ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

THE EFFECTS OF MICROBIAL POLYMERIC SUBSTANCES ON MEMBRANE FOULING

Ph.D. THESIS

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

MİKROBİYAL POLİMERİK MADDELERİN MEMBRAN KİRLİLİĞİ ÜZERİNDEKİ ETKİLERİ

DOKTORA TEZİ

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Necati KAYAALP, a **Ph.D.** student of ITU **Graduate School of Science Engineering and Technology** student ID 501042703, successfully defended the dissertation entitled "**THE EFFECTS OF MICROBIAL POLYMERIC SUBSTANCES ON MEMBRANE FOULING**", which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Date of Submission: **10 May 2012**
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FOREWORD

In the name of Allah, the Beneficent, the Merciful,

"In the heavens and the earth are proofs for the believers. And in your own creation, and in the creatures He scattered, are signs for people of firm faith"

(The Qur'an: 45/3-4).

I have taken *Microbiology* course in the scope of Scientific Preparation for Environmental Engineering Program. I was astonished when I had found out the metabolic activities resembling a complex factory that very tiny microorganisms contain in their structure. This was the most interesting thing that I have learned during my Ph.D. study in Environmental Engineering. I thought that this excellent delicate design can only be the work of a divine mind. Later I learned how these microorganisms helped us to protect our environment. So I recommend for those who want to see *the signs* of divine wisdom to start with observing microbial life.

Firstly, I would like to express my profound gratitude to Allah for He powered and enabled me to finish this work.
Secondly, I would like to thank my thesis advisor Dr. Cumali Kınacı who supported me throughout this work. I am also thankful to the jury members for their valuable contributions they made. I should also thank to BAP Department of ITU for financial support of this work.
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ABBREVIATIONS

μ : Dynamic viscosity [Pa.s]
BAP : Biomass associated products
BPC : Biopolymer clusters
BSA : Bovine serum albumin
$CakeEPS_f$: Specific filterable EPS concentration of cake layer [mg/m^2]
$CakeEPS_t$: Specific total EPS concentration of cake layer [mg/m^2]
$CakeSMP_f$: Specific filterable SMP concentration of cake layer [mg/m^2]
$CakeSMP_t$: Specific total SMP concentration of cake layer [mg/m^2]
CER : Cation exchange resin
ChemBW : Tightly bound pore foulants
CLSM : Confocal laser scanning microscopy
COD : Chemical oxygen demand [mg O_2/L]
CST : Capillary suction time
DGGE : Denaturing Gradient Gel Electrophoresis
DO : Dissolved oxygen [mg/L]
EDTA : Ethylenediaminetetraacetic acid
EDX : Energy-dispersive X-ray spectroscopy
EEM : Exitation-emission matrix
EPS : Extracellular polymeric substances
F/M : Food to microorganisms rate
FT-IR : Fourier transform infrared spectroscopy
GFC : Gel filtration chromatography
HPLC : High-performance liquid chromatography
HRT : Hydraulic retention time [hours]
J : Pure water flux [$m^3/m^2.s$]
MBR : Membrane bioreactor
MLSS : Mixed liquor suspended solids [mg/L]
MLVSS : Mixed liquor volatile suspended solids [mg/L]
MPS : Microbial polymeric substances
NMR : Nuclear magnetic resonance
OLR : Volumetric organic loading rate [g COD/L.d]

P/C : Protein / Carbohydrate ratio
PAC : Powdered activated carbon
PACl : Polyaluminumchloride
PAN : Polyacrylonitrile
PE : Polyethylene
PES : Polyethersulfone
PhysBW : Loosely bound pore foulants
PSD : Particle Size Distribution
PVDF : Polyvinylidenefluoride
$\mathbf{R_c}$: Cake layer resistance [1/m]
$\mathbf{R_{ir}}$: Irrecoverable resistance [1/m]
$\mathbf{R_m}$: Membrane resistance [1/m]
$\mathbf{R_{p1}}$: Pore resistance due to loosely bound foulants [1/m]
$\mathbf{R_{p2}}$: Pore resistance due to tightly bound foulants [1/m]
$\mathbf{R_T}$: Total filtration resistance [1/m]
SEM : Scanning electron microscope
SMP : Soluble microbial products
SNDN : Simultaneous nitrification denitrification
SRT : Sludge retention time [days]
SS : Suspended solids
TMP : Transmembrane pressure
UAP : Utilization associated by-products
UV : Ultraviolet light
VS : Volatile solids
ΔP : Transmembrane pressure [kPa]

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THE EFFECTS OF MICROBIAL POLYMERIC SUBSTANCES ON MEMBRANE FOULING

SUMMARY

The membrane bioreactor (MBR) technology used for wastewater treatment presents many advantages when it is compared to conventional treatment. Substantial reduction in footprint by process intensification and providence of good-quality of effluent and disinfection by complete retention of biomass can be counted among advantages of MBRs. However, this technology has an important disadvantage which is membrane fouling. The membranes used for treatment lose their performance due to fouling as a result of many parameters. This causes reduction in treated water production, discontinuities of the treatment process and increase of operation costs. Therefore, membranes should be cleaned periodically by physical and chemical means.

Membrane fouling is a complex phenomenon on which a lot of measurable and immeasurable parameters play roles. When the literature of membrane fouling is examined, it is noticeable that contradicting results were reported on the effects of the fouling parameters. Although this can be tolerated up to a point because of the complexity of the phenomenon, a part of these contradictions results from inappropriate experimental design and uncontrolled experimental conditions. Especially changing more than one parameter at a time can be said to be the main reason of these contradictions. For example, increasing biomass concentration leaving other controllable parameters such as air scouring efficiency, dissolved oxygen and temperature etc. out of control, which are also influential on fouling, causes considerable problems in obtaining conclusive results. In addition, majority of previous membrane fouling studies is related to the observations made in activated sludge mixed liquor. However, membrane fouling should be investigated directly by observing membrane itself. This is the only way of understanding the real reasons of membrane fouling.

Based on this idea, it was concentrated on membrane itself in the current study. In this study the effect of microbial polymeric substances (Soluble microbial products (SMP) and extracellular polymeric substances (EPS), or shortly microbial polymeric substances (MPS)) on membrane fouling in a submerged MBR – which is the most speculated issue in this field – used for synthetic domestic wastewater was investigated. Small handmade membrane modules were submerged in MBR for different filtration durations. The study was performed under as controllable as possible conditions keeping other related parameters constant. MPSs accumulated in/on fouled membranes were extracted by three steps. While in the first step the cake layer formed on membranes was rinsed with pure water, membranes were backwashed physically with pure water in the second step. In third step membranes were cleaned by chemical backwashing. By this cleaning procedure, foulants accumulated on the membrane surface, foulants loosely attached to the pores and

foulants tightly attached to the pores were extracted and collected separately, and their concentrations were measured in terms of protein and carbohydrate. The extent of fouling that the accumulated foulants caused was determined by measuring membrane filtration resistances.

It was concluded that membrane fouling was mainly resulted from the accumulated foulants in/on the membranes. A strong functional relationship was observed between total extracted foulants and their respective resistances. The great majority of foulants occupied in the cake layer formed on the membrane surface at the very beginning of the filtration. Cake layer was also observed to constitute the greatest filtration resistance. The second greatest fouling source was resulted from MPSs tightly attached to the membrane pores, which was followed by MPSs loosely attached to the pores and permanent (irrecoverable) foulants, respectively. It was observed that protein fraction of MPS accumulated more than carbohydrate fraction in/on the membranes. According to the results of membrane fouling modeling, it was seen that in predicting fouling calibration with the extracted foulants actually accumulated in membrane gave better results than with the cumulative foulant amounts calculated from instantaneous foulant concentration of activated sludge in fouling prediction.

HÜCRE DIŞI POLİMERİK MADDELERİN MEMBRAN KİRLİLİĞİNE ETKİLERİ

ÖZET

Atıksu arıtımında kullanılan membran biyoreaktör (MBR) teknolojisi geleneksel arıtma yöntemine göre pek çok avantaj sunmaktadır. Bunlar arasında arıtma prosesinin yoğunlaştırılarak gerekli tesis alanının önemli oranda azalması, biyokütlenin çıkış akımından tamamen ayrılarak iyi kalitede çıkış suyu ve dezenfeksiyon sağlanması gibi önemli avantajlar sayılabilir. Ancak bu teknolojinin membran kirlenmesi gibi önemli bir dezavantajı bulunmaktadır. Kullanılan membranlar çeşitli koşullara bağlı olarak kirlilikten dolayı bir süre sonra performanslarını kaybederler. Bu durum arıtılmış su üretiminin düşmesine, prosesin kesintiye uğramasına ve işletme maliyetinin artmasına neden olmaktadır. Dolayısıyla membranların belirli periyotlarla fiziksel ve kimyasal olarak temizlenmesi gerekmektedir. Membran kirlenmesi üzerinde etkili ölçülebilen ve ölçülemeyen pek çok parametre rol oynadığından membran biyoreaktörlerindeki membran kirlenmesi olayı oldukça karmaşık bir olaydır. Literatürde membran kirlenmesinin sebepleri araştırıldığında ilk göze çarpan şey parametrelerin etkisi konusundaki birbiriyle çelişen bulgulardır. Olayın karmaşıklığından dolayı bu bir yere kadar normaldir. Ancak bunun bir kısmı da yanlış deney tasarımından ve kontrolsüz deney koşullarından ileri gelmektedir. Örneğin biyokütle konsantrasyonunu arttırıp hava sıyırmasını sabit tutmak, çözünmüş oksijen konsantrasyonu veya sıcaklıkta çok büyük değişimlere izin vermek vs. gibi kirlenmeyi etkileyebilecek diğer kontrol edilebilir parametreleri göz ardı etmek elde edilen sonuçların açıklayıcı olmasında bir hayli problem teşkil etmektedir.

MBR'daki membran kirlenmesinin ana nedenleri üç başlık altında toplanabilir: Aktif çamur özellikleri, işletme koşulları ve membran özellikleri. Membran kirliliğini etkileyen aktif çamur özellikleri arasında biyokütle konsantrasyonu, susuzlaştırma özellikleri ve viskozite, mikrobiyolojik özellikler, mikrobiyal polimerik madde konsantrasyonu ve partikül boyut dağılımı sayılabilir. Membran kirlenmesinde etkili işletme koşulları çamur yaşı (SRT) – veya Besin/Mikroorganizma (F/M) oranı – hacimsel organik yükleme hızı (OYH), akı, hidrolik bekletme süresi (HRT), çözünmüş oksijen (ÇO), sıcaklık ve havalandırma hızı olarak sayılabilir. Pek çok çalışmada bir veya birkaç özelliği birbirinden farklı olan membranlar kullanılmıştır. Örneğin hem membran malzemesi hem de gözenek çapı birbirinden farklı membran aynı çalışmada kullanılıp kirlenme özellikleri birbiriyle karşılaştırılmıştır. Bu özellikleri birbirinden tamamen ayırıp değerlendirmek mümkün değildir. Ancak membran kirliliğini etkileyen membran özellikleri membran malzemesi, gözenek çapı ve hidrofobisite olarak sıralanabilir.

Membran kirliliği konusunda üzerinde en çok çalışılan parametrelerden biri mikrobiyal polimerik maddelerdir. Hücredışı mikrobiyal polimerik maddeler – yani çözünmüş mikrobiyal ürünler (SMP) ve hücredışı polimerik maddeler (EPS) veya ikisini birden ifade etmek için kısaca mikrobiyal polimerik maddeler (MPS) –

polisakkarit, protein, nükleik asit, (fosfo)lipid ve mikrobiyal kütlelerin (aggregates) boşluklarında bulunan diğer polimerik bileşiklerden oluşan mikrobiyal orjinli biyopolimerlerden oluşmaktadır. Bunlar mikrobiyal kütleleri bir arada tutan kohezif kuvvetleri meydana getirirler. Bunların diğer fonksiyonları arasında yüzeylere bağlanmayı (adhesion) sağlama, biyofilm yapısının stabilizasyonun korunması, biyosit ve diğer zararlı etkilere karşı koruma sağlayan koruyucu tabakanın oluşumu, suyun tutulması, besin biriktirmek için çevreden organik maddelerin alınması (sorption) ve dışardaki makromolekülleri parçalayan enzimatik aktivitelerin gerçekleştirilmesi sayılabilir. MPSler aktif çamur süspansiyonunda bulundukları yere, boyutlarına veya oluşum şartlarına göre çözünmüş mikrobiyal ürünler (SMP)/hücredışı polimerik maddeler (EPS), biyopolimer kümeleri (BPC), tüketim yan ürünleri (UAP)/biyokütlesel ürünler (BAP) vb. alt kategorilere ayrılmıştır. MBR'lardaki membran kirlenmesinin mikrobiyal polimerik madde (MPS) ile ilişkisi araştırılırken daha çok MPS oluşum/giderimini etkileyen faktörler incelenmiştir. Bu faktörleri SRT (veya F/M oranı), HRT – akı – OYH, pH – sıcaklık – diğer stres koşulları, havalandırma yoğunluğu/ÇO konsantrasyonu, flokülasyon – koagülasyon – adsorpsiyon eklentileri, mikrobiyal yapı, giriş atıksu bileşimi, nütriyent seviyesi (büyüme fazı) ve reaktör tipi – konfigürasyonu şeklinde özetlenebilir.

Bu çalışmada atıksu arıtımında kullanılan membran biyoreaktörlerdeki membran kirliliği konusunda üzerinde en çok tartışılan mikrobiyal polimerik maddelerin membran kirliliği üzerindeki etkisi incelenmiştir. Çalışma kapsamında laboratuvar ölçekli batık bir membran biyoreaktör düzeneği kurulmuştur. Sistem düz tabakalı 0.2 µm luk mikrofiltrasyon membranlardan oluşan ve sentetik evsel atıksu ile beslenen sürekli bir sistemdir. Literatürdeki membran kirliliği araştırmalarının çoğu aktif çamur süspansiyonunda yapılan gözlemler ile ilgilidir. Yapılan bu çalışmalarda membran kirliliği aktif çamur bileşimindeki değişiklere göre yorumlanmaya çalışılmıştır. Bu çalışmada ise daha çok membranın kendisi üzerine yoğunlaşılmıştır. Kontrollü şartlar altında membran biyoreaktöre daldırılan küçük membran modülleri farklı filtrasyon sürelerinde çalıştırılmıştır. Kirlenmiş membranlarda biriken MPSler üç aşamada ekstrakte edilmiştir. Birinci aşamada membran üzerinde biriken kek tabakası saf su ile sıyrılmıştır. İkinci aşamada membranların saf su ile geri yıkaması yapılmıştır. Üçüncü aşamada ise membranlar kimyasal olarak geri yıkama ile temizlenmiştir. Bu şekilde membran üzerinde biriken kek tabakası kirleticileri, membran gözeneklerine sıkı bağlı bulunan kirleticiler ve membran gözeneklerine gevşek bağlı kirleticiler ayrı ayrı toplanarak kirletici konsantrasyonu protein ve karbonhidrat cinsinden ölçülmüştür. Membranda biriken kirleticilerin ne kadar kirlenmeye sebep olduğu ekstrakte edilen kirleticilerin neden olduğu membran filtrasyon dirençleri ölçülerek belirlenmiştir.

Çalışmadan elde edilen sonuçlara göre membran kirlenmesinin temel olarak membranda biriken kirleticilerden kaynaklandığı belirlenmiştir. Ekstrakte edilen toplam kirletici miktarı ile bunların oluşturduğu dirençler arasında kuvvetli bir fonksiyonel ilişki olduğu görülmüştür. Bu ilişki üstel bir fonksiyon ile ifade edildiğinde korelasyon katsayısı r^2=0.92 çıkmaktadır. Membranda biriken kirleticilerin büyük çoğunluğu filtrasyonun erken devrelerinde oluşan kek tabakasında yer almaktadır. Kek tabakasının aynı zamanda membran filtrasyonundaki en büyük direnci oluşturduğu belirlenmiştir. Kek tabakası direncinin 2.6×10^{12} ile 3.1×10^{12} [1/m] arasında değiştiği ve toplam kirlenme direncine 72.1 % - 76.8 % oranları arasında katkı yaptığı hesaplanmıştır. Kek tabakası direncinden sonra en büyük ikinci direnç 11.8 – 15.6 % arasında değişen membran gözeneklerine sıkı bağlı polimerik maddelerin oluşturduğu geri dönüşümsüz dirençten kaynaklanmıştır.

Bunu sırasıyla 6.0 – 9.9 % ve 2.4 – 5.3 % arasında değişen membran gözeneklerine gevşek bağlı polimerlerin oluşturduğu geri dönüşümlü direnç ve giderilemeyen direnç takip etmektedir. Membranda biriken polimerik madde miktarı büyüklük sıralamasının *kek tabakası kirleticileri* > *gözeneklere sıkı bağlı kirleticiler* > *gözeneklere gevşek bağlı kirleticiler* şeklinde sıralandığı görülmüştür. Membranda MPSnin protein bileşeninin karbonhidrat bileşeninden daha fazla biriktiği görülmüştür. Membran kirliliğinin modellenmesinde membranda biriken kirletici miktarlarının kullanılmasının, aktif çamurdaki anlık kirletici konsantrasyonlarının kullanımıyla hesaplanan toplam süzülen kirletici miktarının kullanılmasından daha iyi sonuç verdiği görülmüştür.

1. INTRODUCTION

1.1 Problem Statement

Wastewaters produced as a result of daily life and industrial activities contain a great number of substances which have adverse effects on environmental and human health. These substances may include organic, inorganic and biological components such as carbon, nitrogen, phosphorus, heavy metals, bacteria, viruses etc. according to their sources. For this reason, wastewaters resulting from any activity should be discharged to the receiving environments after treatment. Conventional wastewater treatment comprises of sequential treatment units such as primary settling where a physical treatment is performed, secondary treatment for organic and nutrient removal, secondary settling for active biomass separation, and tertiary treatment such as sand filtration, disinfection etc. for desired treatment degree. Wastewater discharges and the number of pollutant species it contains have been increasing in parallel to the population and technological developments. Wastewaters discharged to the environment without required treatment cause a lot of problems. Water resources pollution and its adverse effects on human health come in the first place. Almost one billion people are devoid of clean water resources and two million person die every year because of unsafe water, sanitation and hygiene [1]. As a result of precautions taken in the scope of water and soil resources preservation, stricter discharge limits have been applied and wastewater reuse has been encouraged. Accordingly, new and more efficient wastewater treatment techniques required to be developed. Membrane bioreactor (MBR) technology satisfies these aims. MBR technology highly intensifies wastewater treatment process performing the functions of several conventional treatment units. Thanks to this process intensification both smaller area is occupied and treatment efficiency is raised. Since MBR is a modular technology it is possible to scale up and down the capacity of an existing plant. The history of widespread use of MBRs dates back a decade. However, the MBR technology has been improving rapidly and the results of research and development has been quickly transferred to practical application. It is possible to see a lot of

membrane producers and various products on the market. Also, the capacity of the plants increases day by day.

MBR technology may have an important role in resolving environmental problems of Turkey. This technology provides an advantage for both residential places without wastewater treatment plant and upgrading of existing plants. Therefore, the results of this study which investigates the reasons of membran fouling in MBR are considered to be useful for further investigations on the reduction of membrane fouling in MBRs used for wastewater treatment.

1.2 The Aim and Scope of the Study

Membrane fouling is still the biggest problem of MBR technology in spite of improvements in this field. Membranes used in MBR become fouled with time and accordingly the capacity of treated water production decreases and the operation costs, containing energy requirements in the first place, increase. The process of wastewater treatment with MBR is a complex process due to the existence of many interconnected parameters. For this reason, it is quite difficult to predict system's response to changing conditions although an analysis of the system is identified under a predefined set of conditions. This is resulted from immeasurable or uncontrollable parameters causing significant uncertainties in the modeling of system behavior. This is also the cause why membrane fouling could not be clarified in spite of a great number of studies performed. It is even possible to mention a clear contradiction among studies in this field. The effect of biomass concentration on membrane fouling is a typical example of this contradiction.

Membrane fouling in MBR depends on several groups of factors such as influent properties, operating conditions of biological and membrane separation processes and the properties of the membrane used. Yet, it is not possible to determine the effect of individual parameters isolating each one completely. One of the most investigated parameters in terms of its effect on membrane fouling is the parameter of microbial polymeric substances (MPS). These substances are generally classified as soluble microbial products (SMP) and extracellular polymeric substances (EPS). Nonetheless, the majority of the studies are related to the factors affecting production and degradation of MPSs in MBR. The existence of a relationship between MPS and membrane fouling is assumed when a corresponding change in transmembrane

pressure or membrane flux can be observed as a result of a change in MPS concentrations of activated sludge.

The aim of this study is to investigate the existence of a relationship between MPSs and membrane fouling under controlled experimental conditions. On the contrary to the general literature, membrane itself rather than activated sludge suspension was investigated in this study. Therefore, the relationship between membrane fouling and MPS was investigated directly. If a substance present in activated sludge contributes to membrane fouling, it should accumulate either on membrane surface or in its pores. Accordingly, the foulants accumulated in/on membranes were extracted and analyzed to determine which component of MPSs, namely protein and carbohydrate accumulates more at membrane. Since foulant accumulation only does not give sufficient information on membrane fouling, the extent of fouling that the substances extracted cause was determined by measuring filtration resistances before and after foulant extraction. The effect of filtration duration on membrane fouling was also investigated in the study. Also, it was investigated in the study whether membrane fouling can be modeled with the amount of foulants extracted from membranes.

2. LITERATURE SURVEY

2.1 An Overview of Membrane Bioreactors

2.1.1 Definition and history

The word “membrane” has a broad meaning ranging from a cell wall to damp proofing material. In wastewater treatment technique, it means “A material through which one type of substance can pass more readily than the others, thus presenting the basis of a separation process” [2]. All processes integrating a permselective membrane with biological water and wastewater treatment to retain solid matter is called as Membrane bioreactor (MBR) [3]. Although systematic studies on membrane phenomenon can be extended to 18th century, its first widespread usage started with the development of defect-free, high-flux anisotropic reverse osmosis membranes by Loeb-Sourirajan in 1960s. Intensive research and development activities commercialized reverse osmosis and led to the development of microfiltration and ultrafiltration processes **[**4**]**. Continuously improving emission standards required development of new technologies in municipal and industrial wastewater treatment **[**5**]**. MBR utilization in wastewater treatment probably resulted from smaller treatment plants and the need of assuring stricter discharge standards. The first commercialized MBRs substituting settling with an external microfiltration membran module in activated sludge process were put into market by Dorr-Oliver Corp. in 1960s. Full-scale MBR plants entered service in North America in the 1970s, Japan in the 1980s, Europe in the 1990s **[**5**]**. The largest 20 MBR plants in the world by 2010 are shown in Table 2.1. The first pilot-scale studies of MBRs were started in 2005 in Turkey. In these small-scale studies domestic wastewater of university campuses was planned to use in garden watering after treatment with MBR. After small plants having the capacity of 100 – 200 m^3/d the construction of larger plants treating combined domestic and industrial wastewaters was commenced **[**6**]**. In 2010, a HUBER Vacuum Rotation Membrane (VRM®) Bioreactor with a

capacity of 1200 m^3/d was founded to reuse treated wastewaters of a great hotel in Bodrum city in green fields watering. **[**7**]**.

Table 2.1 : The largest 20 MBR plant (May 2010) [3].

Project	**Technology**	**Date**	**PDF (10^3 m^3/d)**
Shending River, China	BOW	2010	120
Wenyu River, China	Asahi K/BOW	2007	100
Johns Creek, GA	GE Zenon	2009	94
Beixiaohe, China	Siemens	2008	78
Al Ansab, Muscat, Oman	Kubota	2010	78
Peoria, AZ	GE Zenon	2008	76
Cleveland Bay, Australia	GE Zenon	2007	75
Sabadell, Spain	Kubota	2009	55
San Pedro del Pinatar, Spain	GE Zenon	2007	48
Syndial, Italy	GE Zenon	2005	47
Broad Run WRF, VA	GE Zenon	2008	47
Beijing Miyun, China	MRE	2006	45
NordKanal, Germany	GE Zenon	2004	45
Tempe Kyrene, AZ	GE Zenon	2006	44
Brescia, Italy	GE Zenon	2002	42
Traverse City, MI	GE Zenon	2004	39
Linwood, GA	GE Zenon	2007	38
North Kent Sewer Authority, MI	GE Zenon	2008	35
Jinqiao Power, China	GE Zenon	2006	31
Dubai Sports City, UAE	GE Zenon	2009	30

PDF, Peak daily flow; BOW, Beijing Origin Water; and MRE, Mitsubishi Rayon Engineering.

2.1.2 Classification of membrane processes

Membrane processes are comprised of microfiltration, ultrafiltration, nanofiltration, reverse osmosis, dialysis and electrodialysis. Membrane processes are classified according to membrane material (organic/inorganic), driving force (hydraulic pressure/concentration difference), separation mechanism (sieving/solution-diffusion/ion exchange) and possible separation size (>50 nm macropore, 2 – 50 nm mesopore, <2 nm micropore). General properties of the membrane processes with

typical operation values and their applications in wastewater treatment field are given, respectively, in Table 2.2 and Table 2.3 **[**8**]**. Separation capabilities of membrane processes are shown in Figure 2.1 **[**3**]**.

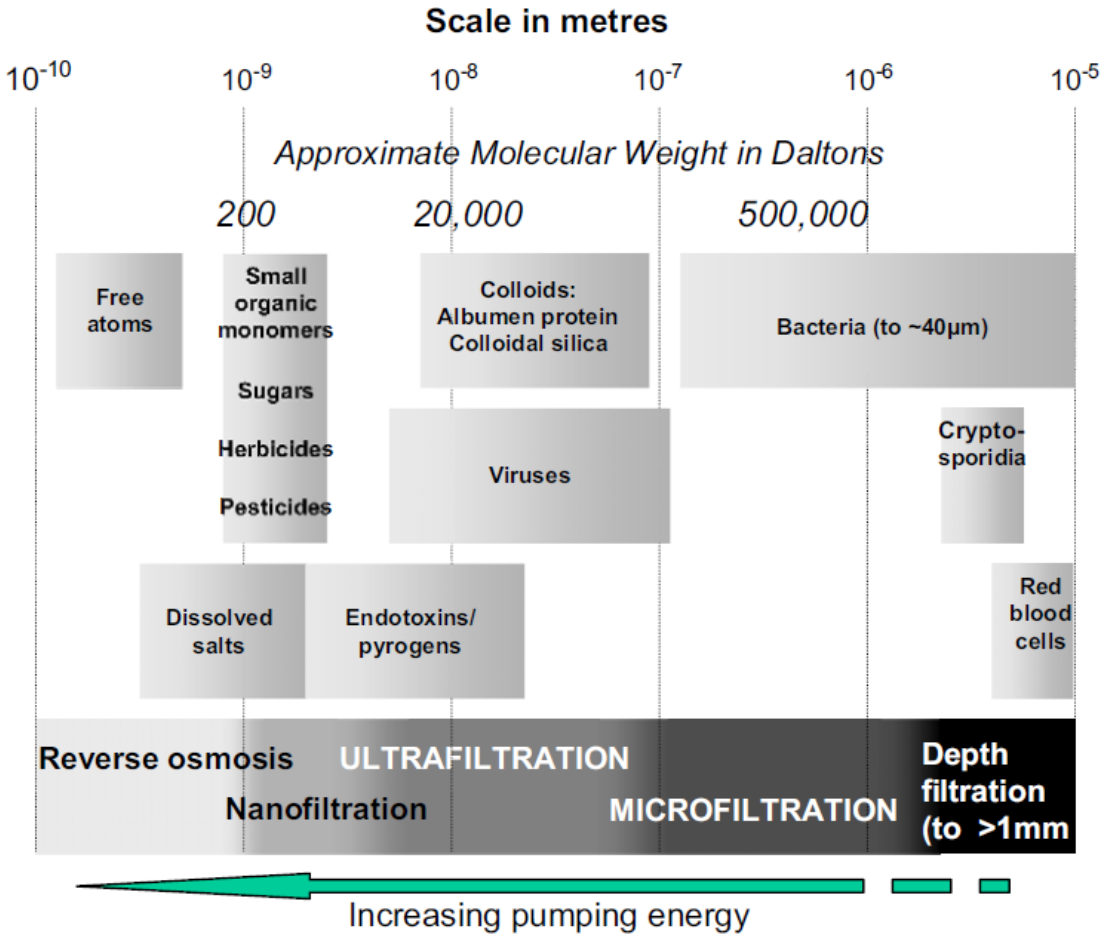


Figure 2.1 : Separation capabilities of membrane processes [3].

2.1.3 MBR Configurations

MBRs are classified as either submerged or external MBR according to whether the membrane module is positioned inside or outside of the biological reactor. Schematic diagram of submerged and external MBRs is shown in Figure 2.2. The first 20 – 30 years of microfiltration and ultrafiltration were based on external configuration using pressurized vessels on recirculation lines. However, later on some developments such as production of low-pressure membranes, elimination the use of cross-flow in case of not very high solids concentration, obtaining desired fluxes with lower pressures than 1 atm. and very efficient control of membrane fouling with bubbling (two-phase) flow, a shift occurred towards submerged MBR configuration **[**9**]**.

Table 2.2 : General characteristics of membrane processes.

Membrane process	Membrane driving force	Typical separation mechanism	Operating structure (pore size)	Typical operating range, µm	Permeate description	Typical constituents removed
Microfiltration	Hydrostatic pressure difference or vacuum in open vessels	Sieve	Macropores (> 50 nm)	0.08 – 2.0	Water + dissolved solids	TSS, turbidity, protozoan oocysts, some bacteria and viruses
Ultrafiltration	Hydrostatic pressure difference	Sieve	Mesopores (2 – 50 nm)	0.005 – 0.2	Water + small molecules	Macromolecules, colloids, most bacteria, some viruses, proteins
Nanofiltration	Hydrostatic pressure difference	Sieve + solution/diffusion + exclusion	Micropores (< 2 nm)	0.001 – 0.01	Water + very small molecules, ionic solutes	Small molecules, some hardness, viruses
Reverse osmosis	Hydrostatic pressure difference	solution/diffusion + exclusion	Dense (<2 nm)	0.0001 – 0.001	Water + very small molecules, ionic solutes	Very small molecules, color, hardness, sulfates, nitrate, sodium, other ions
Dialysis	Concentration difference	Diffusion	Mesopores (2 – 50 nm)	–	Water + small molecules	Macromolecules, colloids, most bacteria, some viruses, proteins
Electrodialysis	Electromotive force	Ion exchange with selective membranes	Micropores (< 2 nm)	–	Water + ionic solutes	Ionized salt ions

Table 2.3 : Typical applications for membrane technologies in wastewater treatment.

Application	Description
	Microfiltration and ultrafiltration
Aerobic biological treatment	Membrane is used to separate the treated wastewater from the active biomass in an activated sludge process.
Anaerobic biological treatment	Membrane is used to separate the treated wastewater from the active biomass in an anaerobic complete-mix reactor
Membrane aeration biological treatment	Membranes are used to transfer pure oxygen to the biomass attached to the outside of the membrane
Membrane extraction biological treatment	Membranes are used to extract degradable organic molecules from inorganic constituents such as acids, bases, and salts from the waste stream for subsequent biological treatment. Such processes are known as extractive membrane bioreactor processes
Pretreatment for effective disinfection	Used to remove residual suspended solids from settled secondary effluent or from the effluent from depth or surface filters to achieve effective disinfection with either chlorine or UV radiation for reuse applications
Pretreatment for nanofiltration and reverse osmosis	Microfilters are used to remove residual colloidal and suspended solids as a pretreatment step for additional processing
	Nanofiltration
Effluent reuse	Used to treat prefiltered effluent (typically with microfiltration) for indirect potable reuse applications such as groundwater injection. Credit is also given for disinfection when using nanofiltration
Wastewater softening	Used to reduce the concentration of multivalent ion contributing to hardness for specific reuse applications
	Reverse osmosis
Effluent reuse	Used to treat prefiltered effluent (typically with microfiltration) for indirect potable reuse applications such as groundwater injection. Credit is also given for disinfection when using reverse osmosis
Effluent dispersal	Reverse osmosis processes have proved capable of removing sizable amounts of selected compounds such as NDMA (N-Nitrosodimethylamine, a highly toxic compound)
Two-stage treatment for boiler use	Two stages of reverse osmosis are used to produce water suitable for high pressure boilers

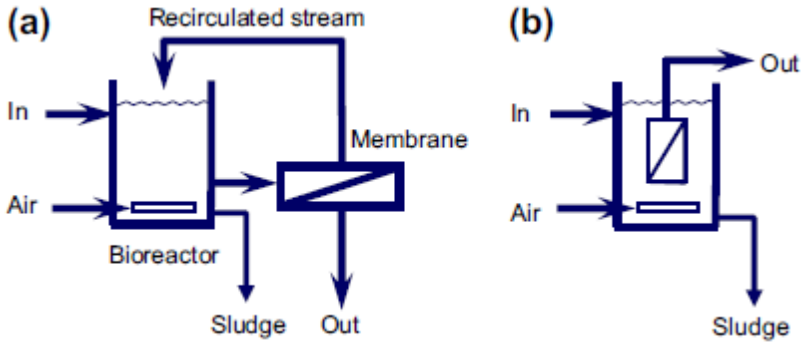


Figure 2.2 : MBR configurations: (a) side-stream, (b) submerged [3].

The first MBR made of hollow fibers was developed by Yamamato et. al in 1989 **[**9**]**. Hollow fibers were shaped in a bundle, and aeration, mixing and induced liquid flow were assured by air bubbles. Although it was met in curiosity in the beginning, submerged MBR became dominant in a decade **[**9**]**. A comparison of some features of submerged and external configurations is made in Table 2.4. It was shown that the fouling propensity of submerged configuration is lower than that of external configuration in a comparison of two configurations **[**5**]**.

2.1.4 Advantages and disadvantages of MBR

There are both advantages and disadvantages of using MBR in water and wastewater treatment. The following features can be mentioned among advantages of MBRs;

- Biomass is retained by membrane instead of sedimentation tank. Therefore, settling problems like sludge bulking do not affect treatment process. Moreover, since biomass concentration in aeration tank is not limited by the capacity of secondary settling, an operation at 10000 – 15000 mg/L biomass concentrations in aeration tank is possible. Higher reaction rates in MBR reduce required plant area due to higher carboneous matter conversion and nitrification rates in lower hydraulic retention time.
- High sludge retention times in MBR enables proliferation of microorganisms degrading special organic pollutants.
- Since membrane serves as a filter, it ensures a better effluent quality in terms of turbidity, bacteria, particular and colloidal organic matter.
- Rapid start up of biological process **[**10**]**

- This reduction of sludge is especially important for industrial sludges belong to hazardous waste class, for cost of treatment and disposal of these sludges is quite high **[**5**]**

In contrast, MBRs have also some disadvantages. Membrane fouling is in the first place among these disadvantages, which is an obstacle in widespread use of MBRs in wastewater treatment. Fouling has a lot of adverse effects on membrane systems such as flux reduction, necessity of substantial increases in transmembrane pressure, degradation of membrane material as a result of biological activity and system failure. Accordingly, high capital costs and routine membrane cleaning due to fouling constitute the disadvantages of MBR utilization in wastewater treatment **[**11**]**.

Table 2.4 : Comparison of external and submerged MF and UF systems [9].

Characteristic feature	**Contained tubular**	**Contained hollow fiber**	**Submerged flat sheet**	**Submerged hollow fiber**
Packing density	Low	High	Moderate	high
Mode of operation	Cross flow	Cross flow and dead-end	Cross flow	Cross flow and dead-end
Piping and valves	High	Modest	Low	Low
Capital cost	High	Modest to low	Modest to low	Low
Energy usage	High (turbulent)	Low (laminar or dead end)	Low (bubbly flow)	Low (bubbly flow or dead end)
Fluid management	Good	Moderate to good	Moderate to good	Moderate to poor
Standardization of module	No	No	No	No
Replacement and repair	Tubes or element	Element	Element	Element or bundle
Cleaning	Good – incl. physical	Backflush; smaller vol. for chemical	Poor (low backflush)	Backflush possible
Turn up/down	Good	Good	Limited by TMP	Limited by TMP

2.2 Membrane Fouling

Membrane fouling develops under a lot of measurable and immeasurable parameters (Figure 2.3). Interrelationships of these parameters make a complete isolation of a parameter to determine its effect on membrane fouling impossible. For example, the impact of SRT only cannot be found keeping all other parameters constant because when SRT is changed, a lot of activated sludge properties also change, including MLSS at first in case of constant feed loading. Nonetheless, this does not mean that

an experiment of membrane fouling cannot be performed under controlled conditions. Detailed information regarding interrelationship of activated sludge properties is given in Section 2.2.5.

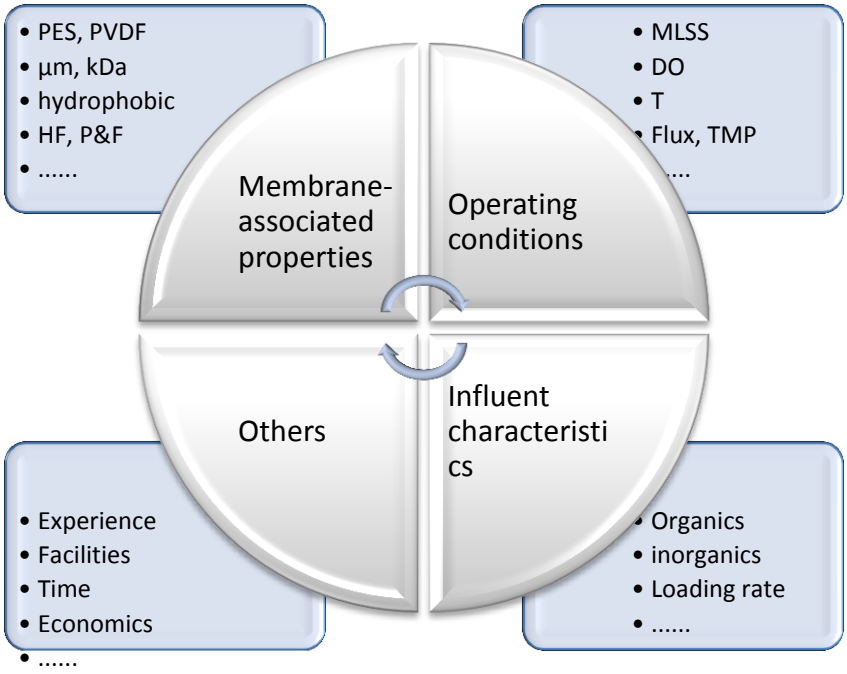


Figure 2.3 : Parameters of membran fouling in MBR.

As it is seen in Figure 2.3 membrane fouling depends on a lot of parameters such as operation parameters of MBR system, reactor configuration, membrane itself, characteristics of influent wastewater, operation time etc.

2.2.1 Factors affecting membrane fouling

The reasons of membrane fouling can be gathered in three major categories, which are activated sludge properties, operating conditions and membrane properties. The studies related to each category are summarized below.

2.2.1.1 Activated sludge properties

MLSS concentration, dewaterability properties and viscosity, microbiological properties and particle size distribution can be mentioned among activated sludge properties influential on membrane fouling.

MLSS concentration

Active microorganism's concentration has an important effect on reaction rates. Since it is not easy to measure active biomass concentration, total biomass

concentration is measured regularly in almost all treatment plants to assure a proper operation. There are a great many of studies regarding the effects of MLSS concentration on membrane fouling. Nevertheless, a consensus has not been reached yet **[**12**]**. The existence of a relationship between membrane fouling and MLSS concentration was investigated by filtering activated sludge taken from two full-scale plants using a method called Delft Filtration Characterization method **[**13**]**. In this method, filterability of different sludge samples is compared by collecting 20 L/m^2 permeate under flux of 80 $L/m^2.h$ using a single tubular, 0.03-µm X-Flow membrane module. By this method a direct correlation between membrane fouling and original MLSS concentrations was not observed. However, it was observed that dilution of samples having original MLSS concentrations greater than 10 g/L impaired filterability. In another study examining the impacts of activated sludge on membrane fouling and critical flux no relation was observed between MLSS concentration and membrane fouling rate **[**14**]** or critical flux **[**15**]**. On the other hand, it was observed that membrane fouling resistance had a strong exponential (r^2=0.97) **[**16**,**17**]** and linear (r^2=0.95) **[**18**]** relationship with MLSS concentration. Similarly, a high linear correlation (r^2=0.91) was observed between MLSS concentration and membrane permeability **[**19**]**. A comparison of different studies reporting the effect of MLSS concentration on membrane fouling is shown in Table 2.5. It is seen that for each MLSS concentration range there are studies on two opposite sides. While data from a group of researchers show the existence of a relation between MLSS concentration and membrane fouling, data from another group show the opposite, which is a clear contradiction. This shows that the effect of this parameter cannot be evaluated independent of other experimental conditions. An evaluation of experimental conditions adopted in membrane fouling studies is presented in Section 2.2.6.

Table 2.5 : Some studies reporting the effect of MLSS concentration on membrane fouling.

MLSS conc. (g/L)	Effect on membrane fouling*	
	Yes	No
2 – 10	[**16,17,19-21**]	[**12-14,20,22**]
10 – 15	[**12,16-21,23**]	[**13-15,22**]
15 – 25	[**12,16-18,22,23**]	[**13**,**15**]

*MLSS concentration of specific studies

[**12**] → 2 – 21 g/L [**13**] → 8.9 – 18.3 g/L [**20**] → 4 – 19 g/L [**18**] → 10.2 – 17.7 g/L
[**19**] → 4.6 – 12.6 g/L [**21**] → 14.0 – 26.9 g/L [**16**] → 2 – 20 g/L [**17**] → 2 – 20 g/L
[**14**] → 5.8 – 12 g/L [**15**] → 10.9 – 21.1 g/L [**22**] → 4 – 12 g/L [**23**] → 3.5 – 12.4 g/L

Dewaterability and viscosity

Capillary suction time (CST) is commonly accepted as a parameter in evaluation of activated sludge dewaterability and a key factor in evaluation of membrane fouling in submerged MBRs [20]. It was observed that CST had a connection with many activated sludge properties, and critical membrane flux was inversely proportional to CST with a high correlation (r^2=0.93) [20,24]. It was pointed out that the CST of a bulking sludge was greater than that of normal sludge, so CST was able to be used as an indicator of membrane fouling [25]. On the other hand, no relationship was observed between membrane fouling and CST in examination of 5 full-scale MBRs [26].

The viscosity of activated sludge was measured in many membrane fouling studies. However, there are very few studies attributing viscosity directly to membrane fouling. Viscosity of activated sludge was generally attributed to parameters such as biomass concentration and concentration of polymeric substances. For example, viscosity increased exponentially with MLSS concentration ranging between 2 and 20 g/L [27] and operation duration [28]. However, while a strong relationship was observed between membrane fouling and activated sludge viscosity [28,29], no direct relationship was also reported [30].

Microbiology

Microbiology of activated sludge is determined by operating properties of the treatment system such as SRT [31], organic loading rate and DO concentration etc. DGGE (Denaturing Gradient Gel Electrophoresis) analyses showed that, although the same inoculation sludge was used, dominant microbial communities shifted within time depending on these parameters [32]. It was stated in various studies that there was a connection between membrane fouling and changing microbiology of activated sludge depending on MBR operation parameters. For example, while it took 434 h for TMP to reach 45 kPa in a normal floc forming sludge, it took only 72 h in bulking sludge dominated by filamentous bacteria developed in low temperature for TMP to reach 45 kPa [33]. This shows that filamentous bacteria may influence membrane fouling. It was reported that cake layer resistance was higher in case of bulking sludge [34]. Microbial community structure impacted on the parameters of activated sludge, e.g. EPS properties, and caused impermeable layer formation [35] and membrane fouling [31]. In another study it was observed that increase of dead

cell ratio (%0 – 100) in activated sludge caused membrane fouling to increase too **[**36**]**.

Microbial polymeric substances

The effect of microbial polymeric substances on membrane fouling is one of the most discussed issues in membrane fouling studies. Since this is the main subject of this thesis, it will be discussed in detail separately in Section 2.2.3.

Particle size distribution

According to Carman's equation established for conventional filtration, specific cake resistance is strongly dependent on particle size; the smaller the particle size is; the greater the specific cake resistance will be **[**37**]**. According to this equation specific cake resistance is inversely proportional to square of particle size. This shows that particle size has an important effect on cake layer resistance. The studies mentioned under the Section 2.2.2.4 which is related to membrane fouling reduction in MBRs by flocculent/coagulant addition are based on this rule in Carman's equation. For example, flocculant addition reduced membrane fouling substantially raising mean particle size 2.5 times (from 62 to 148 μm) **[**38**]**. An analyses of sludge samples taken from 16 MBRs operated under different conditions showed that membrane fouling resistance increased with decreasing particle size distribution (PSD) **[**16**]**. Also A high negative correlation (r = - 0.73) was reported between PSD and membrane fouling in the same study **[**16**]**. In another study it was observed that PSD of foulants accumulated at membrane surface was smaller than that of activated sludge mixed liquor in an external MBR **[**39**]**. While the ratio of particles smaller than 10 μm was % 11 in activated sludge, the ratio of the same particle size was % 49 in foulants accumulated on the membrane surface. Conversely, while the ratio of particles having size 10 – 50 μm was %59 in activated sludge, it was %39 in cake layer deposits. This showed that smaller particles were accumulated on membrane surface preferentially **[**39**]**. It was seen that different type of circulation pumps affected floc size and therefore membrane fouling very much in an external cross-flow MBR **[**40**]**. When centrifuge pump was used for circulation, the ratio of particles smaller than 10 μm increased from %4 to %23 after 24 h, however rotary pump raised the ratio of smaller particles to % 61. After a 6-day operation under 1.5 m/s cross-flow velocity, membrane flux decreased to 36 L/m^2.h with centrifuge pump and to 20 L/m^2.h when rotary pump was used **[**40**]**.

2.2.1.2 Operating conditions

MBR operating conditions impacting on membrane fouling include sludge retention time (or food to microorganism ratio – F/M), volumetric organic loading rate, flux, hydraulic retention time, dissolved oxygen, temperature and aeration rate.

Sludge retention time (or food to microorganism ratio)

Sludge retention time (SRT) is an important biological treatment process design parameter, which affects many activated sludge parameters such as observed biomass yield and MLSS concentration. A great number of studies were performed to investigate the effect of SRT on activated sludge parameters and membrane fouling. Keeping organic loading constant while increasing SRT causes MLSS concentration and viscosity of sludge to increase, which consequently raises membrane fouling **[**41**]**. The rate of membrane fouling at 3-d SRT was nearly 5 times greater than that of 10-d SRT because much higher foulant accumulation was observed on membrane at 10-d SRT **[**42**]**. Although lowering SRT from 40 d to 20 d decreased also MLSS concentration ca. 25%, in contrast membrane cleaning frequency increased nearly two times decreasing from once every 4 – 5 days to once every 2 – 3 days **[**43**]**. Increasing membrane fouling in decreasing SRT is generally attributed to increasing concentration of polymeric substances in lower SRTs **[**44**]** although there are some opposite findings **[**45**,**46**]**. It was reported that specific cake resistance decreased 20 times when SRT increased from 20 d to 60 d **[**47**]**. This was attributed to either reduction of microbial polymeric substances due to high SRT or increase of EPS degradation because of decreased F/M ratio. On the other hand, it was stated that particle flocculation reduced due to decreasing protein and carbohydrate concentration of EPS at longer SRT, accordingly, smaller particles caused more membrane fouling **[**45**]**.

Food to biomass (F/M) ratio is a widely used process parameter to characterize process design and operating conditions. Typical BOD F/M ratios vary between 0.04 – 1.0 g substrate / g biomass for extended aeration and high rate processes **[**8**]**. F/M ratio may affect membrane fouling affirmatively or adversely by changing activated sludge composition. For example, it was observed that the effect of both soluble and suspended components of activated sludge on membrane fouling expressed in terms of modified fouling index (MFI) increased with increasing F/M ratios (0.13, 0.22 and 0.29 g COD/g VSS.d) **[**48**]**. When F/M ratio increased approximately 4 times, the

rate of membrane fouling increased 20 folds [49]. In additon, a SMP producing bacterium *Microbacterium trichotecenolyticum* B4-1 produced less SMP under low F/M ratio (0.7) and did not cause much increase of TMP, while it caused substantial TMP increase under high F/M ratio (8.1) producing high amount of SMP [50].

Volumetric organic loading rate

Membrane fouling substantially increases at higher volumetric organic loading rate (OLR) (0.57, 1.14 and 2.28 g COD/L.d) [32,51]. Two MBRs were operated in parallel to investigate the effects of constant and fluctuating OLR on membrane fouling [52]. While both operated under the same OLR (1.5 kg COD/m^3.d), one of the MBRs were fed with constant flow rate and the other with changing flow rate to simulate diurnal changes observed in a municipal treatment plant. It was observed that fluctuating OLR caused less membrane fouling especially at high fluxes (≥30 L/m^2.h) in long term (~ 45 d) fouling experiments performed under different fluxes. For example, while TMP jump was observed after 15 days at constant OLR, TMP jump was not observed until 28th day at fluctuating OLR [52]. In another study [53], while TMP jump was observed at 35th day when OLR was 0.36 kg TOC/m^3.d, it was observed at 23rd day when OLR increased to 0.9 kg TOC/m^3.d.

Membrane flux

Membrane fouling rate is directly related to membrane flux in MBR operation. It is quite clear that membrane fouls faster at greater fluxes [24]. While TMP jump was observed after 360 h at flux of 30 L/m^2.h, it was observed after 100 h at flux 40 L/m^2.h, and when the flux raised to 50 L/m^2.h TMP jump was observed before 10 h [54]. However, the concept of "critical flux" is somewhat problematic although it was presented [55] as one of the most original idea in testing membrane fouling in tangential filtration. *Critical flux value* is defined as the state of equilibrium between filtration pressure keeping membrane foulants in the vicinity of the membrane and shear forces trying to keep the foulants away from membrane. When permeate flux is below critical flux, particle accumulation does not occur in the membrane region, and if the physicochemical interactions between solute matter and membrane material is ignored, filtration will take place in stabilized conditions as in clean water filtration, in other words, no change is observed in membrane permeability with time [55]. Nonetheless, this description of critical flux caused some problems because it was reported that filtration flux was very influential on membrane fouling, and even

at fluxes such as ~3.3 L/m^2.h far below critical flux, a continuous increase in TMP was observed **[**56**]**. Also an exponential relationship was observed between membrane fouling rate and flux at sub-critical fluxes **[**57**]**. Along with its definition, the measurement of critical flux is also not objective. The protocol chosen (w/o relaxation), peace height, peace duration and total time of the experiment play an influential role on what is called critical flux **[**58**]**. Later the concepts of "critical flux for irreversibility" and "sustainable flux" which was the flux that fouling rate could not be sustained economically and environmentally were added to the critical flux family **[**58**]**. Predominant fouling type on membrane surface was also changed depending on membrane fluxes (13.6, 49.1 and 81.8 L/m^2.h) **[**59**]**. While the fouling was due to biofilm formation at low fluxes, it was resulted from organic matter accumulation at increasing fluxes.

Hydraulic retention time

Hydraulic retention time (HRT) is an important design and operation parameter in activated sludge process. This parameter is briefly related to the degradation rate of a pollutant loading given to a specified reactor. Therefore, if it is possible, choosing a smaller HRT means a reduction in treatment cost due to a reduction in reactor dimensions **[**60**]**. In many studies investigating the effects of HRT on membrane fouling it was seen that a reduction of HRT adversely affected membrane fouling. Reduction of HRT (8, 16 and 24 h) increased membrane cleaning frequency **[**61**]**. While chemical cleaning period was 85 days before HRT reduction from 10 h to 7 h, it became 48 days after HRT reduction **[**62**]**. It was showed that the concentration of polymeric substances and specific cake resistance increased with decreasing HRT (4, 8 and 12 h), and therefore it was concluded that operation at lower HRT than 4 h had adverse effects on membrane fouling and process performance **[**63**]**. HRT reduction (6 – 12 h) elevated the rate of TMP increase **[**64**]** due to increasing biomass production and SMP accumulation **[**45**]**. It was reported that the reduction of HRT from 24 h to 18 h caused more SMP production, increment of smaller sized particles and elevated membrane fouling **[**65**]**.

Dissolved oxygen concentration

Dissolved oxygen (DO), which should be available in amount enough for aerobic treatment, is a component adversely affecting anoxic and anaerobic treatment performances. In addition, dominant microbial species and concentration of

polymeric substances in activated sludge change with dissolved oxygen concentration. These changes in activated sludge structure substantially affect membrane fouling. The rate of membrane fouling in anoxic MBR (DO < 0.3 mg/L) was five times that of aerobic MBR (DO = 6.0 mg/L) despite of much lower biomass concentration in anoxic MBR [66]. It was reported that anoxic biofilm was stickier than aerobic biofilm and since anoxic biofilm was more uniformly distributed on membrane surface, it formed greater fouling resistance than aerobic biofilm [66]. In another study TMP increased faster in anoxic/aerobic MBR than aerobic MBR [67]. The particle size distribution in anoxic/aerobic MBR was found to be lower, which caused smaller particles to form a more compact cake layer on membrane surface providing higher resistance occurrence [67]. Being dominant microbial species in biocake at low DO concentrations (4.0, 2.0 and 0.5 mg/L), the species of *Rhizobiales* and *Paracoccus* belonging to *Alphaproteobacteria* subclass caused severe membrane fouling producing more EPS [68]. Two MBRs, in which DO concentration was set to 4.0 mg/L in one and 0.6 mg/L in the other, were operated in parallel. Low DO concentration (0.6 mg/L) caused filamentous microorganisms to be dominant and less membrane fouling than high DO concentration (4.0 mg/L) by increasing particle size distribution via flocculation of activated sludge [69]. This unusual finding was attributed to the formation of a looser and thinner layer by filamentous sludge.

Temperature

The role of temperature on biochemical reactions is clear. Many activated sludge components and even membrane itself are influenced by temperature changes which consequently affects membrane fouling directly or indirectly. It was observed that thermophilic conditions caused 5 – 10 times more membrane fouling than mesophilic conditions [70]. While a stabilized TMP curve was observed in a MBR operated at 20 ^{0}C, temperature reduction to 10 ^{0}C or elevation to 45 ^{0}C accelerated membrane fouling rate [71]. TMP increase in low and high temperature was attributed to elevated SMP production at these temperatures [71]. Low temperature around 10 ^{0}C also increased EPS production and adversely affected membrane fouling [72] and filterability of activated sludge [73]. Another reason of elevated membrane fouling in low temperatures was reported to be resulted possibly from polysaccharides and/or submicron particles eroding from sludge flocs [74].

Aeration (air scouring) rate

Aeration rate was observed to be quite influential on membrane fouling rate. While the effect of aeration intensity on membrane fouling rate at various fluxes (5 – 25 $L/m^2.h$) was able to be neglected at high aeration rates, a sudden TMP increase was observed when aeration rate reduced from 1.5 to 1.0 m^3 per unit membrane area per hour **[**75**]**. Similarly, doubling air flow scouring the membrane surface provided substantial reduction in membrane fouling by extending filtration duration from 10 – 50 days to over 200 days **[**76**]**. Nonetheless, excessive aeration rate may cause even more fouling. Although elevation of aeration rate from 400 L/h to 800 L/h reduced cake layer accumulation on membrane surface, it caused breakage of flocs and distribution of colloid and solutes previously adsorbed on the sludge flocs to the bulk solution, which resulted in a denser cake layer formation and accordingly, a greater specific cake resistance **[**77**]**. It was observed in another study that the sludge accumulated on membrane surface decreased with increasing air discharge (0 – 25 L/min). However, this effect did not increase linearly with aeration discharge and it became effectless when critical aeration rate is exceeded **[**41**]**. Consequently, lower and higher aeration rates than optimum rate promotes permeability reduction in membrane filtration in MBRs **[**78**]**.

2.2.1.3 Membrane properties

Membranes having one or more different properties were used in MBR studies. For example, the fouling potential of membranes having both different material and pore size were compared in the same study. Since it is not possible to isolate each property separately, the stressed property was reported under proper title.

Membran material

Although PAN, PVA and PTFE are also used, because of their strength and chemical durability PVDF, PP, PE and PES materials constitute the main materials used in the production of commercial MBR membranes. Great majority of these materials are relatively hydrophobic and they are rendered more hydrophilic adding wetting agents such as polyvinylpyrrolidone and methacrylates in the course of production.

PAN membrane was fouled more than PVDF membrane, although both were submerged in the same MBR [**79**]. Though reversible fouling was the predominant fouling type in both membranes, the irreversible fouling of PDVF membrane was greater. The effect of SMP component on the fouling of these membranes was also

different. While hydrophobic part of SMP had a greater share in fouling of PAN membrane, neutral hydrophobics had higher fouling potential for PVDF membrane [79]. The fouling potential of four membranes made of different materials (CA, PES, ME ve PC) was determined by filtering activated sludge through a crossflow filtration cell [80]. ME membrane exhibited the best performance giving the highest flux in steady-state followed by PC, PES and CA, respectively [80]. The fouling potential of three membranes PAN (0.035 μm, the most hydrophilic), PVDF (0.200 μm) and PTFE (0.220 μm, the most hydrophobic) was investigated submerging the membranes in the same MBR. It was observed that the greatest and the most hydrophilic proteins accumulated on PAN membrane while the smallest and the most hydrophobic proteins accumulated on PTFE membrane. It was determined that small proteins caused fouling in membranes having large pores (and vice versa), and the most hydrophobic membrane collected greater amount of proteins [81]. Three membranes made of different materials (PETE, PCTE and PTFE) but having the same pore size (0.1 μm) were used in the same MBR to investigate their fouling potential [82]. It was observed that while PETE membrane having the lowest pure water flux was the fastest fouled membrane, PCTE was the least fouled membrane. On the other hand, after two physical and chemical cleaning periods all three membranes exhibited a similar fouling pattern in the third usage. This was attributed to chemical cleaning, which changed probably the surface properties and porosity of the membranes [82].

Pore size

The steady-state fluxes of ME and PC membranes were reported to increase [80] together with larger pore size (0.10 μm – 0.40/0.45 μm). In contrast, the flux of 0.1-μm PES membrane was greater than fluxes of the same membrane having 0.22 μm and 0.45 μm pore sizes. This was attributed to the closure of greater pore-sized membranes by colloidal material. It was also seen that the flux of 0.22-μm CA membrane was greater than the flux of 0.45-μm membrane made of the same material [80]. In parallel operation of four MBRs made of different pore-sized (0.080, 0.100, 0.200 and 0.300 μm) ceramic membranes it was seen that the membrane having the greatest porosity and the greatest pore size concurrently was the most fouled membrane and vice versa [83]. It was stated that the membrane surface properties were the dominant factors determining the fouling potential of the

membranes [83]. In a membrane fouling study [84] performed with model alginate (100 – 190 kDa) solution in a cross-flow filtration system, it was observed that the initial fouling resistance of 30-kDa PES membrane was greater than that of 100-kDa PES and 0.22-μm PVDF membranes. It was also seen that the fouling rate of 30-kDa membrane was greater than those of other two membranes having greater pore sizes. It was concluded that the rate of resistance increase due to pore blocking or cake layer formation observed in membrane having smaller pore size in proportion to the size of the foulant was greater than the rate of resistance increase due to pore closure observed in membrane having greater pore size in proportion to the size of the foulant [84].

Hydrophobicity

It is expected the membrane fouling in hydrophobic membranes to be greater than that in hydrophilic membranes due to interactions between solutes, EPS resulting from microbial cells and membrane material [3]. Yet, since different additives are added to main membrane material in the production stage, a comparison of membranes according to their main material becomes somewhat irrelevant [3]. Nevertheless, there are many studies about the effect of hydrophobicity on membrane fouling. The performance of two membranes having the same MWCO (30 kDa) was compared to each other by filtering different samples taken from activated sludge under different physiological states in a stirred cell. It was observed that treatment performance of hydrophobic membrane was better despite that its fouling was more [85]. In another study it was observed that the most hydrophobic proteins were accumulated on the most hydrophobic membrane PTFE and the most hydrophilic proteins were accumulated on the most hydrophilic membrane PAN in a comparison of PAN (0.035 μm), PVDF (0.200 μm) and PTFE (0.220 μm) membranes having, respectively, contact angles of 58°, 81° and 127° [81]. On the other hand, the result of a MBR study investigating the fouling potential of three membranes having the same pore size (0.1 μm) but made of different materials showed that hydrophobicity could not be an influential parameter on membrane fouling because of indifferent effluent composition in terms of hydrophobic, hydrophilic and transphilic substances' concentration, despite the fact that PTFE was less hydrophobic than the other two [86].

2.2.1.4 Influent wastewater properties

Influent wastewater characteristic is an important parameter in choosing membrane and operation type. It was observed that nutrient deficiency in influent wastewater reduced membrane fouling by %30 – 40 due to lesser EPS production **[**87**]**. Increasing influent COD concentration (650, 1000 and 1500 mg/L) caused a reduction in steady state fluxes of membranes having different pore size and made of different materials **[**88**]**. Increasing protein / carbohydrate ratio (P/C) ratios caused an increase in SMP production and membrane fouling increased by %18 and %34 when P/C ratios raised twice and four times, respectively **[**89**]**. In addition, Lower Ca^{2+} (0.026 mM) concentration than optimum (2.86 mM) in influent wastewater caused 11 times greater membrane fouling rate **[**90**]**. This was attributed to greater hydrophobic EPS attachment via bioflocculation in case of enough Ca^{2+} presence. In another study while membrane fouling increased with increasing MLSS concentration (0.09 g/L – 0.35 g/L) in an anoxic MBR fed with acetic acid, such a relationship was not observed in MBR fed with ethanol **[**91**]**.

The use of synthetic wastewater in lab-scale experimental studies may have somewhat different effects on membrane fouling, e.g. different microbial communities can develop **[**58**]**. However, the use of synthetic wastewater is also advantageous because a stable influent composition can be sustained during the whole period of experimental study.

2.2.2 Microbial polymeric substances

2.2.2.1 Definition and classification of microbial polymeric substances

Extracellular polymeric substances are comprised of microbial biopolymers such as polysaccharide, protein, nucleic acid, (phospo)lipid, and other polymeric compounds found at intercellular space of microbial aggregates. These substances are responsible for the cohesive forces which keep microbial aggregates together, i.e., biofilms, flocs and sludge **[**92**]**. Adhesion to surfaces, protection of biofilm stabilization, occurrence of protective layer against biocides and other harmful effects, water retention, sorption of nutrients from environment and performing enzymatic activities on disintegration of outside macromolecules can be enumerated among other functions of extracellular polymeric substances **[**93**]**. MPSs were divided into sub-categories such as soluble microbial products (SMP)/extracellular polymeric substances, (EPS)

[93], biopolymer clusters (BPC) [56], utilization associated by-products (UAP)/biomass associated products (BAP) [94]. Different terming or categorization of MPS caused a little confusion. It is seen that the same thing is sometimes called differently (e.g., free or soluble EPS/bound EPS instead of SMP/EPS) or depending on the method used, different definitions such as tightly bound EPS/loosely bound EPS are used. These different names can be said to overlap in some points, e.g., soluble EPS and SMP state actually the same thing [93]. In addition, extraction of MPSs from activated sludge using various techniques and the measurement of extracted polymers by different methods revealed a lot of combinations. Because of these combinations, comparison of the results from different studies becomes more difficult. When different operational and other conditions used in MBRs are added to these measurements, the reason why a common point cannot be reached – although the effect of MPSs on membrane fouling has been one of the most investigated issues – becomes clearer. MPS cycle in activated sludge is shown in Figure 2.4.

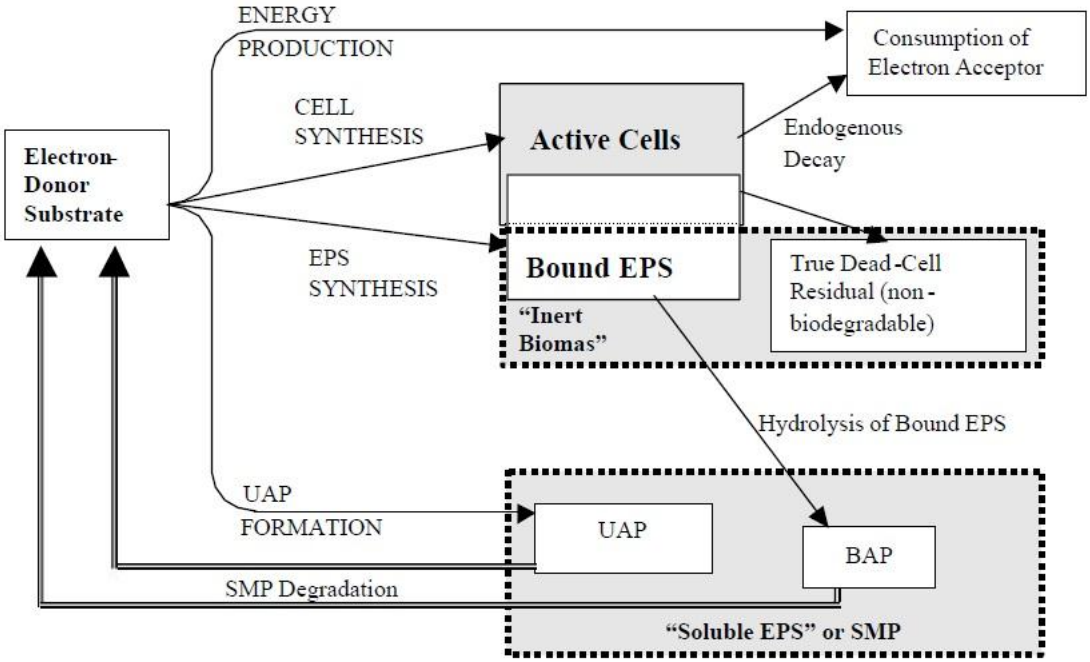


Figure 2.4 : Schematic representation of the unified model for active biomass, EPS, SMP, and inert biomass [93].

It is seen that while cells synthesize active biomass by electrons from electron donating substances, bound EPS and UAP occur concurrently in the process. While active biomass generates dead cell residuals by endogenous decay, bound EPS

converts to BAP via hydrolysis. Since UAP and BAP are biodegradable, they are used as recycled electron donors by active biomass at final stage [93].

Free EPS or SMP can easily be separated from activated sludge by physical methods, while harsh extraction methods should be used to extract (bound) EPS encapsulating the cell. In the following section information related to these extraction methods is given.

2.2.2.2 EPS extraction methods

There are several individual or combination of physical and chemical methods for extraction of polymeric substances encapsulating bacterial cells. These methods use centrifugation, heating, sonication, ion exchange, acid or base treatment according to their procedure. A successful extraction should cause minimal cell lysis and exopolymer disruption [95]. The efficacy of the extraction changes according to the method used. Even if the same method is used the results can be varied due to different intermediate operations such as sludge sample washing, centrifugation speed, and separation of extractant. Five extraction methods regular centrifugation, EDTA extraction, ultracentrifugation, steaming extraction and regular centrifugation with formaldehyde were compared regarding their EPS extraction efficiency of sludge samples from aerobic/sulfate reducing and nitrifying/denitrifying biofilm reactors [96]. Among five methods regular centrifugation with formaldehyde yielded the greatest amount of carbohydrate although the amount of protein was lower compared to other methods, which was caused by hindrance in detection of proteins linked by formaldehyde. In another study, a decreasing order of extraction efficiency was reported [97] for the methods formaldehyde-NaOH, EDTA, formaldehyde-ultrasound, cation exchange resin, formaldehyde, and control, respectively, for aerobic, acidogenic and methanogenic sludges. However, by comparing the amount of extracted EPS to the total roughly interstitial polymers estimated by confocal laser scanning microscopy (CLSM) measurement, it was reported that even the most effective extraction method of formaldehyde-NaOH was able to extract only approximately % 38 of the total EPS [97]. On the other hand, it was observed that in activated sludge flocs there were different EPSs associated with distinct metal ions, which required different extractants for their extraction [98]. For example, CER (cation exchange resin) extraction targeted specifically Ca^{2+} and Mg^{2+} associated EPS while sulfide extraction had good correlation with Fe^{3+} concentration. This

shows that influent wastewater composition should be considered for more efficient extraction of EPS. Several methods including ultrasonication, dialysis, steaming, boiling, CER w/o formaldehyde and NaOH w/o formaldehyde were investigated regarding their EPS extraction efficiency **[**99**]**. It was found that alkaline treatment gave the highest carbohydrate extraction followed by CER addition, steaming and boiling. However, CER addition method was proposed for routine measurements of EPS due to easier separation of CER than other pure chemical extractants. A compilation of 75 data set from different studies in literature on individual EPS components showed that protein/carbohydrate ratio changed between 0.5 and 21.2 (only % 8 was below 1.0) which meant that unlike pure culture, EPS of activated sludge was composed mainly of protein **[**100**]**. This wide range of P/C ratio also suggests that different methods yield different amount of protein or carbohydrate, which results in changes observed in ratios. For example, addition of formaldehyde along with CER caused a decrease of P/C ratio from 3.8 to 2.6 with unproportional decrease in the concentration of both polymers **[**99**]**.

Among all EPS extraction methods three of them have been mostly used by researchers, which are thermal [56,101-106], formaldehyde – NaOH [25,107-109] and cation exchange [47,73,77,95,110-115] methods. Actually a few of studies compared different extraction methods and each reported their arguments to justify their preferred method. For example, a study **[**97**]** proposing the method of formaldehyde-NaOH was adopted by many other studies. In this study it was reported that formaldehyde-NaOH method yielded the greatest extraction while resin method was in the third rank. Nonetheless, the extraction conditions used in resin method were mild extraction conditions which were 600 rpm stirring speed and one hour of extraction time instead of 900 rpm and two hours suggested by Frolund et al. **[**95**]** for better extraction. In contrast, three hours of extraction for formaldehyde-NaOH and EDTA methods were used, and consequently, formaldehyde-NaOH method was chosen as the best method. On the other hand, opponents of thermal extraction methods were concerned about either cellular disruption or protein denaturation, which could cause increased and decreased protein concentrations, respectively. A significant decrease in protein concentration was reported after 10 min steaming, which was attributed to protein denaturation rather than cell lysis **[**99**]**. Moreover, in the same study it was observed that formaldehyde pretreatment decreased protein concentration by nearly 50 % in NaOH and resin extraction

methods. Since there is no standard method with its well defined intermediate operations, it becomes always very difficult to compare different studies, and often contradictory results are reported.

2.2.2.3 Measurement of microbial polymeric substances

It was reported that EPS components of DNA and uronic acids constituted no more than %5 of total EPS amount [110]. Since protein and carbohydrate are the major components of polymeric substances in activated sludge, they are measured more commonly than other components. The literature on the measurement of protein and carbohydrate in wastewater treatment applications is summarized below.

Protein measurement methods

Lowry and Bradford methods are two commonly used methods in protein concentration measurement of wastewater samples. These two methods were compared to each other regarding their measurement performance in wastewater applications [116]. It was reported that Bradford method was not suitable for wastewater application because of its poor capability in particulate protein dissociation. In addition, proteins to be measured by Bradford method should contain at least 8 – 9 peptide bonds. Another serious disadvantage of Bradford method was that it developed only 50 % of the color developed for bovine serum albumin, which was normally used as standard. Although there are many interfering substances in Lowry method, their concentration in wastewater was said to be in tolerable limits [116]. However, the risk of interference from humic substances should be considered [116]. Since the main disadvantages of Bradford method are not present in Lowry method, it has been used more commonly in wastewater applications. In case of high concentrations of humic substances, the interference can be corrected [95].

Carbohydrate measurement methods

Phenol [117] and anthrone [118] methods are two commonly used methods in measurement of wastewater carbohydrate concentration. It was reported that anthrone method developed higher color intensities for hexoses than for pentoses and heptoses, accordingly, the results of this method had to be used carefully [116]. However, it was also notified in the same study that the dominating carbohydrate sources in wastewater were starch- and dairy products, vegetables, fruit and cellulose having a monomeric composition of glucose, fructose and galactose, all being

hexoses. For this reason, the anthrone test was reported presumably not to cause significant errors for measuring carbohydrates in wastewater. Phenol method was observed to show very poor precision in wastewater carbohydrate measurements [116]. This was attributed to inadequate mixing during reaction.

2.2.2.4 Factors affecting MPS production and degradation

Mostly the factors affecting the production and removal of MPSs were examined in investigation of the relationship between membrane fouling and MPSs. If a change in MPS concentration coincided with a change in membrane resistance, then a relationship between membrane fouling and MPSs was assumed to exist. The factors influential on MPS production/degradation are SRT (or F/M ratio), HRT – flux – OLR, pH – temperature – other stress conditions, aeration intensity/DO concentration, flocculant – coagulant – adsorbent addition, microbial structure, influent wastewater composition, nutrient level (growth phase) and reactor type – configuration. A summary of the studies related to these factors are given below.

Sludge retention time (or Food to biomass ratio)

It was reported that increasing SRT (10 – 40 d) caused an important reduction in SMP concentration [46,119,120]. However, further increase of SRT to 60 d changed SMP concentration slightly [119,121]. The existence of an optimum SRT, which made total SMP concentration minimum, was also reported [121]. As SRT raised UAP concentration decreased, in contrast, BAP concentration increased due to higher biomass concentration. Therefore, sum of UAP and BAP, which constituted SMP, became minimum [121]. In a MBR fed with primarily treated municipal wastewater, the effects of SRT (10, 15, 20 and 33 d) on composition and amount of EPS and SMP were investigated [44]. It was reported that 20-d SRT was a threshold value, and while SRT was influential on EPS and SMP below this value, longer SRT values had no effect on production/degradation of these substances [44]. In an anaerobic MBR the greatest MLSS concentration and SMP production were observed at infinite SRT (30, 60 and ∞ d) and low HRT (8, 10 and 12 h) [45]. On the other hand, excitation-emission matrix (EEM) fluorescence spectroscopy examinations showed that SRT was also influential on SMP composition. While protein-like substances were predominant at low SRT (17 d), humic acid-like substances became dominant at high SRT (102 d) and substances of mixed characteristics were dominant at 51-d SRT [122]. It was observed in another study

that 40 times more protein and 4.8 times more carbohydrate accumulated at lower SRT (23 and 40 d) [73]. SRT affects also floc size [43]. Lowering of SRT from 40 d and 20 d reduced also mean floc size from 135 μm to 91 μm and the proportion of high molecular weight SMP (>100 kDa) [43]. In addition, it was reported that a great portion of SMP was hydrophobic (determined by column chromatography) and the proportion of this hydrophobic part would increase with increasing SRT. While it was reported that increasing SRT (10, 30 and ∞ d) elevated EPS production per biomass [31], in another study EPS concentration was reported to be the greatest at the lowest SRT and to decrease with increasing SRT either due to lower production or higher consumption [47].

Hydraulic retention time, flux and organic loading rate

It was stated that lowering HRT caused more membrane fouling due to increasing biomass and SMP production [45,64,110]. While decreasing HRT from 10 to 7 h did not cause a change in EPS concentration, it clearly increased SMP concentrations and caused considerable elevation of membrane fouling rate [110]. For this reason, it was stated that SMP concentration was closely related to membrane fouling and it played a key role in estimation of activated sludge's fouling potential [110]. Three MBRs were operated in parallel at sub-critical (13.6 $L/m^2.h$), critical (49.1 $L/m^2.h$) and sup-critical (81.8 $L/m^2.h$) flux conditions. Interestingly, it was observed that SMP protein concentration of critical- and sup-critical-flux MBRs was lower than that of sub-critical-flux MBR in spite of greater MLSS, volumetric OLR and F/M due to lower HRT in the former two MBRs [59]. On the contrary to this strange situation observed at SMP, the rank of the EPS concentrations in MBRs followed the same rank of membrane fouling observed in MBRs. Accordingly, the amount of EPS was reported to be a good indicator of membrane fouling potential [59]. In another study [32] EPS production decreased with increasing OLR (0.57, 1.14 and 2.28 g COD/L.d). This was attributed to lowering DO (6.9, 5.4 and 0.6 mg/L) concentrations due to higher MLSS observed at higher OLRs [32].

pH, temperature and other stress conditions

As it was mentioned in Section 2.2.2.1, one of the functions of polymeric substances produced by microorganisms is to provide a shelter against stressful environmental conditions. When they face with inappropriate environmental conditions microorganisms produce more EPS for their protection. For example, a study

investigating the effect of stress conditions starvation, high salinity (%5), toxicity (50 ppm $CrCl_3$), low pH (pH=4) and high temperature (55 ^{0}C), which are able to be faced with in wastewater treatment, on SMP production showed that all stress conditions but starvation elevated SMP production **[**123**]**. Moreover, it was observed that different stress conditions were responded in different ways. For example, it was determined that SMP produced in response to high temperature was hydrophilic; while SMP produced in low pH condition was hydrophobic. While microbes facing with high salinity and low pH conditions produced protein- and amino acid-like components, polycarboxilate-type humic acid-like and polysaccharide-like substances were produced at higher salinity, toxicity and high temperature conditions. The effect of stress conditions on SMP production was in the following order: 55 ^{0}C > %5 NaCl > pH=4.0 > 50 ppm $CrCl_3$ > starvation **[**123**]**. Shock pH conditions (8.0, 9.1 and 10) were also reported to increase BPC in an anaerobic MBR. It was observed that especially at high pHs PSD reduced due to floc fragmenting **[**124**]**. An increase in SMP production was observed when temperature was declined from 23 ^{0}C to 17 ^{0}C **[**113**]**. In monitoring a full-scale MBR plant the greatest macromolecular peak intensity was observed at low temperature (~9 ^{0}C), and the peak intensity measured at higher temperatures (~20 ^{0}C) was lower up to %70 than that at low temperatures **[**125**]**.

Aeration intensity/Dissolved oxygen

Aeration in submerged MBR systems performs three functions: providing DO for biomass, surface cleaning by forming a shear stress on membrane surface and mixing reactor content for getting a homogeneous mixture. On the other hand, when it has reached to extreme conditions, aeration increases SMP/EPS production creating a stress effect. High aeration rates cause EPS release by adversely affecting the floc formation. Specific EPS concentration increased from 167 to 238 mg VS/g SS when sludge was exposed to elevated shear stresses (0, 112, 146 and 292 s^{-1}) in a MBR **[**72**]**. Aeration changes also microbial structure of activated sludge. While not observed in low aeration rates (420 L/h), *Aeolosoma hemprichi* ve *Tubificidae* worms proliferated at high aeration rates (630 L/h) and caused reduction in sludge production, elevated SMP production, recovery of settling ability and impairment of dewaterability **[**126**]**. High aeration rate (0.6, 0.8 and 2.2 m/s) increases also

membrane fouling resistance by increasing high molecular weight SMP (30 – 50 kDa) [127].

DO concentration affects activated sludge properties and membrane fouling. It was observed that lowering DO concentration (0.5, 2.0 and 4.0 mg/L) caused substantial increase in EPS protein and carbohydrate concentrations [68]. While there was no relationship between SMP carbohydrates accumulated in biocake and reactor's DO concentration, SMP protein concentration was observed to decrease with declining DO. Interestingly, although the correlations of DO and fouling rate with SMP protein and carbohydrate components were low, they gave a high correlation with P/C ratio [68]. Changing DO concentrations cause also biofilms having different properties from each other. In parallel operation of an aerobic (DO > 6.0 mg/L) and an anoxic MBR (DO < 0.3 mg/L) MBR, it was observed that stickiness of biofilms formed on membrane surface differed from each other [66]. While flocs formed under aerobic conditions were more coherent and the adsorptive forces between flocs and membrane surface were weak, inter-floc connection forces at flocs formed under anoxic conditions were relatively weak, yet the adsorptive forces between flocs and membrane surface were stronger. It was stated that although there was no enough information to completely explain the difference in stickiness of the biofilms formed under different DO conditions, this was able to result from properties of EPS formed under different conditions rather the amount of EPS because more EPS was produced under aerobic conditions than anoxic conditions [66]. Under different DO and NO_3 concentrations, it was observed that EPS uptake increased with raising DO concentration (0.5 – 2.5 mg/L) [128]. In contrast, EPS release was even observed rather than uptake under lower DO concentration than 0.5 mg/L. It was stated that EPS uptake reduced more especially at low DO concentration if nitrate concentration approached to zero at the same time [128].

Flocculation, coagulation and adsorption

When Chitosan and $FeCl_3$ were added in optimum ratio, while SMP concentration of activated sludge reduced by %25 and %23, EPS concentration increased by %18 and %31 [39]. Continuous addition of flocculants (cationic polyelectrolyte and $FeCl_3$) to a jet-loop MBR at optimum ratio though did not cause any change in SMP and EPS concentrations, it reduced membrane fouling by increasing steady state flux of various membranes made of different materials and having different pore sizes [129].

While 0.7 d was enough for control MBR to reach 30 kPa TMP, it took 5.2 d for flocculant (MPE) added MBR to reach to ultimate TMP. This showed that membrane filtration performance of flocculant added MBR was 7.4 times higher than that of control MBR. However, it was reported that membrane filtration performance did not always increase with increasing flocculant addition and that an optimum flocculant dosage were existed. Mean particle size was reported to increase from 70 to 210 μm with increased flocculant dosage, nevertheless it reduced again when further flocculant was added **[**38**]**. While SMP concentration in MBR was 19.4 mg/L, it reduced to 8.2, 9.0 and 11.2 mg/L after addition of $FeCl_3$, PACl and Chitosan **[**130**]**. Flocculant addition decreased the amount of small particles and colloids having size of 0 – 50 μm, and increased the amount of 50 – 100 μm sized particles. This was resulted in lower membrane fouling **[**130**]**. Seven MBRs including one for control were established to investigate the performance of six different flocculants (aluminum sulfate, ferric chloride, polyaluminum chloride, polymeric ferric sulfate, Chitosan, polyacrylamide) in membrane fouling reduction **[**131**]**. It was mostly observed that SMP concentration was reduced and concentration of EPS was increased with flocculant addition at increasing dosages. In Al^{3+} added MBR, excessive Al^{3+} addition increased SMP concentration. Organic polymers (chitosan and polyacrylamide) added provided the occurrence of flocs greater in size and having lower fractal dimension which showed disorderliness of floc structure. Among inorganic flocculants Fe^{3+} salt was observed to be more effective than Al^{3+} salt **[**131**]**. Various adsorbents used in MBR also reduced membrane fouling significantly by reducing the concentration of polymeric substances. PAC, zeolite and Moringa Oleifera (a very valuable plant known since ancient ages in Africa and South Asia regions) addition to a MBR treating effluent wastewaters of a palm oil mill caused % 43 – 85 reduction in membrane fouling decreasing SMP by % 58, 42 and 48, respectively, in short term experiments (120 min) **[**132**]**. In a submerged MBR operated under flux of 10 $L/m^2.h$, while TMP was around 23 kPa before PAC addition, it reduced to around 10 kPa after PAC addition at a ratio of 1.67 g/L **[**133**]**.

Microbial structure

It was observed that predominant microbial community in activated sludge affected the production of microbial polymeric substances and membrane fouling. In a study investigating how sludge bulking affected filtration properties, significant differences

were observed between EPS production of bulking and normal sludges [33]. More protein and carbohydrate production was observed in bulking sludge, and it was observed that % 65 – 70 of EPS was composed of proteins. This excessive EPS production in bulking sludge was reported to result from a probable shift of microbial structure towards more EPS producing nocarida form filamentous bacteria [33]. In another study monitoring a full-scale MBR, it was reported that increasing EPS production at cold seasons could result from concurrent microbial population shift towards nocardia form bacteria [73]. It was stated that due to excessive filamentous bacteria, approximately 3 – 4 times more EPS production was observed at bulking activated sludge than that at normal sludge, and proteins constituted the main part of the EPS produced [34]. Excessively growing filamentous microorganisms caused not only more EPS production but at the same time also increased membrane fouling by decreasing zeta potential and increasing relative hydrophobicity [35].

Influent composition

In a tubular packed bed reactor combined with membrane separation, it was observed that SMP production increased two folds when influent COD concentration was raised from 150 to 600 mg/L. Further increase of COD concentration to 1200 mg/L, caused SMP production to increase 8 folds [134]. Increasing influent P/C ratio caused more membrane fouling resistance because of smaller floc size resulting from lowering EPS protein and carbohydrate concentrations. SMP protein and carbohydrate concentrations increased, respectively, 2.5 and 1.5 times when P/C ratio raised from 2 to 8 [89]. In an anaerobic sequential biofilm reactor 23.6, 13.3, 9.0 and 1.4 mg EPS were produced per g carbon of influent feed glucose, soybean oil, fat acids, and meat extract, respectively [135]. Presence of toxic substances in influent also affects SMP production. Injection of chloroform and chromium (+6) caused 4 and 10 times more SMP accumulation in an anaerobic reactor fed with glucose. The increase of SMP was proportional to the concentration of toxic substance [136].

Nutrient level (Growth phase)

Activated sludge reactors operated at different regimes (log, declining, stationary and endogenous) in practice cause growth of microorganisms under different physiological states [87]. These conditions alter EPS production and membrane fouling. For example, it was observed that the amounts of EPS produced at log

growth and endogenous phases were, respectively, 202 and 271 mg VS/g MLSS. EPS production was observed to considerably increase at endogenous phase [87]. Nutrient level was observed to be influential also on SMP production [137]. While substrate was being used, SMP concentration increased continuously, when it was exhausted, SMP accumulation rate decreased because SMP production was resulted from only microbial decay. Consequently, it was reported that SMP concentration was determined by nutrient concentration in all tests [137]. Nutrient level also affected molecular weight of SMP [138]. While BAP produced at endogenous phase was composed of macromolecular cell fragments having molecular weight of above 4000 kDa, BAP formed at microbial growth phase had molecular weight below 1200 kDa. For this reason, formation rate, chemical structure and biodegradation kinetics were expected to be different for these different molecular-weight SMPs produced [138].

Reactor type and configuration

It was reported that SMP production in a fixed-bed biofilm and a conventional activated sludge system differed from each other. Although there were great differences in SRT and MLSS concentrations (SRT = 300 d vs. 15 d in conventional system, MLSS =11000 vs. 3000 mg/L in conventional system), it was reported that membrane fouling was much lower in fixed-bed biofilm reactor due to much lesser (%71) SMP production [139]. In comparison of a conventional and a simultaneous nitrification and denitrification (SNDN) MBR, it was observed that SMP and EPS concentrations and relative hydrophobicity of EPS were higher in SNDN MBR [140]. It was observed that the concentrations of soluble carbohydrate, protein and humic substances were higher in MBR than those in conventional biomass separation system. The difference in concentrations was increased when OLR was raised [110].

2.2.3 The relationship between microbial polymeric substances in activated sludge and membrane fouling

There are two opposite ideas regarding the effect of microbial polymeric substance's concentration in activated sludge on membrane fouling. One of them claims that there is no relationship between MPS concentration in activated sludge and membrane fouling, and the other is at opponent side. Both of these ideas are based on their experimental data. Studies related to both ideas are summarized below.

Filterability test of samples, which was determined by Delft Filtration Characterization method, taken from a full scale plant at different times showed that there was no relationship between SMP concentration and filterability [13]. It was reported that EPS and SMP concentrations in MBR could not be the cause of TMP increase because although TMP increased from 18 to 32 kPa in 24 h, there were marginal changes at EPS and SMP concentrations in MBR, which was operated under super critical flux conditions [141]. In three systems, namely lab-, pilot- and full-scale, no relationship was observed between bioreactor's polysaccharide concentration and membrane fouling rate [142]. Although SMP concentration was significantly reduced by different coagulants, since no effect of this reduction was observed in TMP development, it was concluded that SMP was not always able to be correlated with membrane fouling [143]. No correlation was observed between polysaccharide concentration and membrane fouling rate for 15-d SRT although a very good correlation was observed for 8-d SRT [113]. In a MBR planned to operate under constant flux conditions, constant flux was changed several times due to instability problems. At the end, such a permeability graph was obtained that even the authors stated that making a conclusion from this graph was very difficult. Even so, it was concluded that no relationship was observed between SMP or EPS and membrane fouling [144]. Since SMP and EPS concentrations did not change when SRT was greater than 20 d and only %10 of TMP recovery was achieved, it was reported that there was no effect of EPS on membrane fouling [44]. In contrast, SMP and ESP had a significant effect on membrane fouling when SRT was lower than 20 d [44]. According to filtration index FI15, which was the ratio of sludge's permeability over that of pure water and measured at a stirred test cell under 10 kPa pressure, there was no relationship between polysaccharide concentration and membrane fouling [125]. It was reported that either FI15 was not a suitable test for measuring membrane fouling or other parameters of full-scale plant prevented exposing this relationship [125]. TMP, in other words membrane fouling, continued to increase although SMP (suspended EPS) concentration decreased in a long term (around 70 h) measurement campaign. Accordingly, it was concluded that SMP concentration alone in MBR was inadequate to clarify TMP development [57]. When MPS content of sludges taken from five different full-scale MBRs were compared with their respective MBR's permeability at the day of sampling, a relationship

between membrane fouling and SMP or EPS was not observed except for a weak correlation between SMP carbohydrate and membrane fouling [26].

In contrast to the studies in previous paragraph reporting no connection between membrane fouling and MPS concentration, there are also other studies reporting the existence of a relation. In the following studies membrane fouling was directly attributed to MPSs. Severe membrane fouling was attributed to elevated SMP concentration [65]. Increasing SMP elevated membrane fouling significantly [145]. The reason of high resistance resulting from pore closure was high molecular-weight SMP retained by membrane [89]. EPS played a significant role in membrane fouling and can be taken as an index showing membrane fouling [32,72,87,146]. Fouling potential was directly correlated with SMP composition and concentration [147]. A considerably high negative correlation was observed between critical flux value and SMP concentration [20]. SMP exhibited a very high negative correlation with membrane fouling [131]. As SMP concentration increased, filtration index showing membrane fouling declined [148]. Carbohydrate concentration of SMP was reported to be the main indicator of membrane fouling [140,149], and have a strong correlation with critical membrane flux [19], very high correlation with filtration resistances [150], a linear correlation with specific cake resistance [151], the highest correlation with fouling rate [14,152] and a negative correlation with permeability [103]. Protein concentration of SMP [153] and EPS [16] was also reported to play an important role in membrane fouling. On the other hand membrane fouling was also attributed molecular weight distribution of polymeric substances. SMP fraction having size of 10 – 100 kDa was the most important fraction causing membrane fouling [154]. Membrane fouling was directly related to high molecular-weight SMP (> 10 kDa) [155]. SUVA, EEM, rapid resin fractionation and HPSEC-UV-DOC analyses of feed and permeate showed that high molecular-weight hydrophilic SMP (40 – 70 kDa) was the main cause of membrane fouling [156].

2.2.4 The relationship of foulants accumulated at membrane with fouling

Considering only the parameters measured at MBR's mixed liquor may be misleading in terms of membrane fouling. For example, in three MBRs operated under sub-, critic and sup-critical flux conditions EPS P/C ratio declined with increasing flux. In a first approach before further investigation, it was supposed that membrane fouling rate increased with increasing average amount of carbohydrate

[59**]**. In contrast, the analysis of biofilm accumulated on membrane surface with CLSM showed that protein accumulated on membrane surface was actually higher than carbohydrate. It was consequently inferred that analysis by CLSM gave more accurate result in characterization of membrane fouling than the parameters measured at MBR sludge, because membrane itself was examined in the former situation. This shows how much the examination of membrane itself rather than activated sludge is important in investigating the reasons of membrane fouling. A similar inference was made in another study **[**68**]**. There are many studies indirectly proving membrane fouling to be closely related to activated sludge components accumulated at membrane. The effects of MBR operating conditions on membrane fouling were investigated in a MBR operated under different F/M ratio and fluxes **[**56**]**. TMP development versus time gave quite different curves for fluxes 0.4 and 0.08 m^3/m^2.d. While under higher flux conditions it took less than 5 d for TMP to reach 80 kPa, it took more than 20 d under lower flux conditions. Even so, since BPC concentrations were close to each other in both cases, previously different TMP curves converged when TMP was plotted versus cumulative permeate volume **[**56**]**. In another study normalized fluxes were plotted versus cumulative EPS delivered to membrane surface by filtration for better comparison of flux curves obtained from filtration of EPS solutions having different concentrations **[**111**]**. It was seen that different flux curves obtained by EPS solutions having different concentrations converged by this way **[**111**]**. Similar results were obtained in a pilot-scale MBR fed with real municipal wastewater. TMP curves converged when they were plotted against total filtered wastewater volume, and this convergence even increased when they were plotted against total organic matter loading **[**62**]**. It is also seen in an empirical equation developed for activated sludge that membrane flux decreases as a function of the amount of protein accumulated at boundary layer on membrane **[**2**]**. Actually in conventional filtration models (standard pore blocking, complete pore blocking and cake filtration) developed for constant pressure conditions, the cumulative volume of foulant solution filtered seems as a term in flux equations **[**157**]**. All these studies show that membrane fouling is related with the amount of total foulant filtered. Nevertheless, the complexity of MBR conditions and sometimes overlooking of controlled experimental conditions prevent this connection to be revealed.

It was seen from the previous paragraph that foulants accumulated at membrane was the reason of membrane fouling, which was indirectly inferred as a result of plotting TMP/flux curves against cumulative amount of foulants filtered. Nevertheless, taking into account only the amount of cumulative foulant filtered or permeate volume may not give always correct result. For example, the fouling at flux of 60 L/m^2.h was more than the fouling at flux of 50 L/m^2.h, although the same amount of permeate was collected **[**158**]**. However, since the same filtration/backwashing periods were used at both fluxes, it was observed that the amount of EPS extracted from the membrane operated under flux of 60 L/m^2.h was higher than that operated under flux of 50 L/m^2.h **[**158**]**. For this reason, the amount of foulants actually accumulated at membrane presents a better approach for membrane fouling. By this way the reasons of membrane fouling can be measured directly and more accurately. To able to do that firstly membrane should be cleaned by extracting the foulants, and then membrane fouling resistances should be measured. In the following, some studies regarding foulant extraction from membranes were cited. However, not all of these studies performed an analysis of extracted foulants and at the same time measured membrane filtration resistances after extraction to prove if actually the extracted material was the cause of membrane fouling. Although there are a few studies performing both of these tasks, only foulant extraction or only resistance measurement was done generally in majority of studies. While in some of these studies membrane integrity was spoiled because of destructive extraction techniques (cutting pieces from membrane or slicing the membrane) used, membrane integrity was preserved in studies using non-destructive techniques (extraction with pure water of chemical solutions). Foulants accumulated on the surface of membrane samples taken from a full scale MBR system were scoured with a plastic sheet and this material was denoted as external foulants **[**159**]**. Remaining foulants were extracted by pure water at 80 °C during 45 min, and these foulants were denoted as internal foulants. These extracted foulants were analyzed by EEM, GFC, FT-IR, SEM and EDX techniques. It was observed that both external and internal foulants were mainly composed of protein-like and soluble microbial by-product-like substances. In addition to these substances it was observed that oily substances and inorganic foulants such as Mg, Ca, Na, Al, K and Si were also accumulated at membranes **[**159**]**. In analyzing cake layer accumulated on membrane surface, which was rinsed with pure water, by a combination of photometric analyzes (Dubois and

Lowry methods) with EEM and NMR, it was observed that foulants accumulated actually on membrane surface were composed of biopolymeric biomacromolecules in size of 100 kDa – 0.45 μm and colloidal biomacromolecules having size of 0.45 μm – 10 μm. It was concluded that high concentration of these substances in supernant contributed directly to the formation of fouling layer on membrane surface **[**160**]**. After rinsing PAN and PVDF membrane surfaces with water, underlying gel layers were scoured with a plastic sheet (removable foulants), and remaining foulants were extracted soaking the membranes into a NaOH solution for 24 h (irremovable foulants) **[**79**]**. EEM analyzes showed that both membranes were fouled mainly (> %90 fouling resistance) due to soluble microbial by-product-like substances accumulated on their surfaces. While hydrophobic humic-like substances were the main components influential in irremovable fouling of PAN membrane, protein-like substances were responsible for irremovable fouling of PVDF membrane **[**79**]**. In another study foulants accumulated on membrane surface were extracted using 0.5 % (v/v) NaOH solution **[**161**]**. It was reported that the ratio of high molecular-weight (> 35 kDa) protein-like substances increased with extending filtration duration (from < 9 d to 20 d), and this was the main reason of blocking **[**161**]**. Foulants were extracted by rinsing, backwashing with pure water and soaking into NaOH solution for 24 h in a study experimenting various filtration modes **[**162**]**. It was observed that a mixing filtration mode comprising of 120-sec high flux (60 $l/m^2.h$) at initial filtration period followed by normal operation at a low flux (10.3 $l/m^2.h$) for 290 sec gave the lowest TMP increase after 24 h operation. By this operation regime, a less compact cake layer formed, and less SMP accumulated on membrane surface **[**162**]**. In analyzes of cake layer scoured with a spatula quite important findings were obtained **[**163**]**. It was observed that organic matter accumulated at cake layer sludge was not SMP because % 70 of this matter was greater than 0.4 μm and accordingly it was termed as biopolymer cluster (BPC). Because of these BPCs the specific resistance of cake layer accumulated on membrane was more than thousand times greater than that of activated sludge. It was observed that specific protein, carbohydrate and humic acid concentration in cake layer was three times higher than those in activated sludge, and for this reason it was stated that cake layer formation was the main cause of membrane fouling in submerged MBRs used for wastewater treatment **[**163**]**. In an anaerobic MBR it was observed that proteins accumulated significantly more than carbohydrates on membrane the surface by examination of membrane surface with

CLSM [164]. PSD analyze showed that the ratio of smaller particles than 5 μm in cake layer accumulated on membrane surface was %18.5, while this ratio was %3 at MBR sludge. This showed that the tendency of small particles to accumulate on membrane surface was greater [164]. It was indicated that membrane fouling depended on the amount of sludge accumulated on the membrane surface. The more sludge accumulated on the membrane surface, the more membrane filtration resistance increased [165]. Three MBRs were operated in parallel to investigate the role of PAC in mitigating membrane fouling [166]. It was seen that the amount of EPS extracted from membranes was increased linearly with total permeate volume obtained [166]. It was observed in an external anaerobic MBR that membrane fouling resistance exhibited a strong logarithmic relationship with the amount of total EPS carbohydrate [54]. When foulants accumulated in HF membrane in a full-scale MBR plant were extracted, it was seen that the rank of fouling matter accumulation was humic acid, carbohydrate, uronic acid and protein in decreasing order [167]. While the ratio of uronic acids in sludge supernant and EPS was % 1 – 3 (w/w), it increased to %10 in foulants accumulated at membrane [167]. When UV-visible absorbance of foulants accumulated on membrane surface was compared with those of SMP and EPS, it was seen that foulants accumulated on membrane surface was mainly resulted from SMP of activated sludge [110]. In an industrial MBR fed by diary wastewater it was seen that the amount of carbohydrate accumulated in gel layer increased linearly with increasing fouling amount [107]. In parallel operation of two pilot-scale MBRs 40 fold and 4.8 fold more EPS protein and carbohydrate accumulated at 23-d SRT than those at 40-d SRT, respectively. Nevertheless, although fibers taken from MBR of SRT 23-d were covered with sludge and white fibers were able to be seen at MBR of SRT 40-d, there was no significant difference between their permeability [73]. Biofilms accumulated on membrane surface in two MBRs (aerobic and anoxic) were collected and analyzed to investigate the reasons of membrane fouling [66]. Membrane fouling was observed to increase with EPS carbohydrate accumulated on membrane in aerobic MBR. Nonetheless, although EPS carbohydrate accumulated on membrane of anoxic MBR was less, its fouling resistance was more than that of aerobic MBR. This contradiction was further examined by analyzing EPS carbohydrate distribution on membrane surface of both MBRs, and it was reported that the reason of higher fouling in anoxic MBR was more uniform distribution of EPS carbohydrate along membrane surface [66].

Nevertheless, although the stickiness of biofilm attached to membrane of anoxic MBR was tested to be greater than that of aerobic MBR, protein concentration – which was able to be responsible for this difference – was not measured. Three MBRs were operated in parallel at various SRTs (17, 51 and 102 d) to investigate the reasons of irreversible fouling in membrane **[**122**]**. At the end of the study, after cleaning membrane surface with a sponge, remaining irreversible foulants were extracted and analyzed by both conventional and other SMP measuring methods (monosaccharide composition with EEM and HPLC). EEM analyzes showed that the greatest protein-like substances' accumulation was occurred at the least fouled membrane, and vice versa. Since EEM did not give information about carbohydrate-like substances, monosaccharide analyzes performed by HPLC showed no difference between MBRs operated at different SRTs in terms of carbohydrate accumulation at membranes except the finding that the least accumulation of Rhamnoz took place at the least fouled membrane. In contrast, conventional SMP analyzes showed a rank of carbohydrate accumulation in membranes at different SRTs, which was conformed completely to the rank of irreversible fouling observed at MBRs operated under different SRTs. Even so, conventional SMP analyzes were stated to be inadequate in investigation of the reasons of membrane fouling **[**122**]**.

2.2.5 Internal connections of some activated sludge parameters

In order that the results of an experimental study can be satisfying, it must be done under conditions as controllable as possible. This means that the number of independent variables should be minimized, and other parameters should be kept constant. Otherwise, it would not be possible to find the influential parameter(s) on the phenomenon among a lot of changing parameters. For example, temperature which has influence on chemical reactions can cause undesired effects on the results if it is not controlled. In a similar way, DO concentration in the bioreactor should be kept constant, if it is not considered as an independent variable. Internal connections of some activated sludge parameters which are generally considered as independent variables in experimental studies are shown in Figure 2.5. This graph shows the internal connections between biological parameters of activated sludge MLSS concentration – SRT – F/M ratio – OLR. Data and procedure used to prepare this graph are given in Section 3.10. The values at this graph are only valid at steady state conditions. In this graph when a SRT value was chosen from right hand axis, the

corresponding F/M ratio can be found going down to horizontal axis from interception point at F/M curve. The corresponding OLR to this F/M ratio can be read at left hand vertical axis according to chosen MLSS concentration. The required feed concentration can be calculated from this volumetric OLR. According to this graph, e.g., while it is possible to study at various MLSS concentrations keeping F/M (or SRT) constant, it is also possible to study at different SRTs (or F/M) keeping MLSS concentration constant at a predetermined value. In nearly all experimental studies in the literature MLSS increases (decreases) with increasing (decreasing) SRT. In contrast, the effects of, e.g., increasing (or decreasing) MLSS concentration on membrane fouling can be studied without changing SRT. As it is seen from Figure 2-5 the concentration of feed to MBR, namely OLR, should be increased in this case. If it is studied with synthetic wastewater, a feed that is more concentrate may be prepared, or in case of real wastewater since concentration is not possible, membrane area can be increased without changing membrane flux. By this way, the effect of various MLSS concentrations on membrane fouling can be studied without changing the most important parameter of biological process SRT and one of the most important parameters of membrane fouling, namely flux. In another case, e.g., the effect of increasing SRT on membrane fouling can be studied without changing MLSS concentration. In this case feed concentration (OLR) should be decreased to keep MLSS concentration unchanged as it is seen from Figure 2-5. In case of synthetic wastewater a more diluted feed may be prepared, or membrane area should be reduced for not to cause a change in flux in case of real wastewater feed. By this way as it is mentioned before, the membrane fouling study can be done under as controllable as possible conditions without changing the most important parameters of both membrane fouling and biological processes. The results of the experiment are expected to be more accurate, meaningful and conclusive in this way.

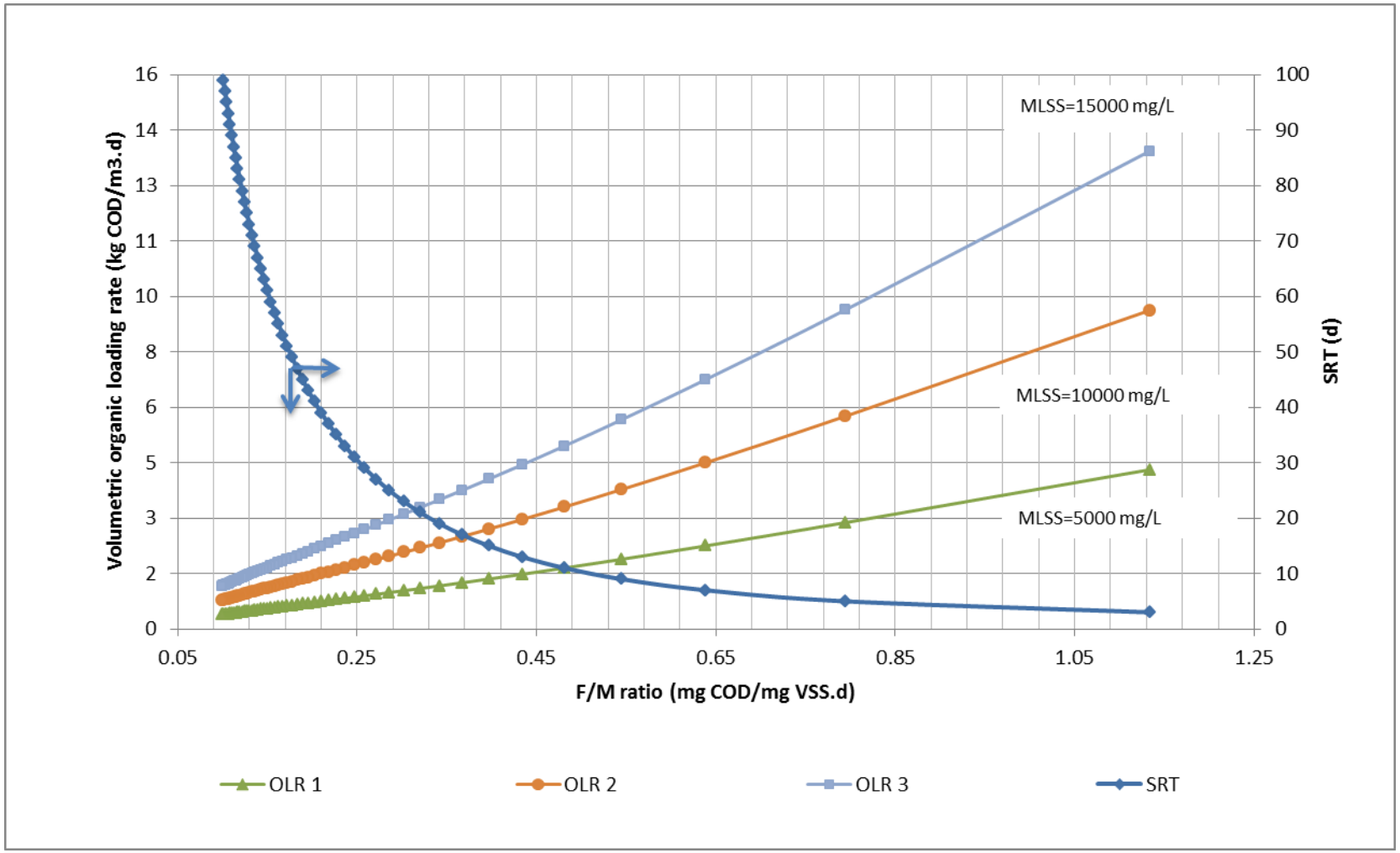


Figure 2.5 : Iso-MLSS curves.

2.2.6 The effects of experimental conditions on membrane fouling

Contradicting findings are frequently reported regarding the effect of any parameter on membrane fouling. The apparent contradictions among studies about the effect of MLSS concentration on membrane fouling were given in related section of 2.2.1.1. Similar to MLSS concentration, contradicting results were also reported for other parameters such as SRT, DO, microbial polymeric substances etc. It should be stated that some of these contradictions result, no doubtly, from inherent complexity of MBR phenomenon. For example, a specific MLSS concentration may cause more membrane fouling in case of inadequate aeration on membrane surface, while its effect may be marginal beyond a specific intensity of aeration. In Section 2.2.1 above, around 20 parameters impacting on membrane fouling were enumerated, nevertheless the number is not limited to this value, and it is not possible either measuring or controlling all these parameters. On the other hand, a scientific experiment should be done under controlled conditions to be able to be sure that a change taking place in the system is really resulted from a change in the parameter what is considered as an independent variable. Otherwise, if the changed parameter causes many uncontrollable changes in the system, in this case this experiment cannot be termed as a controlled experiment. It was observed that many studies examined in the scope of this thesis had problems in terms of controlled experimental technique. The following studies should be evaluated in light of Section 2.2.5.

In a study investigating the relationship between SRT and membrane fouling, SRT was changed between 10 – 60 d. Nevertheless, while MLSS concentration at 10 d-SRT was 2.9 g/L, it raised to 11.8 g/L at 60 d-SRT **[**121**]**. In this case it is very difficult to determine if the changes in fouling potential were resulted from SRT, MLSS or other parameters of activated sludge. Similarly, MLSS concentration increased from 7 to 18 g/L while SRT was changed between 30 – 100 d **[**41**]**. In another study MLSS concentration elevated from 3.5 to 12.4 g/L due to F/M ratio raising from 0.2 to 0.8 kg COD /kg MLSS.d **[**23**]**. Similar examples are seen in other studies, e.g., [44,46,122,148,168]. It is seen that both SRT and MLSS concentration were increased concurrently in these studies investigating the effect of SRT on membrane fouling. In this case there is no option other than observing the combined effect of SRT, MLSS concentration, viscosity, DO and air scouring efficiency on membrane fouling. This is because when MLSS is allowed to increase with elevated

SRT, viscosity increases also, and DO and the efficiency of foulants scouring from membrane surface by aeration decrease at the same time. For example, three MBRs were operated in parallel to investigate the effects of various volumetric OLRs (0.57, 1.14 and 2.28 g COD/L.d) on membrane fouling [32]. Although MLSS concentration can be kept constant as it is seen from Figure 2.5, MLSS concentration was nevertheless allowed to change freely, and it finally became, respectively, 2.2, 4.0 and 8.4 g/L at steady state due to increasing OLR. Consequently DO concentrations measured in MBRs at steady state became 6.9, 5.4 and 0.6 mg/L for each OLR in increasing order. Conversely, it is quite possible to change, though not in the same direction, SRT – F/M ratio – OLR without changing MLSS/DO concentration, which is obviously seen from Figure 2.5. In addition, there are studies, e.g. [169], keeping MLSS concentration constant despite of changing SRT, which was ensured by adjusting HRT.

Another example of uncontrolled experimental conditions is related to unequal/dissimilar initial membrane conditions. Once a membrane is used under a set of conditions, the same membrane cannot be used under different conditions without proper cleaning. In other words, to compare membrane fouling under changing conditions, either new membrane should be used or the used membrane should be cleaned properly to get it back to initial state to use in another experiment. In a study investigating the HRT – membrane fouling relationship, HRT was reduced from 24 to 18 h [65]. Nonetheless, the membrane had been neither cleaned nor replaced before HRT was changed. No important changes were observed in TMP until nearly 20 days after changing HRT. TMP then started to increase. Even so, elevation of membrane fouling was attributed to HRT reduction [65]. Similarly, in a study [170] examining the main factors impacting on membrane fouling, influent COD concentration was doubled, and very naturally some changes were observed in many activated sludge properties. Nevertheless, the membrane used before COD elevation was continued to be utilized after COD increase without replacing or cleaning (this can be seen from TMP development). The previously fouled membrane at the first stage became more fouled at the second stage, and this was attributed to increasing MLSS concentration due to elevated OLR. The question of what the benefit of investigating the fouling potential of a membrane under changing experimental conditions without cleaning is remains to be answered. How the results obtained in this case can be evaluated. Similar problems were seen in another study [146] in

which SRT was set to 10 d, then changed to 40 d and finally reduced to 20 d. At times when SRT was changed, existing membrane was neither replaced nor cleaned, which is again obvious from TMP development. In such a case neither TMP graph can be evaluated nor can the role of SRT on membrane fouling be revealed. In another study investigating the factors affecting membrane fouling, four MBRs made of ceramic membranes were operated in parallel [83]. The membrane having the least surface roughness fouled the least, and vice versa. Yet, the membrane possessing the lowest surface roughness had also the smallest pore size. In this case it seems not possible to determine whether pore size or porosity was the main factor in membrane fouling, or which parameter influenced the fouling at which rate.

Unnecessary membrane flux changes constitute another example of uncontrolled experimental conditions in membrane fouling studies. When the flux is changed to vary another parameter, then the effect of this parameter on membrane fouling cannot be revealed because flux only is very influential on the membrane fouling development. For example, the changes taking place in activated sludge properties and the membrane fouling due to increasing volumetric OLR were investigated [110]. To raise OLR flux increment was preferred. In this case of concurrent changes in both flux and OLR, TMP development cannot be evaluated because the effect of OLR will be shaded by flux change. It is very obvious and natural that membrane fouls very much quickly in the second case where both flux and OLR increased together. Instead of this, flux should be kept constant (which is easily done by increasing membrane area, e.g., [73]) so that the effects of OLR on both activated sludge properties and membrane fouling can be determined. In another study [59] investigating the effects of critical, sub-critical and sup-critical fluxes on membrane fouling, substantial changes were observed at MLSS concentration and HRT as a result of increasing flux. Other components of activated sludge can also be said to have changed with increasing flux. This is a typical example of overlooking controlled experimental conditions. On the other hand, such an experiment can be done accurately preventing uncontrolled effects on experimental conditions. Since membrane area was kept constant, flux increment resulted in lower HRT (from 5.8 to 1 h), higher organic loading rate and elevated MLSS concentration. Conversely, it is quite possible to obtain critical, sub-critical and sup-critical fluxes by changing only membrane area. By this way, a more convincing experiment can be done without

changing either HRT or MLSS concentration. Similar problems were seen in similar studies [45,171] reporting more membrane fouling due to HRT reduction.

Another problem of membrane fouling studies is the transferability of experimental conditions and results. In a study investigating the effects of a toxic pharmaceutical on the properties of activated sludge and membrane fouling, a MBR made of 0.1 μm sized tubular ceramic membrane was operated in cross-flow mode **[**151**]**. Yet, the specific cake resistance of activated sludge and membrane fouling index were determined by a dead-end filtration apparatus made of 0.2-μm cellulose acetate flat sheet membrane. It is obvious that the fouling results obtained at the latter case cannot be used to explain the fouling at the MBR used. In another study **[**153**]** researching the effects of activated sludge components on membrane fouling operation type, membrane material and membrane pore size of the setup used for fouling index determination were all different from those of MBR. In two other studies [24,146] investigating the relationship between rheological and physiological properties of activated sludge and membrane fouling, a pilot scale MBR was operated at flux 22 $L/m^2.h$. A mini MBR was also established to determine membrane fouling rate. However, although material and pore size of the membrane were the same as those of main MBR, the flux used in mini MBR was 45 $L/m^2.h$. In this case the fouling propensity of the membrane used in mini MBR differs substantially from that of the membrane used in main MBR. Accordingly, it would not be possible to compare results obtained from MBRs operated under different conditions.

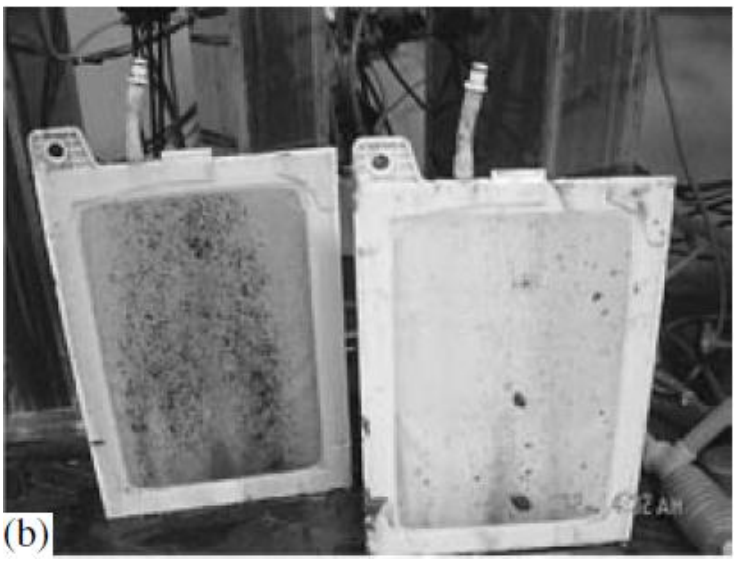
These uncontrolled experimental conditions observed in many membrane fouling studies in MBR were also mentioned in previous studies. For example, the results obtained from a study **[**172**]** investigating the effect of F/M ratio on membrane fouling rate were defined as ambiguous because different MLSS and fluxes were used with varying F/M ratios **[**49**]**. Therefore while changing F/M ratio between 0.34 – 1.41 g COD/g VSS.d (which corresponded to SRT of 2 – 10 d), MLSS concentration and membrane flux were kept constant at around 8 g/L and 30 $L/m^2.h$, respectively, for obtaining unambiguous results regarding the effect of varying F/M ratios on membrane fouling **[**49**]**. To avoid the same problem, in another study [**56**] F/M ratio was changed between 0.16 – 0.40 g COD/g MLSS.d, which corresponded to SRT change of ~15 – 30 d, keeping MLSS concentration constant at 12 g/L. In a study investigating the effects of varying DO concentrations on SMP – EPS

production in activated sludge and biocake and on membrane fouling, DO concentrations were set, respectively, to 4.0, 2.0 and 0.5 mg/L during the study **[**68**]**. At the times when DO concentration was changed, the membrane was chemically cleaned to provide approximately the same initial conditions to ensure controlled experimental conditions.

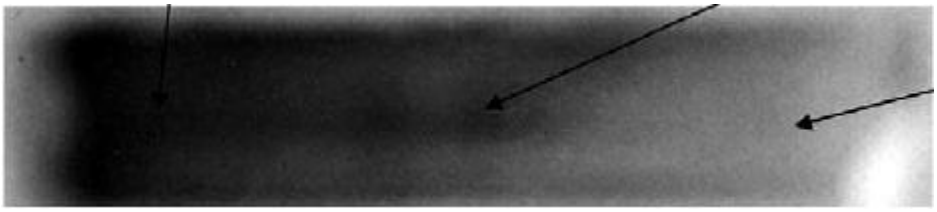
Because of inter-parameter relations in MBR, changing a parameter causes changes in a series of other parameters some of which cannot be controlled. When controllable part of parameters also is allowed to change freely, it would be inevitable to get highly contradicting results, as some of them were already cited above. This case actually prevents research energy to be canalized to a right direction. Experiments planned in a right way and performed under conditions as controllable as possible lead more quickly to the right solution of the membrane fouling phenomenon in MBR.

2.2.7 The effect of membrane module in membrane fouling studies

In some previous MBR studies it was observed that membrane surface fouled not uniformly along both horizontal and vertical directions [54,107,173]. In the former two studies membranes having approximately 28 cm^2 surface area were used. Even these membranes having so small area fouled non-uniformly. In addition, it was reported that hollow fibers fouled very differently depending on their position in module, age of the fibers and the position of the module at filtration line in a full-scale MBR **[**73**]**. Figure 2.6 shows how membranes used in some MBR studies fouled. Non-uniform fouling occurring at membranes can be seen very clearly. This situation resulting mostly from non-optimized air scouring causes a problem in controlled membrane fouling experiments because flux/TMP curve obtained in the case of uniform fouling of membrane surface would be different from that obtained in the case of non-uniform fouling. In other words, in case of non-uniform membrane fouling, a part of membrane is exposed to different conditions, which poses always an additional uncertainty to other independent/dependent variables. Accordingly, evaluation, defining the reasons of membrane fouling and modeling would be more difficult. This shows that membrane fouling studies in MBR should be done with sufficiently small modules to be able to do experiments under more controlled and better defined conditions.



(b)



(c)

(d)

Figure 2.6 : Non-uniform membrane fouling. (a) 35 L/m^2.h flux and >300 h operation, flat sheet membrane (External MBR) [54], (b) flat sheet membrane fouled at SRT = 25 and 250 d (Submerged MBR) [173], (c) Flux 7.9 – 18 L/m^2.h, 89-d operation time (Submerged MBR) [159], (d) 21 L/m^2.h flux, 65-h operation time [174].

2.2.8 MBR modeling studies

In MBR modeling studies membrane fouling or fouling resistance development is generally defined as a function of total permeate volume. In a study researching the effects of hydrodynamic conditions on membrane fouling, membrane resistance was assumed to increase linearly, and the rate of increase was defined empirically by the following equation **[**27**]**;

$$K = (8.933\ x\ 10^{7})X^{0.532}J^{0.376}U_{Lr}^{-3.047} \quad \textbf{(2.1)}$$

where the notations X, J and U denote MLSS concentration, flux and cross-flow velocity, respectively. In another study **[**175**]** membrane surface was divided into 128 equal parts to model non-uniform membrane fouling formed on the membrane surface. It was assumed that cake layer resistance was proportional to the amount of cake material accumulated on membrane surface, and pore resistance was assumed to be proportional to total permeate volume. The amount of biomass accumulated on the membrane surface was defined as $(dM_{sf}/dt)_a$ = ECJ, making a connection to permeate volume again. Here $(dM_{sf}/dt)_a$ denotes biomass accumulation rate on membrane surface, E is a ratio showing accumulation probability and J denotes flux. TMP normalized by total permeate volume (TMP/V_w) was defined in the following form;

$$\frac{\Delta TMP}{V_w} \sim \alpha^{0.78}C^{0.54}J_0^{1.16}q_a^{-0.93} \quad \textbf{(2.2)}$$

as a function of biomass stickiness (α), MLSS concentration (C), membrane flux (J_0) and aeration intensity (q_a) **[**175**]**. It was concluded that flux was the most influential parameter on TMP increase followed by aeration intensity and MLSS concentration, respectively **[**175**]**. In another study **[**176**]** connecting membrane filtration resistance with the concentration of SMP accumulated on membrane, total resistance increase was defined as;

$$R = R_m + \alpha.m \quad \textbf{(2.3)}$$

here R denotes total filtration resistance, α is specific cake resistance, m: SMP accumulated on membrane surface. It was assumed that the amount of SMP accumulated on membrane surface increased with total permeate volume in the following form;

$$\frac{dm}{dt} = J.p - k_{dm}.m \quad \textbf{(2.4)}$$

here J denotes flux, p is SMP concentration of bioreactor and k_{dm} denotes the rate of foulants' detachment **[**176**]**. Simulations by this model showed that flux was the most important parameter of membrane fouling **[**176**]**. In modeling membrane fouling in a full-scale MBR treating municipal wastewater, total resistance was defined in the following form **[**177**]**;

$$R_T = R_m + R_c + R_F \quad \textbf{(2.5)}$$

here R_m, R_c and R_F denote membrane resistance, cake layer resistance and pore fouling resistance, respectively. Cake layer resistance was defined in the form;

$$R_c = k_C c_b e^{(\frac{F}{k_p})} \quad \textbf{(2.6)}$$

as a function of flux. k_p and k_C are coefficients, F and c_b denote flux and bulk concentration, respectively. R_F is defined the following form;

$$R_F = S_F(1 - e^{-k_F \int_0^t F(t)dt}) \quad \textbf{(2.7)}$$

as a function of total permeate volume **[**177**]**. In a pilot-scale MBR fed with real domestic wastewater, membrane fouling was modeled in following form;

$$\frac{dA}{dt} = k(C_f Q).(C_b Q) \quad \textbf{(2.8)}$$

as a function of total SMP and EPS filtered through membrane **[**178**]**. Here notations indicate A: effective membran area, k: constant, C_f: SMP concentration, Q: flowrate and C_b: EPS concentration. In modeling of an external MBR treating oil mill wastewater, membrane fouling was assumed to be resulted only from cake layer, and cake layer resistance was assumed to increase with total filtered activated sludge suspension **[**179**]**. Total membrane filtration resistance was modeled in the following form;

$$R = R_m + R_c(t,z) \quad \textbf{(2.9)}$$

$$R_c = \alpha(t,z).m_c(t,z) \quad \textbf{(2.10)}$$

$$\frac{dm}{dt} = J(t,z).TS(t,z) - k_{dm}(t,z).m_c(t,z) \quad \textbf{(2.11)}$$

here, R, Rm and Rc denote, respectively, total membrane filtration resistance, membrane resistance and cake layer resistance. α, m_c, dm/dt, J and TS denote, respectively, specific cake resistance, total amount of MLSS accumulated on

membrane surface, MLSS accumulation rate, flux and MLSS concentration **[**179**]**. In another study, membrane fouling was defined as a function of filtration time (t), TMP (ΔPs) and volume of permeate (V) as in the following form **[**180**]**;

$$R_t = \frac{10^{25.7809} t^{1.003} \Delta Ps^{1.003}}{V^{1.003}} \quad \textbf{(2.12)}$$

As it is seen from the studies cited above membrane fouling was modeled assuming it to be resulted from fouling material (MLSS, SMP, EPS) accumulated at membrane depending on total permeate volume (in the latter study, depending on filtration time). In other words, it was assumed that membrane fouling increased with increasing amount of foulants accumulated at membrane. Even so, the amount of fouling material accumulated at membrane was measured in quite a limited number of studies.

3. MATERIAL AND METHODS

3.1 Operating Conditions

A 3.9-L lab-scale MBR setup was established to study the effect of microbial polymeric substances on membrane fouling. Schematic diagram and figure of the system constructed are shown in Figure 3.1 and Figure 3.2, respectively. The system comprised of concentrated feed tank, dilution water tank, two peristaltic pumps, bioreactor containing level sensor, air diffusors, electric heater and two membrane modules, two vacuum tanks for permeate collection, two balances connected to a PC, a vacuum pump and a control panel regulating system automation.

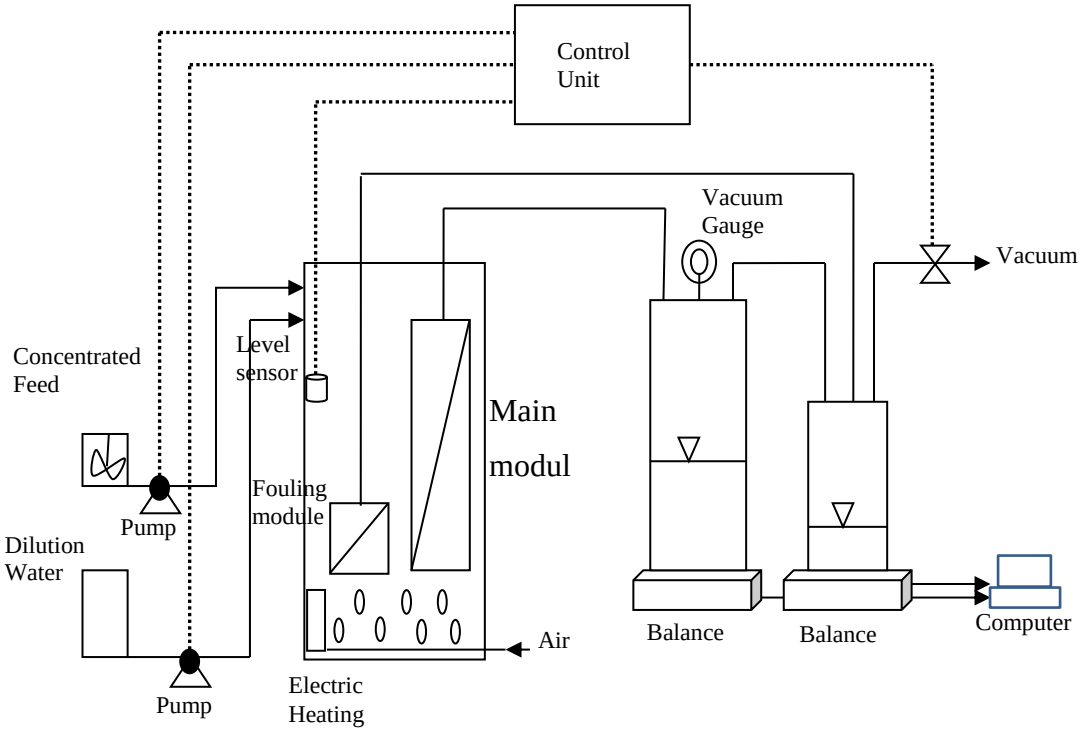


Figure 3.1 : Schematic diagram of MBR.

The system was operated in this way; permeate was withdrawn periodically by vacuum pump operating cyclically 10 min in on position and 1 min in off position by means of solenoid valves mounted on vacuum line. The reduction of hydraulic level in bioreactor due to permeate drawing was compensated by dilution water via a level

sensor and peristaltic pump. Concentrated feed was supplied to the reactor pseudo-continuously by means of the peristaltic pump operating 10 s every 160s. By this way 1.5 L of concentrated feed was supplied to the reactor daily. The separate supply of concentrated feed and dilution water (used also in previous studies [153,181]) has many advantages in terms of experimental conditions especially in MBR, although its operation is somewhat more complex. Firstly, it enables more uniform feeding because especially at constant pressure operation, permeate flux is quite high at first and then decreases with filtration time. If the reactor is supplied with one combined feed solution, the feed must follow the reduction in permeate flux, which causes a non-uniform feeding regime.

Figure 3.2 : Figure of MBR.

Secondly, if feed is supplied to the reactor as combined in dilution water, then HRT and OLR become connected to each other, and it becomes difficult to investigate their separate effects. Then if the effect of varying OLR on the process is investigated without changing HRT, separate supply of feed solution and dilution water would be beneficial. As an alternative, different combined feed solutions having different concentrations can be prepared in this case; nevertheless, non-uniform feeding cannot be avoided by this way. Inoculation sludge was taken from a pilot-scale MBR fed by real municipal wastewater. This sludge was acclimatized to

the synthetic wastewater [181,182] whose properties are given in Table 3.1. This synthetic feed is complex enough to simulate real wastewater. SRT was set to 20 days wasting 195 mL from reactor content daily.

Table 3.1 : Properties of concentrated synthetic feed.

Constituents	**Concentration (mg/L)**
Glucose	1596
Peptone	350
Meat Extract	1050
Starch	2452
Milk Powder	2335
NH_4Cl	257
KH_2PO_4	385
K_2HPO_4	1103
$Cr(NO_3)_3.9H_2O$	15.5
$CuCl_2.2H_2O$	10.8
$MnSO_4.H_2O$	2.2
$NiSO_4.6H_2O$	6.7
$PbCl_2$	2.0
$ZnCl_2$	4.2
Total COD	9387
Soluble COD	5160
Total protein	1014
Filterable protein	989
Total carbohydrate	4607
Filterable carbohydrate	2307
COD/N/P	100/4/2
pH	7.5

The system was operated approximately 6 months. DO concentration was kept above 5 mg/L by pressurized air. Aeration supplied to the reactor performed also the task of membrane cleaning by surface scouring together with mixing reactor content to ensure a homogenous distribution. pH of the reactor was kept between 7.0 – 7.5 with 1N NaOH solution. The temperature in bioreactor was kept at 25 ^{0}C by an electric heater. Two handmade membrane modules shown in Figure 3.3 were submerged to the bioreactor. The main membrane module having 288 cm^2 membrane area was used for MBR operation, while the smaller module having 36 cm^2 membrane area was used for fouling studies. The membrane used in the study was made of PVDF material, had 0.2 μm pore size and was procured from Microdyn-Nadir Company. The membranes were reported to have pure water permeability of 710 L/m^2.h.bar and be resistant to pH = 2 – 12 range and operating temperatures up to 95 ^{0}C by manufacturer. Filtration was performed under 20 kPa constant pressure. Although

constant pressure operation is not widely used in full-scale plants, its application in experimental studies is easier and more sustainable. For example, in a MBR planned to operate at 20 L/m^2.h constant flux, the flux was reduced to 10 L/m^2.h after 5 d due to severe fouling, and even it was reduced to 5 L/m^2.h afterwards **[**144**]**. In other studies it was reported maintaining constant flux when TMP reached 30 kPa was difficult [38,165].

Figure 3.3 : Handmade membrane modules.

It was also reported that constant flux fluctuated by %10 – 15 due to impairment in filterability with time **[**140**]**. In another study constant pressure operation was said to be chosen because of its reliability **[**16**]**. The membranes self-cleaned themselves due to filtration – relaxation periods, which comprised of 10 min filtration and 1 min relaxation. Fluxes were measured via balances connected to a computer. When the flux of main membrane module decreased below 8 L/m^2.h, the module was cleaned by rinsing under tap water followed by soaking into 2000 ppm NaOCl solution for 30 min and put back in bioreactor again after rinsing under tap water.

3.2 Experimental systematic

The small membrane module having effective membrane dimensions of 6 x 6 cm^2 were submerged into MBR to study the effects of different filtration durations on membrane fouling. New membranes were used for different filtration durations to ensure comparable initial conditions. The procedure followed in fouling studies is shown in Figure 3.4. Stepwise information regarding experimental procedure is given as follows;

1. Stage: Conditioning of membranes. In this stage membrane was backwashed with 50 ml pure water in 50 ^{0}C to prepare it for experimental conditions.
2. Stage: Conditioned membrane was fixed in the reactor after specifying the optimum conditions forming a uniform fouling on membrane surface. Although the MBR was established by a small bioreactor, and the membrane module used in fouling studies was relatively small, due to position and place of the module in reactor and the distance from diffuser etc. it was not easy to form a uniform fouling on membrane surface. Small membrane modules were operated at different filtration durations, which were 3, 12 and 24 h.
3. Stage: The gel-like cake layer accumulated on membrane surface in previous stage was rinsed with 50 mL pure water and collected for analyzes. However, the cake layer formed after 24-h filtration was very sticky and was able to be rinsed with 150 mL pure water. Only 100 mL of this rinsing water was collected to prevent further dilution of the solution.
4. Stage: After rinsing cake layer the foulants in membrane pores were collected for analyzes by backwashing with 25 mL pure water
5. Stage: The remaining foulants in membrane pores from previous stage were extracted and collected for analyzes by 25 mL 0.01 N NaOH solution at 50 ^{0}C.

Pure water flux after each stage was measured to determine the resistance resulted from the foulants detached. Rinsing and backwashing water of 3^{th} – 5^{th} Stages were collected and analyzed.

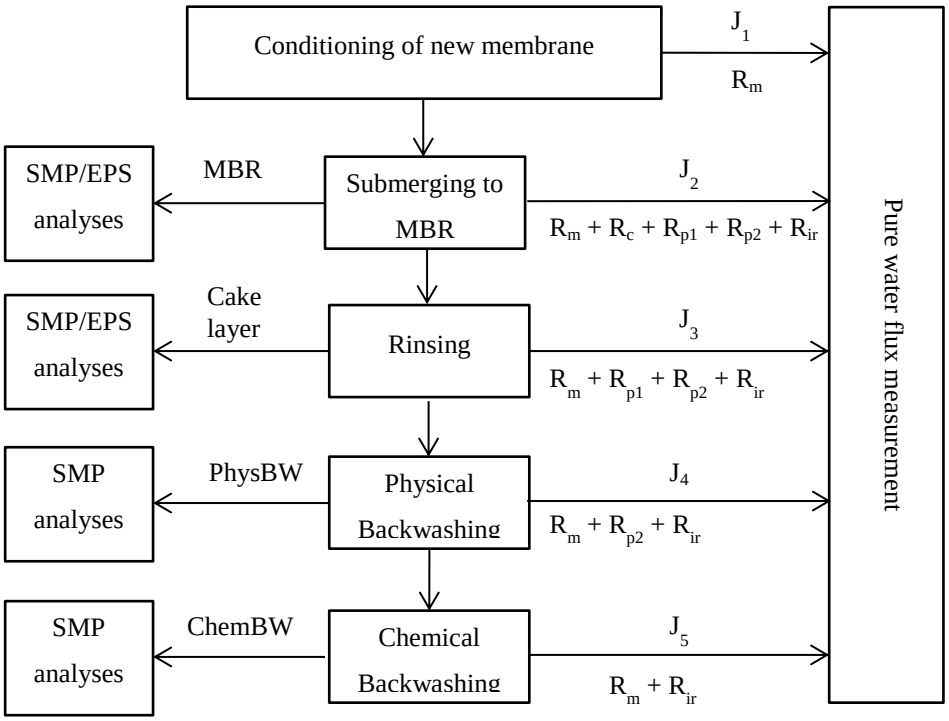


Figure 3.4 : Procedure of membrane fouling study.

3.3 Parameters measured

In influent and effluent of MBR, COD and protein and carbohydrate components of SMP were measured. In bioreactor MLSS, MLVSS and the components of SMP and EPS, namely protein and carbohydrate, were measured. In addition to these, the components of SMP and EPS were also measured in collected foulant solutions from fouling steps between 3 and 5 (Section 3.2). While the components of EPS and SMP were measured in cake layers collected, only the components of SMP were measured in physical and chemical backwash waters because no biomass was observed in these solutions. In this study, EPS term was used to denote microbial polymeric substances which are attached to activated sludge flocs, and SMP was used to denote polymeric substances freely available in activated sludge suspension. Previous studies **[**56**]** showed that not only filterable foulants but also very large polymer clusters caused membrane fouling. For this reason, measuring only filterable part of MPS causes the contribution of remaining part to be ignored. The concentration of MPSs extracted from fouled membranes was measured as *filterable polymer* and *total polymer* separately. For this purpose, a sample taken was firstly divided into two parts by

centrifugation at 7000 g. Then a portion of supernant was measured directly to find the concentration of *total polymer*. The remaining part of supernant was measured after filtering through 0.45-µm PES membran filter to find the filterable polymer concentration of MPS. This procedure was applied for both SMP and EPS measurements of cake layer. However, in effluent, physical backwash waters and chemical backwash waters only the filterable portion of SMP components were measured because there were no settleable particles seen visually.

3.4 Filtration resistances

It was assumed in this study that total membrane filtration resistances were comprised of membrane, reversible, irreversible and irrecoverable resistances. Reversible resistance was further divided into two parts: cake layer resistance (R_c) and reversible pore resistance (R_{p1}). These were able to be removed by rinsing and physical backwashing with pure water, respectively. Irreversible resistance comprised of irreversible pore fouling resistance (R_{p2}) which was able to be removed only by chemical backwashing. Irrecoverable resistance (R_{ir}) was the resistance remained after all the cleaning procedure in the scope of this study. By this classification of membrane filtration resistances, total membrane filtration resistance after each step between 1st and 5th steps, as it is seen from Figure 3.4, can be written in the following form;

$$R_{T_1} = R_m \quad \textbf{(3.1)}$$

$$R_{T_2} = R_m + R_c + R_{p_1} + R_{p_2} + R_{ir} \quad \textbf{(3.2)}$$

$$R_{T_3} = R_m + R_{p_1} + R_{p_2} + R_{ir} \quad \textbf{(3.3)}$$

$$R_{T_4} = R_m + R_{p_2} + R_{ir} \quad \textbf{(3.4)}$$

$$R_{T_5} = R_m + R_{ir} \quad \textbf{(3.5)}$$

where R_{T_i} (i= 1,...,5) and R_m denote total filtration resistance and membrane resistance, respectively. The difference between R_{T_2} and R_m shows *total fouling resistance*. R_{T_i} (i= 1,...,5) can be calculated by pure water fluxes measured after each step using Darcy's equation as follows;

$$R_{T_i} = \frac{\Delta P}{\mu . J_i} \quad \textbf{(3.6)}$$

where, ΔP (Pa), μ (Pa.s) and J_i ($m^3.s^{-1}/m^2$) denote, respectively, TMP, dynamic viscosity and pure water flux. Membrane resistance (R_m), cake layer resistance (R_c), reversible pore resistance (R_{p_1}), irreversible pore resistance (R_{p_2}) and irrecoverable resistance (R_{ir}) are found by piecewise solution of Equations (3-1) – (3-5).

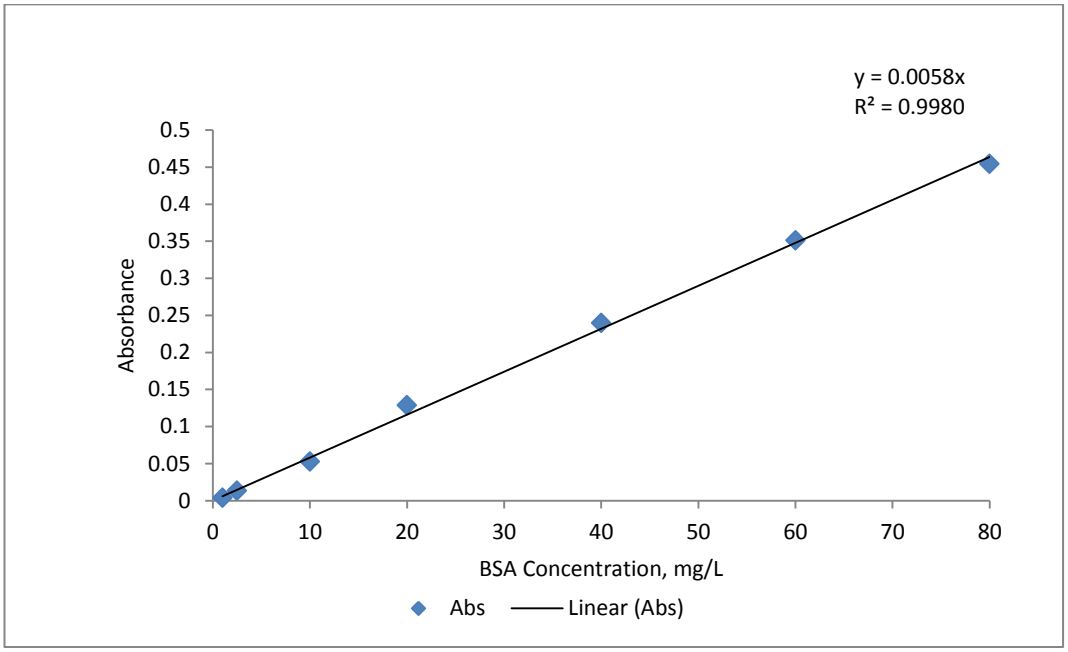
3.5 Extraction of EPS

EPS extraction was performed with cation exchange resin **[**95**]**. Samples taken from MBR and cake layer were centrifuged at 7000 g and 4 ^{0}C for 15 min. As mentioned before in Section 3.3 half of the centrate (supernant of centrifugation) was used to measure total SMP components and the other half was used to measure filterable SMP components. Settled sludge flocs were re-suspended by extraction solution. Extraction solution contained 2 mM Na_3PO_4, 4 mM NaH_2PO_4, 9 mM NaCl and 1 mM KCl. This sludge suspension were transferred into an Erlenmeyer flask and CER (Dowex 50x8, 20-50 mesh in Na form) was added in proportion 75 g CER/g VSS **[**183**]**. Extraction was performed at 4 ^{0}C and 900 rpm for two hours. Extracted EPS was separated from CER and sludge by centrifuging the CER/sludge suspension at 7000 g for 1 min. The centrate was centrifuged again at 7000 g for 15 min. Centrate obtained at this stage was again separated into two parts. In one part total EPS components were measured directly, and in the other part filterable EPS components were measured after filtration through 0.45 μm.

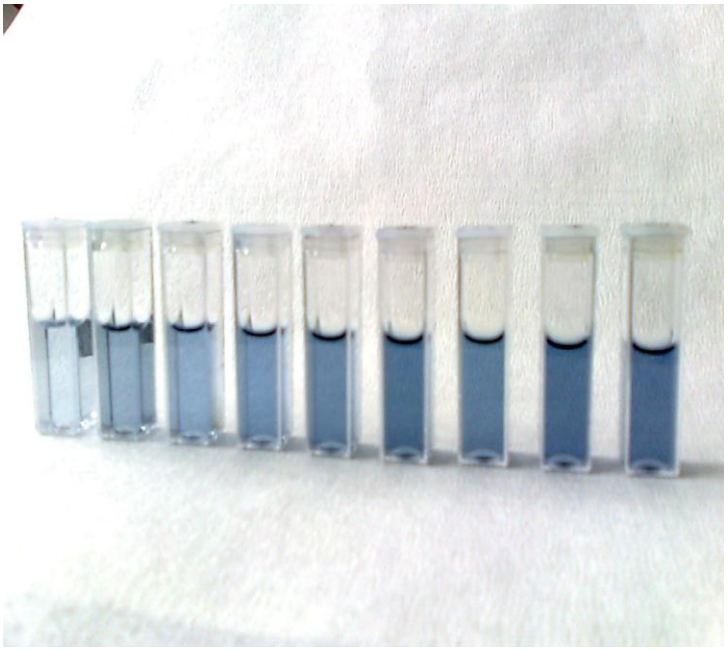
3.6 Protein Concentration Measurement

Protein measurements was performed according to Lowry **[**184**]** method **[**95**]**. No correction was done for humic substances. Bovine serum albumin (BSA) having concentrations varying between 1 – 80 mg/L was used as calibration standard (Figure 3.5). Two replicates were done for each measurement. The reagents used were; *Reagent 1:* 143 mM NaOH, 270 mM Na_2CO_3, *Reagent 2:* 57 mM $CuSO_4$, *Reagent 3:* 124 mM Na-tartrate, *Reagent 4* was made up using reagents 1 to 3 in the proportion 100:1:1, *Reagent 5:* Folin reagent diluted 5:6 with distilled water. Protein measurement procedure was as follows;

1 mL of sample was mixed with 1.4 mL Reagent 4. After adding 0.2 mL Reagent 5, the solution was mixed quickly. After 45 min the absorbance was read against BSA standards at 750 nm spectrophotometrically.



(a)

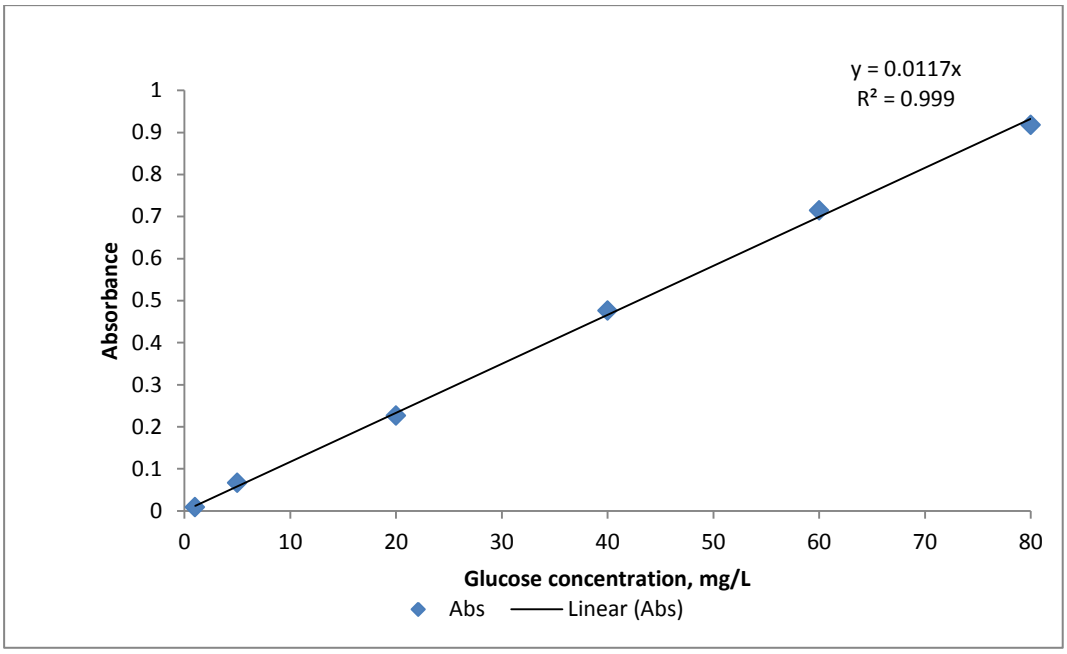


(b)

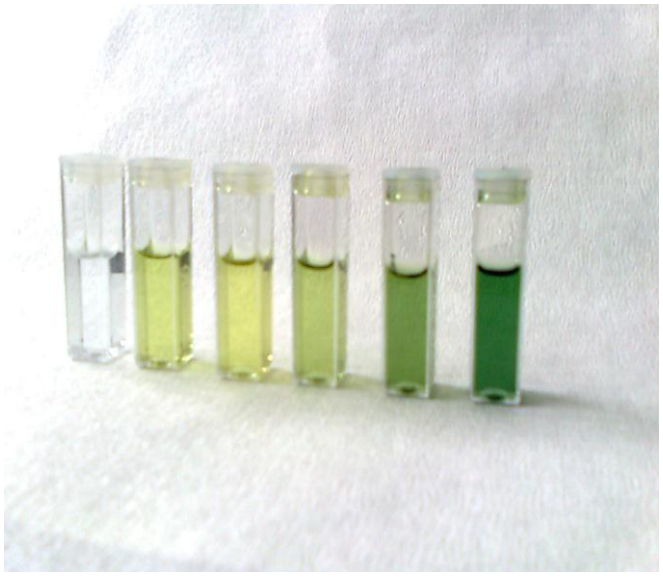
Figure 3.5 : Protein measurement; (a) Calibration curve and (b) BSA standards.

3.7 Carbohydrate Concentration Measurement

Carbohydrate measurement was performed according to anthrone method [95,118,185]. Two replicates were done for each measurement. The reagent used was the solution of 0.2% anthrone at 95 % H_2SO_4.



(a)



(b)

Figure 3.6 : Carbohydrate measurement; (a) Calibration curve and (b) glucose standards.

Procedure: 1 mL of sample was mixed with 2 mL of reagent in a test tube. When the reactant was added, the test tubes were being kept at cold water. Test tubes were then transferred to boiling water. Test tubes were kept at boiling water for 14 min followed by keeping at cold water for 5 min. Later the absorbance of the samples was read at 625 nm spectrophotometrically. Glucose having concentrations between 1 – 80 mg/L was used as standard (Figure 3-6).

3.8 Measurement of Conventional Parameters

MLSS and MLVSS were measured according to standard methods **[**186**]**. COD was measured by Hach test kits (LCK314). pH, temperature and DO were measured by Eutech PCD 650 multimeter.

3.9 Mass balance on bioreactor

The mass balance on a control volume of MBR shown in Figure 3.7 can be written as follows **[**187**]**;

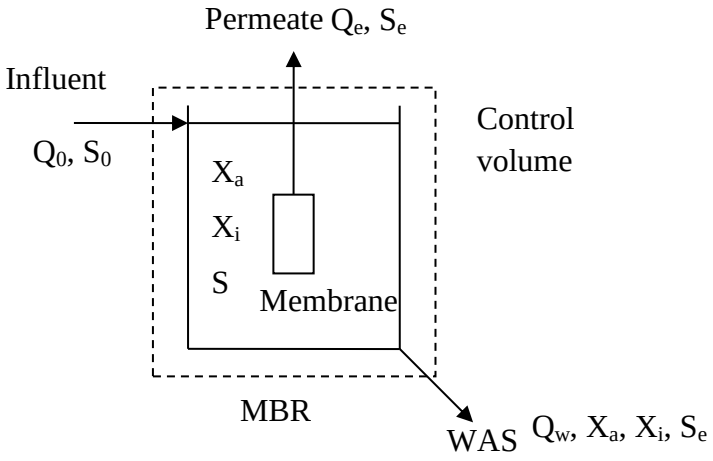


Figure 3.7 : Control volume of MBR.

Mass rate of substrate accumulation in control volume = rate of mass in – rate of mass out + rate of mass generation.

Mass balance of substrate is written as:

$$V.\frac{dS}{dt} = Q.S_0 - (Q_e.S + Q_w.S) + [Y.r_{ut}.V] \quad \textbf{(3.7)}$$

Mass balance of active biomass;

$$V.\frac{dX_a}{dt} = -Q_w.X_a + [Y.(-r_{ut}).V - b.X_a.V] \quad \textbf{(3.8)}$$

Mass balance of inert biomass;

$$V.\frac{dX_i}{dt} = (1 - f_d).b.X_a.V - Q_w.X_i \quad \textbf{(3.9)}$$

Since mass accumulation would be zero at steady state condition,

From Eq. (3-9) X_i can be stated as;

$$X_i = (1 - f_d).b.X_a.\frac{V}{Q_w} \quad \textbf{(3.10)}$$

Sludge retention time (SRT) is defined as follows:

$$\theta_x = \frac{X_a.V}{Q_w.X_a} = \frac{X_i.V}{Q_w.X_i} = \frac{V}{Q_w} \quad \textbf{(3.11)}$$

By this definition of SRT, X_i can be written from Eq. (3-10)

as the following form;

$$X_i = (1 - f_d).b.X_a.\theta_x \quad \textbf{(3.12)}$$

Multiplying Eq.(3-8) by $(\frac{1}{Q_w})$;

$$(\frac{1}{Q_w}).[-Q_w.X_a + [Y.(-r_{ut}).V - b.X_a.V] = 0$$

$$-X_a + \left[Y.(-r_{ut}).\frac{V}{Q_w} - b.X_a.\frac{V}{Q_w}\right] = 0$$

$$-X_a + [Y.(-r_{ut}).\theta_x - b.X_a.\theta_x] = 0$$

$$X_a = \frac{Y.(-r_{ut}).\theta_x}{(1+b.\theta_x)} \quad \textbf{(3.13)}$$

is obtained. Substrate utilization rate is defined as

$$-r_{ut} = \frac{q_{max}.S}{(K+S)}X_a \quad \textbf{(3.14)}$$

Putting Eq. (3-14) in Eq. (3-13) gives;

$$X_a = \frac{\frac{Y.q_{max}.S}{(K+S)}.X_a.\theta_x}{(1+b.\theta_x)}$$

$$1 = \frac{\frac{Y.q_{max}.S}{(K+S)}.\theta_x}{(1+b.\theta_x)} \quad \textbf{(3.15)}$$

From Eq.(3-15) substrate concentration S is obtained as;

$$S = \frac{K.(1+b.\theta_x)}{[Y.q_{max}.\theta_x-(1+b.\theta_x)]} \quad \textbf{(3.16)}$$

MLVSS concentration in the reactor is sum of active and inert biomass.

$$X_{\text{MLVSS}} = X_a + X_i$$

Accordingly MLVSS concentration can be written as;

$$X_{\text{MLVSS}} = X_a + (1 - f_d).b.X_a.\theta_x = [1 + (1 - f_d).b.\theta_x].X_a \quad \textbf{(3.17)}$$

Substrate utilization rate can also be stated in another way;

$$-r_{ut} = \frac{Q.(S_0-S)}{V} \quad \textbf{(3.18)}$$

When Eq. (3-18) is written in its place at Eq 3-13,

active biomass concentration will be in the following form

$$X_a = \frac{Y.Q.(S_0-S).\theta_x}{V.(1+b.\theta_x)}$$

Since HRT is defined as $\theta_h = \frac{V}{Q}$ active biomass concentration takes the form of

$$X_a = \frac{\theta_x}{\theta_h}.\frac{Y.(S_0-S)}{(1+b.\theta_x)} \quad \textbf{(3.19)}$$

Putting Eq (3-19) in its place at Eq. (3-17);

$$X_{\text{MLVSS}} = [1 + (1-f_d).b.\theta_x].\frac{\theta_x}{\theta_h}.\frac{Y.(S_0-S)}{(1+b.\theta_x)} \quad \textbf{(3.20)}$$

is obtained.

MLVSS concentration in the reactor:

$$X_{MLSS} = \frac{X_{MLVSS}}{0.90} \quad \textbf{(3.21)}$$

Nutrient requirement;

Nitrogen:

$$r_N[\frac{mg\ N}{L.d}] = \gamma_n.Y.r_{ut}.\frac{[1+(1-f_d).b.\theta_x]}{(1+b.\theta_x)} \quad \textbf{(3.22)}$$

Phosphorus:

$$r_P[\frac{mg\ P}{L.d}] = r_N.0.2\ \frac{g\ P}{g\ N} \quad \textbf{(3.23)}$$

The definition of parameters used in this section is given in Table 3.2.

3.10 Iso-MLSS curves

Figure 2-5 showing interrelations of some activated sludge properties given in Section 2.2.5 was prepared using Eqs. (3-20) and (3-21). Required influent organic feed concentration for a predetermined MLSS concentration was calculated by means of Eqs. (3-20) and (3-21) as in the following form;

$$S_0 = 0.90X_{MLSS}.\frac{\theta_h}{\theta_x}.\frac{(1+b.\theta_x)}{Y.[1+(1-f_d).b.\theta_x]} + S \quad \textbf{(3.24)}$$

Food to biomass ratio (F/M) was calculated as follows;

$$\frac{F}{M}[\frac{mg\ COD}{mg\ VSS.d}] = \frac{Q.S_0}{V.X_{MLSS}} = \frac{S_0}{\theta_h.X_{MLSS}} \quad \textbf{(3.25)}$$

Volumetric organic loading rate (OLR) was calculated as follows;

$$OLR\ [\frac{kg\ COD}{m^3.d}] = \frac{Q.S_0}{V} = \frac{S_0}{\theta_h} \quad \textbf{(3.26)}$$

Parameter values used in these calculations are given in Table 3.2.

Table 3.2 : Parameters used for mass – balance.

Parameter	Description	Value
V	Reactor volume	3.9 [L]
S	Reactor substrate concentration	
S_e	Effluent substrate concentration	
Q	Inflow rate	
S_0	Influent substrate concentration	
Q_w	Waste flow rate	
Y	True yield for cell synthesis	0.42 [g VSS_a/g COD]
r_{ut}	Rate of substrate utilization	
X_a	Active biomass concentration	
X_i	Inert biomass concentration	
b	Endogenous-decay coefficient	0.2 [1/d]
f_d	Biodegradable fraction of biomass	0.8
θ_x	Sludge retention time	1 – 101 [d]
θ_h	Hydraulic retention time	12 [h]
q_{max}	Maximum specific rate of substrate uitilization	
K	Concentration giving one-half the maximum rate	
X_{MLVSS}	Volatile suspended solids concentration	
X_{MLSS}	Total suspended solids concentration	
r_N	Nitrogen consumption rate	
r_P	Phosphorus consumption rate	
γ_n	Stoichiometric ratio of nutrient mass to VSS for the biomass	0.124 [g N/g VSS]

4. RESULTS AND DISCUSSION

MLSS/MLVSS, pH, DO and temperature parameters measured at activated sludge are given in Figure 4-1, 4-2, 4-3 and 4-4, respectively. The first period, which included the time from startup up to circled one shown in Figure 4-1, was acclimation period. No sludge was wasted at this period. In the second period shown between 1 and 2 time points, while the reactor was operating under steady state conditions, an operation failure took place because of excessive acid injection due to broken pH probe. After three – four hours the appearance of sludge changed very much, accordingly the operation was stopped. In third period shown by 2 – 3 sludge was cultivated again.

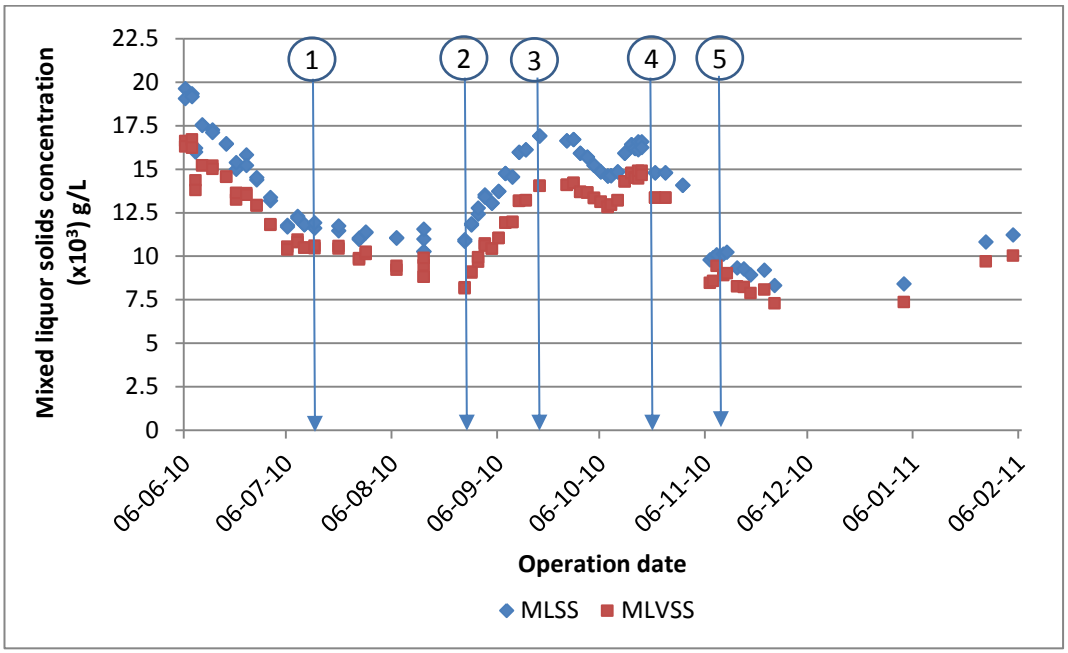


Figure 4.1: MLSS and MLVSS concentration profile during the study.

In the fourth period shown by 3 – 4 time points, the operation was planned to be performed at 15 g/L MLSS concentration, nevertheless, this time some sludge sedimentation occurred at periphery of the circular reactor due to non-optimized aeration and reactor design. Since inhomogeneous reactor content due to sedimentation was seen a serious problem for such a study planned to be done under

as controllable as possible conditions, MLSS concentration was decided to be reduced. After transition period shown between 4 – 5 time points, the reactor was intended to be operated at 10 g/L MLSS concentration, and membrane fouling studies were commenced after the point shown by the point 5.

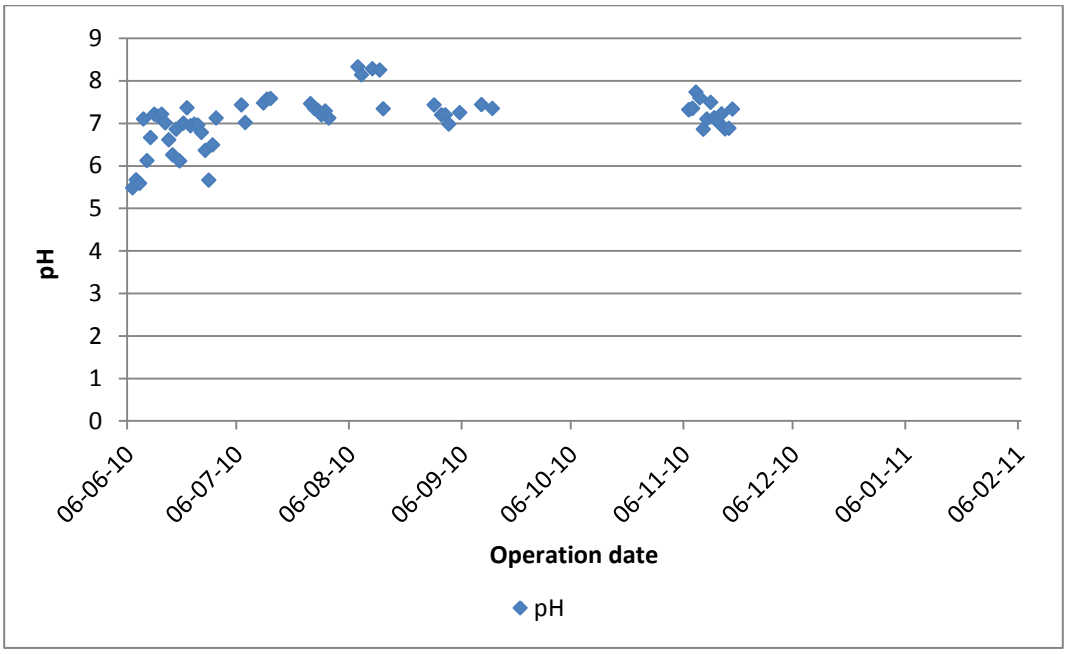


Figure 4.2 : pH values of bioreactor.

The concentrations of the components of SMP and EPS together with COD concentrations measured at the effluent of MBR are given in Table 4.1. The concentrations of SMP protein and carbohdyrate are very close to each other and around 17 mg/L. Protein/carbohdyrate ratios of filterable and total SMP were 0.89 and 1.07 respectively. This shows that filtering the sample causes original protein/carbohydrate ratio to change slightly. Although carbohydrate concentration was five times that of protein at influent (see Tablo 2-1), P/C ratio of around 1.0 in MBR sludge shows that biomass assimilating influent substrate synthesizes mainly proteinous substances.

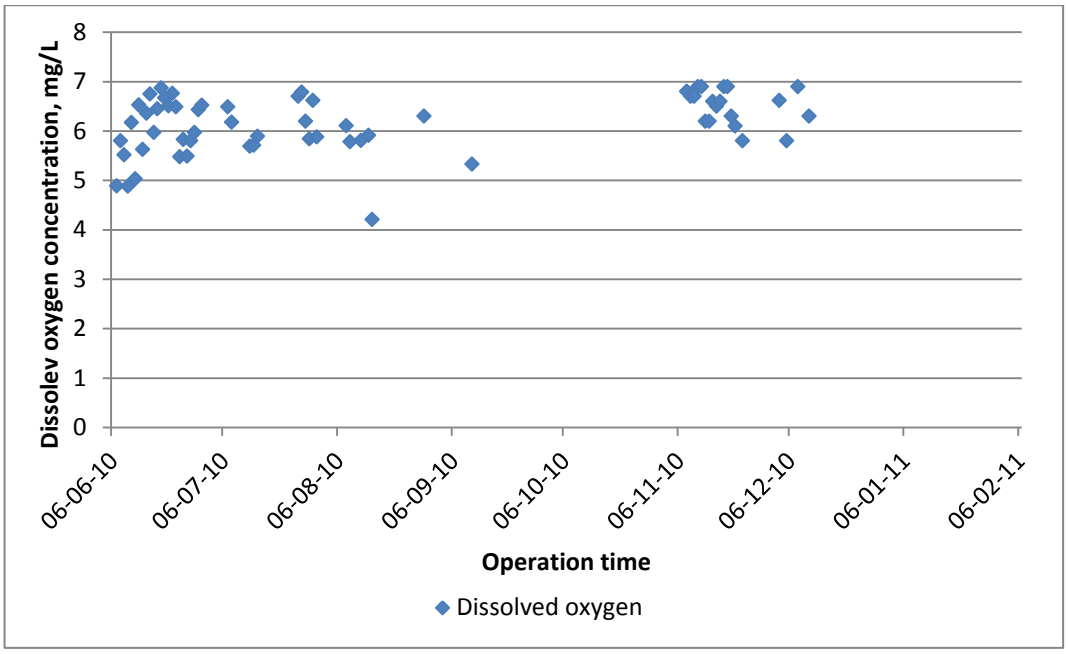


Figure 4.3 : Dissolved oxygen concentration in bioreactor.

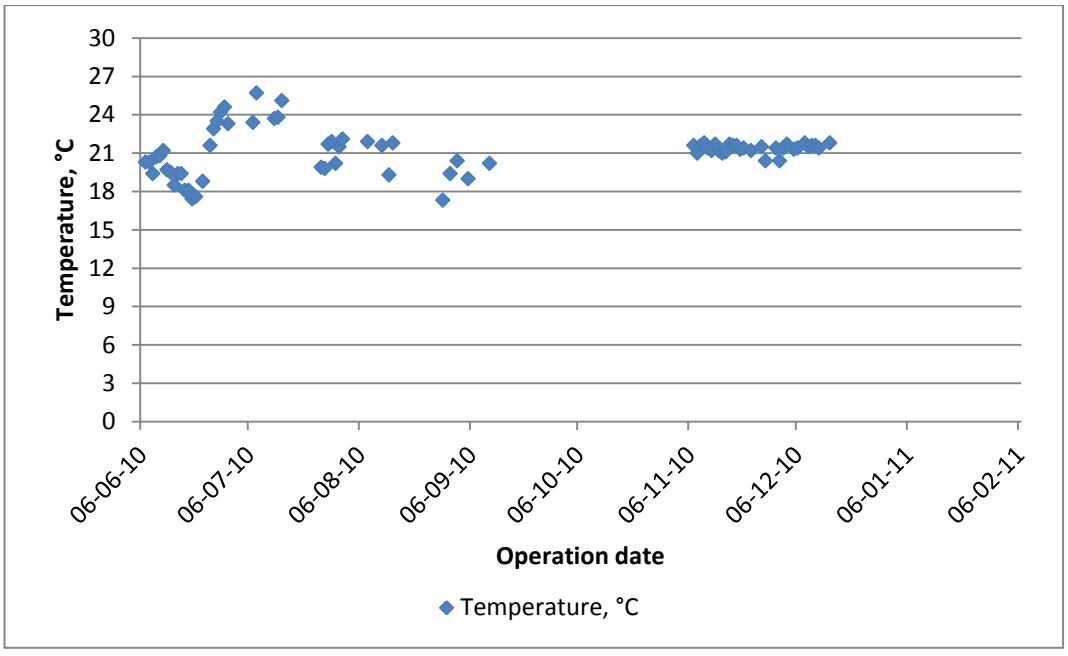


Figure 4.4 : Temperature in bioreactor.

Table 4.1 : Polymeric substances in effluent and mixed liquor of MBR, effluent COD, VSS and TSS concentrations.

Parameter[a]	**Filtration operation time, hours**			**Average**
(mg/L)	**3**	**12**	**24**	**(mg/L)**
SMP_{pf}	13.7	16.1	14.2	14.7
SMP_{pt}	24.7	18.6	14.8	19.4
SMP_{cf}	19.1	15.6	15.2	16.6
SMP_{ct}	-	19.5	16.4	18.0
EPS_{pf}	496.6	502.1	415.3	471.3
EPS_{pt}	514.5	634.6	455.9	535.0
EPS_{cf}	68.3	78.1	63.7	70.0
EPS_{ct}	66.3	87.0	73.0	75.4
Effluent SMP_p	3.5	4.6	5.8	4.6
Effluent SMP_c	2.8	2.4	4.0	3.1
Effluent COD	33.7	25.1	35.5	31.4
MLSS	8980	9580	9260	9273
MLVSS	7920	8520	8000	8147

[a] Lowercase letters denote p: protein,c: carbohydrate, f: filterable fraction, t:total fraction.

This is further supported by EPS protein concentration which is nearly seven times that of carbohydarate (see Table 4-1). These P/C ratios are in a level comparable to the values found in the literature. In previous studies P/C ratios of 0.2 – 0.9 for SMP and 3.3 – 6.6 for EPS were reported **[**110**,**188**]**. Contrary to SMP a great difference is observed between EPS protein and carbohydrate concentrations. While average total EPS protein concentration was 535 mg/L, total EPS carbohydrate concentration was measured to be 75.4 mg/L in average. SMP protein and carbohydrate concentrations measured at effluent were close to each other and low, and changed between 2.4 – 5.8 mg/L. Effluent COD concentrations changed between 25.0 – 36.0 mg/L. This shows that a high organic matter elimination took place in the bioreactor. When COD equivalents of BSA and glucose – being representatives of protein and carbohydrate respectively – are calculated assuming a converison factor of 1.5 **[**189**]** for BSA and 1.07 for glucose, effluent COD resulted from SMP protein and carbohydrate components is seen to vary between 12.9 – 20.3 mg/L. The remaining portion of COD results from organic components other than protein, carbohydrate

and humic substances which are assumed to have been measured together with proteins. Flux curves of membranes exposed to varying fouling durations is given in Figure 4.5.

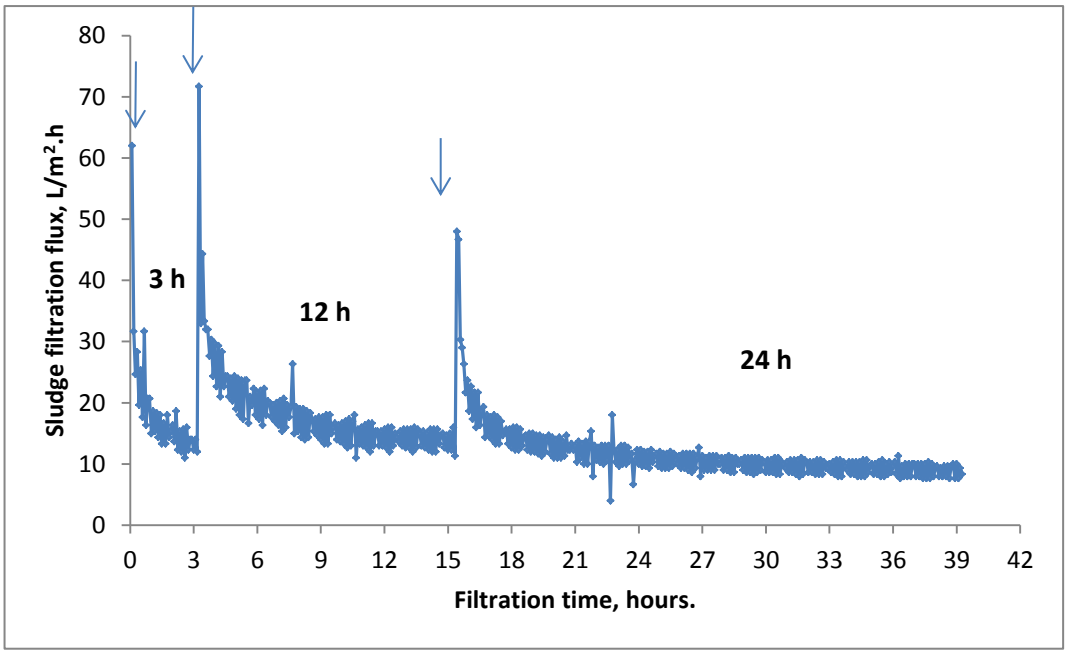


Figure 4.5 : Filtration fluxes vs. fouling duration. Arrows show new membrane submergence.

The arrows in Figure 4.5 show the beginning of a new experiment with a new membrane. These flux curves are typical curves mostly observed at constant-pressure operation. In these curves three phases are observed: a very rapid flux decrease at initial period of filtration, a milder decrease in the second period and a slightly changing flux at the third period. When preparing new membrane modules, special importance was given to pure water fluxes of new membranes to be close to each other. It is seen from Figure 4.5 that all flux curves started to decrease from 30 $L/m^2.h$ except for first several minutes. For this reason it can be assumed that initial state conditions of all the membranes were the same. As it was mentioned before in Section 3.3, not only filterable foulants (<0.45 μm) contribute to membrane fouling. It is seen from the following Table 4.2 that the ratio of filterable MPS to total MPS changes between 54 – 103%. According to the results obtained from Table 4.2 filtering the sample can cause an important portion of MPS to be ignored, which makes more difficult to establish correlation between MPS and membrane fouling.

Table 4.2 : The ratios of filterable MPS/total MPS in mixed liquor and cake sludge on membrane.

Filterable MPS/Total MPS (%)[a]	Filtration operation time, hours		
	3	**12**	**24**
SMP_{pf}/ SMP_{pt}	55	87	96
SMP_{cf}/ SMP_{ct}	-	80	93
EPS_{pf};/ EPS_{pt}	97	79	91
EPS_{cf}/ EPS_{ct}	103	90	87
$CakeSMP_{pf}$/$CakeSMP_{pt}$	79	66	95
$CakeEPS_{pf}$/$CakeEPS_{pt}$	66	69	68
$CakeSMP_{cf}$/$CakeSMP_{ct}$	82	68	79
$CakeEPS_{cf}$/$CakeEPS_{ct}$	-	54	57

[a] Lowercase letters denote p: protein,c: carbohydrate, f: filterable fraction, t:total fraction.

It was reported that filtering a sample through 0.45-µm cellulose acetate filter caused 23% reduction in polysaccharide concentration of original sample **[**113**]**. Further filtration through 0.1-µm PES membran filter together with initial paper filtration resulted in nearly %30 reduction in polysaccharide concentration of original sample **[**113**]**. It was also reported that organic matter content measured after filtration through membrane filter having smaller pore size than 0.5 µm was not be able to be correlated with membrane fouling **[**21**]**. Accordingly it was proposed to measure soluble organic matter content by centrifugation or paper filtration rather than membrane filter (≤ 0.5 µm) **[**21**]**. In another study **[**163**]** it was observed that approximately %70 of organic matter in the supernatant obtained by centrifugation of cake layer sludge accumulated on a 0.4-µm PE membrane was greater than 0.4 µm. It was observed in the same study that %70 of the supernant proteins and carbohydrates and %80 of humic acids were retained by 0.4-µm filter **[**163**]**. In addition, in centrifuged MBR supernant the existence of particulate organic matter reaching up to 50 µm in size was determined by observations made by fluorescent microscope and DAPI staining **[**56**]**. All these findings show that membrane fouling in MBR should be correlated with total organic matter rather than only filterable part.

4.1 The Role Microbial Polymeric Substances in Membrane Fouling

The amounts of total and filterable MPS protein extracted from fouled membranes are given in Figure 4.6 (a). It is seen that the most protein accumulation at membranes occurred at cake layer independent of filtration duration. When filtration duration was raised from 3h to 12h the amount of protein accumulated in cake layer also increased. It is seen that there was a reduction in the amount of protein accumulated on membrane surface when filtration duration was further raised to 24 h. This may have been resulted from the problem observed at rinsing of cake layer occurred on membrane at 24-h filtration experiment as mentioned before in Section 3.2.

One of the important finding is that the ratio of total SMP protein accumulated on membrane surface to total EPS protein decreases with increasing filtration duration. This ratio was calculated to be 6.5, 3.2 and 2.2 for 3-, 12- and 24-h filtration durations, respectively. This may have been resulted from either attachment of SMP accumulated on membrane surface to EPS within time **[**164**]** or EPS production by biomass accumulated on membrane surface as the time passed. When UV-visible absorbance of foulants accumulated on membrane surface was compared to that of SMP and EPS in MBR activated sludge, it was concluded that foulants accumulated on membrane surface was originated mainly from SMP of bulk sludge **[**110**]**. On the other hand, polymer chain reaction – denaturing gradient gel electrophoresis analyzes showed that there was important differences between cake layer sludge and bulk sludge in terms of microbial diversity and intensity **[**164**]**. It was reported that the amount of cake layer EPS was 1.5 times that of bulk sludge in the same study. These studies indicate that the source of cake layer may be different depending on varying MBR conditions. It is seen from Figure 4.6 (a) that the second greatest protein accumulation in/on membrane was resulted from proteins tightly attached to membrane pores. These proteins tightly attached to membrane pores were not removable by physical backwashing and they were able to be removed only by chemical backwashing. The third greatest protein accumulation at membrane was resulted from proteins loosely attached to membrane pores. These proteins loosely attached to membrane pores were easily removed by physical backwashing.

Consequently, the sequence of protein accumulation in/on membrane was observed to occur in the following order;

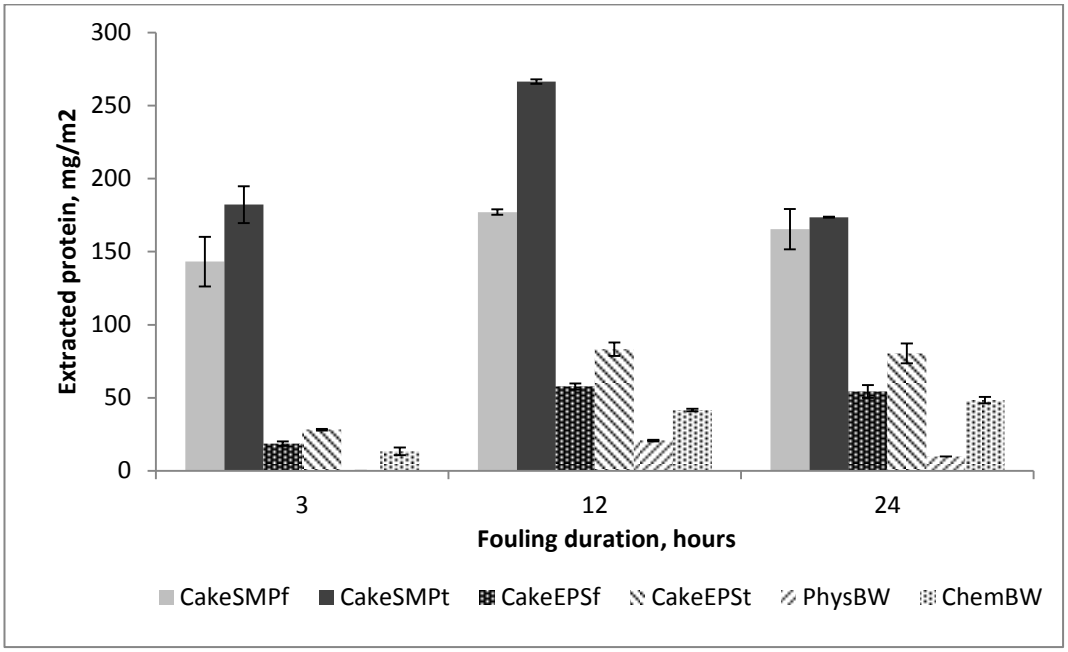
Cake layer proteins > proteins tightly attached to membrane pores > proteins loosely attached to membrane pores

Similar to proteins, analyzes of carbohydrates extracted from fouled membranes showed that the greatest accumulation of carbohydrates took place at cake layer accumulated on membrane (Figure 4.6 (b)). It is seen that carbohydrates tightly attached to membrane pores constituted the second greatest carbohydrate accumulation at membrane. Carbohydrates loosely attached to membrane pores formed the least carbohydrate accumulation at membrane. Interestingly the protein and carbohydrate concentrations of pure water backwashing of 3-h filtration were below 1 mg/L. This shows that polymeric substances accumulated in membrane pores were mainly composed of polymers tightly attached to membrane pores in short term filtration.

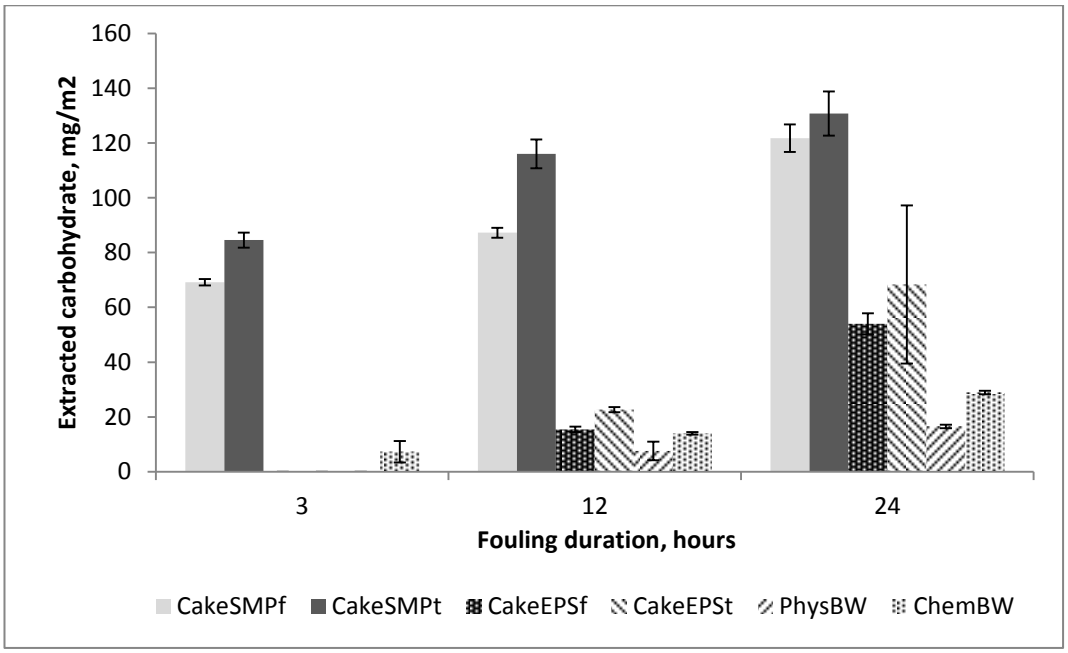
It was seen that EPS carbohydrate also did not accumulate in cake layer at 3-h filtration duration. The order of carbohydrate accumulation followed the same order as protein accumulation;

Cake layer carbohydrates > carbohydrates tightly attached to membrane pores > carbohydrates loosely attached to membrane pores

The ratio of SMP protein accumulated on membrane surface to SMP carbohydrate was calculated as 2.2, 2.3 and 1.3 for 3-, 12- and 24-h filtration durations, respectively. This is one of the important findings of this study, which shows that the amount of protein accumulated on membrane surface was always greater than the amount of carbohydrate independent of filtration duration. It is known that proteins cause membrane fouling attaching to the membrane surface more strongly **[**190**]**. It was also reported that proteins had more potential of adsorption/deposition onto membrane surface and attached more strongly to membrane surface **[**191**]**. Moreover, high stickiness of sludge was attributed to higher P/C ratio of EPS content, which favored more resistant cake layer formation **[**164**]**.



(a)



(b)

Figure 4.6 : Extracted amounts of protein (a) and carbohydrate (b) from fouled membranes in each step. Lowercase letters denote f: filterable (<0.45 μm), t: total amounts.

In this case it can be concluded that the amount of protein accumulated on membrane surface increases as filtration duration extends, and their scouring becomes more difficult. This is further supported by other studies which indicated that high P/C

ratio caused more membrane fouling [89, 192]. As a result, the order of polymeric substance accumulation in/on membrane observed in this study was in the following sequence;

MPSs accumulated in cake layer ≫ MPSs tightly attached to membrane pores > MPSs loosely attached to membrane pores

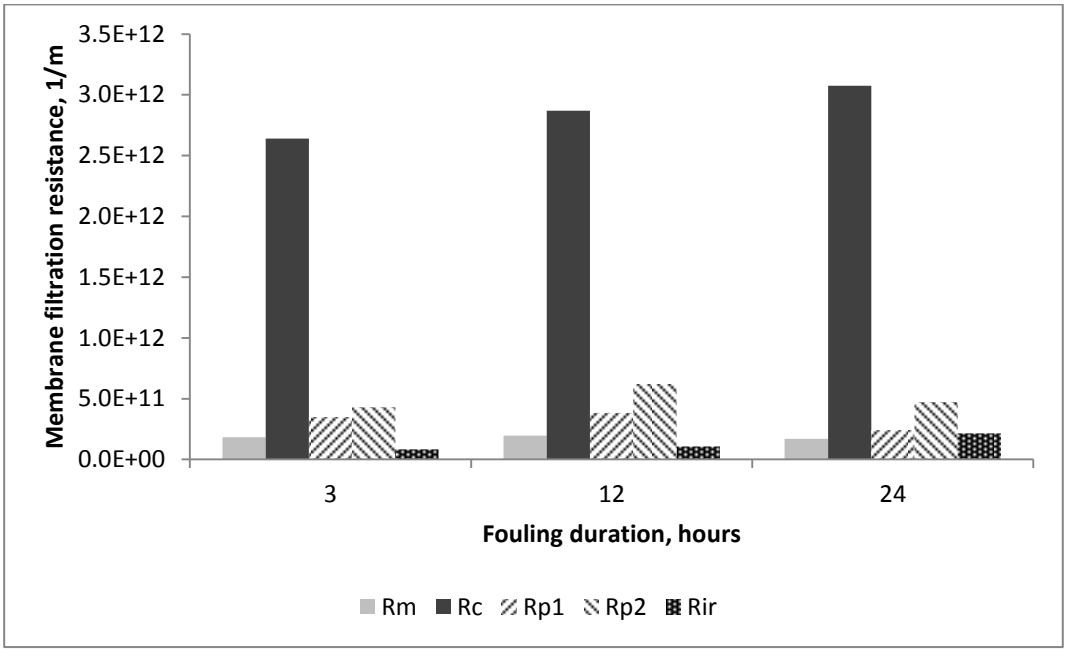
It may be supposed that the constant pressure operation used in this study caused cake layer formation; accordingly the most MPS accumulation was occurred in cake layer. In contrast, as it is discussed in detail in the following paragraph the order of polymer accumulation at membrane is not related to constant pressure or constant flux operation types because the importance of foulants accumulated in cake layer was also reported in the following constant-flux studies. It was reported that the amount of EPS in biocake accumulated on membrane surface was more important than that of mixed liquor in terms of controlling membrane fouling **[**68**]**. It was stated that proteins were the most important membrane foulants, and removal of proteins accumulated on the surface or prevention of their adsorption on membrane surface were possibly able to reduce membrane fouling **[**59**]**. It was reported that the most important reason of membrane fouling in submerged MBRs used for wastewater treatment was the formation of cake layer on membrane surface **[**163**]**. In addition, the reduction of polymeric matter accumulated on the membrane surface by flocculant addition reduced membrane fouling substantially extending 7.5 folds the time to reach final TMP 30 kPa **[**38**]**.

Individual membrane filtration resistances and the contribution of each resistance to total fouling resistance depending on the filtration durations are shown, respectively, in Figure 4.7 (a) and Figure 4.7 (b). As it is seen from Figure 4.7 (a), the highest fouling resistance was resulted from cake layer resistance. Cake layer resistance changed between 2.6 x 10^{12} and 3.1x10^{12} [1/m]. These values are in the same order of magnitude with the values reported in previous studies [70,77,193]. It is seen from Figure 4.7 (b) that the contribution of cake layer resistance to total fouling resistance changed between 72.1 % - 76.8 %. Cake layer caused the highest fouling resistance independent of filtration duration. According to these results it can be said that cake layer formation is the greatest factor in membrane fouling. As it was mentioned in the previous paragraph the predominant role of cake layer in membrane filtration is

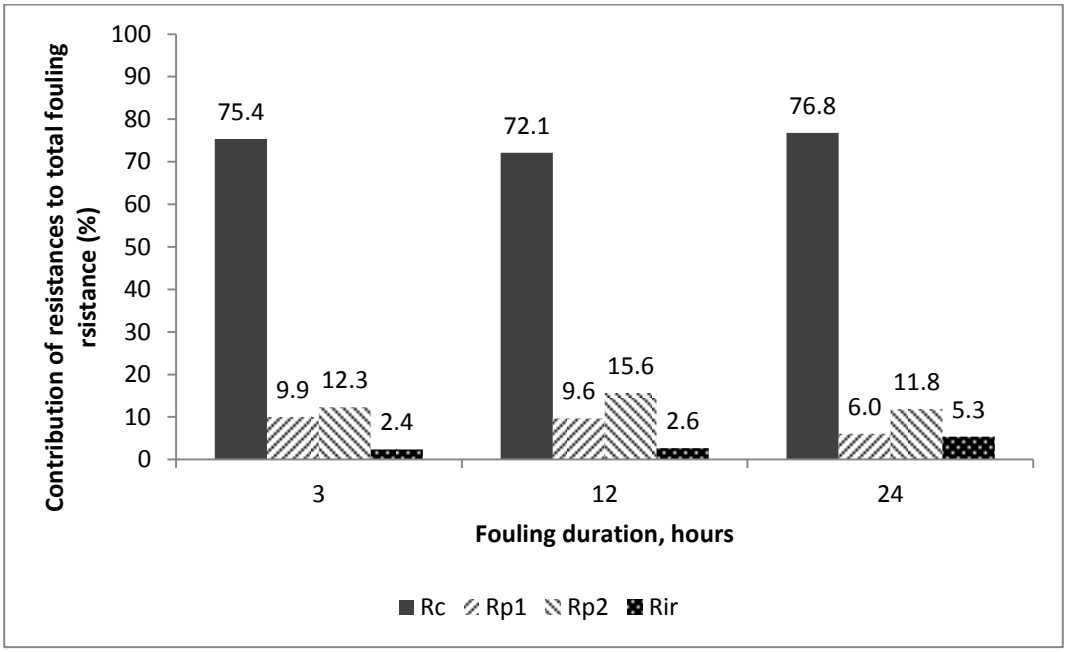
independent of constant pressure or constant flux operation modes. In all of the following studies cited constant-flux operation mode was used. In a conventional and a moving-bed two MBRs made of 0.1-µm hollow fiber polypropylene membranes cake layer resistance constituted 83.7 % and 57.8 % of total resistance, respectively [194]. In a MBR made of 0.1-µm hollow fiber PE membrane cake layer resistance formed the main resistance by %90.9 [190].

It was reported that 98 – 99 % of total resistance resulted from cake layer resistance [188]. In a MBR made by 0.22-µm flat sheet polytetraflouroethylene membrane the contribution of cake layer to total filtration resistance was measured to be 89 % [193]. It was reported that the contribution of cake layer to total filtration resistance was more than 80 % in a MBR made of 0.4-µm hollow fiber membrane [66]. Cake layer resistance constituted the highest resistance in a MBR made of 0.4-µm flat sheet PE membrane [53]. Lastly, even in a MBR made of 90-µm mesh filter cake layer resistance formed the highest resistance by 78.8 % [195]. It was reported that statistically similar specific cake resistances and compressibility were obtained for bacteria, colloidal silica and treated natural water suspensions under constant pressure and constant flux operation modes [196]. It was stated in the same study [196] that the hydraulic resistance of cake layer formed by colloids was dominated by morphology rather than filtration mode. In other words, the hydraulic resistance of foulants accumulated on membrane surface is mainly determined, independent of operation mode (constant flux or constant pressure microfiltration), by instantaneous pressure [197].

This current study together with previous studies cited above indicates that independent of membrane material, pore size, configuration and operation type cake layer formation is the general and the most important problem of membrane fouling in MBRs used for wastewater treatment.



(a)



(b)

Figure 4.7 : Individual resistances versus filtration duration (a) and contribution of each individual resistance to total fouling resistance (R_{TF}) versus filtration (b).

In this study membrane fouling was examined in a time scale changing between 3 – 24 h. However, even at first 30 min of filtration the greatest fouling resistance was resulted from cake layer **[**193**]**. This indicates that cake layer formation takes place at initial period of filtration, and it sustains its predominance during the whole

filtration. After cake layer resistance the second greatest resistance changing between 11.8 – 15.6 % resulted from irreversible resistance (R_{p2}) which was the resistance of polymeric substances that were tightly attached to membrane pores. This was followed by reversible resistance (R_{p1}) which was the resistance imposed by polymeric substances loosely attached to membrane pores and irrecoverable resistance (R_{ir}) which was the remaining resistance after all the cleaning procedure in the scope of this study, which changed between, respectively, 6.0 – 9.9 % and 2.4 – 5.3 %. Therefore, the fouling resistances taking place in the membrane were ordered in the following form;

Cake layer resistance >> irreversible pore resistance >reversible pore resistance > irrecoverable resistance

It seen that the order of the first three resistances followed exactly the same order of foulants accumulated at membranes, which caused these resistances. In other words, a parallelism was observed between the amount of polymeric substances extracted from membrane and corresponding fouling resistances removed. This is one of the most important findings of this study. This mainly indicates that membrane fouling results from the foulants accumulated at membrane. Although this is accepted instinctively, there are very few experimental studies showing this finding. Therefore, the relationship between membrane fouling and the foulants accumulated at membrane was indicated qualitatively. This relationship will be evaluated quantitatively in the following section.

4.2 The Functional Relationship between Microbial Polymeric Substances and Membrane Fouling

The fouling potential of membranes depending on filtration operation time can be evaluated by specific fouling resistance. Specific fouling resistance [m/kg] is defined as the fouling resistance [1/m] resulted from the amount of foulant accumulated at unit membrane area [kg/m^2]. Variation of specific fouling resistance with operation time is shown in Figure 4.8. As it is seen from Figure 4.8 specific fouling resistance of foulants accumulated in both cake layer and membrane pores tends to decrease with extending filtration duration.

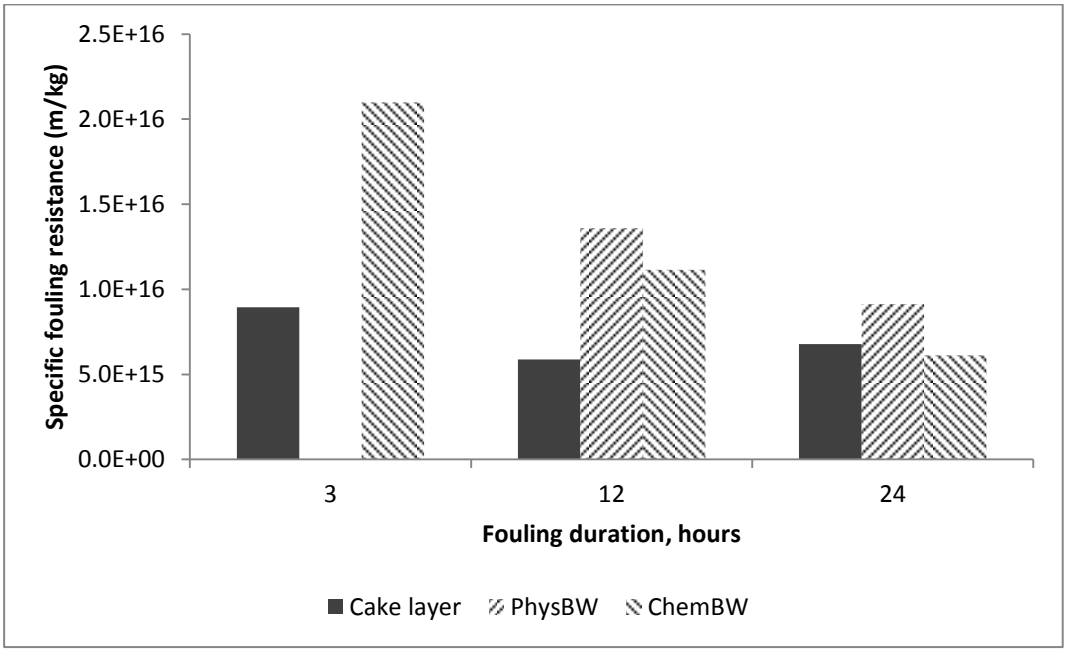


Figure 4.8 : Specific resistances vs. filtration time.

In other words, the resistance of foulants accumulated at membrane at a later time is less than that of previously accumulated foulants. On the contrary to this finding, it was observed that specific fouling resistance of cake layer increased with time in a study performed by model alginate solution **[**84**]**. Yet, while continuous operation was used in that study **[**84**]**, batch filtration (10 min operation and 1 min relaxation) was used in the current study, which might have caused the formation of cake layers different in structure. Secondly, the biological activity taking place in cake layer formed in this study makes direct comparison of the results difficult. As it is seen from Figure 4.8, while specific fouling resistances change between $0.6x10^{16}$ and $2.1x10^{16}$ [m/kg], specific fouling resistance of cake layer varies between $0.6x10^{16}$ – $0.9x10^{16}$ [m/kg]. In literature specific fouling resistance was generally calculated apart from MBR by filtering a predetermined volume of activated sludge mixed liquor by means of a dead-end filtration apparatus **[**32**,**37**,**47**,**164**,**198**,**199**,**200**]**. Nevertheless, it seems more accurate to measure specific fouling resistance by extracting foulants accumulated at membrane after filtration in MBR under investigation. This latter approach was adopted in this study. However, if this is not possible, specific fouling resistance should at least be calculated by activated sludge filtration in MBR, in place. In a previous study **[**54**]** specific fouling resistance measured by extracting foulants accumulated at membrane by phenol solution was

calculated to be $1x10^{16}$ [m/kg]. In another study **[**29**]** specific fouling resistance measured by rinsing cake layer was reported to be between 1.7 $x10^{16}$ and 3.2 $x10^{17}$ [m/kg], which is somewhat higher than those in this study possibly due to much longer operation duration (min 27 days). It is seen from Figure 4.8 that independent of filtration duration the specific fouling resistances of polymers attached to membrane pores tightly and loosely were always greater than specific fouling resistance of cake layer. In a previous study too **[**191**]** it was stated that the specific fouling resistances of intermediate and lower layers were greater than that of upper layer.

The relationship between foulants accumulated at membrane and the resistance they caused is shown in Figure 4.9. It is seen that there is a strong functional relationship (r^2 = 0.92) between foulants accumulated at membrane and their respective resistance. Fouling resistance increases with increasing amount of accumulated foulants. Nevertheless, this increase is not linear. As it is seen from Figure 4.9, resistance formation rate decreases as the amount of polymeric substances accumulated in/on membrane increases. For example, accumulation of approximately 20-mg/m^2 specific foulants in membrane caused a fouling resistance of $3.5x10^{11}$ [1/m]. When foulant accumulation increased averagely 24 times (~470 mg/m^2), the corresponding resistance increased only 8.5 times (~$29.7x10^{11}$ 1/m). In a previous study **[**54**]** it was stated that while a small amount of EPS accumulated at the beginning of the filtration caused a rapid increase in resistance due to pore plugging and closure, EPS accumulated afterwards caused a slower resistance increase forming a cake layer **[**54**]**.

It was also stated in other studies that membrane fouling was directly related to the amount of foulant accumulated at membrane.

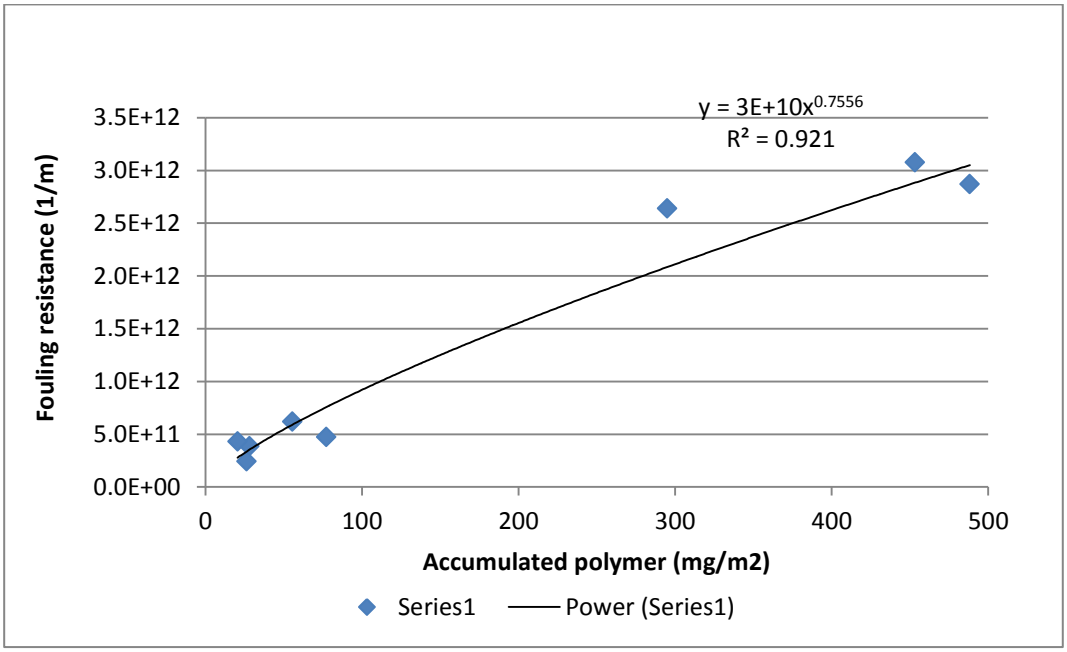


Figure 4.9 : Accumulated total polymer mass (SMP+EPS) vs. respective resistance.

For example, a very high correlation was observed between cumulative organic matter retained by membrane and TMP increase **[**166**]**. A linear relationship was found between cake layer resistance and the amount of sludge accumulated on membrane surface **[**165**]**. It was observed that membrane fouling was proportional to the total amount of bacterial EPS solution filtered through membrane surface **[**111**]**. Similarly, it was observed that irreversible membrane fouling at MBR had a strong exponential relationship with the amount of cumulative organic matter transported to membrane **[**62**]**. As it was explained in detail before in Sections 2.2.4 and 2.2.8, to be able to clarify membrane fouling the amount foulants accumulated at membrane should be considered together with changing conditions. For example, when the same amount of permeate was collected, membrane fouling at flux of 65 $L/m^2.h$ was observed to be greater than that at flux of 50 $L/m^2.h$ **[**158**]**. Yet, since the same amount of permeate was collected, the same amount of foulants should have been accumulated at both membranes, and the fouling of both membranes should have been same. However, when all the relevant conditions are considered it is seen that accumulation of different amount of fouling in these membranes is quite natural. Since the same filtration and backwashing regimes were used at both of fluxes, this probably caused different fouling profiles and backwash efficiencies at different fluxes. Actually, it was observed that the amount foulants accumulated at membrane

operated under flux of 65 L/m^2.h was greater than that under flux of 50 L/m^2.h. Considering the actual amount of foulants accumulated in membrane together with other conditions is especially important in two cases. The first case is that even if there is no change in MBR conditions such as activated sludge properties or membrane flux/TMP etc. membrane resistance is sure to increase to a point where membrane fouling is inevitable. In the second case even if there are substantial changes in MBR conditions, e.g., in activated sludge properties, a noticeable change may not be observed in filtration resistance. It is possible to see both of these cases in MBR studies, and both of these cases caused a lot of confusion in understanding membrane fouling phenomenon. In both of these cases membrane fouling cannot be correlated with a specific parameter, because a noticeable change is not observed in either independent variable (any parameter of MBR) or dependent variable (fouling resistance). For this reason the amount of foulants accumulated at membrane should be considered to be able to explain such cases. If the fouling in two cases is compared to each other, as usually done in the literature, this should be done under comparable conditions and operations. Otherwise, contradictory findings can be reached. Although the amount of foulants accumulated in PVDF membrane was nearly three times greater than that accumulated in PE membrane submerged into the same MBR, irreversible fouling of PE membrane was found to be greater than that of PVDF membrane at the end of the operation **[**115**]**. According to this result the amount of foulants accumulated in membrane cannot be used to evaluate membrane fouling, because there was a controversy between the amount accumulated and the resistance measured. On the other hand, before these measurements were done, PVDF membrane had been physically cleaned on 89th day of the operation (the operation was ceased on 140th day), whereas PE membrane had been chemically cleaned on 76th day of operation **[**115**]**. Nevertheless, if the amount of foulants accumulated in membrane had been compared on, e.g., 60th day when both membranes did not clean yet, this controversial result would have probably not taken place. Yet the reason of this contradictory observation in that study **[**115**]** is different. While the area of PE membrane used was 3 m^2, the area of PVDF membrane used was 1.3 m^2, which shows that the porosities of the membranes were quite different from each other. In this case, that a smaller amount of organic matter accumulated in PE membrane caused more membrane fouling is quite normal.

4.3 Correlation of Membrane Fouling with Microbial Polymeric Substances of MBR Sludge

Accumulation of polymeric substances in/on membrane is an indication of membrane fouling as it was seen in the Section 4.2. The correlation coefficients between the amount of polymers accumulated in membrane and polymer concentrations in MBR can show if there is a connection between membrane fouling and the concentration of polymeric substances in MBR. Table 4.3 shows these correlation coefficients.

Table 4.3 : Correlation coefficients between MPS in MBR sludge and accumulated at membranes.

Parameter	Protein Concentration (mg/L)				Carbohydrate Concentration (mg/L)			
	SMP_f	SMP_t	EPS_f	EPS_t	SMP_f	SMP_t	EPS_f	EPS_t
$CakeSMP_f$	0.875	-0.744			-0.989	-0.807		
$CakeSMP_t$	0.960	-0.052			-0.972	-0.527		
$CakeEPS_f$			-0.381	0.270			-0.518	0.050
$CakeEPS_t$			-0.411	0.238			-0.475	0.100
PhysBW	0.956	-0.590			-1.000	-0.723		
ChemBW	0.513	-0.979			-0.983	-0.827		

[a] Lowercase letters denote f: filterable fraction, t:total fraction.

Some correlations, such as this between PhysBW and EPSf, were not calculated because their values do not mean anything in reality. According to the definition adopted in this study, as it was stated in Section 3.3, the EPS term was used to denote microbial polymeric substances which are attached to activated sludge flocs. For this reason, intrusion of EPS into membrane pores is not possible. Accordingly, the correlation coefficients between EPS concentration of MBR and the amount of polymeric matter accumulated in membrane pores are not meaningful. In spite of this elimination it is seen from Table 4.3 that while there are some high and reasonable correlations (e.g., correlation between CakeSMPf and SMP_f for protein); there are also some unreasonable correlations such as the correlation between $CakeSMP_f$ and SMP_f for carbohydrate, which is -0.989. According to the latter correlation, filterable SMP carbohydrate concentration in cake layer decreases with increasing

concentration of the same parameter in MBR. In other words, as the concentration of filterable SMP carbohydrate in MBR increases, a part of cake layer fouling resulted from carbohydrate accumulation decreases. This shows that the correlation coefficients between membrane fouling and polymeric substances in MBR should be evaluated not only by numbers but also with their physical meaning. The correlation coefficients between polymeric matter concentration of MBR and fouling resistances are shown in Table 4.4.

Table 4.4: Correlation coefficients between MPS of MBR sludge and membrane resistances.

Parameter	Protein concentration (mg/L)				Carbohydrate concentration (mg/L)			
	SMP_f	SMP_t	EPS_f	EPS_t	SMP_f	SMP_t	EPS_f	EPS_t
R_c	0.228	-0.995	-0.819	-0.291	-0.998	-0.665	-0.267	0.319
R_{p1}	0.538	0.620			0.741	0.999		
R_{p2}	1.000	-0.343			-0.183	0.561		

[a] Lowercase letters denote f: filterable fraction, t:total fraction.

It is noteworthy that reversible pore resistance (R_{p1}) had a very high correlation with carbohydrate component of SMP in mixed liquor while irreversible pore resistance (R_{p2}) exhibited a very high correlation with protein component of SMP in mixed liquor. According to these correlations while the contribution of carbohydrates to reversible pore fouling resistance resulted from polymeric substances loosely attached to membrane pores is greater, proteins contribute more to irreversible pore fouling resulted from polymeric substances tightly attached to membrane pores. The amount of cumulative filtered polymers gave higher and more reasonable correlations than the instantaneous concentration of polymeric substance in MBR sludge with the amount of polymeric substances accumulated in/on membrane (Table 4.5). This is very obvious especially for cake layer EPS concentrations. While the correlations between the concentration of EPS protein and carbohydrate in MBR sludge and those in cake layer changed between – 0.518 and 0.270 (see Table 4.3), the correlations obtained by the cumulative filtered polymers changed between 0.739 and 0.999. This shows clearly that membrane fouling in MBR is proportional to the

total amount of microbial polymeric substances transported to membrane surface rather than the concentrations of MBR sludge parameters.

Table 4.5 : Correlation coefficients between total specific filtered MPS and accumulated foulants at membranes[a].

Parameter	Total protein filtered per membrane area				Total carbohydrate filtered per membrane area			
	SMP_f	SMP_t	EPS_f	EPS_t	SMP_f	SMP_t	EPS_f	EPS_t
$CakeSMP_f$	0.898	0.941			0.896	0.802		
$CakeSMP_t$	0.321	0.425			0.997	0.967		
$CakeEPS_f$			0.993	0.999			0.739	0.759
$CakeEPS_t$			0.996	0.998			0.772	0.790
PhysBW	0.787	0.851			0.946	0.873		
ChemBW	0.998	0.983			0.881	0.781		

[a] Lowercase letters denote f: filterable fraction, t:total fraction.

As it is seen from Table 4.5 the amounts of SMP and EPS accumulated on membrane surface exhibits quite high correlations with cumulative amount of SMP and EPS filtered. Similarly, polymeric foulants attached loosely and tightly to membrane pores exhibit quite high correlation with, respectively, cumulative amount of filterable SMP carbohydrate filtered and cumulative amount of filterable SMP protein filtered.

4.4 Temporal Change of Foulant Accumulation at Membrane

The amount of total foulants accumulated at cake layer (M_c) formed on membrane surface is calculated as follows;

$$M_c\ [mg/m^2] = Total\ SMP + Total\ EPS$$

$$Total\ SMP\ [mg/m^2] = total\ SMP\ protein + total\ SMP\ carbohydrate$$

$$Total\ EPS\ [mg/m^2] = total\ EPS\ protein + total\ EPS\ carbohydrate$$

The change of the amount of MPSs accumulated in cake layer with filtration duration is shown in Figure 4.10. It is seen that total amount of polymeric matter accumulated in cake layer increased when filtration duration was raised from 3 to 12 h. A further increase of filtration duration resulted in a decrease in the amount of accumulated

matter. Nevertheless, this may have been resulted from rinsing step of 24-h fouling experiment. It is seen that the amount of microbial polymeric matter accumulated on membrane surface exhibits a good correlation with a power function.

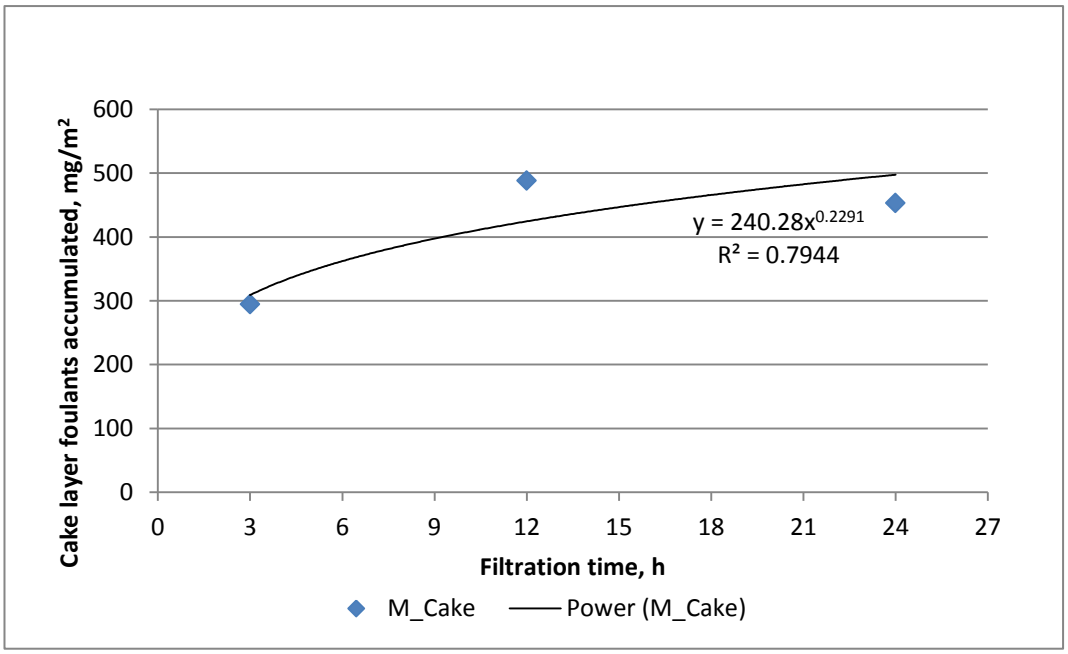


Figure 4.10 : Cake layer foulant accumulation with filtration time.

Temporal change of accumulation of polymeric substances loosely attached to membrane pores (M_PhysBW) is shown in Figure 4.11. It is seen that loosely bound pore foulants did not exhibit a clear relation with filtration duration. The concentration of loosely bound pore foulants was below 1 mg/L at initial filtration times (t = 3h). The amount of tightly bound pore polymers (M_ChemBW) increased with filtration duration (Figure 4.12). It is seen that the increasing rate of polymers tightly attached to membrane pores followed a power function very well. The amount of total MPS accumulated in/on membrane can be stated in the following form;

Total MPS [mg/m^2] = M_Cake + M_PhsyBW + M_ChemBW

By calculation in this way the amount of total polymeric substances accumulated at membrane followed a pattern with filtration duration similar to the accumulation rate observed for cake layer (Figure 4.13).

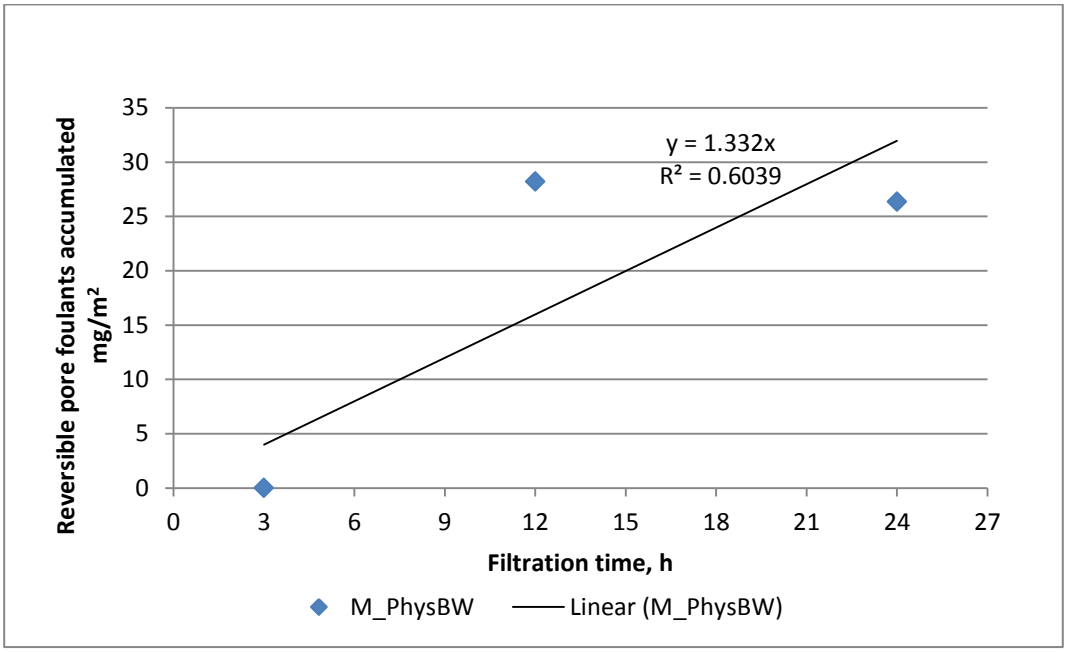


Figure 4.11 : Reversible pore foulant accumulation with filtration time

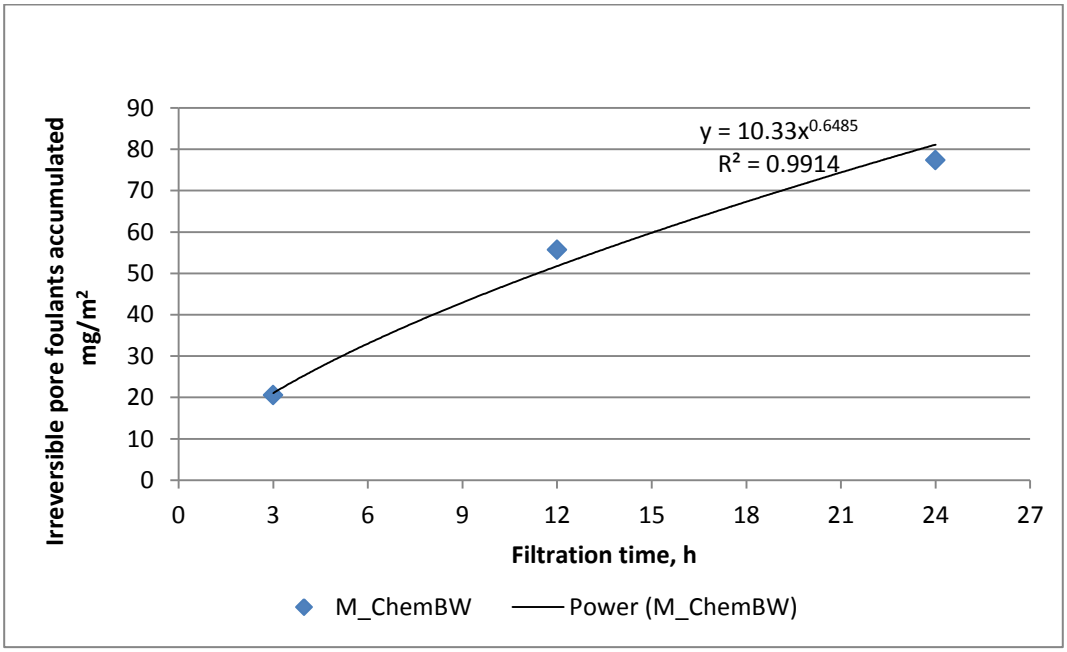


Figure 4.12 : Irreversible pore foulant accumulation with time

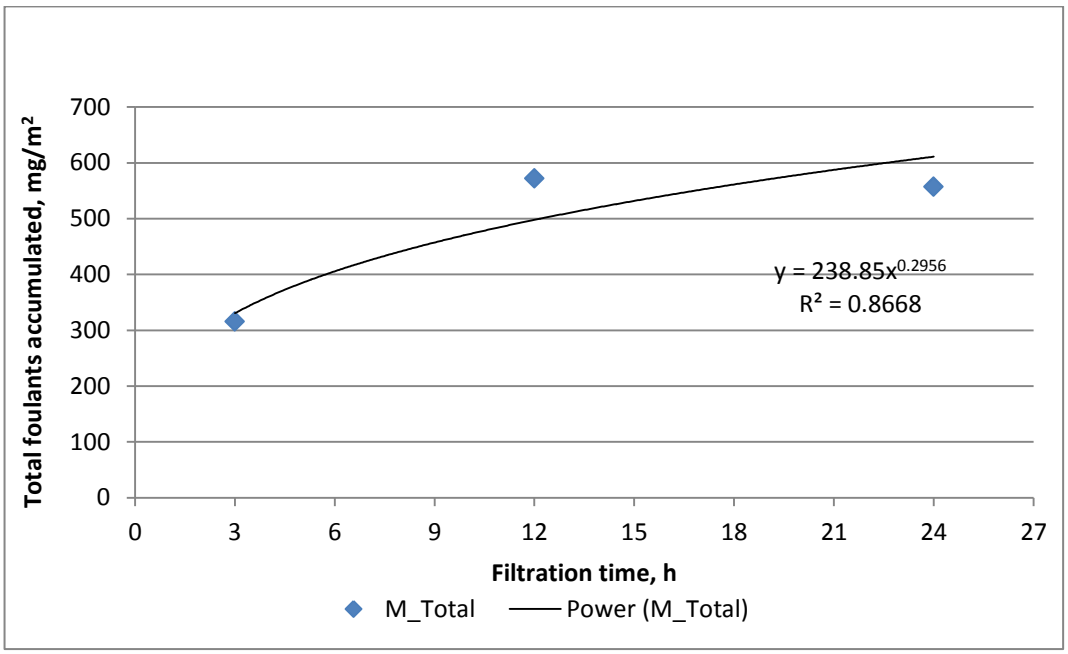


Figure 4.13 : Total polymer accumulation with time.

4.5 The Temporal Change of Filtration Resistances

The change of cake layer resistance with filtration duration is shown in Figure 4.14.

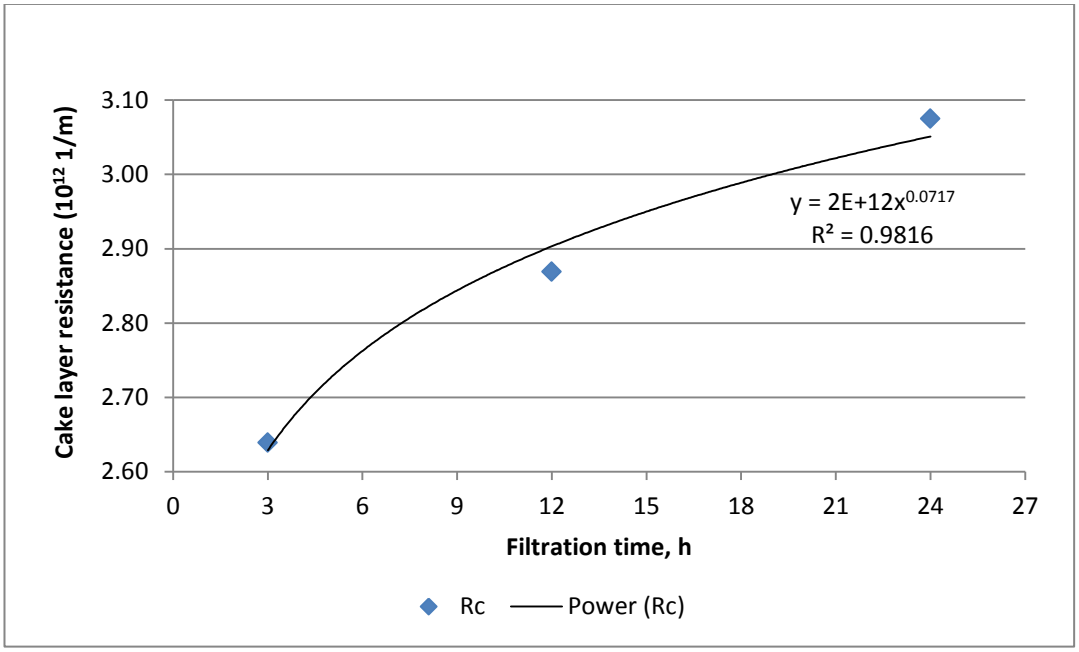


Figure 4.14 : Cake layer resistance with filtration time.

It is seen that cake layer resistance increased with filtration duration following a power function very well. In addition, the rate of cake layer occurrence decreased with increasing filtration duration. It was calculated that while the rate of cake layer

resistance increase was 0.026 [1/m.h] until 12 h, it decreased to 0.018 [1/m.h] after 12 h. When filtration duration increased four times (from 3 to 12 h) cake layer resistance increased by 8.7 %, and when filtration duration was raised eight times (from 3 to 24 h) cake layer resistance increased by 16.7 %. This shows that cake layer did not grow very much after its first formation at initial stage of filtration operation. The change of reversible pore fouling with filtration duration was not orderly (Figure 4.15). However, it stayed between $2.40x10^{11}$ – $3.85x10^{11}$ [1/m], which shows it also did not change very much.

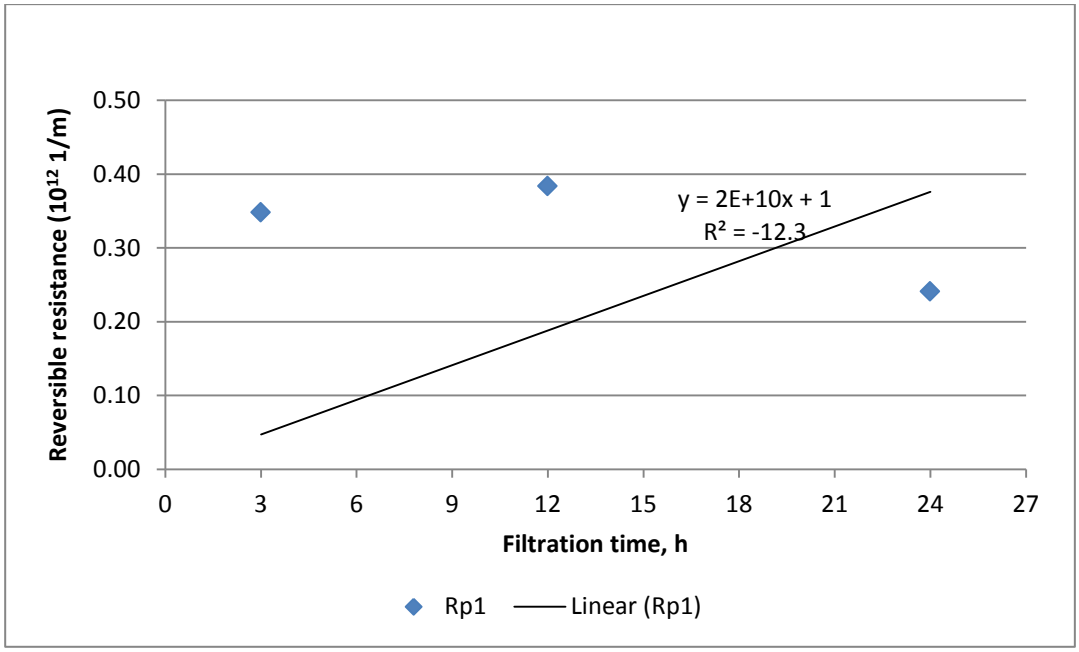


Figure 4.15 : Reversible pore fouling resistance with time.

As it was mentioned in Section 4.4, the amount polymeric substances loosely attached to membrane pores was below 1 mg/L. Nevertheless, as it is seen from Figure 4.7 (b) a 9.9-% reversible resistance was measured due to loosely bound pore foulants. This shows that even a very small amount of foulants accumulated in membrane cause a resistance that cannot be ignored. Similar to reversible pore fouling resistance irreversible pore fouling resistance also did not exhibit a clear correlation with filtration duration (Figure 4.16). This resistance also did not change very much since its initial value. Apart from other fouling resistances irrecoverable resistance that was not able to be removed after all the cleaning procedure used in the scope of this study increased exponentially with filtration duration (Figure 4.17). This irrecoverable resistance followed an exponential function very well.

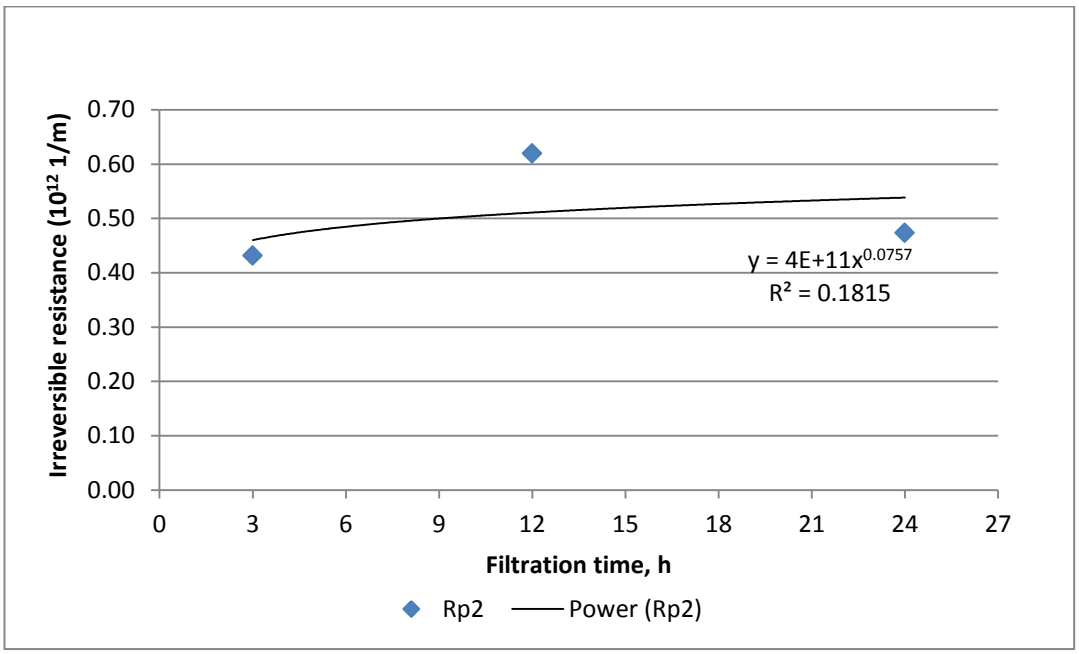


Figure 4.16 : Irreversible pore fouling resistance with filtration time.

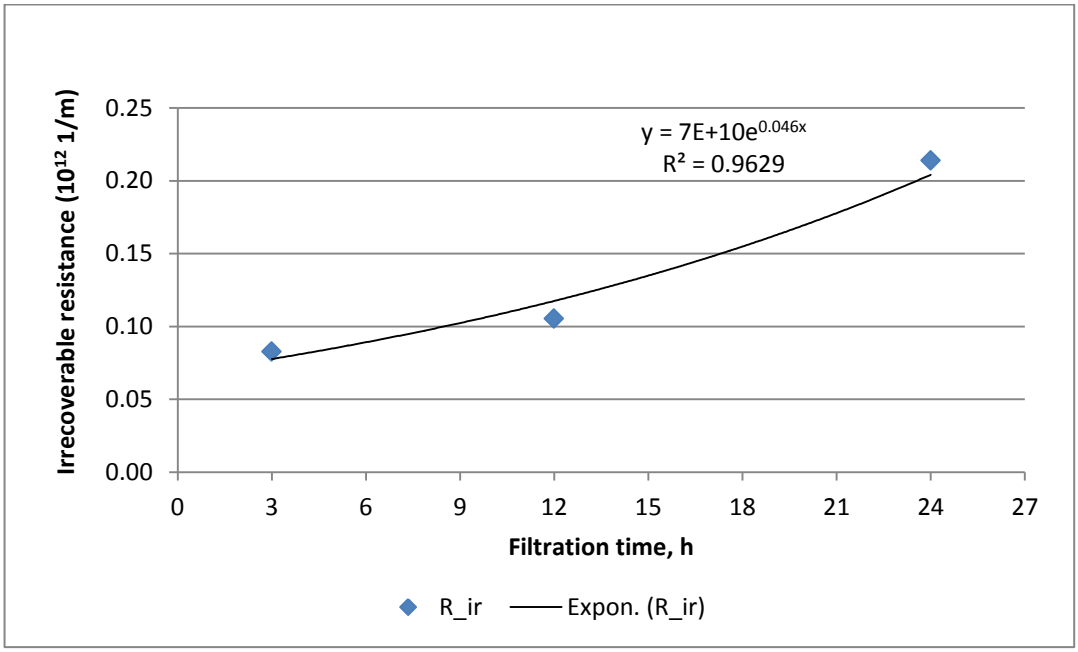


Figure 4.17 : Irrecoverable resistance with time.

As it is seen from Figure 4.18 the change of total filtration resistance can also be stated by a power function. In a previous study too, which was performed under similar conditions (SRT = 30 d, TMP = 15 kPa, T = 25 ℃), total filtration resistance increased with filtration duration following a power function **[**41**]**.

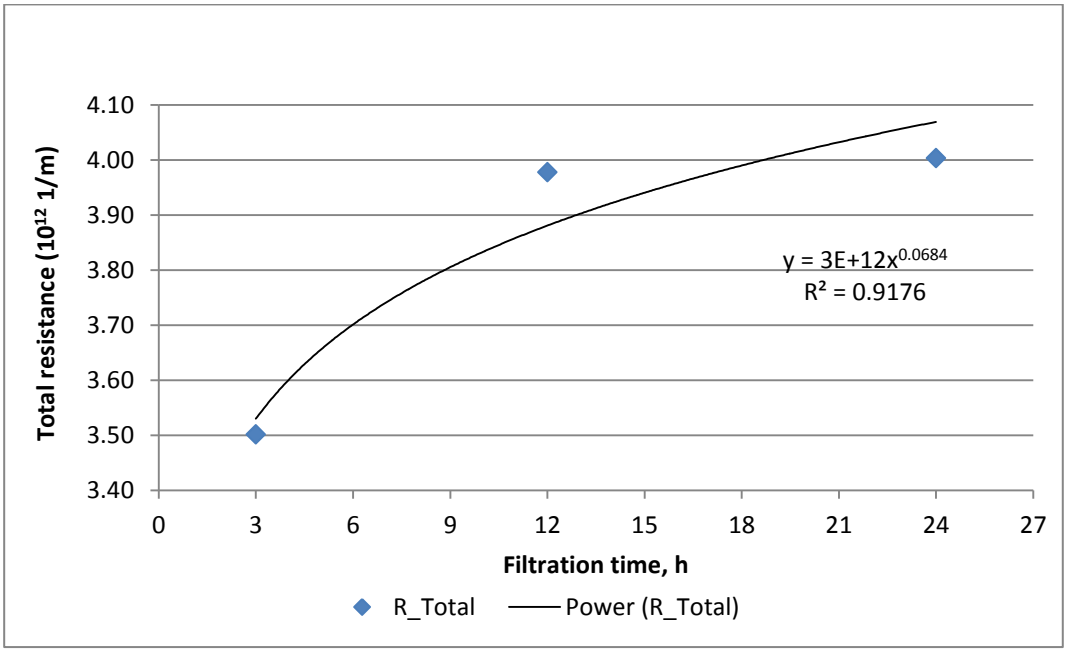


Figure 4.18 : Total filtration resistance with filtration time.

It was seen in Section 2.2.8 that membrane fouling was generally related to the cumulative amount of permeate obtained from membrane. The change of the amount of foulants accumulated in/on membrane and their fouling resistances according to permeate volume collected is given at Figures B1 – B9. This graphs show that the amount of foulants accumulated in/on membrane and their fouling resistances exhibit better correlations with the cumulative amount of permeate collected than those obtained by filtration duration. This shows that the amount of uncertainties in membrane fouling development can be further reduced using the cumulative permeate volume instead of filtration duration as an independent variable.

4.6 Modeling Membrane Fouling

Membrane fouling in MBRs was generally modeled by resistance increase resulted from MLSS accumulated on membrane surface depending on the total volume of sludge suspension delivered to the membrane surface [27,174,175,179,201]. Sometimes EPS accumulated on membrane surface was used instead of MLSS **[**176**]**. Nevertheless, the amount of foulants accumulated in/on membrane was not measured experimentally in these studies. The findings obtained at Sections 4.1 and 4.2 indicated that the main reason of membrane fouling was the accumulation of foulants, which were microbial polymeric substances in this study, in membrane.

While the amount of polymeric substances accumulated at membrane increases, fouling resistances namely membrane fouling also increases. As it was mentioned before in Section 2.2.4 membrane fouling was related to total filtered permeate volume. However, a model of membrane fouling based on experimental measurements of the amount of foulants accumulated in/on membrane would be more compatible with reality. As it was seen in Sections 4.3 and 4.5 both the amount of polymeric matter accumulated in/on membrane and their resistances can be stated by a power function. In this section membrane fouling was modeled using data from both the total amount of polymeric substances delivered to the membrane surface (total amount of MPS filtered) and experimentally measured amount of foulants accumulated in membrane. Fouling was stated in terms of resistance increase. According to this approach fouling resistance is stated with the following equations;

$$R_T = R_m + R_f \quad \textbf{(4.1)}$$

$$R_f = a_1.[a_2.\sum_{t=0}^{t=T} C_{SMP}.V(t) + a_3 \sum_{t=0}^{t=T} C_{EPS}.V(t)]^{a_4} \quad \textbf{(4.2)}$$

where R_T: total membrane resistance [1/m], R_m: membrane resistance, R_f: total fouling resistance, C_{SMP}: SMP concentration of activated sludge, EPS: EPS concentration of activated sludge, V(t): total permeate volume at time t, T: total filtration duration, a_1, a_2, a_3 and a_4 are constants. Constants were found using data of 24-h filtration by least squares method, in other words 24-h filtration data were used for model calibration. By this way the constants found are;

$$\begin{bmatrix} a_1 & a_2 \\ a_3 & a_4 \end{bmatrix} = \begin{bmatrix} 1.28\text{E}+09 & 1.84\text{E}+07 \\ 8.51\text{E}+08 & 3.21\text{E}-01 \end{bmatrix} \quad \textbf{(4.3)}$$

The comparison of the resistances estimated by this model for 3-h and 12-h filtration with the measured resistances is shown in Figure 4.19. By this model while the resistances of 3-h filtration was able to be estimated very well, the resistances estimated for 12-h filtration were somewhat high.

As it was mentioned above, membrane fouling can be modeled better by experimentally measured amount of foulants accumulated in/on membrane in terms of real conditions. When only the amount of foulants delivered to membrane surface is considered, the amount of foulant accumulated in/on membrane calculated by such a model may be very different from the actual amounts of foulants accumulated. This is also valid when membrane resistances have been modeled without measuring

them. A very well fitted model can be established by this way; nonetheless this model does not reflect the reality.

The second modeling approach used in this study was to model membrane fouling by using a foulant accumulation function which was determined by measuring the amount of foulants accumulated at membrane. Accordingly, this second approach is based on the actual amount of foulants accumulated in/on membrane. Fouling resistance here is stated in terms of the amount of foulants accumulated in/on membrane.

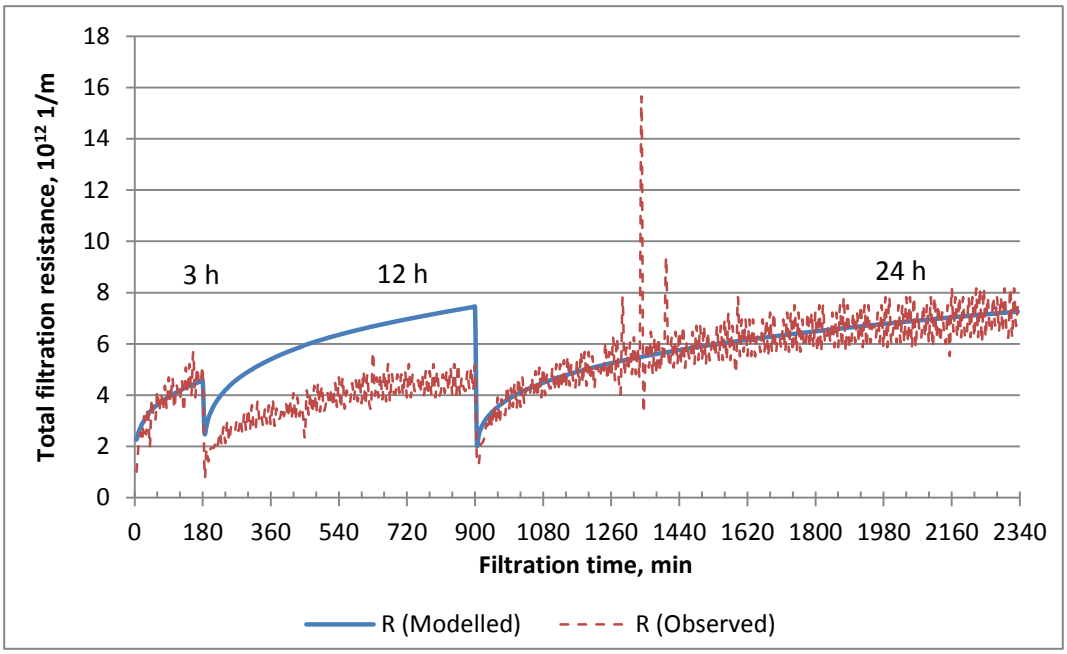


Figure 4.19 : Modeling of membrane filtration based on total filtered MPS.

This model can be stated as follows. Total membrane filtration resistance (R_T) is again defined as in Eq. 4-2. However, R_f is defined as follows;

$$R_f = b_1.\varphi^{b_2} \quad \textbf{(4.4)}$$

$$\varphi = b_3.[V(t)]^{b_4} \quad \textbf{(4.5)}$$

where φ: net MPS accumulation function, b_1, b_2, b_3 and b_4 are constants. The coefficients of MPS accumulation function is found from Figure B – 4 as follows;

b_3 = 38.67 and b_4 = 0.3954. Using these constants fouling function is written as in the following form;

$$R_T = R_m + b_1.[38.67[V(t)]^{0.3954}]^{b_2} \quad \textbf{(4.6)}$$

Using 24-h filtration data for model calibration, the coefficients of this model were found as b_1 = 3.98E+10 and b_2 = 0.8126 by least squares method.

The fouling resistance developments estimated by this model for 3-h and 12-h filtration durations are shown in Figure 4-20 together with their observed values. This model established by an experimentally measured foulant accumulation function estimated 12-h total membrane filtration resistances better than the model based on the amount of MPS delivered to membrane surface, which used the concentration of MPS in activated sludge. Although the zigzagging structure of total membrane resistances is not seen here (Figure 4-20), which can be done by adding more parameters to the model, it can be said that this is quite a good result for such a relatively simple model.

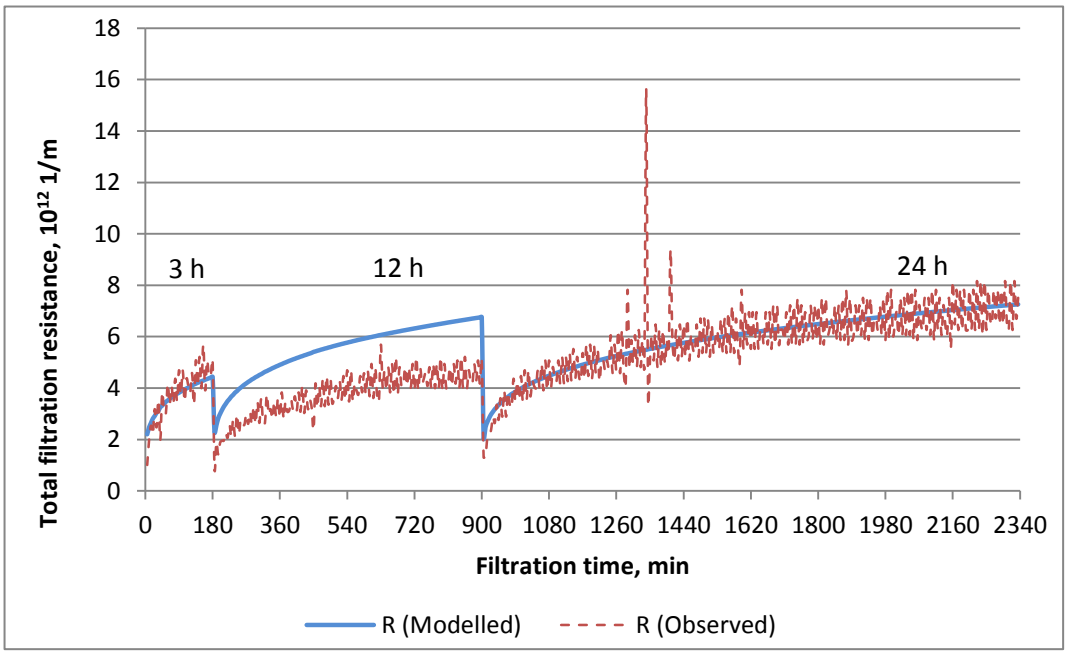


Figure 4.20 : Modeling of membrane filtration based on extracted MPS.

Modeling of membrane fouling with experimentally measured total amount of foulants accumulated at membrane is especially important because relating membrane fouling directly to the amount of foulants accumulated at membrane by this way seems more compatible to the nature of the phenomenon. Actually, that a model contains much more parameters and very complex physical relations does not mean that this model will be successful. For example, in a membrane separation model of a MBR process based on the accumulation of cake layer depending on the

amount of permeate obtained, net biomass accumulation on membrane surface was defined as;

$$\frac{dM_{sf}}{dt} = ECJ - k_d M_{sf} \quad \textbf{(4.7)}$$

where M_{sf}: the amount of sludge on membrane, E: coefficient, J: flux, k_d: sludge scouring ratio **[**175**]**. The complexity of this model was intermediate and it contained 49 model parameters. It was seen that although the model successfully estimated some experimental results, it failed in estimation of remaining substantial portion of results. This was attributed to the complex nature of MBR resulting from changing activated sludge properties within time and variable nature of specific cake resistance in spite of the same amount of cake accumulation on membrane **[**175**]**.

5. CONCLUSIONS AND RECOMMENDATIONS

In this study investigating the membrane fouling in a submerged membrane bioreactor used for treatment of synthetic domestic wastewater the following results were obtained;

1. The greatest microbial polymeric substance accumulation in membrane filtration occurred at cake layer. This was followed by the amount of foulants tightly and loosely attached to membrane pores, respectively.
2. The greatest membrane resistance in other words membrane fouling resulted from the accumulation of cake layer formed on membrane surface. While the contribution of cake layer resistance to total fouling resistance was between 72.1 – 76.8 %, those of irreversible fouling, reversible fouling and irrecoverable fouling were 11.8 – 15.6 %, 6.0 – 9.9 % and 2.4 – 5.3 %, respectively.
3. A very strong functional relationship in the form of $y = 3000x^{0.7556}$ (r^2= 0.92), where x and y denote total fouling resistance and total microbial polymeric substances accumulated in/on membrane respectively, was observed between the amount of total microbial polymeric substances and the fouling resistance they caused.
4. It was observed that the amount of protein accumulated on membrane surface was always greater than that of carbohydrate irrespective of filtration duration. Since the concentrations of protein and carbohydrate in MBR mixed liquor were very close to each other and majority of protein accumulated on membrane surface resulted from SMP accumulated on the membrane surface, it can easily be concluded that proteins attach to membrane surface more tightly than carbohydrates. Otherwise, the proportion of protein and carbohydrate accumulated on membrane surface should have been approximately equal. This shows that proteins are stickier than carbohydrates.

5. The gel-like cake layer accumulated on membrane surface formed in very beginning of the filtration, and it preserved its dominance as the major fouling element throughout the whole filtration duration.
6. Total amount of foulants rather than filterable portion should be taken into consideration in membrane fouling studies in MBRs. Otherwise, a substantial amount of foulants may be omitted from analyzes although they contribute to membrane fouling.
7. The accumulation of foulants in/on membrane with filtration duration or total permeate volume can be stated by a power function with a high correlation. The specific function in this study was $y = 38.367x^{0.3954}$ (r^2= 0.97), where x and y denote total permeate volume and total polymer accumulated in/on membrane, respectively.

In the light of these findings the following recommendations can be enumerated for the better membrane fouling studies to be performed in the future;

1. Since the most important fouling factor is cake layer occurrence, the research of techniques mitigating/delaying the cake layer occurrence or leading to a more porous cake layer will be quite useful. Optimization of flocculant/coagulant/adsorbent addition in terms of efficiency, economy and side effects can be researched.
2. More attention should be paid to the investigation of techniques mitigating the adherence of proteins to membrane surface or providing their scouring in an easier way. In this regard the investigation of the preparation of membranes having less surface porosity or use of enzyme immobilized membranes would be useful.
3. Concentrating on membrane itself rather than reactor inside would be more useful in revealing the reasons of membrane fouling and its mechanism.
4. Utilization of controlled experimental techniques in designing the experimental structure of membrane fouling studies will be quite important in terms of more accurate, convincing and conclusive results.

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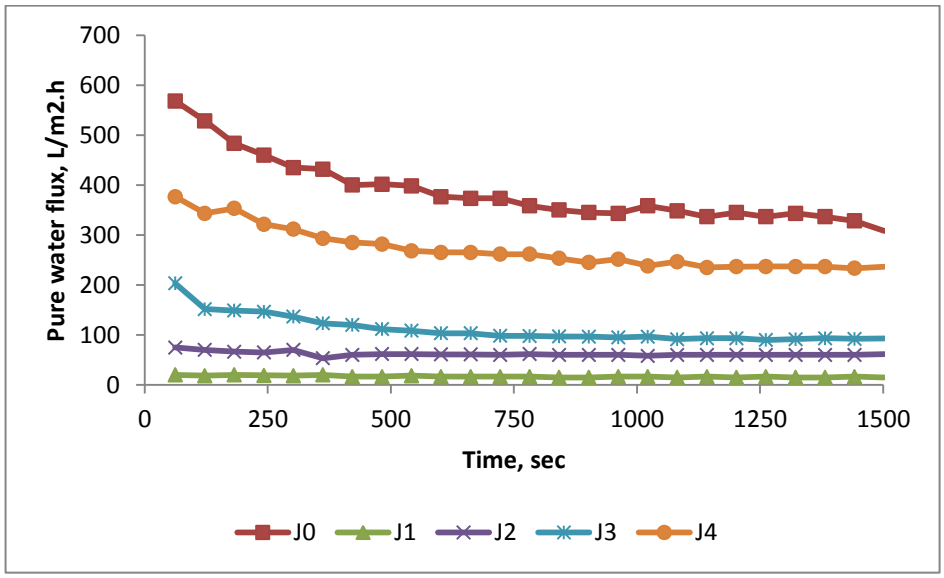
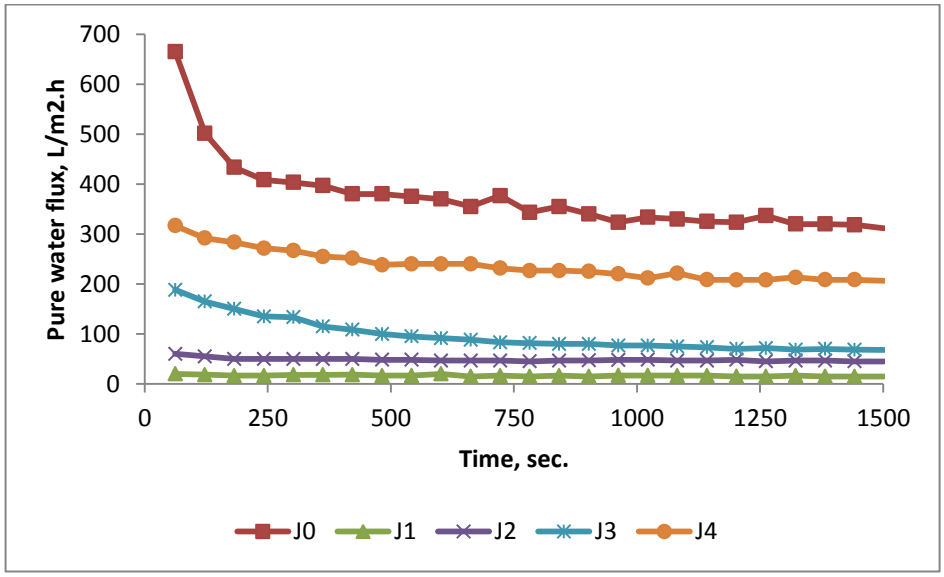
APPENDICES

APPENDIX A **:** Pure water flux measurements for resistance calculations
APPENDIX B **:** Foulant accumulation and individual resistance change with permeate volume
APPENDIX C **:** Protein and carbohydrate concentrations of original samples with their standard errors

APPENDIX A : Pure water flux measurements for resistance calculations

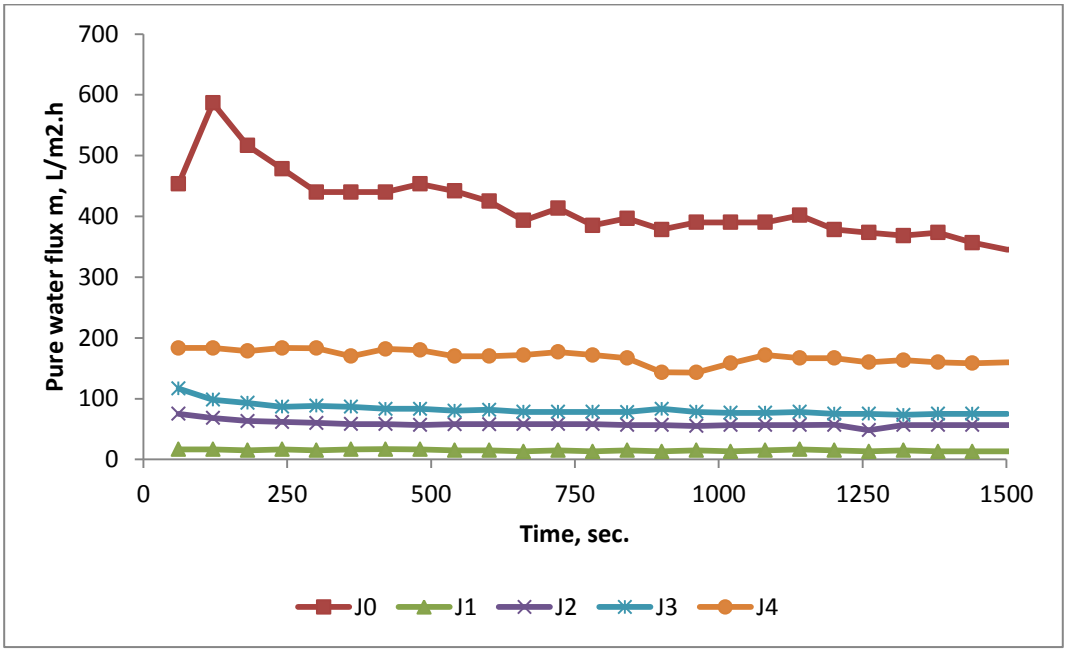


(a)



(b)

Figure A.1 : Pure water fluxes measured in fouling studies (a) 3h, (b) 12h and (c) 24h filtration times. Here J0, J1, J2, J3 and J4 denote pure water fluxes of new membrane, after sludge filtration, cake layer rinsing, pure water backwashing and chemical backwashing, respectively.



(c)

Figure A.1 (Continued) : Pure water fluxes measured in fouling studies (a) 3h, (b) 12h and (c) 24h filtration times. Here J_0, J_1, J_2, J_3 and J_4 denote pure water fluxes of new membrane, after sludge filtration, cake layer rinsing, pure water backwashing and chemical backwashing, respectively.

Table A.1 : Limit fluxes determined for resistance calculations after each fouling measurement step (Section 3.2).

Filtration Time (h)	**Limit flux (L/m2.h)**				
	J_0	J_1	J_2	J_3	J_4
3	345	17	60	90	237
12	320	15	48	68	208
24	368	15	57	73	163

APPENDIX B : Foulant accumulation and individual resistance change with permeate volume

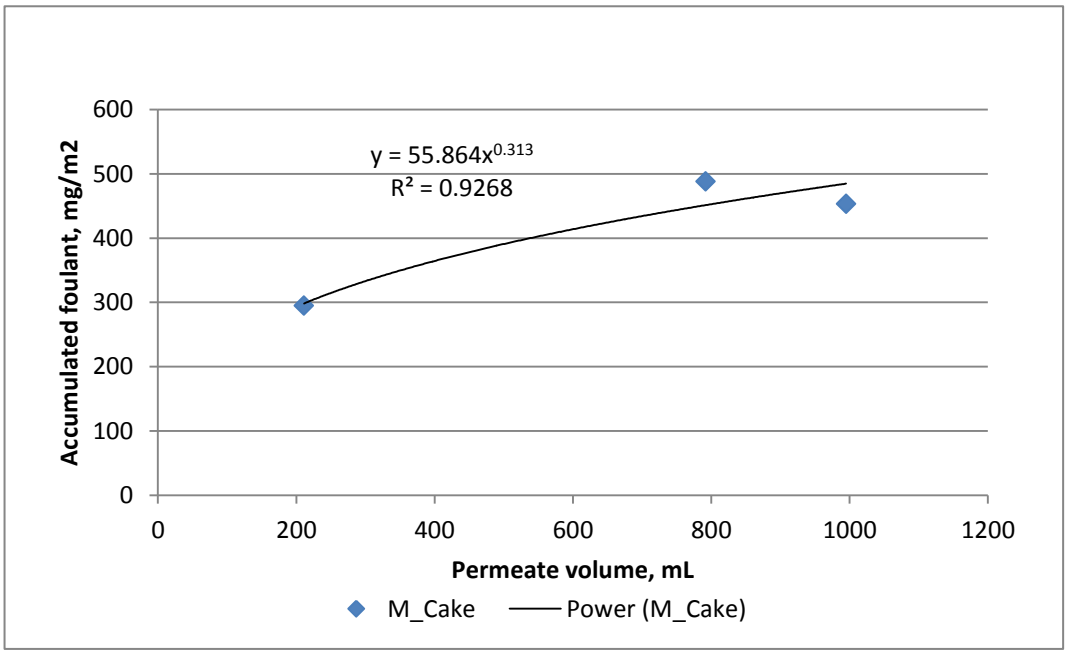


Figure B.1 : Cake layer foulant accumulation vs. cumulative permeate volume.

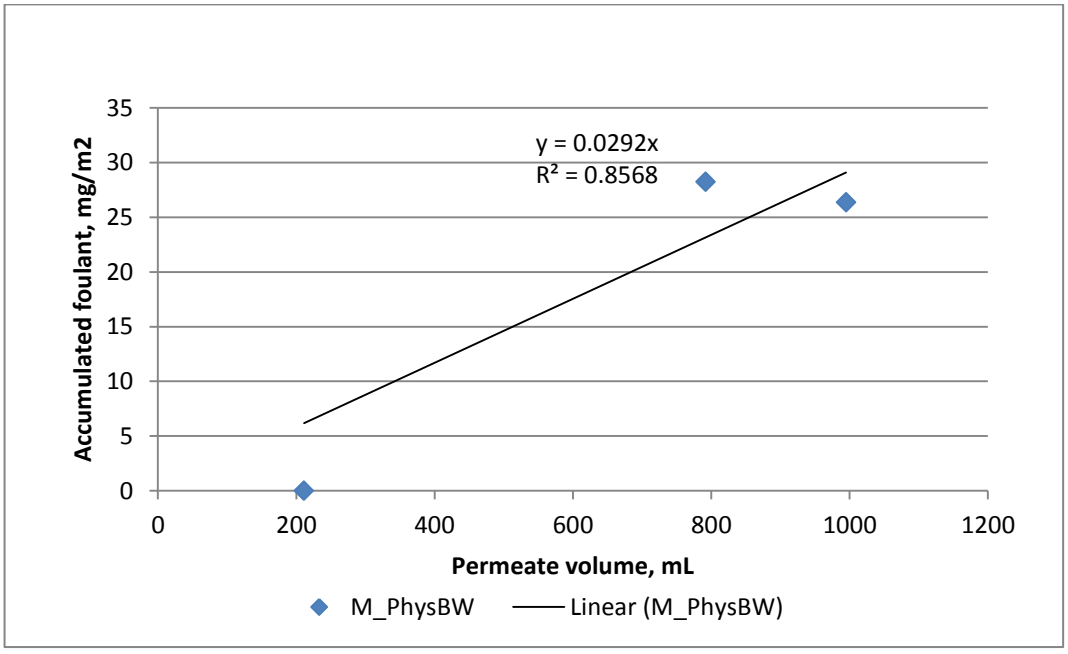


Figure B.2 : Reversible pore foulant accumulation with cumulative permeate volume.

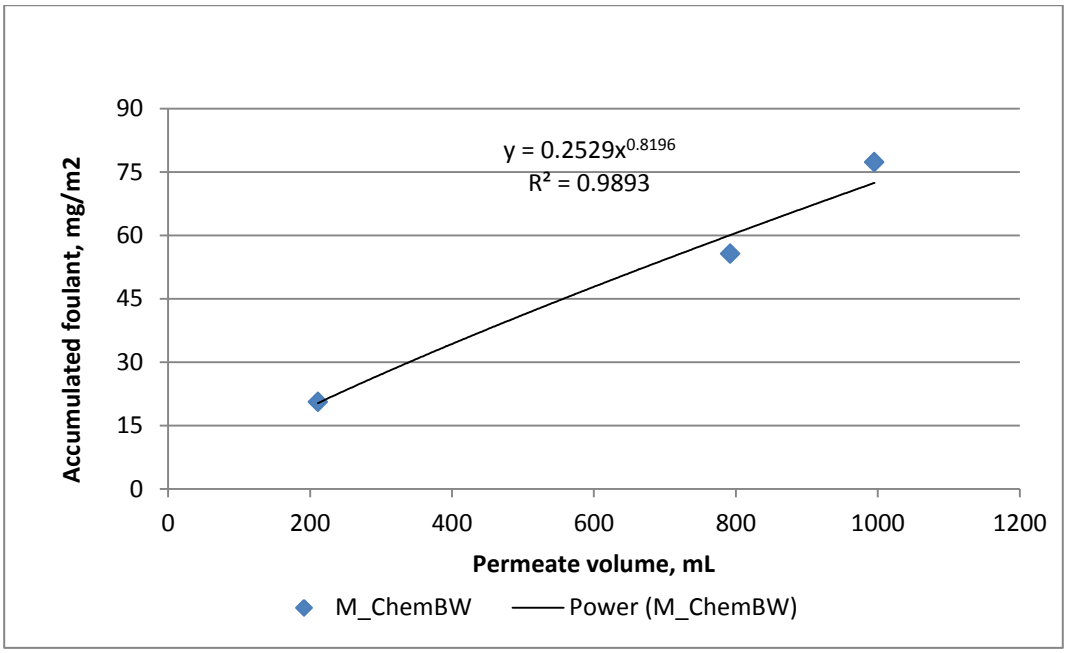


Figure B.3 : Irreversible pore foulant accumulation with cumulative permeate volume.

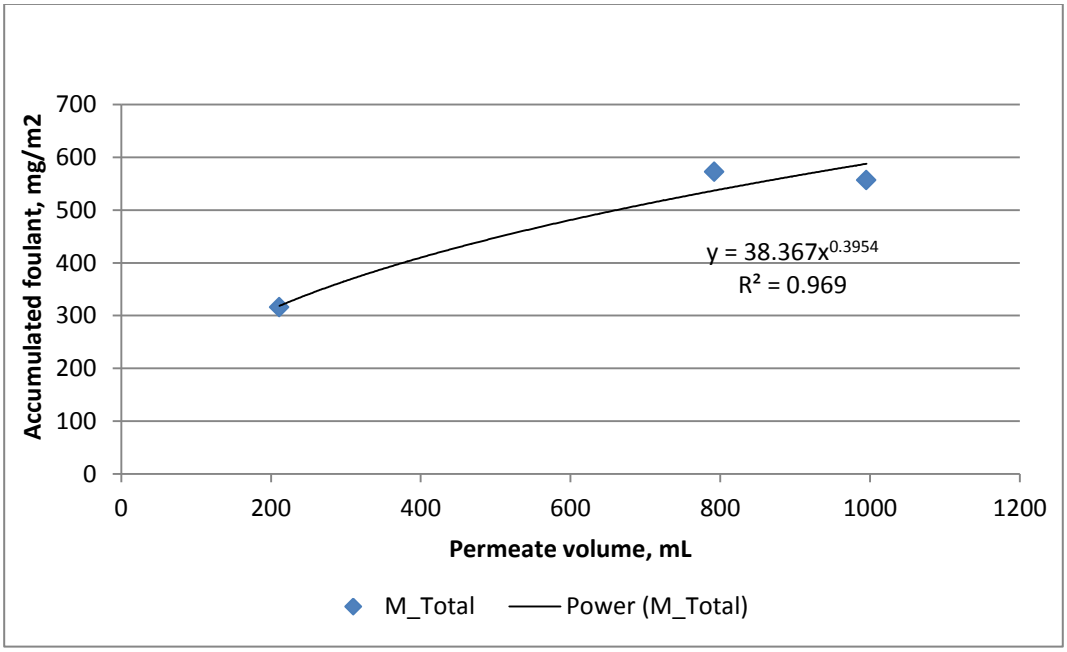


Figure B.4 : Total polymers accumulated at membrane vs. cumulative permeate volume.

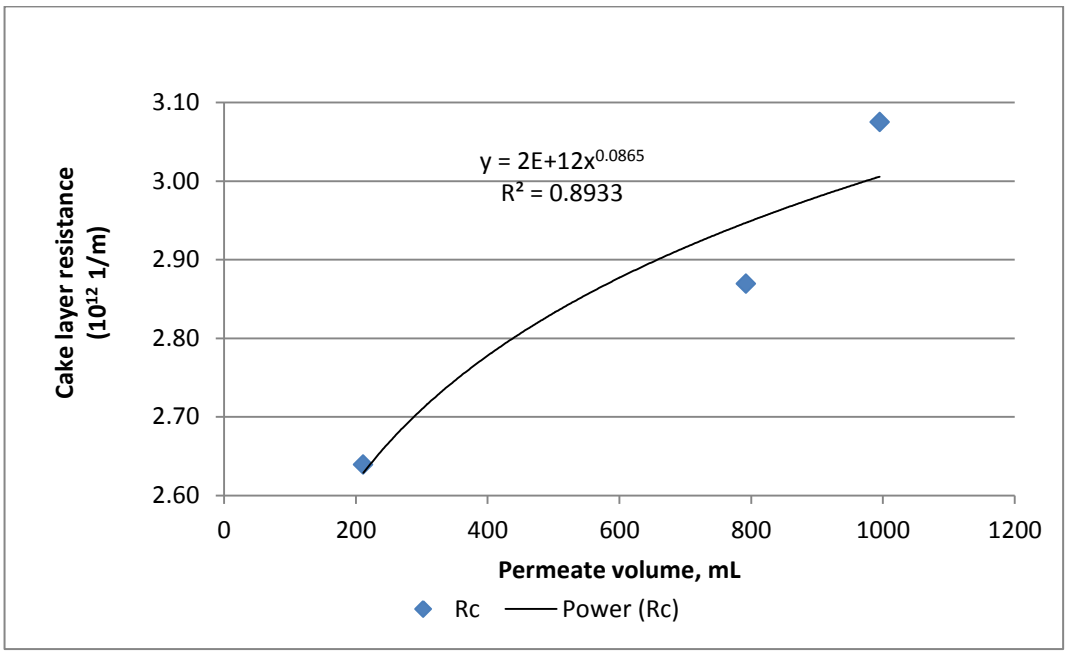


Figure B.5 : Cake layer resistance with cumulative permeate volume.

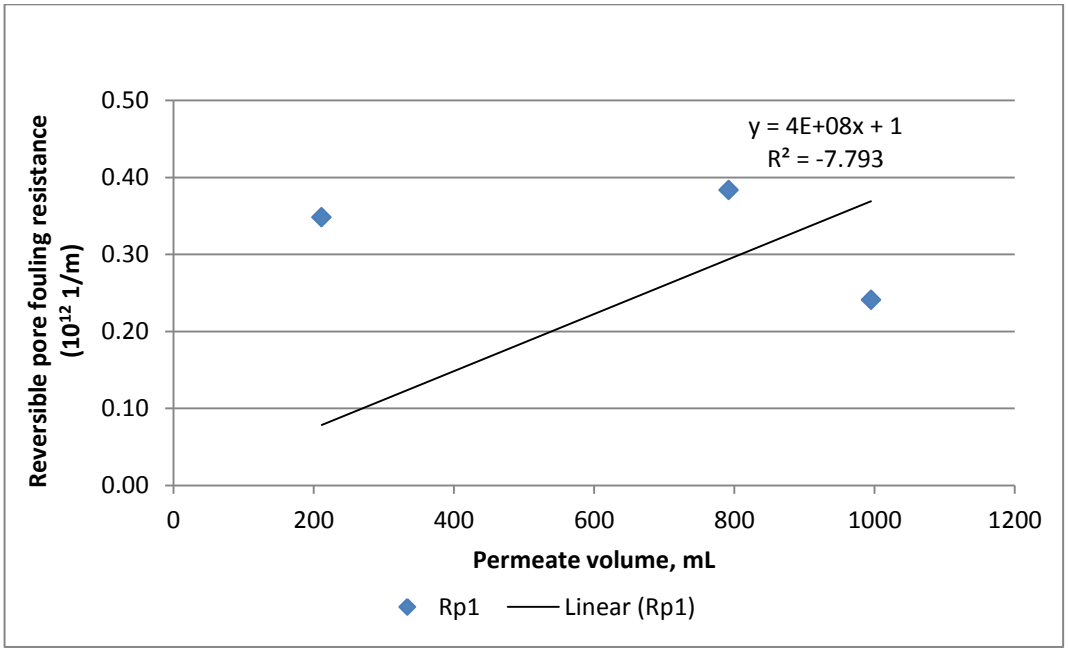


Figure B.6 : Reversible pore fouling resistance with cumulative permeate volume.

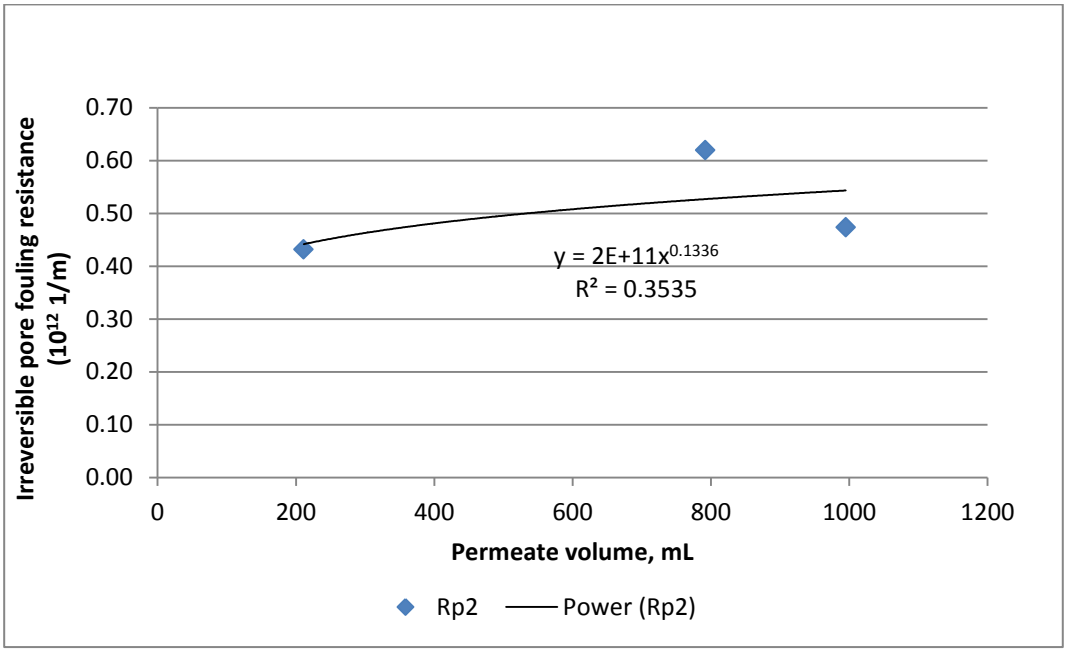


Figure B.7 : Irreversible pore fouling resistance with cumulative permeate volume.

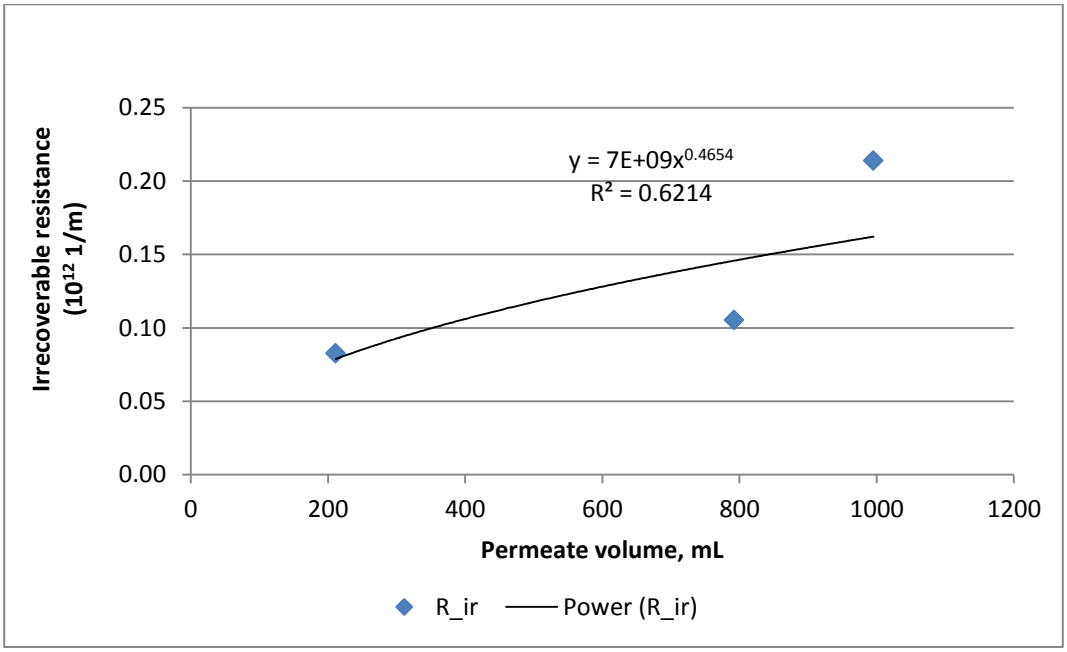


Figure B.8 : Irrecoverable resistance with cumulative permeate volume.

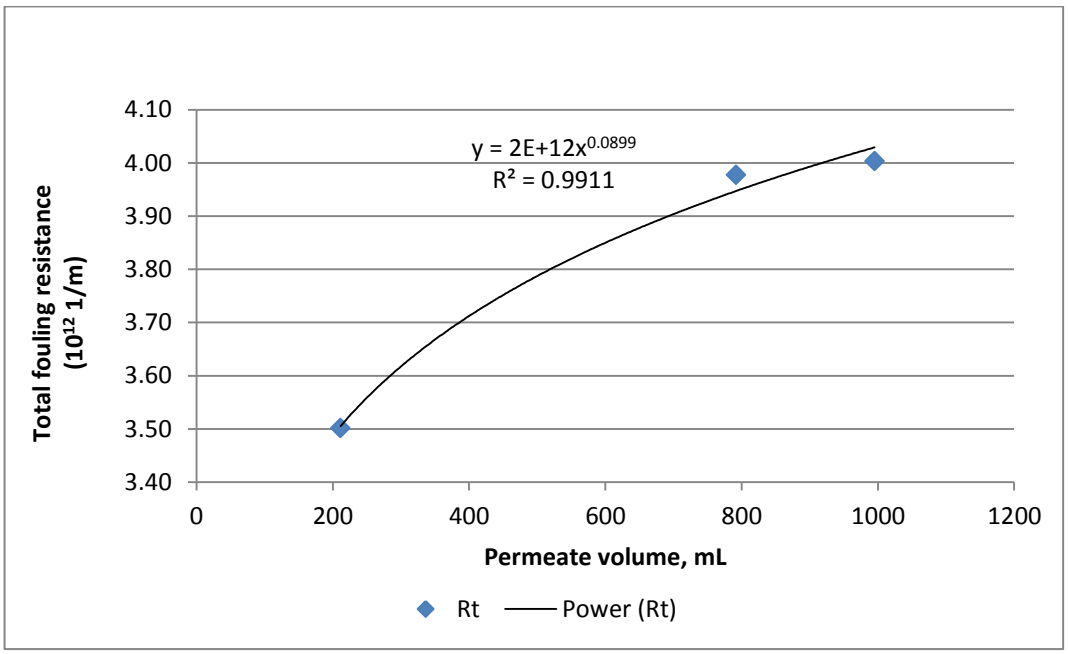


Figure B.9 : Total fouling resistance with cumulative permeate volume.

APPENDIX C : Protein and carbohydrate concentrations of original samples with their standard errors

Table C.1 : MPS protein concentrations and their standard errors with different filtration times.

Parameter	Concentration (mg/L)			Standard errors (%)		
	3	12	24	3	12	24
EFF	3.5	4.6	5.8	0.3	3.7	1.7
CakeEPSf	1.3	4.2	2.0	8.2	3.5	8.1
CakeEPSt	2.0	6.0	2.9	1.8	5.5	8.4
PhysBW	<1.0*	3.0	1.4	<1.0	2.9	0.0
ChemBW	1.9	6.0	7.0	20.4	2.2	4.6
CakeSMPf	10.3	12.8	6.0	11.8	1.1	8.4
CakeSMPt	13.1	19.2	6.3	6.9	0.6	0.2
MBR SMPf	13.7	16.1	14.2	0.9	0.4	1.7
MBR SMPt	24.7	18.6	14.8	21.5	0.1	2.5
MBR EPSf	496.6	502.1	415.3	0.3	0.3	0.1
MBR EPSt	514.5	634.6	455.9	0.3	2.0	0.6

*<1.0 :not detected

Table C.2 : MPS carbohdyrate concentrations and their standard errors with different filtration times.

Parameter	Concentration (mg/L)			Standard errors (%)		
	3	12	24	3	12	24
EFF	2.8	2.4	4.0	0.0	14.0	1.0
CakeEPSf	<1.0	1.1	1.9	-	6.5	7.1
CakeEPSt	<1.0	1.6	2.5	-	4.1	42.2
PhysBW	<1.0	1.1	2.4	-	44.9	3.8
ChemBW	1.0	2.0	4.2	53.7	3.3	2.2
CakeSMPf	5.0	6.3	4.4	1.7	2.1	4.1
CakeSMPt	6.1	8.4	4.7	3.3	4.6	6.2
MBR SMPf	19.1	15.6	15.2	15.9	5.7	7.5
MBR SMPt	a*	19.5	16.4	-	3.2	1.1
MBR EPSf	68.3	78.1	63.7	1.4	1.3	2.1
MBR EPSt	66.3	87.0	73.0	6.3	3.1	3.4

a*:Sample exhausted

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- Kayaalp N. and Kinaci C. (2012). The correlation of membrane fouling with polymeric foulants in MBR. *IWA World Water Congress & Exhibition*, September 16-21, Busan, Korea.