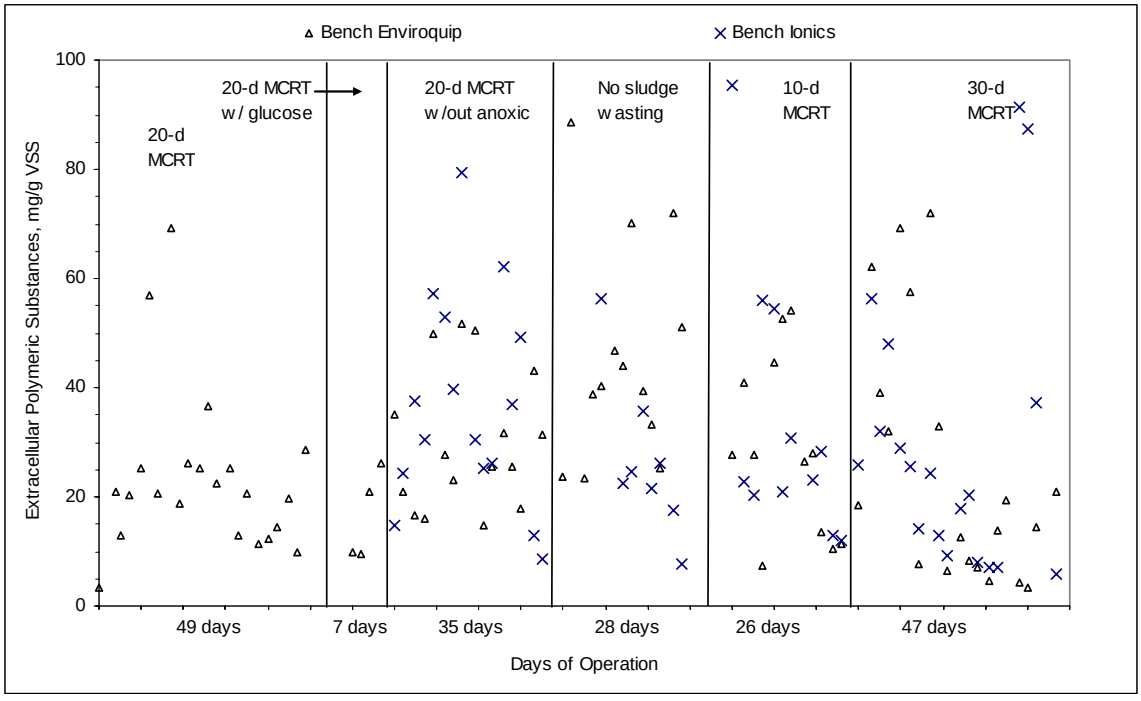


Figure 3.13 Total EPS for the bench Enviroquip and Ionics MBRs

Table 3.15 Total EPS concentrations for bench-scale MBRs

MCRT, d	Mean total EPS concentration, mg/g VSS	
	Bench Enviroquip	Bench Ionics
10	31.3	26.6
20	27.2	----
20[a]	18.1	----
20[b]	32.9	38.7
30	29.3	39.1
No sludge wasting	46.8	26.6

Note:
a. with glucose addition
b. without anoxic tank

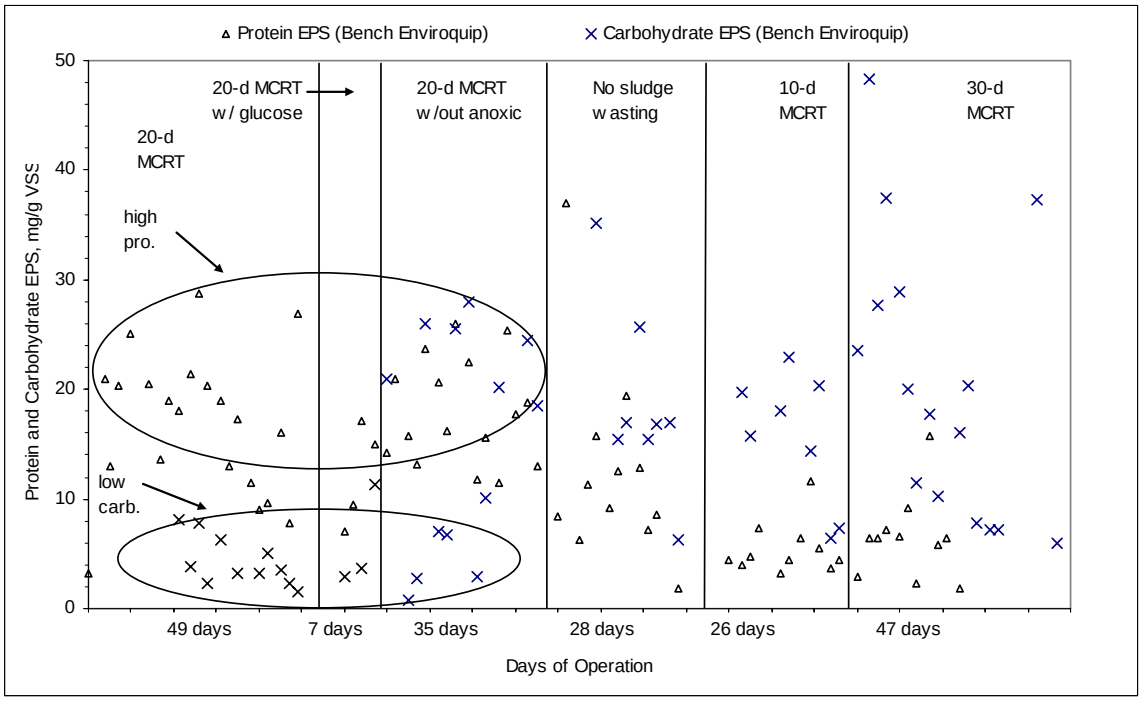


Figure 3.14 Protein and carbohydrate EPS for the bench Enviroquip MBR

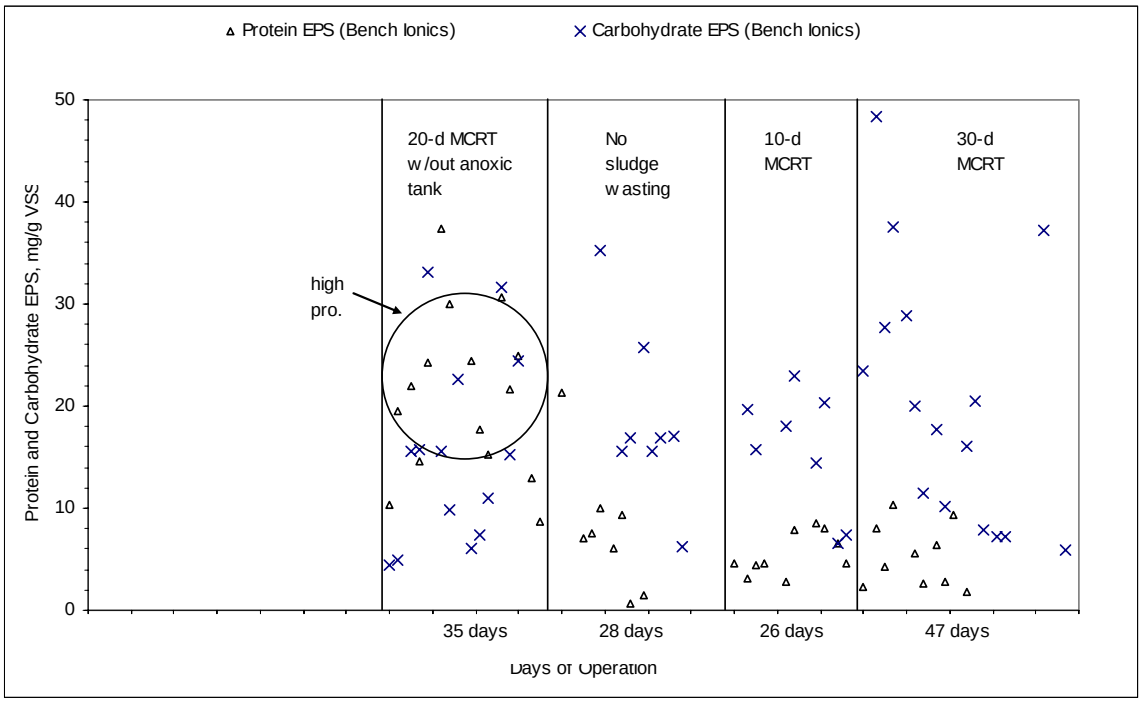


Figure 3.15 Protein and carbohydrate EPS for the bench Ionics MBR

The protein and carbohydrate EPS concentrations for the bench Enviroquip and bench Ionics MBRs are summarized in Table 3.16. Results in Table 3.16 show that the protein EPS concentrations for 20-d MCRT conditions were higher than other MCRT conditions, while the carbohydrate EPS concentrations were lower than other MCRT conditions. Distributions of protein and carbohydrate EPS concentrations are also presented in Table 3.16 and are plotted in Figure 3.16 for the bench Enviroquip MBR and in Figure 3.17 for the bench Ionics MBR to compare the protein and carbohydrate EPS concentration distributions in each MCRT condition. Results indicate that 20-d MCRT conditions showed higher protein EPS to carbohydrate EPS ratios while other MCRT conditions showed lower protein EPS to carbohydrate EPS ratios.

Table 3.16 Protein and carbohydrate EPS concentrations for bench-scale MBRs

MCRT, d	Mean protein and carbohydrate EPS concentration, mg/g VSS							
	Bench Enviroquip				Bench Ionics			
	Protein		Carbohydrate		Protein		Carbohydrate	
10	5.4	17%	25.9	83%	7.9	30%	18.6	70%
20	18.7	69%	8.6	31%	----	----	----	----
20[a]	12.2	67%	6.0	33%	----	----	----	----
20[b]	17.9	55%	14.9	45%	23.2	60%	15.5	40%
30	6.4	22%	22.9	78%	5.3	14%	33.7	86%
No sludge wasting	12.5	27%	34.3	73%	7.9	30%	18.6	70%

Note:
a. with glucose addition
b. without anoxic tank

Low protein and carbohydrate EPS concentrations were obtained form glucose addition condition, which might be an indication that membrane performance will not be affected by the shock loading. Since glucose is a ready to use carbon source for most microorganisms, glucose addition to MBR may simplify metabolism route in some microorganisms and reduce EPS and SMP production.

Research indicated that too short MCRT (less than 10 days) might increase EPS concentration (Cho et al., 2005a; Ng et al., 2006b; Masse et al., 2006) and that too long MCRT (greater than 60 days) also resulted in excessive membrane fouling (Lee et al., 2003; Han et al., 2005). In this study, no clear trend was observed for bench Ionics MBR. However, in bench Enviroquip MBR, total EPS and carbohydrate EPS appeared to increase when MCRT decreased and increased from 20-d MCRT condition.

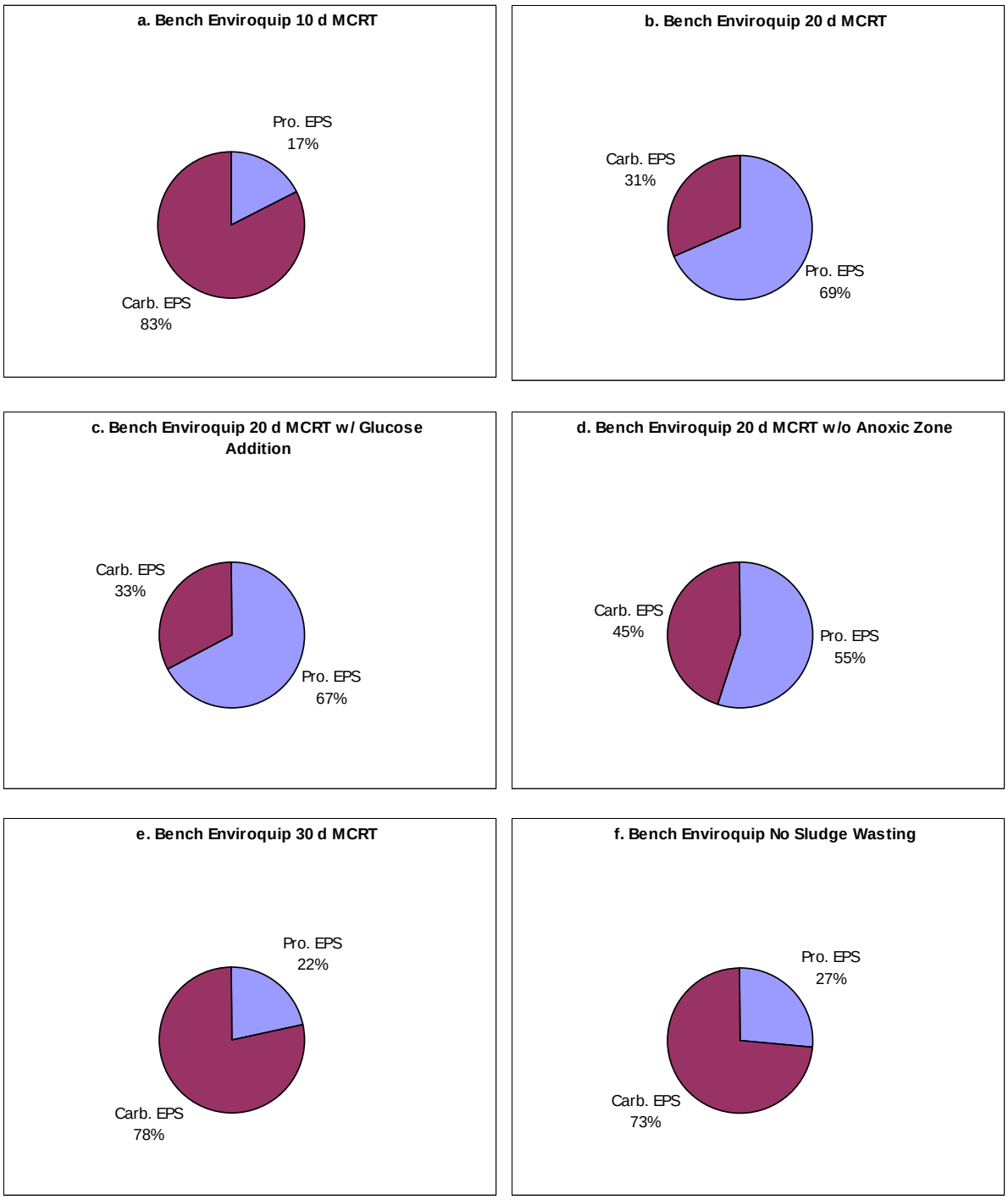


Figure 3.16 Protein and carbohydrate EPS distribution for the bench Enviroquip MBR

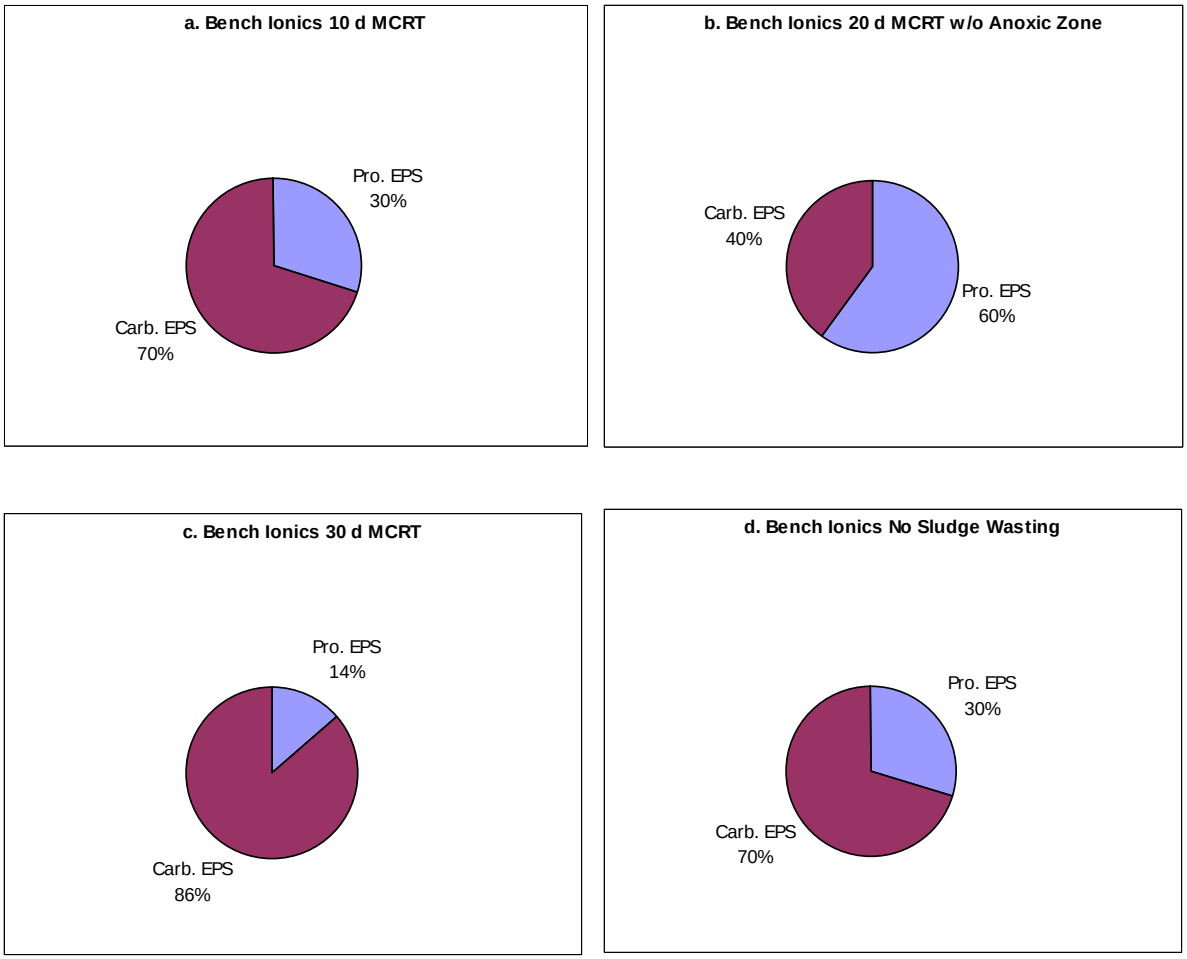


Figure 3.17 Protein and carbohydrate EPS distribution for the bench Ionics MBR

3.3.2 Soluble Microbial Products

SMP concentrations for the bench Enviroquip and bench Ionics MBRs are plotted in Figure 3.18 and are summarized in Table 3.17. Figure 3.18 shows that SMP concentrations appear to be higher at 20-d MCRT than in 10-d, 30-d MCRT, and no sludge wasting condition. The total SMP concentrations for 20-d MCRT conditions of the bench Enviroquip MBR were greater than 12.7 mg/L, while the total SMP concentrations for all other conditions were less than 10 mg/L. Similar trend was also found in the bench Ionics MBR; the total SMP concentration for 20-d MCRT without anoxic zone condition was 15.4 mg/L, while the total SMP concentrations for all other conditions were less than 10.6 mg/L.

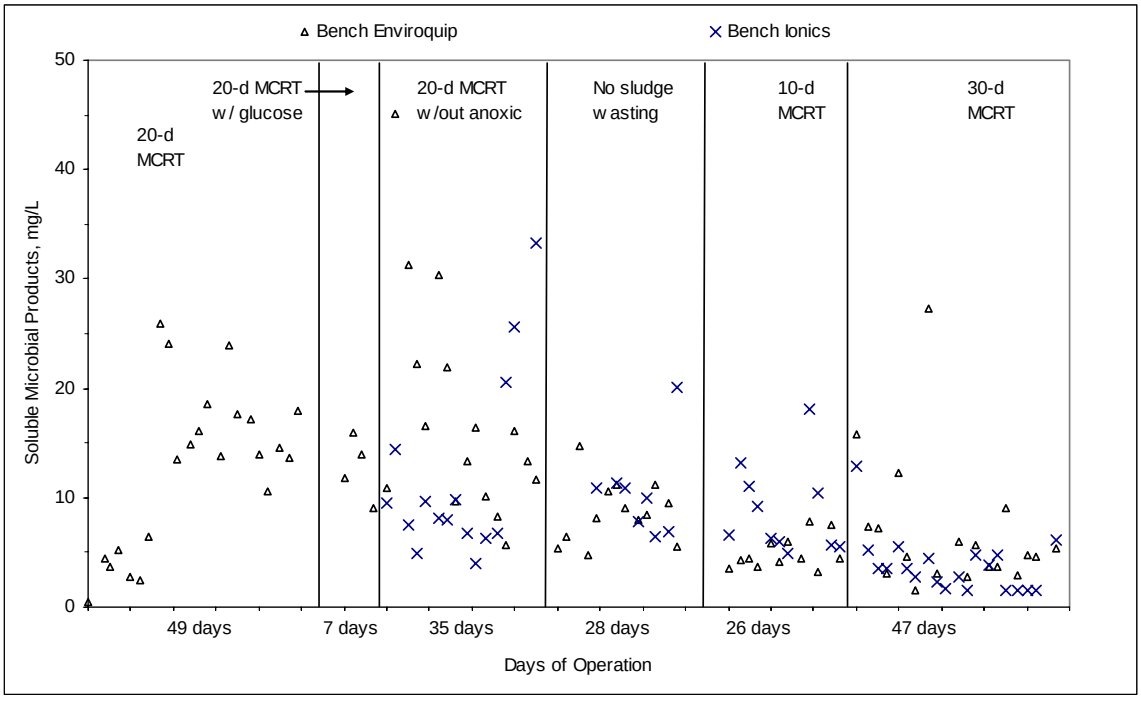


Figure 3.18 Total SMP for the bench Enviroquip and Ionics MBRs

Table 3.17 Total SMP concentrations for bench-scale MBRs

MCRT, d	Mean total SMP concentration, mg/L	
	Bench Enviroquip	Bench Ionics
10	5.2	8.8
20	17.4	----
20[a]	12.7	----
20[b]	19.8	15.4
30	9.9	5.1
No sludge wasting	9.1	10.6

Note:
a. with glucose addition
b. without anoxic tank

The carbohydrate SMP and protein SMP concentrations for the bench Enviroquip and bench Ionics MBRs are plotted in Figure 3.19 and Figure 3.20. In Figure 3.19 and Figure 3.20 it appears that the three 20-d MCRT conditions of the bench Enviroquip MBR were high in carbohydrate SMP concentration compared to other MCRT conditions. Both the protein and carbohydrate SMP concentrations in the bench Ionics MBR were fairly flat. Investigators observed that SMP concentrations decreased with increasing MCRT (Shin and Kang, 2003; Lee et al., 2003; Zhang et al., 2006b; Liang et al., 2007; Rosenberger et al., 2006). In this study, it appears that total SMP and carbohydrate SMP concentration decrease when MCRT decreased or increased from 20-d MCRT.

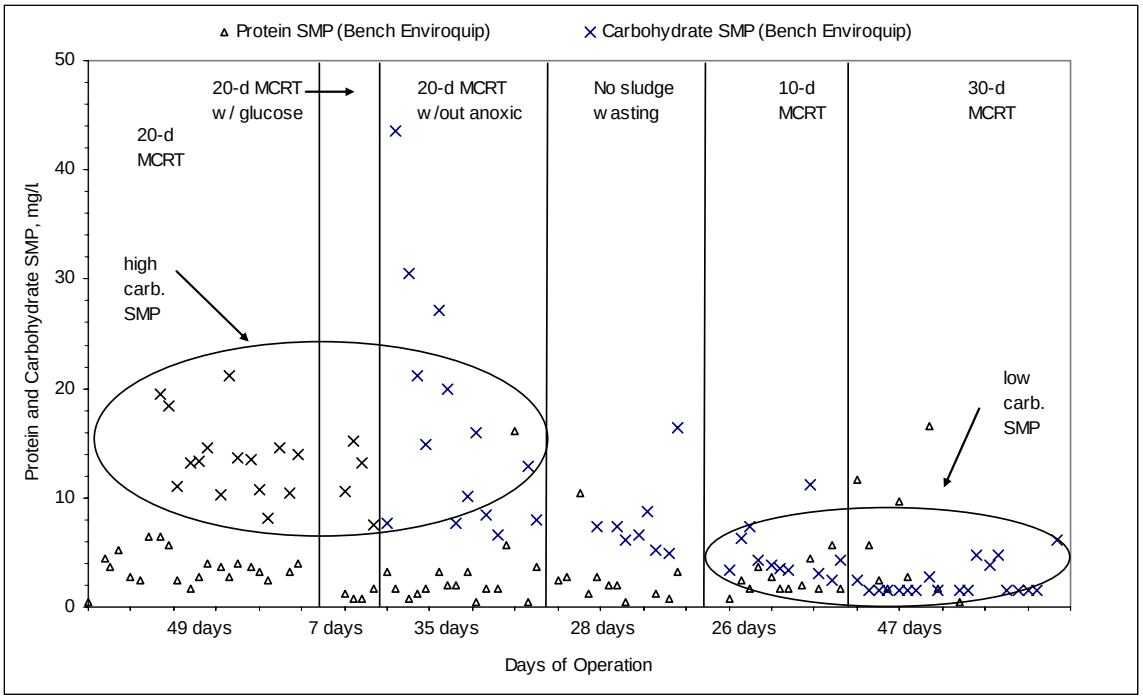


Figure 3.19 Protein and carbohydrate SMP for the bench Enviroquip MBR

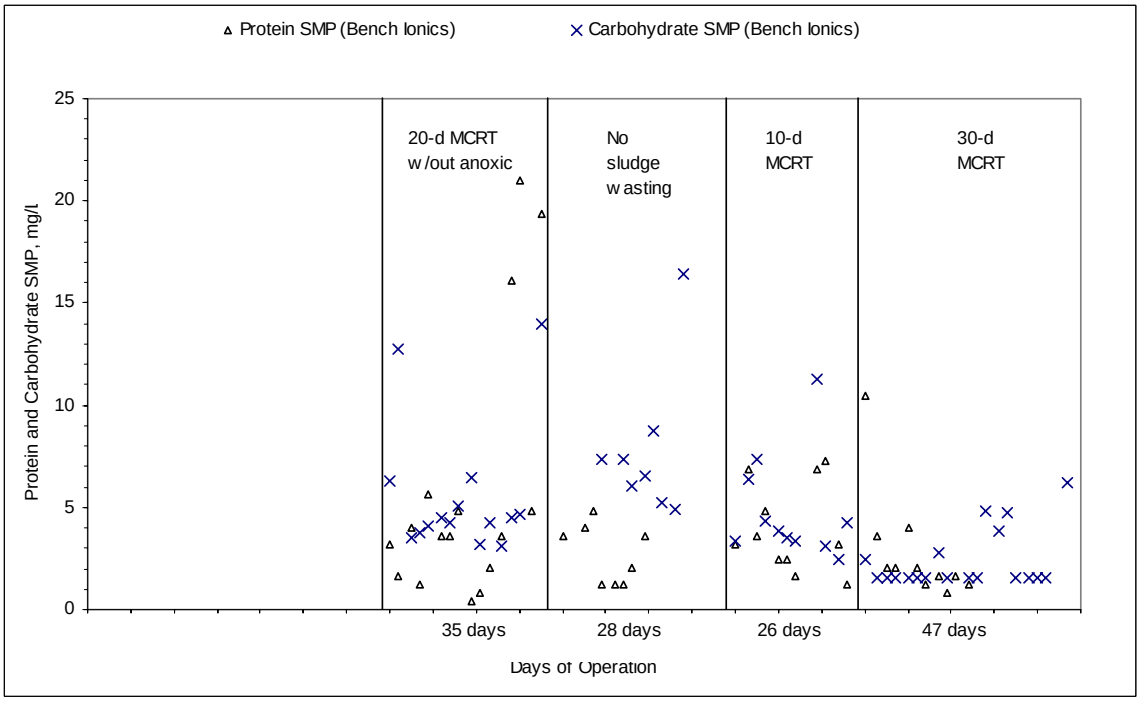


Figure 3.20 Protein and carbohydrate SMP for the bench Ionics MBR

The protein and carbohydrate SMP concentrations for the bench Enviroquip and bench Ionics MBRs are summarized in Table 3.18. Results in Table 3.18 show that the carbohydrate SMP concentrations for 20-d MCRT conditions were higher than other MCRT conditions, while the protein SMP concentrations were similar to other MCRT conditions. Distributions of protein and carbohydrate SMP concentrations are also presented in Table 3.18 and are plotted in Figure 3.21 for the bench Enviroquip MBR and in Figure 3.22 for the bench Ionics MBR to compare the protein and carbohydrate SMP concentration distributions in each MCRT condition. Results indicate that 20-d MCRT conditions of the bench Enviroquip MBR showed significant high carbohydrate to protein SMP ratios, that no sludge wasting condition of both MBRs showed moderate high carbohydrate to protein SMP ratios, and that 10-d and 30-d MCRT conditions showed fairly equal amount of carbohydrate SMP and protein SMP. Similar results to EPS were obtained for SMP, which was discussed previously.

Table 3.18 Protein and carbohydrate SMP concentrations for bench-scale MBRs

MCRT, d	Mean protein and carbohydrate SMP concentration, mg/L							
	Bench Enviroquip				Bench Ionics			
	Protein		Carbohydrate		Protein		Carbohydrate	
10	2.5	48%	2.7	52%	4.0	45%	4.8	55%
20	3.6	21%	13.8	79%	----	----	----	----
20[a]	1.1	9%	11.6	91%	----	----	----	----
20[b]	3.0	15%	16.7	85%	6.0	39%	9.4	61%
30	5.8	59%	4.1	41%	2.8	54%	2.3	46%
No sludge wasting	2.7	30%	6.4	70%	2.7	26%	7.8	74%

Note:
a. with glucose addition
b. without anoxic tank

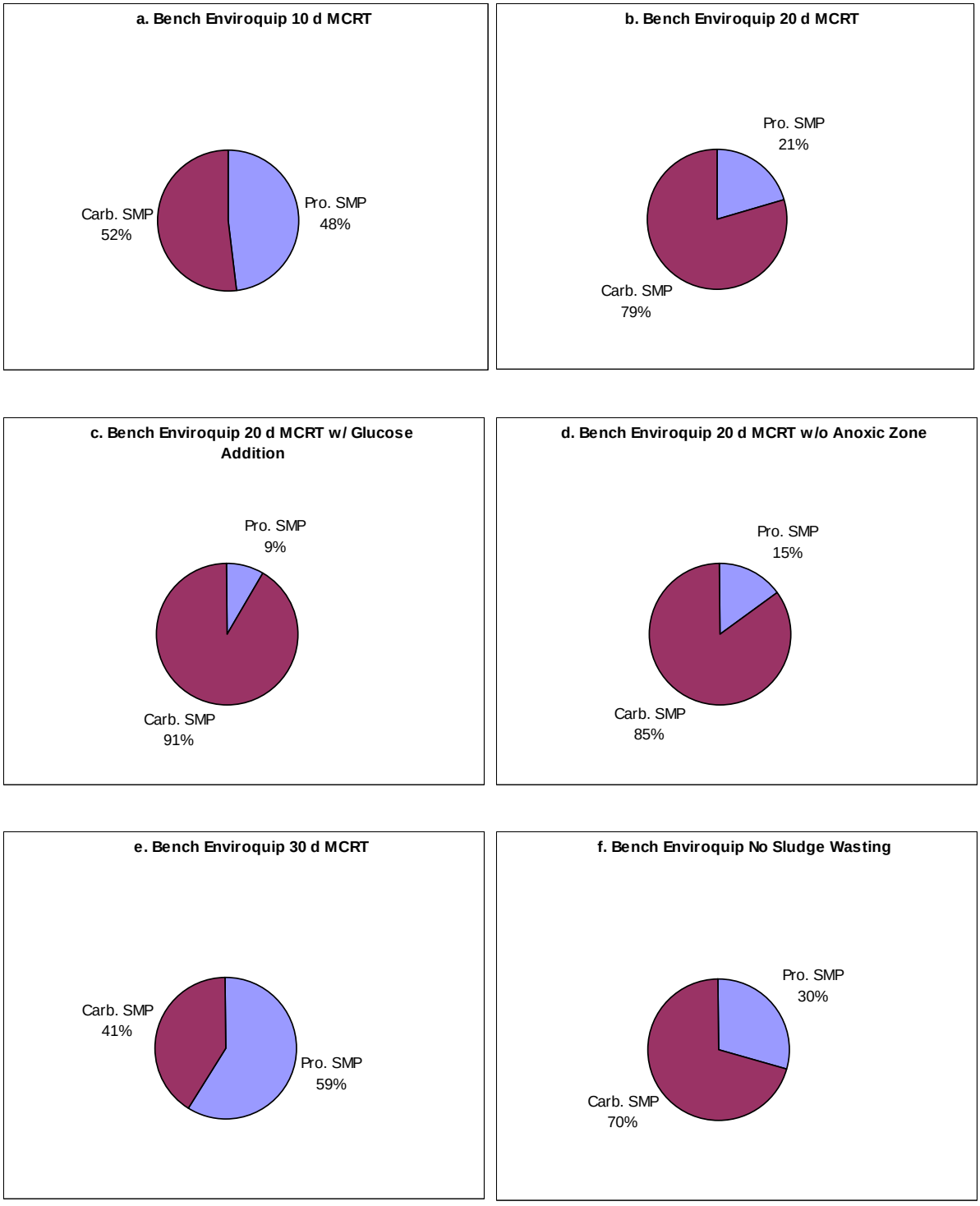


Figure 3.21 Protein and carbohydrate SMP distribution for the bench Enviroquip MBR

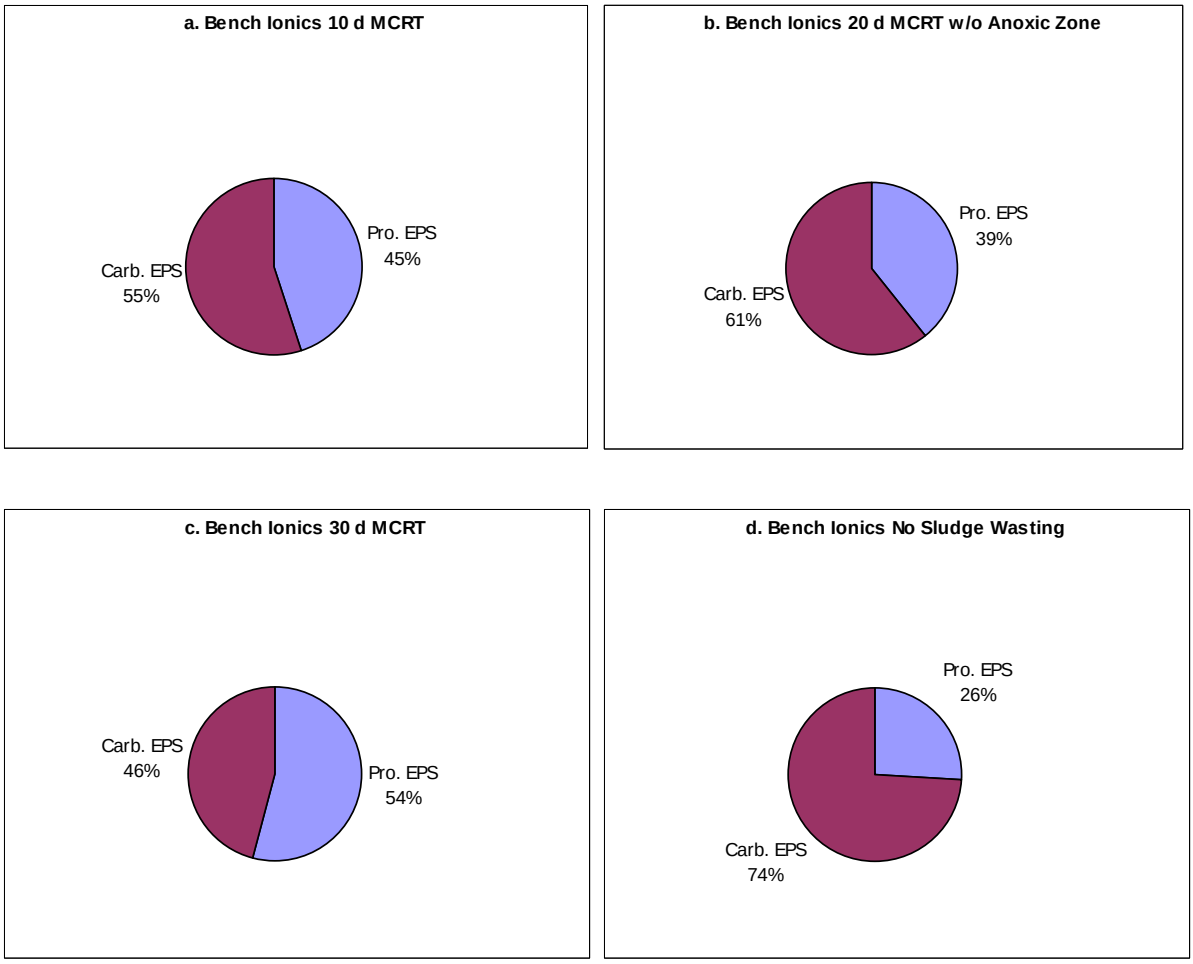


Figure 3.22 Protein and carbohydrate SMP distribution for the bench Ionics MBR

Effluent SMP (carbohydrate and protein) concentrations for the bench Enviroquip and bench Ionics MBRs are plotted in Figure 3.23 and are summarized in Table 3.19. Unlike the mixed liquor total SMP concentrations, the effluent total SMP concentrations for all MCRT conditions did not show significant difference and the average values were in the range of 4 mg/L to 9 mg/L.

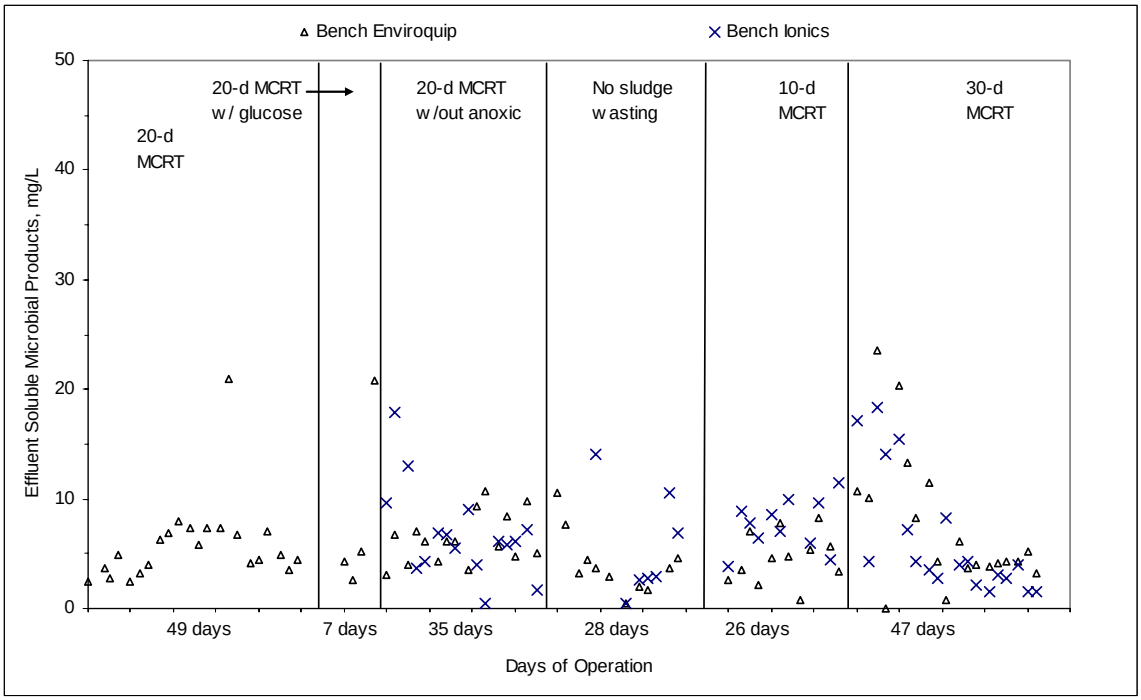


Figure 3.23 Effluent total SMP for the bench Enviroquip and Ionics MBRs

Table 3.19 Effluent total SMP concentrations for bench-scale MBRs

MCRT, d	Mean total SMP concentration, mg/L	
	Bench Enviroquip	Bench Ionics
10	4.9	7.8
20	7.4	----
20[a]	8.3	----
20[b]	6.3	7.2
30	9.4	8.2
No sludge wasting	4.3	6.5

Note:
a. with glucose addition
b. without anoxic tank

The effluent carbohydrate SMP and protein SMP concentrations for the bench Enviroquip and bench Ionics MBRs are plotted in Figure 3.24 and Figure 3.25 and are summarized in Table 3.20. Unlike the mixed liquor 20-d MCRT condition which had a higher carbohydrate SMP concentration, effluent protein and carbohydrate SMP for all MCRT conditions did not show significant differences. The average effluent protein SMP concentrations were between 1 mg/L and 3 mg/L; the average effluent carbohydrate SMP concentrations were between 3.3 mg/L and 7.4 mg/L.

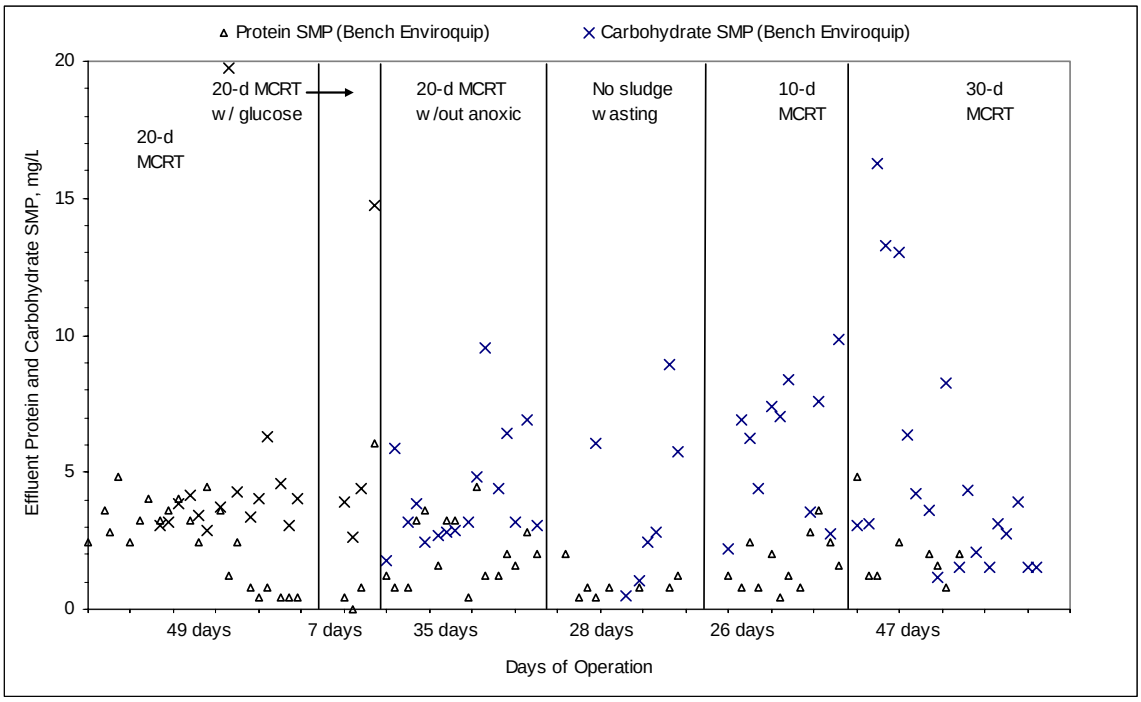


Figure 3.24 Effluent protein and carbohydrate SMP for the bench Enviroquip MBR

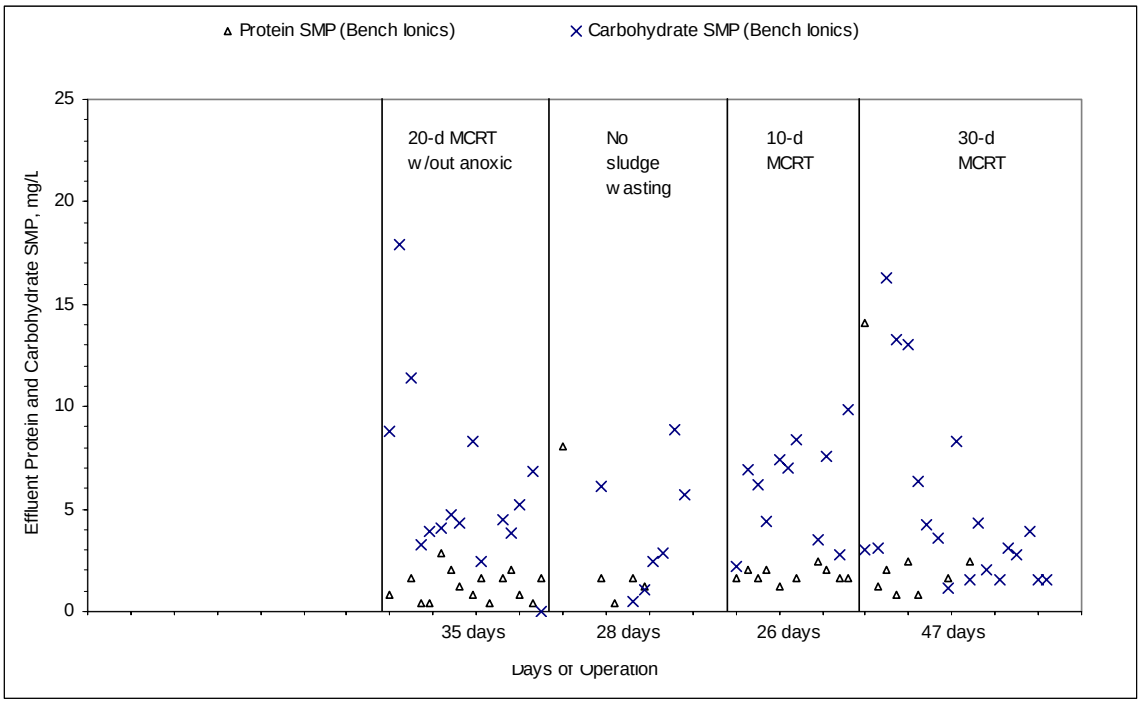


Figure 3.25 Effluent protein and carbohydrate SMP for the bench Ionics MBR

Table 3.20 Effluent protein and carbohydrate SMP concentrations for bench-scale MBRs

MCRT, d	Mean effluent protein and carbohydrate SMP concentration, mg/L			
	Bench Enviroquip		Bench Ionics	
	Protein	Carbohydrate	Protein	Carbohydrate
10	1.7	3.3	1.8	6.0
20	2.5	4.9	----	----
20[a]	1.8	6.4	----	----
20[b]	2.1	4.2	1.2	6.0
30	2.0	7.4	3.2	5.0
No sludge wasting	0.9	3.4	2.6	3.9

Note:
a. with glucose addition
b. without anoxic tank

It is interesting to see that 20-d MCRT conditions had low carbohydrate EPS, high carbohydrate mixed liquor SMP and same level of effluent SMP compared to other

MCRT conditions. The difference between the mixed liquor carbohydrate SMP concentration and the effluent carbohydrate SMP concentration under 20-d MCRT conditions were colloidal soluble microbial products that could not pass through the membranes. It is suspected that the amount of unfilterable mixed liquor SMP (i.e. colloidal SMP) in 20-d MCRT conditions were originated from EPS. It is unknown what caused the transformation of polysaccharides from solid phase to liquid phase, but this change is very important in MBR operations since EPS and SMP may have different impacts on membrane fouling. MCRT is apparently a significant factor in the change of polysaccharides from EPS to SMP, which had been demonstrated in this study.

With different EPS and SMP levels at different MCRT conditions in mixed liquor, membrane fouling caused by SMP and EPS may vary. So, long-term membrane performance was evaluated and results are presented in the following section.

3.4 Membrane Performance

Virgin membrane flux profile and membrane critical flux were analyzed for bench Enviroquip and Ionics MBRs. Long-term membrane performance under each MCRT conditions was evaluated to obtain membrane fouling rate. Fouled membranes were characterized to determine the components of resistance terms.

3.4.1 Virgin Membrane Flux Profile

Virgin membrane flux profiles for the bench Enviroquip and bench Ionics MBRs were analyzed in clear water and the results are presented in Figure 3.26 for the bench Enviroquip MBR and in Figure 3.27 for the bench Ionics MBR. Linear relationships

between TMP and membrane flux in clear water existed in virgin membrane flux profiles. Results indicated that higher TMP was required to obtain 10 gfd to 15 gfd for the bench Ionics MBR compared to the bench Enviroquip MBR. It is very likely the higher TMP requirement for the bench Ionics MBR was due to its membrane module configuration in which not all membrane surface areas were effectively in contact with the liquid phase.

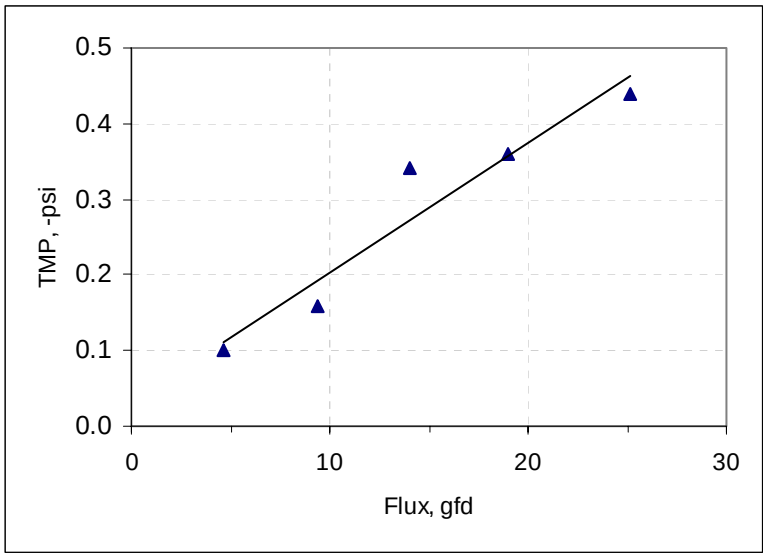


Figure 3.26 Virgin membrane flux profile for the bench Enviroquip MBR

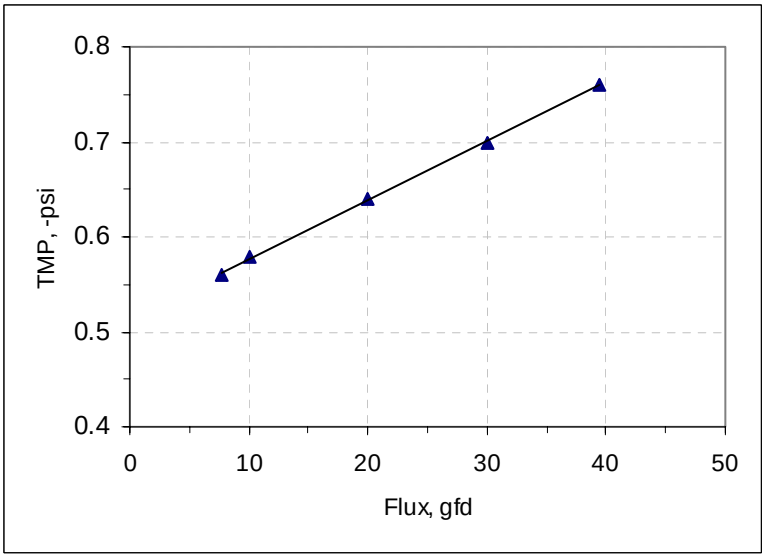


Figure 3.27 Virgin membrane flux profile for the bench Ionics MBR

3.4.2 Membrane Critical Flux

The membrane critical flux concept was first presented by Field et al. (1995) and was defined as "The critical flux hypothesis for microfiltration/ultrafiltration processes is that on start-up there exists a flux below which a decline of flux with time does not occur; above it, fouling is observed". Also two forms of critical flux were classified: strong and weak. The strong form is the flux at which the transmembrane pressure (TMP) starts to deviate from the pure water flux, which is linear to TMP. The weak form is flux below which linear relationship exists between flux and TMP, and above which fouling occurs. These two forms of critical flux has been successfully modeled by Monte Carlo simulation of colloidal membrane filtration (Chen et al., 2005). Slightly different version of critical flux definitions have been proposed depending on the method. Kwon and Vigneswaran (1998) equated critical flux to the lift velocity and some studies have also used mass balance determinations (Kwon et al., 2000).

Due to the difficulties of applying particle hydrodynamics to the identification of critical flux in real MBR systems, experimental methods have been used to determine critical flux. It is possible to observe the transition between the linearly pressure-dependent flux and the occurrence of fouling by plotting flux against TMP. Bouhabila et al. (1998) defined this as secondary critical. Ng et al. (2005) introduced the concept of sustainable flux defined as the flux for which the TMP increases gradually at an acceptable rate such that chemical cleaning is not necessary.

In real MBR system, a common practice to evaluate membrane critical flux is to use the flux-step method in which the flux is incrementally increased for a fixed duration for each increment, resulting in a stable TMP at low flux but an ever-increasing rate of TMP

increase at higher fluxes. The highest flux for which TMP remains stable is defined as critical flux. Due to the TMP jumps in flux-step method, the critical flux can be estimated to be the average of the first nonlinear flux value and the next lower value. TMP-step method can also be used but the flux-step method is preferred since it provides a better control of the flow of material deposition on the membrane surface (Delfrance and Jaffrin, 1999). In determining critical flux, hours of each flux step operation is required to determine a long-term critical flux, which is the true critical flux for a microfiltration or ultrafiltration process. This long-term critical flux determination normally requires several days of filtration operation. A practical alternative to the long-term critical determination is to estimate critical flux by determining short-term critical flux with 10 to 40 minutes of each critical flux step operation (Guo et al., 2005; Le-Clech et al., 2003) and no significant differences were found between short-term and long-term critical fluxes. The short-term critical flux determination provides an alternative to long-term critical flux determination but only requires several hours of filtration operation. 10 minutes of step flux operation were adopted in this research to estimate critical fluxes for bench Enviroquip and Ionics MBRs.

Critical flux analysis data are plotted in Figure 3.28 for the bench Enviroquip MBR and in Figure 3.29 for the bench Ionics MBR. In the bench Enviroquip MBR, when flux reached approximately 25 gfd, TMP response was not stable and appeared to increase continuously. The critical flux for the bench Enviroquip MBR was estimated to be 22 gfd (37.4 LMH), the average of this flux (25 gfd) and the next low flux (19 gfd). Similarly, the critical flux for the bench Ionics MBR was estimated to be 23.1 gfd (39.3 LMH). Results indicated that critical fluxes for the bench Enviroquip MBR and the

bench Ionics MBR are similar, but TMP requirement of 0.9 psi for the critical flux of the bench Ionics MBR was higher than the TMP requirement of 0.3 psi for the bench Enviroquip MBR. Similarly to the result of virgin membrane flux profile, the higher TMP requirement for the bench Ionics MBR might be caused by the membrane module configuration in which not all membrane surface areas were effectively in contact with the liquid phase.

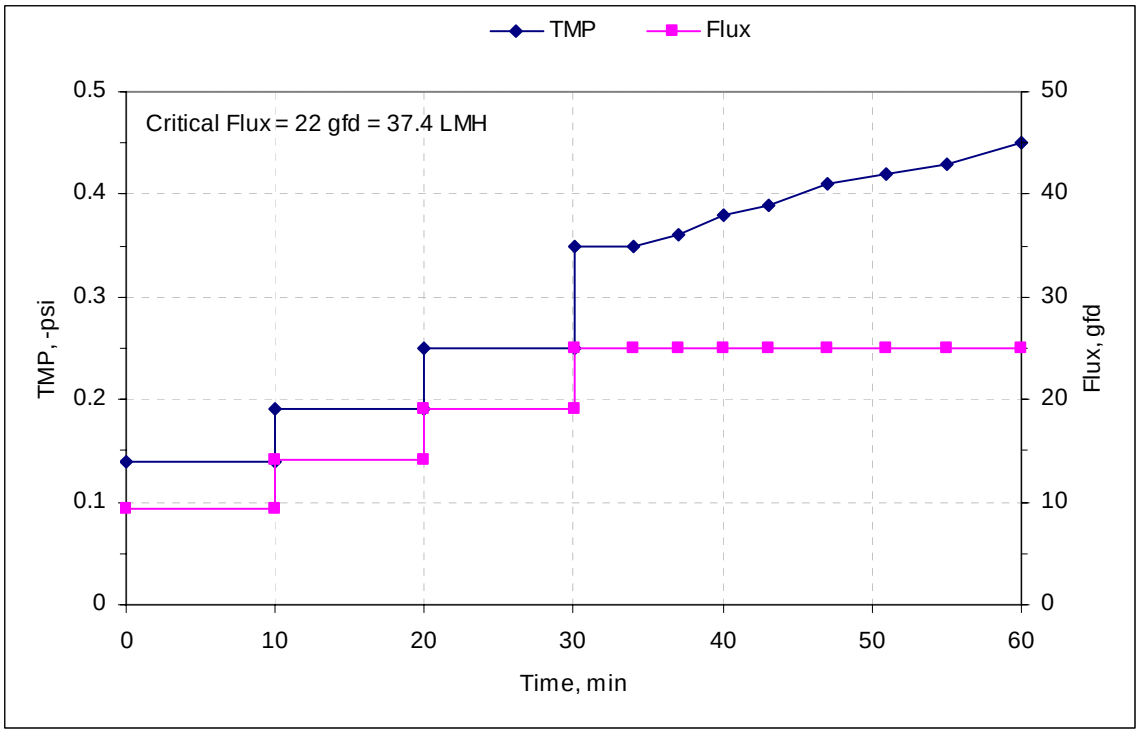


Figure 3.28 Critical flux analysis for the bench Enviroquip MBR

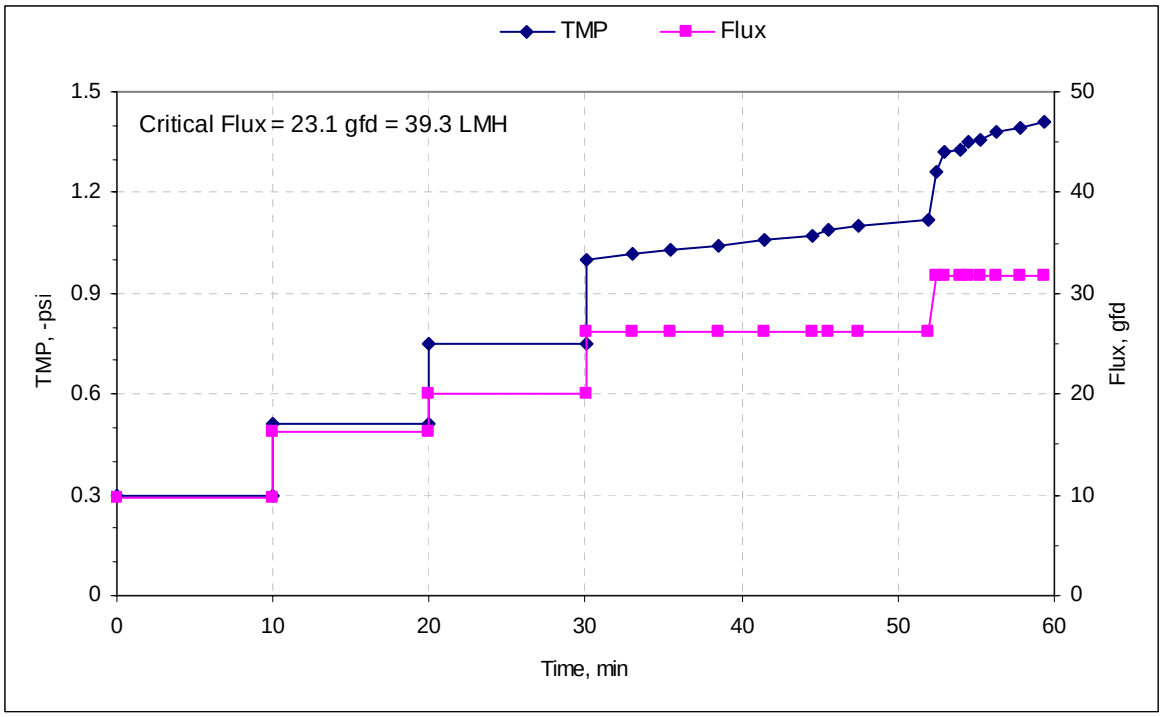


Figure 3.29 Critical flux analysis for the bench Ionics MBR

3.4.3 Long-Term Membrane Performance

Long-term membrane performance of the bench-scale MBRs was evaluated at each MCRT conditions by monitoring the membrane flux and TMP response. Bench MBRs were operated at constant subcritical fluxes, 15.7 gfd (26.7 LMH) for bench Enviroquip and 14.7 gfd (25 LMH) respectively. TMP normally increases with constant flux operation. Though intended to be kept at constant, operating fluxes started to decline after a certain period of operation. This was most likely caused by excessive membrane fouling and inability of the pump to maintain constant flux. Specific flux is plotted against time of operation to determine membrane fouling rate in terms of LMH/bar/day. Specific flux is the membrane flux obtained from an applied driving force and is defined by equation 14.

Figure 3.30 shows the membrane performance of the bench Enviroquip MBR at 10-d MCRT. Membrane flux was maintained at 26 LMH. The specific flux started from approximately 1000 LMH/bar and decreased to approximately 500 LMH/bar after 26 days of operation. The conditioning fouling rate was 17 LMH/bar/day. It is apparent that the reactor did not reach steady state. Based on the operation of other MCRT conditions for which the steady state specific flux was between 100 and 200 LMH/bar, it is estimated that the reactor needed at least another 20 days of operation to reach steady state based on the conditioning fouling rate.

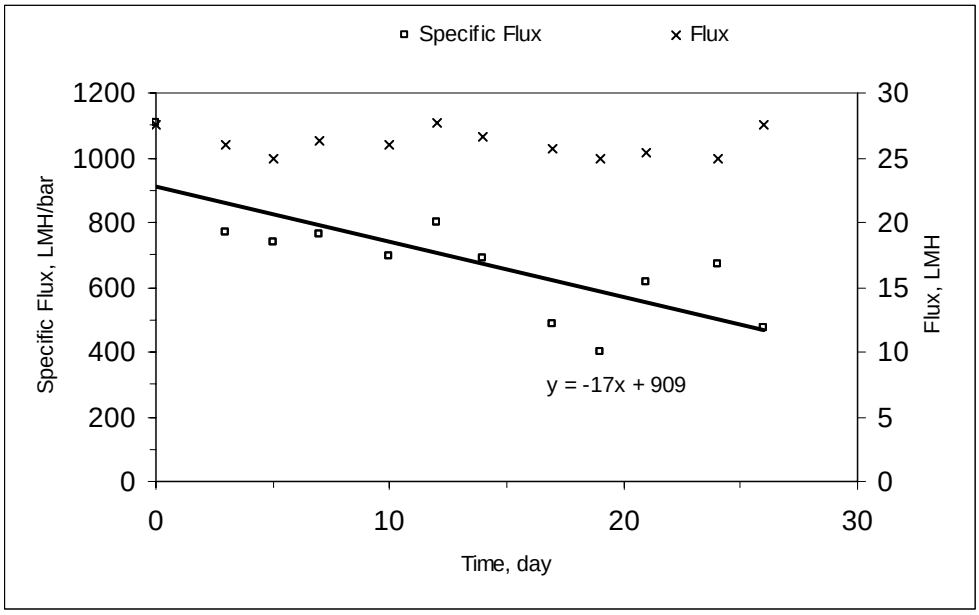


Figure 3.30 Membrane performance for the bench Enviroquip MBR at 10-d MCRT

Figure 3.31 presents the membrane performance of the bench Ionics MBR at 10-d MCRT. Membrane flux was maintained at approximately 21 to 26 LMH. The specific flux started from approximately 1000 LMH/bar and decreased to approximately 150 LMH/bar after 26 days of operation. The conditioning fouling rate was 17 LMH/bar/day.

It appears the reactor did not reach steady state. Based on the operation of other MCRT conditions for which the steady state specific flux was between 100 and 200 LMH/bar, it appeared that the reactor was running near steady state at the end of 25 days of operation.

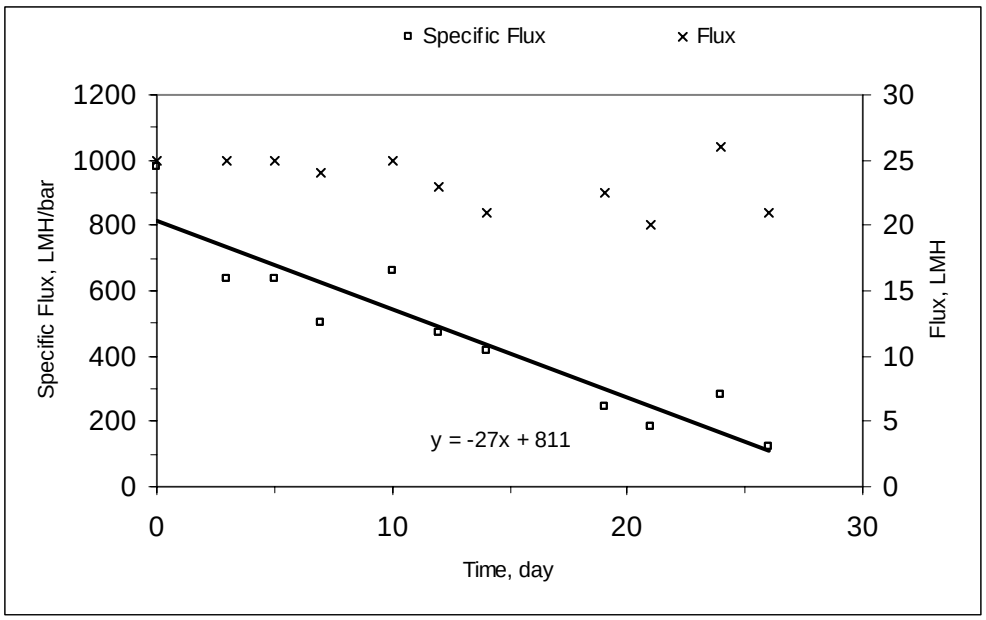


Figure 3.31 Membrane performance for the bench Ionics MBR at 10-d MCRT

Figure 3.32 shows the membrane performance of the bench Enviroquip MBR at 20-d MCRT. Membrane flux was maintained at 25 to 26 LMH for about 40 days in the beginning of the operation and then gradually decreased to 22 LMH at the end of 50 days of operation. The specific flux started from over 1000 LMH/bar and decreased to approximately 150 LMH/bar after 21 days of operation. The conditioning fouling rate was 61 LMH/bar/day. It is apparent that the reactor had reached steady state fouling condition, which is characterized as a steady slow decline of membrane fouling rate, after 21 days of operation. The steady fouling rate was approximately 3.2 LMH/bar/day. The flux decline near the end of operation was also an indication of membrane fouling.

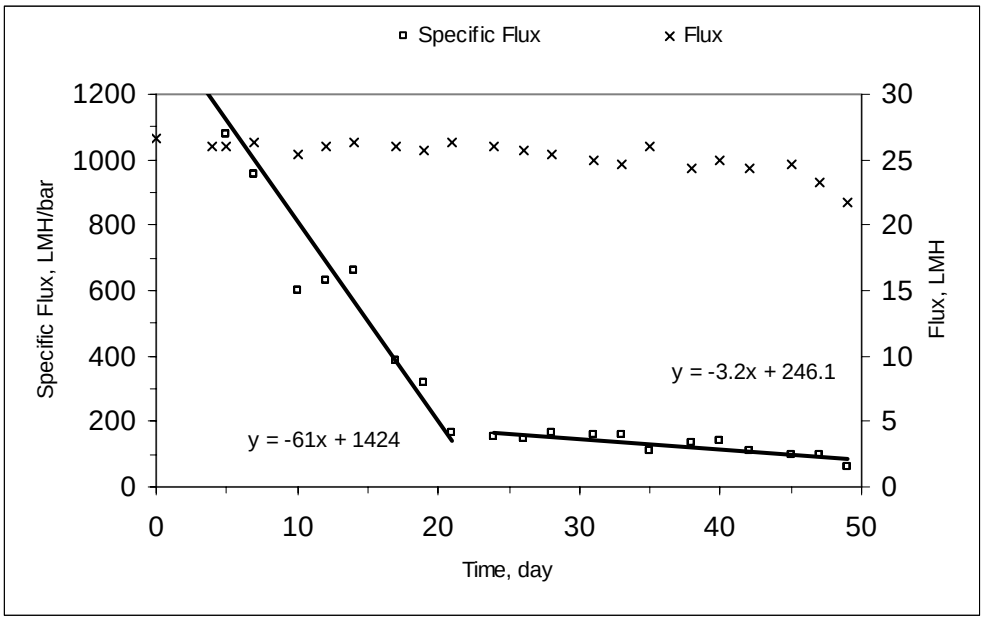


Figure 3.32 Membrane performance for the bench Enviroquip MBR at 20-d MCRT

Figure 3.33 shows the membrane performance of the bench Enviroquip MBR at 20-d MCRT with glucose addition. Membrane flux was maintained at approximately 26 LMH. The specific flux started from 1000 LMH/bar and decreased to approximately 600 LMH/bar after 7 days of operation. The conditioning fouling rate was 54 LMH/bar/day. It is apparent that the reactor did not reach steady state fouling stage. It is estimated that the reactor needed another 7 to 10 days of operation to reach steady state based on the conditioning fouling rate. It seems addition of glucose to MBR does not increase membrane fouling rate, which is good for MBR operation. When look at mixed liquor EPS and SMP concentrations as presented in Tables 16 and 18, slightly lower EPS and SMP concentrations were obtained for glucose addition condition compared to regular 20-d MCRT operation, which provided a basis for the results obtained here. This implies that bench MBRs are capable of handling shocking loading without compromising membrane performance.

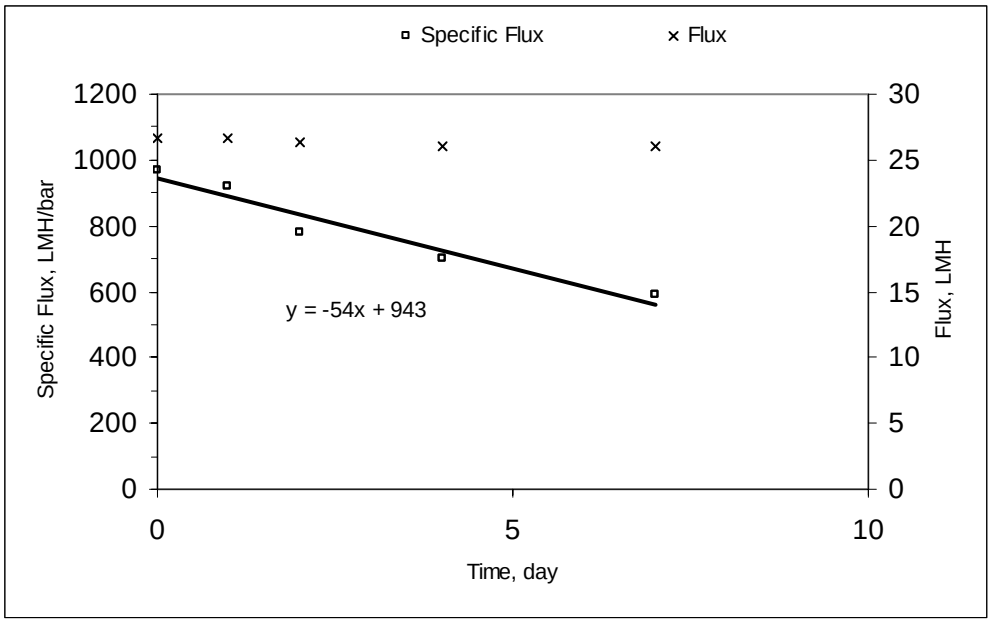


Figure 3.33 Membrane performance for the bench Enviroquip MBR at 20-d MCRT with glucose addition

Figure 3.34 shows the membrane performance of the bench Enviroquip MBR at 20-d MCRT without anoxic zone. Membrane flux was maintained between 25 to 26 LMH. The specific flux started from approximately1000 LMH/bar and decreased to approximately 300 LMH/bar after 35 days of operation. The conditioning fouling rate was 20 LMH/bar/day. It is apparent that the reactor did not reach steady state fouling stage. It is estimated that the reactor needed another 5 to 10 days of operation to reach steady state based on the conditioning fouling rate. By comparing the conditioning fouling rates between 20-d MCRT under anoxic/aerobic operation and without anoxic zone operation, the without anoxic zone operation showed even lower conditional fouling rate and it barely reached steady state fouling stage after 35 days of operation, while the anoxic/aerobic operation reached steady state fouling stage after 20 days of operation. This implies that the MBR operation without anoxic zone does not increase membrane

fouling and that a MBR operation could change to aerobic zone operation only without compromising membrane performance to reduce MBR operational cost incurred by maintaining anoxic zone operation such as the need for sludge mixing and sludge recirculation. However, anoxic zone operation does provide activated sludge process with certain benefits such as low effluent nitrate concentration and system alkalinity restoration for which less sodium bicarbonate is needed to maintain system pH.

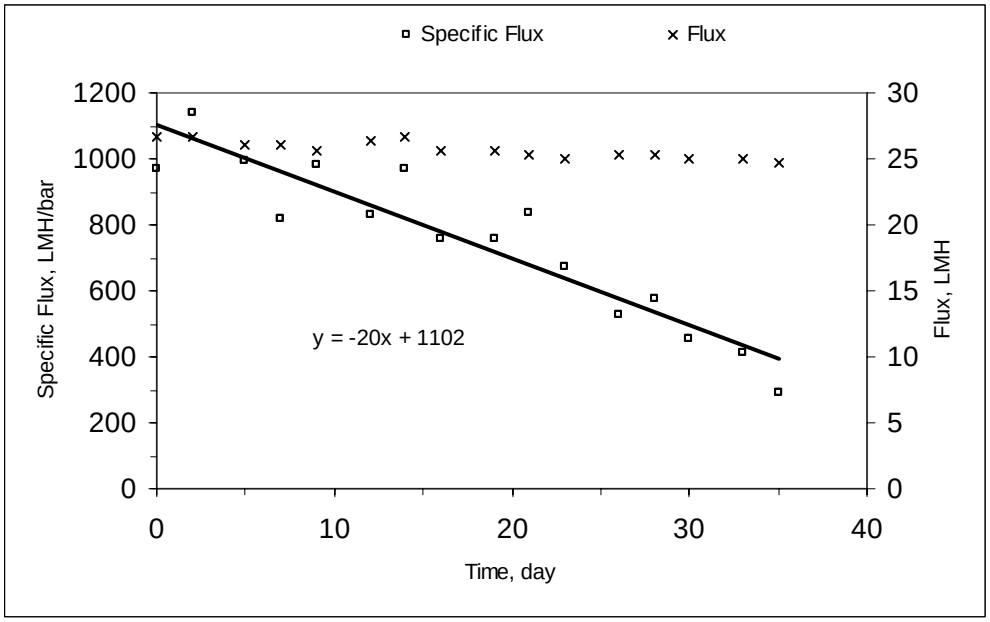


Figure 3.34 Membrane performance for the bench Enviroquip MBR at 20-d MCRT without anoxic zone

Figure 3.35 presents the membrane performance of the bench Ionics MBR at 20-d MCRT without anoxic zone. Membrane flux was maintained at 25 LMH for about 15 days in the beginning of the operation and then gradually decreased to 26 LMH at the end of 35 days of operation. The specific flux started from approximately 1000 LMH/bar and decreased to approximately 200 LMH/bar after 20 days of operation. The conditioning

fouling rate was 49 LMH/bar/day. It is apparent that the reactor had reached steady state after 20 days of operation. The steady fouling rate was approximately 6.1 LMH/bar/day. The flux decline near the end of operation was also an indication of membrane fouling.

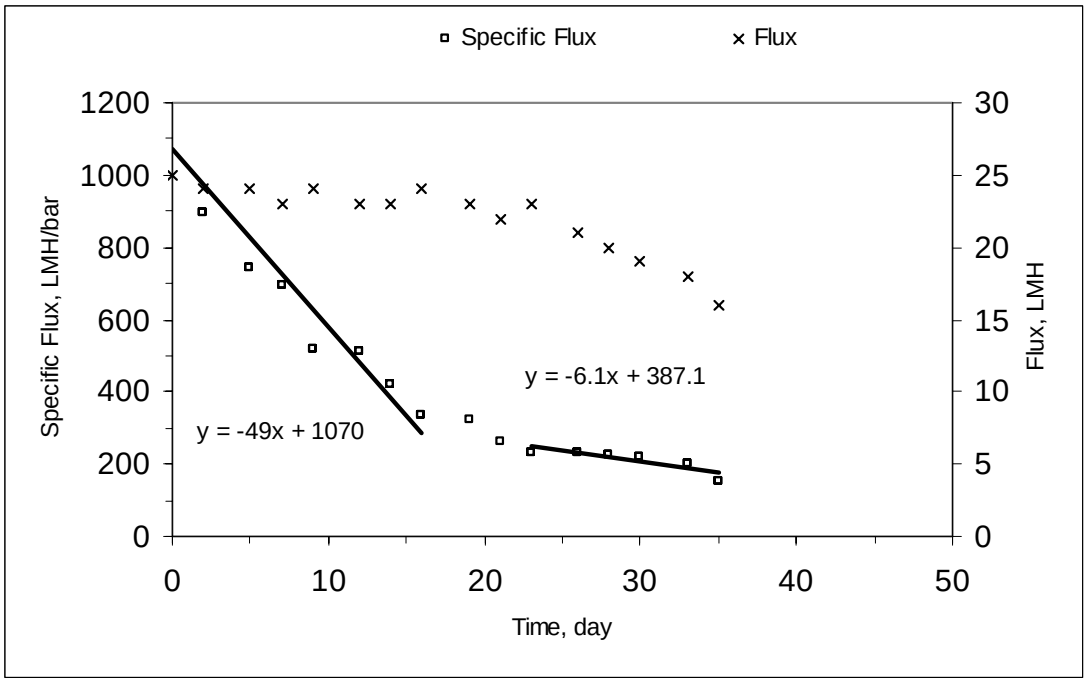


Figure 3.35 Membrane performance for the bench Ionics MBR at 20-d MCRT without anoxic zone

Figure 3.36 shows the membrane performance of the bench Enviroquip MBR at 30-d MCRT. Membrane flux was maintained at 25 LMH for about 40 days in the beginning of the operation and then started to decrease to 22 LMH at the end of 50 days of operation. The specific flux started from approximately 1000 LMH/bar and decreased to approximately 150 LMH/bar after 40 days of operation. The conditioning fouling rate was 19 LMH/bar/day. It appears that the reactor had reached steady state after 40 days of operation. The steady fouling rate was approximately 5.7 LMH/bar/day. The flux decline near the end of operation was also an indication of membrane fouling.

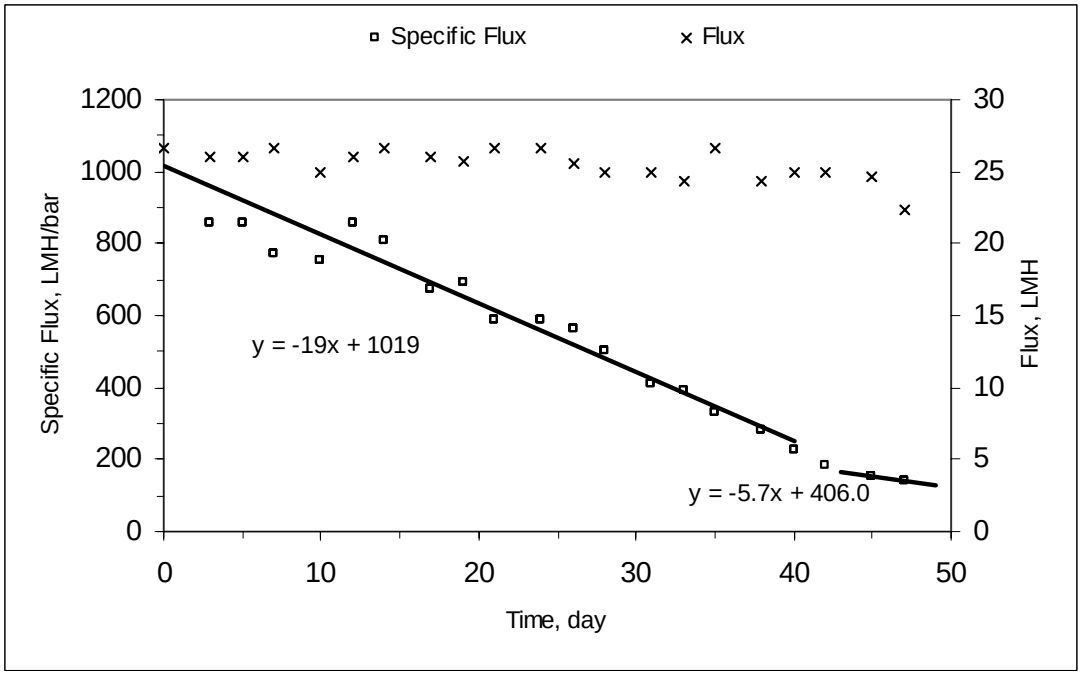


Figure 3.36 Membrane performance for the bench Enviroquip MBR at 30-d MCRT

Figure 3.37 presents the membrane performance of the bench Ionics MBR at 30-d MCRT. Membrane flux was maintained at 25 LMH for about 20 days in the beginning of the operation and then started to decrease to 10 LMH at the end of 50 days of operation. The specific flux started from approximately 800 LMH/bar and decreased to approximately 100 LMH/bar after 20 days of operation. The conditioning fouling rate was 41 LMH/bar/day. It is apparent that the reactor had reached steady state after 20 days of operation. The steady fouling rate was approximately 3.1 LMH/bar/day. The flux decline starting at 20 days of operation was clearly an indication of membrane fouling.

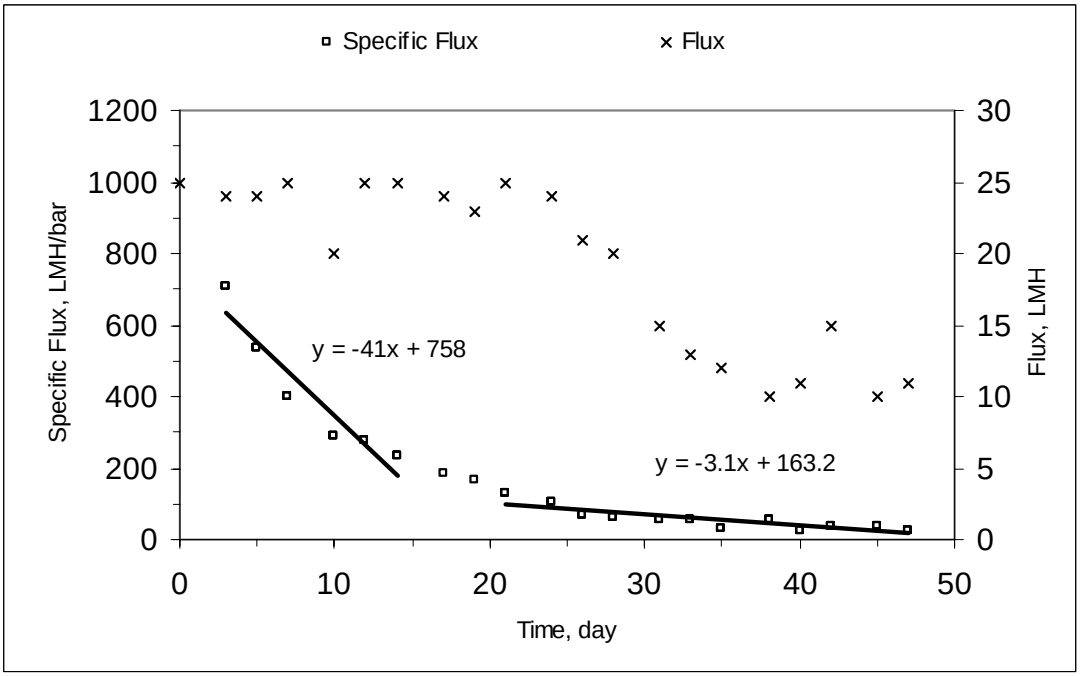


Figure 3.37 Membrane performance for the bench Ionics MBR at 30-d MCRT

Figure 3.38 shows the membrane performance of the bench Enviroquip MBR at 40-d MCRT. Membrane flux decreased from 26 LMH in the beginning of the operation to 20 LMH at 30 days of operation. The membrane then underwent physical cleaning by removing deposited sludge on membrane surface to restore permeability, and the cycle repeated. For each cycle, the specific flux started from approximately 1000 LMH/bar and decreased to approximately 100 LMH/bar after 10 days of operation. The conditioning fouling rate was 80 LMH/bar/day. The reactor then reached steady state after 10 days of operation. The steady fouling rate was estimated to be 3.1 LMH/bar/day.

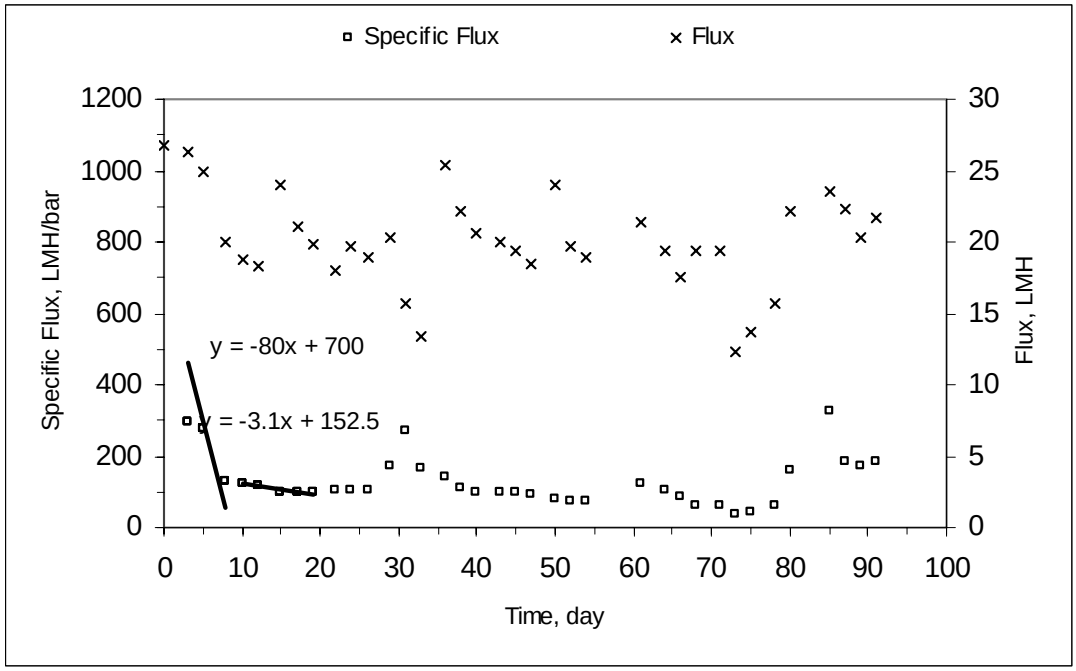


Figure 3.38 Membrane performance for the bench Enviroquip MBR at 40-d MCRT

Figure 3.39 presents the membrane performance of the bench Ionics MBR at 40-d MCRT. Membrane flux decreased from 26 LMH in the beginning of the operation to 5 LMH at 30 days of operation. The membrane then underwent physical cleaning by removing trapped sludge between membrane fibers to restore permeability, and the cycle repeated. For each cycle, the specific flux started from approximately 1000 LMH/bar and decreased to approximately 50 LMH/bar after 15 days of operation. The conditioning fouling rate was 38 LMH/bar/day. The reactor then reached steady state after 15 days of operation. The steady fouling rate was estimated to be 1.7 LMH/bar/day.

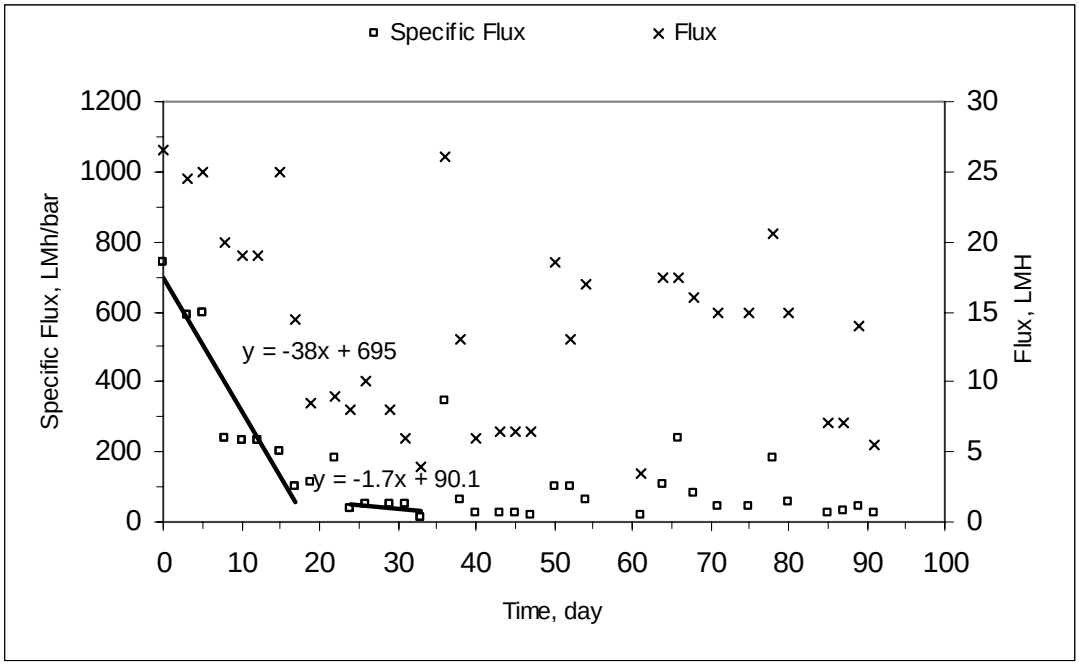


Figure 3.39 Membrane performance for the bench Ionics MBR at 40-d MCRT

Figure 3.40 shows the membrane performance of the bench Enviroquip MBR under no sludge wasting condition. Membrane flux decreased from 26 LMH in the beginning of the operation to 20 LMH at 15 days of operation. The membrane then underwent physical cleaning by removing deposited sludge on membrane surface to restore permeability. For each cycle, the specific flux started from approximately 800 LMH/bar and decreased to approximately 150 LMH/bar after 5 days of operation. The conditioning fouling rate was 88 LMH/bar/day. The reactor then reached steady state after 5 days of operation. The steady fouling rate was estimated to be 12 LMH/bar/day.

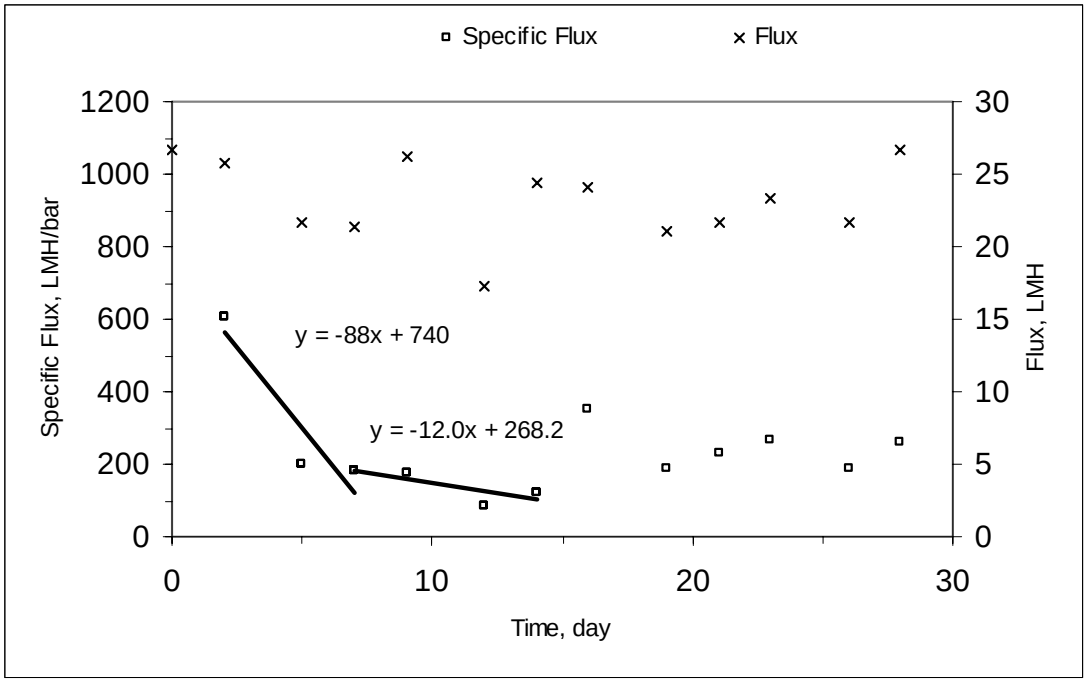


Figure 3.40 Membrane performance for the bench Enviroquip MBR no sludge wasting

Figure 3.41 presents the membrane performance of the bench Ionics MBR under no sludge wasting condition. Membrane flux decreased from 25 LMH in the beginning of the operation to 15 LMH at 20 days of operation. The specific flux started from over 800 LMH/bar and decreased to approximately 150 LMH/bar after 10days of operation. The conditioning fouling rate was 74 LMH/bar/day. The reactor then appeared to reach steady state after 10 days of operation. The steady fouling rate was estimated to be 9.9 LMH/bar/day.

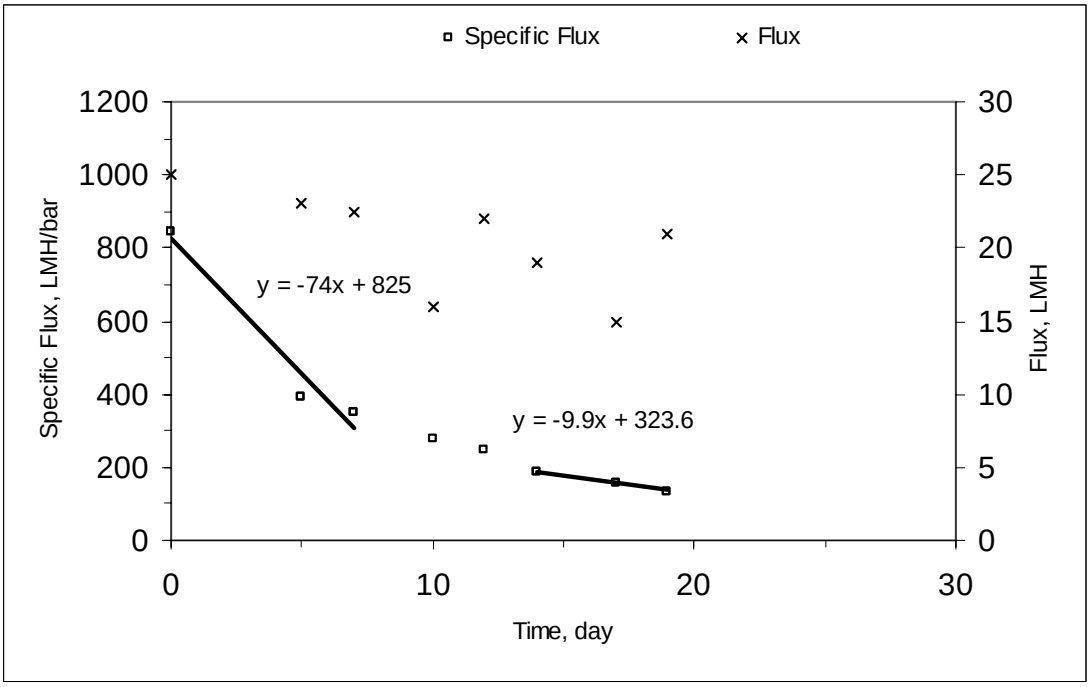


Figure 3.41 Membrane performance for the bench Ionics MBR no sludge wasting

Table 3.21 summarized the membrane fouling data for the bench Enviroquip and bench Ionics MBRs. It appears that when MCRTs were 30 days or less, conditioning fouling rate was low compared to MCRTs 40 days or more and it took longer to reach steady state and the MBR system could take advantage of the low operating pressures. Though 40-d MCRT had high conditioning fouling rate, low steady fouling rate was observed and thus there should be no significant difference of overall fouling rate between the 10- to 30-d MCRT conditions and the 40-d MCRT condition. However, when the MBR system was operated under no sludge wasting condition, both high conditioning fouling and steady fouling rates were obtained. Therefore, MBRs should not be operated under no sludge wasting condition to avoid high fouling rate and consequently high frequency of physical and chemical cleaning.

Table 3.21 Summary of membrane fouling data for bench-scale MBRs

MCRT, d	Bench Enviroquip				Bench Ionics			
	Condi-tioning fouling rate, LMH/bar/ day	Time to steady state, day	Steady fouling rate, LMH/ bar/day	Steady state specific flux, LMH	Condi-tioning fouling rate, LMH/bar/ day	Time to steady state, day	Steady fouling rate, LMH/ bar/day	Steady state specific flux, LMH
10	17	45*	----	----	27	25*	----	----
20	61	20	3.2	150	----	----	----	----
20[a]	54	15*	----	----	----	----	----	----
20[b]	20	35*	----	----	49	20	6.1	200
30	19	40	5.7	150	41	20	3.1	150
40	80	10	3.1	100	38	15	1.7	50
No sludge wasting	88	5	12.0	150	74	10	9.9	150

Note:
a with glucose addition
b without anoxic tank
* estimated time

3.4.4 Effect of SMP and EPS Concentrations on Membrane Performance

SMP concentrations are plotted against steady-state fouling rate in Figure 3.42 and Figure 3.43 for the bench Enviroquip and bench Ioncis MBRs to see the effects of SMP concentrations. Results show that steady state fouling rate in the bench Enviroquip MBR decreased with the increasing SMP concentrations with low correlations, whereas steady state fouling rate in the bench Ionics MBR increased with the increasing SMP concentrations with low correlations. The low correlations between steady state fouling rates and SMP concentrations indicate that SMP concentrations had little or no effect on membrane fouling. Contrary to this study, some studies indicated SMP had greater

impact on membrane fouling (Germain et al., 2005; Fan et al., 2006; Rosenberger et al., 2005, 2006; Jeong et al., 2007; Drews et al., 2008; Paul and Hartung, 2008).

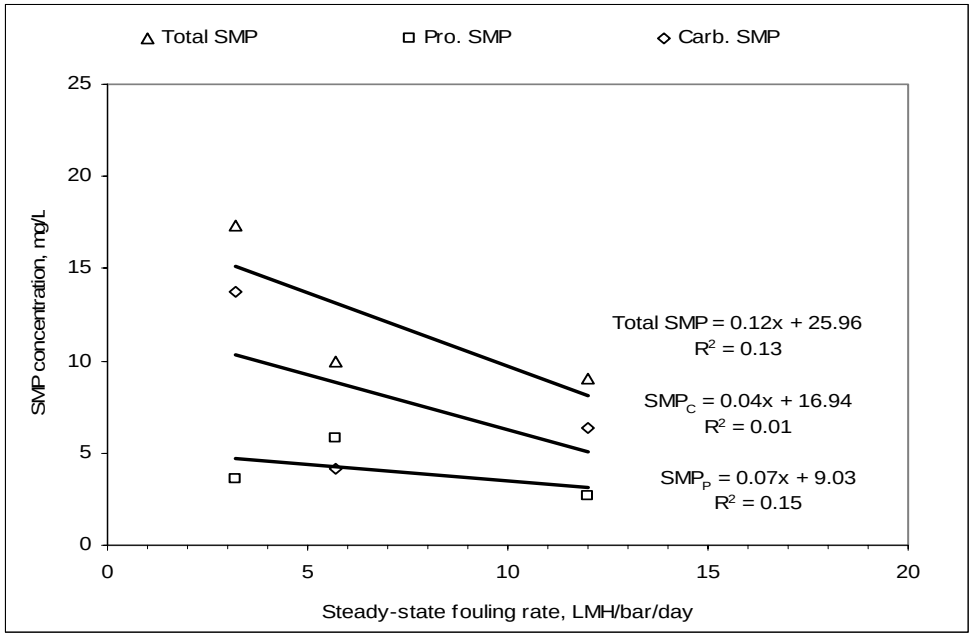


Figure 3.42 Correlations of SMP concentrations to steady-state membrane fouling rates for the bench Enviroquip MBR

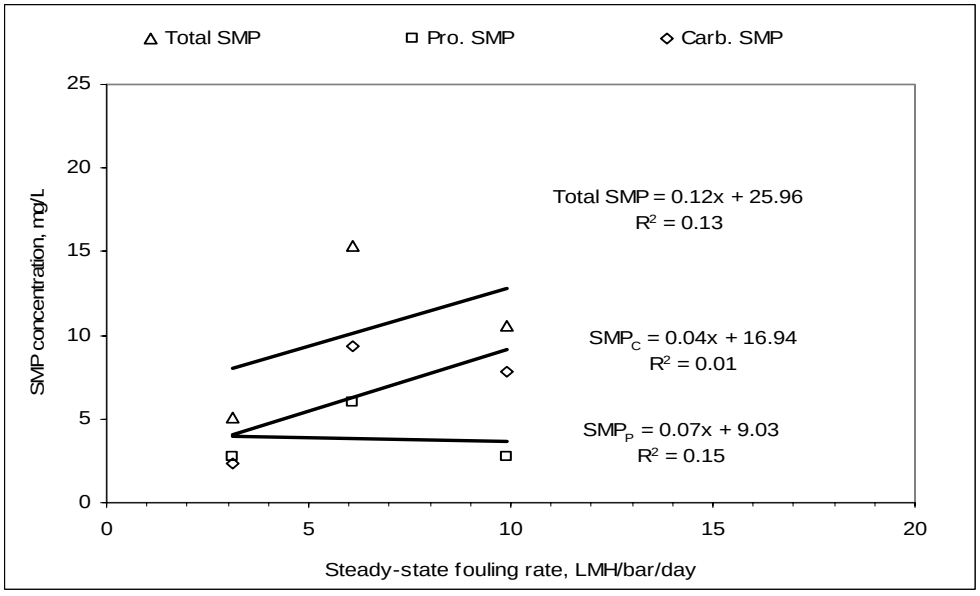


Figure 3.43 Correlations of SMP concentrations to steady-state membrane fouling rates for the bench Ionics MBR

EPS concentrations are also plotted against steady-state fouling rate in Figure 3.44 and Figure 3.45 for the bench Enviroquip and bench Ioncis MBRs to see the effects of EPS concentrations. Results show that steady state fouling rate in the bench Enviroquip MBR increased with the increasing total and carbohydrate EPS concentrations with very good correlations (R^2=0.97 and R^2=0.91, respectively). The steady state fouling rate in the bench Ionics MBR also increased with the increasing Carbohydrate EPS concentrations but with low correlation. It appears that carbohydrate EPS had a significant impact on steady-state membrane fouling rate. Some studies also found that increasing EPS levels directly increase fouling resistance and hence impact membrane fouling (Drews et al., 2006; Cho et al., 2005b; Lesjean et al., 2005; Chae et al., 2006; Ramesh et al., 2007).

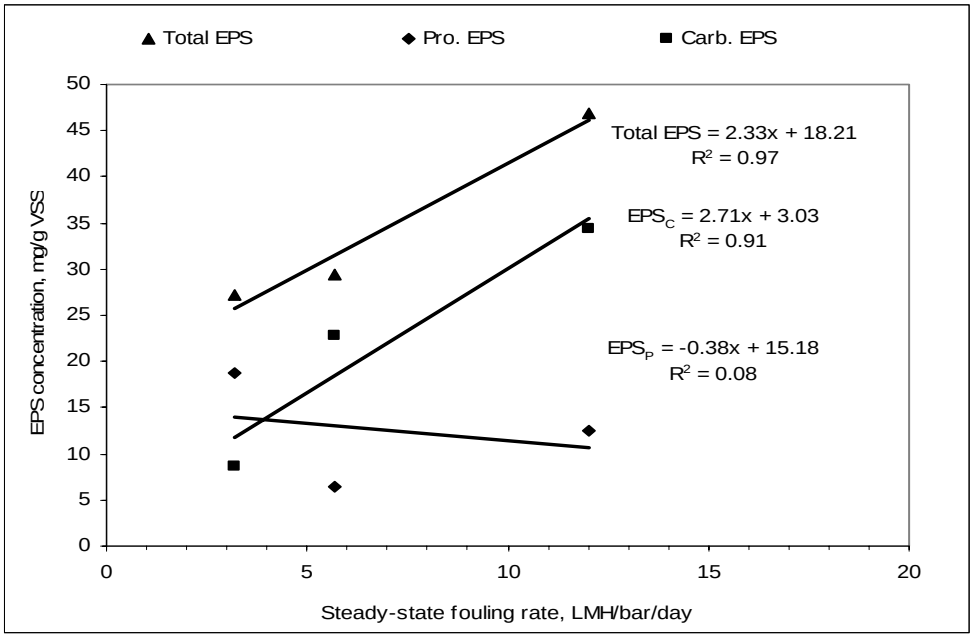


Figure 3.44 Correlations of EPS concentrations to steady-state membrane fouling rates for the bench Enviroquip MBR

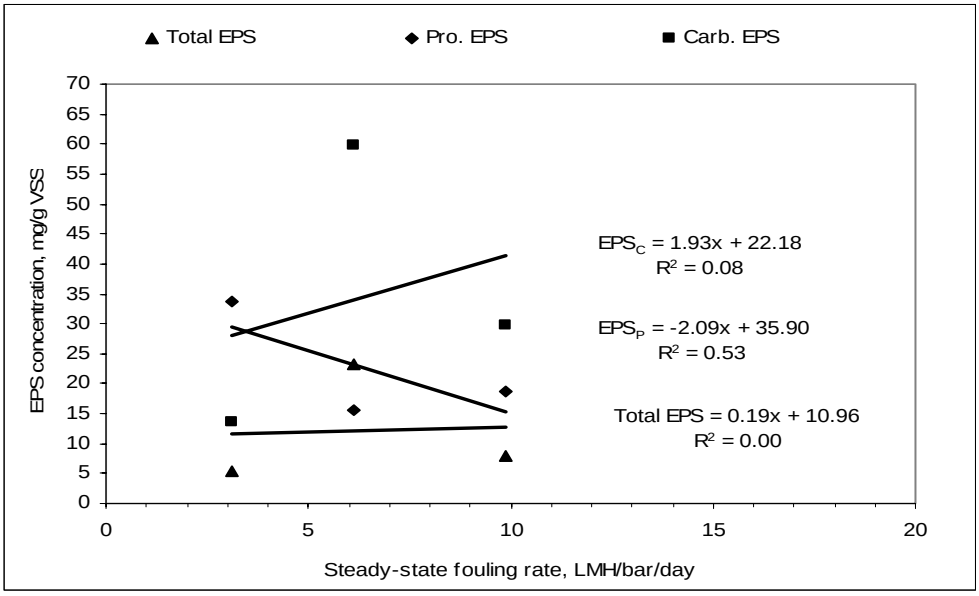


Figure 3.45 Correlations of EPS concentrations to steady-state membrane fouling rates for the bench Ionics MBR

3.5 Fouled Membrane Resistance Terms

At the end of each MCRT operating condition, the membrane was measured to determine total resistance (R) along with cake resistance (R_C), foulant resistance (R_F), and membrane resistance (R_M) for 10-d, 20-d, 40-d, and no sludge wasting MCRT conditions. The results are presented in Table 3.22 for both the bench Enviroquip and bench Ionics MBRs. The R value at the end of each MCRT condition depends on if the reactor has reached steady state or how long it has operated if it has reached steady state.

For the bench Enviroquip MBR, the R value was between $1x10^{-12}$ m^{-1} to $2.5x10^{-12}$ m^{-1}, except for the 20-d MCRT and 20-d MCRT with glucose addition, which does not represent the true R value. The R value of the 20-MCRT was $6.38x10^{-12}$ m^{-1} due to its longer period of operation, while the R value of the 20-d MCRT with glucose addition was only $0.62x10^{-12}$ m^{-1} due to its very short period of operation. For the bench Ionics MBR, the R value was slight higher compared to bench Enviroquip MBR and was

between $1.4x10^{-12}$ m^{-1} and $3x10^{-12}$ m^{-1} except for the 40-d MCRT. The R value for the 40-d MCRT was over $14x10^{-12}$ m^{-1} due to its very long period of operation. It should be noted that the R value for the 40-d MCRT was not very high even after 91 days of operation because at the time the reactor was stopped for resistance components measurement, it did not reach steady state.

By comparing each resistance component of the bench Enviroquip and bench Ionics MBR, it appears the higher R value for the bench Ionics MBR was largely attributed to relatively higher R_C value due to the membrane module configuration of bench Ionics MBR in which cake formation and large particles interference were more likely to occur.

Table 3.22 Resistance components at the end of each MCRT for bench-scale MBRs

MCRT, d	Bench Enviroquip					Bench Ionics				
	Days of operation	Resistance, 10^{-12} m^{-1}				Days of operation	Resistance, 10^{-12} m^{-1}			
		R	R_C	R_F	R_M		R	R_C	R_F	R_M
10	26	1.07	0.06	0.80	0.21	26	2.83	0.59	2.03	0.21
20	49	6.38	1.72	4.33	0.32	----	----	----	----	----
20[a]	7	0.62	0.08	0.22	0.32	----	----	----	----	----
20[b]	35	1.16	0.28	0.66	0.21	35	1.41	0.92	0.29	0.21
40	91	2.42	1.41	0.80	0.21	91	14.66	12.42	2.03	0.21
No sludge wasting	28	1.46	0.04	1.21	0.21	19	2.69	2.69	0.26	0.21

Note:
a. with glucose addition
b. without anoxic tank

To further investigate the components of membrane fouling, percentages of each fouling term for the bench Enviroquip MBR is plotted in Figure 3.46 and in Figure 3.47 for the bench Ionics MBR. R_M terms were generally less than 20% of total resistance,

except for the bench Enviroquip 20-d MCRT with glucose addition in which steady state was not reached. It appears that R_C terms in the bench Ionics MBR were higher than that in the bench Enviroquip MBR which was anticipated due to the configuration of bench Ionics membrane module.

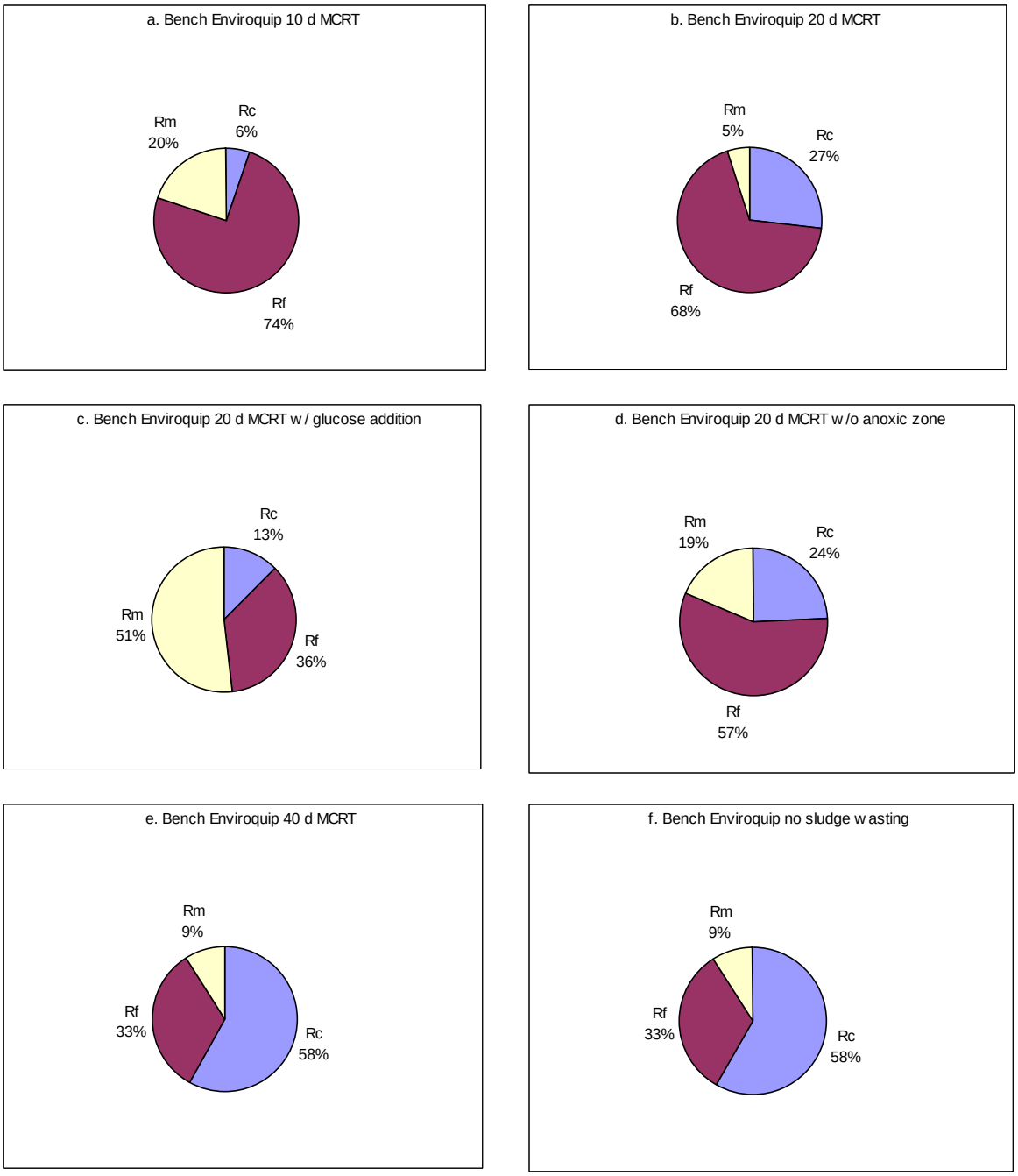


Figure 3.46 Fouled R distributions for the bench Enviroquip MBR

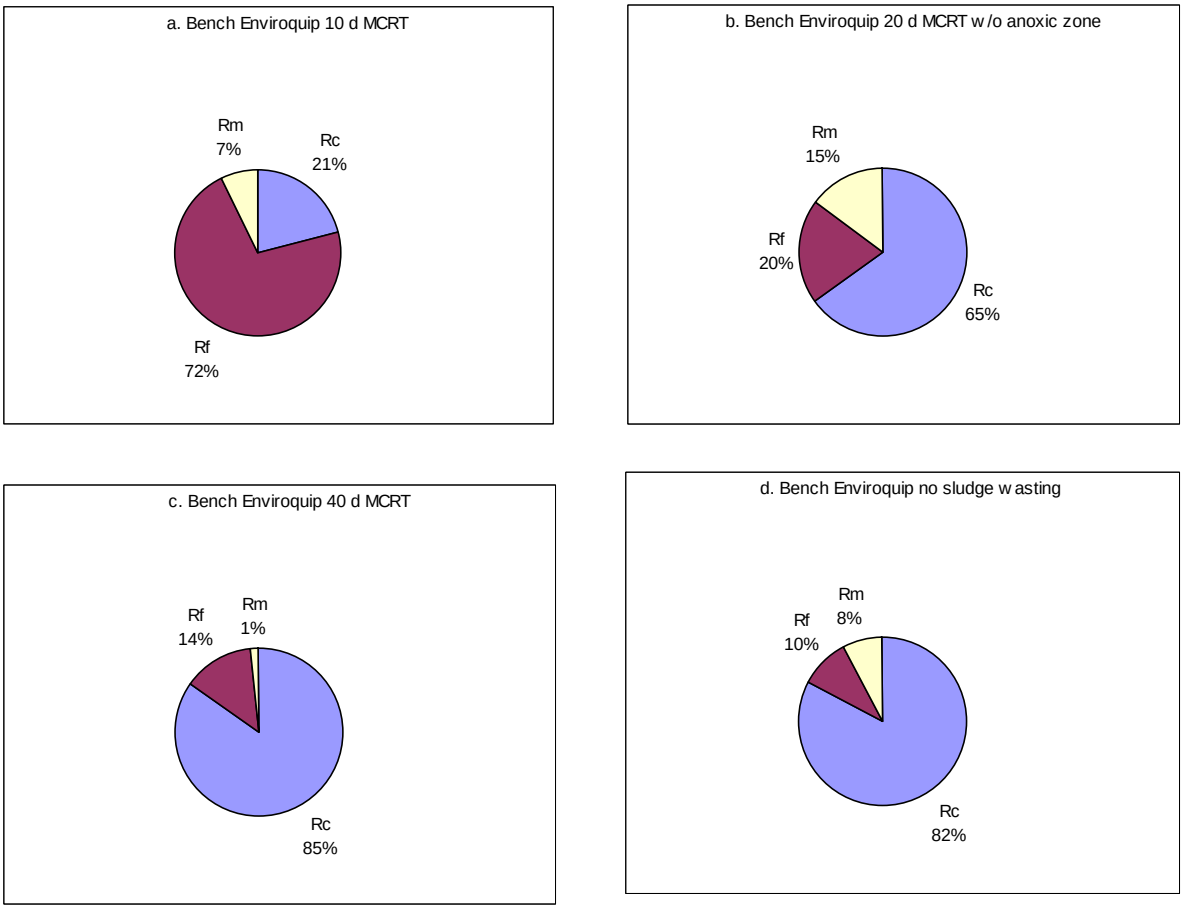


Figure 3.47 Fouled R distributions for the bench Ionics MBR

3.6 Conclusions for Chapter 3

Yield coefficient, Y, and decay coefficient, k_d, for bench Enviroquip and Ionics MBRs were determined to be in the lower range of typical values in municipal wastewater treatment, which implies biomass accumulation is slow in bench MBRs. Because microfiltration membranes are used for solid-liquid separation, the bench Enviroquip and Ionics MBRs at all MCRT conditions produced superior effluent water in terms of water quality parameters such as TSS, BOD_5, COD, TOC, TP removal, TN removal, turbidity and UTV_{254}. The four pilot MBRs also obtained similar effluent water quality as the bench MBRs.

Carbohydrate EPS appeared to be increasing when MCRT increased or decreased from 20-d MCRT, while protein EPS at 20-d MCRT condition were substantially higher than other MCRT conditions. Higher carbohydrate mixed liquor SMP were found for 20-d MCRT condition than other MCRT conditions, but there is no difference in protein mixed liquor SMP at all MCRT conditions. No differences in effluent protein and carbohydrate SMP were found all MCRT condition, resulting in higher unfilterable SMP existed in 20- MCRT condition.

Due to the membrane module configuration, bench Ionics MBR had higher TMP resistance than bench Enviroquip MBR. The critical flux for bench Enviroquip and Ionics MBRs were estimated to be 37.4 LMH and 39.3 LMH, respectively. Long term fouling process was monitored for each MCRT condition and it took 5 to 45 days for bench MBRs to reach steady state fouling state. No significant difference in conditioning and steady state fouling rates existed in 10 to 40-d MCRT conditions. However, no sludge wasting condition had higher fouling rates in both conditioning and steady state periods. The shocking loading condition and no anoxic zone condition following the normal 20-d MCRT operation did not show any increase of membrane fouling, indicating MBRs are capable of handling shocking loading and variance in operating schemes as well without compromising membrane performance. It was found that SMP concentrations had little or no effect on membrane fouling process, while carbohydrate EPS concentrations appeared to have a significant impact on steady-state fouling rate. At fouled condition, majority of bench Enviroquip MBR resistance attributed to foulant resistance, while majority of bench Ionics MBR resistance attributed to cake resistance due to the membrane module configuration that tends to trap more sludge cakes between membrane fibers.

The major findings in this chapter are listed below: 1) carbohydrate EPS appeared to be increasing when MCRT increased or decreased from 20-d MCRT and SMP concentrations had little or no effect on membrane fouling process, while carbohydrate EPS concentrations appeared to have a significant impact on steady-state fouling rate; 2) no significant difference in conditioning and steady state fouling rates existed in 10 to 40-d MCRT conditions. However, no sludge wasting condition had higher fouling rates in both conditioning and steady state periods; 3) the shock loading condition and no anoxic zone condition following the normal 20-d MCRT operation did not show any increase of membrane fouling, indicating MBRs are capable of handling shock loading and variance in operating schemes as well without compromising membrane performance.

CHAPTER 4 MOLECULAR BIOLOGY STUDY OF MICROBIAL COMMUNITIES FOR BENCH SCALE AND PILOT MBRS

4.1 DGGE Fingerprinting Analysis

The DGGE fingerprinting profile of the bench MBRs under different operating conditions and the four pilot MBRs are presented in Figures 4.1. 24 DGGE bands were cut for PCR and DNA sequencing to determine their species. The results are presented in Table 4.1. Results and findings of DGGE fingerprinting analysis are discussed in the following sections.

4.1.1 DGGE profile for bench-scale MBRs at 20-d MCRT

In addition to aerobic/anoxic operating condition at 20-d MCRT, glucose addition and no anoxic zone conditions were also operated for bench Enviroquip MBR to evaluate the impact on membrane performance. Bench Ionics MBR was only operated with no anoxic zone condition.

Lanes 1 and 2 represent microbial communities for mixed liquor and biofilm for bench Enviroquip MBR at 20-d MCRT. It can be seen from lanes1 and 2 in Figure 4.1 that the mixed liquor at the end of 20-d MCRT operation was more abundant in microbial species, but none were absolutely dominant. In contrast, two bright bands (3 and 4) were identified in the biofilm DGGE fingerprinting profile, indicating the two microorganisms were dominant species and were selected to accumulate and reproduce on membrane surfaces. DNA sequencing results as presented in Table 4.1 indicate that the two dominant species in biofilm were *Herminiimonas sp.* and *Methylibium sp.*

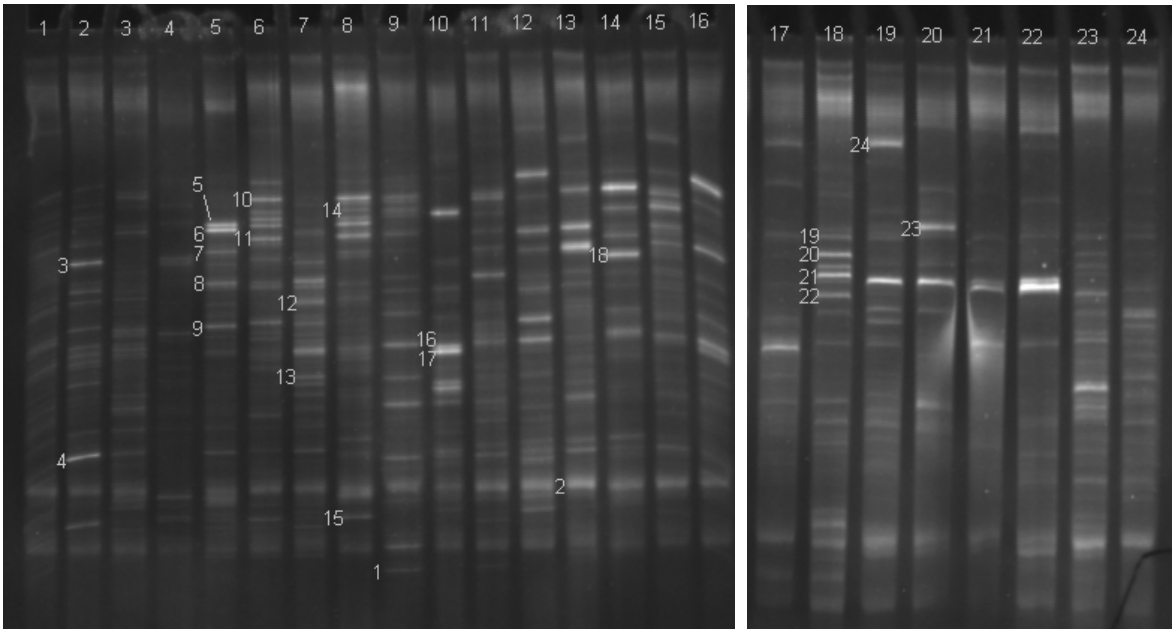


Figure 4.1 DGGE fingerprinting profiles of bench-scale and pilot MBRs

Lane 1, bench Enviroquip ML, 20-d MCRT;
Lane 2, bench Enviroquip biofilm, 20-d MCRT;
Lane 3, bench Enviroquip ML, 20-d MCRT w/ glucose addition;
Lane 4, bench Enviroquip biofilm, 20-d MCR w/ glucose addition;
Lane 5, bench Enviroquip ML, 20-d MCRT w/o anoxic zone;
Lane 6, bench Enviroquip biofilm, 20-d MCR w/o anoxic zone;
Lane 7, bench Ionics ML, 20-d MCRT w/o anoxic zone;
Lane 8, bench Ionics biofilm, 20-d MCR w/o anoxic zone;
Lane 9, bench Ionics ML, no sludge wasting;
Lane 10, bench Ionics biofilm, no sludge wasting;
Lane 11, bench Enviroquip ML, no sludge wasting;
Lane 12, bench Enviroquip biofilm, no sludge wasting;
Lane 13, bench Ionics ML, 10-d MCRT;
Lane 14, bench Ionics biofilm, 10-d MCRT;
Lane 15, bench Enviroquip ML, 10-d MCRT;
Lane 16, bench Enviroquip biofilm, 10-d MCRT;
Lane 17, bench Ionics ML, 30-d MCRT;
Lane 18, bench Ionics biofilm, 30-d MCRT;
Lane 19, bench Enviroquip ML, 30-d MCRT;
Lane 20, bench Enviroquip biofilm, 30-d MCRT;
Lane 21, pilot Ionics ML;
Lane 22, pilot Huber ML;
Lane 23, pilot Enviroquip ML;
Lane 24, pilot Koch ML.

Table 4.1 Sequence alignment analysis of DGGE bands in bench and pilot MBRs

DGGE band	Gene bank comparison results		
	Bacteria species	**Length of DNA sequence, bp**	**Similarity**
1	*Mitsuaria sp.*	149	88%
2	*Brevundimonas sp.*	128	91%
3	*Herminiimonas sp.*	153	89%
4	*Methylibium sp.*	155	97%
5	*Bacteroidetes sp.*	115	93%
6	*Uncultured bacterium*	159	99%
7	*Uncultured bacterium*	145	93%
8	*Burkholderiales sp.*	164	93%
9	*Chitinophagaceae sp.*	147	96%
10	*Pseudomonas sp.*	154	99%
11	*Pseudomonas sp.*	154	100%
12	*Desulfobacterales sp.*	132	94%
13	*Clostridiales sp.*	137	96%
14	*Pseudomonas sp.*	155	100%
15	*Nitrospira sp.*	152	91%
16	*Frigoribacterium sp.*	138	98%
17	*Actinobacterium*	146	97%
18	*Rhodobacteraceae sp.*	98	85%
19	*Pseudomonas sp.*	149	99%
20	*Pseudomonas sp.*	154	99%
21	*Pseudomonas sp.*	156	99%
22	*Arcobacter sp.*	140	98%
23	*Firmicutes sp.*	*120*	*95%*
24	*Chitinophaga sp.*	165	90%

Lanes 3 and 4 represent microbial communities for mixed liquor and biofilm for bench Enviroquip MBR at 20-d MCRT with glucose addition. Similar to the result as discussed for 20-d MCRT condition, microbial species in mixed liquor was abundant but none of them were dominant species. On the contrary, microbial species in biofilm were relatively limited. It is suspected that the glucose addition only selected a few certain microorganisms onto membrane surface, and in the meantime the microorganisms that were able to grow on membrane surface under normal operating conditions could not compete with the newly selected biofilm microbial species. It is interesting to see that band 4, which was clearly shown in almost all other biofilm DGGE profiles, did not show up in the biofilm of 20-d MCRT with glucose addition. This could be due to the inhibitory effect of glucose addition or the relatively short operating time (7 days). Unlike other biofiom DGGE profiles that all had at least one or two bright bands, the biofilm DGGE profile of 20-d MCRT with glucose addition did not show any bright bands, which might be attributed to the short operating period. Membrane fouling results as presented in Figure 3.33 indicated very limited membrane fouling occurred at this condition. Therefore, the brightness of DGGE bands in biofilm DDGE profile is an indication of the extent of membrane fouling.

4.1.2 DGGE profile for bench-scale MBRs without anoxic zone

Lanes 5 and 6 represent ML and biofilm DGGE profiles of bench Enviroquip 20-d MCRT without anoxic zone. Lanes 7 and 8 represent ML and biofilm DGGE profiles of bench Ionics 20-d MCRT without anoxic zone. It appeared ML DGGE profiles of bench Enviroquip and bench Ionics MBRs were similar except bench Enviroquip showed two

dominant species in upper DGGE profile region (band 5, *Bacteroidetes sp*.; and bands 6 and 7, uncultured bacteria), while bench Ionics showed a few dominant species in lower DGGE profile region (bands 12 and 13, *Desulfobacterales sp*. and *Clostridiales sp*.). Bands 8 and 9 were common to bench Enviroquip and bench Ionics and were sequenced as *Burkholderiales sp*. and *Chitinophagaceae sp*. Membrane biofilms of bench Enviroquip and bench Ionics selected several dominant species in upper DGGE profile region (bands 10, 11, and 14 *Pseudomonas sp*.) and one dominant species in lower DGGE profile region (band 15, *Nitrospira sp*.). It appeared *Nitrospira sp*. showed up in most biofilm DGGE profiles. Different than other operating conditions, no anoxic zone condition selected more bacteria, which evolved to dominant species, on membrane surface than other operating conditions did.

Burkholderiales sp. is a gram negative aerobe responsible for biodegradation of pesticide and polychlorinated biphenyls (PCBs); it also has the ability of antibiotics resistance and could be detected with large quantity in bioreactors treating municipal wastewater containing antibiotics. The existence of *Burkholderiales sp.* in the sludge plays an important role in biological degradation of increasing amount of antibiotics and PCBs in wastewater. *Pseudomonas sp.* is capable of degrading many organic compounds such as nitro-bezene. *Pseudomonas sp.* has been detected in studies of membrane fouling (Xia *et. al*., 2008 and 2010), indicating its presence may increase membrane fouling. It can be seen from Figure 4.1 *Pseudomonas sp*. existed in every condition. The presence of *Bacteroidetes sp.* and *Nitrospira sp.* is important in nitrogen and phosphorus removals.

4.1.3 DGGE profile for bench-scale MBRs without sludge wasting

Lanes 9 and 10 represent ML and biofilm DGGE profiles of bench Ionics MBR without sludge wasting. Lanes 11 and 12 represent ML and biofilm DGGE profiles of bench Enviroquip MBR without sludge wasting. It appeared ML DGGE profiles for bench Ionics and Enviroquip MBRs are similar. One microorganism (band 1, *Mitsuaria sp.*) identified in the very bottom region of ML DGGE profile was unique to no sludge wasting condition. Unlike other operating conditions, totally different dominant species were selected on membrane surfaces of bench Ionics and bench Envirouip MBRs. Band 1 represented *Mitsuaria sp.;* bands 16 and 17 represented *Frigoribacterium sp.* and *Actinobacterium*., respectively.

4.1.4 DGGE profile for bench-scale MBRs at 10-d and 30-d MCRT

Lanes 13, 14, 15, and 16 represent ML and biofilm DGGE profiles of bench Ionics and EnviroquipMBRs at 10-d MCRT condition (see Figure 4.1). Similar ML and biofilm DGGE profiles were found for bench Enviroquip and bench Ionics MBRs with a few dominant species. Compared to 20-d MCRT condition, more dominant species (bright bands) existed in 10-d MCRT condition. Band 2 and 18 was selected for sequencing and represented *Brevundimonas sp.* and *Rhodobacteraceae sp*., respectively. *Brevundimonas sp.* plays an important role in bio-degradation of organic compounds and nitrogen and phosphorus removal (Liu et al., 2005; Ryu et al., 2007).

Lanes 17, 18, 19, and 20 represent ML and biofilm DGGE profiles of bench Ionics and Enviroquip at 30-d MCRT condition (see Figure 4.2). ML DGGE profiles of bench Ionics and Enviroquip were similar but biofilm DGGE profiles appeared to be different.

More dominant species existed on membrane surface of bench Enviroquip than that of bench Ionics. Bands 19 to 24 were selected for sequencing. Bands 19, 20, and 21 from bench Ionics biofilm DGGE profile represented *Pseudomonas sp*., indicating *Pseudomonas sp.* intensively involved in membrane fouling. Bands 22, 23, and 24 represented *Arcobacter sp*., *Firmicutes sp*., and *Chitinophaga sp*., respectively.

4.1.5 DGGE profile for pilot MBRs

Lanes 21, 22, 23, and 24 represent ML DGGE profiles of pilot MBRs (see Figure 4.1). It can be seen that pilot Ionics and pilot Huber had similar DGGE profiles while pilot Enviroquip and pilot Koch had similar profiles. In comparison, pilot Ionics and pilot Huber had less dominant species. It is suspected that the difference in two types of DGGE profile was partly attributed to possible difference in raw wastewater characteristics. Pilot Ionics and pilot Huber were sampled in September 2005 which was during dry season in Hawaii, while pilot Enviroquip and pilot Koch were sampled in November 2005 and January 2006 which fell in wet season in Hawaii. During wet season, higher wastewater flows caused by inflow/infiltration tend to flush sewer lines and carry more food source and anaerobic type microorganisms to WWTP. The DGGE profile differences observed in each bench-scale MBR operating conditions might partly due to this effect, but in a lesser extent since less and non-continuous raw wastewater was fed to bench-scale MBR. The difference in DGGE profile may also partly attributed to different configurations adopted for each pilot MBR.

4.2 Bench-Scale MBR 16S rRNA Clone Library Analysis

Lane 17 (bench Ionics MBR at 30-d MCRT condition) was selected for 16S rRNA clone library analysis to study bacteria phylogenetic characteristics. The phylogenetic distribution statistical analysis is presented in Figure 4.2 and the phylogenetic relationship is presented in Figure 4.3. Similar to the results of DGGE band sequencing analysis, *proteobacteria* were dominant in the microbial community. Of the *proteobacteria* detected in the clone library, *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* represented 8%, 47% and 10%, respectively. No *Deltaproteobacteria* was dectected in the clone library. *Azobacter sp.* and denitrifying bacteria were identified in *Betaproteobacteria*, indicating the presence of denitrification process in the bench-scale MBR. *Thauera aromatica* (1%) is a type of nitrifying bacteria. Other bacteria species detected in the clone library included *Firmicutes* (3%), *Acidobacteria* (5%), *Paucibacteria* (11%), *Rhodococcus* (3%), *Planctomycetes* (1%) and some other unclassified bacteria. It is apparent that *Betaproteobacteria* is the absolutely dominant species in the clone library. The detected Betaproteobacteria included *Ideonella sp.*, *Comamonadaceae bacterium, Rhodocyclaceae bacterium* and some *uncultured Betaproteobacteria bacterium.* The detected *Alphaproteobacteria* included *Sphingopyxis sp.* and *Pedomicrobium sp. etc. and the detected Gammaproteobacteria* included *Cellvibrio sp* and *Aeromonas sp.* etc. It was reported that *Bacteroidetes* (50%) and *Proteobacteria* (40%) were found to be dominant in MBR systems (Du et al., 2008). It was also reported that Alpha and Betaproteobacteria and Bacteroidetes dominating the gene library of a full scale MBR system (Wan et al., 2011). Miura found that

Betaproteobacteria and *Gammaproteobacteria* were dominant (61% and 22 %, respectively) species on a MBR membrane surface (Miura et al., 2007).

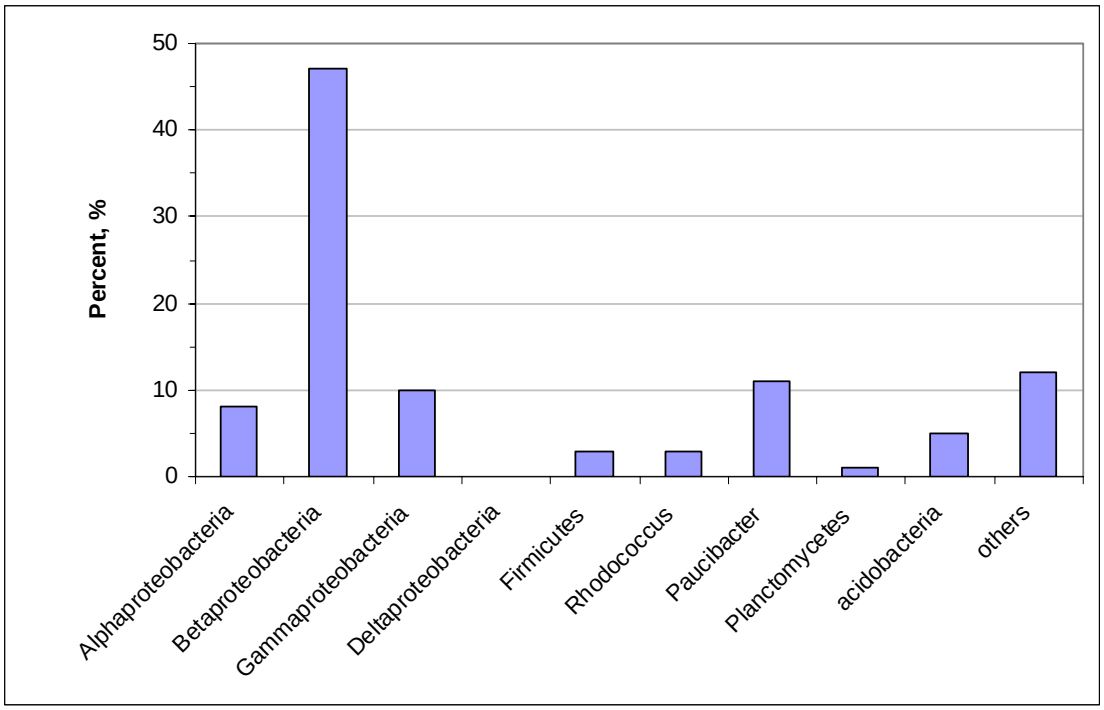


Figure 4.2 Phylogenetic distribution of the OTUs in clone libraries for bench Ionics MBR

Since *Betaproteobacteria* accounted for 47% clones in the clone library, it deserves a further breakdown analysis, which is presented Figure 4.4. As shown in Figure 4.4, except for 13% of unidentified species, the remaining clones represent *Comamonadaceae* (1%), *Variovorax sp.* (1%), *Rhodocyclaceae* (1%), *Denitratisoma sp*. (1%), *Thauera aromatica* (1%), *Ideonella sp*. (3%), *Aquabacerium sp*. (5%), *Dechloromonas sp*. (5%), *Oxalobacterace* (6%), *Herbaspirillum sp.* (10%). These species are commonly encountered in MBR systems (Zhang et al., 2006c; Wan et al., 2011; Miura et al., 2007; Xia et al., 2010; Du et al., 2008). Many of these identified microbial species are involved

in nitrogen removal process. Denitrifying bacteria *Denitratisoma sp*. and *Thauera aromatica* were detected in denitrifying environments (Wu et al., 2008; Chang et al., 2011). *Aquabacterium sp*. and *Nitrosomonas sp*. are also important in nitrogen removal (Iasur-Kruh et al., 2010). *Dechloromonas sp*. was reported as a nitrate reducer (Wan et al., 2011; Frankenberger and Zhang, 2007). Herbaspirillum sp. is known to be a nitrogen-fixing bacterium.

Figure 4.3 Phylogenetic tree of activated sludge microorganisms in bench Ionics MBR

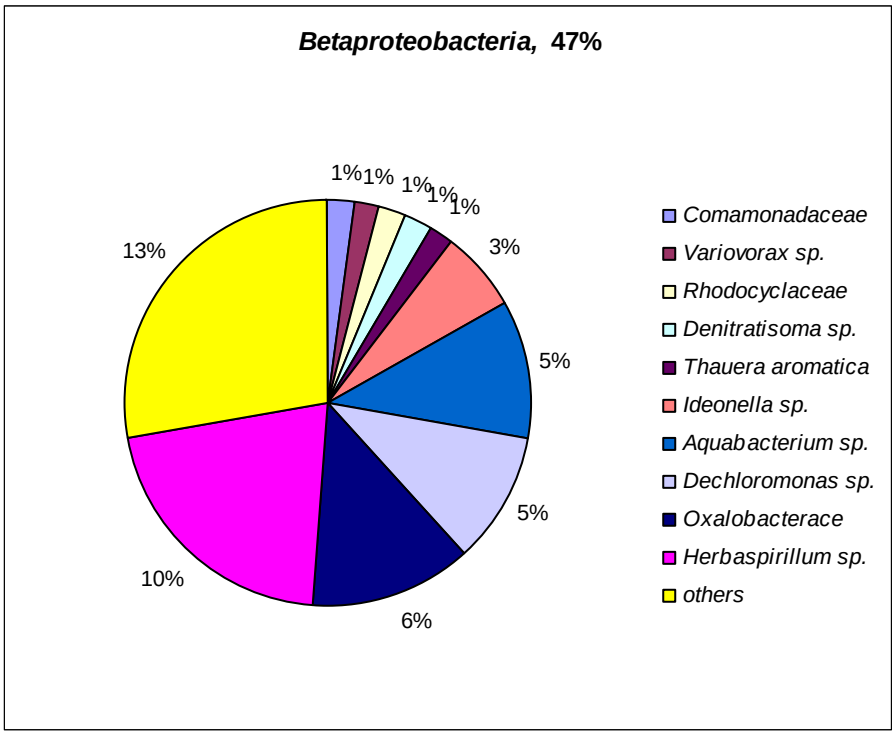


Figure 4.4 *Betaproteobacteria* in bench-scale Ionics MBR clone library

4.3 Conclusions for Chapter 4

Brightness of DGGE bands in biofilm DGGE profile is an indication of the extent of membrane fouling. Glucose addition to MBR could inhibit growth of certain type of microorganisms that can grow on membrane surface under normal operating conditions. Compared to other operating condition, bench MBR with no anoxic zone condition selected more microorganisms, which eventually evolved to dominant species on membrane surface.

Pseudomonas sp. was the most often detected species in biofilm, which indicate *Pseudomonas sp* might intensively involve in membrane fouling. *Rhodobacteraceae sp. and Brevundionas sp.* were also found and their presence could increase membrane fouling. No sludge wasting condition allowed accumulation of some specific type of

microorganisms in mixed liquor. Different microbial species, such as *Mitsuaria sp.*, *Frigoribacterium sp.* and *Actinobacterium*. sp., were identified in membrane biofilm. Clone library analysis indicated that *Betaproteobacteria* was the dominant mixed liquor microbial class, which represented 47% of the clone library.

The most important findings for this chapter are listed below: 1) Glucose addition to MBR could inhibit growth of certain type of microorganisms that can grow on membrane surface under normal operating conditions; and no anoxic zone condition selected more microorganisms, which eventually evolved to dominant species on membrane surface; 2) the presence of *Pseudomonas sp. Rhodobacteraceae sp. and Brevundionas sp. on membrane surface* could increase membrane fouling; 3) *Betaproteobacteria* was the dominant mixed liquor microbial class and represented 47% of the entire microbial community.

CHAPTER 5 MBR PVDF AND PVC ULTRAFILTRATION MEMBRANE CLEANING STUDY

The purpose of this subject was to study the cleaning method of ultrafiltration (UF) membranes in lab bench-scale MBR, to evaluate membrane flux restoration after different combinations of cleaning method, and to monitor the post-cleaning membrane fouling process to determine an optimal UF membrane cleaning method. Scope of this study also included evaluation of mixed liquor microbial community in the lab bench MBR and investigation of surface structure for attached microorganisms on PVDF membrane surface. Results are presented in the following sections.

5.1 Membrane Flux Recovery

5.1.1 Distribution of Membrane Resistance Components

Membrane resistance distribution was determined prior to cleaning of the PVDF and PVC membranes and results are presented in Figure 5.1. Figure 5.1 shows that the percentages of Rm and Rf in PVDF membrane module (8.65% and 3.97%, respectively) were slightly less than those in PVC membrane module (9.82% and 4.22%, respectively), which might be attributed to the larger nominal pore size of PVDF membrane (0.02 μm) in comparison with the smaller nominal pore size of PVC membrane (0.01 μm). Results indicate that majority of membrane resistances attributed to Rc (87.38% for PVDF membrane and 85.96% for PVC membrane, respectively) and the slightly higher Rc valuce for PVDF membrane was partly due to its higher flux.

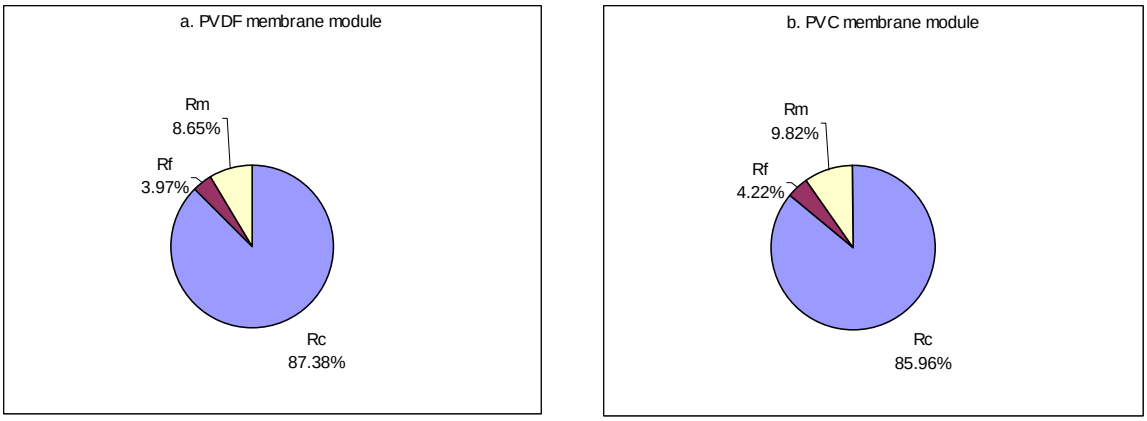


Figure 5.1 Membrane resistance distributions in PVDF and PVC UF membranes

Particle size of activated sludge in MBR is normally smaller than that in conventional biological treatment due to the lack of selection pressure of sedimentation. Smaller particle size of activated sludge tends to increase membrane resistance. In UF membrane applications, bulk of membrane resistance results from cake resistance and concentration polarization which can be effectively controlled by air scouring or periodic physical cleaning. Though accounted for only a small portion of total membrane resistance, foulant resistance plays an important role in membrane fouling. If can not be effectively controlled in membrane operation or chemical cleaning maintenance, foulant resistance distribution in membrane total resistance will increase over time and will greatly effect operating life of membranes. Using regular inorganic cleaning reagents can not effectively remove all foulants, therefore further study on membrane cleaning becomes necessary in order to completely remove foulants deposited in membrane pores.

5.1.2 Recovery of Flux after Membrane Cleaning

Common practice of soaking membrane in inorganic cleaning reagents cannot completely remove foulant particles deposited in membrane pores. During next cycle of operation, these foulant residuals could increase membrane fouling within short period of operation through chelating between metal ions and organics, bonding between organic compounds, and adsorption of organic/inorganic foulant to microorganisms. Therefore, vacuum permeation cleaning was implemented as the last step of membrane cleaning to improve cleaning performance. Results of membrane flux recovery are presented in Figure 5.2 and Figure 5.3. Membrane flux can not be 100% recovered which was possibly due to the configuration of hollow fiber membrane module, for which sludge residues in the two ends of membrane fiber could not be completely removed.

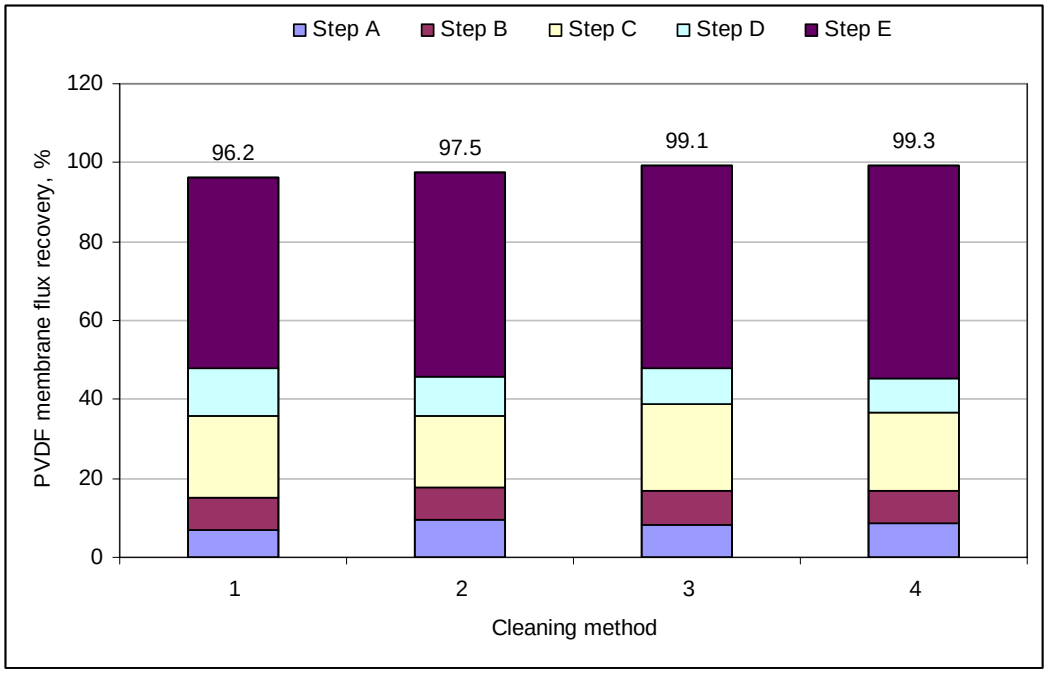


Figure 5.2 Recovery of PVDF membrane flux with different cleaning methods

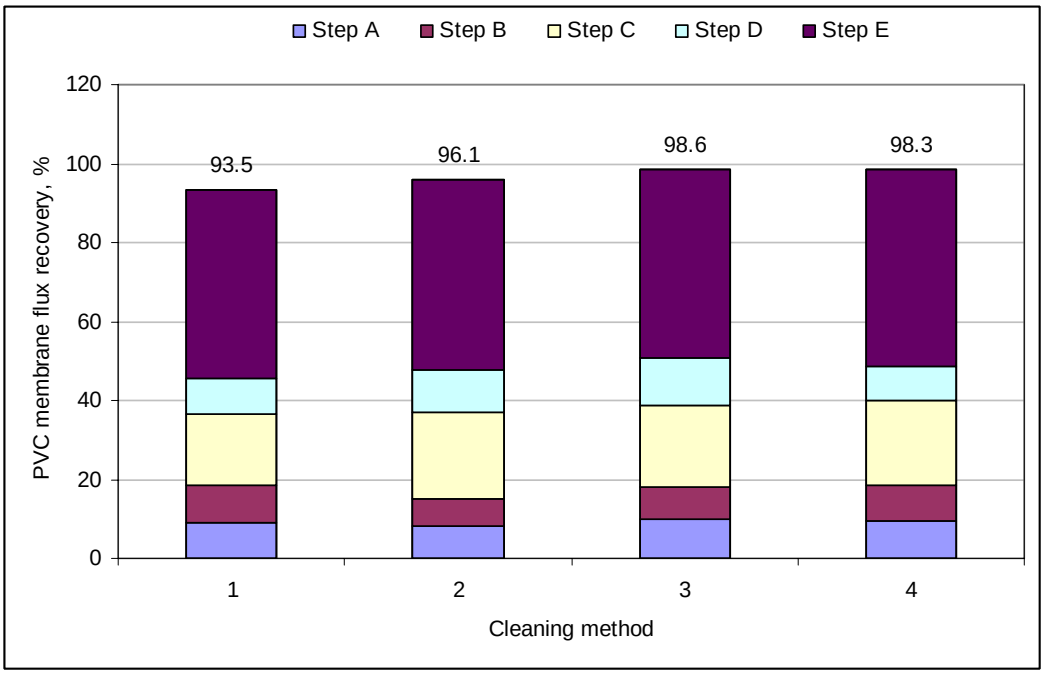


Figure 5.3 Recovery of PVC membrane flux with different cleaning methods

It can be seen from Figure 5.2 and Figure 5.3 that citric acid cleaning contributed the most to the fist four steps of cleaning. The first four cleaning steps (A, B, C, and D, i.e. clean water rinsing, mechanical cleaning, citric acid cleaning, and NaOH solution cleaning) in the four cleaning methods totally accounted for 47.8%, 45.6%, 47.7% and 45.5%, respectively, for the PVDF membrane; and for the PVC membrane, 45.5%, 47.7%, 50.8% and 48.8%, respectively. The following step E of NaClO cleaning, Clorox® disinfectant cleaning, Clorox® disinfectant + industrial alcohol, or Clorox® disinfectant + propylene glycol solution pumping permeation cleaning in the four cleaning methods provided 48.4%, 51.9%, 51.4% and 53.8%, respectively, for the PVDF membrane; and for the PVC membrane, 48%, 48.4%, 47.8% and 49.5%, respectively. It is apparent that the performance of cleaning step E was better than the total performance of the first four cleaning steps (A, B, C and D). In PVDF membrane cleaning, method 4

had the best performance and step E of method 4 had the best performance among the four cleaning methods. Similar results were also obtained for the PVC membrane cleaning. Hence, the above comparison analysis indicates that step E with disinfectant and organic solvent solution pumping permeation cleaning provided better performance than soaking cleaning only, and method 4 using the combination of disinfectant and propylene glycol solution cleaning performed better than the other three methods. The mechanism of using the combination of disinfectant and propylene glycol solution cleaning deserves further study. In addition, it needs to further evaluate the membrane flux characteristics in the next operation cycle.

5.1.3 Monitoring of Re-Fouling Process for the Post Cleaning UF Membrane

PVDF and PVC UF membranes underwent chemical cleaning to restore membrane permeability were reset in the lab bench MBR for solid-liquid separation process. Daily water back pulsing was applied to the membranes and TMP was monitored. TMP responses in the lab bench MBR for the PVDF and PVC membranes in post cleaning operating condition are plotted in Figure 5.4 and Figure 5.5. Results show that higher TMP values were observed for cleaning method 1 due to its low flux recovery rate. In the PVDF membrane operation, TMPs started to increase exponentially from day 10 of operation and reached between 45 kPa and 55 kPa in the following four days of operation. Compared to cleaning methods 3 and 4, higher TMP increase was observed for cleaning method 2, which indicate utilizing Clorox® disinfectant as a single cleaning reagent did not provide the best performance. It appears that cleaning method 4 had the best performance in operation under post membrane cleaning condition.

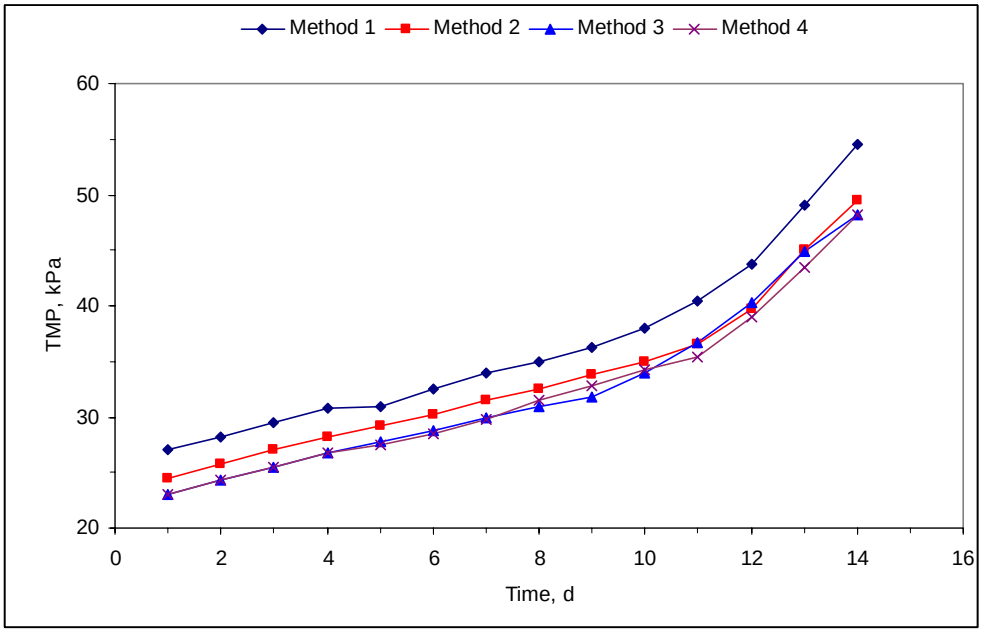


Figure 5.4 TMP response for post cleaning PVDF membrane

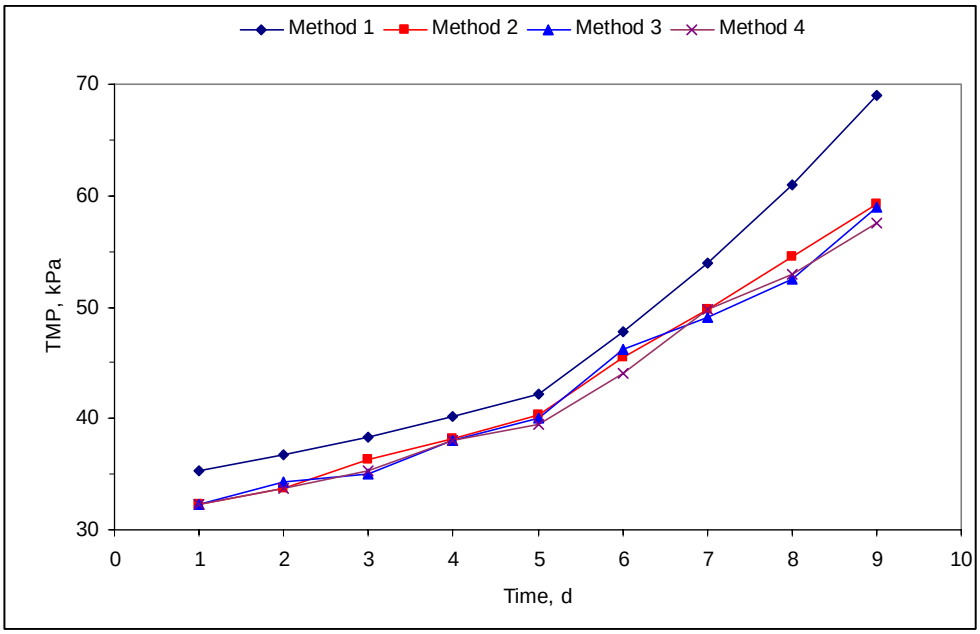


Figure 5.5 TMP response for post cleaning PVC membrane

Similar results were obtained for the PVC membrane. The TMPs for the PVC membrane started from between 32 kPa and 35 kPa in the beginning of the operation and reached approximately 40 kPa at day 5 of operation, and then were monitored with a faster increase after day 5 of operation.

It can be seen from the results that cleaning method 4 had the best performance with relatively complete removal of residual foulants and had the best performance in the post membrane cleaning operation. Further study is needed to evaluate if there were residual foulants remained on membrane surface and what type of foulant residues impact the membrane performance in the next operation cycle.

5.2 FTIR, SEM and EDX Analysis

This study also conducted FTIR, SEM and EDX analysis to study the property of membrane surface structure. Results are presented in the following sections.

5.2.1 Membrane FTIR Spectroscopy Analysis

The results of Fourier transform infrared spectroscopy (FTIR) analysis for membrane surface are presented in Figure 5.6 and Figure 5.7. It can be seen from Figure 5.6 and 5.7 that the FTIR spectra of fouled membrane were relatively flat and no characteristic wave for membrane material was detected due to large quantity of deposited foulants on membrane surface, whereas cleaned membrane showed characteristic wave for membrane material. The absorbance peak below 1000 cm^{-1} represented characteristic wave for alkene (C=C bond), which is the material used in PVDF and PVC. The vibration at 1400 cm^{-1} and 1380 cm^{-1} in fouled membrane FTIR spectra indicated the

presence of nitrogen containing amide and nitrate pollutants, respectively, on membrane surface; the small vibration at 1000 cm^{-1} in fouled membrane FTIR spectra indicated the presence of phosphate and silicate pollutants on membrane surface. It should be pointed out that the vibration at 1651 cm^{-1} and 1029 cm^{-1} represented C=O bond and Glycoside, a representative spectrum of polysaccharide, indicated the presence of polysaccharide type microbial products on membrane surface. The vibration at 1050 cm^{-1}, 1100 cm^{-1}, and 1150 cm^{-1} in cleaned membrane FTIR spectra indicated the presence of ethanol and propylene glycol cleaning reagents, on membrane surface. The FTIR spectroscopy results showed better characteristic peaks existed in cleaned membrane with the selected organic cleaning reagents, indicating methods 3 and 4, especially method 4 had better cleaning performance over other cleaning methods.

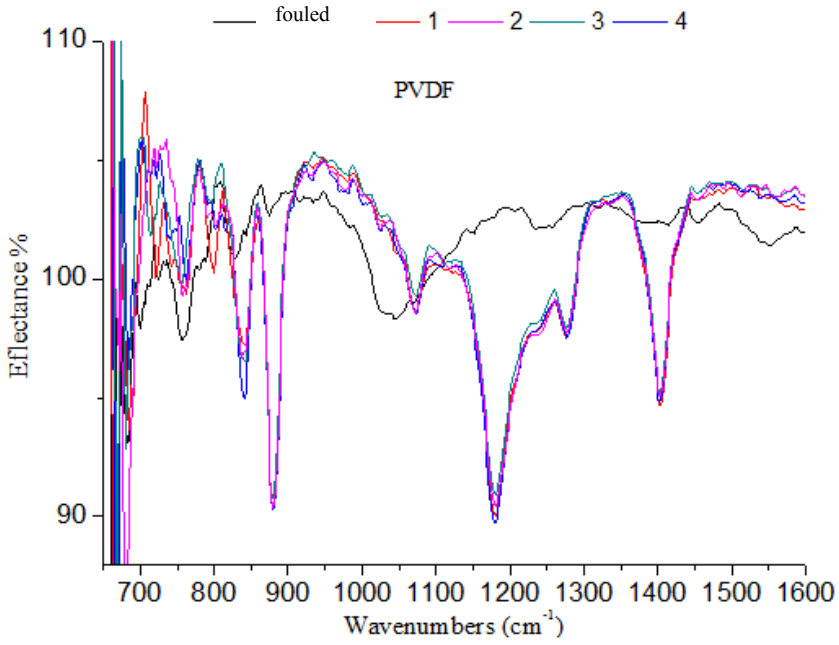


Figure 5.6 FTIR spectrum of PVDF membranes with different cleaning methods

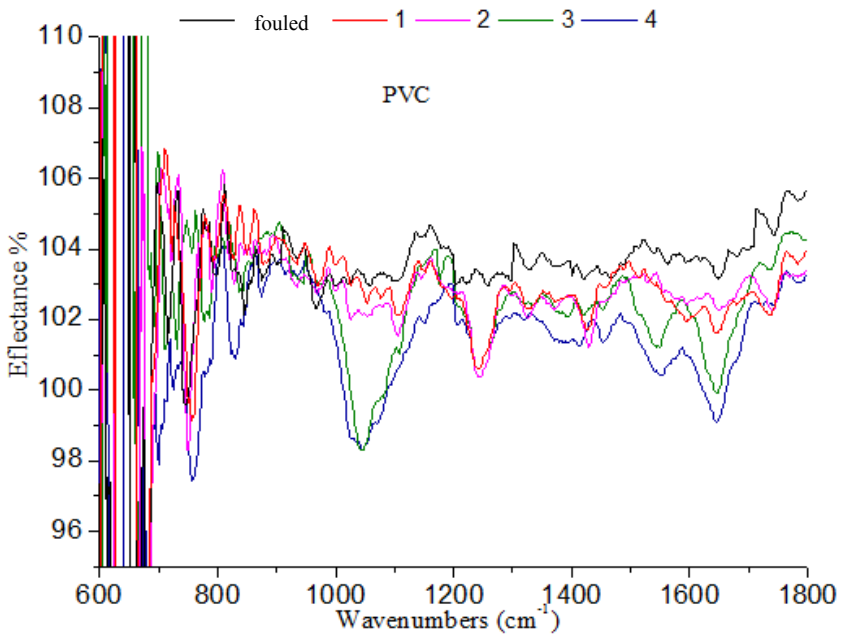
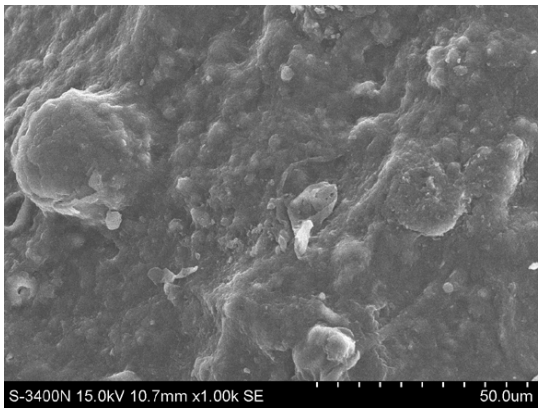


Figure 5.7 FTIR spectrum of PVC membranes with different cleaning methods

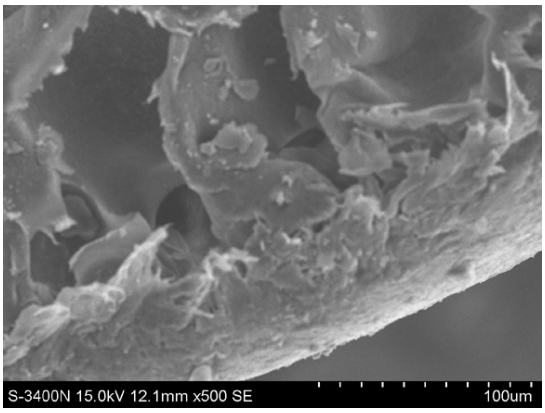
5.2.2 Membrane SEM Analysis

Surface morphology under scanning electron microscopy (SEM) for PVDF and PVC UF membranes before and after cleaning are presented in Figures 5.8 to 5.10. It can be seen from Figure 5.8 and Figure 5.10 that the entire fouled membrane surfaces were covered with a thick layer of pollutants and that there were some foreign particles existed within membrane pores. Based on cross section view of cleaned membranes, three layers could be identified in membrane structure. These three layers can be described as outside dense layer, middle irregular macrovoid support layer and internal dense layer. No foreign pollutants were observed on cleaned membrane surface and within the membrane irregular macrovoid layer for membranes treated with cleaning method 4; indicating pollutants can be completely removed from membrane pores. However for membranes

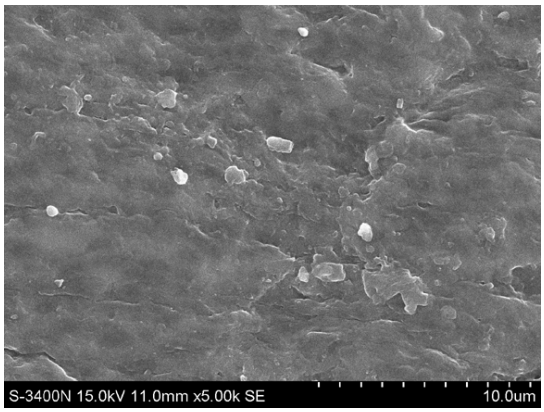
treated with cleaning methods 1 and 2, some small particles were still visible on membrane surfaces, especially for method 1 using soaking solution for cleaning, indicating membrane cleaning utilizing soaking solution only could not completely remove foulants. According to the cleanness on membrane surface with method 1, some residual pollutants might still remain within the pores. Cleaned membrane surface for cleaning method 2 utilizing commercial Clorox disinfectant appeared to be better than method 1, but it still cannot completely remove all the pollutants.



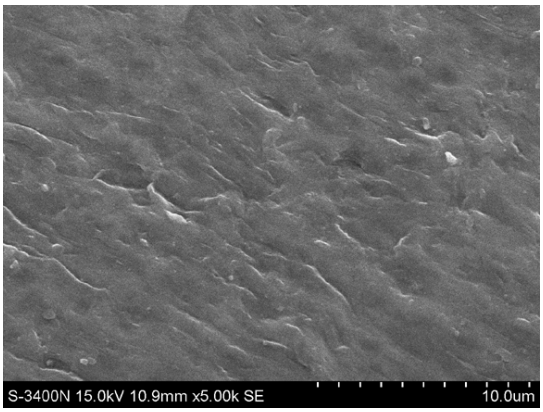
a. PVDF fouled membrane surface 1000x SEM



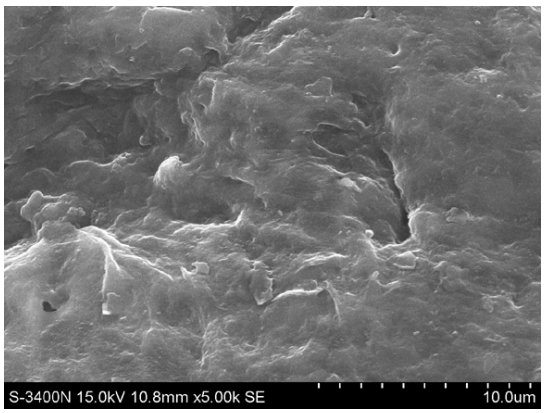
b. PVDF fouled membrane cross section 500x SEM



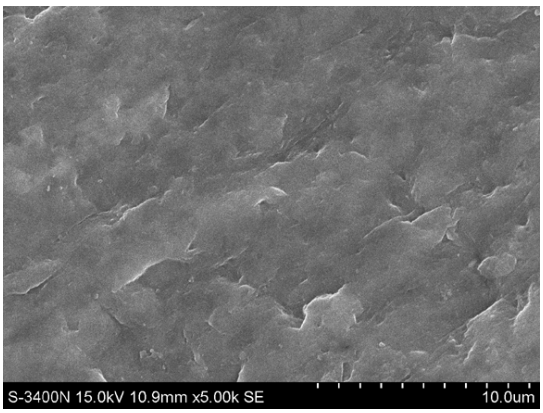
c. Method 1 post cleaning PVDF membrane surface 5000x SEM



d. Method 2 post cleaning PVDF membrane surface 5000x SEM

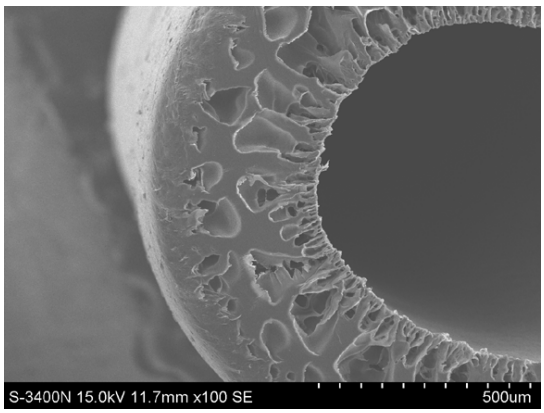


e. Method 3 post cleaning PVDF membrane surface 5000x SEM

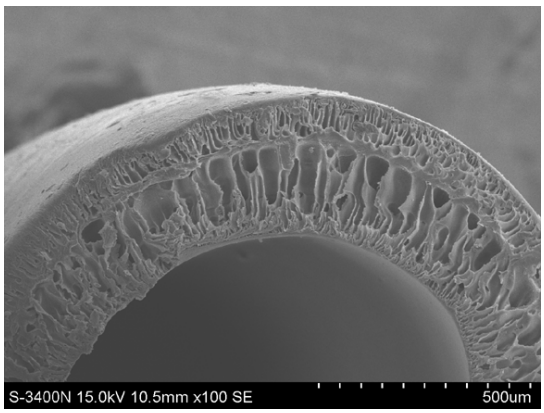


f. Method 4 post cleaning PVDF membrane surface 5000x SEM

Fig. 5.8 SEM pictures of PVDF membranes with different cleaning methods



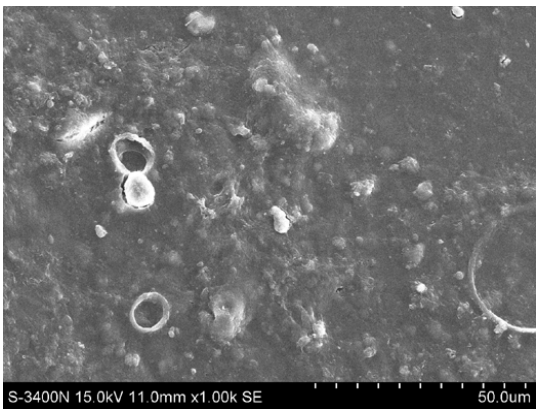
a. Post cleaning PVDF membrane cross section 100x SEM



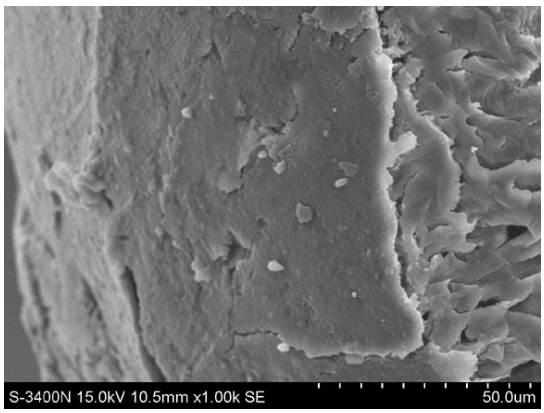
b. Post cleaning PVC membrane cross section 100x SEM

Fig. 5.9 SEM cross section pictures of PVDF and PVC membranes after cleaning

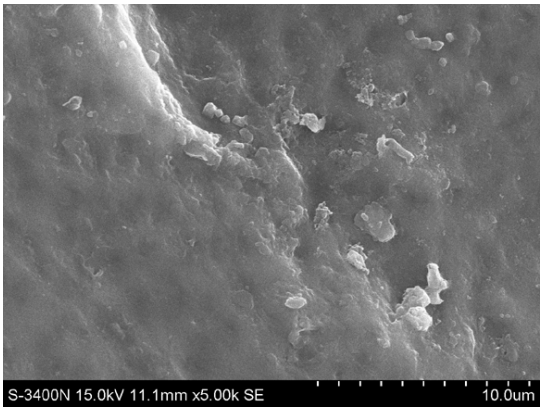
In contrary, membrane surfaces for cleaning methods 3 and 4 were very clean without any apparent visible deposited foreign particles. It should be noted that even the membrane surface were not smooth (in the case of PVDF membrane treated with cleaning method 3), methods 3 and 4 were able to remove all pollutants on surface and within pores. All these observations indicate that organic cleaning reagents had better performance compared to inorganic cleaning reagents. When commercial Clorox disinfectant + industrial alcohol or propylene glycol were used in the cleaning process with pumping permeation operation, oxidant and surfactant in the Clorox disinfectant can effectively remove majority of organic and inorganic pollutants on membrane surface and within pores, while the organic solvent will further dissolve all the organic compound residues, and thus completely remove all the pollutants. Therefore, methods 3 and 4 were the best cleaning methods based on the observation from SEM pictures.



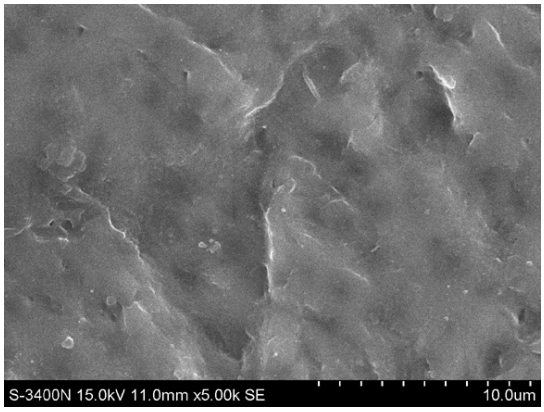
a. PVC fouled membrane surface 1000x SEM



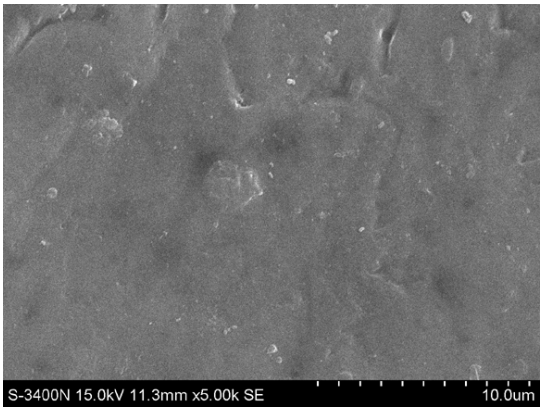
b. PVC fouled membrane cross section 1000x SEM



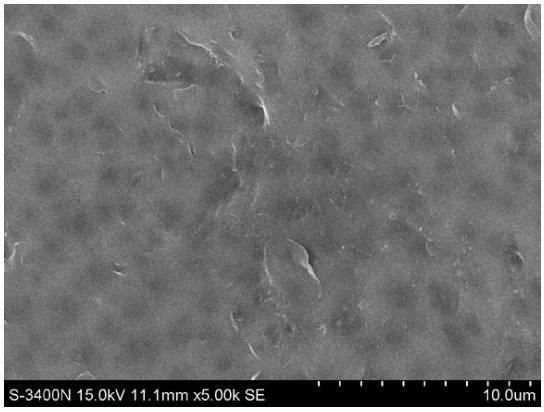
c. Method 1 post cleaning PVC membrane surface 5000x SEM



d. Method 2 post cleaning PVC membrane surface 5000x SEM



e. Method 3 post cleaning PVC membrane surface 5000x SEM



f. Method 4 post cleaning PVC membrane surface 5000x SEM

Fig. 5.10 SEM pictures of PVC membranes with different cleaning methods

5.2.3 Membrane EDX Analysis

Energy-dispersive X-ray spectroscopy (EDX) analysis was also performed for the PVDF and PVC membranes to study contaminant composition on membrane surface. The results are presented in Table 5.1 and Table 5.2. Table 5.1 and Table 5.2 show that almost all the elements commonly encountered in wastewater were detected for fouled membranes. In the fouled PVDF and PVC membranes, the compositions of O were 52.75% and 54.64%, respectively. This is apparent that large amount of organic contaminants were presented on fouled membrane surfaces. Similar to the results in FTIR analysis, contaminant elements N, P, S, and Si were also detected in the fouled membranes, indicating the presence of organic compound and inorganic salt contaminants.

Table 5.1 Contaminant composition on PVDF membrane

Contaminant Element	Fouled membrane	Method 1	Method 2	Method 3	Method 4
	Percentage (%)	Percentage (%)	Percentage (%)	Percentage (%)	Percentage (%)
C	34.07	20.42	21.51	25.45	21.68
O	52.75	0.71	0.71	/	/
F	/	73.47	73.48	72.24	75.42
Na	1.18	2.91	1.40	1.04	1.40
Mg	0.87	/	/	/	0.75
Al	1.09	/	/	/	/
Si	1.81	/	/	/	/
P	3.31	/	/	/	/
S	1.29	/	/	/	/
Cl	0.22	2.20	1.06	0.39	0.33
K	1.00	0.28	1.84	0.87	0.43

Table 5.2 Contaminant composition on PVC membrane

Contaminant Element	Fouled membrane	Method 1	Method 2	Method 3	Method 4
	Percentage (%)	Percentage (%)	Percentage (%)	Percentage (%)	Percentage (%)
C	34.35	52.91	26.57	29.04	32.02
O	54.64	17.04	15.61	13.74	13.73
Na	0.16	1.95	0.63	1.22	/
Mg	0.36	/	/	/	/
Al	0.79	/	F 1.59	F 2.80	/
Si	1.62	/	/	/	/
P	3.21	N 7.52	N 3.71	/	N 3.55
S	1.18	/	/	/	/
Cl	0.27	20.58	50.48	52.44	50.70
K	0.18	/	/	0.76	/
Ca	1.49	/	/	/	/

After cleaning with the four methods, compositions of F in PVDF membranes increased to between 72.2 % and 75.4% and compositions of Mg, Al, Si, P, and S were not detectable which indicates they were removed in the cleaning process. In PVDF membrane, compositions of O were reduced to near zero with cleaning methods 1 and 2, and to zero with methods 3 and 4, indicating all organic contaminants were removed. It appears cleaning method 4 had the best cleaning result.

In post cleaning PVC membrane, no F was detected, which indicates F is not a component of PVC membrane material. With cleaning method 1, high O composition and low Cl composition indicated soaking the membrane in inorganic cleaning solution only did not provide a thorough organic contaminant removal; whereas in post cleaning PVC membranes with cleaning methods 2, 3 and 4, low or non-detectable metal contaminant compositions, low O contaminant composition, and high Cl composition indicated

majority of inorganic contaminants and most but not all organic contaminants were removed, with method 4 having the best performance.

Similar to the findings in FTIR and EDX analysis, cleaning method 4 provided the best cleaning performance for both the PVDF and PVC UF membranes.

5.3 Molecular Biology Study of Microbial Communities for Lab Bench-Scale MBR

PCR-DDGE analysis was conducted for the lab bench-scale MBR to study microbial diversity in mixed liquor and on membrane surface. Clone libraries were also generated to document microbial species and study their relationship. Results are presented in the following two sections.

5.3.1 Lab Bench-Scale MBR DGGE Fingerprinting Analysis

To study microbial diversity changes over time, PCR-DGGE analysis was conducted for mixed liquor and PVDF and PVC membranes in both the beginning and the end of the operation. The DGGE fingerprinting profile is presented in Figure 5.11. Lanes h1, A1 and B1 represent the microbial communities for mixed liquor, PVDF membrane and PVC membrane in the beginning of operation; lanes h2, A2 and B2 represent the microbial communities for mixed liquor, PVDF membrane and PVC membrane at the end of operation. It should be noted that DGGE bands were clear and bright enough with naked eyes but were not very bright in the picture due to problems of gel documentation system. However this did not impact the microbial diversity analysis.

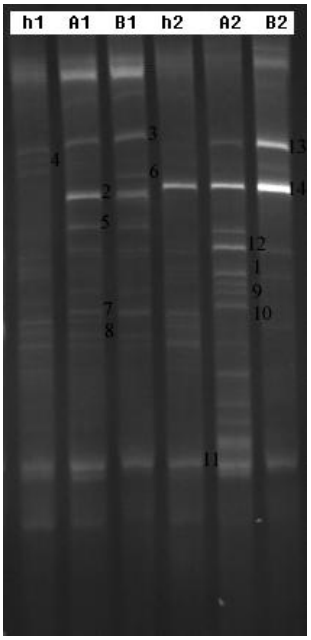


Figure 5.11 DGGE profile of mixed liquor and membrane surface bacteria communities in lab bench-scale MBR

Lane h1, mixed liquor in the beginning of operation;
Lane A1, PVDF membrane in the beginning of operation;
Lane B1, PVC membrane in the beginning of operation;
Lane h2, mixed liquor at the end of operation;
Lane A2, PVDF membrane at the end of operation;
Lane B2, PVC membrane at the end of operation.

It can be seen from lanes h1 and h2 in Figure 5.11 that the seeded mixed liquor from Honouliuli WWTP in the beginning of operation was more abundant in microbial species, but none were absolutely dominant. At the end of two months of operation, one DGGE band in the upper portion of the profile was very bright, indicating one microorganism was the dominant species in mixed liquor.

After one week of operation, microbial communities on PVDF and PVC membrane surface (lanes A1 and B1) were similar to that in seeded mixed liquor except two bands (2 and 3) were getting brighter, indicating these two types of microorganisms were selected to accumulate and reproduce on membrane surfaces. At the end of the two

month operation, microbial community on PVDF membrane was more abundant while that on PVC membrane was less abundant than the beginning operating condition; in addition, several microbial species were dominant on PVDF membrane surfaces while only two microbial species were absolutely dominant on PVC membrane surfaces. The difference in microbial diversity and abundance on PVDF and PVC membrane surfaces could be caused by the flux variation between PVDF and PVC membranes; higher flux in PVDF membrane allowed more nutrients passing through and thus selected more microorganisms to adsorb and survive, while lower flux in PVC membrane allow limited nutrients passing through and thus was only able to support the growth of limited species.

14 DGGE bands were cut for PCR and DNA sequencing to determine their species. The results are presented in Table 5.3. It should be noted that results for bands 7, 8 and 12 were negative due to technical problems.

Table 5.3 Sequence alignment analysis of DGGE bands in lab bench-scale MBR

DGGE band	Gene bank comparison results		
	Bacteria species	Length of DNA sequence, bp	Similarity
1	*Aquabacterium sp.*	168	97%
2	*Pseudomonas sp.*	154	100%
3	*Pseudomonas sp.*	164	97%
4	*Pseudomonas sp.*	156	92%
5	*Acidovorax sp.*	165	99%
6	*Pseudomonas sp.*	119	95%
7,8	----	----	----
9	*Brevundimonas sp.*	140	97%
10	*Caulobacteraceae sp.*	141	95%
11	*Stenotrophomonas sp.*	156	96%
12	----	----	----
13	*Methylobacillus sp.*	146	99%
14	*Methylophilaceae sp.*	166	98%

Table 5.3 shows that bands 2 and 3 in the beginning of operation were *Pseudomona* sp. in *Gammaproteobacteria*, and bands 13 and 14 at the end of operation were *Methylobacillus sp*. and *Methylophilaceae* in *Betaproteobacteria*. The two species in *Betaproteobacteria* mainly use sugar type substances as their media, indicating polysaccharides were involved in membrane fouling. The brightness of bands 13 and 14 may indicate the severity of membrane fouling.

5.3.2 Lab Bench-Scale MBR 16S rRNA Clone Library Analysis

Clone libraries were created for mixed liquor (h2) and PVDF membrane (A2) at the end of the two month operation. 100 clones were selected for DNA sequencing; Clustalx software was used first to conduct a preliminary comparison; then MDGA4 software was used to do the final comparison. Based on the criteria of 97% sequence similarity for each OTU, 49 and 47 OTUs were identified for h2 and A2, respectively. Phylogenetic trees for h2 and A2 were generated by MEGA4 software; statistical analysis for h2 and A2 are presented in Figure 5.12 and phylogenetic trees are presented in Figure 5.13 and Figure 5.14, respectively.

In the MBR mixed liquor microbial community, 100 clones were grouped to 49 OTUs and then were further classified to 7 different classes, of which *Proteobacteria* was the most abundant, accounting for 79%. Other minor microbial classes included *Firmicutes*, *Chlorobi*, *Streptococcus*, *Prosthecobacter*, *Gemmatimonas* and *Acidobacteria*. In *Proteobacteria*, *Alphaproteobacteria* accounted for 25%, *Betaproteobacteria* 40% and *Gammaproteobacteria* 14%. No *Deltaproteobacteria* were identified. The most abundant bacteria class in mixed liquor was *Betaproteobacteria*;

this result is similar to findings in other studies (Duan et.al, 2009, Xia, et al, 2010, and Miura et.al. 2007). *Methylophilaceae sp*., *Betaproteobacteria*, accounted for 17% in the clone library, indicating its absolutely dominant standing in mixed liquor microbial community. This result is consistent with DGGE analysis in which *Methylophilaceae sp*. was dominant species. In addition, *Pseudomonas sp.*, *Gammaproteobacteria*, accounted for 4% in the clone library, indicating the dominant in mixed liquor microbial community. This result is also consistent with DGGE analysis in which *Pseudomonas sp*. was detected with higher chance.

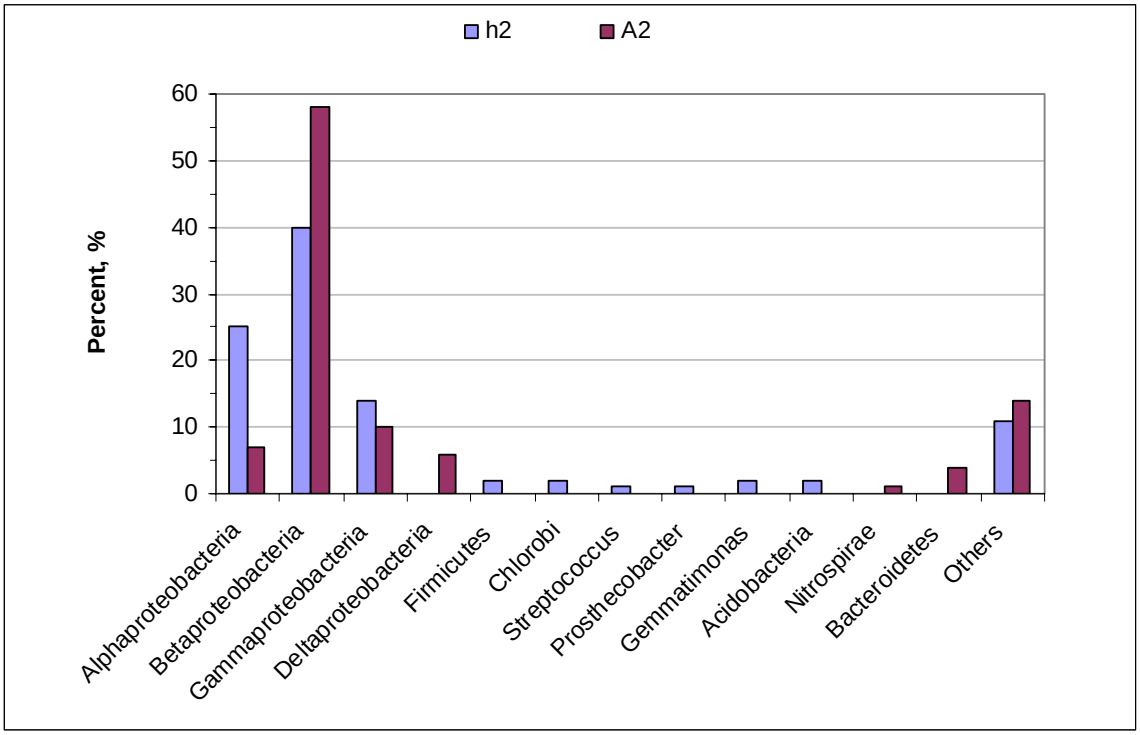


Figure 5.12 Phylogenetic distribution of the OTUs in clone libraries for lab bench MBR

Note:
h2, mixed liquor at the end of operation;
A2, PVDF membrane at the end of operation;

In the PVDF membrane microbial community, 100 clones were grouped to 47 OTUs and then were further classified to 8 different classes, of which *Proteobacteria* was the most abundant, accounting for 81%. In *Proteobacteria*, *Alphaproteobacteria* accounted for 7%, *Betaproteobacteria* 58%, *Gammaproteobacteria* 10% and *Deltaproteobacteria* 6%. *Betaproteobacteria* quantity increased, which may be an indication of membrane fouling. It should be noted that the minor microbial species identified in mixed liquor microbial community were not encountered on PVDF membrane surface; instead *Nitrospirae* and *Bacteroidetes* were found, indicating these two bacteria species found a niche and could reproduce on membrane surface.

It can be seen from Figure 5.12 that major differences between mixed liquor and PVDF membrane microbial communities were the extremely high percentage of *Betaproteobacteria* and the unique *Deltaproteobacteria* and *Bacteroidetes* on PVDF membrane surface. The large amount of *Betaproteobacteria* as well as *Deltaproteobacteria* and *Bacteroidetes* might result in the increase of EPS and SMP, and thus further increased membrane fouling rate (Urgun-Demirta et al., 2005; Lee et al., 2008). *Betaproteobacteria* and *Bacteroidetes* played an important role on UF membrane fouling. Therefore, the phylogenetic relationship study provided a direction in effectively controlling membrane fouling in MBR.

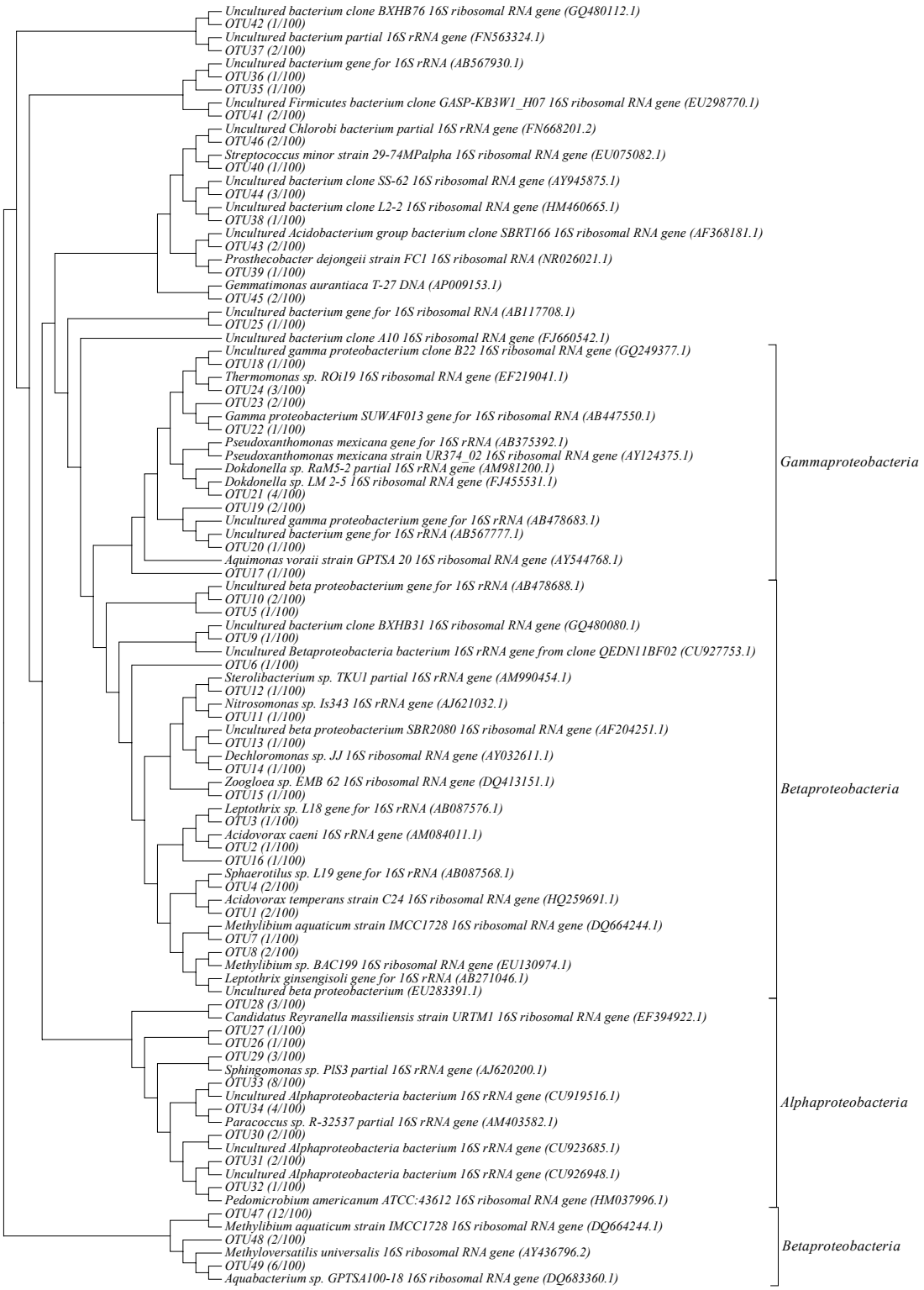


Figure 5.13 Phylogenetic tree of activated sludge microorganisms (h2) in lab bench MBR

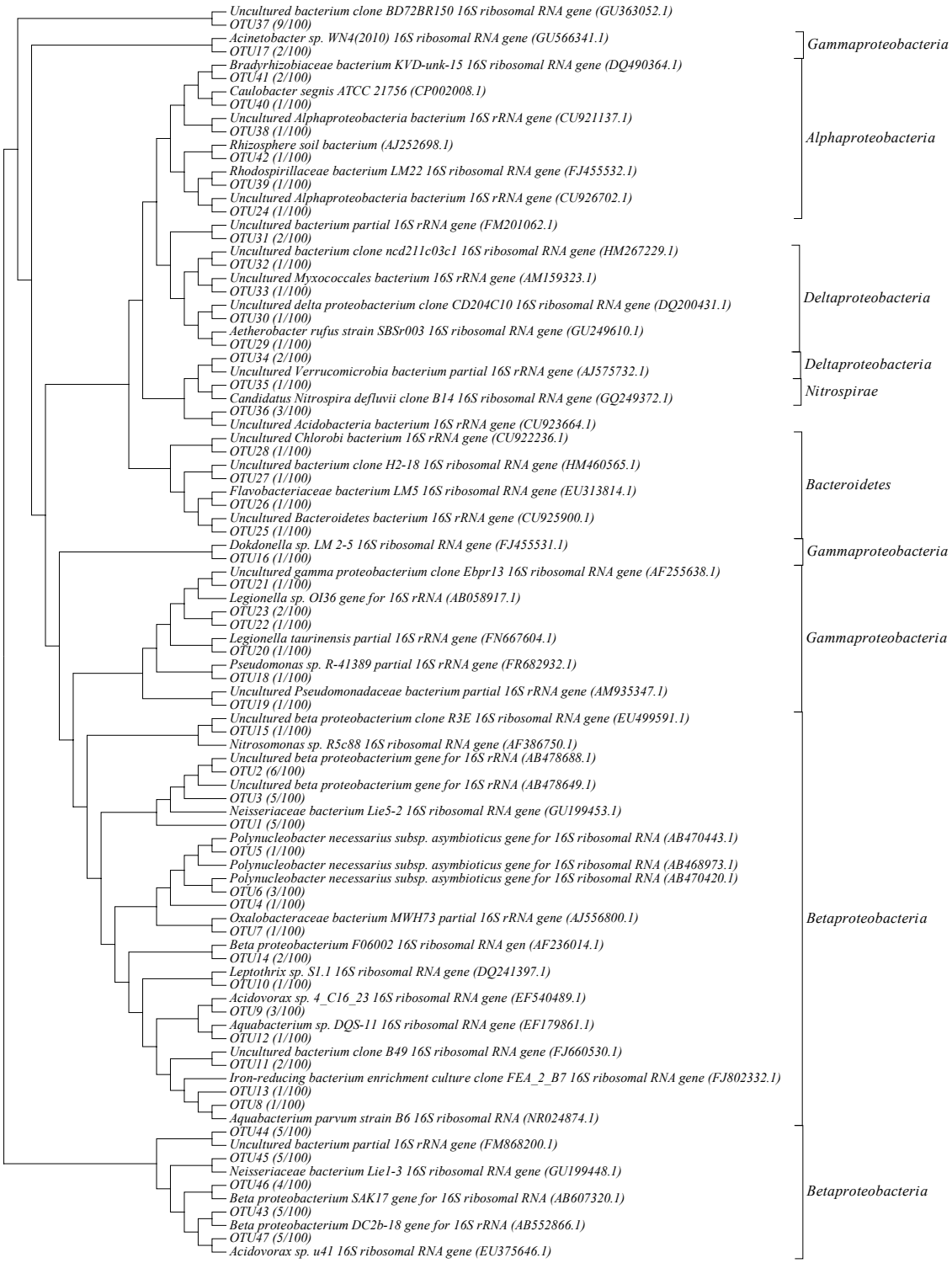


Figure 5.14 Phylogenetic tree of activated sludge microorganisms on PVDF membrane surface (A2) in lab bench MBR

5.5 Conclusions for Chapter 5

Cake resistance was found to be the major resistance component for ultrafiltration membranes and intrinsic membrane resistance and foulant resistance were relatively minor resistance components. Soaking in inorganic cleaning reagent (cleaning method 1) had the worst cleaning performance with the requirement of long soaking time and contaminants on membrane surface and within membrane pores were not completely removed. While vacuum permeation with Clorox® disinfectant + propylene glycol solution (cleaning method 4) had the best cleaning performance with the highest membrane flux recovery. Monitoring of TMP response of post cleaning PVDF and PVC membranes indicated that the re-fouling process with cleaning method 1 was the fastest and re-fouling process with cleaning method 4 was the slowest and thus longer life of operation.

FTIR, SEM and EDX analysis for pre and post cleaning PVDF and PVC membranes indicated that different cleaning performance existed between different cleaning methods. The FTIR spectra for ethanol or propylene glycol cleaning method were more representative than inorganic cleaning methods, which means ethanol or propylene glycol cleaning method can thoroughly remove contaminants. SEM results showed that no contaminant were observed on membranes surface with ethanol or propylene glycol cleaning method but some contaminants were still existing on membrane surface with inorganic cleaning methods. EDX analysis is consistent with SEM results and Clorox® disinfectant + propylene glycol solution (cleaning method 4) is more suitable for PVDF and PVC UF membrane cleaning.

DGGE fingerprinting profile analysis indicated that major changes in microbial community were found between mixed liquors, and membrane surfaces as well, in the beginning of the operation and at the end of the operation. During the membrane fouling process, certain type of microorganisms adhered to and eventually developed to dominant species on membrane surfaces. These microorganisms utilize sugar type organic compounds as their primary media source and belong to *Betaproteobacteria*, *Deltaporteobacteria* and *Bacteroidetes*. More dominant species were found on PVDF membrane surface compared to mixed liquor due to high membrane flux and thus high nutrients passing through membrane. 16S rRNA clone library analysis indicated that extremely high percentage of *Betaproteobacteria* and unique *Deltaproteobacteria* and *Bacteroidetes* were observed on PVDF membrane surface. *Betaproteobacteria* and *Bacteroidetes* might play an important role on UF membrane fouling.

Major finding for this chapter are listed below: 1) vacuum permeation with Clorox® disinfectant + propylene glycol solution (cleaning method 4) had the best cleaning performance with the highest membrane flux recovery: 2) all foreign contaminants on membrane surfaces were completely removed as evidenced by FTIR, SEM and EDX analysis; 3) high percentage of *Betaproteobacteria* and unique *Deltaproteobacteria* and *Bacteroidetes* were observed on PVDF membrane surface. *Betaproteobacteria* and *Bacteroidetes* might play an important role on UF membrane fouling.

CHAPTER 6 APPLICATION TO MBR OPERATION

This research demonstrated that bench MBRs produce superior effluent water quality in terms of TSS, BOD_5,, COD, TOC, TP removal, TN removal, and UVT_{254} over the MCRT range from 10-d to 40-d MCRT and no sludge wasting condition. It is strongly recommended that MBR should be operated in an optimal MCRT range with consideration of both low membrane fouling rate and low operating and maintenance effort. This research indicated high conditioning and steady membrane fouling were observed when MBRs were operated at very long MCRT (no sludge wasting condition in this research); even when MBRs were operated at 40-d MCRT, indication of increased conditioning fouling rate started to show up. On the other hand, low MCRT operation (10-d or less) will result in excessive operating and maintenance cost and high membrane fouling tendency as well as demonstrated in some researches. Therefore, the practical MCRT operating range as demonstrated in this research is between 10-d and 40-d MCRT, or the optimal MCRT range to be between 20-d and 30-d MCRT.

Membrane fouling is inevitable in a membrane filtration process even MBR is operated at optimal MCRT range and hence chemical cleaning is a necessary step to restore membrane permeability as demonstrated in this research. Various inorganic cleaning solutions soaking only was proven to not effective in membrane cleaning, while the combination of inorganic and organic solutions cleaning with vacuum permeation cleaning was proven to be much more effective as proven by flux recovery analysis, FTIR analysis, SEM analysis, and EDX analysis. Therefore, the combination of inorganic and organic cleaning solution under vacuum permeation is strongly recommended for MBR membrane chemical cleaning process.

CHAPTER 7 RECOMMENDATIONS FOR FUTURE RESEARCH

This study evaluated the effects of MCRT on membrane fouling and microbial communities of MBR treating municipal wastewater. PVDF and PVC ultrafiltration membrane cleaning was also evaluated in this study. Mixed liquor properties (EPS and SMP concentrations) and *Betaproteobacteria* are believed to be associated with membrane fouling and the combination of inorganic and organic cleaning reagents cleaning are demonstrated to perform better than inorganic cleaning solutions only. The following research is recommended in this area of study:

1) In-depth study of the effect of mixed liquor properties (EPS and SMP concentrations) on membrane fouling. In this study, only EPS or carbohydrate EPS was found to be associated with membrane fouling process. While discrepancy researches had shown that either EPS or SMP concentrations could be related to membrane fouling. This discrepancy could be caused by different wastewater sources, operating conditions (MCRT, etc.), local weather conditions, or types of MBR technologies. Therefore, in-depth studies should be conducted to answer this question.

2) In-depth study of fouling microorganisms in order to develop biofouling control strategies. *Betaproteobacteria* and some other species such as *Pseudomonas sp.* and *Bacteroidetes* were identified to be intensively involved in membrane fouling as demonstrated in this study and many other studies. Most efforts in identifying membrane fouling microorganisms only focused on the response of fouling microorganisms to changing operating conditions, while very limited research has been done to study the physiology of these fouling microorganisms. It is believed by the author that once knowledge about fouling microorganisms' physiological properties is known,

corresponding effective biofouling control stratagies could possibly be developed to control biofilm formation on membrane surface.

3) In-depth study of ultrafiltration membrane cleaning and its application to pilot and full scale MBR systems. This study found that the combination of inorganic and organic cleaning solutions under vacuum permeation operation is an effective way to clean fouled membrane. However, this is only a preliminary study and its effectiveness on fouling membrane cleaning should be confirmed by repeated bench-scale MBR operation and cleaning processes. The recommended cleaning method should also be applied to pilot and full scale MBR system to determine its optimal cleaning process since many factors need to be considered in membrane cleaning such as cost of cleaning solutions, effort of cleaning process, system operation downtime, etc.

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